

**JOURNAL
OF
ASSOCIATED
MEDICAL
SCIENCES**

THE OFFICIAL PEER-REVIEWED
ONLINE JOURNAL

Volume 58 Number 2 May - August 2025 E-ISSN: 2539-6056



Journal of Associated Medical Sciences

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2. Radiologic Technology
3. Occupational Therapy
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- Issue 1: January-April
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The *Journal of Associated Medical Sciences* is supported by the Faculty of Associated Medical Sciences, Chiang Mai University, Thailand.

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The *Journal of Associated Medical Sciences* was established in 1968 as the *Bulletin of Chiang Mai Associated Medical Sciences*. For over 48 years, printed versions from Vol. 1, No. 1 to Vol. 49 were published. To advance internationally and strengthen academic quality, the journal title was changed to *Journal of Associated Medical Sciences (J Assoc Med Sci, JAMS)* in 2017 with Vol. 50, No. 1 and onwards, with online access only.

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Journal website

Homepage <https://www.tci-thaijo.org/index.php/bulletinAMS/index>

Journal E-ISSN:

2539-6056

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Faculty of Associated Medical Sciences, Chiang Mai University

110 Inthawaroros Road, Suthep, Muang, Chiang Mai, 50200

Phone 053 935072 Facsimile 053 936042

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***In vitro* activity of ethanolic extract of propolis (EEP) against *Candida albicans* pathogenicity mechanisms**

Supakit Khacha-ananda¹, Patcharin Thammasit², Anupon Iadnut³, Sirikwan Sangboonruang^{4,5}, Yingmanee Tragoolpua⁶, Khajornsak Tragoolpua^{5*}

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ARTICLE INFO

Article history:

Received 21 November 2024

Accepted as revised 26 December 2024

Available online 3 January 2025

Keywords:

Candida albicans, propolis, adhesion, yeast-to-hyphal transition, invasion, virulence enzymes.

ABSTRACT

Background: Candidiasis is an opportunistic fungal infection mainly caused by an overgrowth of *Candida albicans*. Several virulence factors, including the ability to change its morphology from yeast to hyphae and the secretion of hydrolytic enzymes, contribute to and promote the pathogenesis of the disease.

Objective: We aimed to investigate the efficacy of ethanolic extract of propolis (EEP) on growth and some major virulence factors of *C. albicans* that involved pathogenesis development, such as adhesion, hyphal germination, invasion, and virulence enzyme activities.

Materials and methods: *C. albicans* DMST 21424 was treated with various concentrations of EEP, and the growth of yeast cells was determined by colony-forming unit assay. An investigation of morpho-transformation ability was carried out using a hyphal germination assay and a hyphal length measurement. The adhesion ability of EEP-treated yeast cells was determined on both abiotic and biotic materials using acrylic discs and HeLa cell surfaces, respectively. The infected cells were treated with EEP to study invasion ability, and the cell damage was indicated by lactate dehydrogenase (LDH) activity assay. The effect of EEP on virulence enzyme activity was evaluated on sheep blood agar for hemolysin, egg yolk agar for phospholipase, and bovine serum albumin (BSA) agar for proteinase. Then, the mRNA expression levels of virulence enzyme-related genes such as *ALS1*, *SAP1*, *SAP2*, *SAP3*, and *SAP6* were assessed by quantitative reverse transcriptase polymerase chain reaction (RT-PCR).

Results: The growth of EEP-treated yeasts was suppressed in a dose-dependent manner. The yeast-to-hyphae transition property was significantly reduced in EEP-treated yeast. EEP treatment also significantly decreased adhesion, invasion, and proteinase activity. However, there was no difference in hemolysin and phospholipase activities between EEP-treated yeast and the control. Moreover, EEP also remarkably down-regulated agglutinin-like-sequence 1 (*ALS1*) and secreted aspartyl proteinase (*SAPs*) genes.

Conclusion: The findings revealed that EEP exhibited potent anti-*C. albicans* virulence factors associated with pathogenesis. Therefore, this study suggested that propolis might be an effective complementary medicine alternative for candidiasis treatment.

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doi: 10.12982/JAMS.2025.032

E-ISSN: 2539-6056

Introduction

Infection of *C. albicans* into host cells is supported by several virulence factors such as adhesion, hyphal germination, hydrolytic enzyme secretion, and invasion that contribute to pathogenesis. Adhesion, the first critical step of infection, requires adhesion molecules on the yeast cell wall, including agglutinin-like sequence (ALSp), hyphal wall protein1 (HWP1p), and other proteins, to adhere to host cell ligands and is implicated in biofilm formation. By this step, the adherent *C. albicans* release various hydrolytic enzymes to invade the epithelial cell and break down the interepithelial cell junction, enhancing the pathogenicity of infection.^{1,2}

The hydrolytic enzymes that contribute to pathogenicity are phospholipases (PLs), secreted aspartyl proteinases (SAPs), and hemolysin. Phospholipase B is the critical virulence for candida infection. It plays a role in hydrolyzing the glycerophospholipid of the host cell membrane to penetrate, invade, and interact with the host signal transduction.¹ The SAPs play a role in hydrolyzing the host cells' collagen, laminin, fibronectin, immunoglobulin, and cytokines that support adhesion, invasion, and evasion of phagocyte killing. Furthermore, these proteins provide yeast cell growth and biofilm formation nutrients.^{1,3} The hemolysin is responsible for the acquirement of the host iron, which is essential for yeast metabolism through the interaction between hyphal yeast mannoproteins and complement receptor-like molecules on the surface of the erythrocyte.^{3,4}

Recently, the typical treatment of candidiasis has been based on antifungal drugs, especially the polyene and the azole groups. The main functions of the drugs are to inhibit the ergosterol enzyme and interact with ergosterol on the cell membrane to disrupt the membrane integrity.⁵ However, all chemical synthetic medicines with prolonged treatment are associated with serious adverse effects. There is an urgent requirement for novel bioactive chemicals that exhibit antifungal activities and offer safety properties to gain better therapeutic outcomes. Propolis is a natural product obtained from honeybees, and it has been extensively identified as having components rich in phenolic and flavonoid compounds. Importantly, propolis also exhibits several biological properties, including antimicrobial activities against bacteria, viruses, and fungi, particularly antifungal activity against *Candida* sp.⁶⁻⁹ However, the evidence-based mechanism associated with virulence factors is necessarily explored.

This study aimed to evaluate the anti-candida activities of an ethanolic extract of propolis (EEP) on growth, adhesion, hyphal germination, invasion, and virulence enzyme activities, as well as the mRNA expression level of virulence genes. The findings of this work would be beneficial for further application of EEP in treating candidiasis.

Materials and methods

Microorganisms, cell culture, growth conditions, and propolis

C. albicans DMST 21424 was obtained from the

Department of Medical Sciences, Ministry of Public Health (MOPH), Thailand. This strain has been previously proven for biofilm production.^{9,10} The yeast was cultured in Sabouraud dextrose agar (SDA) (Becton Dickinson, NJ) at 37 °C. A yeast colony was picked and cultured in Sabouraud dextrose broth (SDB) (Becton Dickinson, NJ) within a shaking incubator at 37 °C overnight. The yeast cells were then harvested and adjusted to the proper cell number before treatment with various concentrations of propolis.

Prof. André Lieber from the Division of Medical Genetics, University of Washington, Seattle, USA, kindly provided HeLa cells. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, CA) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL), and streptomycin (100 µg/mL). The cell lines were maintained in a humidified atmosphere of 5% CO₂ at 37 °C.

Ethanolic propolis (EEP) powder extract was kindly gifted from Bee Product Industry Co., Ltd., Lamphun, Thailand. The EEP powder was dissolved in dimethyl sulfoxide (DMSO) to obtain a stock concentration of 500 mg/mL. A hundred microliters of a stock concentration were aliquoted and stored at -20 °C until use. The DMSO dissolved in the medium corresponding to the final concentration of EEP in each experiment was used as a solvent control.

Growth of *C. albicans*

To determine the viability of yeast cells after treatment with EEP, the colony-forming unit (CFU) assay was performed. The yeast cell suspension was adjusted to the 1×10⁶ cells and treated with various concentrations of EEP for 2 hrs at 37 °C. The treated yeasts were then collected and subjected to serial two-fold dilutions technique. The growth of yeast cells was quantified by determining the colony on an SDA plate after 48 hrs incubation at 37 °C. The yeast colonies were counted and expressed as log CFU/mL.

Hyphal germination

Yeast cells (1×10⁶ cells) were treated with EEP for 2 hrs and washed thrice with sterile PBS. Viable yeast cells were adjusted to the same amount in each experimental group and subjected to 1:4 human diluted plasma. After the incubation, the germ tube-forming cells were counted, and the hyphal length was measured under a light microscope (Olympus, Japan).

Adhesion assay

The adhesion ability of EEP-treated yeast cells was determined on both abiotic and biotic materials. The EEP-treated yeasts and solvent control groups were adjusted to an equal number at 4×10⁵ cells and placed on a sterile acrylic disc as an abiotic material model in a 96-well tissue culture plate. After cell attachment for 90 min at 37 °C, the planktonic yeast cells were removed and replaced with 100 µL of RPMI1640-MOPs. The adherent yeast cells on the acrylic discs were continuously incubated at 37 °C for another 48 hrs. The adhesion of yeast cells on abiotic

material was then subjected to sample preparation and observed by scanning electron microscope (SEM). The adhesion of the EEP-treated yeast cells was observed and photographed under an SEM (JEOL, MA).

For the study on a biotic material, the treated yeasts were cultured on the HeLa cell monolayer. In brief, HeLa cells were plated in a 24-well tissue culture plate at 37 °C, 5%CO₂ for 24 hrs. Before the co-culture condition, the EEP-treated yeasts were pre-stained with concanavalin A-Alexa Fluor® 488 conjugate (ConA-Alexa 488) (Molecular Probes, NY) for 1 hr. Then, the stained yeast cells were subjected to co-culture with HeLa cells for 90 min. After removing the planktonic yeast cells, the fluorescent-stained yeasts attached to HeLa cells were observed using the fluorescence microscope (Zeiss, Oberkochen, Germany). A fluorescent microplate reader quantified the fluorescent intensity at excitation and emission wavelengths of 495 nm and 519 nm (BioTek, Winooski, VT). The percentage of adhesion was quantified with some modification and then calculated using the following formula.¹¹

$$\% \text{Adhesion} = [(MFI_{\text{test}} - MFI_{\text{control}}) / (MFI_{\text{background}} - MFI_{\text{control}})] \times 100$$

MFI_{test} : Mean fluorescent intensity of a tested group

MFI_{control} : Mean fluorescent intensity of a control group

MFI_{background} : Mean fluorescent intensity of the background

Invasion assay

HeLa cells (2×10⁴ cells) were cultured in a 24-well tissue culture plate and maintained in a humidified atmosphere of 5%CO₂ at 37 °C. The EEP-treated yeasts (10 MOI) were added to HeLa cells for 90 mins. Then, the unattached yeast cells were removed, and DMEM medium without phenol red (100 µL) was added. After 24 hrs incubation, LDH released in a cultured supernatant of infected cells was elucidated using the CytoTox 96® Non-Radioactive cytotoxicity assay according to the manufacturer's instructions (Promega, WI). One percentage of Triton X-100 was used as the positive control. The percentage of LDH activity was calculated using the following formula.¹²

$$\% \text{LDH activity} = [(Abs_{\text{test}} - Abs_{\text{background}}) / (Abs_{\text{control}})] \times 100$$

Abs_{test} : Absorbance of HeLa cells infected with propolis-treated yeast

Abs_{background} : Absorbance of EEP-treated yeast

Abs_{control} : Absorbance of HeLa cells infected with solvent-treated yeast

Virulence enzyme activities

The influence of EEP on major virulence enzymes, including hemolysin, phospholipase, and proteinase, was investigated based on qualitative enzyme activities of clear zone production. The hemolysin and phospholipase activities were evaluated as described elsewhere. Briefly, the yeast cells (1×10⁶ cells) were incubated with EEP for 2 hrs. To examine hemolysin production, an equal amount of viable yeast cells was dropped on sheep blood agar and incubated at 37 °C for 2 days, while detection of phospholipase activity was determined on egg yolk agar at 37 °C for 4 days.¹³ The yeast cells were grown on bovine serum albumin (BSA) agar to assess proteinase activity and incubated at 37 °C for 5 days. Then, the BSA agar was fixed with 20% trichloroacetic acid, stained with 1.25% amido black, and de-stained with 15% acetic acid for 15 hrs.¹⁴ The colonies and the clear zone diameter were measured to calculate the enzyme activities.

Virulence enzyme-related gene expression

Virulence enzyme-related genes, including *ALS1*, *SAP1*, *SAP2*, and *SAP3*, were further investigated. To digest the yeast cell wall, the EEP-treated yeasts were resuspended in binding buffer (1 M sorbitol, 100 mM EDTA, 14 mM β-mercaptoethanol, and 5 U zymolyase) and incubated at 30 °C for 60 mins. Then, the total RNA of the treated yeast was extracted using NucleoSpin RNA II (MACHEREY-NAGEL GmbH & Co. KG, Germany). The cDNA was reverse transcribed following the RevertAid First Strand cDNA Synthesis Kit instruction (Thermo Fisher Scientific, MA). For quantification, real-time PCR was performed by maxima SYBR Green qPCR Master Mix (Thermo Fisher Scientific, MA) using primers for *ALS1*, *SAP1*, and *SAP2*. The expression of the mRNA level was analyzed using the 2^{-ΔΔCT} method, using *ACT1* as an internal control. The sequences of the primer and PCR reaction conditions are shown in Table 1. The PCR reaction for *ALS1* amplification was 50 cycles (initial denaturation at 95 °C, 10 mins; denaturation at 94 °C for 15 sec; annealing at 60 °C for 30 sec; extension at 72 °C for 1 min; final extension at 72 °C for 10 mins.¹⁵ For *SAPs* amplification, the PCR reaction was 60 cycles (initial denaturation at 94 °C for 3 mins; denaturation at 94 °C for 15 sec; annealing at 50 °C for 1 min; extension at 72 °C for 1 min; final extension at 72 °C for 10 mins.¹⁶

Table 1. *ALS1*, *SAPs*, and *ACT1* primers sequences.

Gene	Forward primers	Reverse primers	References
<i>ALS1</i>	5'-att ggt aaa gta act gta cca-3'	5'-cac att gaa tat gcc atg tg-3'	15
<i>SAP1</i>	5'-agg gaa agg tat tta cag t-3'	5'-gat ttg ctt aca tag taa gta-3'	16
<i>SAP2</i>	5'-tga ttg tca agt cac tta tag t-3'	5'-ctt agg tca agg cag aaa tac tg-3'	16
<i>SAP3</i>	5'-tgg att gga aca ttg cta att c-3'	5'-caa tct cca gag gag tac ttc c-3'	16
<i>ACT1</i>	5'-agc ttt gtt cag acc agc tga tt-3'	5'-gga gtt gaa agt ggt ttg gtc aa-3'	15

Statistical analysis

The data are presented as the mean $\pm$ SEM of three independent experiments for each test. An independent sample t-test was performed for paired comparisons between the untreated and the treated cells. Statistically significant differences are expressed with $p < 0.05$.

Results

Suppression of *C. albicans* growth

The result demonstrated a significant growth reduction of *C. albicans* by about 1.99 log CFU, 7.62 log CFU, and 10.06 log CFU after treatment with EEP at concentrations of 5, 10, and 20 mg/mL for 2 hrs, respectively, as compared to the solvent control. The complete inhibition of *C. albicans* survival was observed in the highest concentration of EEP at 40 mg/mL, as shown in Figure 1. This finding suggests that EEP had a dose-dependent effect on yeast cell viability.

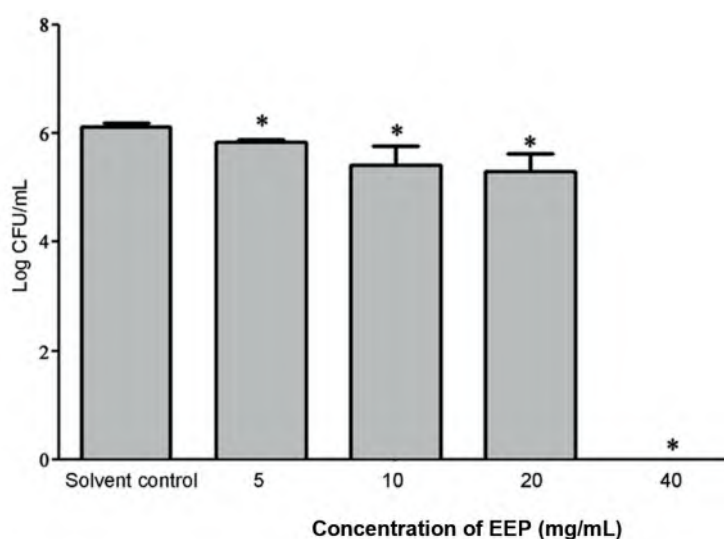


Figure 1. Inhibitory effect of EEP against *C. albicans* growth. The propolis-treated yeasts were spread on the SDA plate and determined by colony-forming unit assay. The result was expressed as log CFU/mL of a triplicate experiment. The data are represented as mean $\pm$ S.E.M. Significant differences (* $p < 0.05$) were determined by independent sample t-test and compared with the solvent control.

Inhibition of *C. albicans* adhesion

It was shown that EEP-treated yeasts demonstrated slight colonization and adhesion to the abiotic surface. The remarkable inhibition of hyphal formation was also observed in the EEP-treated condition (Figure 2a). On the other hand, yeast cells could colonize, attach, and form the condensed hyphae in the solvent control condition. Furthermore, a biological HeLa cell surface was employed as a model for examining the ability of yeast to adhere to a biotic material. From the result, EEP-treated yeasts exhibited a significant decrease in the number of fluorescent-stained yeast and the percentage of yeast

adhesion on HeLa cells when compared to the solvent control group (Figure 2b). According to these results, the ability of the yeasts to adhere to both abiotic and biotic materials was also remarkably reduced upon the treatment of EEP in a dose-dependent way. This outcome was then confirmed by the study of *ALS1*, the *C. albicans* cell wall adhesin. The transcriptional level of *ALS1* was down-regulated in EEP-treated yeasts, as shown in Figure 2c. The most significant suppression of *ALS1* mRNA expression was observed at EEP concentration of 5-20 mg/mL. These results indicated that propolis might inhibit yeast adhesion by reducing the expression of *ALS1* mRNA.

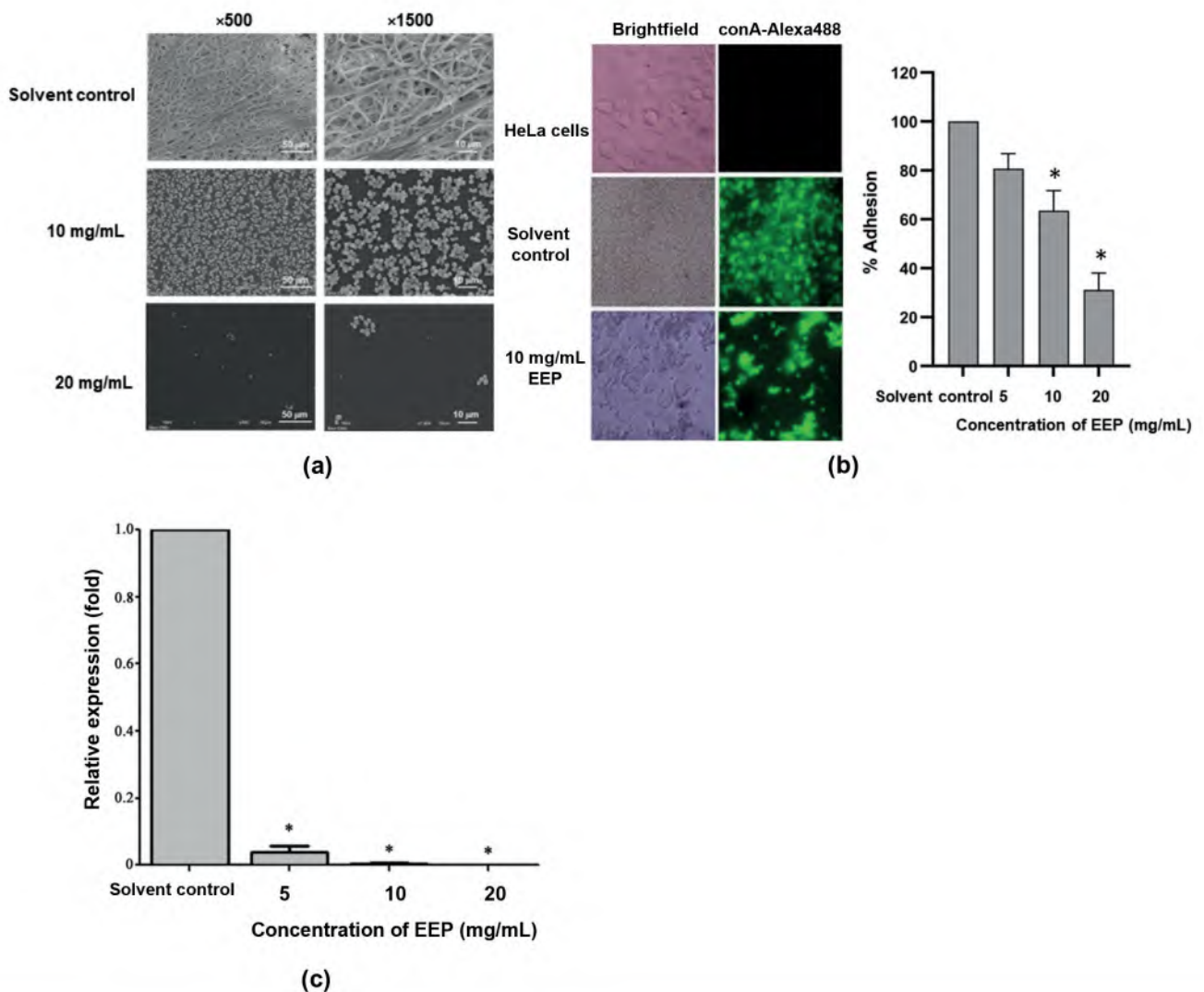


Figure 2. EEP reduced the adhesion ability of *C. albicans*. (a) The EEP-treated yeasts were cultured on the acrylic disc and imaged by a scanning electron microscope (SEM) with a magnification of 500 $\times$; scale bar 50 μ m, and 1,500 $\times$; scale bar 10 μ m. (b) The adhesion activity of EEP-treated yeast on epithelial cells was stained with conA-Alexa488. The fluorescent-stained yeasts were analyzed by photography, fluorescent intensity quantification, and the percentage of adhesion. (c) The relative fold change of the ALS1 mRNA expression level of the EEP-treated yeast. The data are represented as mean $\pm$ S.E.M. Significant differences (* $p < 0.05$) were determined by independent sample t-test and compared with the solvent control.

Decrease in hyphal germination

Based on earlier results demonstrating the inhibition of yeast adhesion, we hypothesized that it would then influence hyphal germination. EEP significantly and dose-dependently decreased the hyphal development in *C. albicans* compared to those treated with solvents (Figure 3). In *C. albicans* treated with EEP at concentrations of

5, 10, and 20 mg/mL, the rate of hyphal formation was approximately 80, 19, and 3%, respectively. The percentage of hyphal formation and hyphal length are shown in Table 2. Our findings revealed that EEP reduces the yeast adhesion ability, which results in a diminished yeast-to-hyphal transition in *C. albicans*.

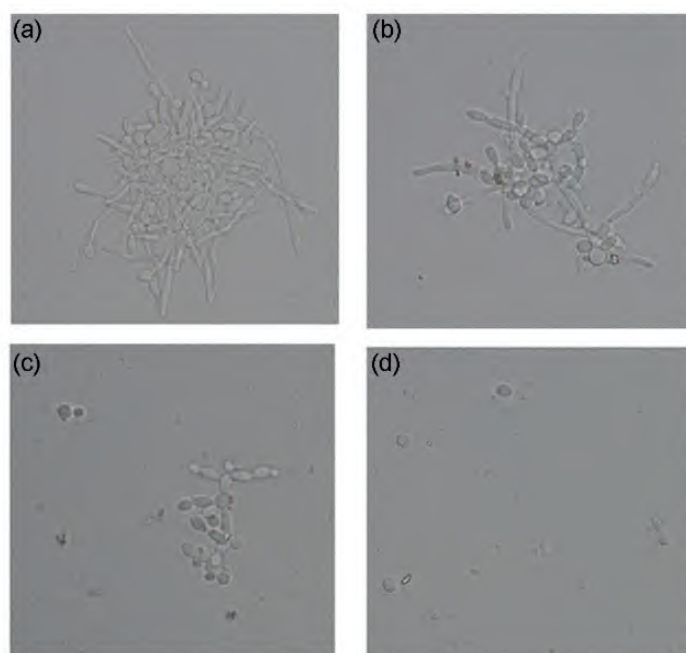


Figure 3. EEP interfered with the ability of *C. albicans* to transition from yeast to hyphal growth. The hyphal formation was visualized and photographed under a light microscope (40× magnification) on (a) solvent control, (b) 5 mg/mL, (c) 10 mg/mL, and (d) 20 mg/mL of EEP. Five hundred yeast cells were counted and measured the hyphal length by imaging software Image J.

Table 2. Percentage of hyphal germination and length in EEP-treated yeasts.

EEP concentration (mg/mL)	Hyphal formation (%)	Hyphal length (μm)
Solvent control	96.70 ± 0.08	26.78 ± 0.18
5	80.30 ± 0.34	18.94 ± 3.01
10	19.00 ± 0.30*	10.53 ± 2.34*
20	3.00 ± 0.16*	8.65 ± 5.79*

The data are represented as mean ± S.E.M. Significant differences (* $p < 0.05$) were determined by independent sample t-test and compared with the solvent control.

Reduction of invasion on propolis-treated yeasts

Given that EEP diminished the yeast-to-hyphal transition, we speculated whether it might have a consequence on *C. albicans*'s invasion step. The LDH activity was assessed to demonstrate host cell damage

induced by yeast invasion. LDH activity was reduced by almost 80% when propolis-treated yeasts and HeLa cells were co-cultured, as shown in Figure 4. This result implied that propolis effectively prevents *C. albicans* invasion *in vitro*.

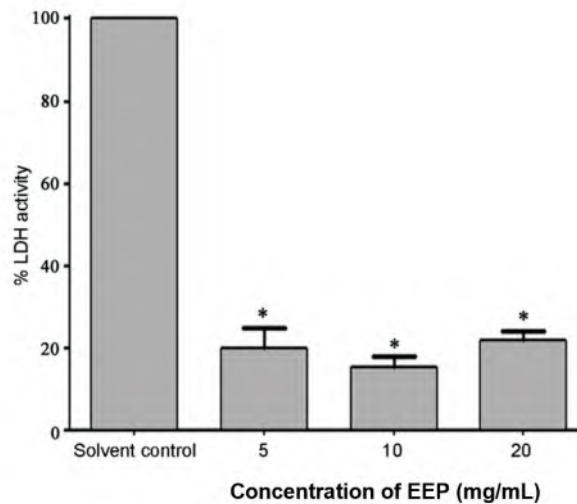


Figure 4. EEP reduced the invasion ability of *C. albicans*. The percentage of LDH activity of invading HeLa cells was measured after treatment with EEP for 24 h. The data are represented as mean ± S.E.M. Significant differences (* $p < 0.05$) were determined by independent sample t-test and compared with the solvent control.

Inhibition of virulent enzyme activities by propolis

The virulence enzyme activities were determined since they are implicated in yeast adhesion, hyphal germination, and invasion. It was shown that EEP did not affect the hemolysin and phospholipase activities of *C. albicans*. Remarkably, all EEP concentrations significantly decreased the proteinase activity by 1.00 ± 0.00 , indicative

of negative proteinase activity, as shown in Table 3. Then, qRT-PCR was carried out to verify the effect of propolis on the *SAPs* mRNA. The results showed that propolis significantly down-regulated mRNA expression of *SAP1*, *SAP2*, *SAP3*, and *SAP6* (Figure 5). According to these results, EEP exhibited an ability to suppress the transcriptional and translational levels of proteinase enzymes.

Table 3. Virulence enzyme activities of EEP-treated yeasts.

Propolis concentration (mg/mL)	Virulence enzymes activities (mean ± S.E.M)		
	Hemolysin	Phospholipase	Proteinase
Solvent control	0.281 ± 0.054	0.775 ± 0.178	0.733 ± 0.058
5	0.320 ± 0.122	0.876 ± 0.034	1.00 ± 0.00*
10	0.345 ± 0.103	0.842 ± 0.144	1.00 ± 0.00*
20	0.269 ± 0.012	0.559 ± 0.328	1.00 ± 0.00*

The data are represented as mean ± S.E.M. Significant differences (* $p < 0.05$) were determined by independent sample t-test and compared with the solvent control.

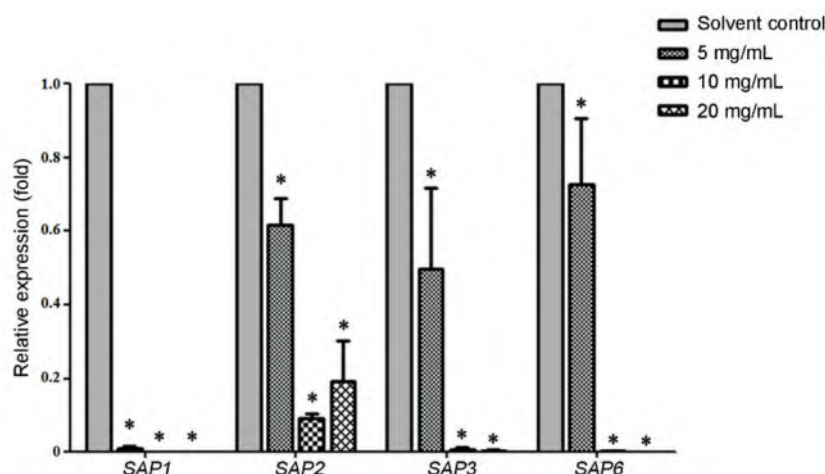


Figure 5. EEP down-regulated SAPs mRNA expression. The expression level of SAPs mRNA was determined by qRT-PCR normalized to the ACT1 mRNA level and expressed as a fold change with the solvent control. The data are expressed as mean $\pm$ S.E.M. Significant differences (* $p < 0.05$) were determined by independent sample t-test and compared with the solvent control.

Discussion

Even though antifungal drugs are designated standard treatment for *Candida* infection, various adverse effects and the chance of resistance development remain concerns. In recent decades, several studies have highlighted the potential of natural products and their derivatives as alternative therapeutic agents. Of them, propolis has been suggested as a candidate for therapeutic use.^{9,17,18} It has been utilized in traditional medicine worldwide to treat and prevent many diseases, and its safety profile has been reported in animal models and humans.¹⁹⁻²¹ In treating *Candida* infection, controlling invasive candidiasis necessitates deactivating the essential stage of pathogenesis and virulence. Hence, the effect of propolis on major phenotype characteristics and virulence factors is explored. This present study assessed the anti-*Candida* activities of EEP on adhesion, yeast-to-hyphae transition, invasion, and virulence enzymes. Our findings demonstrated that EEP inhibited the growth of *C. albicans* in a dose-dependent manner, which might have resulted from the combined actions of several chemical components in propolis, particularly polyphenol compounds.²² Compared to other natural extracts, such as curcumin, a natural polyphenol known for antimicrobial and anti-inflammatory activities, EEP is more effective against *Candida albicans*. In contrast, curcumin has less effect on *Candida* strains.²³

However, different types of propolis present diverse properties due to collection time and geographical origin, and they might offer different biological activities depending on their chemical compositions and characteristics.²⁴ In this study, several phenolic chemical classes of propolis were previously characterized such as gallic acid, quercetin, pinocembrin, chrysin, and galangin.⁹ Gallic acid and its derivatives have lipophilicity and hydrophilicity, which makes it easier to facilitate substances to pass through the cell wall and membrane, allowing the distribution and accumulation of bioactive molecules in

the cytoplasm and mitochondria.²⁵ Hence, it is likely that phenolic chemicals exerted an effect on the damaged cell membrane and interfered with cell respiration and the production of highly reactive molecules. Li *et al.*, 2017 found that gallic acid decreased ergosterol in the cell membrane and the survival of *C. albicans* by interfering with the sterol biosynthetic pathway.²⁶ Moreover, several types of phenolic compounds disturb the function of mitochondria, involving cell respiration and energy production.²⁷ Likewise, the lipophilic group of phenolic compounds was reported to have an inhibitory effect on ATPase activity in *Saccharomyces cerevisiae*, resulting in interfering cellular respiration.²⁸

The adherence of *C. albicans* is the initial step for yeast pathogenicity and contributes to the structural integrity of biofilms, which are frequently found on implanted medical devices. It causes persistent infections and promotes drug resistance. Therefore, limiting biofilm formation is the ultimate goal in preventing invasive candidiasis.²⁹ In another study, we also found that EEP suppressed the adhesion ability of *C. albicans* to the surface of acrylic discs and HeLa cells.³⁰ There are various mechanisms underlying the inhibition of yeast adhesion, such as disrupting the fungal cell membrane by inhibiting the biosynthesis of cell wall components, degrading enzyme production, downregulating adhesin genes, and blocking adhesin and host cell receptor interactions.³¹ In this study, we further investigated whether the reduction of yeast adhesion was related to the down-regulation of the *ALS1* gene, a virulence gene of the adhesion family. The result demonstrated a significant down-regulation of the *ALS1* gene of *C. albicans* after treatment with EEP. The role of *ALS1* in adhesion was confirmed since the mutant *ALS1* resulted in a deficiency in adhesion on epithelial cells, and the fungal burden of mice was decreased.³² Additionally, some flavonoids with long hydrophobic chains are associated with reduced contact between microbial and epithelial cells.^{33,34} Besides, the components

in propolis can also suppress phospholipase, restricting the adhesion of fungal cells to epithelial cells.³⁵

Yeast morphogenesis is crucial for the development of invasive candidiasis. The yeast-to-hyphal transition in *C. albicans* is essential in developing the disease process. This hyphal phase can cause tissue damage by invading mucosal epithelial cells and leading to systemic infection.³⁶ According to our findings, the hyphal formation and length of EEP-treated yeasts were decreased in a dose-dependent manner. It has been reported that propolis might induced the alteration of cell membrane structure and cytoskeleton reorganization that led to the loss of plasma membrane integrity and morpho-transformation of fungal cells.³⁷ As antioxidant substances, these compounds might decrease the ROS production generated during an initial stage of germ tube formation and filament elongation.³⁸ Reversible cellular morphogenesis is an important virulence factor that facilitates the invasion of host tissues. We revealed that administrating EEP significantly reduced the production of LDH from infected cells, indicating that treating with EEP inhibits the yeast invasion process and might have a protective effect against cell damage.

Since different pathogenesis steps are involved and regulated by several hydrolytic enzymes, we investigated whether EEP affected the virulence enzymes that influence yeast morphogenesis. SAPs are key extracellular hydrolytic enzymes encoded by a family of 10 *SAP* genes of *C. albicans* that play a pivotal role in colonization and invasion. In different phases of yeast cells, *SAP1-SAP3* genes are predominately expressed in yeast form, *SAP4-SAP6* are responsible for the hyphae stage, and *SAP1* and *SAP3* were mainly expressed during phenotypically switched cells.^{1,2} Herein, the EEP-treated yeast showed down-regulated levels of *SAP1,2,3* and *6* mRNA expression, suggesting that EEP might inhibit proteinase production. These results support the inhibition of yeast-to-hyphae transition characteristic of *C. albicans* by the down-regulation of *SAP1-3* genes and eventually lacking colonization and invasion ability. Moreover, suppression of *SAP6* was associated with cell wall function loss, as correlated with the previous finding.³⁹ Propolis acts by blocking the interaction between the functional group in phenolic compounds and proteinase enzymes by forming hydrogen bonds with aspartic acid 32 (Asp32), threonine 221 (Thr221), and threonine 222 (Thr222) in the active site region of SAP enzymes.⁴⁰ Additionally, it might act on the ATPase in the plasma membrane, leading to a decrease in enzymatic activity and the efflux of SAP enzyme throughout the cell membrane.⁴¹ Furthermore, the mode of action of propolis against *Candida* planktonic and biofilm was also previously mentioned. These effects were found in *C. albicans* and non-*albicans Candida* species, such as *C. tropicalis* and *C. parapsilosis*.⁴² Thus, propolis could be one of the candidates for antifungal natural products for *Candida* infection.

However, EEP did not affect the phospholipase and hemolysin activities. This is consistent with previous reports showing no effects of the combinations of phenolic acid and azoles on phospholipases and hemolytic activities

in *C. albicans* and *C. bovina* strains.⁴³ To strengthen the potential of EEP for therapeutic use, other *Candida* species and drug-resistant strains would be additionally investigated. Moreover, an evaluation of the EEP effect in animal models is still required to support its therapeutic application in *Candida* infection.

Conclusion

We provided more insights into the underlying mode of propolis on *C. albicans* pathogenesis and virulence genes. According to this study, EEP reduced critical pathogenicity steps involved in adhesion, morpho-transition, and invasion to the host cells through the down-regulation of several virulence enzymes. These findings reveal the potential of EEP as an anti-virulence substance. However, further application of EEP in invasive candidiasis treatment necessitates an *in vivo* investigation.

Conflict of interest

The authors declare no conflict of interest.

Funding

This research was funded by Research and Researcher for Industry (RRI) fund from The Thailand Research Fund, grant No. PHD56I0018 and CMU Research Fellowship Program 2555, Chiang Mai University, Thailand. This research work was also partially supported by Chiang Mai University.

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The short effect of sports liniment on muscular physiological responses and physical performance among male Muay Thai fighters: Case study

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ARTICLE INFO

Article history:

Received 4 November 2024

Accepted as revised 24 December 2024

Available online 3 January 2025

Keywords:

Massage, Thai boxing, sports liniment, oxygen, tape stripping.

ABSTRACT

Background: Sports liniments are commonly used as a preparatory phase before an activity. Muay Thai fighters, such as Namman Muay (NM), have generally been used, but the scientific evidence on physiological and sports performance effects has not been studied.

Objective: This case study aimed to investigate the effects of NM sport 'boxing' liniment on muscular physiological responses and physical performance outcomes among male Muay Thai fighters.

Materials and methods: Four Muay Thai fighters were recruited into the case study and equally divided into two groups: the tape stripping (TS) (N=2) and the massage (MG) groups (N=2). The TS group received 20 tape strips across the muscle before receiving 3 mL of NM, while the MG group received 3 mL of NM with an effleurage massage for 5 minutes. Two tests were performed on Day 1) with no application of NM, and on Day 2), 3 mL NM was applied and randomly allocated into the TS group or MG group. Physiological responses such as skin temperature (ST), muscle oxygen (SmO₂), myoglobin (Mb), and hemoglobin (Hb) were evaluated by Fluke thermography and Moxy Monitor (Near Infrared Spectroscopy; NIRS). Physical performance as knee extensor and hip flexor strength was assessed using a Lafayette handheld dynamometer and isometric endurance hip flexion test as a time to failure (TTF). Data analysis was performed using a descriptive study, evaluating the data results between two days of the experiment.

Results: ST did not increase after application of NM or receiving either TS or MG. SmO₂ Mb percentage for the TS group increased between day 1 and day 2, with a mean difference of 10.78. The MG group observed only one participant increasing Mb with a group mean difference of 7.56. The Hb percentage observed little change and remained relatively constant between the two days. There were no observational differences in knee extension strength; however, Hip flexion strength observed more significant increases in the TS group, with a mean of 2.45 kg, while the MG group observed a mean increase of 0.65 kg after receiving the NM. The isometric endurance test observed increases in both groups, with higher increases in the MG group mean of 66.43 sec and TS group mean of 37.33 sec, after having NM applied. The 3 mL NM applied onto the Rectus Femoris muscle as a precursor to a warm-up increased SmO₂ values.

Conclusion: Tape stripping with NM enhances its permeation and may improve isometric strength, while massage with NM appears to increase isometric muscular endurance among male Muay Thai fighters.

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doi: 10.12982/JAMS.2025.033

E-ISSN: 2539-6056

Introduction

The study delves into the widespread presence of Muay Thai gyms in Thailand and globally, underlining the popularity of sports liniment such as Namman Muay (NM) among athletes, with 30% of sales attributed to its use. Namman Muay, a product containing 31% Methyl Salicylate and 1.25% menthol, known as active pharmaceutical ingredients (APIs), has a rich historical and cultural significance within Muay Thai.¹ With an estimated three hundred million Muay Thai practitioners worldwide,² the product's association with the sport has been instrumental in its success. Also, Muay Thai training camps in Thailand provide both fighting and cultural insights.³

Despite the everyday use of warm-up rubs like NM by Muay Thai athletes to enhance performance and aid in injury recovery, there is a noticeable lack of academic studies focusing on the specific use of sports rubs in Muay Thai. However, general warm-up and recovery principles suggest that such rubs can benefit fighters by improving their performance and aiding recovery. Over-the-counter sports rubs in Thailand contain two key APIs: methyl salicylate and L-menthol. These APIs serve as counterirritants on the skin, inducing sensations of warmth and soothing effects,⁴ dilating surface blood vessels, ultimately desensitizing sensory neurons, and alleviating pain.⁵

The challenge lies in determining how effectively sports liniments penetrate the skin to warm underlying tissues, potentially increasing muscle oxygenation or acting as an analgesic. Quantifying the penetration and absorption of APIs, particularly in the dosage form, poses difficulties for pharmaceutical researchers. The skin, the body's largest organ, presents a significant intrinsic and extrinsic barrier, with the stratum corneum acting as a formidable obstacle for APIs. Pharmaceutical companies have explored using nanoparticles, including prodrugs like methyl salicylate, to enhance permeation, especially for drugs with poor solubility or permeability.⁶

In the pursuit of enhancing permeation for topical applications, various extrinsic methods have been proposed. While some techniques require expensive equipment, cost-effective methods like massage and tape stripping have shown promise. A recent study demonstrated that local perfusion and systemic Hemodynamics using effleurage, comparing massage limbs, showed significant differences with increases in two outcome measurements, using laser Doppler flowmetry and reflection photoplethysmography.⁷ Recent research has highlighted the efficacy of tape stripping in enhancing drug delivery,⁸ percent for substances like caffeine (28.2%), sorbic acid (21.6%), and testosterone (7%) when employing tape stripping compared to non-tape stripping methods.⁹ Thereby, tape stripping demonstrates notable enhancements in drug uptake percentages for various substances.

This study focuses on the Muay Thai kicking technique, specifically the roundhouse kick (RHK), known for heavily engaging the rectus femoris hip flexor and knee extensor muscles.¹⁰ RHK is a favored technique among fighters due to its combat effectiveness.¹¹ Nevertheless, the efficacy

of topically applied sports rubs for warm-up purposes remains inconclusive. By pinpointing the rectus femoris muscle, it becomes possible to isolate and concentrate on strength testing, underscoring the significance of overall muscle strength in optimizing athletic performance. In general, valuable muscular strength values can be obtained by identifying specific muscle groups for strength evaluation, such as through isometric strength testing using handheld dynamometers. This is essential as muscular strength is vital in improving athletic performance by enhancing force-time characteristics, which are crucial for an athlete's ability to execute various sports-specific skills effectively. The pursuit of muscular strength values in sports is driven by the desire to optimize performance and prevent injuries, tailor training programs, facilitate long-term athlete development, and contribute to the body of scientific knowledge in sports medicine science.¹² The rectus femoris muscle, serving as both a hip flexor and knee extensor, can lead to unusual strength values during assessments due to its biarticular nature crossing the hip and knee joints. This complexity arises from its ability to simultaneously influence movements at both joints, affecting strength measurements depending on the hip and knee positions during testing. Studies indicate that muscle length during exercises impacts muscle damage and strength output, with variations in strength values observed based on different activities such as sprinting or walking.^{13,14} Factors such as joint angles and assessment methods like isometric and isokinetic testing contribute to the uniqueness of strength measurements in the rectus femoris muscle. This study measures isometric strength and endurance. The handheld dynamometer measures the maximum force output (strength), while the muscular hold for time to failure measures the duration of sustained contraction (endurance). While both tests are valuable, they serve different purposes in assessing muscle function. Strength assessments can help diagnose conditions related to muscle weakness, whereas endurance tests can inform training programs and rehabilitation strategies focused on improving stamina.¹⁵

Raising skin temperature is crucial for preparing for combat sports such as Muay Thai. This process enhances muscle flexibility and performance, reducing the risk of strains and injuries during intense activities. Backed by research, effective warm-up routines can reduce injury rates by up to 50% in various sports.^{16,17} A proper warm-up readies the neuromuscular system, improving coordination and reaction times for executing combat sports techniques. It also primes the cardiovascular system for the physical demands of Muay Thai, ensuring endurance during training sessions. Dynamic stretching and sport-specific drills boost muscle blood flow, elevating skin temperature and enhancing flexibility. Starting a warm-up at a lower intensity and gradually increasing it helps minimize the risk of cardiac stress and muscle damage. Elevated skin temperature is vital for peak performance and injury prevention in Muay Thai. A structured warm-up routine incorporating dynamic movements and intensity progression effectively prepares

athletes for the rigors of their training.¹⁸

Perrey's emphasis on the complexity of muscle oxygenation in human physiology during exercise underscores the importance of understanding this process.¹⁹ Near Infrared Spectroscopy (NIRS) has emerged as a valuable tool for measuring physiological oxygen indicators in sports and exercise.²⁰ The Moxy monitor, enabling real-time and continuous monitoring of muscle oxygen levels, is vital in enhancing aerobic capacity without compromising speed, essential attributes for Muay Thai practitioners. Studies have shown that muscle isometric voluntary contractions may be linked to increased SmO₂ levels, underscoring the importance of monitoring athletes to aid their development and objective achievement.²¹

Thus, the primary objectives of this case study were to evaluate the effects on muscle oxygenation, myoglobin (Mb) and hemoglobin (Hb) oxygen levels, knee extension/hip flexion strength, and isometric muscle endurance following the application of 3 mL of NM to the muscle, administered through either tape stripping or massage techniques, among male Muay Thai fighters.

Materials and methods

Participants

The research protocols underwent thorough evaluation and approval by the Centre for Ethics in Human Research at Khon Kaen University, Thailand (Ref No: HE662203). The study involved Muay Thai trainers from a gym in Khon Kaen, Thailand, who met specific criteria: actively training fighters daily and having engaged in fights within the past 18 months. They agreed to refrain from consuming alcohol and energy drinks before the study. Exclusion criteria encompassed allergies to aspirin, heart

conditions, recent injuries, and sensitivity to tape. Each participant's dominant leg, used for kicking, was determined. The research was conducted in a controlled setting between 9:00 am and 3:00 pm, maintaining a temperature of around 21 °C.

Experimental procedures

The experimental design comprised two days of testing. On Day 1, participants underwent assessments without using sports liniment or any interventions. Measurements included oxygen values, skin temperature, isometric strength tests for knee extension and hip flexion, and assessing isometric hold time to failure (TTF).

On Day 2, participants were randomly assigned to either the TS group (black card) or the MG group (red card) through a selection process using playing cards. Both groups received the application of 3 mL of sports liniment. Before the experiment, participants underwent health screenings and physical anthropometrics. They then engaged in the same experimental measurement protocol as seen on Day 1. i.e., oxygen and skin temperature were recorded, and various strength tests were performed using a handheld dynamometer and a kettlebell.²²

Interventions

The tape stripping (TS) group had 20 tape strips applied to the rectus femoris muscle of their dominant kicking leg within a 9x6 cm patch (Figure 1), followed by using 3 mL of sports liniment inside the patch. Conversely, the massage group (MG) had the same 9x6 cm patch applied over the rectus femoris muscle of their dominant kicking leg, with the sports liniment applied within the patch, followed by a 5-min session of massage/effleurage.



Figure 1. The 9x6 cm patch used to contain the Namman Muay.

Outcome measurements

Skin temperature

Skin temperature was measured using a Fluke Thermography camera (TiS20+ Max thermal imaging camera, Fluke®, Washington, USA), known for its reliability and accuracy within the specified temperature range. The camera was positioned at a standardized distance and angle from the rectus femoris muscle, ensuring consistent and precise recordings. Infrared thermographic cameras showed excellent reliability in both intra and inter-measurements.²³⁻²⁵

Muscle oxygenation

Muscle oxygenation was assessed using the Moxly Monitor (Moxly 5, Fortiori Design, Minnesota, USA) with Near Infrared Spectroscopy technology (NIRS), capturing wavelengths between 670 and 810 nm. The monitor was positioned in the center of the designated patch (Figure 1), recording Mb and Hb levels post-sports liniment application and post-experiment. At the same time, the participants were in a supine position. The validity and reliability of this method have been confirmed, making it a suitable and

accurate tool for measuring muscle oxygen levels in sports settings.^{19,26}

Muscle strength

Muscle strength was evaluated using a Lafayette handheld dynamometer (01165A, Lafayette, Indianapolis, USA), measuring in kilograms (kg). Knee extension and hip flexion tests were conducted with one leg secured to the plinth, and three measurements were taken to calculate the mean score. The study compared the results of the tests performed without the sports liniment and with the application of 3 mL of sports liniment. During the tests, stabilization with a belt on the non-dominant leg showed reliable outcomes for assessing knee and hip strength. It is advisable to have consistency in examiners to avoid discrepancies in results, emphasizing the importance of using the same tester throughout the evaluations.²⁷

Isometric hold during 95° hip flexion

Participants in the study were required to lift kettlebells weighing 8 kg, 10 kg, or 12 kg from the floor using their foot, achieving a hip flexion angle of 95° as

measured by a handheld goniometer. The participants were instructed to maintain this position for as long as possible, with the "Time to Failure" stopwatch recording the duration in seconds. The stopwatch was immediately stopped if participants exhibited backward leaning or dropped their hip below 90° of flexion. Each participant underwent this test once per day, totalling two tests throughout the protocol. Previous research has suggested that isometric tests can provide insights into the muscle's oxygen saturation levels.²¹

Statistical analysis

A descriptive statistical analysis was conducted to investigate the impact of tape stripping and massage interventions on various dependent variables among four participants divided into two groups. Each participant received 3 mL of Namman Muay on the rectus femoris muscle of their dominant kicking leg. The dependent variables included skin temperature, myoglobin, and hemoglobin levels, as well as isometric strength tests for hip flexion and knee extension and an isometric endurance test measuring the time to failure of the hip flexor in the dominant leg.

The analysis compared measurements taken on Day 1 (pre-intervention) with those on Day 2 (post-intervention). The study focused on calculating the differences in each dependent variable between the two days to assess the effects of the interventions. Descriptive statistics were utilized to present the changes observed in each variable on both days, offering a comprehensive summary of the outcomes.

Results

In this case study, the baseline characteristics Table 1 revealed that 4 male Muay Thai fighters were recruited, with an average age of 34.5 years old, height of 1.65 meters, weight of 65.6 kg, and BMI of 23.95 kg/m². No participants left the study. Table 2 reveals ST between the TS and MG groups, with temperatures decreasing within groups from Day 1 to Day 2 after applying 3 mL Namman Muay. The results of Mb and Hb oxygen percentile levels without NM (Day 1) and with 3 mL NM (Day 2) show that Mb percentage levels increased in both TS participants (64.0 to 78.85% and 79.28 to 86.00%); however, only 1 participant in the MG group (78.28 to 86.71%). The mean increase in the TS group was 10.78, while the MG group was 7.56. The Hb percentage reported in Table 2 also shows that the mean MG group value observed a slight increase of 0.57, while the TS group observed a slight decrease of 0.88 after 3 mL NM was applied.

Table 3 depicts the variations in hip flexion strength between Day 1 (NM) and Day 2 (NM) across the 3 mL TS and MG groups. The findings indicate that the TS group observed more significant increases with a mean value of 2.47 kg, while the MG group's mean was 0.65 kg. Table 3 also shows the Isometric endurance test time to failure (TTF) observing increases on Day 2 compared to Day 1, with the TS group reporting a mean increase of 37.33 sec (30.52 and 44.14), while the MG group observed a mean rise of 66.43 sec (64.84 and 68.03).

Table 1. Participants baseline characteristics (N=4).

Participant	No. 1	No. 2	No. 3	No. 4	Mean
Age (years)	33	30	31	44	34.5
Height (cm)	163.0	167.0	159.0	173.0	165.0
Weight (kg)	62.2	64.0	57.2	78.9	65.6
BMI (kg/m ²)	22.6	23.4	23.4	26.4	23.95

Note: These are the characteristics of the four participants who were Muay Thai fighters with an observation that participant No.4 is not consistent with other three participants.

Table 2. Physiological outcomes differences in skin temperature (°C), myoglobin (%), and hemoglobin (%) between the tape stripping group (N=2) and massage group (N=2).

Physiological outcome tests	Day 1 No treatment					Day 2 Treatment Tape stripping (TS) or massage (MG) with 3 mL Namman Muay [change]			
		No.1	No. 3	No. 2	No. 4	No. 1 (TS)	No. 3 (TS)	No. 2 (MG)	No. 4 (MG)
Skin temperature (°C)	Pre	36.8	35.6	34.8	34.9	32.5	33.2	32.3	34.9
	5-min Post	35.1	35.2	35.5	36.7	32.3	32.5	32.9	33.8
	Post (End)	35	34.9	35.7	36.7	32.6	32.4	31.8	33.9
Myoglobin, Mb % (Post end test)	64.00	79.28	86.57	78.28	78.85 [+14.85]	86.00 [+6.72]	85.7 [-0.87]	86.71 [+8.43]	
Hemoglobin, Hb % (Post end test)	12.55	12.17	11.84	11.71	11.57 [-0.98]	12.27 [+0.1]	11.91 [+0.2]	12.21 [+0.5]	

Table 3. Physical outcomes reporting mean testing values, Tape stripping group (N=2) and Massage group (N=2) without Namman Muay and 3 mL of Namman Muay.

Physical outcome tests	Day 1 No treatment				Day 2 Treatment Tape stripping (TS) or massage (MG) with 3 mL Namman Muay [change]			
	No. 1	No. 3	No. 2	No. 4	No. 1 (TS)	No. 3 (TS)	No. 2 (MG)	No. 4 (MG)
Hip flexor strength (kg)	9.9	10.1	11.1	13	13.3 [+3.4]	12.1 [+1.5]	11.8 [+0.7]	13.6 [+0.6]
Knee extension strength (kg)	8.2	9.8	7.3	9.2	8.5 [+0.3]	10.23 [+0.4]	7.3 [0.0]	9.8 [+0.6]
Isometric (Time to failure, sec)	17.2	21.9	26.3	22.2	47.71 [+30.52]	66.04 [+44.14]	91.14 [+64.84]	90.23 [+68.03]

Discussion

The results of this case study suggest the potential benefits for Muay Thai fighters when 3 mL NM is used as part of the warm-up training. The two mechanical enhancers used to promote penetration of the liniment, massage (effleurage) and tape stripping, could be challenged in a real-life situation, with massage being the more likely method to be used.

The ST was a surprise, with a lack of increases being observed after the interventions, in particular within the massage group due to the friction of massage exerting pressure on the stratum corneum, assuming an increase in both skin temperature and enhancing the distribution of the API or Transpennedgeal across the skin pathway would take effect.^{28,29} Elevating skin temperature through a warm-up routine before engaging in physical activities has garnered support from numerous studies that underscore its performance enhancement and injury prevention advantages.¹⁶⁻¹⁸ The physiological benefits of warming up are substantial, as it triggers changes in the body that prime it for exercise. A key effect is the elevation of muscle temperature, which boosts muscle elasticity and diminishes the risk of injuries. This temperature increase can also

optimize metabolic processes, ultimately improving performance outcomes.³⁰ Furthermore, the skin and muscle temperature escalation during warm-up enhances muscle function, including increased muscle contractility and quicker reaction times. Research has demonstrated that higher temperatures can elevate the rate of force development and enhance overall muscle performance, which is particularly crucial in sports that necessitate explosive movements like Muay Thai.³¹

Sports liniments containing Methyl Salicylate and L-Menthol being applied before warm-ups may have an inhibitory effect on increases in skin temperature due to their vasodilatory effects, the body's response to cold, altered sensory perceptions, and potential impacts on muscle activation.^{5,32} In practice, the inhibition of skin temperature increases can significantly affect athletic performance, increase injury risk, and alter psychological readiness. The penetration of methyl salicylate first acts on the enzyme cyclooxygenase by inhibiting it. This prevents prostaglandin and thromboxane A2 from reacting as inflammatory mediators and counteracting the increase in skin temperature. Increasing skin temperature has beneficial effects; however, as seen in this case study,

reducing skin temperature may be counterproductive. Athletes and coaches should be mindful of these effects when incorporating topical sports liniments containing methyl salicylate and L-menthol into their routines. Future studies may consider increasing the liniment dosage and a more extensive study population to ascertain whether this raises skin temperature.

The potential impact of sports liniment containing methyl salicylate and L-menthol on muscular myoglobin levels has not been extensively studied. However, the vasodilatory properties of these compounds could improve blood flow to muscles. This enhanced perfusion may lead to increased oxygen delivery, indirectly affecting myoglobin levels.⁵ The protein myoglobin binds and stores oxygen, and when Muay Thai fighters begin to perform, oxygen is delivered to these muscles. Helping muscles to contract further and produce energy. However, if the intensity is too high or lasts too long, the muscles may not receive enough oxygen from myoglobin, leading to fatigue and injury.¹⁹ If the APIs in NM can increase myoglobin, this will benefit Muay Thai fighters. The TS group's mean increase was 2.45 kg after 3 mL NM with methyl salicylate reacting to the muscle to increase blood flow.^{33,34} This value may have a significant clinical effect on the hip flexor strength among Muay Thai fighters, stability, and agility while also playing a role in injury prevention.

Hemoglobin saturation levels were negligible, which was an unexpected outcome. Hemoglobin levels tend to remain steady during physical performance, such as Muay Thai fights, due to several physiological mechanisms that regulate oxygen transport and utilization in the body. Factors contributing to this stability are adaptations to training: Over time, athletes develop physiological adaptations that enhance their ability to utilize oxygen efficiently. These adaptations include improved muscle oxidative capacity and increased capillary density, which facilitate better oxygen extraction from the blood, thereby allowing hemoglobin levels to remain stable even during strenuous activity.³⁵

Muay Thai fighters require various subcategories, including maximal endurance and explosive strength. The specific demands for these types of strength depend on the intensity, duration, and frequency of muscle contractions during training and competition.³⁶ A study found no significant differences in knee extension strength when measured in a seated position, raising questions about this testing position's effectiveness. It was suggested that measuring knee extension strength in a supine position with the knee flexed at a 10° angle might yield different results.³⁷ As aforementioned, the rectus femoris muscle may yield atypical strength values^{13,14,38} due to its unique anatomical and functional characteristics. Effectively addressing the atypical nature of the rectus femoris when measuring strength values and standardizing the testing protocol helps to mitigate against inconsistent, unreliable, and invalid measurements. This reflects the muscles' dual role as hip flexor and knee extensor are considered and managed.³⁹

The application of sports liniments containing the

compounds Methyl Salicylate and L-Menthol, again with limited previous research on muscular strength implications, may be attributed to a combination of vasodilation, pain relief, enhanced muscle activation, improved breathing comfort, and psychological benefits.^{5,43} Regarding hip flexion strength, both groups in the study showed increases, with the TS group demonstrating a significant increase of 2.45 kg compared to 0.65 kg in the other group. This indicates that the TS group had a more significant improvement in hip flexion strength. The tape stripping technique is a non-invasive method that effectively removes the stratum corneum, which consists of three significant lipids that form a barrier on the skin.^{8,9,40} This barrier can impede the absorption of substances, but tape stripping enhances skin permeability, possibly allowing for better results in strength training applications. In contrast, the massage technique used in the study required a pressure of no more than 25 kPa to achieve similar increases in skin permeability.⁴¹

The isometric endurance strength test showed significant results: The TS group mean increased its holding ability to 37.33 seconds, while the MG group increased its holding ability to 68.03 sec. All participants used an external mass of 17% of their body mass (low intensity) and sustained the isometric holding force to exhaustion with 80% to 100% voluntary muscle contraction. Variables such as muscle mass size can be expected to favor one participant over another, especially in weight-categorized sports such as Muay Thai. Lower muscle mass may be able to sustain an isometric contraction longer, as larger muscles require more oxygen and energy to maintain contraction. MG group, penetration of the APIs, was the last performance test, so the Methyl Salicylate should be effective now. This increases SmO₂, which correlates with an increase in strength.^{19,21} Future studies may want to include measurements of the muscle being explored, thereby observing whether muscle mass correlates with any increase or decrease in muscular endurance capability.

Muscular strength and endurance increase can be attributed to various physiological mechanisms. Sports liniments are formulated with ingredients that promote vasodilation, resulting in increased blood flow to the muscles. This improved circulation enhances oxygen delivery and nutrient supply to the muscle tissues, thereby supporting enhanced performance and quicker recovery.

Moreover, many liniments possess analgesic properties that help relieve pain and reduce muscle stiffness. This can contribute to a more fantastic range of motion and strength, particularly in hip flexion activities.⁴² When liniment is applied with massage, it can boost neuromuscular activation. This synergy enhances the communication between the nervous system and muscles, ultimately improving muscle contraction and strength output during movements.⁴³ The effects of sports liniment are typically immediate, offering a short-term performance enhancement. This quick boost can be especially advantageous in sports, where athletes must rapidly optimize their strength and flexibility before competitions or training sessions.

The present study is the first to apply sports liniment

to Muay Thai fighters' physiological and athletic performance outcomes. Secondly, the study design as a case study allowed the experienced Muay Thai fighters to compare baselines with their intervention. Finally, field testing devices were high-precision instruments that enabled the validity and reliability of data collection. The study was not without limitations. Firstly, the trial duration was short at 35 minutes, which may have limited the effect of permeation of the API into the underlying tissues.⁴⁴ A more extended period of 90 minutes is recommended to allow for an impact and is realistic for a Muay Thai fight or training duration.⁴⁵ Secondly, the dosage form was limited to only 3 mL NM. Lower or higher dosage forms may result in either less, better, or equal results, one recommendation being 1 mL and 5 mL.⁴⁶ Participants' nutritional and hydration status was not considered or recorded, which should be regarded as due to possible correlations with impaired hemoglobin levels.⁴⁷ However, it is essential to note that this study had a small sample size and relied on descriptive analysis, limiting the generalizability of the findings to other populations. The descriptive nature of the analysis also restricts the ability to establish causal relationships or underlying mechanisms. While this type of research is valuable for exploratory purposes, future studies with larger sample sizes, control groups, and more sophisticated analytical methods are necessary to validate or challenge the results of this study.

Conclusion

This case study indicates that sports liniment may positively impact physiological outcomes such as increasing oxygen myoglobin percentages and specifically enhancing muscular strength and endurance when applied to the hip musculature as part of the warmup in Muay Thai. The use of tape stripping may enhance the permeation of the sports liniment into the underlying tissue more effectively, potentially further increasing myoglobin within the muscle.

Conflicts of Interest

The authors declare no conflict of interest.

Funding

The author(s) disclose receipt of the following financial support for the research, authorship, and/or publication of this article: Doctoral research was granted from the Faculty of Associated Medical Sciences, Khon Kaen University, Thailand, and Namman Muay was supported by LLC, Devakam Apothecary Hall Co., Ltd., Thailand, however, the sponsor did not have any conflict of interest in the study.

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Impact of increasing tube potential and additional filtration on image quality and radiation dose for digital chest radiography

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ARTICLE INFO

Article history:

Received 23 November 2024

Accepted as revised 4 January 2025

Available online 7 January 2025

Keywords:

High kV, additional filtration, radiation dosage, image quality, digital chest radiography

ABSTRACT

Background: Chest radiography is one of the most commonly performed examinations as routine check-ups in radiology departments. Radiographers should be concerned with minimizing patient radiation dose while maintaining high diagnostic image quality.

Objective: This study aimed to investigate the effect of increasing tube potential (kV) and adding filtration on image quality and radiation dose for posteroanterior (PA) chest radiography using a digital radiography (DR) system.

Materials and methods: Eighty-five kV with no filter was used as the reference exposure technique. Subsequently, the kV was increased to 96, 117, and 133, and additional filtrations of 2 mm Al, 1 mm Al+0.1 mm Cu, and 1 mm Al+0.2 mm Cu were applied. A total of sixteen images were produced. The entrance surface air kerma (ESAK) was measured and evaluated. Signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR) were accessed for objective image quality. Five independent radiographers assessed a subjective image quality (IQ) score using two alternative forced choices (2AFC).

Results: Increasing kV and adding filtration reduced the ESAK while enhancing the SNR and CNR. However, the IQ score declined relative to the reference image when higher kV and additional filtration were applied except 85 kV. The IQ score indicated that an image acquired at 85 kV with 1 mm Al+0.2 mm Cu showed superior quality compared to the reference image. Notably, the SNR for this image was significantly higher ($p < 0.05$). Additionally, this image resulted in a lower radiation dose (13.44 mGy) compared to the reference image (24.97 mGy). Furthermore, the image quality (IQ) score was higher than the reference images.

Conclusion: This study's findings indicate that using an 85 kV with 1 mm Al+0.2 mm Cu additional filtration for digital PA chest radiography can reduce the radiation dose while improving image quality. However, this study used an anthropomorphic chest phantom; further clinical research is recommended.

Introduction

Diagnostic medical imaging procedures and technologies have increased over the past decade,¹ raising concerns regarding patient radiation doses.^{2,3} Chest radiography is one of the most common examinations in the radiology department, typically in routine check-ups. It is approximately 30-40% of all radiographic procedures performed.⁴ A single chest radiograph delivers an ionizing radiation dose equivalent to three days of natural environmental exposure, which is minimal. However, the cumulative dose from repeated imaging can be significant.⁵

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doi: 10.12982/JAMS.2025.034

E-ISSN: 2539-6056

Atiyyah TAE-R *et al.* indicates that cumulative exposure from diagnostic imaging, including PA chest radiography, may not cause acute toxicity but could increase long-term malignancy risk due to chronic exposure. Despite the relatively low radiation dose associated with chest radiography, approximately 0.07 mSv^2 , there is still a potential for biological effects through a stochastic effect, particularly cancer.⁶ The probability of this effect is directly related to the increase in radiation dose, and it is essential to note that there is no threshold dose for this effect.⁷

In recent years, optimizing radiation dose has become a considerable challenge, particularly since the introduction of digital radiography. Digital image receptors have a wider dynamic range than traditional radiographic film, which can lead to a phenomenon called “dose creep,” where excess radiation is used without awareness. Radiation dose optimization is one of the fundamental principles for radiation protection established by the International Commission on Radiological Protection (ICRP), which states that all exposures should be maintained at a level as low as reasonably achievable (ALARA) without compromising the diagnostic quality of the examination.⁷

One strategy for optimizing radiation dose is X-ray beam hardening. This technique influences photon interactions, particularly photoelectric absorption. Increasing kVp and adding filtration can increase the effective photon energy and reduce the patient’s radiation dose.⁸⁻¹⁰ However, it is crucial to consider that these adjustments may also impact image quality.

Radiographers should carefully consider specific

exposure parameters for each patient. Furthermore, appropriate technical exposure factors must be established to ensure the production of images that meet diagnostic purposes.

This study investigated the effect of high kV with additional filtration on the radiation dose and image quality for digital chest radiography. Moreover, the study aimed to identify the optimal exposure parameter, providing essential guidance in digital chest X-ray examinations.

Materials and methods

Radiographic equipment and data acquisition

This study was performed using a flat plate detector (Digital GOS (Gadox); Philips Healthcare) operating with a Digital Diagnost X-ray machine (Philips Healthcare). The quality controls of the X-ray unit, including kV and time accuracy and precision, radiation output reproducibility and linearity, and collimator and beam alignment, were within acceptable limitations.

An anthropomorphic chest phantom (RS-330, Radiology Support Devices, Inc., USA) was radiographed in the posteroanterior (PA) position using a large focal spot with a 180 cm source-to-image distance (SID) (Figure 1A). Two outer AEC chambers with an anti-scatter grid (85 lines per centimeter, 10:1 ratio) were used. Tube potentials increased from 85 to 133 kV, with approximate 15% kV rule increments.¹¹ The image with 85 kV was used as a reference image calculated by $\text{kV} = (\text{thickness} \times 2) + 40$.^{12,13} The additional filtrations built in the X-ray machine were: no filtration, 2 mm Al, 1 mm Al+0.1 mm Cu, and 1 mm Al+0.2 mm Cu. Sixteen images were produced.

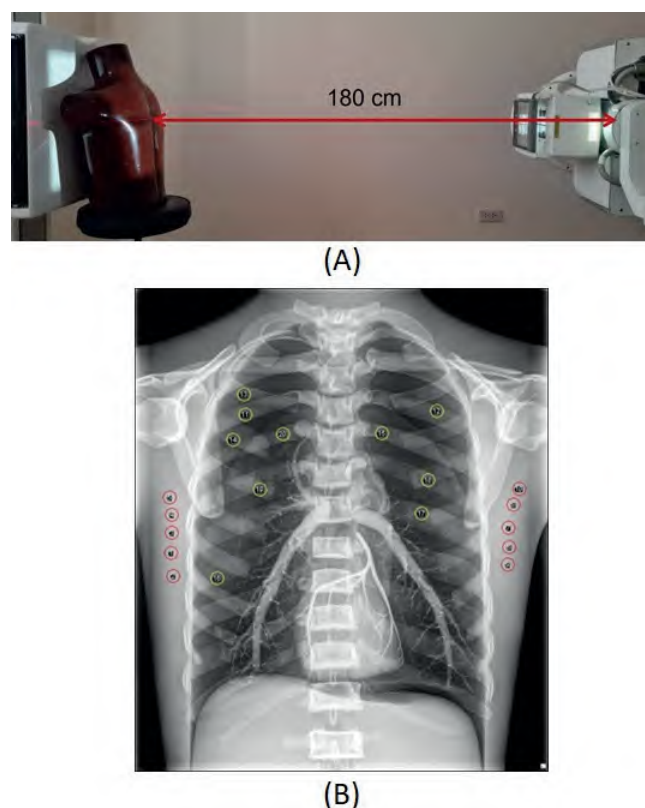


Figure 1. A simulation of phantom’s chest radiography setup. A: ROIs on the phantom image using the ImageJ software, B: the yellow circle is the object, the red circle is the background.

Radiation dose measurement

The entrance surface air kerma (ESAK) is defined as the kerma to air measured on the central beam axis at the position of the phantom, including the backscattered radiation. The ESAK is a dosimetry quantity recommended for use as a patient dose by IAEA. The ESAK was calculated by multiplying an incident air kerma with an appropriate backscatter factor for each HVL according to TRS No. 457.¹⁴ An ionization dosimeter (Radcal ionization chamber, model 10X5-6 with Radcal electrometer, model 9010, Radcal Corporation, Monrovia, CA, USA) was used to measure the incident air kerma (IAK).

$$K_e = K_i \times B \quad \text{Equation 1}$$

When K_e : entrance surface air kerma (ESAK)

K_i : incident air kerma

B : backscatter factor (BSF) for selected field size

Objective assessment of image quality

To calculate the signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR), twenty regions of interest (ROIs) were drawn by an experienced radiographer: 10 ROIs in the ribs and lungs (object) and 10 ROIs in the soft tissue (background) as shown in Figure 1B. The size and location of ROIs were kept constant for all images using ImageJ software (National Institutes of Health, Bethesda, MD). The SNR and CNR were calculated using the following Equation 2¹⁵ and 3.^{15, 16}

$$SNR_i = \frac{PV_i}{\sigma_i} \quad \text{Equation 2}$$

$$CNR = \frac{PV_i - PV_{bg}}{\sigma_{bg}} \quad \text{Equation 3}$$

When PV_i and σ_i : mean pixel value and the standard deviation of ROI , respectively.

PV_{bg} : mean pixel value of the ROI background

σ_{bg} : standard deviation of the ROI background.

Subjective assessment of image quality

Visual grading analysis (VGA) was performed using two alternative forced choices (2AFC). 2AFC assesses the responses of the observers who are presented with two separate images displayed side by side.¹⁷ Images were displayed on two Coronis 5MP monitors (Barco MDCG-5121, Kortrijk, Belgium; 2048 by 2560 pixels). The reference image (85 kV with no added filtration) was permanently displayed on one monitor, and the experimental images were shown in random order on the other monitor to be scored against the reference image.

Five radiographers with at least 5 years of clinical experience evaluated the image using a 5-point Likert scale. The image quality (IQ) criteria were adapted from the European Guidelines on Quality Criteria for Diagnostic Radiographic Images,¹⁸ shown in Table 1.

Radiographers were not permitted to manipulate the windowing and magnification of the image. The overall score for each image was calculated by adding the scores from all six IQ criteria. The final IQ score for each image was determined by taking the average IQ score from the five radiographers.

The intra-class correlation coefficient (ICC) and its 95% Confidence level (CI) were used to assess the reliability based on a single measurement, absolute agreement, and two-way mixed-effects model. The ICC value was interpreted as follows: poor (<0.5), moderate (0.5 to <0.75), good (0.75 to 0.9), and excellent (>0.9) reliability.¹⁹⁻²¹

Table 1. Criteria evaluation tool for the visual grading analysis.

No.	Criteria	Image Score
1	Visually sharp reproduction of the vascular pattern in the whole lung, particularly the peripheral vessels	5 = much better than
2	Visually sharp reproduction of the trachea and proximal bronchi	4 = slightly better than
3	Visually sharp reproduction of the borders of the heart and aorta	3 = equal to
4	Visually sharp reproduction of the diaphragm and lateral costophrenic angles	2 = slightly worse than
5	Visualization of the retrocardiac lung and the mediastinum	1 = much worse than
6	Visualization of the spine through the heart shadow	

Statistical analysis

All results are presented as means±standard deviation (SD). Tests showed that DAP and IQ were normally distributed. The data were compared using an ANOVA-like test. The inter-observer correlation was also evaluated by the intra-class correlation coefficient (ICC), and the result was considered significant at the 95% confidence level by using. Differences were considered to be statistically significant at $p<0.05$.

Results

Radiation dose

When using the AEC, the results indicated that with both increasing in kV and adding filtrations, there was a gradual reduction in ESAK (Figure 2). The most significant decrease in ESAK (60.76%) was found at 133 kV with a 1

mm Al+0.2 mm Cu filter compared to the reference image at 85 kV without filtration. In contrast, the minimum reduction of 21.10% occurred at 96 kV without filtration. Furthermore, the ESAK at 117 kV and 133 kV, both with and without additional filtration, showed similarities (Figure 2).

Objective image quality

With an increase in kV and adding filtration, both SNR and CNR increased. When compared with the reference image, it was found that there were statistically significant increases in SNR and CNR ($p<0.05$) except 85 kV with 2 mm Al, 85 kV with 1 mm Al+0.2 mm Cu, and 96 kV without filtration (Figure 3). The highest SNR and CNR, achieved from 133 kV combined with 1 mm Al+0.2 mm Cu, were 12.52 ± 1.12 and 14.85 ± 0.66 , respectively.

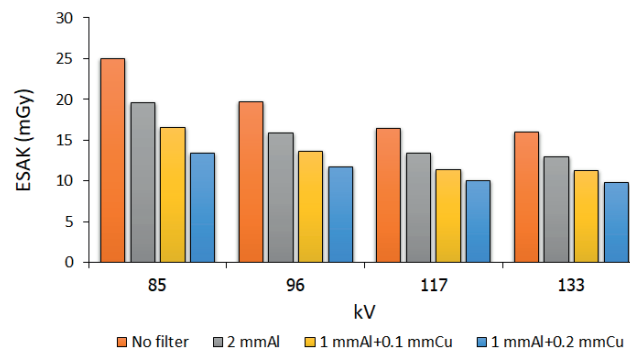


Figure 2. Decrease in ESAK as kV increases and filtration added.

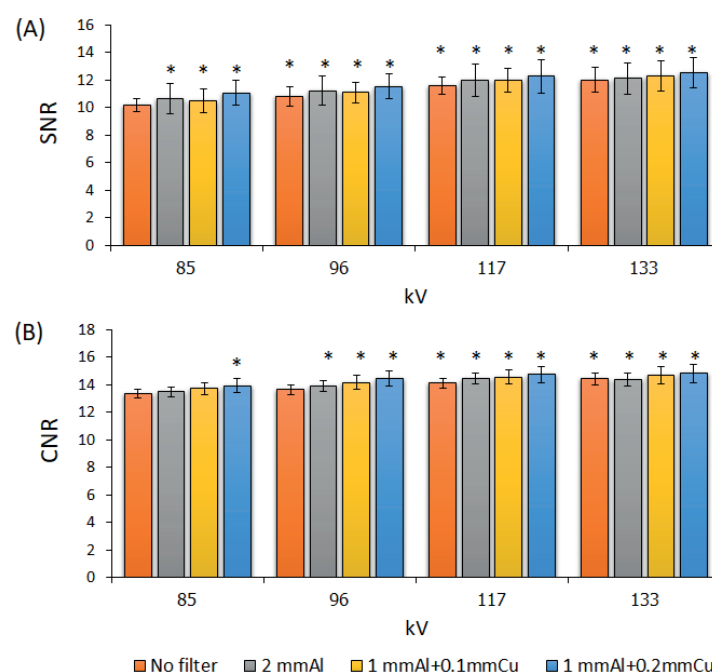


Figure 3. Objective image quality assessment as kV increases and filtration was added. A: SNR increase, B: CNR increase (* $p<0.05$).

Subjective image quality

The average subjective image quality scores compared to the reference image are shown in Table 2 (>3: higher subjective image quality than the reference image, 3: the same subjective image quality as the reference image, <3: lower subjective image quality than the reference image). According to the data, the images

obtained from 85 kV with 2 mm Al, 85 kV with 1 mm Al+0.1 mm Cu, 85 kV with 1 mm Al+0.2 mm Cu, and 96 kV with 2 mm Al showed more excellent IQ scores than the reference image. The highest IQ score was 3.60 ± 0.81 from 85 kV with 2 mm Al. The reliability of the observer was good (ICC was 0.78) ($p < 0.05$).

Table 2. Average subjective image quality score (IQ score) by 5 radiographers.

kV	Filter	mAs	IQ score
85	No filter	5.36	Reference image
	2 mm Al	6.38	3.60 ± 0.81
	1 mm Al+0.1 mm Cu	7.09	3.07 ± 0.87
	1 mm Al+0.2 mm Cu	8.14	3.13 ± 0.73
96	No filter	3.38	2.77 ± 0.50
	2 mm Al	3.98	3.13 ± 0.57
	1 mm Al+0.1 mm Cu	4.34	2.73 ± 0.45
	1 mm Al+0.2 mm Cu	4.97	2.93 ± 0.74
117	No filter	2.03	2.43 ± 0.50
	2 mm Al	2.24	2.63 ± 0.61
	1 mm Al+0.1 mm Cu	2.38	2.20 ± 0.76
	1 mm Al+0.2 mm Cu	2.63	2.30 ± 0.60
133	No filter	1.63	2.37 ± 0.49
	2 mm Al	1.77	2.37 ± 0.56
	1 mm Al+0.1 mm Cu	1.85	2.17 ± 0.75
	1 mm Al+0.2 mm Cu	1.97	2.30 ± 0.53

Discussion

The findings of this study indicate that increasing kV and adding filtration is a feasible radiation dose-optimization tool for digital chest radiography. In this study, the kV was adjusted following the 15% rule,²² starting from an initial value of 85 kV and increasing to 96, 117, and 133 kV. Various filtration combinations were used, including no filtration, 2 mm Al, 1 mm of Al plus 0.1 mm Cu, and 1 mm of Al+0.2 mm of Cu. As the kV increased and filtration was added, the radiation dose (ESAK) decreased (Figure 1). These results support the findings from earlier studies.^{16,23-26} Except for the increase from 117 kV to 133 kV, comparable ESAK values were recorded with the same levels of filtration. This similarity is attributed to the sufficient photon energy penetrating the chest phantom.

The variation in kV impacts both the quantity and quality of X-rays. Higher kV produces more X-ray photons with higher X-ray energy. However, adding filtration into the X-ray beam reduces the quantity of X-rays across all energy levels, resulting in a hardened X-ray energy through the absorption of low-energy X-ray photons over high-energy X-ray photons.²⁷

Both kV and filtration significantly affect both patient dose and image quality. This study found that increasing the kV and adding filtration substantially reduces the ESAK while notably enhancing both the SNR and the CNR. Higher kV and added filtration produce X-rays with

greater energy, which improves the beam's penetration through the patient. This enhanced penetration results in a lower patient dose (ESAK). In terms of image quality, the increased energy allows a greater number of X-ray photons to reach the image receptor, thereby improving both the SNR and CNR of the image.

However, the subjective image quality indicates that increased kVp combined with the same additional filtration results in a decreased IQ score. The results also show that using 85 kV with all filter combinations and 96 kV with 2 mm Al results in a higher IQ score than the reference image. Notably, among these techniques, the 85 kV with 1 mm Al+0.2 mm Cu provides the lowest ESAK with a reduction of 46.18%.

This study has several limitations. Firstly, it was conducted using an anthropomorphic phantom, meaning the results may not accurately reflect actual human anatomy. Secondly, the investigation was based on a single X-ray machine coupled with one image receptor, indicating the necessity for additional research involving other systems. Lastly, the subjective image quality scores were assessed by experienced radiographers who have worked in the diagnostic radiology department for over five years. Further study should include real human subjects, different X-ray systems, and a wider range of radiographers to confirm the findings and ensure they apply to various settings. It should also evaluate the

diagnostic accuracy of images produced with the proposed technique. In clinical practice, the results could guide the development of updated radiography protocols, routine equipment calibration, and specialized radiographer training to optimize radiation dose and image quality, improving patient safety and diagnostic outcomes across diverse patient populations.

Conclusion

The ESAK decreases as the kV increases and with the addition of filtration. Regarding objective image quality, both the SNR and the CNR improve with higher kV and additional filtration. The IQ score suggests that an image taken at 85 kV with 1 mm Al+0.2 mm Cu has better image quality than the reference image, achieving a radiation dose reduction of 46.18%. Additionally, this image demonstrates significantly better SNR than the reference image ($p < 0.05$).

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This study, which involved human participants, complied with the ethical principles of the Declaration of Helsinki. The research protocol was reviewed and approved by the Ethics Committee of Naresuan University, with the approval number COA No. 211/2020.

Funding

This research study was supported by the Thailand Institute of Nuclear Technology (Public Organization), Thailand, through its program TINT to University (2023).

Acknowledgements

The authors would like to acknowledge the Thailand Institute of Nuclear Technology (a Public Organization) for their support and kind assistance. The authors also thank the radiographers for assessing the image quality.

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Intuitionistic fuzzy RFE-based prognostic model for liver transplantation

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ARTICLE INFO

Article history:

Received 1 September 2024

Accepted as revised 2 January 2025

Available online 8 January 2025

Keywords:

Artificial neural network (ANN),
transplantation of liver (LT), survival
prediction, probability of survival.

ABSTRACT

Background: Survival prediction after liver transplantation is a very challenging but complex task. LT is often the best treatment for advanced liver disease, provided no other medical conditions contraindicate it. This article explores clinical predictors, such as MELD scores and hormone levels, along with computational algorithms for forecasting post-transplant survival.

Objective: This study evaluates the performance of machine learning models for predicting survival outcomes in liver transplant recipients using UNOS data. It develops and validates a donor and recipient-based prognostic model.

Materials and methods: The UNOS database contains 65,535 donor-recipient pairs in transplants conducted in the U.S. between October 1987 and June 2021, with 421 attributes. The top 24 features, including logistic regression, random forest, artificial neural networks, XGBoost, CART, and K-nearest neighbors, were used to train the models upon feature selection. Models were compared using AUROC, accuracy, specificity, sensitivity, and precision.

Results: ANN outperformed other models for the UNOS dataset, with an AUROC of 0.98–0.99. Validated results in the KCH dataset are robust at AUROC: 0.94–0.95.

Conclusion: The model offered exceptional generalizability performance to guide clinical decisions in transplantation support, yet variability in patients' characteristics may differ significantly among the cohorts and impact the results.

Introduction

Liver disease represents a significant global health issue, causing approximately 2 million deaths annually, with conditions such as cirrhosis, viral hepatitis, and liver cancer contributing to these statistics. Despite being the eleventh leading cause of death,¹ liver-related mortality often remains underreported,² with notable variations in regional impact. Women comprise 34% of liver-related deaths. Liver transplantation is the primary treatment for end-stage liver disease; however, patient outcomes significantly rely on factors such as organ quality, donor availability, and illness severity.³ Accurate pre-transplant mortality prediction is crucial in clinical decision-making and organ allocation, which currently employs the MELD score system. Nonetheless, there are inconsistencies in the MELD score's predictive efficacy^{4,5} due to its variability and the exclusion of certain patient factors.^{6,7} The field recognizes the need for advanced modeling techniques, such as machine learning, to enhance predictive accuracy by analyzing extensive patient data.^{8,9} Integrating varied demographic and clinical information is essential for improving risk assessments and optimizing organ use.¹⁰

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doi: 10.12982/JAMS.2025.035

E-ISSN: 2539-6056

Table 1. describes the abbreviations used in this study.
Table 2. describes significant works in the field, emphasizing

the need for more reliable and interpretable models.

Table 1. Nomenclature.

Abbreviation	Description
PTIME	Patient survival time in days
GSTATUS	Graft failed (1=yes)
LIST_MELD	Patient listed prior to meld/ peld?
GRF_STAT	Recipient graft status
EXTRACRANIAL_CANCER_DON	Deceased donor-extra cranial cancer
MALIG	previous malignancy
DIABETES_DON	Diabetes duration
PREV_TX_ANY	previous transplant any organ
BILIARY	biliary tract complication
HTLV2_OLD_DON	donor-antibody to htlv
HBV_CORE_DON	Donor hbv core antibody
PRETREAT_MED_DON_OLD	Deceased donor pre-recovery medications
INOTROPES	Recipient life support type
TX_MELD	Transplant occurred prior to meld/peld?
PSTATUS	Boolean Patient Status(1=Dead,0=Alive)

Table 2. Overview of existing work.

Findings	Dataset used	Limitations	Reference
ANN achieved 99.74% accuracy in survival prediction.	United Network for Organ Sharing data.	Clinical interpretability.	Raji & Chandra ⁶
Neural networks outperformed for 10-year survival prediction.	62,294 patients with 97 predictors.	Handling large datasets and model interpretability.	Kantidakis <i>et al.</i> ⁷
RF achieved AUROC (0.85).	Liver transplantation patients with perioperative data.	Specific to kidney injury.	Yeh <i>et al.</i> ⁸
Predicted postoperative morbidity and mortality.	242 patients in Korea.	Potential for selection bias.	Jung <i>et al.</i> ⁹
ML models outperformed in predicting survival after liver transplantation.	Multi-institutional liver transplantation.	Ethical and logistical challenges in clinical implementation.	Tran <i>et al.</i> ¹⁰
Lasso regression identified novel biomarkers which improved the MELD score for 90-day survival predictions.	Data from a European liver transplant cohort.	External validation needed.	Gibb <i>et al.</i> ¹¹
Light GBM achieved AUC 0.740.	Multicenter liver transplant registry data.	No validation on external datasets.	Yanagawa <i>et al.</i> ¹²

Motivation and goals of the research

The study addresses the limitations of current machine learning models in predicting liver transplant survival by developing a novel method that utilizes intuitionistic fuzzy and recursive feature elimination techniques. Key contributions include a hybrid feature extraction methodology aimed at discarding less relevant and ambiguous data and a ranking system based on entropy values to calculate feature weights effectively. This process ensures that the selected features are refined and appropriately scaled for use in machine learning classifiers, ultimately enhancing mortality risk assessment in LT based on pre-transplant data.

Materials and methods

This process involves high risks that require assessment. The block diagram describes the model along with its feature selection criteria.

Study flow

Initially, data pre-processing steps such as transformation and cleaning were applied. Then, different feature extraction strategies were used to identify the key features. Finally, six machine learning models were selected for classification.

Intuitionistic fuzzy RFE-based prognostic model for liver transplantation

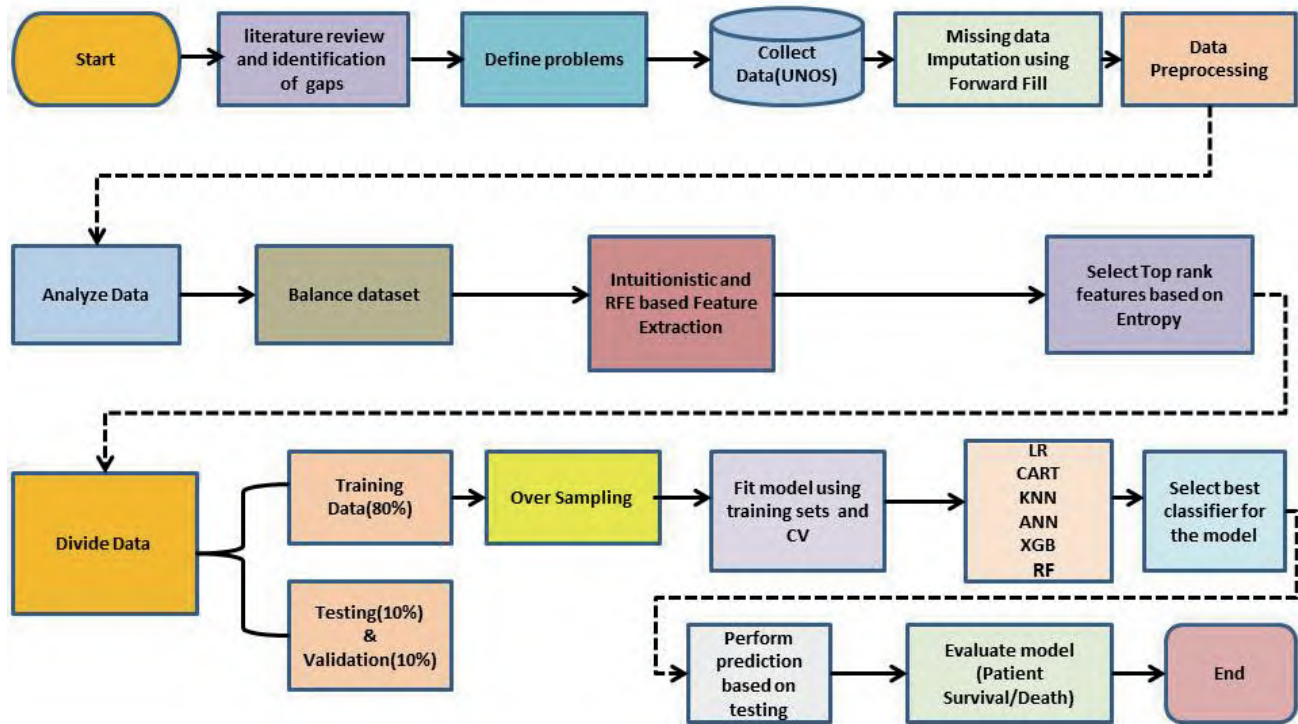


Figure 1. Block diagrams of the proposed model.

Data collection

Data sources used the United Network for Organ Sharing data from 65,535 liver transplants from October 1, 1987, to June 30, 2021. The dataset included 421 variables, including 105 recipient variables, 59 donor variables, 157 clinical data points, 14 waiting list data points, 56 patient status data points, and 30 post-transplant data points.

Missing values-imputation method

This study focused on estimating missing values.¹¹ Missing data can be caused by the failure of laboratory devices, clinician oversight, or even patient visit cancellation. We compared several imputation methods, including forward filling, mean filling, median filling, and random filling.¹² The forward-filling method is employed as it provides the best AUROC on this dataset.

Pre-processing

Firstly, irrelevant features were removed based on the hepatologist's judgment, reducing the dataset from 421 to 396 characteristics. Features irrelevant to graft survival, such as patient code and admission date, were excluded. Transplants performed after 2016 were retained to ensure a 5-year follow-up period for all LT cases used to build our models. The number of patients with LT was reduced from 65,535 to 64,635. The dataset contained continuous and categorical data. The categorical data were transformed into constant data using label encoding.

The feature extraction method

The proposed method enhances ML algorithm

performance in predicting LT mortality risk by using Recursive Feature Elimination (RFE) with Intuitionistic Fuzzy Logic (IFL) for feature extraction. RFE removes irrelevant and poor features but preserves strong features. IFL assigns weights based on belongingness, non-belongingness, and hesitancy and introduces information about the importance of features. The entropy metric ranks the features. Lower entropy means higher relevance, whereas intuitionistic fuzzy sets create thresholds for feature inclusion. This results in better accuracy and interpretability of the model.^{13,14}

Intuitionistic fuzzy concept

Non-belongingness and reluctance grades are included with intuitionistic fuzzy logic, which is quite beneficial for inconsistent, ambiguous, and uncertain datasets.¹⁴

Definition 1: An IFS F over a universal set $X = \{x_1, x_2, \dots, x_n\}$ is defined as:

$$F = \{[x, \mu_F(x), \nu_F(x)] \mid x \in X\}, \quad (1)$$

Where $\mu_F(x): X \rightarrow [0,1]$ the membership degree is $\nu_F(x): X \rightarrow [0,1]$ is the nonmembership degree, and they satisfy:

$$0 \leq \mu_F(x) + \nu_F(x) \leq 1 \quad (2)$$

The hesitation degree is given by:

$$\pi_F(x) = 1 - \mu_F(x) - \nu_F(x) \quad (3)$$

Definition 2: Entropy for IFS

Its entropy $E(x_j)$ quantifies the uncertainty within the intuitionistic fuzzy framework for a feature. RFE iteratively fits a model using progressively smaller feature sets until

the termination condition is met. Each iteration ranks the attributes according to their relevance.¹⁴ The feature selection algorithm is shown in Figure 2

Require: Dataset $D = \{(x_{ij}, y_i) \mid i = 1, 2, \dots, m; j = 1, 2, \dots, n\}$

1: **Initialization:** Set $F = \{x_1, x_2, \dots, x_n\}$.

2: Define weights α , β , and γ for membership, non-membership, and hesitation components.

3: **function** COMPUTEENTROPY (F)

4: **for** each feature $x_j \in F$ **do**

5: **for** each sample $i = 1$ to m **do**

6: Compute membership: $\left(\mu_{F_j}(x_{ij})\right) = e^{-\frac{|x_{ij} - \text{mean}_j|}{\sigma_j}}$

7: Compute non-membership: $\left(v_{F_j}(x_{ij})\right) = 1 - \mu_{F_j}(x_{ij})^\gamma$

8: Compute hesitation: $\left(\pi_{F_j}(x_{ij})\right) = 1 - \mu_{F_j}(x_{ij}) - v_{F_j}(x_{ij})$

9: **end for**

10: Compute intuitionistic fuzzy entropy for feature x_j :

$$E_{F_j} = \frac{1}{m} \sum_{i=1}^m \left[\alpha \mu_{F_j}(x_{ij}) \ln \left(\mu_{F_j}(x_{ij}) \right) + \beta v_{F_j}(x_{ij}) \ln \left(v_{F_j}(x_{ij}) \right) + \gamma \pi_{F_j}(x_{ij}) \ln \left(\pi_{F_j}(x_{ij}) \right) \right]$$

11: **end for**

12: **return** $E_F = \{E_{F_1}, E_{F_2}, \dots, E_{F_n}\}$

13: **end function**

14: **function** RECURSIVEFEATUREELIMINATION (F, E_F)

15: **while** $|F| > k$ **do**

16: Rank feature: Rank (F) = sort (E_F)

17: $F \leftarrow F \setminus \{x_{\arg \max E_F}\}$

18: Recompute E_F using COMPUTEENTROPY (F).

19: **end while**

20: **return** F

21: **end function**

22: Compute $E_F = \text{COMPUTEENTROPY}(F)$

23: Perform $F_{\text{selected}} = \text{RECURSIVEFEATUREELIMINATION}(F, E_F)$

24: **return** Ranked features and selected subset F_{selected} .

Figure 2. Algorithm showing feature selection.

Terminal classification of the model

Death within 90 days post-transplantation is a binary event represented as [0,1]. "PTIME" measures the time from transplantation to death or censoring, with PSTATUS = 1 being death post-transplantation.

Overview of methods

Different methods were used to develop the classification models. Logistic regression was first used due to its simplicity and interpretability, assuming linearity and no interactions. Random forests and ANN models were then used to model nonlinear relationships.

Logistic regression

Logistic regression, a type of generalized linear model (GLM), is described by¹⁵

$$g(y) = \theta_0 + \theta_1 x_1 + \dots + \theta_n x_n \quad (4)$$

Where y is the dependent variable, x_i are predictors, and θ_i 's are coefficients.

$$g(y) = 0 + 1x_1 + \dots + nx_n \quad (5)$$

Random forest classifier

Random forests capture interactions and nonlinearity, often outperforming simpler models, though they lack interpretability.¹⁶

$$y = f(x_1, \dots, x_n) \quad (6)$$

CART classification

CART creates decision trees based on the Gini impurity index. Nodes split according to attribute thresholds and result in homogeneous sub-nodes. The process is called tree pruning, and it continues until pure subsets or leaves are produced.¹⁷

XGBoost classification

XGBoost is a gradient-boosting technique. It is used to improve the performance of the weak model predictions. At iteration, it minimizes the regularization and loss functions t:

XGBoost is efficient, scalable, and good for large datasets.^{18,19}

$$L^{(t)} = \sum_{i=1}^n l(\hat{y}_i, \hat{y}_i^{(t-1)} + f_t(x_i)) + (f_t) \quad (7)$$

ANN classifier

A fully connected multilayer perceptron (MLP) with ReLU activation functions was used. Dropout and batch normalization were used to prevent overfitting, while training was performed using backpropagation with a cross-entropy loss function.²⁰ Features were standardized, missing values imputed, and training performed over 50 epochs with 5-fold cross-validation. The best model, selected based on validation loss, was compared using average AUROC scores.²¹ Mini-batch gradient descent with the Adam optimizer was used.

K nearest neighbor (KNN)

KNN classifies new instances based on similarity to previous cases. It offers quick and accurate results after capturing existing data.²²

K-fold cross-validation

In k-fold CV, the dataset is divided into k equal-sized, mutually exclusive folds (DS_1 , DS_2 , and DS_k). CV accuracy is calculated using the following formula.²³

$$CV = \frac{1}{k} \sum_i A_i \quad (8)$$

Where A_i is the accuracy of each fold, and k denotes the number of folds. Tenfold CV was adopted in the present research. It distributed the data into 10 equally sized folds. Stratified CV maintained that the percentage distribution

of predictor labels in all folds would be identical to that in the original data.

Results and discussion

Classifier's ability to classify the patient survival: Class "1" or "0" was checked using the area under the receiver operating characteristic curve, AUROC.

The following are the formulas adopted to check performance:

$$\text{Sensitivity} = \frac{TP}{TP+FN} \quad (9)$$

$$\text{Accuracy} = \frac{TP+TN}{TP+TN+FP+FN} \quad (10)$$

$$\text{Precision} = \frac{TP}{TP+FP} \quad (11)$$

$$F1 \text{ Score} = 2 * \frac{\text{Precision} * \text{Recall}}{\text{Precision} + \text{Recall}} \quad (12)$$

$$\text{Specifity} = \frac{TN}{TN+FP} \quad (13)$$

The above expressions represent the equations for true positive, false negative, true negative, and false positive, respectively. Feature selection employed RFE and intuitionistic fuzzy set. The key features were established by LR, KNN, RF, CART, ANN, and XGBoost classifiers.²⁴ The crucial features, GSTATUS and GRF_STAT, are in Figures 3A-3G. For example, RF-Intuitionistic Fuzzy and ANN ranked PTIME, GSTATUS, and GRF_STAT as the top rank. In contrast, RFE-Intuitionistic Fuzzy, as well as CART, ranked the features as GSTATUS, followed by PTIME, and finally GRF_STAT. Features concerning entropy are present in Figure 3G.

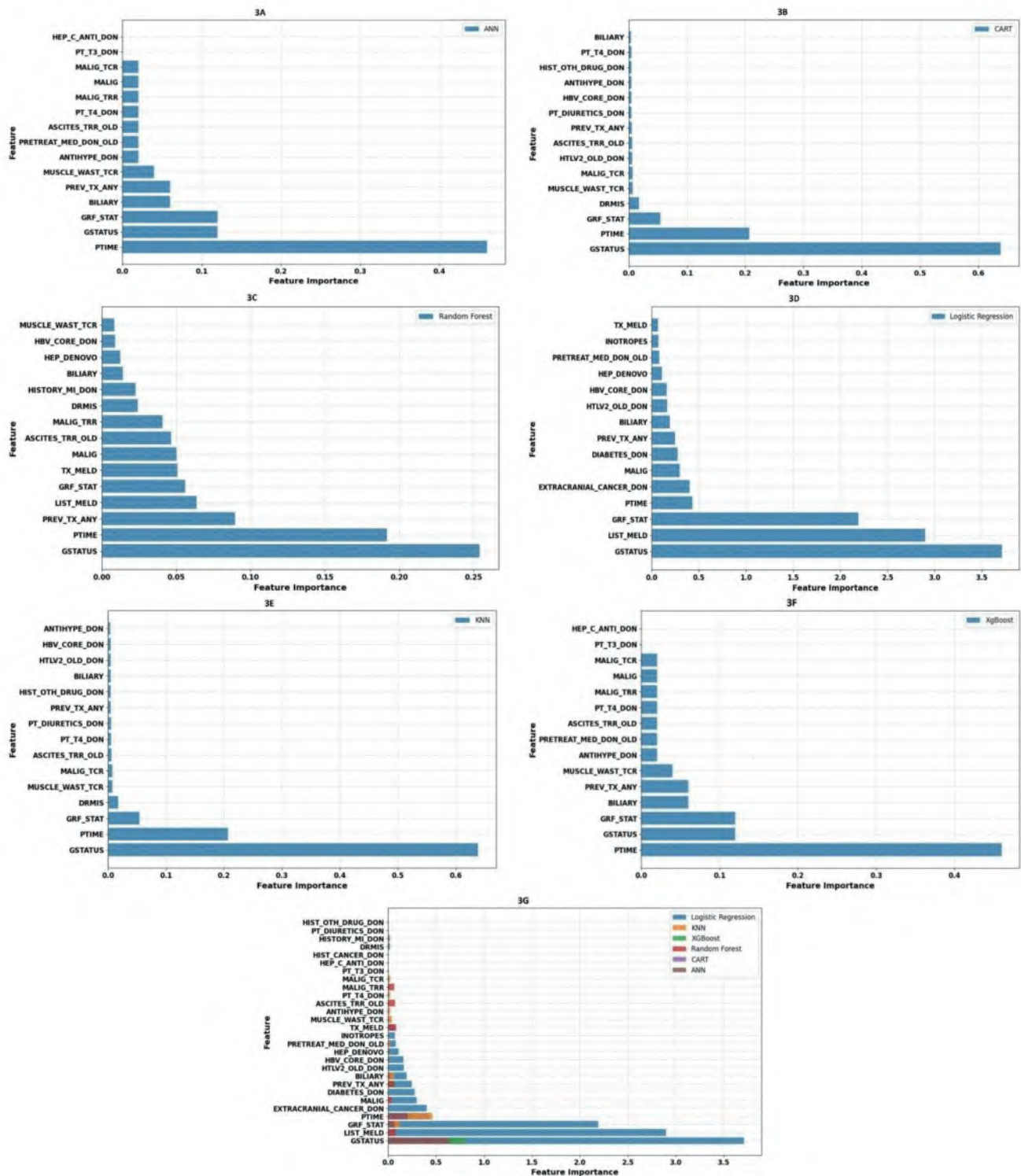


Figure 3. Top-ranked features using different classifiers. 3A: artificial neural network (ANN), 3B: classification and regression tree (CART), 3C: random forest (RF), 3D: logistic regression (LR), 3E: K nearest neighbor (KNN), 3F: extreme gradient boosting (XGB), 3G: selected top rank features.

ML methods' performance

Figure 4 shows a graph displaying the performance of different feature importance fed to the different classifiers and comparing different algorithms.²⁵ Finally, the

combination of all the feature importance (24 features) is fed to different classifiers like RF, LR, KNN, XGBoost, ANN, and CART. The ANN classifier performs better than the other models.

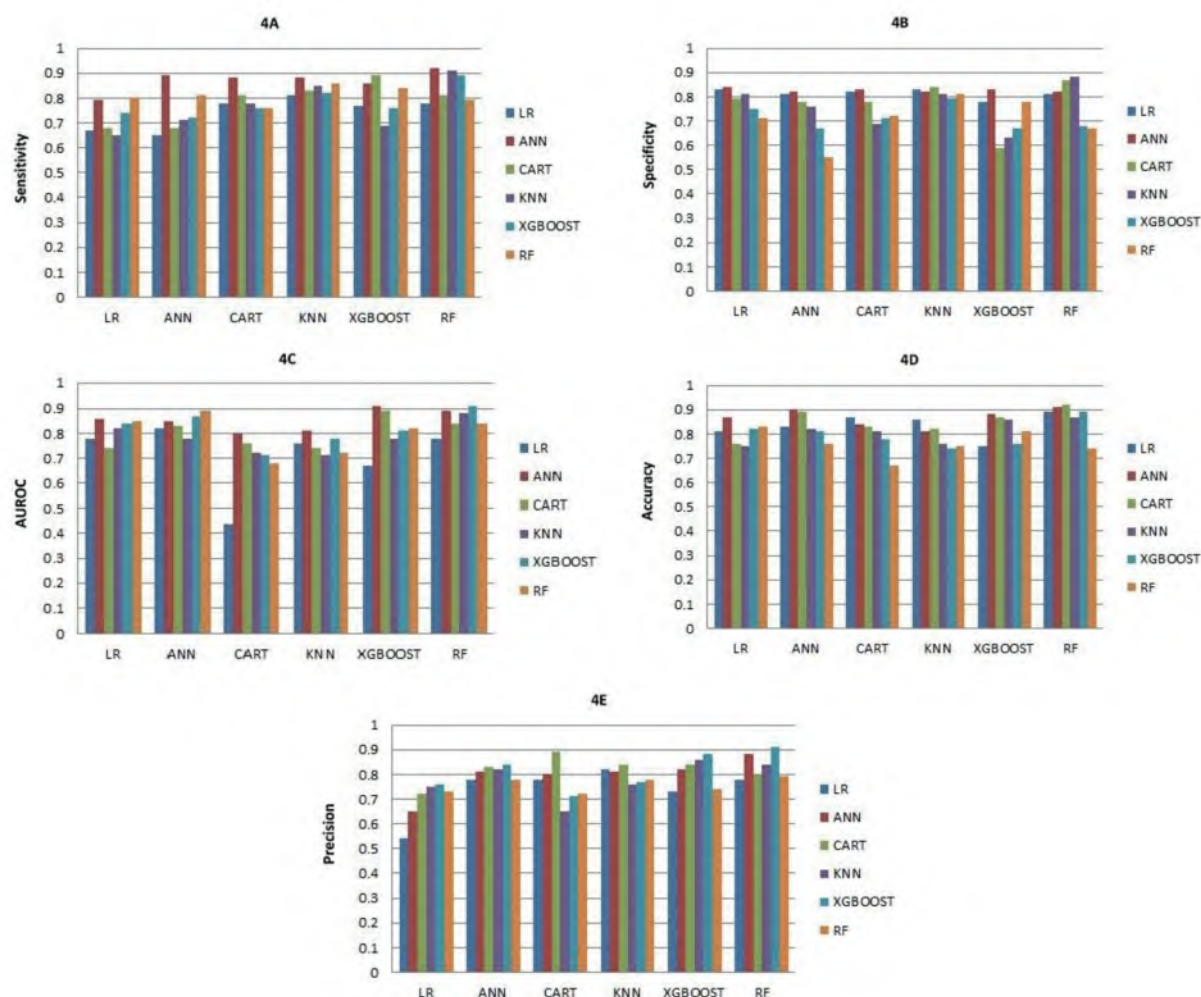


Figure 4. Performance comparison of different feature importance applied to different classifiers. 4A: sensitivity, 4B: specificity, 4C: AUROC, 4D: accuracy, 4E: precision.

Table 3 depicts the top-ranked variables detected in multiple studies. The predictors do not match the methods used previously; however, this will align with the indication provided by the SHAP values.

Table 3. Key variables identified in various studies.

Key variables identified	Study
Gstatus, List_MELD, frequency of graft, PTIME, Cancer condition, Diabetes of the donor.	Proposed method
MELD Score, Donor age, cold ischemia time, Liver condition.	Yanagawa <i>et al.</i> ¹⁵
Donor age, recipient age, MELD score, cold ischemia time, donor BMI.	Kantidakis <i>et al.</i> ⁵
Donor age, recipient age, MELD score, cold ischemia time, donor BMI.	Wang <i>et al.</i> ⁸
Age, gender, ethnicity, BMI, blood type, MELD score, presence of HCC.	Guijo-Rubio <i>et al.</i> ²⁴

Comparison to previous studies

Our proposed ANN model outperformed previous studies in terms of accuracy, AUROC, specificity, precision, and sensitivity in predicting survival after LT, with an AUROC of 0.99 (Figure 5A).²⁶ The model’s generalizability

was validated by external validation using the KCH dataset, where feature importance was calculated and applied similarly to the UNOS dataset. Validation results are shown in Figure 5B.

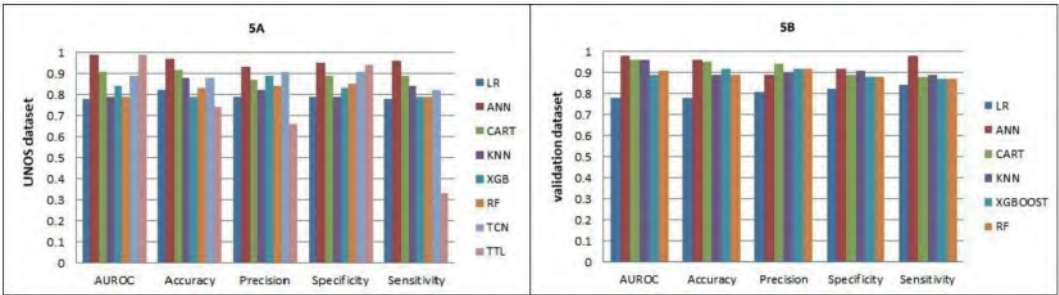


Figure 5. Proposed model training, testing, and validation. 5A: UNOS Dataset, 5B: KCH dataset.

SHAP values

Figure 6. Illustrates feature importance using SHAP values. Positive values increase the target prediction, while negative values decrease it. The x-axis indicates feature influence, with warmer dot colors for higher amplitudes

and more remarkable for lower. The y-axis ranks features by importance, with higher positions indicating a more significant impact.²⁷ The algorithm performance can be measured using two parameters.

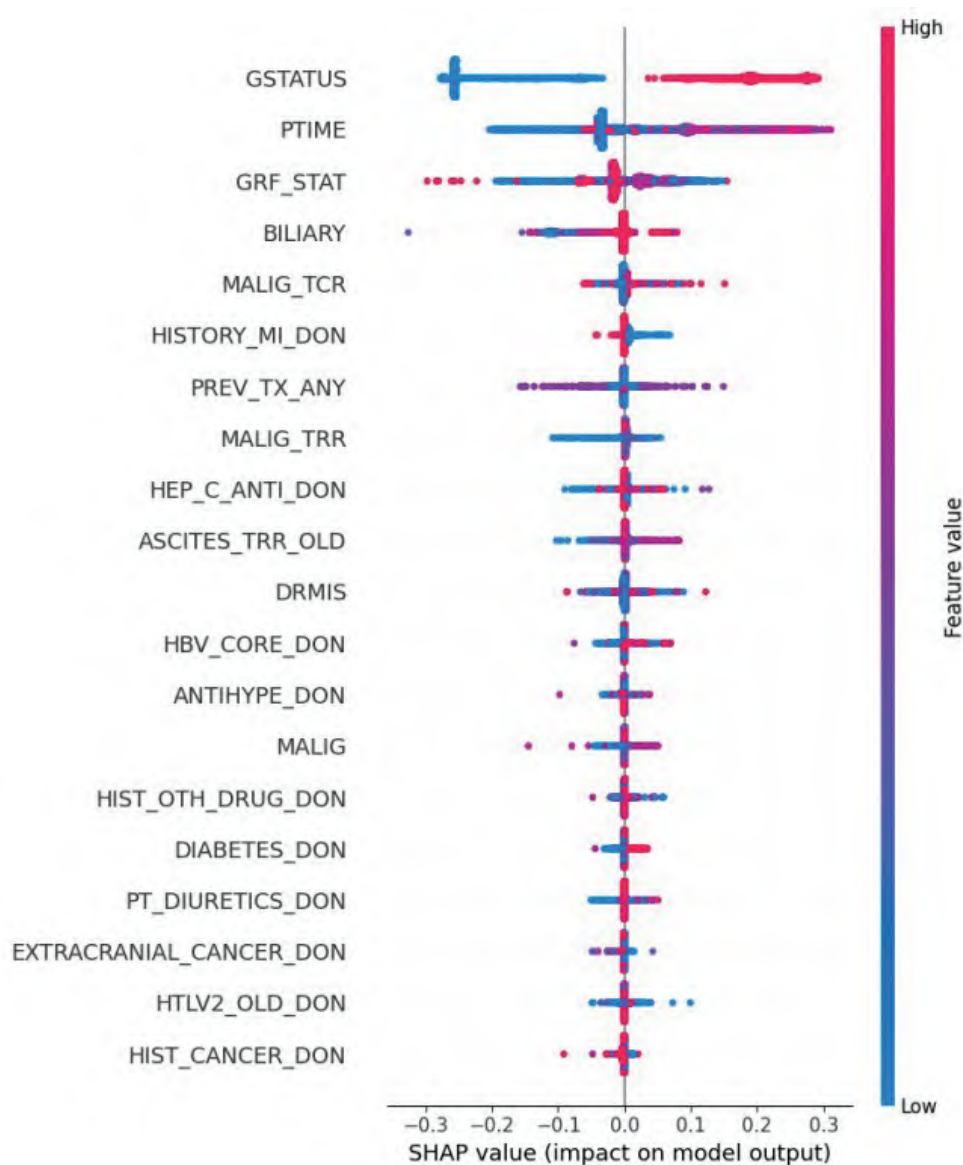


Figure 6. Feature importance among different classes.

Calibration curve

In machine learning and predictive modeling, a calibration curve is a helpful tool for better understanding and adjusting the projected probabilities from classification models. Figure 7A shows the calibration curve for the different algorithms used in this study.²⁸ It can be seen that KNN, ANN, and logistic regression are overconfident, whereas CART is underconfident in this study.

Discrimination

The best measure for evaluation is the concordance statistic, the c-index. For a prediction model to be helpful in medical decision-making, it must be able to distinguish between those who experience the outcome and those who do not.²⁸ Figure 7B presents the discrimination analysis of the proposed model.

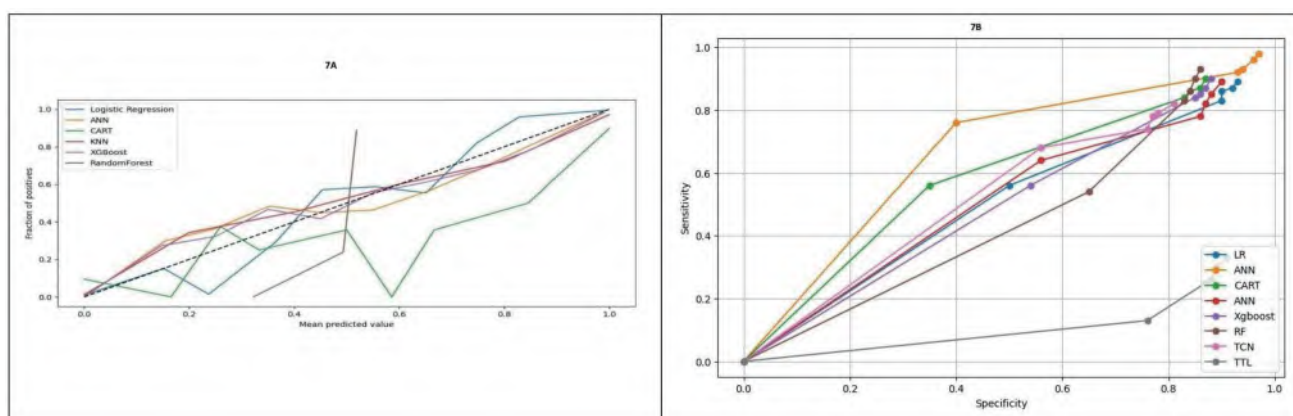


Figure 7. Proposed model's performance. 7A: calibration plot, 7B: discrimination plot.

Ablation analysis

Ablation analysis is performed to determine the effect of a component in a system. The result of this proposed

model is compared with the performance of the model when the meld score was used for survival prediction. Figure 8 presents the comparison of the ablation study.

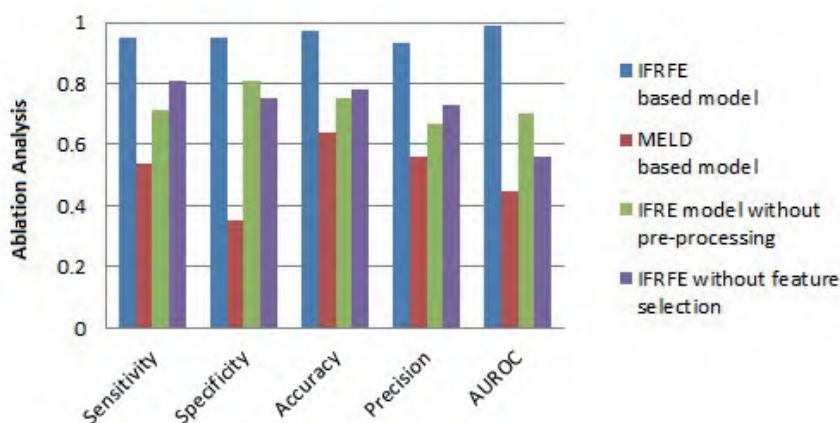


Figure 8. Ablation analysis results.

Strengths and limitations

This study has the advantage of a large dataset with many patients and attributes, advanced machine-learning techniques, and SHAP values for model interpretation. However, it relies on potentially biased and error-prone UNOS database data.

Conclusions

Machine learning is increasingly used in healthcare to identify patterns, though many algorithms lack interpretability. This study's main contribution is applying interpretable machine learning for prediction. Among the six models, ANN best predicts mortality after liver transplantation, supporting the development of early

warning systems. Future research could focus on survival prediction for other transplants with improved optimization methods.

Acknowledgements

I am grateful to the co-author for her insight and to United Network Organ Sharing for supplying the dataset.

Conflict of interest

There are no conflicts of interest.

Funding Statement

Nil

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Evaluation of the modified indirect hemagglutination assay using chicken red blood cells as a routine melioidosis detection in Maharaj Nakorn Chiang Mai Hospital

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ARTICLE INFO

Article history:

Received 12 August 2024

Accepted as revised 3 January 2025

Available online 8 January 2025

Keywords:

Melioidosis, *Burkholderia pseudomallei*, indirect hemagglutination assay, chicken red blood cells

ABSTRACT

Background: Melioidosis is a life-threatening illness caused by *Burkholderia pseudomallei*, which is endemic throughout Thailand. The indirect hemagglutination assay (IHA), a serological test, is widely used to diagnose melioidosis. However, the conventional IHA available in Thailand still requires more than 4 hours for measurement time, limiting its efficiency in clinical settings. This study addresses the need for a quicker and more efficient diagnostic method for melioidosis.

Objective: The study aimed to evaluate the agreement between a modified indirect hemagglutination (modified IHA) assay and the commercially available IHA kit for routine melioidosis diagnosis at Maharaj Nakorn Chiang Mai Hospital. Chicken red blood cells were used instead of sheep red blood cells to reduce diagnostic time and costs while maintaining high accuracy and reliability.

Materials and methods: A total of 368 serum samples were tested using the modified IHA assay, which utilized chicken red blood cells instead of sheep red blood cells used in the commercial IHA kit. The results were compared with those of the commercial IHA kit for melioidosis detection.

Results: The modified IHA assay showed 99.46% agreement, with an excellent kappa value of 0.98, compared to the commercial kit. In the validation step, the modified IHA assay correctly identified 100% (54 out of 54) of positive samples and 100% (54 out of 54) of negative samples. However, 0.63% (2 out of 320) false positives were observed in the diagnostic samples with the modified IHA assay.

Conclusion: The modified IHA assay may serve as a valuable alternative for the routine diagnosis of melioidosis because it is less time-consuming and more cost-effective than the commercial IHA kit. However, further studies with more clinical samples are warranted to confirm its utility.

Introduction

Melioidosis is an infectious disease of humans and animals caused by the environmental bacterium *Burkholderia pseudomallei* (*B. pseudomallei*), a Gram-negative rod-shaped bacterium. It is a severe disease that is endemic with a high mortality rate in Northern Australia and Southeast Asia.¹ In Thailand, this disease is endemic in several regions, including the Northeast, Central, East, North, and South.^{2,3} Patients are infected via skin contact, ingestion, and inhalation of *B. pseudomallei* in

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doi: 10.12982/JAMS.2025.036

E-ISSN: 2539-6056

environmental sources, particularly soil, groundwater, and stagnant water.¹

The clinical manifestations of melioidosis vary widely, ranging from asymptomatic infection to abscesses, pneumonia, disseminated disease, and death.⁴ Notably, a high mortality rate of melioidosis, accounting for 42.3% (11 out of 26 patients), has been reported at Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand.⁵ The disease incubation period generally ranges from 1 to 21 days (mean 9 days).⁶ However, this organism can remain latent in the human body for long periods.⁷

Standard laboratory diagnostic tests for investigating melioidosis include Gram's staining, bacterial culture, and the indirect hemagglutination assay (IHA).

Gram's staining can detect *B. pseudomallei* as a Gram-negative rod-shaped bacterium, but this test lacks sensitivity and specificity.⁸ Bacterial culture remains the mainstay method for diagnosing melioidosis.⁹ However, *B. pseudomallei* can sometimes be misidentified as other bacteria or dismissed as a contaminant. Although microbiological culture is the gold standard for diagnosis, it is a complex and time-consuming method. The IHA, a serological test, is widely used for the diagnosis of melioidosis by detecting antibodies raised against *B. pseudomallei*.⁹

Although it lacks standardization, it can be valuable for diagnosing febrile illness in people who have not lived in an endemic region. In Thailand, the diagnostic sensitivity of the IHA for melioidosis at admission has been reported as 73% and specificity as 64%.¹⁰ The IHA has been used for the diagnosis of melioidosis for over 50 years, which remained a primary unchanged protocol. Nowadays, conventional and commercial IHA protocols use sheep red blood cells for detection, but the detection time remains more than 4 hours.

Chicken red blood cells are commonly used in hemagglutination assay (HA) and hemagglutination inhibition (HI) tests to measure hemagglutinating antibodies against viruses with lower cross-reactivity with human antibodies than mammalian red blood cells (e.g. sheep, horse, or cow).^{11,12} Some publications on HA and HI protocols for quantifying influenza-specific antibody titers have reported that using chicken red blood cells can

reduce the measurement time to less than 2 hours.¹³ This study used chicken red blood cells to detect melioidosis in a modified indirect hemagglutination (modified IHA) assay.

This study evaluated the correlation between the modified IHA assay and the commercial IHA kit used for routine melioidosis diagnosis at Maharaj Nakorn Chiang Mai Hospital.

Materials and methods

Study population

This study used 368 serum samples from suspected melioidosis patients at Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, collected between January 2021 and December 2022. The residual specimens from routine IHA for melioidosis detection were utilized to test the modified IHA assay. They compared it to the commercial IHA kit utilizing sheep red blood cells provided by the National Institute of Health, Department of Medical Science, Thailand, for melioidosis detection.

Chicken red blood cells preparation for modified indirect hemagglutination assay

Collection and storage of chicken red blood cells

Chickens, species *Gallus gallus domesticus*, were kindly supported by the Research Unit, Faculty of Medicine, Chiang Mai University. Five milliliters of chicken blood were collected from the brachial wing vein of a chicken aged 8-9 months by a veterinarian (AK.) under aseptic conditions (Figure 1). The chicken blood was then placed in a sterile tube containing an equal volume of Alsever's solution. The mixed blood was transported to the Immunology laboratory at a controlled temperature of 4 °C for further processing. Next, the mixed blood was washed in phosphate buffer saline (PBS) three times. The packed chicken red cells were suspended in PBS containing 0.05% glutaraldehyde to prepare the working solution as 1% washed cells (i.e., 10 µL of blood cells in 990 µL of PBS). The prepared 1% glutaraldehyde-treated chicken red blood cell suspension can be stored for up to three months at 4 °C, maintaining cell integrity for extended use in assays.



Figure 1. Blood collection site at the brachial wing vein chicken.

Chicken red blood cell sensitization

B. pseudomallei was isolated from a positive blood culture. The positive blood culture was Gram-stained, and the remaining blood culture was routinely processed by subculture onto MacConkey, blood, and chocolate agar plates. The bacteria grown on the plates were assessed for colony morphology, purity, and Gram-stain reaction. The isolated colony was then subjected to biochemical characterization (e.g., oxidase, indole, catalase, motility, triple sugar iron, urease, and carbohydrate fermentation tests).

The confirmed *B. pseudomallei* was subcultured on trypticase soy agar and then incubated at 37 °C for 24 to 48 hrs. For crude *B. pseudomallei* antigen preparations, the confirmed *B. pseudomallei* cells were optimized for the bacterial antigen dilution using McFarland number 3 (9×10^8 cells/mL) in 5 mL of PBS. The adjusted bacteria were then inactivated by autoclaving at 121 °C under a pressure of 15 psi for 15 minutes. Next, the optimal bacterial cell suspension was diluted to make the final dilutions of the antigens of 1:20, 1:40, 1:60, 1:80, 1:100, 1:120, 1:140, and 0 (no antigen) in PBS for titration of the sensitized red cells. The 1% washed chicken red blood cells were added to crude *B. pseudomallei* antigen suspension at a ratio 1:1, and the mixture was incubated at 37 °C for 1 hr. After incubation, the mixture was washed and stored at 4 °C until the modified IHA assay was used. The optimal bacterial antigen dilution was established for each new batch of bacterial antigen, representing a process of standardization of antigen concentration based on previous batches. The concentration of each antigen preparation was standardized with reference pooled sera before use in the assay to prevent batch-to-batch variation.

Titration assay

The positive control serum, pooled from three patients with culture-proven melioidosis who had established IHA titers around 1:1,280, was diluted two-fold, ranging from 1:20 to 1:10,240. Then, 25 µL of 1% washed chicken red blood cells from each antigen dilution were incubated with the positive control serum. Non-sensitized red cells were added as negative control. Then, the mixtures were incubated at room temperature for 2 hrs, following the commercial kit protocol, with observations taken every 15 minutes. The optimal antigen concentration was identified as the one that gives the correct (previously known) IHA titer for the pooled serum. The highest antibody dilution demonstrating complete or partial agglutination was recorded as the IHA titer. This study identified the optimal antigen concentration for the modified IHA assay as 1:100, ensuring accurate titer determination in subsequent analyses.

Validation of modified indirect hemagglutination test

To validate the modified IHA assay, 54 known positive and 54 known negative samples, including positive and negative controls from the commercial kit and patient samples, were evaluated for serum titers against *B. pseudomallei* antigen. Serum samples were prepared as detailed in Table 1, followed by incubation with either 1% sensitized chicken red blood cells for the modified-IHA assay or 1% sensitized sheep red blood cells for the commercial IHA kit. Non-sensitized red blood cells served as negative controls for both methods. The mixtures were incubated at room temperature for 2 hrs, with observations taken every 15 mins under the commercial kit protocol. An optimized incubation time of 45 minutes for the modified IHA assay was established based on results from known positive samples. The measurement steps for the commercial IHA kit and the modified IHA assay across four samples are shown in Table 1.

Table 1. Comparison of measurement steps between the commercial IHA kit and the modified IHA assay.

Steps	Commercial IHA kit	Modified indirect hemagglutination test
1. Serum preparation	Yes	No
- Serum inactivation	(At least 1 hour, depended on the number of samples)	
- Incubation of 5% uncoated cells with serum		
- Centrifugation for collecting the absorbed serum		
2. Sample dilution	Yes	Yes
- Two-fold dilution of absorbed serum	(At least 1 hour, depended on the number of samples)	(At least 1 hour, depended on the number of samples)
- Addition of sensitized red blood cells, diluted serum, and negative and positive controls into a microplastic 96-well plate		
3. Indirect hemagglutination test (incubation time)	Yes	Yes
	2 hours	45 mins
Total measurement time*	At least 4 hours	At least 1 hours 45 mins
(The measurement time depended on the number of samples)		

Performance of modified indirect hemagglutination test

The serums were diluted two-fold, ranging from 1:20 to 1:10,240, to evaluate the titers of patient serum against *B. pseudomallei* antigen. Twenty-five microliters (μL) of each diluted serum were added to a 96-well plate. Then, 25 μL of 1% sensitized chicken red blood cells were added to the 96-well plate. Next, the mixtures were incubated for 45 minutes at room temperature (optimized time from validation assay). The reaction titer was the last dilution that presented 50% agglutination. Non-sensitized red cells were used as negative controls. A titer of $\geq 1:160$ was considered positive.

Statistical analysis

Cohen’s kappa was used to test the measure of inter-rater reliability between the commercial IHA kit (provided by the National Institute of Health, Department of Medical Science, Thailand) and the modified IHA assay. A $p<0.05$

was considered statistically significant. All statistical analyses were performed using STATA version 16 (STATA Corp., Texas, USA).

Results

Validation of modified IHA assay in the diagnosis of melioidosis

The modified IHA assay offers a reduced measurement time, providing reliable readings at 45 mins post-incubation, unlike the commercial IHA kit, which requires a complete 2-hr incubation for accurate results (Figure 2). In validation tests, the modified IHA assay achieved a 100% positive detection rate (titer $\geq 1:160$) for all known positive samples (54 out of 54). Additionally, the assay demonstrated 100% specificity by accurately yielding negative results for all known negative samples (54 out of 54), as summarized in Table 2.

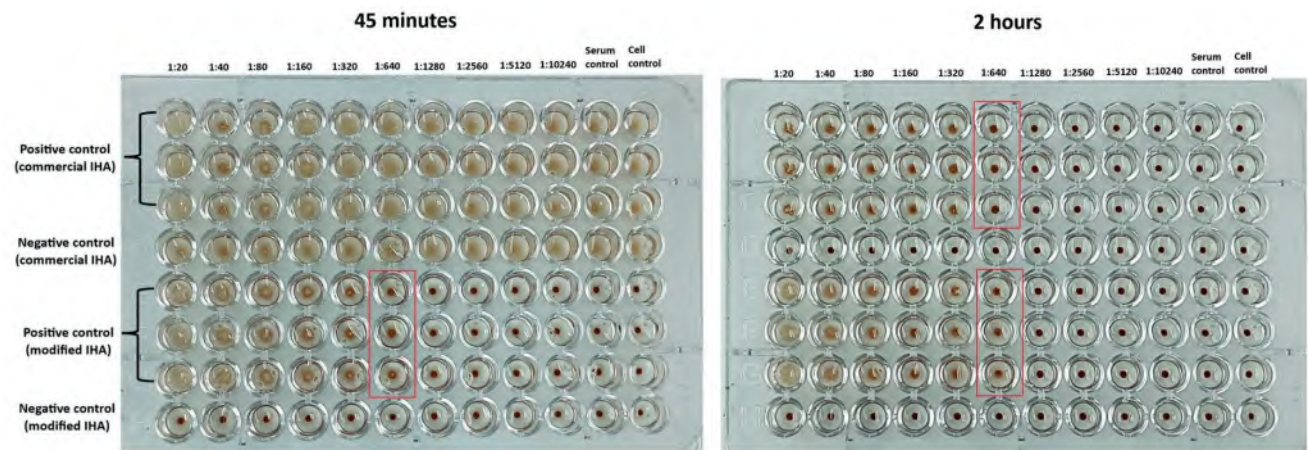


Figure 2. Comparison of measurement time between the commercial IHA kit and the modified IHA assay.

Table 2. Comparison of indirect hemagglutination results between the commercial IHA kit and modified IHA assay using known negative and positive samples.

Validation of samples	Modified indirect hemagglutination assay		Total
	Negative	Positive	
Known negative	54	0	54
Known positive	0	54	54
Total	54	54	108

Diagnostic samples

Among the 368 patients, 86.96% (320 out of 368) were found negative for melioidosis, and 13.04% (48 out of 368) were found positive using the commercial IHA kit. The titers of the 320 negative samples were distributed as follows: <1:20 (56.00%), 1:20 (29.50%), 1:40 (12.00%), and 1:80 (2.50%). Among the 48 positive samples, the titers were distributed as follows: 1:160 (18.75%), 1:320 (18.75%), 1:640 (25.00%), 1:1280 (16.67%), 1:2560 (10.42%), 1:5120 (6.25%), and 1:10240 (4.16%).

Using the modified IHA assay, 86.41% (318 out of 368) were classified as negative, and 13.59% (50 out of 368) were classified as positive for melioidosis. The titers of the 318 negative samples were distributed as follows:

<1:20 (55.60%), 1:20 (32.40%), 1:40 (10.12%), and 1:80 (1.88%). Among the 50 positive samples, the titers were distributed as follows: 1:160 (20.00%), 1:320 (26.00%), 1:640 (32.00%), 1:1280 (8.00%), 1:2560 (8.00%), 1:5120 (4.00%), and 1:10240 (2.00%) (Figure 3).

The concordance between the commercial IHA kit and the modified IHA assay was 99.46%, with a kappa value of 0.98, indicating excellent agreement (Table 3). However, there were two discordant results between the two assays. Two patients tested positive using the modified IHA assay, with titers of 1:160, while the commercial IHA kit classified these samples as negative with titers of 1:40 and 1:80, respectively (Table 4).

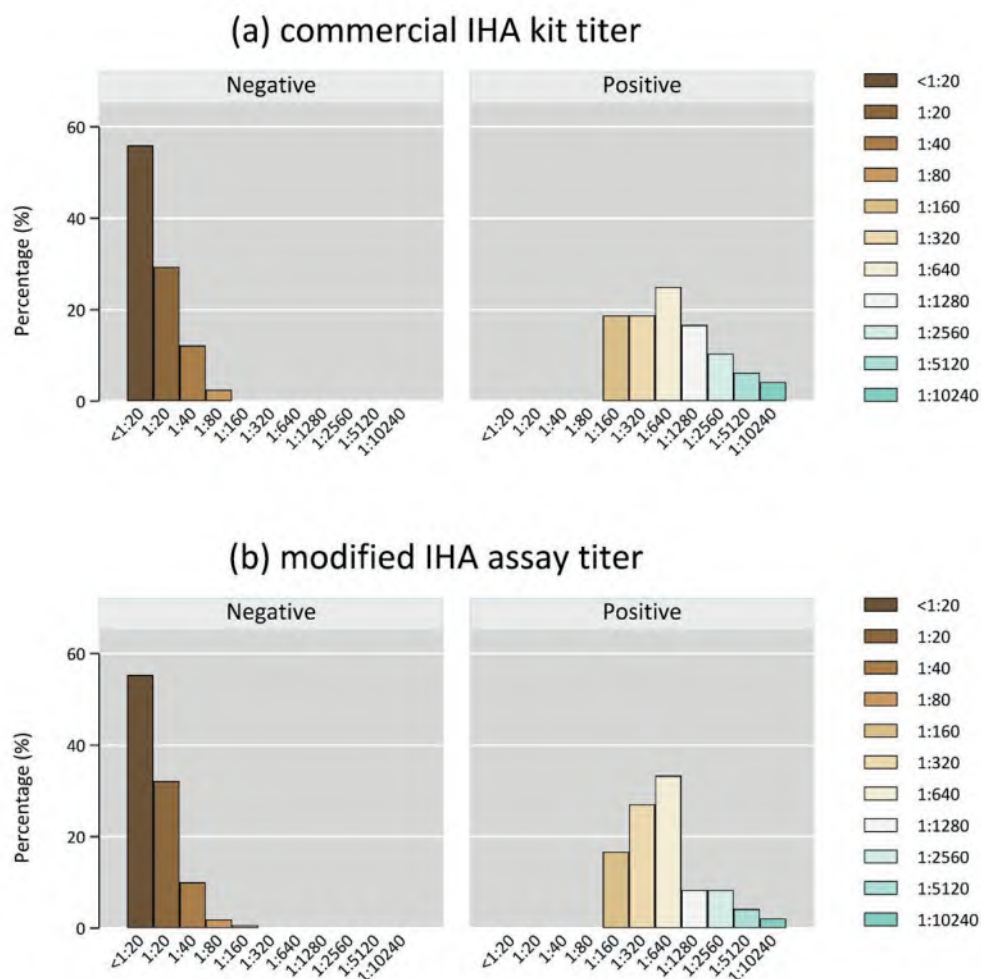
**Figure 3.** The proportion of each IHA titer level for each method stratified by IHA positive status.

Table 3. Comparison of indirect hemagglutination from the commercial IHA kit and modified IHA assay using diagnostic samples.

Commercial IHA kit	Modified IHA assay		Total
	Negative	Positive	
Negative	318	2	320
Positive	0	48	48
Total	318	50	368

Table 4. Clinical relevance of discordance results between commercial IHA kit and the modified-IHA assay.

Discordant case	Commercial IHA kit	Dilution	Modified IHA assay	Dilution	Diagnosis
Case 1	Negative	1:40	Positive	1:160	Afebrile
Case 2	Negative	1:80	Positive	1:160	<i>Klebsiella pneumoniae</i> mixed <i>Streptococcus</i> spp. infection

Discussion

The IHA is one of the most widely used methods for diagnosing melioidosis in disease-endemic countries, particularly Thailand.³ It has remained unchanged since it was first described over 50 years ago due to its low cost and simplicity. However, despite its longevity and cost-effectiveness, the method remains time-consuming, with detection times exceeding 4 hours when using sheep red blood cells, as seen in various commercial IHA kits currently available in Thailand. In contrast, chicken red blood cells are commonly used in virology tests such as hemagglutination assay and hemagglutination inhibition tests for quantifying influenza-specific antibody titers. Using chicken red blood cells, the measurement time for HA and HI protocols is less than 2 hours.^{13,14} This improved efficiency is attributed to the unique properties of chicken red blood cells, including reduced non-specific binding due to lower cross-reactivity with human antibodies compared to mammalian red blood cells.^{11,12} Chicken red blood cells have a shorter lifespan of approximately 1-2 months¹⁷ than sheep red blood cells, which can last 3-6 months¹⁸ due to differences in metabolic activity and cell structure. Despite the shorter lifespan, the reduced detection time and efficiency associated with chicken red blood cells present a potential advantage for improving the IHA method for melioidosis diagnosis.

This study adapted the IHA protocol using chicken red blood cells to detect melioidosis, showing excellent agreement with the commercial IHA kit. Notably, the modified protocol reduced measurement time, improving diagnostic workflow efficiency in clinical settings. This advancement facilitates faster decision-making and potentially enhances patient outcomes, particularly in resource-limited environments. In contrast, a small false positive rate of 0.63% (2 out of 320) was observed in the modified IHA assay compared to the commercial IHA kit. Despite this, the results suggest that the modified IHA assay could be viable for routine melioidosis diagnosis. However, the small sample size in this study underscores the need for further prospective studies with larger clinical cohorts. Additionally, exploring the heat inactivation of serum in future research may help reduce false positives further.

The limitations of this study include the variation in the strains (typically local clinical strains) used for crude antigen preparation compared to the commercial IHA kit. The uncharacterized nature of the antigenic epitopes and antibodies might contribute to the false positive observed. False positives can occur due to high background seroconversion rates in endemic areas, where titers of 1:160 to >1:1,280 have been reported in non-melioidosis patients at rates of 33% and in afebrile patients at rates of 60%.¹⁵ In Thailand, a titer of 1:160 is generally considered positive, with the IHA sensitivity for melioidosis at admission reported as 73% and a specificity as 64%.¹⁰ The unstandardized antigen content and varying host responses may further complicate interpretation. Addressing these issues through additional measures, such as heat inactivation of sera, could help mitigate false positive results.

Bacterial culture remains the gold standard for diagnosing melioidosis, though it can sometimes be misidentified or dismissed as contamination. Notably, *B. pseudomallei* is classified as a hazard group 3 pathogen globally and in Thailand (https://tbrcnetwork.org/riskgroup.php?riskgroup_h=3), handling this bacterium requires strict biosafety measures and regular training.¹⁶

Despite the limitations, the modified IHA method improves by reducing measurement time to less than two hours while maintaining comparable sensitivity and specificity to the commercial IHA kit. Furthermore, the cost of the modified IHA is approximately 3.5 times lower than the commercial assay (10 Baht/test vs. 35 Baht/test). These findings suggest that the modified IHA assay has the potential to enhance routine diagnostics, particularly in resource-limited settings. Future studies with larger clinical sample sizes will be essential to validate this method's robustness and clinical utility.

Limitation

One limitation of this study is using local clinical strains of *B. pseudomallei* to prepare crude antigens, contrasting with the standardized commercial IHA kit. The uncharacterized nature of the antigenic epitopes and corresponding antibodies may have contributed to the variability in results. Additionally, the impact of heat

inactivation of sera should be further explored in future studies to reduce false positives.

Conclusion

The modified IHA assay using chicken red blood cells offers advantages such as cost-effectiveness and improved laboratory detection time while maintaining sensitivity and specificity comparable to those of a commercial IHA kit for melioidosis. However, further studies with larger clinical sample sizes are needed to validate these findings and confirm the method's utility in routine diagnostics.

Conflict of interest

The authors declare no conflict of interest.

Funding

No funding was received to conduct this study.

Ethical approval

This study was approved by the institutional ethics committee of the Faculty of Medicine, Chiang Mai University (Research ID: 8830/ Study code: PAT-2565-08830). Additionally, this study was approved for biosafety (Research ID: CMUIBC02023/2564) and use of animals (Protocol number: 30/2565) by the institutional biosafety committee and the institutional animal care and use committee of the Faculty of Medicine, Chiang Mai University, respectively.

Acknowledgements

The authors gratefully acknowledge the Faculty of Medicine, Chiang Mai University, in completing this study.

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Effect of structured exercise program on fundamental motor skills in children with Down syndrome: A randomized controlled trial

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ARTICLE INFO

Article history:

Received 14 November 2024

Accepted as revised 7 January 2025

Available online 9 January 2025

Keywords:

Structured exercise program, FMS proficiency, children with Down syndrome.

ABSTRACT

Background: Down syndrome (DS) is one of the most common chromosomal abnormalities, and it has a deficiency in fundamental motor skills (FMS).

Objective: This study aimed to determine how a structured exercise program impacted children with Down syndrome's fundamental motor skills.

Materials and methods: This study was conducted in a randomized controlled trial at the School for Disabled Children, Yangon. Thirty participants (15 in each group) with DS were divided into the experimental and control groups. The experimental group participated in a structured exercise program (focusing on stability, object control skills, and locomotor skills) for six weeks, while the control group did artistic and recreational activities (singing, dancing, drawing, and coloring). Lower extremity functional strength (five times sit-to-stand test), static balance (modified Stroke test), and FMS proficiency (TGMD-2) were assessed by blinded assessors at the start of the intervention, three weeks later, and six weeks later. Independent sample t-tests and repeated measure ANOVA were used to compare the two groups and within each group.

Results: Post-test analyses of all outcome measures between the two groups revealed statistically significant differences. In the third- and sixth-weeks following intervention, the FMS, static balance, and lower extremity functional strength were significantly higher in the experimental group than in the control group.

Conclusion: The current study's findings highlight that a structured exercise program focused on FMS can be applied in educational and clinical settings to improve the FMS of children with DS.

Introduction

Individuals with Down syndrome (DS) often delay developing fundamental motor skills (FMS), such as locomotor skills, object control skills, and stability skills, which are the building blocks for more complex, specialized movements and sport-specific skills.^{1,2} Children with DS may have phenotypic characteristics such as hypotonia, joint laxity, balance dysfunctions, and incoordination, making it more difficult to perform these activities.^{3,4} These phenotypic features led to compensatory movement patterns in acquiring FMS.^{5,6} Many muscles or muscle groups need to be activated to perform the FMS, which is crucial for children's cognitive, social, and psychological development.^{1,7,8}

The FMS is the foundation for the successful performance of sporting and physical activities and socialization in later life.^{1,9} The children will be delayed in

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doi: 10.12982/JAMS.2025.037

E-ISSN: 2539-6056

developing more complex, specialized, and sports-specific skills if they are deficient in the FMS.¹ Proficiency in the FMS has been identified as a major contributing factor to improving physical activity,⁹⁻¹¹ physical fitness,¹² a healthy weight,¹³ and mastering more complex motor skills for present and advanced age.^{11,14} Furthermore, the FMS correlates with lifetime involvement in physical activity, which is essential to maintain physical health and support cognitive and social development during childhood.¹⁵ Therefore, every child had to be proficient in their FMS. Even so, the number of children with FMS deficiency was still growing, mainly among children with special needs. In addition, a recent systematic review stated that the FMS level of 6- to 10-year-old TDC around the globe was “below average” when compared with normative data from Ulrich’s TGMD-2.¹⁶

Previous research recommended that FMS would need to be taught and trained by children to achieve proficiency in these skills because their development is not a natural process. The currently available literature has highlighted that free play will not contribute to the FMS.^{8,17} Therefore, sufficient practice opportunities, intervention programs, and quality instructions and feedback are vital to enhance FMS proficiency.⁸

Several studies, systematic reviews, and meta-analyses concerning children and youth with typical development,^{8,17-21} children with developmental coordination disorder (DCD),²² and youth with intellectual disabilities (ID)²³ have approved that motor skill training effectively enhances their FMS. These studies have examined TDC, children with DCD, and ID. Indeed, studies are still needed to explore the effect of motor skill intervention on the FMS in children with DS, according to our knowledge. In this study, the researchers attempted to implement a structured exercise program as an intervention to enhance FMS. The intervention program is based on theoretical literature and several previous studies that have successfully implemented intervention programs. Moreover, the design aimed to improve the FMS of children with DS, aged 7 to 10 years, by focusing on three FMS domains: locomotor, object control, and stability skills.

A structured exercise program has not been proven to have any positive effects on the FMS of children with DS in Myanmar. However, numerous studies are being done on FMS training in TDC and children with special needs. Additionally, research into physical therapy treatments for people with DS is growing. However, there are still many important issues about how structured exercise programs enhance FMS proficiency in children with DS, and their findings are slightly contradictory. Most physical therapy treatments for children with DS also focus on improving physical fitness, strength, endurance, and balance. A systematic physical therapy program was still required to raise the FMS proficiency of DS children in Myanmar. Therefore, the objective of the current study was to assess how a structured exercise program improved DS children’s FMS proficiency.

Materials and methods

This randomized controlled trial was conducted at the School for Disabled Children (SDC), Yangon, and included 30 children with DS (22 boys and 8 girls). The participants, diagnosed with DS by their pediatrician, could follow two-step commands considered inclusion criteria. The participants who met the exclusion criteria had severe neurological, medical, and musculoskeletal problems. The principal, the classroom teachers, the children with DS, and their parents or guardians at the SDC, Yangon, were invited to participate in this study. Afterward, the participant was chosen and provided an in-depth verbal and written explanation of the study’s objectives, methodologies, advantages, and safety precautions. Then, all parents and guardians gave their written and informed agreement, and all participants gave verbal assent. The Institutional Review Board (IRB) of the University of Medical Technology, Yangon, granted ethical approval (ethical approval number IRB/UMTY/2-2020/002). The study followed the rules set by the Consolidated Standards of Reporting Trials (CONSORT) and was registered with the Thai Clinical Trials Registry (TCTR20230129001). Figure 1 displays the CONSORT flow diagram of the investigation.

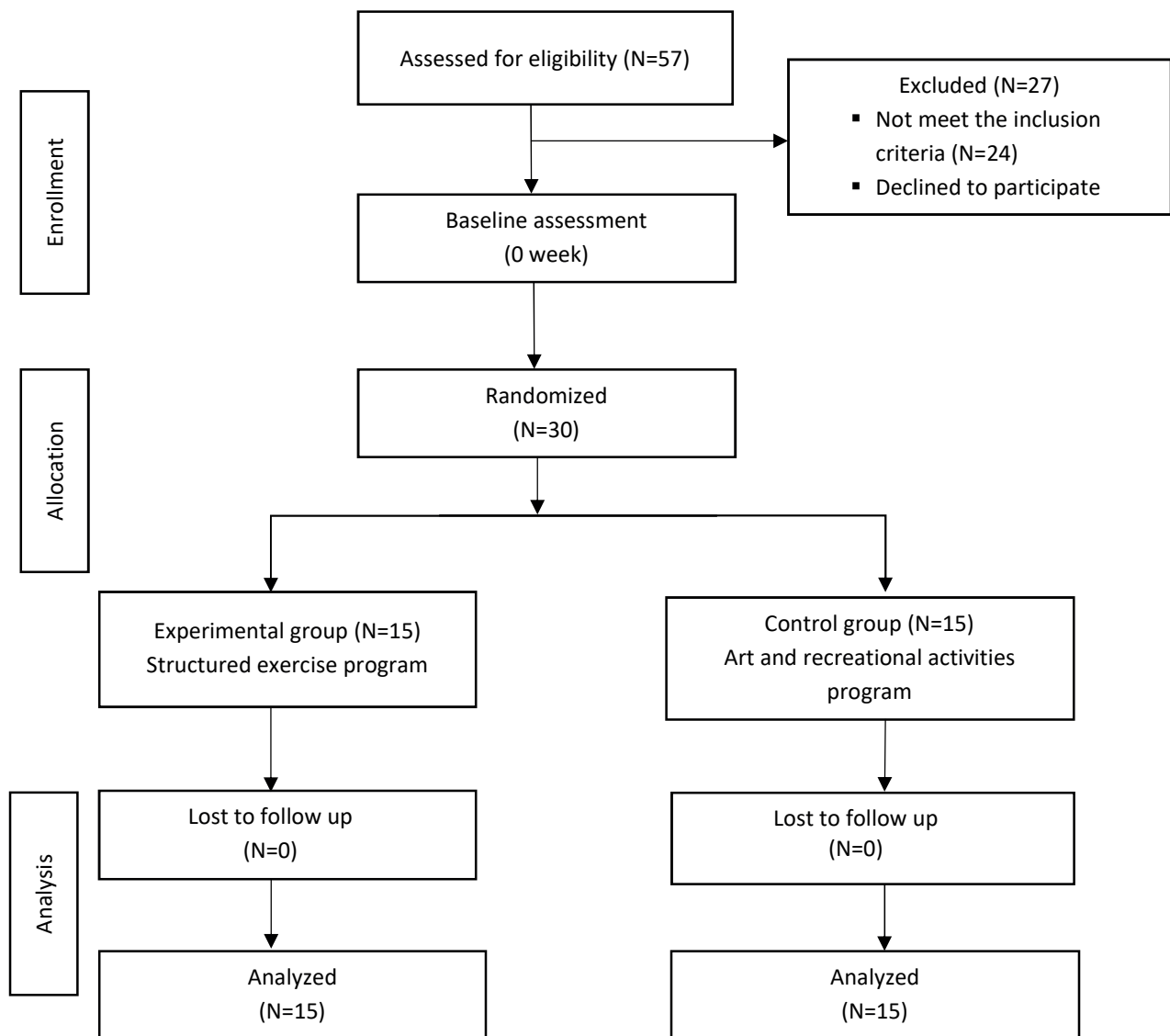


Figure 1. The CONSORT flow diagram of children with Down syndrome recruitment.

Children with DS were stratified by sex to ensure that the experimental and control groups involved equal numbers of boys and girls. Three permuted blocks of 10 participants were created using a computer-generated program to allocate children with DS into two trial arms. The participants were allocated via the sequentially numbered opaque sealed envelopes (SNOSE) and assigned a class teacher. The principal researcher and one research assistant gave the experimental group, and two research assistants provided the control group. Another two research assistants served as blind assessors for the outcome measures.

In the experimental group, the participants were given a structured exercise program (stability, object control skills, and locomotor control skills). It was developed and contained 24 exercises and activities in a functional, goal-oriented approach for FMS proficiency.

Physiotherapists commonly prescribe these exercises in pediatric rehabilitation. Exercises and activities were categorized and arranged into steps (I and II) based on task complexity and balancing challenging activities. Each step was comprised of 12 exercises that were related to playing activities and improving FMS proficiency. Step I; exercises and activities were performed in the first three weeks, and Step II; exercises and activities were performed in the next three weeks, respectively. These activities were divided into three parts: warming up (5 minutes), structured exercises (40 minutes), and cooling down (5 minutes), and the total exercise duration lasted approximately 50 minutes for each session. Furthermore, skill instruction, practice, set-up, and explanation for activities lasted for 15 minutes. The comprehensive depiction of the structured exercise program is outlined in Supplementary Table 1.

Table 1. Demographic characteristics of experimental and control groups.

Demographic characteristics	Experimental group (N=15)	Control group (N=15)	p value
Mean age (years)	9.27±1.03	9.33±0.97	0.85
Mean age (months)	119.87±14.35	120.47±14.20	0.90
Mean height (cm)	128.35±12.47	123.21±12.78	0.27
Mean weight (kg)	38.47±9.12	32.026±8.40	0.06
Mean BMI (kg/m ²)	23.04±2.65	20.87±3.26	0.06
Mean BMI percentiles	94.16±3.53	83.46±25.61	0.31
Weight status according to BMI percentiles			
Underweight (<5 th percentile), N (%)	0	0	-
Healthy weight (5 th - <85 th percentile), N (%)	1 (6.7%)	0	
Overweight (85 th - <95 th percentile), N (%)	7 (46.6%)	7(46.6%)	
Obese (≥95 th percentile), N (%)	7 (46.6%)	8 (53.4%)	
Gender			
Boy, N (%)	11 (73.3%)	11 (73.3%)	-
Girl, N (%)	4 (26.7%)	4 (26.7%)	

Note: Significant difference tested by independent sample t test.

In the control group, the participants were given an art and recreational activities program (singing, dancing, drawing, and coloring). Each session in the control group also lasted for around 50 minutes, which is equivalent to the duration of the sessions in the experimental group. The participants in both groups were informed of the details of the intervention program. Each program was scheduled for 6 weeks with 3 sessions per week (18 sessions). These exercises and activities were performed in groups containing five participants. Participants in both groups were given similar warm-up and cool-down activities. These activities were conducted separately.

The primary outcome measure was the test for gross motor development second edition (TGMD-2), and the secondary outcome measures were the modified stork test and the five times sit-to-stand test (FTSTS). The inter-rater agreement (between the principal investigator and the blinded assessor) of outcome measures was also explored before starting the assessment. The inter-rater reliability of TGMD-2 was found to be excellent, as shown by the high Cronbach's alpha and intra-class correlation coefficient (ICC) values. The ICC for the GMQ was 0.96, while the raw scores for locomotor and object control were 0.97 and 0.97, respectively.²⁴ The inter-rater reliability of the modified Stork test and five times sit-to-stand test was 0.89 and 0.86, respectively. All outcome measures were taken at baseline, the third week after, and the sixth week after intervention by the blinded assessors. The physiotherapist scored the TGMD-2, and the principal researcher recorded the video. The secondary outcome measures were assessed by the class teacher from the SDC. Both the assessors and participants were blinded to the participants' intervention groups.

The static balance of the participants was assessed using a modified Stork test. The participants stood with one leg on the flat floor, and the other was lifted above the ankle joint with the arms at the sides (Figure 2). The participants had to perform once for each leg, and the time (seconds) was recorded using a stopwatch. The reliability and objectivity of this test were 0.87 and 0.99, respectively.²⁵

**Figure 2.** Modified Stork test.

Using a stopwatch, the FTSTS measured the time (seconds) required to complete five successive sit-to-stand cycles as quickly as possible. A chair with no armrest was used, and the barefoot test was done on a floor (Figure 3). A child was instructed to sit with their

arms folded across their chest and their back against the chair, stand up and sit down as quickly as possible five times. The FTSTS is a functional lower limb muscle strength measurement with excellent reliability in children with spastic diplegic cerebral palsy.²⁶



Figure 3. Five times sit-to-stand test.

The FMS assessment process followed the TGMD-2's standard operating procedures. The performance criteria for the TGMD-2 were 3-5, comprising 12 skills (six locomotor and six object control skills). The participants received a score of 1 if they were successful in doing the skill and a score of 0 if they were unsuccessful. A skill score was calculated by adding the sum of the performance criteria's total criterion scores. The six skill scores were added to get the raw subtest scores. The locomotor and object control skills subtest had a maximum raw score of 48. The subtest's raw score was changed for the locomotor and object control standard scores. In addition, the subtest standard scores were totaled up and converted into the gross motor quotient (GMQ); the maximum was 160. This test had excellent reliability and validity.⁷

The statistical analysis was conducted using the Statistical Package for the Social Sciences software version 25.0 for Windows (SPSS, Inc. Chicago, IL, USA). The Shapiro-Wilk test was used to test whether the data were normally distributed. Descriptive statistics, independent samples t-tests, and repeated measure ANOVA were used

to verify the differences between and within groups and the group-by-time interaction effects of interest. The effect sizes between groups were determined using partial eta squared (η_p^2), with values of less than 0.10, between 0.10 and 0.06, and greater than 0.14, which were categorized as small effect size, medium effect size, and large effect size, respectively.²⁷ The Bonferroni correction was employed for conducting post hoc pairwise comparisons. An independent t-test was used to compare the groups' significant differences in the mean score. A $p < 0.05$ was considered statistically significant.

Results

There was no statistically significant difference between the mean scores of the two groups in demographic characteristics such as age and BMI percentiles, as shown in Table 1.

To examine the effects of time and group as well as the interaction effect of time and group on FMS (TGMD-2), a two-way mixed repeated measure ANOVA was conducted, and the detailed results are shown in Table 2.

Table 2. Comparison of FMS within each group among baseline, 3rd week, and 6th week after intervention between experimental and control groups.

FMS	Groups	Baseline Mean±SD	3 rd week Mean±SD	6 th week Mean±SD	p value ^a	p value ^b	p value ^c
LSS	Experimental	1.80±1.37	5.00±1.51	8.80±1.85	<0.001	<0.001	<0.001
	Control	2.00±1.36	2.07±1.48	2.27±1.87	1.00	0.566	0.493
	p value ^d	0.692	<0.001	<0.001			
OCSS	Experimental	2.33±1.79	6.07±2.52	9.07±1.43	<0.001	<0.001	<0.001
	Control	2.00±1.41	2.00±1.41	2.13±1.76	-	1.000	1.000
	p value ^d	0.577	<0.001	<0.001			
GMQ	Experimental	52.60±7.53	73.20±10.84	93.60±8.47	<0.001	<0.001	<0.001
	Control	52.00±7.52	52.20±7.89	53.20±10.19	1.000	0.713	0.566
	p value ^d	0.829	<0.001	<0.001			

Note: Pairwise comparison tested by Bonferroni Post hoc test at $p < 0.05$, Within-group comparisons; a: comparison of FMS between baseline and 3rd week after intervention, b: comparison of FMS between 3rd week and 6th week after intervention, c: comparison of FMS between baseline and 6th week after intervention, Between-group comparison; d: comparison of FMS between experimental and control groups, FMS: fundamental motor skills; LSS: locomotor standard score; OCSS: object control standard score; GMQ: gross motor quotient.

The significant effect of time ($F[2,56]=187.38$, $p < 0.001$, effect size=0.87) and the considerable influence of group ($F[1,28]=32.85$, $p < 0.001$, effect size=0.54) were found for FMS (TGMD-2: LSS). Furthermore, the effects of time and group LSS interacted to produce a score of ($F[2,56]=160.60$, $p < 0.001$, effect size=0.85). For FMS (TGMD-2: OCSS), a significant effect of time ($F[2,56]=93.45$, $p < 0.001$, effect size=0.76), and a significant effect of group ($F[1,28]=42.85$, $p < 0.001$, effect size=0.61), were found. Likewise, there was an interaction effect of time and group ($F[2,56]=86.61$, $p < 0.001$, effect size=0.76) on the OCSS. A significant effect of group ($F[1,28]=48.03$, $p < 0.001$, effect size=0.63) and a significant effect of time ($F[2,56]=193.98$, $p < 0.001$, effect size=0.87) were found for FMS (TGMD-2: GMQ). Additionally, there was a time-group interaction impact on the GMQ score ($F[2,56]=172.57$, $p < 0.001$, effect size=0.86).

Table 3 displays the results obtained from the analysis of balance and lower extremity functional strength among children with DS.

For static balance (right side), a significant effect of time ($F[2,56]=240.71$, $p < 0.001$, effect size=0.89) was found, as well as a significant effect of group ($F[1,28]=44.83$, $p < 0.001$, effect size=0.62). Likewise, there was an interaction effect of time and group ($F[2,56]=182.94$, $p < 0.001$, effect size=0.86) on the balance score. Both a significant effect of time ($F[2,56]=287.51$, $p < 0.001$, effect size=0.91) and a significant effect of group ($F[1,28]=43.39$, $p < 0.001$, effect size=0.61) were discovered for static balance (left side). The interaction between time and group also had an impact on the balance score ($F[2,56]=195.91$, $p < 0.001$, effect size=0.87).

Table 3. Comparison of static balance and strength within each group among baseline, 3rd week, and 6th week after intervention between experimental and control groups.

Variables	Groups	Baseline Mean±SD	3 rd week Mean±SD	6 th week Mean±SD	p value ^a	p value ^b	p value ^c
MST (Rt)	Experimental	6.33±1.04	8.00±1.19	14.07±1.79	<0.001	<0.001	<0.001
	Control	6.73±1.03	7.07±0.88	7.33±0.81	0.058	0.122	0.021
	p value ^d	0.301	0.022	<0.001			
MST (Lt)	Experimental	5.33±0.81	7.07±0.96	12.13±1.64	<0.001	<0.001	<0.001
	Control	5.67±0.90	6.13±0.83	6.40±0.63	0.011	0.311	0.009
	p value ^d	0.297	0.008	<0.001			
FTSTS	Experimental	16.93±1.62	15.67±1.71	11.33±1.29	0.094	<0.001	<0.001
	Control	17.33±1.58	16.87±1.72	16.73±1.75	0.011	1.000	0.008
	p value ^d	0.501	0.067	<0.001			

Note: Pairwise comparison tested by Bonferroni Post hoc test at $p < 0.05$, *negative score in FTSTS test (seconds) means better improvement, Within-group comparisons; a: comparison of variables between baseline and 3rd week after intervention, b: comparison of variables between 3rd week and 6th week after intervention, c: comparison of variables between baseline and 6th week after intervention, Between-group comparison; d: comparison of variables between experimental and control groups, MST (Rt): modified Stork balance test (right side); MST (Lt): modified Stork balance test (left side); FTSTS: five times sit to stand test.

Both a significant effect of group ($F[1,28]=20.58$, $p<0.001$, effect size=0.42) and a significant effect of time ($F[2,56]=78.17$, $p<0.001$) were reported for strength. The interaction between time and group also had an impact on the strength score ($F[2,56]=55.11$, $p<0.001$, effect size=0.66).

Discussion

This study has examined the effect of a structured exercise program on the FMS of children with DS after participating in a 6-week institutional-based, group-based, and fun-based exercise program. The structured exercise program was expected to be superior to the arts and recreational program. This study also showed that a structured exercise program can help children with DS improve their FMS performance, static balance, and lower extremity functional strength at all post-training assessments compared to baseline. Based on strong theoretical evidence, the current study confirmed a clear difference between a structured exercise program and art and recreational programs in a real-world setting.

The improved FMS performance of the children with DS in the experimental group was greater than that of the control group after the 3rd and 6th weeks of the intervention. Moreover, there were also significant differences in FMS (TGMD-2) between baseline and the 3rd week after the intervention and between the 3rd week and the 6th week after the intervention in the experimental group compared to the control group.

There are several possible explanations for these results. All exercises and activities in the experimental group were significant and specific tasks based on FMS domains with augmented feedback, quality instructions, and practice opportunities.^{28,29} Additionally, the researcher and one research assistant trained the group of participants, emphasizing the skillful performance of FMS for 50 minutes and 10 minutes of instruction in a 1-hour training session, three sessions a week for six weeks in this study. During exercise training, the researcher and research assistant manually assisted or reinforced the participant with appropriate verbal or visual feedback to get the skillful performance of the FMS.

In this study, all participants in the experimental group received repetitive skilled training and quality feedback because of the supervised exercise therapy and reinforcement provided by the researcher and research assistant. In addition, a structured exercise program was performed in a group; therefore, all participants achieved the effects of group exercises, such as mutual encouragement and stimulation from other group members, and did not feel bored with the exercises.³⁰ Furthermore, exercises and activities were performed station by station; therefore, they could achieve a sense of achievement and completion. Therefore, all participants were physically healthy and had fun performing the exercises and activities. All these effects might enhance the FMS proficiency of participants. The enhancement in TGMD-2 scores was indicative of these effects.

These results align with previous studies, systematic

reviews, and meta-analyses that investigated the effects of motor skills training in various study populations such as TDC,^{8,17,20,31,32} children with DS,^{33,34} and children with ID.²³ These studies revealed that the various motor skill intervention programs significantly enhanced FMS levels among preschoolers, typically developing youth populations, and children with special needs after the intervention. The interventions were effective when the training programs lasted 5 to 24 weeks, and the training sessions were done three times a week for 20 to 30 minutes. Hence, the findings of this study further support that a 6-week (18-hour) structured exercise program effectively enhances FMS performance in children with DS.

The current study's results seemed consistent with other research, including the previous systematic review conducted by Maïano and coworkers in 2019. They reviewed the effects of a motor skill intervention program on FMS in individuals with ID. They found that the intervention programs positively enhanced balance and overall FMS in individuals with ID. Moreover, their findings also mentioned that a developmental physical education program, therapeutic sensorimotor training, and intensive motor skills training significantly advanced FMS proficiency among individuals with ID.²³

Several systematic reviews and research studies stated that the motor skills interventions (various types and durations) significantly improved the FMS performances of TDC or children with special needs.^{8,17,20,31,33,34} Thus, based on these results, the various motor skills training and exercise programs seem effective regardless of the intervention variants used, different training durations, and training sessions per week.

During the early childhood period, youngsters are most likely to be skilled in the FMS, and several studies have shown that FMS proficiency has significantly improved in the middle childhood and youth populations.^{28,32} Moreover, it is crucial to introduce motor skill training as early as possible in children with or without disabilities.¹⁶ According to the current systematic reviews, meta-analyses, and global level studies that confirmed that various FMS trainings have been shown to improve FMS at a young age significantly, these programs should be applied in different educational settings, such as primary schools and special schools, to enhance the FMS levels of children.¹⁶ The results of the present study further support the idea behind their findings and experiments.

This study's findings also support the value of structured exercise programs in increasing the static balance and lower extremity functional strength of children with DS. This may be because a structured exercise program consists of stability skills and weight-bearing exercises.

The results match those observed in earlier systematic reviews and experimental studies. A recent systematic review by Stander et al. stated that VRT combined with standard physiotherapy or occupational therapy interventions improved motor proficiency (balance, coordination, strength, and agility) in the DS

population. They found that the balance and strength of the DS showed significant improvement after 6 weeks of the therapy program.³⁵ They also advised that it could be used in children with DS to improve balance, and the duration of the intervention is 5–24 weeks.³⁵

The current study's findings are consistent with a previous systematic analysis of several balancing exercise regimens that improve balance in the DS population.³⁶ These reviews reported that the intervention group significantly improved the balance and strength of the children with DS. Therefore, the current study's findings broadly support the work of other studies in this area. Additionally, no serious adverse effects of the intervention were found in this study. Therefore, the structured exercise program used in the current study represents a safe and realistic form of intervention for children with DS.

The limitation is the small sample size and limited time in this study; there is no long-term follow-up of the participants. So, further recommendations for future studies must be made with a large group and extended follow-up periods. Lastly, the present study has shown the effectiveness of a structured exercise program based on objective outcome measures. Thus, further investigation using product outcome measures (e.g., velocity and distance of running) and technology-based measures (e.g., the Biodex Balance System for balance and the isokinetic dynamometer for strength) to explore the changes in DS is still required.

Conclusion

In conclusion, this study revealed that the structured exercise program impacted the lower extremities' FMS, static balance, and functional strength. The present study suggests that a structured exercise program has measurable effects on FMS proficiency, static balance, and lower extremity functional strength in children with DS. Furthermore, after 6 weeks (approximately 18 hours) of exercises and activities, a statistically significant improvement in FMS proficiency was found. Lastly, this study can support the use of a structured exercise program to improve FMS performance despite some limitations on the generalizability of the results. These findings can be applied as a practical standard and/or alternative use of structured exercises in routine treatment programs for physiotherapists, physical educators, and other health care professionals to improve the FMS performance of children with DS.

Acknowledgements

We would like to express our sincere thanks to all participants.

Conflict of interest

There was no conflict of interest, according to the authors.

Funding

Nil

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Supplementary Table 1. Structured exercise program for the experimental group.

Components	Step I (first three weeks)	Step II (next three weeks)	Duration
Warming up	a) Introduction to group members with saying “Mingalarbar” and “Hand waving” b) Standing together in a group c) Clapping the hands and singing nursery rhyme together d) Marching together for 20 steps	As same as step I	5 minutes
Exercises and activities for stability	a) Walking up and down stepping foam (12-inch width x 12-inch length x 6-inch height) - 10 times b) Marching in place 10 repetitions for each leg c) Walking on balance beam (6-inch width x 144-inch length x 3-inch height) with hand support (to and fro 3 times) d) Walking along the straight line (2-inch width x 3 meters) (to and fro 3 times)	a. Walking up and down 3 step stairs - 5 times b. Walking on steppingstones with hand free. Set up steppingstone-scatter (To and fro x 3 times) c. Walking on balance beam (6-inch width x 144-inch length x 3-inch height) with hands holding the light plastic ball (to and fro 3 times) d. Walking, turning around the obstacles (5 cones x 12 inches between every two cones) (to and fro 3 times)	10 minutes
Exercises and activities for locomotor skills	a. Walking on the footprint forward direction - 10 steps (3 times) b. Jumping in hoops forward direction (2 feet diameter x 5 hoops) both feet together (to and fro 3 times) c. Running between two straight line (2 feet width x 25 feet length) (to and fro 2 times) d. Hop in hoops with therapist's hand support (2 feet diameter x 5 hoops) 5 times for each leg	a. Walking on the footprint sideward direction - 10 steps (to and fro 3 times) b. Jumping in hoops sideward direction (2 feet diameter x 5 hoops) both feet together (to and fro 5 times) c. Running, turning around the obstacles (5 cones x 24 inches between every two cones x 25 feet length) (to and fro 2 times) d. Hop in hoops with hand free (2 feet diameter x 5 hoops) 5 times for each leg	15 minutes
Exercises and activities for object control skills	a. Throwing a bean bag to the target. Distance between starting point and target is 10 feet (5 times) b. Bowling: Roll bowling x 5 times. Distance between starting point and target is 10 feet c. Ball kicking: Running and kicking a stationary ball 5 times. Distance between stationary ball and target is 20 feet d. Playing Basketball: 4 bounces with one hand and catch with two hands x 5 times	a. Throwing a bean bag to the target. Distance between starting point and targets is 20 feet (5 times) b. Bowling: Roll bowling x 5 times. Distance between starting point and target is 20 feet c. Ball kicking: Running and kicking a stationary ball 5 times. Distance between stationary ball and target is 30 feet d. Playing Basketball: 4 bounces with one hand and catch with two hands and throw with two hands into overhead x 5 times	15 minutes
Cooling down	a. Self- stretching demonstrated by the researcher b. Singing Good-Bye songs	As same as step I	5 minutes

Detection and characterization of SARS-CoV-2 variants in Thailand from 2020-2023 using next-generation sequencing

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ARTICLE INFO

Article history:

Received 6 October 2024

Accepted as revised 5 January 2025

Available online 10 January 2025

Keywords:

SARS-CoV-2, genomic variants, Thailand, whole genome sequencing, COVID-19.

ABSTRACT

Background: The evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to multiple variants with significant public health implications.

Objective: This study aimed to detect and characterize SARS-CoV-2 genomic variants in Thailand using next-generation sequencing (NGS) from 2020 to 2023.

Materials and methods: A total of 2,592 oro/nasopharyngeal swab specimens with high viral loads (cycle threshold; Ct≤25.0) were collected from SARS-CoV-2-positive patients in Nonthaburi, Nakhon Phanom, and Tak Provinces. NGS was performed using Ion Torrent, Oxford Nanopore Technologies, and Illumina platforms to identify and analyze SARS-CoV-2 variants.

Results: The sequencing revealed various SARS-CoV-2 variants circulating in Thailand, with 2,592 lineages identified. The Omicron variant was predominant, accounting for 93.44% (2,422/2,592). The most common Omicron subvariants were BA.2 (54.86%, 1,422/2,592), BA.5 (20.22%, 524/2,592), and XBB (14.51%, 376/2,592). The Delta variant constituted 2.66% (69/2,592) of the cases, with AY.30 (1.20%, 31/2,592) and AY.85 (1.27%, 33/2,592) being the most frequent sub-lineages. The Alpha variant (B.1.1.7) was detected in 33 cases (1.27%), and the Beta variant (B.1.351) in 1 case (0.04%).

Conclusion: The findings reveal a significant shift in the SARS-CoV-2 variant landscape in Thailand, with Omicron becoming the dominant strain post-2022. Continuous genomic surveillance is critical for tracking viral evolution, informing public health strategies, and supporting pandemic control efforts in Thailand and Southeast Asia.

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the virus responsible for COVID-19, has resulted in a global pandemic with significant public health implications. Since its emergence in December 2019, SARS-CoV-2 has undergone numerous mutations, leading to the emergence of various genomic variants with differing transmission rates, virulence, and impacts on vaccine efficacy.^{1,2} Monitoring these variants is essential for understanding viral evolution guiding public health intervention.

Next-generation sequencing (NGS) has become a pivotal tool in identifying and tracking the spread of SARS-CoV-2 variants. By analyzing the complete viral genome, WGS provides detailed insights into the virus's genetic changes and evolutionary patterns.³ This technique has been instrumental in detecting novel variants and

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doi: 10.12982/JAMS.2025.038

E-ISSN: 2539-6056

monitoring their spread across different regions.^{4,5} World Health Organization (WHO) recommended using targeted NGS to determine SARS-CoV-2 variants, including Ion Torrent and Oxford Nanopore Technologies (ONT).⁶

In Thailand, the first confirmed case of COVID-19 was reported in January 2020. Since then, the country has faced multiple waves of infection, each associated with different SARS-CoV-2 variants. Understanding the genomic landscape of SARS-CoV-2 in Thailand is essential for developing targeted public health strategies and mitigating the impact of future outbreaks.⁷⁻⁹

Thailand is a significant area of study for several reasons. Its geographical location and status as a major travel hub in Southeast Asia mean it plays a crucial role in regional epidemiology. The high volume of international travel and trade through Thailand increases the likelihood of the importation and exportation of new SARS-CoV-2 variants, which can spread throughout Southeast Asia. Secondly, the country's diverse population and varied public health responses provide a unique opportunity to study how different factors influence the evolution and spread of the virus. Monitoring SARS-CoV-2 variants in Thailand can provide valuable insights into the virus's behavior in an epidemiologically and socioeconomically diverse region.^{10,11}

Recent studies highlight the importance of variant monitoring in Thailand and its implications for Southeast Asia. The research conducted by Sooksawasdi Na Ayudhya et al. demonstrated the emergence and spread of multiple SARS-CoV-2 variants in Thailand, emphasizing the need for continuous genomic surveillance to track viral mutations and inform public health responses.¹² Furthermore, the study by Bunyong *et al.* underscored the role of WGS in identifying and characterizing new variants, which is crucial for effective vaccine deployment and containment strategies in the region.¹³ Additionally, the research by Tongsima *et al.* provided insights into the regional dynamics of SARS-CoV-2 spread, highlighting the interconnectedness of Southeast Asian countries and the necessity of coordinated surveillance efforts.¹⁴

This study aims to detect and monitor SARS-CoV-2 variants in Thailand using NGS. The NGS was chosen for

this study due to its high throughput, accuracy, and ability to provide comprehensive genomic data. These qualities make it an invaluable tool for tracking variants and understanding the dynamics of SARS-CoV-2 spread.^{15,16} This study was intended to support global efforts against COVID-19 while providing data to enhance local surveillance and response strategies. This approach will help Thailand and neighboring Southeast Asian countries improve their public health responses and control measures against COVID-19.

Materials and methods

Specimen collection

Oropharyngeal or nasopharyngeal swab specimens were collected from 2,592 SARS-CoV-2-positive cases tested at the Bamrasnaradura Infectious Diseases Institute (Nonthaburi) and the Division of Global Health Protection, Thailand Ministry of Public Health - US CDC Collaboration, Thailand (Tak and Nakhon Phanom). The total number of specimens from each site corresponds to the number of SARS-CoV-2 sequencing submissions (N=2,592) in Table 1. These specimens, stored in a viral transport medium (VTM), were retrieved from storage and included in this study. All specimens originated from patients in the three sites from June 2020 to May 2023. We conducted a retrospective surveillance study of SARS-CoV-2 infections. The criteria for selecting SARS-CoV-2 positive specimen selection were based on a nucleocapsid protein (N Gene) cycle threshold (Ct) value of ≤ 25.0 on the qRT-PCR, with the average Ct value of 15 across all DNA samples. A Ct value of ≤ 25.0 on the qRT-PCR, corresponding to approximately 1,500 to 50,000 copies, was used to determine the copy number of a sample based on the known copy number in control reactions. Selecting the appropriate Ct value from real-time PCR is vital for obtaining high-quality SARS-CoV-2 genome data using the NGS platform.¹⁷ The specimens were collected from patients with symptoms including cough, runny nose, sore throat, anosmia, tachypnea, shortness of breath, or difficulty breathing, and/or having a temperature ≥ 37.5 °C, or having traveled to/from a foreign country, or having contact with a confirmed COVID-19 case.

Table 1. The number of SARS-CoV-2 sequencing submissions (N=2,592)

Year	Submission month	Sequencing protocol	Nonthaburi	Nakhon Phanom	Tak
1 (N=1,349)	Jan-22	Ion AmpliSeq	-	10	15
	Feb-22	Ion AmpliSeq	54	-	-
	May-22	Ion AmpliSeq	76	23	16
		ARTIC-ONT	100	-	-
	Jun-22	Illumina COVID Seq	96	-	-
	Jul-22	Ion AmpliSeq	-	81	10
		Illumina MiniSeq RVOP	82	125	19
		Illumina COVID Seq	257	-	-
	Aug-22	Illumina MiniSeq RVOP	-	64	15
	Sep-22	Illumina MiniSeq RVOP	-	226	80
Total			665	529	155
2 (N=538)	May-23	Ion AmpliSeq	-	218	36
	Jun-23	Ion AmpliSeq	-	150	42
	Jul-23	Ion AmpliSeq	-	39	25
	Aug-23	ONT Midnight	-	24	4
Total			-	431	107
3 (N=705)	Sep-23	ONT Midnight	-	10	72
	Nov-23	ONT Midnight	-	269	146
	Dec-23	ONT Midnight	-	68	16
	Feb-24	ONT Midnight	-	52	17
	Jun-24	Ion AmpliSeq	-	15	40
Total			-	414	291
Total			2,592		

Nucleic acid extraction

Following the manufacturer's protocol, total nucleic acid was extracted from the oropharyngeal or nasopharyngeal swab specimens using the automated MagNA Pure 96 platform (Roche, Switzerland). Then, a QC step is performed by use the Qubit dsDNA High Sensitivity Quantification Assay (Thermo Fisher Scientific, USA).¹⁸

Library preparation and whole genome sequencing

Total nucleic acid was reverse transcribed into RNA, followed by amplification of the cDNA targets and barcode tagging using a specific reagent kit. The barcoded samples were pooled and sequenced. Sequencing libraries were prepared using the Ion AmpliSeq SARS-CoV-2 Insight Research Assay for the Ion Torrent platform and loaded onto Ion 540 Chips (Thermo Fisher Scientific, USA) according to the manufacturer's instructions.¹⁹ Sequencing was performed on the Ion Torrent S5 System (Thermo Fisher Scientific, USA). For the Oxford Nanopore Technologies (ONT) platform: (1) ARTIC libraries were prepared using a ligation-based sequencing kit (SQK-LSK109), and (2) Midnight libraries were prepared using the Midnight RT PCR Expansion (EXP-MRT001) kit. Both were sequenced on the MinION Mk1B and Mk1C (Oxford Nanopore Technologies, UK) following the manufacturer's protocol.²⁰ For the Illumina platform, sequencing was performed using the COVIDSeq Assay and Respiratory

Virus Oligo Panel (RVOP) according to the manufacturer's instructions.^{21,22}

Variant calling and data processing

The sequence was interpreted using package software, including variantCaller and SARS-CoV-2_coverage analysis. The collected FASTA data were uploaded to two online databases, including Pangolin COVID-19 Lineage (<https://pangolin.cog-uk.io>)²³ and Nextclade (<https://clades.nextstrain.org>).²⁴ The findings of this investigation were then examined using descriptive statistics, which included the percentage of variants detected in all samples. Confidence interval for a proportion was calculated by Sample Size Calculator (<https://sample-size.net/confidence-interval-proportion>).²⁵

Ethical considerations

The study was approved by the Institutional Review Board of the Bamrasnaradura Infectious Disease Institute (approval ID: BIDI S033h/63_ExpD) and the National Institute of Health in Thailand (approval ID: DMSc-EC002).

Results

Between January 2020 and May 2023, 2,592 oro/nasopharyngeal swab specimens were collected from SARS-CoV-2-positive patients in Nonthaburi, Nakhon Phanom, and Tak, Thailand. These specimens were tested

using Next-Generation Sequencing (NGS) platforms, including Ion Torrent, Oxford Nanopore Technologies, and Illumina. A retrospective surveillance study of SARS-CoV-2 infections was conducted, with specimens selected based on a nucleocapsid protein (N Gene) cycle threshold (Ct) value ≤ 25.0 , indicating a high viral load.

The sequencing results revealed a wide variety of SARS-CoV-2 variants circulating in Thailand during the study period, with a total of 2,592 SARS-CoV-2 lineages identified. These lineages were distributed across several variants, including Alpha, Beta, Delta, and Omicron, as shown in Table 2 and Figure 1. The Omicron variant emerged as the predominant strain, accounting for 93.44% (2,422/2,592) of the total sequenced samples. Within the Omicron lineage, BA.2 was the most common subvariant, representing 54.86% (1,422/2,592) of the cases, followed

by BA.5 (524/2,592, 20.22%) and XBB (376/2,592, 14.51%).

The Delta variant contributed 2.66% (69/2,592) of the cases, with AY.30 (31/2,592, 1.20%) and AY.85 (33/2,592, 1.27%) as the most frequently detected sublineages. The Alpha variant, represented by B.1.1.7, was detected in 33 cases (1.27%), while the Beta variant (B.1.351) was found in only 1 case (0.04%).

These findings suggest a significant shift in the SARS-CoV-2 variant landscape in Thailand, with the Omicron lineage becoming the dominant strain after early 2022. The NGS platform, combined with careful specimen collection and variant calling using the GISAID database, provided a comprehensive overview of the circulating SARS-CoV-2 variants and their distribution in these three regions of Thailand.

Table 2. The number of 2,537 SARS-CoV-2 lineage sequenced by NGS platform in Nonthaburi, Nakhon Phanom and Tak during January 2020 to May 2023.

SARS-CoV-2 variants and lineages		Number of detections	95% CI
Delta	A	35	1.35 (0.94-1.87)
	B	32	1.23 (0.85-1.74)
	AY.125	1	0.04 (0.0-0.22)
	AY.30	31	1.20 (0.81-1.69)
	AY.79	3	0.12 (0.02-0.34)
	AY.85	33	1.27 (0.88-1.78)
	B.1.617.2	1	0.04 (0.0-0.22)
Total		69	
Alpha	B.1.1.7	33	1.27 (0.88-1.78)
Beta	B.1.351	1	0.04 (0.0-0.22)
Omicron	BA.1	80	0.007 (0.025-0.038)
	BA.2	1422	0.019 (0.539-0.578)
	BA.4	20	0.003 (0.004-0.011)
	BA.5	524	0.016 (0.188-0.219)
	XBB	376	0.013 (0.118-0.145)
	Total	2422	
Total		2592	

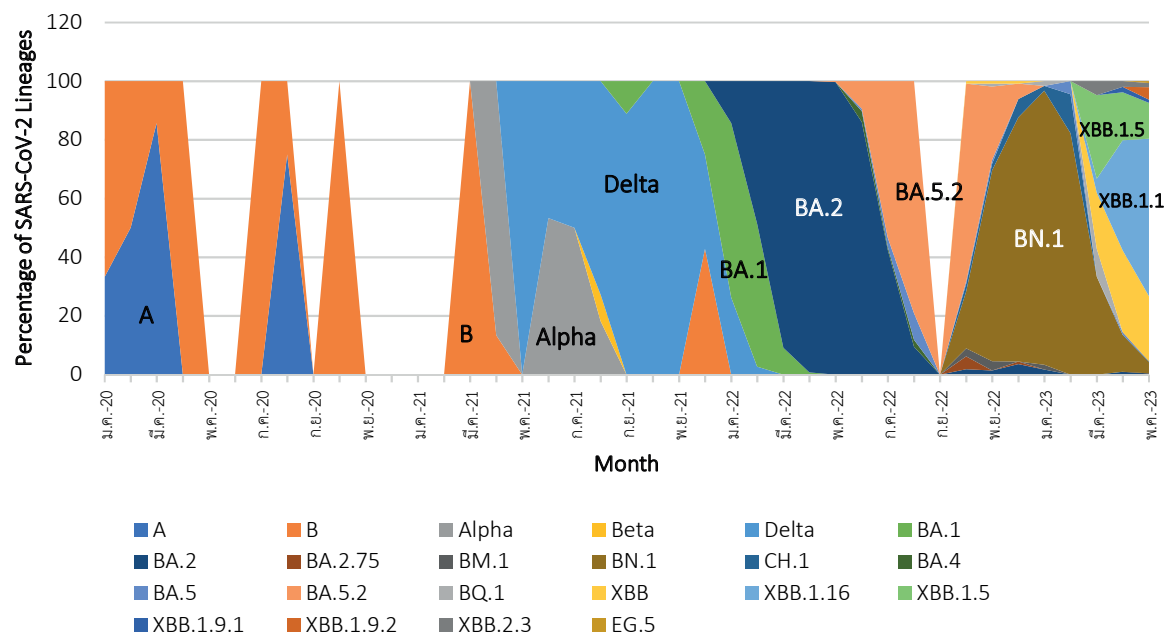


Figure 1. The distribution percentage of 2,592 SARS-CoV-2 lineage in Nonthaburi, Nakhon Phanom, and Tak from January 2020 to May 2023. A: A, A.6, B: B, B.1, B.1.1, B.1.1.142, B.1.524, B.41, B.1.36.6, Delta: AY.125, AY.30, AY.79, AY.85, B.1.617.2, Alpha: B.1.1.7, Beta: B.1.351.

Discussion

The detection and characterization of SARS-CoV-2 variants in Thailand from 2020 to 2023 provide valuable insights into the country's viral evolution and transmission dynamics. The study utilized next-generation sequencing (NGS) platforms, including Ion Torrent, Oxford Nanopore Technologies, and Illumina, to identify and track various SARS-CoV-2 lineages. A total of 2,592 specimens were analyzed.

From January 2020 to May 2023, the Omicron variant emerged as the predominant strain, accounting for 93.44% of the sequenced samples (2,422/2,592). Within the Omicron lineage, the BA.2 subvariant was the most prevalent, constituting 54.86% (1,422/2,592) of cases, followed by BA.5 (20.22%, 524/2,592) and XBB (14.51%, 376/2,592). The 95% confidence interval (CI) for the proportion of Omicron cases ranged from 92.52% to 94.36%, reflecting a high confidence level in this variant's dominance. The Delta variant was identified in 2.66% (69/2,592) of the cases, with AY.30 and AY.85 as the most frequently detected sub-lineages, accounting for 1.20% (31/2,592) and 1.27% (33/2,592) of the total samples, respectively. The 95% CI for Delta cases ranged from 2.04% to 3.28%, highlighting its lower but notable presence compared to Omicron. The Alpha (B.1.1.7) and Beta (B.1.351) variants were present in much smaller proportions, with Alpha representing 1.27% (33/2,592) of cases and Beta a mere 0.04% (1/2,592). The 95% CI for Alpha ranged from 0.85% to 1.69%, and for Beta ranged from 0.00% to 0.12%, underscoring their limited detection during the study period.

This study indicated a shift in the SARS-CoV-2 variant landscape in Thailand, particularly with the emergence

and subsequent dominance of the Omicron lineage after early 2022. This trend is consistent with global observations, where Omicron's high transmissibility and immune escape capabilities have led to its widespread prevalence despite vaccination efforts.^{22,23} The use of NGS platforms in this study was instrumental in providing comprehensive genomic data and identifying SARS-CoV-2 variants. The selection of specimens with a nucleocapsid protein (N Gene) cycle threshold, $Ct \leq 25.0$, ensured high viral loads, enhancing the reliability of sequencing results. This methodological rigor allowed for the accurate detection of multiple variants and subvariants, offering a detailed overview of the circulating strains in Thailand.

Comparing this data with global trends, it is evident that Thailand's SARS-CoV-2 variant distribution aligns with broader patterns observed worldwide. The dominance of Omicron and the decline of earlier variants such as Alpha and Beta reflect similar dynamics seen in other regions, underscoring the interconnected nature of viral spread.^{26,27} The implications of these findings are significant for public health strategies in Thailand and the broader Southeast Asian region. Continuous genomic surveillance is essential for early detection of new variants, understanding their epidemiological impact, and informing public health interventions. The high throughput and accuracy of NGS platforms are invaluable for tracking viral mutations and aiding in developing targeted response strategies.^{28,29} In addition, from the study to track the changes of SARS-CoV-2 strains in Thailand by Puenpa J *et al.*, it was found that in June 2021, both Alpha and Delta variants were reported, with a higher proportion of Alpha than Delta variants. In July-August 2021, the Alpha variant decreased, but the Delta variant increased. From September 2021 to

November 2021, the Delta variant was 100%. In December 2021, the Omicron BA.1 variant was detected as the main variant from January 2022 to March 2022. The Omicron BA.2 variant was also identified in January 2022.³⁰ The strain report's period and the proportion of variations discovered are compatible with this study. Except for June 2021, the small number of samples utilized in the study may have influenced the fraction of variations at that time. The Omicron variety has been found since December 2021, with reports of its proliferation in Thailand.⁷

Most infections in the community with omicron BA.2, BA.4/5, and XBB subvariants were moderate, but this did not change when omicron subvariants evolved in this highly vaccinated population. Because many sick people did not have fevers, more sensitive screening than fever alone may be required to prevent community transmission. The trend of a steadily decreasing correlation of vaccination and an increasing connection of previous infection with the symptom profile supported the idea of vaccine escape or immunological fading when additional SARS-CoV-2 omicron subvariants emerged.³¹

Due to the limited sample volume used in this study and the fact that most patients were from Nonthaburi, Nakhon Phanom, and Tak Province, the detected variants were not diverse. Although the detected variants tended to be in the same direction as the Department of Medical Sciences reports, the proportion of variants detected each month may change if the study is conducted with various sites.⁶⁻⁸ Decoding the SARS-CoV-2 genome using NGS techniques is essential for monitoring the changes in variants. It allows us to know the origin and source of the virus that spreads in each period. It could be linked to molecular epidemiology, and the data could be used for disease prevention and control measures, including selecting appropriate strains for vaccine development in Thailand.

Conclusion

This study provides the SARS-CoV-2 variant in Thailand from 2020 to 2023, highlighting shifts in variant prevalence. The early dominance of the original lineage was succeeded by the rapid emergence of more transmissible and immune-evasive variants such as Alpha, Delta, and Omicron subvariants. These findings underscore continuous genomic surveillance's importance in informing public health strategies and vaccine development. The emergence and dominance of variants like Delta and Omicron subvariants illustrate the virus's adaptive capabilities, impacting the efficacy of public health measures and vaccines. Ongoing genomic monitoring, combined with data sharing, is crucial for early detection of new variants and for guiding effective public health responses and control measures against COVID-19.

Conflict of interest

The authors declare no conflict of interest.

Funding

This research was supported by the Division of Global

Health Protection, Thailand-U.S. CDC Collaboration.

Acknowledgements

We extend our gratitude to Dr. Beth Skaggs, Dr. Pongpun Sawatwong, and Dr. Saithip Bhengsri from the Division of Global Health Protection, Thailand MOPH-U.S.CDC. Collaboration (TUC), for their contributions. We would like to thank Dr. Navin Horthongkham, Dr. Piroon Jenjaroenpun, and Dr. Thidathip Wongsurawat from the Faculty of Medicine, Siriraj Hospital, Mahidol University, for their technical support.

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Core stabilization exercise can improve lumbar joint position sense in healthy individuals

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ARTICLE INFO

Article history:

Received 5 November 2024

Accepted as revised 13 January 2025

Available online 15 January 2025

Keywords:

Core stabilization exercise, joint position sense, smartphone application, sensory-motor integration, lumbar spine.

ABSTRACT

Background: Core stabilization exercises (CSE) are based on motor learning concepts focusing on sensorimotor function. It has been proposed that CSE can improve core stabilizer muscle function, leading to enhanced joint position sense. However, evidence to support the effects of CSE on joint position sense is still limited.

Objective: To compare the immediate effect of CSE on joint position sense of the lumbar spine using a smartphone application in healthy individuals.

Materials and methods: Fifty-six healthy individuals (9 males and 47 females) were recruited in the study. Two smartphones with the application were attached to the lumbar spine (L1) and sacrum (S2). They were asked to perform 45-degree lumbar flexion and return to neutral position, while lumbar joint position errors were recorded in each direction. They were then randomly assigned to CSE and control groups. After the intervention, they were asked to perform 45-degree lumbar flexion and return to a neutral position.

Results: The CSE group demonstrated significant improvement ($p < 0.05$) in lumbar joint position sense during the 45-degree flexion, whereas the control group did not show improvement. Both groups did not show significant improvement during the return to a neutral position.

Conclusion: Findings suggested that one session of the CSE can improve lumbar joint position sense, which could be due to the increased activities of muscle spindles and Golgi tendon organs. In addition, our findings support the clinical utility of this smartphone application in measuring lumbar joint position sense.

Introduction

Clinical observation of trunk movement is one critical part of physical therapy examination aiming to identify patterns that deviate from typical movement, including altered lumbopelvic rhythm, out-of-sagittal plane deviation, or instability catch.^{1,2} Several studies found these abnormal movements were associated with poor muscle coordination.³ Muscle coordination is necessary to achieve dynamic stability, particularly in the middle of the movement (loose-packed positions), where the ligaments and joint capsules have minimal contribution to lumbar stability.² This would suggest that the level of muscle contraction depends on feedback from muscle spindles and Golgi tendon organs within the muscles themselves rather than those ligaments and joint capsules. Muscle spindles and Golgi tendon organs are mechanoreceptors in the muscles and tendons, respectively.⁴⁻⁶ They can detect changes in muscle length and tensile load and send sensory

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doi: 10.12982/JAMS.2025.039

E-ISSN: 2539-6056

information to the central nervous system to estimate position and movement in space.⁴⁻⁶ Therefore, they are critical in the lumbar joint position sense, contributing to static and dynamic stability. Making efficient movements and maintaining lumbar stability during dynamic tasks are complex actions requiring muscle coordination to provide the right force at the right time, the right duration, and the right amount of combined forces.⁷⁻⁹ Such coordination results from synergistic muscle actions with sensory, biomechanics, and motor-processing strategies with experiences and abilities to anticipate changes.^{4,10,11}

Motor control relies on proprioception from the muscles as a primary sensory mechanism.⁴ The proprioception provides three main proprioceptive inputs for sensorimotor processing.⁴ The first input is the position and orientation of joints during static position and movement. Another proprioceptive information is the perceived timing of muscle contractions necessary to optimize muscle contraction. The last key input component is the information regarding forces and efforts to handle the workload. These functions suggest the critical role of proprioception in sensorimotor integration to optimize muscle recruitment and timing for static and dynamic stability of the lumbar spine.^{4,10,11}

Core stabilization exercises (CSE) are therapeutic exercises based on the motor learning approach, which emphasizes the co-activation of the lumbar multifidus and transversus abdominis muscles.^{12,13} These stabilizer muscles are attached to the thoracolumbar fascia and produce a stabilizing effect in the lumbar spine through increased intra-abdominal pressure, further providing lumbar segmental stability.¹⁴ CSE can be used to re-train the critical functions of those stabilizer muscles and improve the accuracy of the sensory integration process for spine stability.^{4,10,11} However, evidence to support the effects of CSE on joint position sense remains limited.

In addition, studies investigating joint position sense have used advanced lab-based equipment, including an electromagnetic tracking system, a 3D kinematic motion analysis system, and an isokinetic dynamometer, which could limit clinical application.¹⁵⁻¹⁷ Recently, the growing popularity of smartphone applications in assessing joint position sense highlights their ease of use and wide availability, making them an appealing choice for measuring joint position sense.¹⁸⁻²¹ The use of a smartphone application would allow generalizability in clinical practice.

Therefore, this study aimed to determine the immediate effects of CSE on joint position sense during active trunk flexion and return to neutral position using a smartphone application in healthy individuals. We hypothesized that CSE would improve joint position sense. Our findings would provide evidence to support CSE's usefulness in improving joint position sense and help design appropriate interventions for patients with LBP. In addition, our findings would provide the clinical utility of the smartphone application in measuring joint position sense.

Materials and methods

Study design

This study was a single-blind, randomized controlled trial to determine the immediate effects of CSE on joint position sense during active trunk flexion and return to neutral position using a smartphone application in healthy individuals. The study protocol was approved by the university institutional review board (MU-CIRB 2016/047.0704) and registered to clinicaltrial.gov (NCT02974062). Participants provided written informed consent before collecting data.

Participants

Fifty-six participants (9 males and 47 females) were recruited from the Faculty of Physical Therapy, Mahidol University. The inclusion criteria were age between 18 and 25 years, male and female, and no low back pain in the past six months. Exclusion criteria were current musculoskeletal pain, clinical signs of systematic disease, definitive neurologic deficit, experience of CSE or Pilates, or body mass index greater than 25 kg/m². This study was part of another research project; therefore, we did not perform sample size calculations. However, post-hoc power analysis was conducted to determine the effect size and number of participants required to replicate our study.

Instruments and measures

Two smartphones (iPhone® is a trademark of Apple Inc., Cupertino, CA, USA) with a goniometer application (Goniometer Pro, Version 2.7, 5fuf5 Co., Bloomfield, NJ, USA) were attached by positioning the center of the smartphone to the lumbar spine at the L1 spinous process and sacrum at the S2 spinous process. The angle between the lumbar spine and sacrum was used to represent lumbar motion. The lumbar position error (PE) from the targets was derived. These data represented lumbar position during forward flexion and return to the starting position. Greater PE value indicated poorer lumbar position sense. Several studies validated the smartphone application while concurrently measuring the lumbar motion with a lab-based motion-tracking system. Our preliminary study demonstrated excellent test-retest reliability ($ICC_{2,k}=0.95$; $CI=0.92-0.97$) of the smartphone application to measure lumbar position sense.²² Therefore, the smartphone application is suitable for clinical use in measuring lumbar position sense in this study.

Procedure

Participants first completed demographic data questionnaires. Then, the researcher exposed the participant's lower back. Each smartphone was inserted in a transparent pocket, allowing the researcher to see the screen. These pockets were attached to the participant's lumbar spine and pelvis using Velcro straps (the center of the smartphone was aligned with L1 and S2 spinous processes).

The researcher, blinded to the group assignment, performed data collection. The participant was asked to stand with feet shoulder-width apart on the drawing paper, and the researcher drew a footprint. This footprint was used to re-position the participant at the post-test. The participant was asked to stand up straight to record starting position data, which is operationally defined as the position in which the participant feels comfortable without trying to maintain it. The participant performed three repetitions of active trunk flexion, while peak lumbar flexion for each repetition was recorded (Figure 1). Then, the researcher provided three practice trials of lumbar flexion at 45 degrees, and return to the starting position with verbal feedback to familiarize with the task. The participant was asked to perform six repetitions of 45-degree lumbar flexion and return to the starting position without verbal

feedback while the researcher recorded the lumbar angles. These data were used as a baseline pre-test. Simple randomization was performed to assign the participants to the control (CON) or CSE group. Based on the anatomy of the lumbar spine's superior and inferior articular facets, the significant movement in the lumbar spine is flexion-extension rather than lateral flexion and axial rotation.²³ Therefore, sagittal plane investigation should be relevant to the biomechanics of the lumbar spine. According to systematic reviews, most studies focused on the sagittal plane.^{24,25} However, few studies investigated lateral flexion and axial rotation and found no significant differences between healthy individuals and patients with low back pain.^{24,25} In addition, studies reported that reposition error measurement in axial rotation was unreliable.^{26,27} Therefore, we selected the sagittal plane for our study.

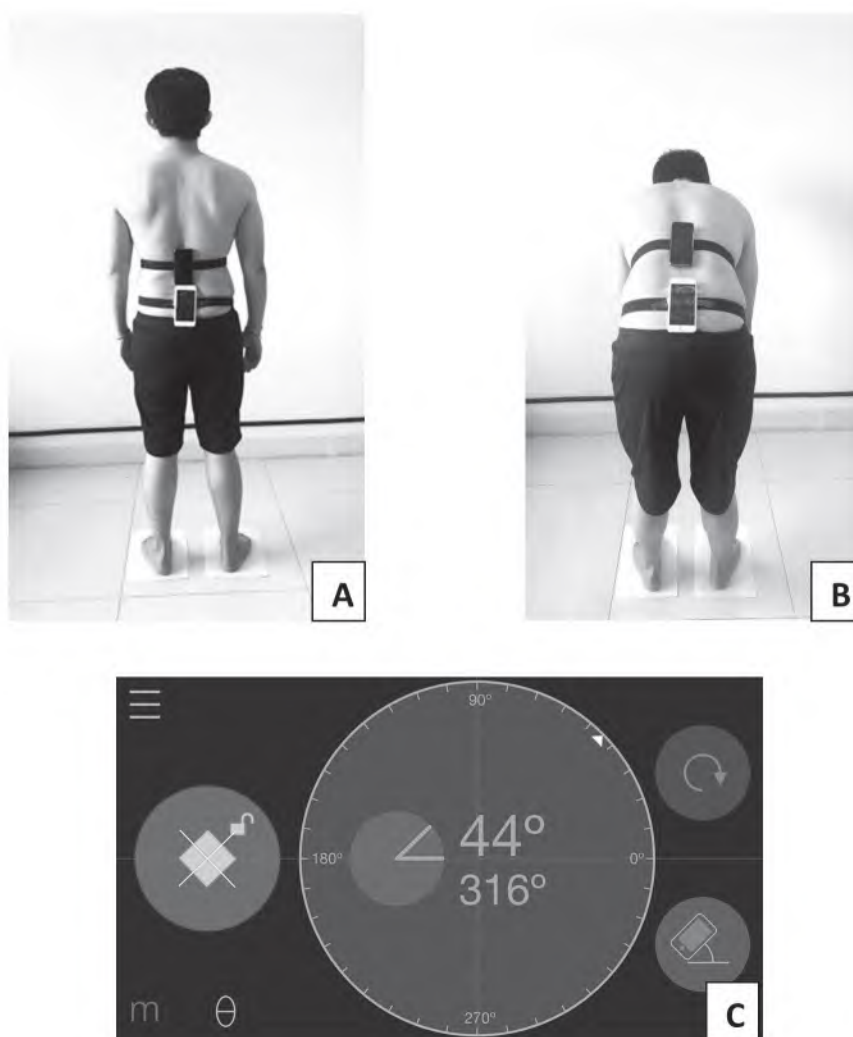


Figure 1. Smartphone placement and starting (A) and end positions during 45-degree flexion (B) and smartphone application screen (C).

The CSE group received a 30-minute CSE based on motor control learning (Figure 2), while the CON group was instructed to rest by lying down for 15 minutes. Based on our pilot study to determine changes in lumbar joint position sense between 15 and 30 minutes. We found no significant differences in lumbar position sense; therefore, we used a 15-minute protocol to minimize our data collection time. The exercise comprised three stages: 1) co-contraction of transverse abdominis and lumbar multifidus muscles, 2) co-contraction with limb movement, and 3) co-contraction with functional movement. Each participant's performance was assessed to determine the highest ability to perform CSE. Then, the researcher used that maximum performance as an initial exercise. If the participants achieved the target, they progressed to the next exercise.

Stage 1: Co-activation of the transverse abdominis and lumbar multifidus muscles. If the participants could hold co-contraction for

60 seconds without holding their breath or compensation from other muscles, they proceeded to stage 2.

Stage 2: Co-contraction with limb movement. Participants were asked to co-contraction the transverse abdominis and lumbar multifidus muscles while slowly performing upper and/or lower limb movements. If the participants could perform co-contraction with limb movements in the supine position for ten repetitions without substituting other muscles, they could proceed to the next stage.

Stage 3: Co-contraction with functional movement. Participants were asked to perform co-contraction and functional movements (e.g., sit to stand, walk in the hallway, etc.) for ten repetitions.

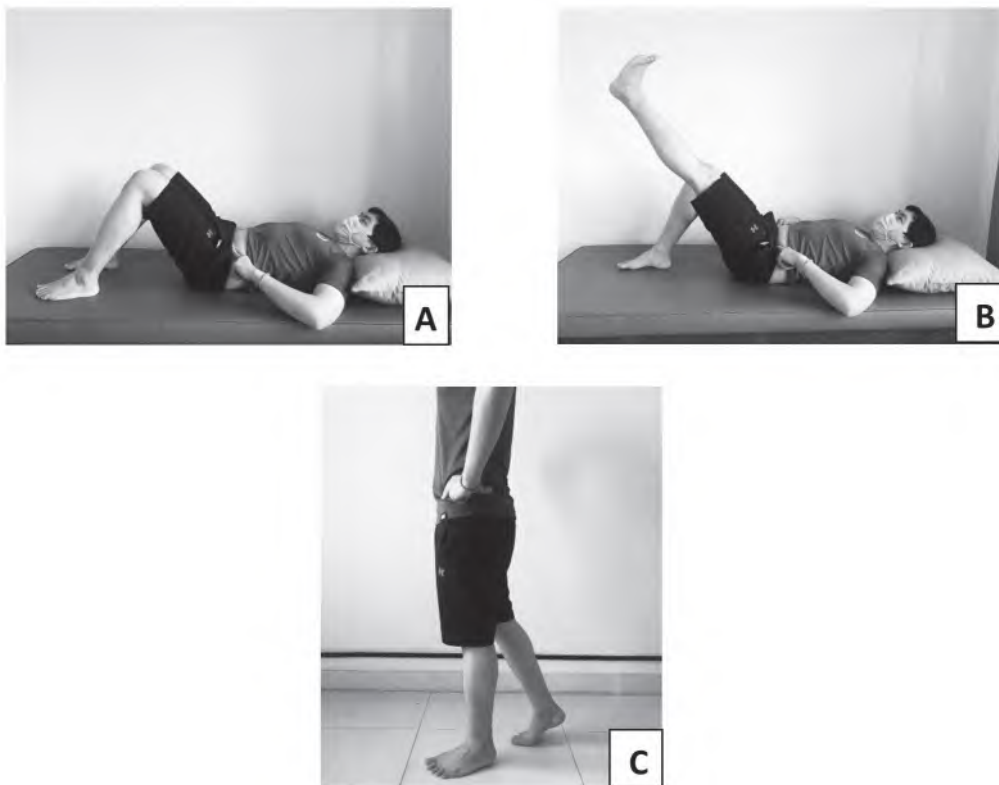


Figure 2. Core stabilization exercise. Example of level 1 (A) co-contraction of the transverse abdominis and lumbar multifidus muscles, level 2 (B) co-contraction with limb movement, and level 3 (C) co-contraction with functional movement

These low-intensity exercises aimed to develop the ability to perform precise co-contraction of the transverse abdominis and lumbar multifidus muscles via sensory-motor integration. After performing CSE in the CSE group

and resting in the CON group, post-test data collection was performed using the same protocol as pre-test data collection. Figure 3 demonstrates a flow chart of the study.

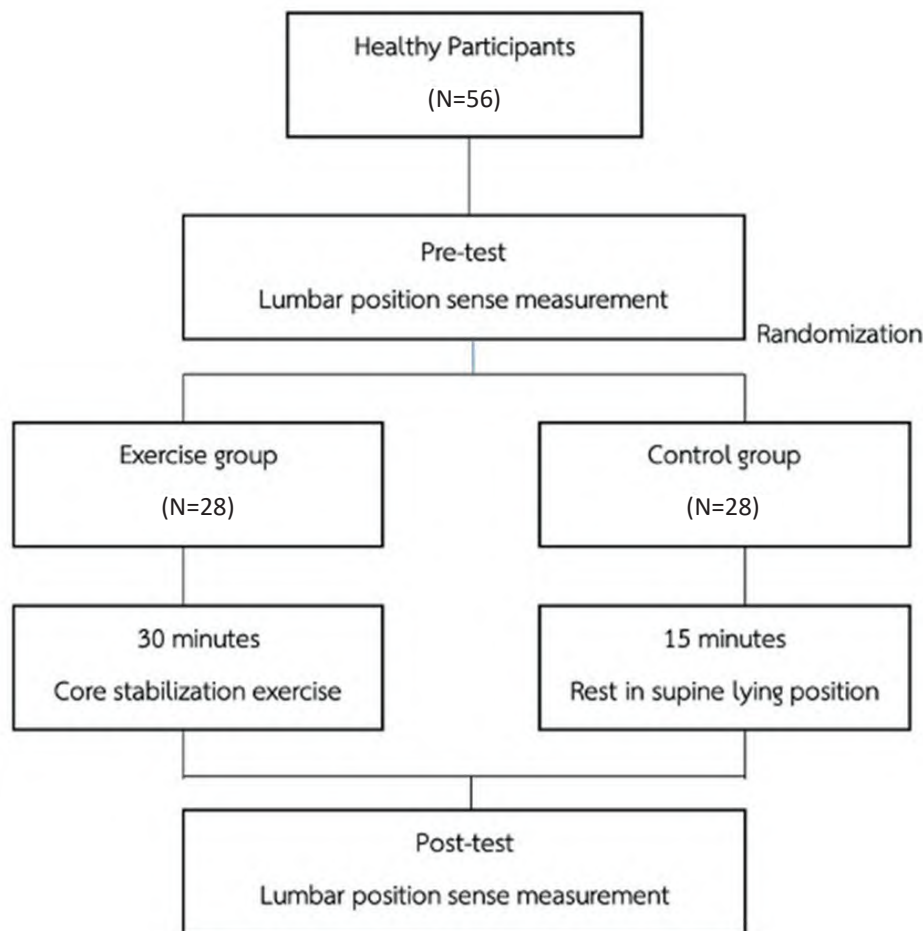


Figure 3. Flow chart of this study.

Data analysis

Lumbar position error was calculated by taking the absolute value of the difference between the actual lumbar flexion and 45-degree target angle ($PE_{flexion}$) and the actual position during upright and starting (PE_{start}). These parameters were used to represent lumbar position sense. Averaged $PE_{flexion}$ and PE_{start} across six repetitions were used for statistical analyses.

Statistical analysis

Statistical analyses were performed using the SPSS Program (IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY, USA). The Shapiro-Wilk test was conducted to determine the normality of the data. An independent t-test was used to determine demographic (age and BMI) and baseline (lumbar range of motion, $PE_{flexion}$, and PE_{start}) data, while the chi-square test was used to assess sex between groups. A two-way mixed ANOVA with post-hoc pairwise comparison was used to determine the effect of group (CON vs. CSE) and time (Pre vs. Post) on lumbar

position sense. The significance level was held at 0.05 for all statistical analyses.

Results

Demographic data are presented in Table 1. Statistical analysis demonstrated no significant differences ($p>0.05$) between groups regarding sex, BMI, lumbar range of motion, or position errors during flexion and return to neutral position. However, CSE group members were significantly older than CON group members ($p<0.05$).

Our data were normally distributed; therefore, a two-way mixed ANOVA was performed for statistical analysis. Results demonstrated no Time*Group interaction ($p=0.085$), while the significant main effect of Time was found ($p<0.05$) in $PE_{flexion}$. Post-hoc pairwise comparison (Table 2) demonstrated significant improvement in $PE_{flexion}$ in the CSE group but not in the CON group. However, no significant Time*Group interaction and the main effect of Group and Time were found ($p>0.05$) in PE_{start} .

Table 1. Demographic data.

Variable	CON (N=28)	CSE (N=28)	p value
Age (years)	20.5±1.3	21.4±1.0	0.01
Gender (%female)	93	75	0.07
BMI (kg/m ²)	21.2±2.7	21.3±2.4	0.81

Note: CON; control group, CSE: core stabilization exercise group.

Table 2. Post-hoc pairwise comparisons between groups (CON vs CSE) and times (Pre vs Post) during forward flexion at 45 degrees and return to neutral position.

		Pre Mean±SD (deg)	Post Mean±SD (deg)	Difference (deg)
PE _{flexion}	CON	5.31±3.80	4.76±3.78	0.55
	CSE	6.65±3.21	4.52±4.12	2.13*
	Difference	-1.34	0.24	
PE _{start}	CON	3.00±1.98	2.44±1.72	0.56
	CSE	2.74±2.63	1.94±1.48	0.80
	Difference	0.26	0.50	

Note: CON: control group, CSE: core stabilization exercise group, PE_{flexion}: position error during flexion, PE_{start}: position error during return to starting position, SD: standard deviation, deg: degrees, *significant difference between pre and post-test ($p < 0.05$).

Discussion

These results partially support our hypothesis that one-session CSE can improve joint position sense during trunk flexion at 45 degrees. The CSE is designed to improve co-contraction of the transverse abdominis and lumbar multifidus muscles following three stages from beginning to challenging levels. CSE can enhance proprioceptive sense from the muscle spindle, which is necessary to increase the precision of muscle contraction, thereby decreasing absolute errors. Our finding provides evidence to support improved sensory-motor integration mechanisms. According to the spinal reflex pathway, co-contraction of the transverse abdominis and lumbar multifidus muscles would cause increased muscle tension, thereby enhancing the sensitivity of muscle spindles in the muscles. This would increase proprioceptive signals or information to the spinal reflex pathway to provide a more precise co-contraction at the target angle.⁴

Improved joint position sense during trunk flexion was consistent with the study by Kim *et al.*, in which they found that 8 weeks of CSE could improve the proprioception of passive lumbar motion at 20 degrees flexion and 10 degrees extension.¹⁵ Although our study investigated the immediate effects of CSE, we found that participants could actively perform trunk flexion with less position error. Our findings and their studies imply that CSE could enhance joint position sense in active and passive movements.

In contrast, a previous study showed no significant difference in lumbar position sense after 8-week CSE.¹⁶ The discrepancy between studies could be because their study assessed the absolute error of passive lumbar rotation (passive structure), while our study focused on the ability of muscles (active structures). We selected trunk flexion at 45 degrees to differentiate muscle feedback from ligaments and joint capsule feedback. CSE was designed

to improve muscle performance, including joint position sense from muscle spindles and Golgi tendon organs. This should result in improved joint position sense during active movement. On the other hand, their study measured joint position sense during passive lumbar rotation, where trunk muscles have minimal contribution to proprioception.¹⁶ In this case, proprioceptive signals from active muscles would be minimal or inadequate to determine joint position sense.

Regarding joint position sense at the neutral position, our result did not show significantly improved joint position sense when returning to the starting position. Our result differed from a previous study that found improved joint position sense during the upright sitting position after CSE.¹⁷ However, our study aimed to determine the effect of one-session CSE which might have been inadequate to induce change in joint position sense. Further analysis may be required to determine the most appropriate amount of CSE to improve joint position sense. Another potential explanation for such different findings is that our healthy participants had no low back symptoms or were familiar with the neutral position, evidenced by fewer position errors than flexion. This reduced error could also have caused the ceiling effect or limited space for improvement. Therefore, applying CSE in patients with impaired joint position sense can clarify this issue.

Although we found a significant difference in age between the CON and CSE groups, they were relatively young, implying that they were still in homogeneous groups. In addition, no significant difference was observed in absolute errors between groups during 45-degree flexion and return to upright position at baseline. These would imply that both groups were comparable, and changes in absolute error could be interpreted as the effect of one-session CSE.

We used a smartphone with a goniometer application to measure lumbar joint position sense during forward flexion and return to the starting position. Our preliminary study established excellent test-retest reliability of the smartphone application, suggesting that changes in lumbar joint position sense were likely to be actual changes rather than changes from measurement errors. One study determined the concurrent validity of a smartphone application to measure shoulder joint position sense using an electromagnetic tracking system as a reference standard. The findings indicated that the smartphone application could replace the electromagnetic tracking system to measure shoulder joint position sense.¹⁹ One study attached a smartphone to the right arm rather than the lumbar spine.¹⁸ Therefore, their joint position sense was the output from several body segments.¹⁸ We attached the smartphone to the lumbar spine and pelvis and used the difference between the two segments, which should better represent the lumbar joint position sense. Our findings support the clinical utility of smartphone applications in measuring lumbar joint position sense.

The study encountered limitations. First, we investigated the effects of CSE among healthy individuals who may have better proprioceptive sense than patients with LBP. Further, our sample size was underpowered, which could explain nonsignificant findings in the joint position sense when returning to the upright position. Therefore, further studies should recruit a larger sample size and include patients with LBP to confirm our findings. Second, this study aimed to determine the immediate effect of CSE on the lumbar position sense of trunk flexion at 45 degrees and return to the starting position. This would limit the generalizability to other movements or angles. However, our finding at 45-degree flexion could represent movement in the middle range where the contribution of proprioceptive sense from muscles is critical to minimize the risk of injury. Further study should include CSE in other movements and angles to explore the potential effects of CSE on joint position sense before applying it to clinical practice. In addition, we investigated the immediate effects of CSE without following up on the residual effect. Therefore, it would limit the insight into the optimal frequency to provide CSE to induce permanent improvement in joint position sense. Therefore, future studies should include the retention effects of CSE to provide insight into the impact of CSE.

Conclusion

Our study aimed to determine the immediate effects of CSE on absolute position error at 45-degree flexion and return to an upright position. Our findings demonstrated a significant decrease in absolute position error during 45-degree flexion, while no significant difference was found when returning to the upright position. Our findings suggest the ability of CSE to improve joint position sense in the middle of the movements, implying the potential clinical utility of CSE in minimizing the risk of low back injury.

Conflict of interest

The authors acknowledge that we do not have any financial or personal relationships with other people or organizations that could inappropriately influence the work described in the current manuscript.

Funding

This study was partially funded by the Thailand Research Fund (TRG5880133, 2015).

Acknowledgements

We would like to thank the Faculty of Physical Therapy, Mahidol University, for providing the data collection space and equipment. We would also like to thank all participants for contributing to this study.

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Effect of pre-exposure to low-dose radiation followed by H₂O₂ treatment on leukemic cells proliferation

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ARTICLE INFO

Article history:

Received 12 November 2024

Accepted as revised 18 December 2024

Available online 15 January 2025

Keywords:

Low-dose radiation, free radical, radiation, cancer, cell death.

ABSTRACT

Background: Leukemia is a blood cancer illness that causes morbidity and mortality around the world. A new approach to treatment is challenging.

Objective: The objective was to investigate the effects of pre-exposure to low-dose radiation (LDR) followed by H₂O₂ treatment on cell proliferation in leukemic cells.

Materials and methods: The human leukemic doxorubicin-sensitive K562 and doxorubicin-resistant K562/*adr* cells were exposed to 0.1 mGy of gamma radiation from radioactive ¹³⁷Cs at a dose rate of 0.001 Gy/min followed by treatments with various concentration of H₂O₂ at 4 hrs-post irradiation. The cell morphology and metaphase cells in treated groups were compared with a control group at 72 hrs post-irradiation. The cell proliferation was determined at days 1-8 post irradiation.

Results: The results showed that the number of cells in treated groups with 50, 100, and 200 µM of H₂O₂ was less than the control group (0 µM H₂O₂). The pattern of the cells rapidly increased at 24-48 hrs and then decreased until the 5th and 6th days. This was found in all treated groups, except those treated with 200 µM of H₂O₂ alone and with pre-exposure to LDR followed by H₂O₂ groups. The microscopic images showed rough cells, shrinking cells, irregular-shaped cells, and swelling cells in H₂O₂ alone and pre-exposure to LDR followed by H₂O₂ groups. The metaphase cells were significantly decreased in H₂O₂ alone groups in a concentration-dependent manner. In addition, the metaphase cells were also considerably reduced in pre-exposure to LDR, followed by H₂O₂ groups when compared with control and LDR alone groups.

Conclusion: This data provides a basis for additional studies to help clarify the potential use and benefits of pre-exposure to LDR followed by H₂O₂ treatment in cancer cells.

Introduction

Reactive oxygen species (ROS) are commonly used to define molecules with high oxidative reactivity. ROS are produced exogenously following exposure to xenobiotics like radiation, chemotherapy, or ultraviolet light. They are also physiologically produced due to aerobic metabolism and cellular respiration. Pathologically enhanced ROS are seen in disorders such as cancer and inflammation. ROS are a group of highly reactive oxygen-containing molecules such as hydroxyl radicals (HO[•]), superoxide radicals (O₂^{•-}), and hydrogen peroxide (H₂O₂).^{1,2} Under normal physiological conditions, intracellular ROS is scavenged

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doi: 10.12982/JAMS.2025.040

E-ISSN: 2539-6056

using antioxidants to control the dynamic redox balance in cells.³ Evidence suggests that the high amounts of ROS typically detected in cancer cells are almost incompatible with cell viability, making these cells more sensitive to ROS-induced cell death than normal cells.^{4,5}

Leukemia is a blood cancer illness that causes morbidity and mortality around the world.⁶ Chemotherapy is routinely used to treat this condition. However, treatment with anticancer drugs for leukemia is frequently limited by the development of multidrug resistance (MDR) in cancer cells.^{7,8} As a result, developing a new approach to overcoming MDR in cancer cells that involves MDR-reversing drugs that decrease undesired side effects is a formidable challenge.

Evidence shows several biological responses to low doses of low linear energy transfer radiation (i.e., gamma- and X-rays).⁹⁻¹¹ Chen Z. and Sakai K. found that LDR did not produce hormesis in human leukemic MOLT-4 cells.¹² However, pre-exposure to LDR could induce apoptosis when subjected to a high radiation dosage. These LDR effects were not observed in normal cells compared to tumor cells under identical circumstances. Furthermore, the LDR-induced cell growth has been shown in normal cells, but they did not cause the cell growth of cancer cells, including leukemic K562 cells.^{13,14}

Based on these facts, we decided to evaluate the effect of pre-exposure to LDR followed by H₂O₂ treatment on human leukemic doxorubicin-sensitive K562 and doxorubicin-resistant K562/*adr* cells by investigating the cell proliferation, cell morphology, and metaphase cells. The objective was to investigate the effect of pre-exposure to LDR followed by H₂O₂ treatment on cell proliferation in leukemic cells.

Material and methods

Chemicals

Fetal bovine serum (FBS) and penicillin/streptomycin were bought from Capricorn Scientific. RPMI-1640, hydrogen peroxide, and trypan blue were received from Caisson Labs Colcemid (GIBCO-BRL).

Cell line and cell culture

The human leukemic doxorubicin-sensitive K562 cell and doxorubicin-resistance K562/*adr* (P-glycoprotein-overexpression) cell were used in the study. The cells were grown in a complete RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin in a 5% CO₂, 37 °C incubator. The density of cells was initially at 1×10⁵ cells/mL and increased to 8-10×10⁵ cells/mL 72 hrs later, followed by sub-cultured cells.^{15,16}

For the experiment, cells initially had a density of 5×10⁵ cells/mL and increased to 8-10×10⁵ cells/mL 24 hours later. Cells were in an exponential growth phase.

Irradiation

The irradiation was performed as previously described.¹⁷ The ¹³⁷Cs radioactive standard source located at the Department of Radiologic Technology, Faculty

of Associated Medical Sciences, Chiang Mai University, Thailand, was used as a gamma radiation source. Cells were given a dose of 0.1 mGy gamma rays at a dose rate of 0.001 Gy/min. The radiation dose was calculated by using the equation;

$$A_t = A_0 e^{-\lambda t}$$

$$\text{And } D = \frac{A_t \times \Gamma}{d^2}$$

When A₀ and A_t : activity of radioactive present at t=0 and time=t

λ : decay constant,

D : radiation dose

d : distance from radioactive

Γ : specific gamma ray constant.

Cell morphological observation

A number of cells at 1×10⁵ cells/mL were irradiated to 0.1 mGy of gamma radiation from radioactive ¹³⁷Cs, followed by treatments with and without 50, 100, and 200 μM of H₂O₂ in a complete RPMI-1640 medium at 4 hrs post-irradiation. The cells were placed into a humidified atmosphere having 5% CO₂ at 37 °C incubation. After 72 hrs post-irradiation, the cells were observed under a light microscope without any cell disruption.

Cell proliferation

The cell proliferation was measured by using the trypan blue exclusion method. Cells (1×10⁵ cells/mL) were irradiated to 0.1 mGy of gamma radiation from radioactive ¹³⁷Cs followed by treatments with and without 50, 100, and 200 μM of H₂O₂ in a complete RPMI-1640 medium at 4 hrs-post irradiation. They were placed into a humidified atmosphere with 5% CO₂ at 37 °C incubation. The number of cells was counted under a light microscope by staining with trypan blue solution at a ratio of 1:1 (volume: volume). The cell proliferation was determined at days 1-8 post irradiation.

Metaphase assay

Cells (1×10⁶ cells/mL) were irradiated to 0.1 mGy of gamma radiation from radioactive ¹³⁷Cs, followed by treatments with and without 50, 100, and 200 μM of H₂O₂ in a complete RPMI-1640 medium at 4 hrs-post irradiation. They were placed into a humidified atmosphere with 5% CO₂ at 37 °C incubation. After 72 hrs-post irradiation, a colcemid (30 μL of 10 μg/mL) was added to each cell. After 45 min incubation in a 37 °C, 5% CO₂ humidified incubator, cells were centrifuged and washed twice in 1 mL of phosphate buffer saline (PBS). Five hundred μL of 0.075 M KCl was added to each cell, followed by 45 min of additional incubation. Afterward, cells were centrifuged and washed twice, then added 1 mL of fixatives (Carnoy's solution, 3:1 v/v methanol:acetic acid). Fixed cells were dropped gently on a clean microscope slide, air-dried, and stained with Wright Giemsa for 3 min. The slides were coded, and the codes were not disclosed until after the slides had been scored. The metaphase cells are shown in the Figure 1.

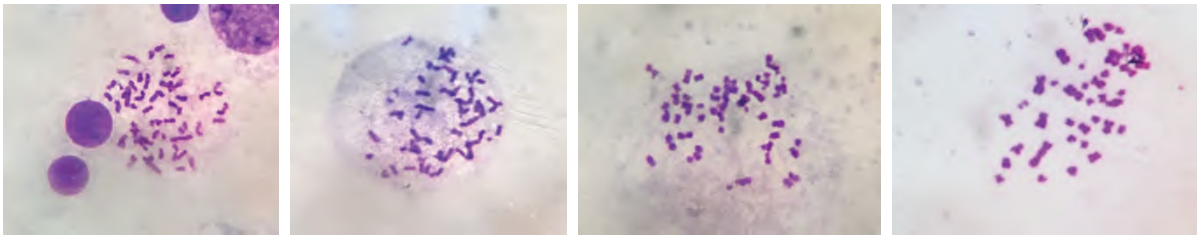


Figure 1. Metaphase cells under a light microscope (40X magnification).

Statistical analysis

The results were shown as the mean $\pm$ SEM. Student's t-test was used independently to evaluate statistical differences in the mean values between the treated and non-treated groups. The $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Cell morphology

Figure 2 shows the morphology of K562 and K562/*adr* cells under a light microscope. The microscopic images showed rough cells, shrinking cells, irregular-shaped cells, swelling cells, and decreases in cell density in K562 and K562/*adr* cells treated with H_2O_2 alone and having pre-exposure to LDR followed by H_2O_2 groups. These phenomena in treated cells were linked to characteristics of cell death.

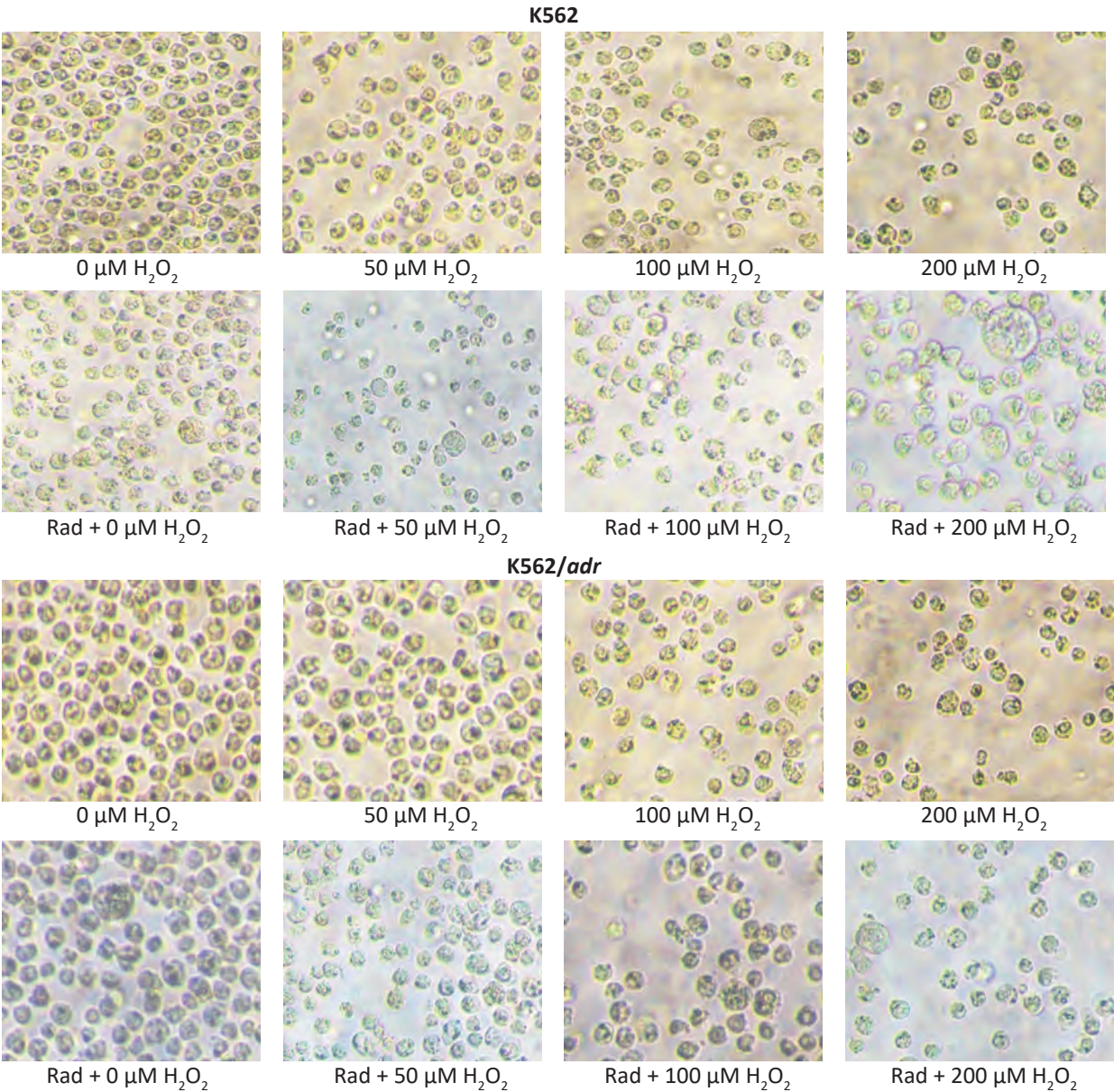


Figure 2. Effect of H_2O_2 and pre-exposure to LDR followed by H_2O_2 on K562 and K562/*adr* cells, light microscope, 20X magnification. Rad: radiation.

Cell proliferation

The cells rapidly increased at 24-48 hrs and then decreased until 5-6 days. This pattern was found in all treated groups, except cells treated with 200 μM of H_2O_2

alone and with pre-exposure to LDR followed by 200 μM of H_2O_2 groups, as indicated in Figure 3. In addition, the number of cells in treated cells with 50, 100, and 200 μM of H_2O_2 was less than in non-treated cells (0 μM H_2O_2).

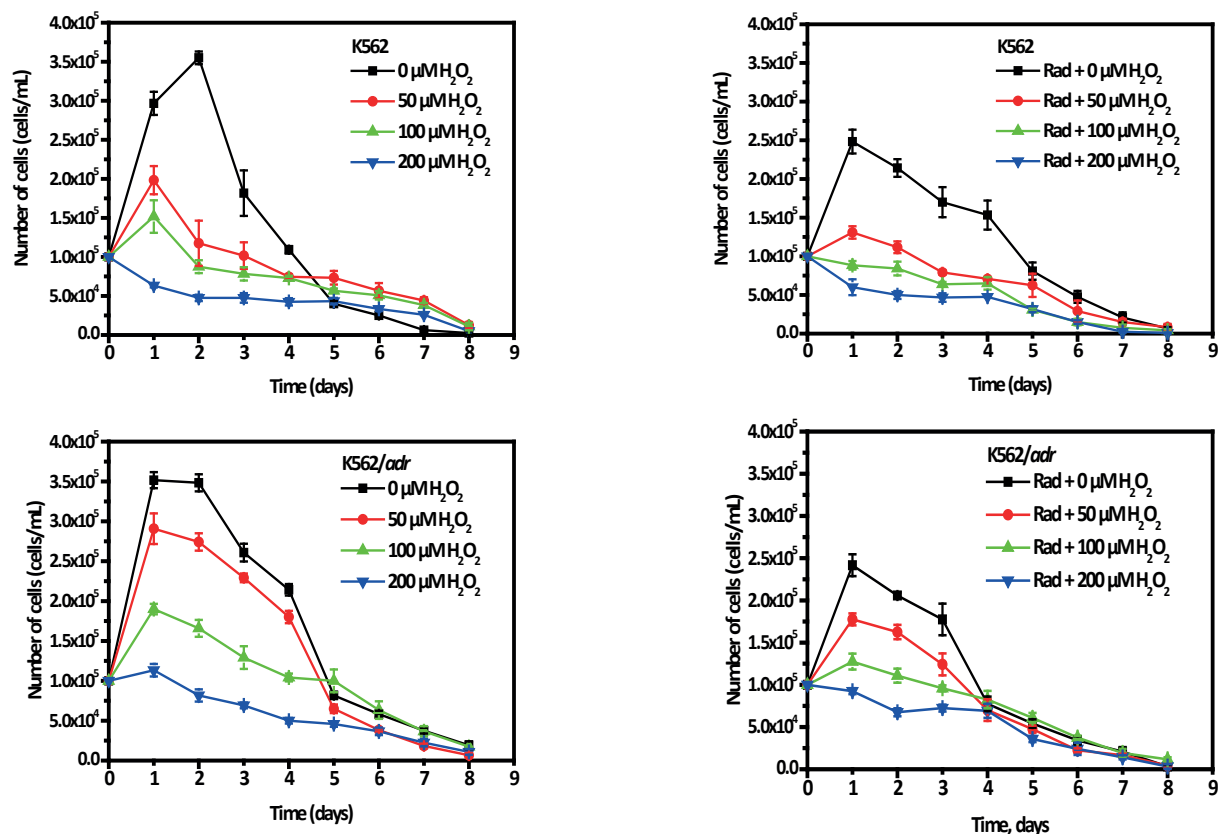


Figure 3. Effect of H_2O_2 alone and pre-exposure to LDR followed by H_2O_2 on cell viability of K562 and K562/adr cells. Rad: radiation.

Metaphase cells

Figure 4 shows the number of metaphase cells in treated and non-treated K562 and K562/*adr* cells. This data showed that the metaphase cells significantly decreased in both treated K562 and treated K562/*adr* cells with 50, 100, and 200 μM of H_2O_2 compared to a control group.

Similarly, the metaphase cells in K562 and K562/*adr* cells decreased in pre-exposure to LDR, followed by treatments with 50, 100, and 200 μM of H_2O_2 groups compared to a control group. Of note, the number of metaphase cells in irradiated K562 and irradiated K562/*adr* cells significantly decreased compared to the control group.

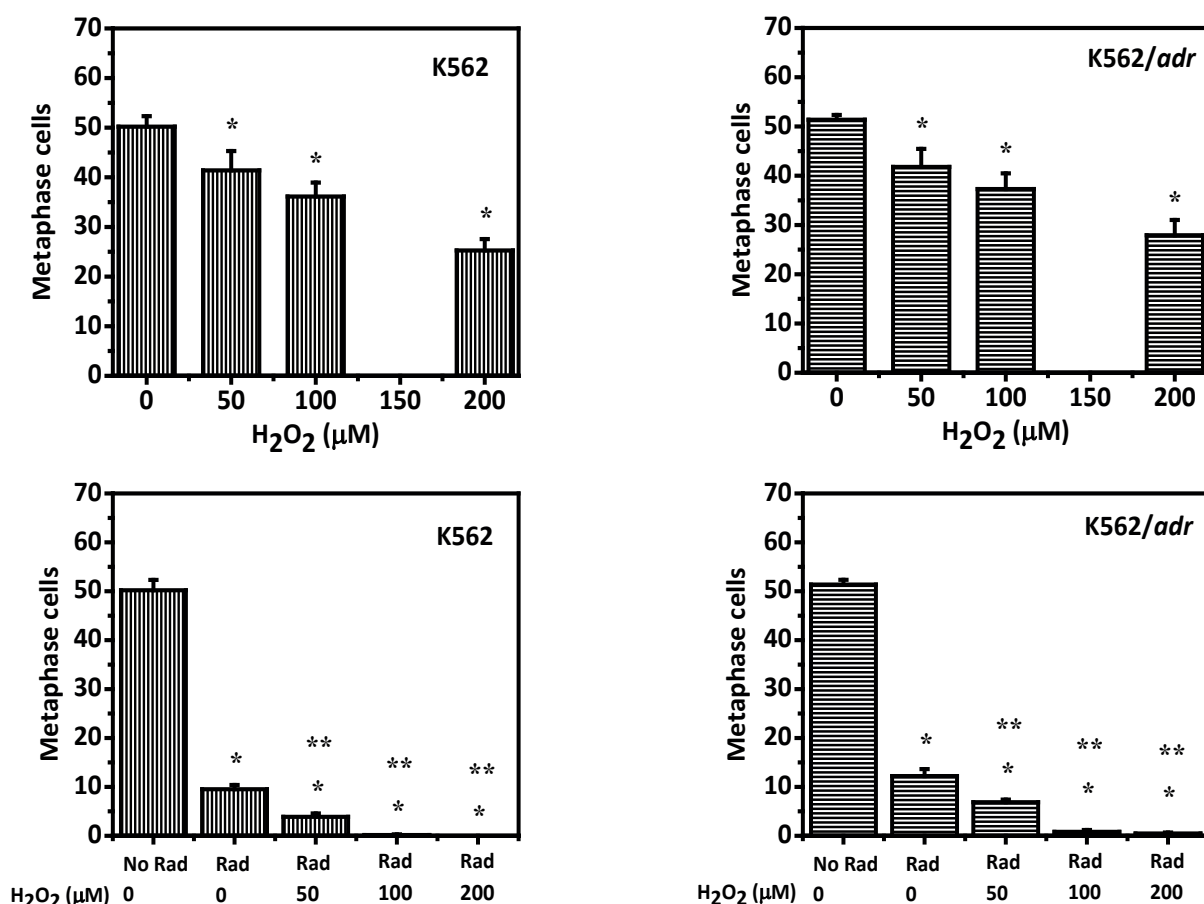


Figure 4. The number of metaphase cells in 1,000 treated and non-treated K562 and K562/*adr* cells. * $p < 0.05$ as compared with No Rad, H_2O_2 0 μM group. ** $p < 0.05$ as compared to Rad, H_2O_2 0 μM group, Rad: radiation.

Discussion

Our study provides additional evidence regarding the effect of pre-exposure to LDR followed by H_2O_2 on the morphology, proliferation, and metaphase cells of the K562 and K562/*adr* cells. The microscopic images showed rough cells, shrinking cells, irregular-shaped cells, and swelling cells in pre-exposure to LDR, followed by the H_2O_2 groups and H_2O_2 alone group. These morphological changes and reduction of the number of metaphase cells are the typical signs of cell death. Therefore, this data suggests pre-exposure to LDR followed by H_2O_2 -induced K562 and K562/*adr* cells deaths. Consequently, the number of cells in pre-exposure to LDR followed by H_2O_2 groups and in 50, 100, and 200 μM of H_2O_2 groups were less than non-treated cells (0 μM H_2O_2).

We have not examined the mechanisms by which pre-exposure to LDR followed by H_2O_2 induced cell death in the cells. However, it is well known that hydrogen peroxide is a potent pro-oxidant that can generate and

accumulate intracellular ROS, resulting in cell death and apoptosis or necrosis.¹⁸⁻²² Kohshour MO *et al.* studied the antiproliferative effects of H_2O_2 against human acute myelogenous leukemia KG1 cell lines.²³ The authors showed that H_2O_2 significantly decreased cell proliferation and led to the induction of apoptosis via activation of caspase-3, affecting the expression of Bcl-2 and Bax. Datta K. *et al.* investigated the induction of cell death by H_2O_2 and its relation to p53 in human glioma cells.²⁴ The authors showed that H_2O_2 induced p53-dependent apoptosis in glioma cells in a dose-dependent manner. Moreover, H_2O_2 could cause an increase in the level of DNA damage, resulting in apoptotic cell death in Jurkat T-cells.²⁵ Dumont A. *et al.* showed H_2O_2 -induced T-cell death that involved the formation of mitochondrial permeability transition pores, resulting in a rapid decrease of the mitochondrial transmembrane potential and the release of cytochrome C.²⁶ In addition, it has been well established that biological effects of radiation result from energy

deposition in irradiated cells in which ROS, including H_2O_2 , are produced.²⁷ We have reported that ROS statistically significantly increased.

In contrast, mitochondrial activity decreased,¹⁷ and lysosomal pH changed²⁸ in the K562 and K562/*adr* cells after exposure to LDR from ^{137}Cs in a dose-dependent manner.

Taken together, for these reasons, it is plausible to hypothesize that the effect of pre-exposure to LDR from ^{137}Cs followed by H_2O_2 treatment observed in our *in vitro* study is due to its pro-oxidative activity. However, the limitation of our study was that only one dose of LDR (0.1 mGy) was used in our research. Therefore, it remains unknown to what extent LDR can sustain the effect of pre-exposure to LDR followed by H_2O_2 in K562 and K562/*adr* cells. Consequently, further investigation is required to determine the magnitude of the impact of pre-exposure to LDR followed by H_2O_2 in K562 and K562/*adr* cells, using different treatment protocols.

Conclusion

These findings suggest that pre-exposure to LDR followed by H_2O_2 treatment significantly decreased cell proliferation in leukemic doxorubicin-sensitive K562 and doxorubicin-resistant K562/*adr* cells in a H_2O_2 concentration-dependent manner. Therefore, this data provides a basis for additional studies to help clarify the potential use and benefits of pre-exposure to LDR followed by H_2O_2 treatment in cancer cells.

Acknowledgements

This research was partially supported by Chiang Mai University. The authors would like to thank the Faculty of Associated Medical Sciences, Chiang Mai University, for their support. We also want to thank all staff and students who participated in this study.

Ethical approval

This article contains no studies with human subjects or animals performed by any authors.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Fibroblast growth factors in female reproductive disorders: A systematic review

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ARTICLE INFO

Article history:

Received 4 November 2024

Accepted as revised 16 January 2025

Available online 21 January 2025

Keywords:

Female infertility, in vitro fertilization, ovarian reserve, polycystic ovary syndrome, reproductive medicine.

ABSTRACT

Background: Fibroblast Growth Factors (FGFs) are a family of signaling proteins that play key roles in various biological processes, including cell proliferation, differentiation, and tissue repair. In recent years, there has been growing interest in the role of FGFs in reproductive biology, particularly in the context of female fertility and assisted reproductive technologies (ART).

Objective: This systematic review aimed to evaluate the role of Fibroblast Growth Factors (FGFs) in female reproductive disorders with a focus on repeated implantation failure (RIF), diminished ovarian reserve (DOR), polycystic ovary syndrome (PCOS), and premature ovarian insufficiency (POI).

Materials and methods: A comprehensive literature search was conducted across PubMed, Web of Science, and Scopus databases for studies published between January 2014 and December 2024, yielding 8 eligible studies.

Results: The review found: reduced FGF-2 levels in PCOS patients' serum and follicular fluid, contrasting with earlier research; lower serum FGF-1 levels and specific genetic variants in RIF patients; elevated FGF-13 levels associated with increased androgen levels and ovarian volume in PCOS; altered FGF-5 expression in DOR cases; and unexpectedly elevated FGF-2 levels in biochemical POI.

Conclusion: FGF isoforms may be a contributor to the pathophysiology of female reproductive disorders. However, these findings while important, the review identified important limitations including small sample sizes and the tendency to focus on individual FGF isoforms. For future studies, there's a need for larger and more comprehensive studies examining the entire FGF family and their connections in reproductive dysfunction.

Introduction

Fibroblast Growth Factors (FGFs) are a family of signaling proteins that play key roles in various biological processes, including cell proliferation, differentiation, and tissue repair.¹ In recent years, there has been growing interest in the role of FGFs in reproductive biology, particularly in the context of female fertility and assisted reproductive technologies (ART). The FGF family consists of 22 members in humans, classified into seven subfamilies based on their sequence homology and functional properties.² Among, these several FGF isoforms have been implicated in ovarian function, folliculogenesis, and embryo development. FGF-2 (basic FGF) and FGF-1 (acidic FGF) are perhaps the most studied in this context, with roles in promoting angiogenesis and regulating granulosa cell function.^{1,3,4} Other isoforms, such as FGF-5, FGF-13, and FGF-21, have also appeared to contribute to reproductive processes.⁵⁻⁷

The complex relationship between FGFs and

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doi: 10.12982/JAMS.2025.041

E-ISSN: 2539-6056

reproductive function has been increasingly reported in many female reproductive disorders. These include repeated implantation failure (RIF), defined as the failure to achieve clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles,⁸⁻¹⁰ diminished ovarian reserve (DOR), characterized by reduced quantity and quality of oocytes,^{5,11} biochemical Premature Ovarian Insufficiency (bPOI), a condition of ovarian dysfunction before age 40 characterized by irregular menses and elevated follicle-stimulating hormone levels,¹²⁻¹⁴ and polycystic ovary syndrome (PCOS), an endocrine disorder marked by irregular menstruation, elevated androgens, and polycystic ovaries.^{15,16} Despite the growing body of research, the role of FGFs in female reproductive disorders remains a subject of large debate, particularly when some reports show controversial findings. Thus, this review aims to systematically evaluate the current evidence on the role of FGFs in female reproductive disorders, with a focus on RIF, DOR, PCOS, and POI.

Materials and methods

Literature search

A systematic literature search was conducted to find studies on the relationship between Fibroblast Growth Factor (FGF) and female reproductive disorders, with a focus on *in vitro* fertilization (IVF) outcomes. Four electronic databases, including PubMed, Web of Science, Scopus, and Embase, were used for relevant articles published between January 2014 and December 2024 (10-year period). The search strategy combined terms related to FGF (e.g., "Fibroblast Growth Factor" and "FGF") with terms associated with female reproductive disorders and IVF (e.g., "in vitro fertilization", "IVF", "ICSI", "embryo transfer", "repeated implantation failure", "RIF", "diminished ovarian reserve", "DOR", "polycystic ovary syndrome", "PCOS", "premature ovarian insufficiency", "POI"). In addition, the reference lists of included studies and relevant review articles were manually searched

to identify any potentially missed studies. The search was limited to English-language publications on human subjects.

Eligibility criteria

Studies were considered eligible if they were observational studies (prospective cohort, retrospective cohort, case-control) or randomized controlled trials involving women of reproductive age (18-45 years) with RIF, DOR, PCOS, or POI, undergoing IVF or ICSI procedures. Included studies must have measured FGF levels (any isoform) in biological samples such as follicular fluid, serum, culture media, granulosa cells, or endometrial tissue. The studies needed to report at least one of the following outcomes: clinical pregnancy rate, live birth rate, implantation rate, embryo quality, fertilization rate, miscarriage rate, or oocyte yield. Only English-language publications on human subjects published between January 2014 and December 2024 were considered. Studies were excluded if they were case reports, reviews, or animal studies, or if they did not provide sufficient statistical data for effect size calculation.

Results

The systematic literature search yielded a total of 5,757 records across three databases: Scopus (N=2,579), PubMed (N=2,122), and Web of Science (N=1,056). After the removal of 4,677 records marked as ineligible by automation tools and 539 duplicate records, 541 unique articles remained for screening. The initial screening process, based on titles and abstracts, resulted in the exclusion of 522 records that did not meet our inclusion criteria. Of the 19 full-text articles assessed for eligibility, 10 were not retrievable, and 1 was excluded due to non-relevance to the research question. Ultimately, 8 studies met all inclusion criteria and were included in this systematic review. The study selection process is illustrated in the PRISMA flow diagram (Figure 1).

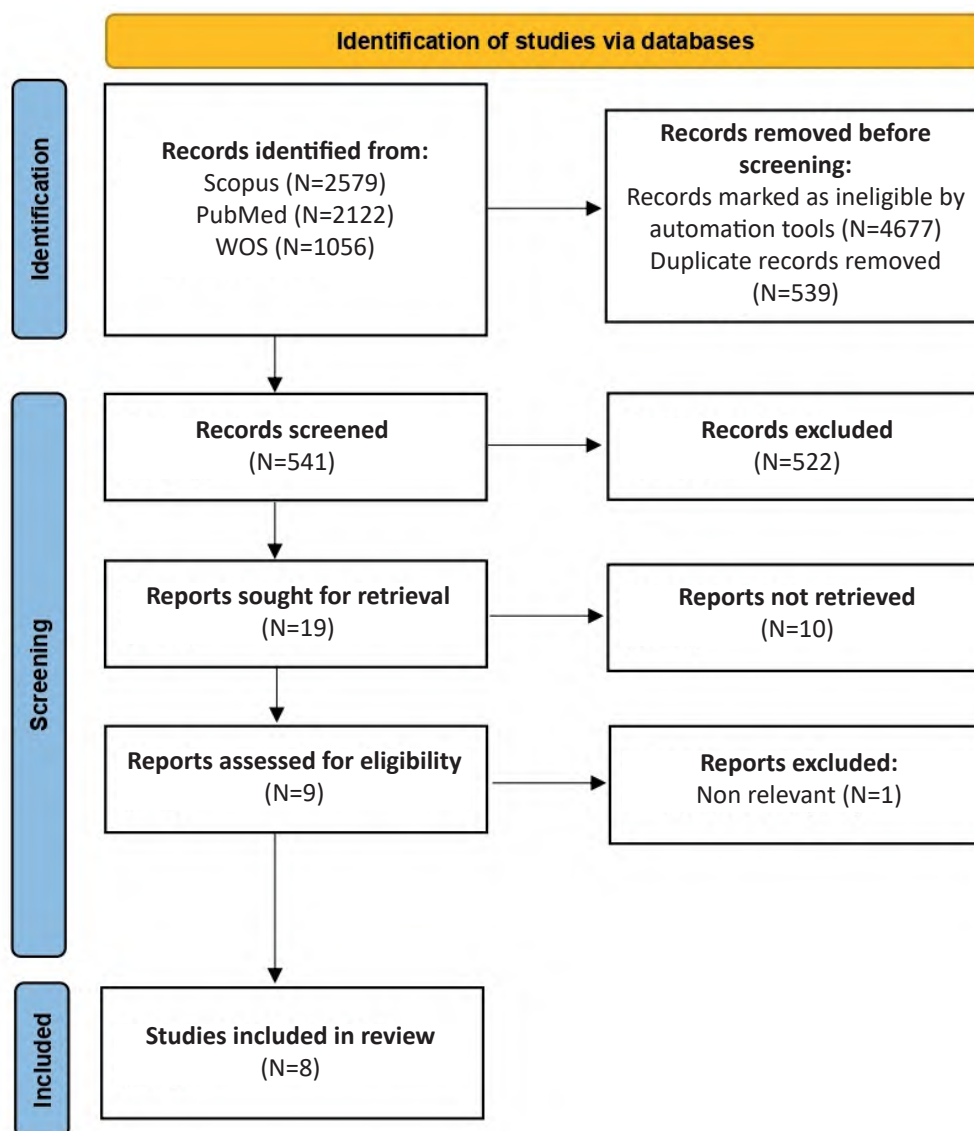


Figure 1. PRISMA flow diagram of study selection process.¹⁷

Table 1 presents a comprehensive overview of the included studies, their design, population, sample size,

FGF isoforms studied, biological samples used, detection methods, and key conclusions.

Table 1. Comprehensive overview on fibroblast growth factor (FGF) studies in reproductive medicine.

Study	Design	Population	Sample size	FGF	Biological sample	Method of detection	Conclusion
Aghajani <i>et al.</i> ⁸	RCT	Infertile women with unexplained RIF undergoing IVF	20 (10 in RIF group, 10 controls)	FGF1, FGF2, FGF13 (genes)	Endometrial biopsy	PCR array and quantitative reverse transcriptase PCR	Endometrial scratching induced significant upregulation of <i>FGF1</i> , <i>FGF2</i> , and <i>FGF13</i>
Kharamani <i>et al.</i> ⁹	Cross-sectional	Infertile women with RIF history and healthy women undergoing first IVF-ET with successful delivery	800 (400 RIF, 400 control)	FGF-1	Serum	ELISA	- FGF-1 was significantly reduced in RIF compared to controls - Significant association between <i>FGF1</i> (rs34011) promoter polymorphism and its serum concentration with RIF - The AA genotype was linked to lower FGF-1 serum levels and may be a risk factor for RIF
Abhari <i>et al.</i> ⁵	Case-control	Women undergoing ovarian stimulation for IVF	131 (56 DOR cases, 75 controls)	FGF-5	Follicular fluid	Multiplex immunoassay (Quantibody® Human Cytokine Antibody Array)	- FGF-5 was significantly lower in DOR group
Patil <i>et al.</i> ¹⁵	Case-control	Women undergoing controlled ovarian hyperstimulation for IVF	60 (30 PCOS cases, 30 controls)	FGF-2	Follicular fluid, serum and granulosa-lutein cells	ELISA for serum and follicular fluid levels, and qRT-PCR for gene expression in granulosa-lutein cells	- FGF-2 levels were significantly lower in both serum and follicular fluid levels of PCOS women compared to controls - FGF-2 transcript levels showed positive correlation with oocyte fertilization rate - <i>FGF2</i> expression was significantly lower in PCOS patients compared to controls
Liu <i>et al.</i> ¹²	Case-control	Women undergoing IVF/ICSI	72 (35 bPOI cases, 37 controls)	bFGF	Follicular fluid	Magnetic beads multiplex immunoassay (Luminex)	- bFGF was significantly higher in bPOI group compared to controls - bFGF in follicular fluid was significantly correlated with FSH, AMH, and AFC
Yland <i>et al.</i> ¹⁸	Prospective cohort	Women presenting for oocyte retrieval	34 (10 with unilateral endometrioma, 24 without endometriosis)	FGF-β	Follicular fluid	Multiplex immunoassays and Luminex bead-based antibody capture and recognition arrays	Non-significantly different between the groups

Table 1. Comprehensive overview on fibroblast growth factor (FGF) studies in reproductive medicine (*continued*).

Study	Design	Population	Sample size	FGF	Biological sample	Method of detection	Conclusion
Liu <i>et al.</i> ⁶	Cross-sectional	Infertile women undergoing first IVF/ICSI, aged 20-37 years	75 (43 PCOS cases, 32 non-PCOS controls)	FGF-13	Follicular fluid	ELISA	- The prevalence of elevated FGF-13 was significant among PCOS patients - FGF13 is associated with androgen levels and oocyte developmental competence in PCOS patients - MII oocyte rate negatively correlated with FGF-13 in PCOS patients
Vithoulkas <i>et al.</i> ¹⁹	Randomized prospective comparative study	IVF patients aged 35-40 (mean age 36.5)	90	FGF-1, FGF-2	Supernatants from embryo cultures	Milliplex factor-specific magnetic-bead panels with phycoerythrin fluorescent factor-specific antibodies using immunofluorescence cytometry technology	- FGF-2 was not detected in samples of group II (standard culture) but was present in samples of group I (co-culture) and C (cumulus cells alone) - FGF-1 was only detectable in very low concentrations in group I and C, ranging from 0.5 to 1.5 pg/mL

Note: RCT: randomized controlled trial, RIF: repeated implantation failure, IVF: in vitro fertilization, ET: embryo transfer, ELISA = enzyme-linked immunosorbent assay, DOR: diminished ovarian reserve, PCOS: polycystic ovary syndrome, qRT-PCR: quantitative reverse transcription polymerase chain reaction, bPOI: biochemical premature ovarian insufficiency, FSH: follicle-stimulating hormone, AMH: anti-Müllerian hormone, AFC: antral follicle count, ICSI: intracytoplasmic sperm injection, MII: metaphase II.

Discussion

The role of FGFs, since past two decades till nowadays, in female reproductive conditions is a complex and area of rich research. In the current systematic review, the involvement of several FGF isoforms in conditions such as polycystic ovary syndrome (PCOS), repeated implantation failure (RIF), diminished ovarian reserve (DOR), and biochemical premature ovarian insufficiency (bPOI) was evidenced based on the overall assessment of the included studies.

The molecular mechanisms underlying FGF signaling in reproductive tissues involve complex, interconnected pathways (Figure 2). Three main FGF isoforms, FGF-1, FGF-2, and FGF-13, work together with their specific receptors (FGFR1-3) to activate specific but overlapping signaling cascades. Upon ligand binding, these receptors trigger three primary pathways: the MAPK pathway,

which regulates cell proliferation, migration, and survival; the PI3K/AKT pathway, crucial for angiogenesis; and the STAT pathway, which controls cellular differentiation.²⁰⁻²³ These molecular cascades ultimately influence three key reproductive processes: follicular development, corpus luteum function, and oocyte maturation.^{24,25} This complex signaling network helps explain these systematic review findings, particularly the diverse effects observed when specific FGF isoforms are dysregulated. For instance, the reduced FGF-2 levels observed in PCOS patients may compromise both MAPK-mediated cellular survival and PI3K-dependent angiogenesis, potentially contributing to the observed luteal phase deficiencies.²⁶⁻²⁸ Similarly, the association between FGF-13 and androgen levels in PCOS may be mediated through STAT-dependent differentiation pathways affecting steroidogenic cells.⁶

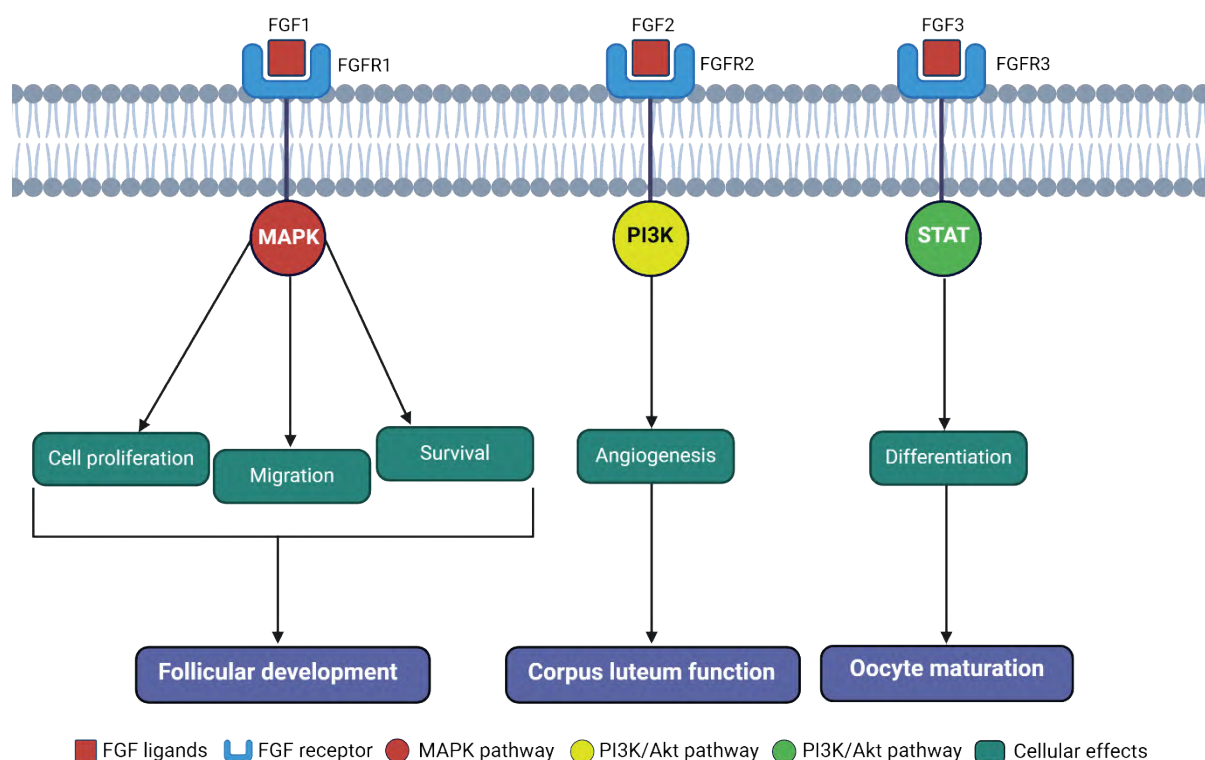


Figure 2. Molecular mechanisms of FGF signaling in reproductive tissues. These cascades regulate cellular processes, including proliferation, angiogenesis, and differentiation, ultimately influencing follicular development, corpus luteum function, and oocyte maturation (purple boxes). Arrows indicate signaling direction.

One of the most important findings relates to the role of FGF-2 (also known bFGF) in ovarian function and PCOS. The FGF-2 was differently reported in some previous studies,^{27,29} however, in the last 10 years, research has observed significantly lower levels of FGF-2 in both serum and follicular fluids of women with PCOS compared to controls.¹⁵ Patil *et al.*, in their study, showed that this downregulation extends to the gene expression level in granulosa-lutein cells (GLCs) of PCOS patients.¹⁵ Moreover, the concurrent reduction in FGFR1 protein expression in these cells hints a possible disturbance in the FGF-2 signaling pathway in PCOS.¹⁵ Taken together, these findings oppose earlier notions about FGF-2's role in

PCOS and draw attention to the need for more research into the complex interaction between FGFs and ovarian function in this disorder. The involvement of reduced FGF-2 signaling in PCOS are especially interesting when considering its known roles in angiogenesis and corpus luteum function. The observed reduction of FGF-2 and FGFR1 in PCOS patients may correlate with decreased vascularization of the corpus luteum, possibly explaining some of the luteal phase deficiencies and recurrent miscarriages observed in this population.¹⁵ Furthermore, the positive correlation between FGF-2 transcript levels in GLCs and oocyte fertilization rates rise attention to the probable role for FGF-2 in determining oocyte quality and

developmental potential.¹⁵ The relationship between FGF signaling and reproductive outcomes has been further supported by recent follicular fluid studies. Vithoulkas *et al.* (2017) examined women undergoing IVF and reported the presence of FGF-2 in embryo co-culture systems may contribute to improved embryo development and quality.¹⁹ These findings together suggest that FGF isoforms, besides their usefulness as biomarkers, play active roles in maintaining embryo development during assisted reproductive procedures. The observation that FGF-2 levels are reduced in co-culture conditions, possibly due to embryo consumption or metabolism, and this indicates a direct functional role in early embryonic development.

In the context of repeated implantation failure (RIF), this review found a possibly necessary role for FGF-1. Studies have concluded lower serum FGF-1 levels in women with RIF compared to controls, and a specific SNP in the *FGF1* promoter (rs34011) was associated with increased RIF risk.⁹ The correlation between this genetic variant and lower serum FGF-1 levels provides a potential mechanistic link between FGF-1 dysregulation and implantation failure. Additionally, the upregulation of *FGF1*, *FGF2*, and *FGF13* after endometrial scratching in RIF patients confirms that these growth factors may have a role in the reported benefits of this procedure.⁸ These findings collectively point to the potential of FGF-targeted therapies in improving implantation rates in RIF patients.

The role of FGF-13, a less-studied member of the FGF family, has also come to light in this review. High expression levels of FGF-13 in adult female ovaries and its detection in human follicular fluid indicate an important function in ovarian physiology.^{6,8} Attractively, elevated follicular fluid FGF-13 levels were associated with higher androgen levels and increased ovarian volume in PCOS patients while negatively correlating with mature oocyte rates.⁶ These observations direct that FGF-13 may be involved in the hormonal and morphological changes characteristic of PCOS, although the exact mechanisms require deeper elucidation.

Another interesting isoform of the FGF family that has been mentioned in recent studies is FGF-5. The analysis of follicular fluid from women with diminished ovarian reserve showed significantly different levels of FGF-5 in DOR patients compared to controls.⁵ This may point to its probable role in ovarian reserve regulation. However, as current evidence regarding FGF-5's specific function in reproductive disorders remains limited, more in-depth work is needed to fully understand its mechanistic involvement and potential clinical significance in ovarian pathologies.

In cases of diminished ovarian reserve (DOR) and biochemical premature ovarian insufficiency (bPOI), the review has revealed some unexpected findings. Contrary to what might be expected in conditions of reduced ovarian function, FGF-2 (or bFGF) levels were found to be significantly elevated in the follicular fluid of women with bPOI compared to controls.¹² This elevation positively correlated with FSH levels and negatively with AMH and antral follicle count, the matter draw attention

to a potential compensatory mechanism in response to declining ovarian function.¹² These findings emphasize the complex and sometimes paradoxical nature of growth factor regulation in reproductive disorders and underline the need for careful interpretation of such biomarkers in clinical settings.

This review suggests several possible clinical applications: serum FGF-1 levels may be used as a predictive biomarker for implantation failure risk, particularly in patients carrying specific genetic variants. In PCOS cases, monitoring FGF-2 and FGF-13 levels might help identify patients at higher risk for luteal phase deficiency. For DOR patients, FGF-5 assessment could complement existing ovarian reserve markers. These molecular markers could enhance current diagnostic options. However, it is necessary to acknowledge that standardization of measurement techniques and establishment of clear clinical thresholds is important before routine clinical implementation.

Still, and while these findings help us better understand FGFs in reproductive disorders, we must acknowledge some important limitations. First, many studies only show that certain changes happen together, but don't prove that one causes the other. Second, several studies looked at small groups of patients, which makes it harder to be sure the results apply to everyone. Third, since most research focused on just one or two types of FGF at a time, we might be missing the bigger picture of how all FGFs work together in reproductive health. Moving forward, we need larger studies that look at all types of FGFs at once and how they interact with other growth factors in reproductive tissues.

Conclusion

Fibroblast Growth Factors (FGFs) play various and important roles in female reproductive disorders. This systematic review showed several significant findings across different conditions. In PCOS, contrary to earlier research, FGF-2 levels are significantly reduced in both serum and follicular fluid which suggests its possible role in the pathogenesis of the syndrome. For patients with repeated implantation failure (RIF), lower serum FGF-1 levels and specific genetic variants in the *FGF1* promoter appear to increase risk, which points to possible therapeutic targets. Moreover, the elucidation of FGF-13's association with androgen levels and ovarian volume in PCOS patients and FGF-5's altered expression in diminished ovarian reserve (DOR) cases further demonstrates the broad involvement of different FGF isoforms in reproductive dysfunction. Future research should prioritize three key methodological improvements: 1) multi-center studies with larger sample sizes, 2) comprehensive proteomic analysis examining all FGF isoforms simultaneously rather than individual molecules, and 3) integration of genomic and proteomic data through systematic pathway analysis. These enhancements would provide more robust and complete understanding of FGF networks in reproductive disorders. To summarize, FGF levels could be useful for the monitoring/management of reproductive disorders

and possibly predict IVF outcomes. However, limitations such as small sample sizes and the focus on individual FGF isoforms indicate the need for larger, more comprehensive studies examining the entire FGF family and their interactions.

Conflict of interest

The author has no conflict of interest.

Funding

None

Acknowledgements:

Nil

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Improving the effectiveness of DHF prevention: Lessons from Pariaman City

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ARTICLE INFO

Article history:

Received 4 September 2024

Accepted as revised 12 January 2025

Available online 21 January 2025

Keywords:

Aedes aegypti, DHF, G1R1J, vector control, mosquito Larvae-monitoring, students.

ABSTRACT

Background: The *Aedes aegypti* mosquito transmits the viral disease dengue hemorrhagic fever (DHF), a significant public health problem in many tropical countries. The 1 House 1 Larva Monitoring (G1R1J) Movement, which involves every household as a mosquito larva monitoring agent, is one of the efforts to help communities control mosquito growth. Dengue Hemorrhagic Fever (DHF), transmitted by the *Aedes aegypti* mosquito, remains a significant public health issue in many tropical regions. The “1 House 1 Larva Monitoring” (G1R1J) movement, which mobilizes households as mosquito larvae monitoring agents, represents a community-based approach to controlling mosquito proliferation.

Objective: This study highlights a knowledge gap regarding the effectiveness of the G1R1J program with larvae-monitoring students compared to a program solely relying on larva-monitor cadres.

This study addresses a knowledge gap by comparing the effectiveness of the G1R1J program integrated with larva-monitoring students against the traditional approach relying solely on larva- monitor cadres.

Materials and methods: This study used a comparative design to compare the efficiency of the G1R1J program with the number of DHF cases in two DHF-endemic areas in Pariaman City, West Sumatra. A comparative study was designed to evaluate the efficiency of the G1R1J program concerning the incidence of DHF cases in two endemic areas in Pariaman City, West Sumatra. The researchers focused on field surveys and community attitudes because these aspects are directly linked to the implementation and success of the G1R1J program.

Results: The main results, with $p=0.000$ and OR 0.03, showed that comprehensive field-based interventions can significantly reduce the risk of vector presence. The study also demonstrated the importance of community attitudes in vector control, with $p=0.002$ and OR=0.15, suggesting that positive attitudes towards vector control practices can enhance the program's effectiveness. Statistical analysis revealed significant results, with a p -value of 0.000 and an odds ratio (OR) of 0.03, indicating that comprehensive, field-based interventions markedly reduce vector presence. Additionally, the study highlighted the role of community attitudes, with $p=0.002$ and OR=0.15, underscoring that positive perceptions and practices towards vector control significantly enhance program effectiveness.

Conclusion: This study shows that integrating the G1R1J program with larva-monitoring students can reduce the density of *Aedes Aegypti* mosquito vectors and dengue fever incidence. In addition, it offers strategic guidance for building more sustainable and efficient vector control policies in other endemic areas. Integrating the G1R1J program with larvae-monitoring students reduces *Aedes aegypti* vector density and DHF incidence. This approach offers strategic insights for developing sustainable and efficient vector control policies in other endemic regions.

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doi: 10.12982/JAMS.2025.042

E-ISSN: 2539-6056

Introduction

Dengue hemorrhagic fever (DHF) is a viral disease transmitted by the *Aedes aegypti* mosquito, which has become a serious public health problem in many tropical countries. Including Indonesia.¹ DHF can cause symptoms such as high fever, headache, joint and muscle pain, and rash. In severe cases, it can develop into DHF, which is characterized by bleeding and blood vessel damage.² Recent studies show that increasing dengue incidence is often associated with environmental factors such as changes in rainfall patterns and temperature, which influence the distribution and density of mosquito populations.^{3,4} Apart from that, public perception and understanding of the dengue vector and appropriate preventive measures also play an essential role in controlling this disease.⁵ Efforts to reduce the incidence of dengue fever require an integrated approach involving vector monitoring, public education, and environmental control.⁶

Dengue hemorrhagic fever (DHF) is a viral disease transmitted by the *Aedes aegypti* mosquito, which has become a significant public health issue in many tropical countries, including Indonesia.¹ DHF presents symptoms such as high fever, headache, joint and muscle pain, and rash. In severe cases, it may progress to a critical condition characterized by bleeding and vascular damage.² Recent studies have highlighted that the rising incidence of dengue is often associated with environmental factors, including changes in rainfall patterns and temperature, which influence the distribution and density of mosquito populations.^{3,4} public awareness and understanding of the dengue vector and appropriate preventive measures are crucial in disease control.⁵ Efforts to reduce dengue incidence require an integrated approach encompassing vector surveillance, community education, and environmental management.⁶

Dengue Hemorrhagic Fever (DHF) continues to increase in various regions, including in big cities, which shows an alarming global trend. In Indonesia. Data from the last five decades shows an increase in the incidence of dengue fever with increasingly widespread distribution in urban areas due to urbanization and climate change, which expands the habitat of the *Aedes aegypti* mosquito.^{7,8} Research shows that climatic conditions such as high temperatures and erratic rainfall contribute significantly to the increase in dengue cases, as observed in several countries along the Belt and Road route.^{9,10} In big cities such as Jakarta, high population density and environmental conditions that support mosquito breeding are additional factors that worsen the situation.^{11,12} With the increasing incidence of dengue fever, more effective and sustainable prevention efforts are needed, including environmental management, public education, and the development of public health strategies adaptive to climate change.¹³ These findings emphasize the importance of an integrated approach in dengue control to minimize future health and economic impacts.¹⁴

Dengue Hemorrhagic Fever (DHF) continues to rise globally, including in major cities, indicating an alarming trend. In Indonesia, data from the past five decades shows

a consistent increase in dengue cases, with an increasingly widespread distribution in urban areas driven by urbanization and climate change, which expand the habitat of the *Aedes aegypti* mosquito.^{7,8} Research highlights that climatic factors, such as high temperatures and erratic rainfall, significantly contribute to the rise in dengue cases, as observed in several countries along the Belt and Road route.^{9,10} In metropolitan areas like Jakarta, high population density and environmental conditions conducive to mosquito breeding exacerbate the situation.^{11,12} Given the rising incidence of dengue fever, more effective and sustainable prevention efforts are urgently needed. These include environmental management, public education, and the development of adaptive public health strategies to address climate change.¹³ Such findings underscore the importance of an integrated approach to dengue control to mitigate future health and economic impacts.¹⁴

Mosquito vector control is one of the main strategies for preventing DHF and involves various methods such as fogging, the use of insecticides, and environmental management.¹⁵⁻¹⁷ Widespread and targeted use of insecticides can suppress adult mosquito populations and reduce disease transmission, although it must be balanced with efforts to prevent insecticide resistance.¹⁸ Another method that is gaining increasing attention is biological control, including the use of *Wolbachia* bacteria, which can reduce the ability of mosquitoes to transmit the dengue virus.¹⁵ Additionally, odor-based strategies, such as the use of attractants that attract mosquitoes to special traps, have also shown potential as part of integrated vector management.¹⁹ Environmental management, such as eliminating mosquito breeding sites through draining and cleaning water containers, remains a key component in practical control efforts.²⁰ These approaches are supposed to be integrated, significantly reducing the risk of dengue transmission in endemic areas.

The 1 House 1 Larva Monitoring (G1R1J) is a community initiative that aims to empower the community to control the development of mosquitoes by involving every household as a larva monitoring agent. This program is based on the concept that every household can contribute to preventing DHF by actively monitoring and managing mosquito breeding sites around them.²¹ This initiative prioritizes community participation in fighting the spread of dengue fever through education and empowerment, where each family is trained to identify and destroy mosquito breeding sites. Studies show that this approach not only increases public awareness about the importance of dengue prevention. However, it also significantly reduces vector mosquito populations in areas where this program is implemented.⁷ Implementing the G1R1J program in various regions has proven its effectiveness in reducing dengue cases and developing a better health culture in the community.²² This program exemplifies how community-based interventions can be integral to a sustainable and efficient vector control strategy.^{22,23}

The G1R1J program is a community-based initiative to empower households to control mosquito populations by

actively engaging each family as a larva monitoring agent. This program operates on the premise that every household can contribute to preventing DHF by monitoring and managing mosquito breeding sites in their surroundings.²¹ Central to this initiative is the emphasis on community participation in combating the spread of dengue fever through education and empowerment, wherein families are trained to identify and eliminate mosquito breeding sites. Studies indicate that this approach raises public awareness about the importance of dengue prevention and significantly reduces vector mosquito populations in areas where the program is implemented.²¹ Implementing the G1R1J program in various regions has effectively reduced dengue cases and fostered healthier community behaviors.²² This initiative exemplifies how community-driven interventions can be vital to sustainable and efficient vector control strategies.^{23,24}

This research is focused on evaluating the effectiveness of the G1R1J program in reducing the incidence of DHF in endemic areas. This research aims to provide empirical evidence supporting this program's implementation in other affected areas. So that it can be used as a broader prevention model. Previous studies have shown that this community-based approach involves the active participation of residents in monitoring and eliminating mosquito breeding sites. It can reduce vector populations and significantly reduce the dengue incidence rate.²¹ The success of G1R1J in several regions in Indonesia has provided essential insights into how community empowerment can contribute to sustainable disease control efforts.^{7,25} Thus, this research not only seeks to assess the direct impact of the G1R1J program. But also, to identify success factors that can be adopted across different geographic and social contexts.⁹ It is hoped that the results of this research can become the basis for developing more effective and evidence-based public health policies in dealing with dengue fever.

This research is vital because Pariaman City recorded the highest DHF incidence in West Sumatra Province. Data shows that the distribution of dengue fever cases in urban areas reaches 65%, while in rural areas, it is around 35%. The school-age group (<12 years) accounts for 40-50% of cases. The high prevalence of dengue fever in this region, especially among children, demands effective and targeted interventions. The G1R1J as a community empowerment program is relevant for implementing and evaluating its effectiveness in these local conditions. The findings from this research are significant because they focus on solutions that touch the root of the problem-namely, controlling mosquito vectors in the home environment by reducing mosquito populations through monitoring and eliminating breeding sites. This program has the potential to reduce the incidence of dengue fever significantly.

Therefore, this research aimed to explore the effectiveness of the G1R1J program in reducing the incidence of dengue fever in endemic areas, especially in Pariaman City. Researchers will compare the results in the intervention area (Puskesmas Kota Pariaman Working Area) with the control area (Puskesmas Marunggi Working

Area) to evaluate the impact of the program on mosquito populations and the incidence of dengue fever. This research also aims to identify factors that influence the program's success and obstacles to its implementation by providing empirical evidence. Hopefully, this research can become the basis for developing more effective and evidence-based public health policies and supporting program replication in other regions with similar conditions.

Based on initial data analysis. This research proposes several hypotheses. First, it is hypothesized that implementing G1R1J activities without Larva-monitoring students' support will have a higher level of vector density. Greater population mobility, more mosquito breeding sites, and lower vector surveillance activities compared to G1R1J activities equipped with Larva-monitoring students. Second. The larvae-free rate in areas that implement G1R1J plus Larva-monitoring students is estimated to be higher than in areas that only implement G1R1J by Larva Monitoring cadres. Third. It is hypothesized that increasing community knowledge will have a positive influence. Attitudes and actions after implementing G1R1J plus Larva-monitoring students on reducing vector density (CI reduction) in Pariaman City in 2023. Fourth, physical environmental and climate factors included air temperature and humidity, rainfall, mosquito breeding areas, population density, and population mobility. The presence of trees and vector surveillance activities after implementing G1R1J plus Larva-monitoring students are also hypothesized to contribute significantly to reducing vector density (CI reduction) in Pariaman City in 2023.

Materials and methods

Research design and population

This research uses a comparative design to compare the effectiveness of the G1R1J program on the incidence of DHF in two dengue-endemic areas in Pariaman City, West Sumatra. The working areas of the Pariaman City Health Center and Marunggi Health Center were chosen because both regions have already implemented the One House One Larva Monitoring (G1R1J) program. Selecting areas directly relevant to the study program is crucial to enhancing the relevance and validity of the research findings. In community-based epidemiological research, areas with existing programs allow researchers to evaluate the program's implementation, challenges, and effectiveness based on more measurable data. In the intervention areas, in addition to G1R1J cadres, Larva-monitoring students (Sismantik) were also involved in regularly monitoring larvae in households, whereas in the control areas, Sismantik was not involved. The research population consisted of housewives who had received G1R1J training and had children who were in grade 4 and grade 5 of elementary school. These inclusion criteria ensure the data's relevance and consistency and support the program's sustainability by integrating children's education and parental participation. The selection of children in grades 4 and 5 reflects an evidence-based approach that considers cognitive, social, and operational

aspects, aligning with international research standards emphasizing contextual relevance and feasibility in implementing community-based studies. The total population consisted of participants divided into two groups based on region. All had undergone the G1R1J program.

This study aimed to assess the reduction in larva presence through the container Index (CI) or an increase in the larva-free index (ABI) and the decrease in DHF cases. Before the intervention, the number of DHF cases in the intervention area (Pariaman Health Center) was 28, while the control area (Marunggi Health Center) recorded 19 cases. After the intervention, the number of DHF cases in the intervention area decreased to 17, whereas the control area experienced an increase, reaching 21 cases.

Inclusion and exclusion criteria

The inclusion criteria for this study were housewives who:

1. Have received G1R1J training.
2. Have a child in grade 4 or grade 5 of elementary school.
3. Willing to participate in research by signing a written consent.

The exclusion criteria included

1. Housewives who relocated from the study area before the research was concluded.
2. Participants who did not complete the G1R1J training program.

Data collection

Data was collected through structured questionnaires and direct interviews. The questionnaire measures knowledge, attitudes, and practices related to dengue prevention and recording reported incidents. Interviews were used to deepen participants' understanding and experiences of the G1R1J program. In addition, secondary data from local health facilities is used to verify dengue case reports.

Data were collected using structured questionnaires and direct interviews. The questionnaires assessed participants' knowledge, attitudes, and practices related to dengue prevention and documented self-reported incidents. Direct interviews were conducted to gain deeper insights into participants' understanding and experiences with the G1R1J program. Additionally, secondary data from local health facilities validated the reported dengue cases. The study initially calculated a sample size of 130 respondents for the intervention group and 130 for the control group, resulting in 260 respondents. However, some samples were excluded for not meeting the criteria,

leaving 126 respondents in the intervention group and 127 in the control group. The sampling method used was purposive sampling, where regions (sub-districts/villages) were selected based on their status as endemic areas for DHF.

Data analysis

Data analysis was carried out using the chi-square test to identify differences in the incidence of dengue fever between the two regions. Odds ratio (OR) and 95% confidence interval (CI) were used to assess the relative risk of dengue fever associated with demographic and behavioral variables. All analyses were carried out using SPSS Version 23 statistical software-efforts to Reduce Bias to reduce potential bias in this study. Several steps have been taken with Randomization: Participants were selected randomly to minimize selection bias. Blinding: Researchers who collect and analyze data do not know the participant's origin group to avoid analytical bias.

Data Validation

Data collected from questionnaires and interviews was verified with secondary data from health facilities to ensure the accuracy of dengue incident reports.

Efforts to reduce bias

To minimize potential bias, several measures were implemented

1. *Randomization*: Participants were randomly selected to reduce selection bias.
2. *Blinding*: Data collectors and analysts were blinded to the participants' group assignments to avoid analytical bias.
3. *Data Validation*: Data collected through questionnaires and interviews were cross-verified with secondary data from health facilities to ensure the accuracy of reported dengue cases.

Results

Based on the research results, the following is the demographic information of the respondents based on the variables used in the distributed questionnaire. Based on univariate data (Table 1), this study involved 253 participants divided into two groups: intervention (126 participants or 49.8%) and control (127 participants or 50.2%). Most of the participants were under 45 years old (75.1%). While the rest were over 45 years old (24.9%). This distribution indicates that most of the study population is in the productive age range, which may impact activity patterns and exposure to dengue risk factors.

Table 1. Social demographic variables.

Variables	Category	N (%) (Total 253)
Group	Intervention	126 (49.8)
	Control	127 (50.2)
Age	(≤45 years old)	190 (75.1)
	(>45 years old)	63 (24.9)
Level of education	High (high school>)	127 (50.2)
	Low (elementary/middle school)	126 (49.8)
	General	92 (45.5)
Work	PNS/TNI/Polri/BUMN/BUMD	15 (5.9)
	Private employees	3 (1.2)
	Self-employed	24 (9.5)
	Fisherman	3 (1.2)
	Laborer	5 (2.0)
	Not working/IRT	195 (77.1)
	Other	8 (3.2)
Type of house floor	ceramic/tile/marble/cement	213 (84.2)
	Cracked stucco cement	40 (15.8)
Types of house walls	Wall	186 (73.5)
	Wood/board/plywood	65 (25.7)
	Bamboo	2 (8)
Type of ceiling/ceiling of the house	Concrete	19 (45.5)
	Gypsum/asbestos/GRC board	38 (15.0)
	Plywood	166 (65.6)
	Banbu Woven	29 (11.5)
	There isn't any	1 (4)
Fogging has been done.	Yes	62 (24.5)
	No	191 (75.5)
Have you ever used insecticides at home?	Yes	15 (5.9)
	No	238 (94.1)
The presence of houses in slum areas	Yes	18 (7.1)
	No	235 (92.9)
Mobility	Low risk	87 (34.4)
	High risk	166 (65.6)
Trees	There are no trees	106 (41.9)
	There are trees ≥5 m	147 (58.1)
Reporting	Full report	48 (19.0)
	Incomplete report	205 (81.0)
Number of House Occupants	>5 people	99 (39.1)
	≤5	153 (60.5)
	6	1 (4)
Condition of the bedroom	No risk	221 (87.4)
	Risky	32 (12.6)
Condition of kitchen room	No risk	209 (82.6)
	Risky	44 (17.4)
Condition of family room	No risk	214 (84.6)
	Risky	39 (15.4)

Table 1. Social demographic variables (*continued*).

Variables	Category	N (%) (Total 253)
Knowledge	Low	71 (28.1)
	High	182 (71.9)
Attitude	Positive	106 (41.9)
	Negative	147 (58.1)
Action	Not enough	115 (45.5)
	Good	138 (54.5)
3M	Not enough	95 (37.5)
	Good	158 (62.5)
G1R1J Socialization	Not enough	121 (47.8)
	Enough	132 (52.2)
Number of Cases Jan to Dec 2023	≤49/100000 Pddk	221 (87.4)
	>49/100000 Pddk	32 (12.6)

Social and economic characteristics

The educational level of the participants was quite balanced, with 50.2% having higher education (high school or above) and 49.8% having low education (elementary/middle school). Regarding employment, most participants did not work or were housewives (77.1%). While only a small portion worked as civil servants or in the private sector. This data can influence access to information and resources for dengue prevention and participation rates in health programs.

Home environmental conditions

Most participant homes had floors made from ceramic, tile, marble, or cement (84.2%). Only 15.8% use cement floors with cracked plaster. The type of house wall is dominated by walls (73.5%). While the use of wood/board/plywood is 25.7%. And bamboo is only 0.8%. This condition shows variability in building quality, which can influence the potential development of dengue vector mosquitoes.

Dengue prevention practices

Only 24.5% of participant homes had ever been fogged, and 5.9% had used insecticides. This indicates a low adoption of insecticide-based dengue prevention methods in the community. Most homes were also in areas not considered slums (92.9%), which may reduce the risk of disease transmission.

Social and cognitive factors

Participants' knowledge about dengue fever was relatively high, with 71% having adequate knowledge. Positive attitudes towards dengue prevention were recorded in 106 participants (41.9%). Meanwhile, 147 participants (58.1%) showed negative attitudes. Preventive actions such as 3M (covering, draining, burying) also show sufficient awareness, although some are still less than optimal in practice (95 people). The outreach regarding the "1 house 1 mosquito larvae monitor movement" program was quite successful, with more than half of the participants receiving adequate outreach (132 participants).

From Table 2, the bivariate analysis results show a significant relationship between several environmental and social factors and the incidence of DHF in the study area. One of the main findings was the risky condition of the bedroom and kitchen. This was correlated with an increase in the incidence of dengue fever. In the group with risky bedroom conditions, no cases of dengue fever were identified, while in the group with risky kitchen conditions, 15.3 experienced dengue fever, with a *p* value of 0.011 and an odds ratio (OR) of 0.87. This shows that good environmental conditions play an essential role in preventing dengue fever. Considering that rooms not at risk tend to have a lower incidence (Table 3).

Table 2. Analysis of the effectiveness of dengue control through G1R1J and system integration.

Variable	Dengue fever				p value	OR (95%CI)
	>49/100.000		≤49/100.000			
	N	(%)	(n)	(%)		
Age						
< 45 Years	23	12.1	167	87.9	0.816	0.826 (0.361-1.894)
>45 Years	9	14.3	54	85.7		
Work						
PNS/TNI/Polri/BUMN/BUMD	0	0.0	15	100.0	0.313	-
Private employees	0	0.0	3	100.0		
Self-employed	6	25.0	18	75.0		
Fisherman	0	0.0	3	100.0		
Laborer	0	0.0	5	100.0		
Not Working/IRT	25	12.8	170	87.2		
Other	1	12.5	7	87.5		
Education						
Tall	13	10.2	114	89.8	0.332	0.642 (0.302-1.364)
Low	19	15.1	107	84.9		
Number of house occupants						
>5 people	15	14.3	85	85.7	0.596	-
<=5	17	11.1	136	88.9		
Condition of the bedroom						
No Risk	32	15.0	189	85.0	0.044	0.855 (0.810-0.903)
Risky	0	0.0	32	100.0		
Kitchen condition						
No Risk	32	15.3	177	84.7	0.011	0.87 (0.799-0.897)
Risky	0	0	44	38.4		
Condition of family room						
No Risk	32	15.0	182	85.0	0.02	0.850 (0.804-0.900)
Risky	0	0	39	100		
Type of house floor						
Ceramic/tile/marble/cement	20	9.4	193	90.6	0.001	0.242 (0.107-0.548)
Cracked stucco cement	12	30.0	28	70.0		
Types of house walls						
Wall	19	10.2	167	89.8	0.107	-
Wood/board/plywood	13	20.0	52	80.0		
Bamboo	0	0.0	2	100.0		
Type of ceiling/ceiling of the house						
Concrete	2	10.5	17	89.5	0.129	-
Gypsum/asbestos/GRC board	5	13.2	33	86.8		
Plywood	21	12.7	145	87.3		
Woven bamboo	3	12.7	26	87.3		
There isn't any	1	100.0	0	0.0		
Have you ever done fogging at home?					0.772	1.240 (0.541-2.845)
Have you ever done fogging at home?	9	14.5	53	85.5		
Never do fogging at home	23	12.0	168	88.0		

Table 2. Analysis of the effectiveness of dengue control through G1R1J and system integration (*continued*).

Variable	Dengue fever				p value	OR (95%CI)
	>49/100.000		≤49/100.000			
	N	(%)	(n)	(%)		
Have you ever used insecticides at home?						
Have you ever used insecticides at home?	5	33.3	10	66.7	0.037	3.907 (1.242-12.289)
Never use insecticides at home	27	11.3	211	88.7		
The existence of houses in slum areas						
the presence of houses in slum areas	4	22.2	14	77.8	0.368	2.112 (0.650-6.869)
Not in the home area	28	11.9	207	88.1		
Mobility						
Low risk	26	29.9	61	70.1	0.000	11.366 (4.460-28.964)
High risk	6	3.6	160	96.4		
Water reservoir trees						
There are no trees	24	22.6	82	77.4	0.000	5.085 (2.184-11.844)
There are trees ≥5 m	8	5.4	139	94.6		
Reporting						
Complete report	22	45.8	26	54.2	0.000	16.500 (7.037-38.688)
Incomplete report	10	4.9	195	95.1		
The presence of larvae in landfill						
No	11	7.3	140	92.7	0.000	-
There are no larvae in the landfill	20	29.0	49	71.0		
There are larvae in the landfill	1	3.1	31	96.9		
Attitude						
Positive	21	19.8	85	80.2	0.007	3.055 (1.403-6.651)
Negative	11	7.5	136	92.5		
Knowledge						
Low	11	15.5	60	84.5	0.522	1.406 (0.640-3.089)
High	21	11.5	161	88.5		
Action						
Less action	16	13.9	99	86.1	0.717	1.232 (0.587-2.5880)
Good action	16	11.6	122	88.4		
3M						
Not enough	14	14.7	81	85.3	0.562	1.344 (0.635-2.846)
Good	18	14.7	140	85.3		
G1R1J socialization						
Not enough	8	6.6	113	93.4	0.010	0.319 (0.137-0.740)
Enough	24	18.2	108	81.8		

Table 3. Full model.

Variables	B	p value	OR	95% of CI for OR	
				Lower	Upper
Age	0.36	0.591	1.44	0.37	5.56
Education	-0.44	0.479	0.63	0.18	2.20
Work	0.28	0.182	1.32	0.87	2.01
Type of house floor	2.20	0.005	9.08	1.93	42.60
Types of house walls	-0.32	0.647	0.72	0.18	2.88
Type of ceiling/ceiling of the house	0.29	0.364	1.33	0.71	2.49
Have you ever done fogging at home	-0.24	0.725	0.78	0.19	3.10
Have you ever used insecticides at home	-1.85	0.055	0.15	0.02	1.04
The existence of houses in slum areas	-0.12	0.894	0.88	0.14	5.26
Mobility	-2.16	0.001	0.11	0.03	0.43
The existence of trees that collect water	-2.23	0.003	0.10	0.02	0.47
Field survey	-3.68	0.000	0.02	0.006	0.10
Knowledge	-0.08	0.894	0.91	0.25	3.32
Attitude	-2.34	0.001	0.09	0.02	0.39
Action	0.69	0.365	2.00	0.44	9.04
3M+ activities	-0.62	0.396	0.53	0.12	2.26
1 house 1 larva monitor socialization	0.20	0.750	1.23	0.34	4.41

The final model results were obtained after obtaining the full results from the modeling using SPSS analysis. The following table shows the final model results.

In this study, multivariate analysis showed that several variables significantly influenced the presence of the *Aedes aegypti* mosquito vector in residential environments. The type of house floor is one of the most influential factors, with a $p=0.002$ and an OR of 7.88 (Table 4). This suggests that certain types of flooring are due to different materials or hygiene conditions. This may increase the risk of vector presence. The use of insecticides at home, although only

marginally significant ($p=0.05$), shows a protective effect with an OR value of 0.21. This means that homes that use insecticides have a lower risk of becoming mosquito breeding sites. Population mobility was also found to have a significant effect on vector presence. With $p=0.001$ and OR 0.13. Indicating that lower mobility correlates with more effective vector control. The presence of water-bearing trees was also a significant risk factor, with $p=0.005$ and OR 0.17. Indicating that these trees may provide a potential habitat for mosquitoes to breed.

Table 4. Final model.

Variable	B	Sig.	OR	95 % CI for OR	
				Lower	Upper
Type of house floor	2.06	0.002	7.88	2.19	28.32
Have you ever used insecticides at home?	-1.56	0.05	0.21	0.04	1.00
Mobility	-1.99	0.001	0.13	0.04	0.45
The existence of trees that collect water	-1.75	0.005	0.17	0.05	0.59
Field survey	-3.35	0.000	0.03	0.01	0.12
Attitude	-1.86	0.002	0.15	0.04	0.51

Field survey results provide additional support for these findings. By showing a highly significant effect on vector control. With $p=0.000$ and OR 0.03. This indicates that comprehensive field-based interventions can significantly reduce the risk of vector presence. Community attitudes were also crucial in vector control, with $p=0.002$ and OR 0.15. Indicating that positive attitudes toward vector control practices can increase program

effectiveness. An unexpected finding in this study was the effect of insecticide use. Although marginally significant. It shows the potential for more effective vector control if its use can be optimized.

This research emphasizes the importance of a multifactorial approach to controlling dengue vectors, including physical factors in the home, community behavior, and structured field-based interventions. These

findings can be used to develop more comprehensive and effective vector control strategies in the future.

Discussion

The interventions in the G1R1J program were implemented by integrating larva-monitoring students and traditional larva-monitor cadres as part of a community-based dengue control strategy. The evaluation involved a comparative study design conducted in two DHF-endemic areas of Pariaman City, West Sumatra. The study measured the incidence of dengue cases and the density of *Aedes aegypti* mosquitoes, using statistical analysis to assess the program's effectiveness.

Key metrics included the presence of mosquito larvae (as a direct indicator of vector density) and community attitudes toward vector control. Data collection methods likely involved field observations, surveys, and interviews to gauge household participation and attitudes. Statistical tools were used to compare outcomes between the student-integrated and cadre-only approaches, with significant results ($p=0.000$ for vector reduction and $p=0.002$ for community attitude impact) supporting the program's effectiveness. By explicitly detailing these methods, the discussion can better demonstrate the study's scientific foundation and provide a replicable framework for similar interventions.

The results of this study confirm the initial hypothesis that implementing G1R1J without larva-monitoring students integration has a higher mosquito vector density. The low intensity of vector surveillance and high population mobility support this condition. Which worsens the situation.²⁶ In contrast, the G1R1J plus larva-monitoring students intervention effectively identified and eliminated mosquito breeding sites and increased surveillance activities. This aligns with vector control theory, which states that comprehensive and structured interventions are more effective in reducing vector populations than partial approaches.²⁷ Therefore, Integrating the larva-monitoring students' program in G1R1J is the right step to increase the effectiveness of dengue control in endemic areas.

The finding that larvae-free rates were higher in areas that implemented G1R1J plus larva-monitoring students compared to places that only relied on larva-monitoring cadres underscores the importance of advanced technology and methodology in vector surveillance.^{21,28} The larva-monitoring students program, with intensive training and technological support. It has proven more effective in detecting and eliminating mosquito breeding sites. This is based on expert opinion, which states that using technology in public health can increase the accuracy and efficiency of intervention.^{13,29} Therefore, the results of this study strengthen the argument that the use of advanced technology and methodology is essential in a comprehensive dengue vector control program.

Local environmental and cultural factors are crucial in determining the scalability and applicability of the findings from the G1R1J program. Environmental factors such as climate, urbanization, water storage practices, and waste

management significantly influence mosquito breeding habitats. For instance, regions with higher rainfall or poor drainage systems may require adaptations to the program to address increased mosquito proliferation. Additionally, the availability of resources, such as larvicide, monitoring tools, and trained personnel, can vary across regions, impacting program implementation.

Cultural factors, including community attitudes toward health interventions, trust in government programs, and existing practices for mosquito control, also affect scalability. In some communities, resistance to external interventions or a lack of awareness about dengue prevention may hinder program adoption. Conversely, communities with strong social cohesion and a tradition of collective action may more readily embrace the program.

Tailoring interventions to local contexts is essential to ensure the program's broader applicability. This may involve modifying educational materials to align with local languages and cultural norms, addressing region-specific mosquito breeding sites, and involving community leaders to foster trust and participation. These adjustments would enhance the program's effectiveness and sustainability in diverse endemic areas.

The enhanced G1R1J + larva-monitoring student intervention showed a statistically significant impact, with a p value 0.000 and an odds ratio (OR) of 0.03. This indicates that households supported by student involvement were substantially less likely to have mosquito larvae than those relying solely on larva-monitoring cadres. The study also revealed that integrating students positively influenced community attitudes toward vector control, as evidenced by a p value of 0.002 and an OR of 0.15. This suggests that the involvement of students not only improved participation but also fostered more proactive and sustained mosquito control practices among households.

Multivariate analysis shows that attitudes after the implementation of G1R1J plus Larva-monitoring students have a significant influence on reducing vector density. This indicates that public education and increasing awareness play an essential role in the success of vector control programs.^{11,27} According to behavior change theory. Positive knowledge and attitudes encourage practical actions. Which can ultimately reduce the risk of disease transmission.^{14,30} Thus. These findings emphasize the need to focus on vector control programs' educational and behavioral change components.

Physical environmental and climatic factors have also been proven to significantly influence the presence of mosquito vectors. as found in this study. Variables such as floor type, insecticide use. and the presence of water-bearing trees provide a clear picture of how environmental conditions influence the ecology of the *Aedes aegypti* mosquito.²⁴ This supports the view that vector control interventions must consider local environmental factors to achieve maximum results.^{9,31} Thus, this research underlines the importance of a multifactorial approach that includes the physical condition of the house and the surrounding environment in dengue control strategies.

The finding that vector surveillance and community

attitudes are essential factors in reducing vector density strengthens the view that a community-based approach is the key to success.^{19,23} Effective surveillance enables early detection and rapid response to increase vector populations. Meanwhile, positive public attitudes increase participation in control activities. This is because the literature shows that public health interventions involving active community participation are more sustainable and effective.²⁹ In conclusion, this research confirms that an approach that integrates surveillance, education and community participation is key to effective and sustainable vector control.

To avoid all the gaps or problems identified in this research. It is recommended that mosquito vector control programs. G1R1J should be integrated with more sophisticated monitoring systems such as Larva-monitoring students. This integration will increase the effectiveness of vector surveillance and enable more efficient detection and elimination of mosquito breeding sites. In addition, increasing public education and awareness about the importance of vector control, dengue prevention practices and environmental factors such as the type of flooring must be prioritized. Using insecticides and water-holding trees must also be considered in control efforts. Ensuring environmental conditions support the prevention of mosquito vectors.

If these gaps or problems have already emerged. There is no need to worry because solutions have been identified in the results section of this research, for example. The use of insecticides that are proven to be effective can be applied more widely to reduce mosquito populations. A multifactorial approach should be used, including community education and environmental monitoring. Community-based interventions can also be adopted to address existing problems. Implementing more stringent and regular vector surveillance is also recommended to ensure potential risks can be appropriately managed by implementing the solutions found. It is hoped that the incidence of dengue fever can be reduced, and public health can be better maintained.

This research concludes that integrating the G1R1J program with larva-monitoring students significantly increases the effectiveness of controlling the *Aedes aegypti* mosquito vector, which is the leading cause of the spread of DHF-environmental factors such as floor type. The use of insecticides, water-holding trees, and social variables such as community knowledge are also significant. Attitudes and actions were proven to have a substantial effect on reducing vector density. Effective vector surveillance and a proactive attitude of the community are the keys to the success of dengue control programs. These findings provide strong empirical evidence for developing more comprehensive and sustainable vector control strategies in endemic areas.

Theoretically, these findings strengthen the concept of an integrated approach involving technology, education, and community participation can effectively control disease vectors. This research adds to the literature on infectious disease epidemiology and vector control, mainly related

to dengue fever. These findings guide policymakers and public health practitioners in designing more effective vector control programs, including the importance of sustainable vector surveillance and community empowerment. Implementation of this strategy can help reduce the incidence of dengue fever. Improve people's quality of life. And reduce the public health burden.

The study acknowledges several potential limitations that may influence the interpretation of its findings. First, reliance on self-reported data from households and students about their participation in larva monitoring activities introduces the possibility of bias, such as overreporting compliance or underreporting lapses. Additionally, sustaining community participation over time poses a significant challenge, as initial enthusiasm may wane without continuous reinforcement or incentives. Environmental factors, such as seasonal variations in rainfall or temperature, could also independently affect mosquito breeding, making it difficult to attribute changes solely to the interventions. Resource constraints, including the need for training, educational materials, and monitoring tools, may limit the feasibility of replicating the G1R1J + larva-monitoring students approach in resource-limited settings. Finally, while the program succeeded in two endemic areas, its scalability to larger or culturally diverse regions remains uncertain. Addressing these limitations in future research through objective data collection, strategies for sustained engagement, and testing in varied contexts could enhance the program's effectiveness and generalizability.

Research limitations

This study has several limitations, including generalizing the findings to other regions with different characteristics. In addition, this study did not explore other factors, such as insecticide resistance and climate change, that may also play a role in the spread of dengue fever; for future research, it is recommended to explore further the effects of additional variables such as mosquito genetics. Use of information technology in surveillance. And environment-based interventions. Further research can also evaluate the long-term effectiveness of the G1R1J plus larva-monitoring students program to ensure its sustainable positive impact.

Conclusion

This research shows that integrating the G1R1J program with larva-monitoring students can effectively reduce the density of the *Aedes aegypti* mosquito vector and the incidence of DHF-environmental factors such as floor type. The use of insecticides, water-holding trees, and social aspects such as community knowledge are also significant. Attitudes and actions contribute significantly to the success of vector control programs. Good surveillance and active community participation are essential in this effort. These findings strongly support developing more comprehensive and sustainable vector control strategies, which can be implemented in other endemic areas. However, this study provides important

insights. Several limitations need to be acknowledged. Such as limitations in generalizing the findings to different regions and a lack of exploration of other factors that may influence the spread of dengue fever. For future research. It is recommended to focus on additional variables, such as insecticide resistance, climate change, and the use of information technology in surveillance. In addition, a long-term evaluation of the effectiveness of the G1R1J+ larva-monitoring students' program is needed to ensure its sustainable positive impact. Thus, future research can further contribute to controlling dengue fever and improving public health.

Ethical approval

The University of Indonesia approved this research Ethics Committee with ethical review number 39/UN2.F10.D11/PPM.00.02/2023. Participants were provided complete information about the study's aims, procedures, and rights. Written informed consent was obtained before participation. Research ethical standards maintain the privacy and confidentiality of participant data. Privacy and confidentiality of participant data were safeguarded through adherence to strict research ethical standards, including secure data storage and limited access. These measures ensured compliance with ethical guidelines and protected participant welfare throughout the study. Protected participant welfare throughout the study.

Author contribution

HR, TYN: Conception and designing of work, data acquisition, and analysis, along with manuscript writing and revision. RD, AM: Conception of work and data acquisition. DS, CAY: Conception of work, data analysis, and data acquisition. All authors critically reviewed the manuscript and gave final approval of the manuscript.

Conflict of interest

The authors declared no conflicts of interest relevant to this manuscript.

Funding

Nil

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Swallowing problems and oral health status among dwelling older adults in the community

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ARTICLE INFO

Article history:

Received 28 October 2024

Accepted as revised 24 January 2025

Available online 29 January 2025

Keywords:

Swallowing problems, older adult, oral health status.

ABSTRACT

Background: Oral health factors affecting oral food processing may comprise swallowing. Swallowing problems are commonly observed in older adults and are associated with various complications such as malnutrition, dehydration, and an increased risk of aspiration pneumonia. Age-related physiological changes in the swallowing mechanism, along with chronic diseases and dry mouth, further elevate the risk of dysphagia in this population.

Objective: This study aimed to investigate the relationships between oral health status and swallowing problems in community-dwelling older adults from Sukhothai Province, Thailand.

Materials and methods: A cross-sectional study was conducted with 297 older adults aged 60 years and above, and a community-based study was conducted among the residents of Sukhothai province. Participants were assessed using the 10-item Eating Assessment Tool (EAT-10) questionnaire to evaluate swallowing problems. In addition, demographic data, underlying diseases, oral health status, and the incidence of dry mouth were recorded. The Thai Mental Status Examination (TMSE) was used to screen for cognitive function. Logistic regression analysis examined the association between these factors and the swallowing problem.

Results: The prevalence of swallowing problems was 48.8%. Swallowing problems were reported significantly with age, particularly in those aged ≥ 70 years, with a higher prevalence of swallowing issues at 84.7%, compared to 24.7% in participants aged 60-69 years (OR=25.56, $p < 0.001$). Dry mouth was also strongly associated with swallowing problems. Participants suffering from dry mouth had higher odds of experiencing swallowing issues (OR=3.31, $p < 0.01$). Underlying diseases further increased the risk of dysphagia (OR=4.41, $p < 0.001$). No significant association was found between swallowing problems and gender, occlusal pairs, or denture use.

Conclusion: 48.8% of community-dwelling older adults suffer from swallowing problems. This study highlights age, underlying diseases, and dry mouth as critical factors contributing to swallowing problems in older adults. Screening for swallowing issues in those aged ≥ 70 years, underlying disease, or dry mouth should be integrated into routine health assessments.

Introduction

The global aging population is a major demographic shift of the 21st century, driven by healthcare, sanitation, and living standards advances. The United Nations Department of Economic and Social Affairs (UN DESA) projects that 2050 the global population aged 65 and over will surpass 1.5 billion.¹ Thailand faces a similar trend; in 2021, 20% of its population was aged 60 and older, with this figure expected to reach 28% by 2030, marking Thailand as one of Southeast Asia's fastest-aging societies.² In 2023,

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doi: 10.12982/JAMS.2025.043

E-ISSN: 2539-6056

the Sukhothai Provincial Statistical Office reported that 23.05% of its population was elderly.

As individuals age, bodily systems decline, and significant economic and social changes create various challenges. This increases the healthcare burden on nations, requiring them to provide more services for the older population.³ A key concern in this demographic is the prevalence of age-related medical conditions, known as geriatric syndromes, which include falls, loss of mobility, urinary incontinence, cognitive impairments, adverse drug reactions, and swallowing difficulties.⁴ Dysphagia, or difficulty swallowing, is common among older adults due to the deterioration of various organs involved in the process, including weakened pharyngeal muscles, reduced saliva production, tooth loss, decreased oral sensation, and slower swallowing reflexes.⁵ It can also arise from comorbidities like stroke, Parkinson's disease, dementia, and cancers of the larynx or esophagus. In older adults, swallowing issues can lead to malnutrition and dehydration, as well as the risk of aspiration of food particles and bacteria, potentially resulting in aspiration pneumonia and even death.⁶

Oral health significantly impacts chewing ability in older adults. Common issues like tooth loss and untreated dental caries often lead to extractions, which correlate with a decline in quality of life and increased oral health problems in this population.⁷ The loss of teeth essential for chewing and conditions like dry mouth can reduce food intake.⁸ Studies show that 59.9% of elderly nursing home elderly experience swallowing problems.⁹ Additionally, Sura *et al.* found that the prevalence of dysphagia among community-dwelling older adults ranges from 5% to 72%.¹⁰

In Thailand, however, research on swallowing problems among community-dwelling older adults and its relationship with oral health remains limited. This data collection study may lead to preventive strategies and interventions for addressing dysphagia among older adults. Screening and referral of elderly individuals with swallowing problems for appropriate treatment can help reduce complications of dysphagia in this population.

This study aimed to investigate the relationship between swallowing problems and oral health status among older adults living in the community.

Material and methods

This cross-sectional study examined the association between swallowing problems and oral health status among older adults living in the community. It received ethical clearance from the Human Research Ethics Committee of the Sukhothai Provincial Public Health Office, Thailand, with the approval codes COA No. 16/2023 and IRB No. 10/2023.

Participants

A participant in this study was selected through systematic sampling to ensure representative inclusion of community-dwelling individuals aged 60 years and older from Bandanlanhoi district, Sukhothai province, Thailand. The target population was clearly defined, and a sampling

interval was calculated to recruit participants from each sub-district proportionately. Every individual from a pre-determined list of eligible participants was selected to maintain sample representativeness. The sample size was determined using a population proportion formula. Individuals with diabetes (uncontrolled diabetes), stroke, dysphagia (diagnosed and under treatment or rehabilitation), Parkinson's disease, or cognitive impairment were excluded from the study. To assess cognitive function, participants completed the Thai Mental Status Examination (TMSE), a cognitive screening tool. Participants were required to have TMSE scores exceeding 23, 17, or 14, depending on their educational background (higher than primary school, primary school, or no formal education, respectively). Only those who met these cognitive criteria and provided informed consent were included in the study. According to the two age groups (60-69 years and >70 years) and with the ratio of male to female 1:1, all data from 297 participants were collected by cross-sectional analysis in a singular evaluation, of which 148 were female, and 149 were male.

Data collection

Using a structured questionnaire, data on variables such as age, gender, height, body weight, underlying health conditions, marital status, living arrangements, education level, occupation, income, and sources of income were collected.

Assessments of swallowing problems

Assessments of swallowing problems screening tool utilized were Thai-version of the Eating Assessment Tool (EAT-10), a 10-item questionnaire designed to screen for oropharyngeal dysphagia. The EAT-10 consists of 10 questions, with a maximum total score of 40 points. Participants with an EAT-10 score of 3 or more were classified as having swallowing problems. The internal consistency of the EAT-10 was found to be 0.96, and the test-retest intra-item correlation coefficient ranged from 0.72 to 0.91.¹¹ The accuracy of the Thai version of the EAT-10 was reported to be high, with sensitivity at 96.9% and specificity at 93.4%.¹²

Oral health status

All participants underwent a comprehensive oral examination conducted by a dentist. The examination assessed the number of teeth, occlusal pairs (categorized as fewer than 4 occlusal pairs or 4 or more occlusal pairs)¹³, wear dentures, and the presence of dry mouth. The Clinical Oral Dryness Score (CODS) was used to evaluate oral dryness. CODS consists of 10 features, each scored as 1 point, yielding a total score ranging from 0 to 10, where a higher score indicates more significant oral dryness. The reproducibility of CODS was confirmed to be excellent, with an intraclass correlation coefficient (ICC) ranging from 0.89 to 0.96.¹⁴ Ethical approval was obtained, and informed consent was secured from all participants before the study.

Data analysis

Descriptive statistics were employed to summarize the general characteristics of the older adults in the study. Continuous variables, such as age, height, and weight, were reported as mean values. Categorical variables, including gender, underlying diseases, marital status, living arrangements, education level, occupation, average monthly income, and sources of income, were presented as percentages.

To assess the prevalence of swallowing problems, the EAT-10 questionnaire was used, and results were also reported as percentages using descriptive statistics.

The relationship between swallowing problems and variables such as gender, age, presence of underlying diseases, occlusal pairs, denture wear, and oral dryness were analyzed using the Chi-square test. The adjusted odds ratio (adjusted OR) was calculated to measure the strength of associations between these factors and

swallowing problems. Statistical significance was set at $p < 0.05$ for all tests.

Result

The study included 297 participants, evenly split by gender (50.2% male, 49.8% female). Most were 60-69 (61.3%), while 38.7% were 70 or older. The mean BMI was 21.51 ± 3.57 . A majority (59.9%) had underlying conditions, mainly hypertension (45.8%) and hyperlipidemia (23.6%). Most were married (93.3%), lived with relatives (90.2%), had completed elementary school (91.2%), and over half (53.9%) were unemployed. About 86.9% had a monthly income below 5,000 THB. Participants averaged 13.67 ± 9.89 natural teeth, with 2.95 ± 3.06 occlusal pairs; 60.9% had fewer than four occlusal pairs, and 20.5% used dentures. Dry mouth was reported by 84.2%, with 72.1% experiencing mild and 12.1% moderate symptoms (Table 1).

Table 1. Demographic data (N=297).

	N	Percent
<i>Gender</i>		
Male	149	50.2
Female	148	49.8
<i>Age</i>		
60-69	182	61.3
≥70	115	38.7
BMI=21.51±3.57		
<i>Underlying disease</i>		
None	119	40.1
Present	178	59.9
Hypertension	136	45.8
Controlled diabetes	27	9.1
Hyperlipidemia	70	23.6
<i>Marriage status</i>		
Single	20	6.7
Marriage	277	93.3
<i>Living characteristics</i>		
Alone	29	9.8
With relative (spouse or other)	268	90.2
<i>Education graduate</i>		
None	26	8.8
Elementary school	271	91.2
<i>Occupation</i>		
None	160	53.9
Agriculture	90	30.3
Employee (Merchant/Government)	47	15.8
<i>Salary</i>		
≤5,000 THB.	258	86.9
>5,000 THB.	39	13.1

Table 1. Demographic data (N=297) (continued).

	N	Percent
Number of natural teeth 13.67±9.89		
Number of occlusal pair 2.95 ±3.06		
Number of dental caries teeth 2.42±3.31		
<i>Occlusal pair teeth</i>		
<4 occlusal pair teeth	181	60.9
≥4 occlusal pair teeth	116	39.1
<i>Denture ware</i>		
Yes	61	20.5
No	236	79.5
<i>Dry mouth</i>		
No	47	15.8
Yes	250	84.2
Mild	214	72.1
Moderate	36	12.1

Table 2 shows the distribution of swallowing problems based on the EAT-10 questionnaire. Most participants had minimal issues: 96.3% did not report weight loss due to swallowing, and 98.7% had no trouble going out for meals. However, 13.5% felt food sticking in their throat, 10.4% coughed while eating, and 4.0% found

swallowing stressful. Swallowing liquids was challenging for 3.4%, while 4.7% found swallowing solids and pills difficult, 4.4% experienced painful swallowing, and 2.4% reported reduced enjoyment of eating due to swallowing issues (Table 2).

Table 2. Number of responders for each item of EAT-10.

Item	No problem (Score 0)	Have problem (Score 1-4)
	N (%)	N (%)
1. My swallowing problems has caused me to lose weight	286 (96.3)	11 (3.7)
2. My swallowing problem interferes with my ability to go out for meal	293 (98.7)	4 (1.3)
3. Swallowing liquids takes extra effort	287 (96.6)	10 (3.4)
4. Swallowing solids takes extra effort	283 (95.3)	14 (4.7)
5. Swallowing pills takes extra effort	283 (95.3)	14 (4.7)
6. Swallowing is painful	284 (95.6)	13 (4.4)
7. The pleasure of eating is affected by my swallowing	290 (97.6)	7 (2.4)
8. When I swallow food sticks in my throat	257 (86.5)	40 (13.5)
9. I cough when I eat	266 (89.6)	31 (10.4)
10. Swallowing is stressful	285 (96.0)	12 (4.0)

The prevalence of swallowing problems was 48.8%. The factors associated with swallowing problems (a total EAT-10 score ≥3) were significantly linked to age, underlying disease, and dry mouth. Participants aged ≥70 years had a substantially higher prevalence of swallowing problems (84.7%) compared to those aged 60-69 years (24.7%), with an adjusted odds ratio (OR) of 25.56 (95% CI: 11.55-48.07, $p<0.001$). Additionally, underlying diseases increased the likelihood of experiencing swallowing problems (59.0%

versus 33.6%, OR=4.41, 95% CI: 2.27-8.57, $p<0.001$). Participants with dry mouth were also more prone to swallowing problems (54.8% versus 27.7%, OR=3.31, 95% CI: 0.14-7.94, $p<0.01$). No significant associations were observed for gender, the number of occlusal pair teeth, or denture use (Table 3). This comprehensive analysis highlights the significance of age, underlying diseases, and dry mouth in contributing to swallowing problems in older adults (Table 3).

Table 3. Factors associated with swallowing problems (Total EAT-10 score 3).

Factor	Swallowing problem		<i>p</i> value	Adjusted OR (95%CI)	<i>p</i> value
	No N (%)	Yes N (%)			
<i>Gender</i>					
Male	77 (51.7)	72 (48.3)	0.908 ^a	1.02 (0.55-1.89)	0.952 ^b
Female	75 (50.7)	73 (49.3)			
<i>Age (year)</i>					
60-69	137 (75.3)	45 (24.7)	<0.001 ^{a*}	25.56 (11.55-48.07)	<0.001 ^{b*}
≥70	15 (15.3)	100 (84.7)			
<i>Underlying disease</i>					
No	79 (66.4)	40 (33.6)	<0.001 ^{a*}	4.41 (2.27-8.57)	<0.001 ^{b*}
Yes	73 (41.0)	105 (59.0)			
<i>Occlusal pair teeth</i>					
<4 occlusal pair teeth	88 (48.6)	93 (51.4)	0.286 ^a	1.04 (0.54-2.00)	0.919 ^b
≥4 occlusal pair teeth	64 (55.2)	52 (44.8)			
<i>Denture ware</i>					
Yes	30 (49.2)	31 (50.8)	0.775 ^a	0.88 (0.39-1.96)	0.752 ^b
No	122 (51.7)	114 (48.3)			
<i>Dry mouth</i>					
No	34 (72.3)	13 (27.7)	0.002 ^{a*}	3.31 (0.14-7.94)	0.007 ^{b*}
Yes	118 (45.2)	132 (54.8)			

Note: a: Chi-square test, **p*<0.05, b: binary logistic regression, **p*<0.05.

Discussion

This study found that the prevalence of swallowing problems among community-dwelling older adults was about 48.8%. Higher than the study in Northeastern Thailand, Thai healthy community-dwelling elders were assessed as about 11%.¹⁵ This study found a higher prevalence than a study of elderly people in the Northeastern region because of the demographic differences. The older adults we studied had higher underlying disease and dry mouth levels. This comprehensive analysis highlights the significance of age, underlying diseases, and dry mouth contributing to swallowing problems in older adults. The study explored the prevalence of swallowing issues among older adults and the factors associated with these difficulties. The results demonstrated that swallowing problems were relatively uncommon for most participants; however, a significant proportion of older adults experienced some difficulty, especially those aged 70 and above. These findings are consistent with previous studies, which have shown that swallowing problems tend to increase with age due to physiological changes in the swallowing mechanism, also known as presbyphagia.¹⁶ In our study, participants aged ≥70 years had an odds ratio of 25.56 for developing swallowing problems compared to those aged 60-69 years (*p*<0.001), highlighting age as a critical risk factor for dysphagia in older adults. In the current study, 84.7% of participants aged ≥70 reported swallowing problems, compared to only 24.7% of those aged 60-69. This may be attributed to the degeneration of the neural and muscular systems involved in swallowing, which is a major cause of dysphagia in older adults. Such changes are commonly associated with aging, leading

to muscle weakness and decreased coordination in swallowing.¹⁶

The findings of this study demonstrate that older adults, mainly those aged 70 and above, are more likely to experience swallowing problems. A previous study also observed a similar trend, highlighting that swallowing issues increase with age, particularly among older adults with comorbidities. The study pointed out that the decline in swallowing ability is closely linked to complications such as malnutrition and respiratory infections, which significantly affect the quality of life of elderly individuals.¹⁷

This study found that underlying disease also emerged as a significant predictor of swallowing problems, consistent with research suggesting that chronic conditions such as hypertension, diabetes, and hyperlipidemia, which were common in our sample, can exacerbate the development of dysphagia.¹⁸ The impact of comorbidities on swallowing function emphasizes the importance of comprehensive health assessments in older adults to identify and manage these risks early.

Our study found that participants with dry mouth were significantly more likely to experience swallowing problems, with 54.8% affected compared to 27.7% without dry mouth. A dry mouth impairs saliva production, which is crucial for moistening and lubricating food during swallowing. This aligns with existing literature that shows a strong association between dry mouth and dysphagia in older populations.¹⁹ Additionally, saliva is essential for swallowing and protecting oral and pharyngeal tissues, preventing infections, and ensuring the smooth passage of food.¹⁷ Consequently, dry mouth increases the risk of complications like choking and malnutrition. Given its high

prevalence in older adults, dry mouth should be closely monitored in geriatric assessments.

This study found no significant association between swallowing problems and gender, occlusal pairs, and denture use. The findings differ from earlier research suggesting denture use could affect swallowing function.²⁰ The lack of association in our sample might be due to the relatively small number of participants using dentures (20.5%), which could have limited the statistical power to detect differences.

In this study, participants experiencing dry mouth were significantly more likely to face swallowing difficulties than those with fewer occlusal pairs. This finding is consistent with previous research, highlighting saliva's pivotal role in the swallowing process. A study by Ship *et al.* emphasized that saliva plays a critical role in lubricating food and facilitating smooth passage through the oropharynx.²¹ Thus, the absence of saliva due to dry mouth is more pressing than dental conditions like reduced occlusal pairs. While sufficient occlusal pairs are vital for efficient mastication, research has shown that even individuals with fewer teeth or occlusal pairs can manage adequate nutrition and oral function if saliva production remains normal.²² In contrast, dry mouth patients are more prone to experiencing discomfort during swallowing, food sticking in the throat, and an increased risk of aspiration, regardless of the number of occlusal pairs.²³ Moreover, dry mouth is linked to systemic factors like medication use, dehydration, and autoimmune diseases, which disproportionately affect older adults. While fewer occlusal pairs can reduce chewing efficiency, it doesn't impact swallowing as directly as dry mouth.²⁴ Turner and Ship confirmed that dry mouth increases dysphagia risk more than dental status by affecting food bolus lubrication and formation, which are essential for safe swallowing.²⁵ Thus, managing dry mouth should be a priority in geriatric care to prevent swallowing problems. Saliva substitutes, hydration strategies, and managing underlying health conditions could significantly reduce dysphagia incidence. Addressing dental issues like occlusal pairs is also essential, though the immediate focus should be on the severe effects of dry mouth on swallowing.

These findings emphasize the importance of regular screening for swallowing problems in older adults to prevent complications and improve their quality of life. This is especially significant in geriatric care, particularly in Thailand, where the older adult population is rapidly increasing. Integrating screening for swallowing problems into routine health assessments is crucial, especially for individuals aged 70 and older or those with chronic diseases and dry mouth. Preventive strategies, such as maintaining optimal oral health and managing underlying medical conditions, are critical for reducing the risk of dysphagia and ensuring better outcomes for this vulnerable population.

The limitation of this study was that it could not be done in the clinical evaluation of swallowing, so there was no confirmatory evidence for swallowing disorder, nor was swallowing pathology and physiology identified.

The questionnaire will be assessed to screen and monitor patients and refer them to a comprehensive clinical evaluation.

Conclusion

The prevalence of swallowing problems in community-dwelling older adults was 48.8%. This study showed the significant role of age, underlying diseases, and dry mouth in contributing to swallowing issues among older adults. The findings reveal that swallowing problems are particularly prevalent in individuals aged 70 and above, with dry mouth being a key risk factor. These results highlight the need for regular screening and early detection of dysphagia, especially in those with dry mouth and chronic health conditions. Incorporating assessments for swallowing problems into routine healthcare for older adults can help prevent complications, improve quality of life, and reduce the risk of serious health issues.

Conflict of Interest

The authors declare no conflicts of interest. The research was conducted independently, with no financial or personal relationships influencing the results.

Ethical approval

The study received ethical approval from the Human Research Ethics Committee of the Sukhothai Provincial Public Health Office, Thailand (COA No. 16/2023, IRB No. 10/2023). All participants gave informed consent, and the study adhered to ethical guidelines.

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Holistic intelligence and smart organization affecting the performance of standard medical laboratory guidelines of medical technologists at community hospitals in Health Region 7

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ARTICLE INFO

Article history:

Received 13 December 2024

Accepted as revised 1 February 2025

Available online 7 February 2024

Keywords:

Holistic intelligence, smart organization, medical laboratory standards, medical technologist, community hospital.

ABSTRACT

Background: Medical laboratories are vital components of healthcare systems in community hospitals, serving as frontline facilities in public healthcare. The development of laboratory quality standards requires comprehensive improvement in personnel and organizational aspects to ensure public confidence and safety in service delivery.

Objective: This cross-sectional descriptive research aimed to study the effects of holistic intelligence and intelligent organization on medical laboratory standard practices among medical technologists in community hospitals within Health Region 7.

Materials and methods: The sample consisted of 187 medical technologists from community hospitals in Health Region 7, selected through stratified random sampling proportionally by province, followed by simple random sampling within each province. Data were collected between November 13-23, 2024, using an online questionnaire validated for content by three experts, with an overall reliability coefficient of 0.988. Data analysis employed descriptive statistics, Pearson's correlation coefficient, and stepwise multiple linear regression.

Results: The results revealed that overall holistic intelligence was at a high level (Mean=4.12, SD=0.45), overall intelligent organization was at a high level (Mean=3.80, SD=0.65), and overall adherence to medical laboratory standards was at a high level (Mean=4.27, SD=0.49). Both holistic intelligence and intelligent organization showed statistically significant moderate positive correlations with medical laboratory standard practices ($r=0.599$, $p<0.001$ and $r=0.557$, $p<0.001$, respectively). Predictive factor analysis identified organizational learning system co-creation ($B=0.266$), moral and ethical intelligence ($B=0.262$), and emotional intelligence ($B=0.213$) as significant predictors, collectively explaining 47.2% of the variance in medical laboratory standard practices ($R^2_{adj}=0.472$).

Conclusion: Based on these findings, relevant agencies should develop organizational learning systems through the establishment of learning centers and mentoring systems, enhance moral and ethical intelligence through training and knowledge exchange, and improve personnel emotional management skills to elevate the quality and safety standards of medical laboratory operations in community hospitals.

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doi: 10.12982/JAMS.2025.044

E-ISSN: 2539-6056

Introduction

The 20-Year National Strategy (2018-2037) encompasses strategic initiatives to create social opportunities and equality, address disparities, and ensure equitable access to quality healthcare services across all regions. It emphasizes a citizen-centric approach, prioritizing

responsive, efficient, and transparent service delivery.¹ Medical laboratories in community hospitals provide essential analytical services and facilitate specimen referrals for specialized testing to accredited external facilities. These laboratories play a crucial role in public health systems, generating vital data for diagnosis, treatment monitoring, and disease surveillance. Furthermore, their findings inform healthcare policy development and public health service planning. Quality and standardization in medical laboratory operations significantly enhance public health service system efficiency and outcomes. The 2019 Medical Laboratory Standards of the Ministry of Public Health comprise 12 critical evaluation domains.² The evolving healthcare landscape demands continuous improvement in medical laboratory standards, particularly in community hospitals serving as frontline healthcare facilities. This study examines how holistic intelligence and competent organization characteristics influence the implementation of medical laboratory standards in community hospitals within Health Region 7. Holistic intelligence represents an integrated approach to professional capability, encompassing multiple dimensions essential for comprehensive performance in modern healthcare settings. Simultaneously, smart organization characteristics reflect organizational capabilities necessary for maintaining high-quality standards in rapidly changing environments.

Recent studies indicate that maintaining medical laboratory standards requires individual competence and organizational support systems. However, the relationship between these factors and laboratory standards implementation, particularly in community hospital settings, remains understudied.

Holistic intelligence is key to achieving operational objectives, encompassing multiple components essential for comprehensive goal attainment. Successful performance requires more than singular competency, necessitating the development of diverse capabilities across six dimensions. First, Emotional intelligence emerges as a fundamental component in healthcare settings, particularly in laboratory environments where precision and stress management are crucial.³ Emotional intelligence significantly influences decision-making accuracy and professional resilience in high-stakes medical environments. Second, moral and ethical Intelligence plays a vital role in medical laboratory operations, where decisions directly impact patient care and safety.⁴ Third, adversity intelligence shows better adaptation to changing protocols and maintains performance standards even under resource constraints.⁵ This capability becomes particularly relevant in community hospital settings where resources may be limited. Fourth, social intelligence contributes to effective knowledge sharing and professional networking, which are crucial elements in maintaining laboratory quality standards.⁶ Fifth, creative intelligence facilitates the development of novel solutions to operational challenges and contributes to continuous quality improvement initiatives.⁷ Lastly, digital intelligence has become increasingly critical in modern

laboratory operations, particularly with the advancement of automated systems and digital record-keeping.⁸ The concept of smart organization has emerged as crucial in the current era of rapid globalization and technological advancement. This organizational paradigm comprises five key dimensions: 1) shared vision creation has been identified as fundamental for aligning organizational efforts toward quality excellence, 2) human resource development significantly influences an organization's ability to maintain high operational standards and adapt to changing requirements, 3) operational process co-creation shows that collaborative process development leads to better implementation outcomes and sustained quality improvements, 4) organizational learning system development demonstrate that robust learning systems contribute significantly to maintaining high laboratory standards and fostering innovation, and 5) organizational culture formation provides the foundation for sustainable quality practices.⁹ Research indicates that strong organizational cultures supporting quality and continuous improvement lead to better long-term outcomes in laboratory operations.

A survey of medical laboratories in Health Region 7, encompassing Kalasin, Khon Kaen, Maha Sarakham, and Roi Et Provinces, revealed 71 community hospitals employing 240 medical technologists.¹⁰ Of these facilities, 23 have received Ministry of Public Health certification,¹¹ 17 have obtained Medical Technology Council quality accreditation,¹² and one has achieved ISO:15189 certification.¹¹ This represents a 57.75% accreditation rate, leaving 42.25% without formal certification, potentially affecting service quality and patient safety. Previous research has identified correlations between emotional intelligence and healthcare personnel performance and between organizational climate and medical technologists' work-life quality.^{13,14} However, no direct studies have examined the relationship between holistic intelligence, smart organization, and medical laboratory standards implementation in this region. This study aims to identify factors that could enhance medical laboratory service standards in Health Region 7 and provide insights for future quality improvement initiatives.

The 20-Year National Strategy (2018-2037) emphasizes creating social opportunities and equality, ensuring equitable access to quality healthcare services across all regions through citizen-centric approaches prioritizing responsive and efficient service delivery.¹ Medical laboratories in community hospitals serve as essential components of public health systems, providing analytical services and facilitating specimen referrals for specialized testing. These laboratories generate vital data for diagnosis, treatment monitoring, and disease surveillance while informing healthcare policy development and public health service planning. Quality and standardization in medical laboratory operations significantly enhance public health service system efficiency and outcomes, with the 2019 Medical Laboratory Standards of the Ministry of Public Health comprising 12 critical evaluation domains.²

Successful laboratory operations require comprehensive

capabilities across multiple dimensions. Holistic intelligence represents a key factor in achieving operational objectives, encompassing six essential components: emotional intelligence,³ focusing on self-awareness and relationship management; moral and ethical intelligence,⁴ ensuring professional integrity and accountability, adversity intelligence,⁵ enabling effective problem-solving under pressure; social intelligence⁶, facilitating teamwork and communication; creative intelligence,⁷ promoting innovative solutions; and digital intelligence,⁸ supporting technological adaptation and data management.

In parallel, the concept of smart organization has emerged as crucial in the current era of rapid technological advancement. This organizational paradigm comprises five key dimensions: shared vision creation, establishing common goals and direction; human resource development, enhancing staff capabilities, operational process co-creation, optimizing workflow efficiency, organizational learning system development, facilitating

knowledge transfer; and organizational culture formation, building sustainable quality practices.⁹

A survey of medical laboratories in Health Region 7, encompassing Kalasin, Khon Kaen, Maha Sarakham, and Roi Et provinces, revealed that among 71 community hospitals employing 240 medical technologists,¹⁰ only 57.75% have achieved various forms of quality certification.^{11,12} Previous research has identified correlations between emotional intelligence and healthcare personnel performance¹³ and between organizational climate and medical technologists' work-life quality.¹⁴ However, no direct studies have examined the relationship between holistic intelligence, smart organization, and medical laboratory standard compliance in this region. This study aims to identify factors that could enhance medical laboratory service standards in Health Region 7 and provide insights for future quality improvement initiatives. Figure 1 displays the conceptual framework.

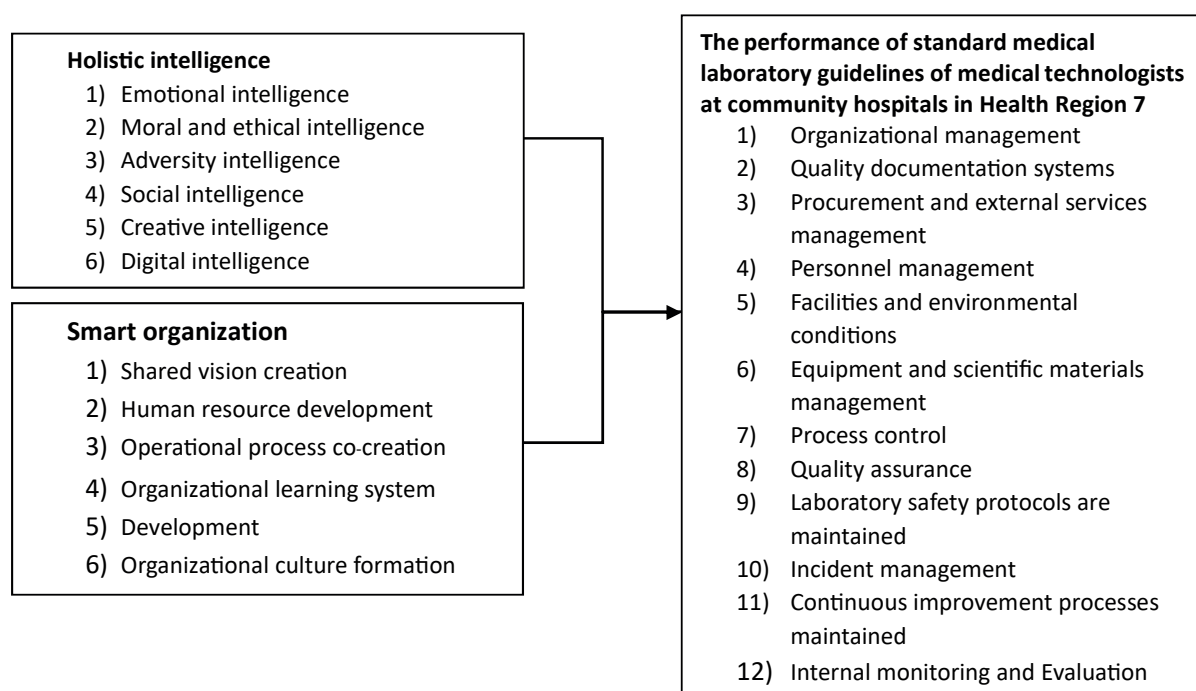


Figure 1. The conceptual framework.

Methodology

Research design and sample

This research employed a cross-sectional descriptive design to examine medical technologists working in community hospitals within Health Region 7. The sample size was determined using Cohen's formula¹⁵ for stepwise multiple linear regression analysis, drawing from a previous study on personal characteristics and leadership aesthetics skills affecting physiotherapists' performance in Health Region 8.¹⁶ This calculation yielded a required sample size of 187 participants.

The study utilized stratified random sampling to ensure proportional representation across the region's provinces.¹⁷ This sampling method was selected to enhance the sample's representativeness by accounting for geographical distribution and organizational characteristics.

The sampling framework encompassed four provinces within Health Region 7: Khon Kaen Province: 54 participants selected from a population of 70 medical technologists; Kalasin Province: 53 participants selected from a population of 69 medical technologists; Roi Et Province: 45 participants selected from a population of 57 medical technologists, Maha Sarakham Province: 35 participants selected from a population of 44 medical technologists

Research instruments

The research utilized a comprehensive questionnaire comprising four sections designed to gather data on medical technologists' characteristics, competencies, and operational practices. The first section focused on personal characteristics, incorporating eight key demographic and

professional items. These included gender, age, marital status, educational level, academic position, income, duration of service in community hospitals within Health Region 7, and participation in medical laboratory standards training. The second section assessed holistic intelligence through 30 items consisting of 1) emotional intelligence,³ 2) moral and ethical intelligence,⁴ 3) adversity intelligence,⁵ 4) social intelligence,⁶ 5) creative intelligence,⁷ and 6) digital intelligence.⁸ The third section evaluated smart organization characteristics using 25 items⁹ consisting of 1) shared vision creation, 2) human resource development, 3) operational process co-creation, 4) organizational learning system development, and 5) organizational culture formation. The fourth and final section contained 60 items specifically addressing medical laboratory standards 1) organizational management, 2) quality documentation systems, 3) procurement and external services management, 4) personnel management, 5) facilities and environmental conditions, 6) equipment and scientific materials management, 7) process control, 8) quality assurance, 9) laboratory safety protocols maintained, 10) incident management, 11) continuous improvement processes maintained, and 12) internal monitoring and Evaluation.⁸

Instrument quality assessment

The development and validation of the research questionnaire followed a rigorous process to ensure both content validity and reliability. The instrument underwent comprehensive quality assessment through multiple stages of evaluation and refinement. Content validity was established through expert review by three qualified professionals. The Item Objective Congruence (IOC) assessment required scores exceeding 0.66 from at least 2 out of 3 qualified professionals to retain each item, confirming the questionnaire's content validity. This validation process ensured that each item appropriately measured its intended construct and aligned with the research objectives. Reliability testing was conducted through a pilot study with 30 medical technologists working in community hospitals within Health Region 8. This region was selected for the pilot study due to its similar operational environment and geographical proximity to the study area. The pilot sample provided valuable insights into the instrument's performance under real-world conditions. Statistical analysis using Cronbach's alpha coefficient demonstrated strong internal consistency across all questionnaire sections. The holistic intelligence section achieved a reliability coefficient of 0.962, while the smart organization section showed a reliability coefficient 0.979. The medical laboratory standards section demonstrated a reliability coefficient of 0.981. The overall questionnaire exhibited exceptional reliability with a coefficient of 0.988.

Data interpretation

The research employed a comprehensive scoring system to evaluate levels of holistic intelligence, smart organization characteristics, and implementation of medical

laboratory standards. The interpretation framework was based on established guidelines for five-level rating scales.¹⁸ The questionnaire utilized a five-point rating scale across all sections, measuring holistic intelligence, smart organization characteristics, and medical laboratory standards among medical technologists in community hospitals within Health Region 7. The rating scale assigned numerical values from 1 to 5, corresponding to performance levels from lowest to highest: highest performance (5 points), high performance (4 points), moderate performance (3 points), low performance (2 points), and lowest performance (1 point). Following standardized interpretation guidelines,¹⁹ the mean scores were categorized into five distinct levels of performance: highest level: mean scores of 4.50-5.00; high level: mean scores of 3.50-4.49, moderate level: mean scores of 2.50-3.49, low level: mean scores of 1.50-2.49, lowest level: mean scores of 1.00-1.49.

For correlation analysis, the interpretation of Pearson's correlation coefficients (r) followed established guidelines with values ranging from -1 to +1.²⁰ The strength of relationships was classified as follows: no correlation ($r=0$), low correlation ($r=\pm 0.01$ to ± 0.30), moderate correlation ($r=\pm 0.31$ to ± 0.70), high correlation ($r=\pm 0.71$ to ± 0.99), and perfect correlation ($r=\pm 1$).

Data collection and analysis

Data was collected through online questionnaires between November 13-23, 2024, after obtaining approval from the Khon Kaen University Human Research Ethics Committee. The process began with authorization from the Faculty of Public Health Dean, followed by coordination with Provincial Public Health Officers in Health Region 7 (Roi Et, Khon Kaen, Kalasin, and Maha Sarakham). Survey links were distributed to randomly selected participants through LINE network groups of provincial Medical Technology Professional Networks. Participant privacy and confidentiality were maintained throughout the data collection process, with a 15-day response window provided.

Data analysis employed both descriptive and inferential statistics. Descriptive statistics included frequency distributions, percentages, means, SD, and range values to summarize data characteristics. Pearson's correlation coefficient examined relationships between variables for inferential statistics, while stepwise multiple linear regression analysis identified significant predictors of medical laboratory standard implementation.

Ethical considerations

This study was approved by the Khon Kaen University Human Research Ethics Committee (Protocol No. HE672178, October 8, 2024). The research protected participant privacy by excluding personally identifiable information and using only aggregated data for analysis. Research data were securely stored, accessible only to the primary researcher, and will be destroyed one year after study completion.

Results

Personal characteristics of medical technologists in community hospitals, Health Region 7

The demographic analysis of medical technologists working in community hospitals within Health Region 7 revealed significant insights into the workforce composition. Female professionals constituted the majority of the sample, with 124 participants (66.31%). The age distribution showed a concentration in the 31-40 age bracket, comprising 102 participants (54.55%). The mean age was 36 years (SD=7.23), ranging from 24 to 60 years. Regarding marital status, single professionals formed the largest group, with 97 participants (51.87%). Educational attainment data showed that most participants held

bachelor's degrees or equivalent qualifications, accounting for 160 individuals (85.56%). Regarding professional classification, medical technologists at the specialist level represented the largest segment, with 101 participants (54.01%). The financial analysis indicated an average monthly income of 34,062 Baht (SD=8,277). Professional experience metrics showed an average tenure of 11.15 years (SD=7.75), ranging from 1 to 41 years in service. Professional development data revealed that 118 participants (63.1%) had received training in medical laboratory standards, with most participants (104 individuals) having attended one to two training sessions as presents in Table 1.

Table 1. Percentage, mean, SD, median, minimum, and maximum personal characteristics of medical technologists in community hospitals, Health Region 7 (N=187).

Personal characteristics	frequency (N=187)	Percentage
Gender		
Male	63	33.69
Female	124	66.31
Age (years)		
21-30	46	24.60
31-40	102	54.55
41-50	30	16.04
51-60	9	4.81
Mean=36.25, SD=7.23, Minimum=24, Maximum=60		
Marital status		
Single	97	51.87
Married	84	44.92
Widowed/divorced/separated	6	3.21
Educational level		
Bachelor's degree or equivalent	160	85.56
Master's degree or higher	27	14.44
Academic position		
Medical technologist	25	13.37
Medical technologist practitioner level	61	32.62
Medical technologist professional level	101	54.01
Income (Baht)		
≤20,000	14	7.49
20,001-30,000	54	28.88
30,001-40,000	88	47.06
≥40,001	31	16.58
Mean=34,061, SD=8,277, Minimum=15,000, Maximum=56,000		
Duration of service in community hospitals with in Health Region 7 (years)		
1-10	108	57.75
11-20	57	30.48
≥21	22	11.16
Mean=11.15, SD=7.75, Median= 10, Minimum=1, Maximum= 41		
Participation in medical laboratory standards training		
No	69	36.90
Yes	118	63.10
Number of times participation in medical laboratory standards training (N=118)		
1-2	104	88.13
3-4	13	11.01
≥5	1	0.85

Holistic intelligence levels in medical laboratory standards operations

The analysis of holistic intelligence among medical technologists in community hospitals within Health Region 7 revealed consistently high levels across multiple dimensions. The overall holistic intelligence score achieved a mean of 4.12 (SD=0.44), indicating strong performance across the measured domains. Moral and ethical intelligence emerged as the most vigorous dimension, with a mean score of 4.31 (SD=0.49), demonstrating medical technologists' robust commitment to ethical practice and professional integrity. Social intelligence was followed closely by a mean score of 4.25 (SD=0.53), reflecting

strong interpersonal capabilities within the professional environment. Emotional intelligence also showed strong results with a mean score of 4.14 (SD=0.50), indicating well-developed emotional awareness and management skills. The assessment of adversity intelligence revealed a mean score of 4.08 (SD=0.51), suggesting effective resilience and problem-solving capabilities among the practitioners. Digital intelligence and creative intelligence, while still achieving high-level ratings, showed slightly lower mean scores of 3.99 (SD=0.54) and 3.98 (SD=0.56), respectively, indicating potential areas for professional development (Table 2).

Table 2. Holistic intelligence levels in medical laboratory standards operations of medical technologists at community hospitals in Health Region 7 (N=187).

Holistic intelligence in medical laboratory standards operations of medical technologists at community hospitals in Health Region 7	Score	SD	Interpretation
1. Emotional intelligence	4.14	0.50	High
2. Moral and ethical intelligence	4.31	0.49	High
3. The assessment of adversity intelligence	4.08	0.51	High
4. Social intelligence	4.25	0.53	High
5. creative intelligence	3.98	0.56	High
6. Digital intelligence	3.99	0.54	High
Holistic intelligence overview	4.12	0.44	High

Smart organization levels in medical laboratory standards operations

The assessment of smart organization characteristics in community hospitals within Health Region 7 demonstrated strong organizational capabilities across multiple dimensions. The overall smart organization score achieved a mean score of 3.80 (SD=0.65), indicating robust institutional frameworks supporting medical laboratory operations. Operational process co-creation emerged as the most vigorous dimension, with a mean score of 3.87 (SD=0.74), reflecting practical collaborative

approaches to developing and implementing laboratory procedures. Organizational culture formation followed closely with a mean score of 3.83 (SD=0.72), suggesting well-established shared values and professional practices within the institutions. Vision creation and alignment showed similarly strong performance with a mean score of 3.79 (SD=0.64), indicating clear strategic direction and purpose. The organizational learning system development achieved a mean score of 3.79 (SD=0.69), while human resource development and support scored 3.73 (SD=0.71), as detailed in Table 3.

Table 3. Smart organization levels in medical laboratory standards operations of medical technologists at community hospitals in Health Region 7 (N=187).

Smart organization in medical laboratory standards operations of medical technologists at community hospitals in Health Region 7	Mean	SD	Interpretation
1. Shared vision creation	3.79	0.64	High
2. Human resource development	3.73	0.71	High
3. Operational process co-creation	3.87	0.74	High
4. Organizational learning system development	3.79	0.69	High
5. Organizational culture formation	3.83	0.72	High
Smart organization overview	3.80	0.65	High

Implementation of medical laboratory standards

The analysis of medical laboratory standards implementation among medical technologists in community hospitals within Health Region 7 revealed strong performance across all operational domains. The overall implementation level achieved a score of 4.28 (SD=0.49), indicating robust adherence to established standards. Organizational management emerged as the highest-performing area, with a mean score of 4.50 (SD=0.51), demonstrating effective administrative practices. Quality documentation systems followed closely with a mean score of 4.41 (SD=0.53), reflecting substantial documentation and record-keeping practices. Laboratory safety protocols maintained high standards with a mean score of 4.36 (SD=0.56), while equipment and scientific materials

management achieved a mean score of 4.35 (SD=0.54). Process control and quality assurance demonstrated consistent performance with identical mean scores 4.30 (SD=0.53 and 0.58, respectively). Procurement and external services management showed strong implementation with a mean score of 4.28 (SD=0.61), followed by internal monitoring and evaluation at 4.26 (SD=0.62). Continuous improvement processes maintained a robust score of 4.22 (SD=0.59). Areas showing relatively lower scores while still maintaining high-performance levels included facilities and environmental conditions (Mean=4.09, SD=0.64), incident management (Mean=4.10, SD=0.70), and personnel management (Mean=4.15, SD=0.54), as detailed in Table 4.

Table 4. Levels in medical laboratory standards operations of medical technologists at community hospitals in Health Region 7 (N=187).

Medical laboratory standards operations of medical technologists at community hospitals in Health Region 7	Mean	SD	Interpretation
1. Organizational management	4.50	0.51	High
2. Quality documentation systems	4.41	0.53	High
3. Procurement and external services management	4.28	0.61	High
4. Personnel management	4.15	0.54	High
5. Facilities and environmental conditions	4.09	0.64	High
6. Equipment and scientific materials management	4.35	0.54	High
7. Process control	4.30	0.53	High
8. Quality assurance	4.30	0.58	High
9. Laboratory safety protocols maintained	4.36	0.56	High
10. Incident management	4.10	0.70	High
11. Continuous improvement processes maintained	4.22	0.59	High
12. Internal monitoring and Evaluation	4.26	0.62	High
Medical laboratory standards overview	4.28	0.49	High

Relationships between holistic intelligence, smart organization, and medical laboratory standards implementation

The analysis revealed significant moderate correlations between both holistic intelligence and smart organization characteristics with medical laboratory standards implementation. The overall holistic intelligence demonstrated a mild positive correlation ($r=0.599$, $p<0.001$), while smart organization showed a similar moderate positive relationship ($r=0.557$, $p<0.001$) with laboratory standards implementation.

Within the holistic intelligence dimensions, social intelligence emerged as the strongest correlate ($r=0.570$, $p<0.001$), closely followed by emotional intelligence ($r=0.569$, $p<0.001$). Moral and ethical intelligence also

substantially correlated ($r=0.537$, $p<0.001$). Adversity intelligence ($r=0.477$, $p<0.001$) and creative intelligence ($r=0.476$, $p<0.001$) demonstrated similar moderate relationships, while digital intelligence showed the lowest, though still significant, correlation ($r=0.450$, $p<0.001$).

Regarding smart organization characteristics, the organizational learning system development showed the most substantial relationship with standards implementation ($r=0.575$, $p<0.001$). Operational process co-creation ($r=0.547$, $p<0.001$) and organizational culture formation ($r=0.540$, $p<0.001$) also demonstrated robust correlations. Vision creation and alignment ($r=0.482$, $p<0.001$) and human resource development ($r=0.416$, $p<0.001$) showed moderate positive relationships, as detailed in Table 5.

Table 5. Pearson's correlation coefficients between holistic intelligence, smart organization, and medical laboratory standards operations of medical technologists at community hospitals in Health Region 7 (N=187).

<i>Holistic intelligence and smart organization</i>	<i>Medical laboratory standards operations</i>		
	<i>Correlation coefficient (r)</i>	<i>p value</i>	<i>Relationship level</i>
Holistic intelligence overview	0.599***	<0.001	moderate
Emotional intelligence	0.569***	<0.001	moderate
Moral and ethical intelligence	0.537***	<0.001	moderate
The assessment of adversity intelligence	0.477***	<0.001	moderate
Social intelligence	0.570***	<0.001	moderate
Creative intelligence	0.476***	<0.001	moderate
Digital intelligence	0.450***	<0.001	moderate
Smart organization overview	0.557***	<0.001	moderate
Shared vision creation	0.482***	<0.001	moderate
Human resource development	0.416***	<0.001	moderate
Operational process co-creation	0.547***	<0.001	moderate
Organizational learning system development	0.575***	<0.001	moderate
Organizational culture formation	0.540***	<0.001	moderate

***Correlation is significant <0.001 level.

Holistic intelligence and smart organization affecting medical laboratory standards implementation of medical technologists at community hospitals in Health Region 7

The study employed stepwise multiple regression analysis to examine the influence of holistic intelligence and smart organization characteristics on medical laboratory standards implementation among medical technologists in community hospitals within Health Region 7. Initial statistical assumptions were verified through rigorous testing procedures. The Kolmogorov-Smirnov test confirmed the normal distribution of subjective data ($p=0.083$), exceeding the established threshold of 0.05. The Durbin-

Watson statistic of 1.541 confirmed the independence of residuals, which falls within the acceptable range of 1.50-2.50. The homoscedasticity assumption was met, indicating consistent variance in prediction errors across all predictor variables. Multicollinearity testing showed no concerning relationships between variables (tolerance=0.574-0.765, variance inflation factor (VIF)=1.307-1.742), meeting the regression analysis requirements where Tolerance should be greater than 0 but not exceeding 1, and VIF should be less than 10. These results confirmed that all statistical assumptions for multiple regression analysis were satisfied. As detailed in Table 6.

Table 6. A stepwise multiple regression analysis examining factors influencing medical laboratory standards implementation among medical technologists in community hospitals within Health Region 7 (N=187).

Factor	B	95%CI		SE	Beta	T	p value	R	R ²	R ² adj	R ² change
		lower	Upper								
The organizational learning system co-creation	0.266	0.181	0.351	0.405	0.376	6.168	<0.001	0.575	0.330	0.327	-
Moral and ethical intelligence	0.262	0.122	0.402	0.366	0.259	3.680	<0.001	0.675	0.456	0.450	0.126
Emotional intelligence	0.213	0.170	0.357	0.358	0.220	2.938	0.004	0.693	0.480	0.472	0.024

Note: Constant 1.255, $F=56.366$, $p<0.001$, $R=0.693$, $R^2=0.480$, $R^2adj=0.472$.

Discussion

The study findings demonstrate high overall adherence to medical laboratory standards among medical technologists (Mean=4.28, SD=0.49), with organizational management and administration emerging as the most substantial domain (Mean=4.49, SD=0.51). This exemplary performance reflects the successful implementation of Hospital Accreditation (HA) quality systems, which have established clear administrative structures and strategic development plans in community hospitals. However, facilities and environmental conditions received the lowest mean score (Mean=4.09, SD=0.64), highlighting significant infrastructural challenges. Community hospitals face constraints in upgrading aging facilities and maintaining appropriate environmental conditions due to budget limitations and increasing service demands. These findings align with previous research.^{13,21,22}

Medical laboratory operations demand highly specialized knowledge and skills, making effective knowledge management systems essential for operational excellence. A robust organizational learning system enables personnel to efficiently access and apply critical knowledge while facilitating valuable knowledge transfer between staff members. This systematic approach to learning supports continuous knowledge development and innovation within the laboratory environment. The dynamic nature of laboratory standards, which evolve continuously with technological advancements, further emphasizes the importance of practical learning systems. These systems enable personnel to monitor standard modifications, adapt procedures promptly, and implement changes efficiently, thereby reducing operational errors and enhancing service quality. Previous research has demonstrated that effective organizational learning in medical settings significantly improves diagnostic accuracy and operational efficiency, particularly in high-precision environments like medical laboratories.²³ The collaborative nature of laboratory work underscores the value of organizational learning systems. These systems foster a shared understanding of operational standards and facilitate the exchange of best practices among team members, leading to comprehensive staff development.

Previous research has identified organizational learning systems as key drivers of medical laboratory standards implementation, enabling superior adaptation to change and maintenance of innovation standards.¹³ Studies have consistently supported these findings. Research has shown that organizations with effective knowledge management systems achieve superior quality development outcomes, particularly in staff skill development and service innovation.²⁴ Additionally, evidence suggests that establishing a learning culture in healthcare organizations is crucial for service quality improvement, enabling continuous professional development and effective technological adaptation.²⁵ The strong correlation between organizational learning systems and laboratory standard implementation indicates that investing in knowledge management infrastructure yields significant operational quality and sustainability returns.

This relationship suggests that medical laboratories should prioritize developing comprehensive learning systems to maintain high standards and adapt to evolving healthcare requirements.

Medical laboratory work's direct impact on patient health and lives underscores the critical importance of moral and ethical intelligence among medical technologists. This foundational aspect of professional competence ensures adherence to operational standards, accountability for analytical results, and unwavering focus on patient welfare. The laboratory environment demands exceptional integrity and reliability in all operations. Medical technologists with well-developed moral and ethical intelligence consistently demonstrate honesty in result reporting, maintain strict patient confidentiality, and adhere rigorously to professional ethics. Their commitment to compassionate care drives continuous quality improvement and maintains high service standards. Research has established strong positive correlations between moral intelligence and ethical work behaviors, directly affecting medical service quality.²⁶ The four fundamental dimensions of moral intelligence—integrity, responsibility, compassion, and forgiveness—align precisely with medical laboratory requirements, where precision, accountability, and patient-centered care are paramount.²⁷

Previous research has demonstrated that moral intelligence correlates significantly with organizational commitment, enhancing operational efficiency and patient satisfaction.²⁸ Professionals exhibiting high moral intelligence consistently show more remarkable dedication to quality improvement and adherence to professional standards. Additional studies have identified positive relationships between administrative skills, work ethics, and intrinsic motivation.²⁹ The results suggest enhanced management capabilities strengthen ethical practice and operational standards. Laboratory professionals regularly encounter situations requiring complex ethical decision-making, from prioritizing urgent tests to managing limited resources and reporting errors. Strong moral and ethical intelligence enables appropriate decision-making that balances ethical principles, procedural accuracy, and patient benefit. This capacity becomes particularly crucial in challenging situations where standard protocols may not provide clear guidance.

In medical laboratory operations, emotional intelligence is critical in maintaining professional standards under high-pressure conditions. Medical technologists face multiple pressures, including high workload volumes, urgent reporting deadlines, and expectations for precise analytical results. High emotional intelligence enables these professionals to effectively manage these pressures while maintaining the focus and attention to detail essential for laboratory work. Previous research demonstrates that healthcare personnel with developed emotional intelligence show improved work efficiency, particularly in service delivery and quality assurance.³⁰ This improvement stems from enhanced stress management capabilities, leading to better concentration and methodical adherence to

established standards. Supporting these findings, previous research reveals that emotional intelligence development enhances communication skills and interprofessional collaboration, critical elements in maintaining laboratory standards through effective coordination and information exchange.³¹ The impact of emotional intelligence extends beyond individual performance. Previous research found that personnel with high emotional intelligence consistently deliver superior service quality with fewer operational errors, attributing this to better emotional regulation and stress management.³² This directly influences adherence to laboratory safety and quality standards. Moreover, research indicates that emotional intelligence correlates strongly with learning ability and self-development capacity, essential qualities for adapting to evolving laboratory standards and continuous quality improvement initiatives.³³

Implementing holistic intelligence and smart organization concepts in medical laboratories encounters several significant challenges that require systematic consideration and strategic solutions. Understanding these challenges and developing appropriate mitigation strategies is crucial for successful implementation. Resource and financial constraints represent fundamental obstacles in implementing quality improvement initiatives. Budget limitations frequently affect the acquisition of new technologies and staff training programs in community hospitals. Research indicates that successful quality improvement initiatives typically require 2-5% of total operating budgets for effective implementation.^{23,24} A phased implementation approach, prioritizing low-cost, high-impact activities and establishing educational institution partnerships, has been identified as an effective strategy for resource optimization.²⁵ Organizational resistance to change presents a significant behavioral challenge in implementation efforts. Studies have documented consistent staff concerns regarding increased workload and systemic changes. Organizational culture transformation typically requires 3-5 years and sustained executive support.^{26,27} Effective change management strategies emphasize comprehensive stakeholder engagement, transparent communication channels, and well-structured incentive systems.²⁸

Personnel expertise limitations significantly impact quality development initiatives. The scarcity of professionals with technical expertise and mentoring capabilities has been identified as a critical implementation barrier.²⁹ Successful approaches include developing multi-tiered mentoring systems, implementing technology-supported learning platforms, and establishing inter-institutional collaboration networks.³⁰ Inter-departmental coordination complexity presents substantial systemic challenges to implementation efforts. Successful implementation requires coordinated efforts across all organizational sectors.³¹ Effective solutions include establishing clear governance structures, implementing regular progress monitoring mechanisms, and developing standardized reporting systems.³² Future research directions should focus on qualitative investigations to understand context-specific

barriers and enabling factors. This includes examining successful implementation cases, evaluating strategy effectiveness, and monitoring the long-term sustainability of quality improvement initiatives.³³ Developing successful medical laboratory standards requires comprehensive planning that addresses resource constraints, change management processes, personnel development strategies, and inter-departmental coordination mechanisms. Understanding these implementation challenges enables organizations to develop context-appropriate strategies that promote efficient and sustainable laboratory standards development.

Based on the research findings, three key factors—organizational learning systems, moral and ethical intelligence, and emotional intelligence—significantly influence the implementation of medical laboratory standards. At the policy level, the Ministry of Public Health should establish learning centers at the regional level and develop mentoring systems to facilitate knowledge transfer among medical technologists. These centers would serve as hubs for operational standard exchange and professional development across community hospitals. Institutions should implement comprehensive knowledge management systems and ethical consultation mechanisms for organizational development. Healthcare facilities should establish emotional support structures and foster an organizational culture that promotes staff well-being. Performance monitoring systems should be developed to evaluate and maintain the implementation of medical laboratory standards, with indicators encompassing learning systems, ethical practice, and emotional management competencies. Professional development initiatives should focus on continuing education programs that enhance laboratory standards implementation. The Medical Technology Council should create clear career paths linked to medical laboratory standards implementation capabilities and increase medical technologists involvement in policy development. These systematic developments at both organizational and professional levels will strengthen medical laboratory standards implementation and enhance healthcare service quality in community hospitals.

At the hospital administration level, organizational learning system development requires systematic implementation. Hospital administrators should establish digital knowledge repositories designed explicitly for medical technologists, containing standardized operating procedures, case studies, and best practices that can be easily accessed and updated. To enhance knowledge transfer and professional development, structured mentoring programs should pair experienced medical technologists with newer staff members. Additionally, hospitals should implement regular professional development programs focusing on moral, ethical, and emotional intelligence through monthly ethics case discussions, emotional intelligence workshops, and professional supervision sessions. Resource allocation should prioritize staff development through dedicated annual budgets for training programs, conference

attendance, and continuing education opportunities.

We recommend establishing Regional Learning Centers within Health Region 7 for policymakers and healthcare authorities to serve as hubs for knowledge exchange, professional development, and quality improvement initiatives. These centers should have simulation facilities, digital learning resources, and expert facilitators to support continuous professional development. A comprehensive standard competency framework should be developed, integrating technical skills with holistic intelligence components to guide career progression and professional development planning. Furthermore, a unified quality monitoring system should be implemented across the region, incorporating traditional quality metrics and newer indicators related to organizational learning and staff development.

Educational and training institutions are crucial in developing comprehensive curricula integrating technical competencies with holistic intelligence development. These programs should include practical modules on emotional intelligence, ethical decision-making, and organizational development, emphasizing case-based learning methods to connect theoretical knowledge with practical application. Professional development courses should have flexible delivery modes, including online and face-to-face components, and precise assessment criteria aligned with workplace requirements. Formal collaborative partnerships between training institutions and hospitals should be established through memoranda of understanding to ensure continuous alignment between educational content and workplace needs.

Through this comprehensive approach, with clearly defined roles for each stakeholder group, we can create a sustainable framework for enhancing medical laboratory standards while developing individual capabilities and organizational effectiveness.

Limitation

This study faced several methodological limitations that warrant consideration. The primary constraint was the cross-sectional design, which collected data simultaneously, limiting our ability to establish causal relationships between variables. While the study revealed significant associations between holistic intelligence, smart organization characteristics, and laboratory standards implementation, the temporal nature of data collection prevents definitive conclusions about cause-and-effect relationships. Another notable limitation concerns our statistical approach using stepwise multiple regression, which may have inherent limitations, including potential overfitting and model instability. While widely used, this method could potentially exclude essential predictor variables and may not fully capture the complex interrelationships among our study variables. The study's geographical scope, confined to Health Region 7's community hospitals, may limit generalizability to other regions.

Additionally, our reliance on self-reported questionnaire

data, though validated and reliable, may be subject to response bias. While employing a cross-sectional design provided valuable insights, it restricted our ability to establish causal relationships between variables, and our regression model's explanatory power ($R^2_{adj}=0.472$) indicated that approximately 52.8% of the variance in medical laboratory standards implementation remains unexplained. Future research would benefit from employing more robust statistical methods, such as hierarchical regression or structural equation modeling (SEM), alongside longitudinal or experimental designs to provide more substantial evidence of relationships between variables and confirm the stability of our findings.

Conclusion

Implementing medical laboratory standards among medical technologists in community hospitals within Health Region 7 is significantly influenced by three main components: collaborative organizational learning system development, moral and ethical intelligence, and emotional intelligence. These components necessitate integrated development across multiple levels.

Research findings support the development of a comprehensive capacity-building system at the policy level through the establishment of essential competency frameworks, integrated care system development, and sustainable resource allocation. At the regional and provincial health levels, emphasis should be placed on developing training curricula, mentoring systems, and efficient information systems. Meanwhile, community hospitals should prioritize the development of context-appropriate care systems and practical guidelines.

Implementation at the organizational level should emphasize personnel participation and strengthening professional networks. Operational strategies should be adapted to align with the resources and capabilities of each area, along with the development of effective monitoring and evaluation systems to ensure continuous operational improvement and growth. This approach will strengthen and sustain medical laboratory standards in Thailand, enabling efficient response to public health needs.

Conflict of interest

The authors declare no potential conflict of interest.

Funding

Nil

Acknowledgements

The authors gratefully acknowledge the faculty members of the Public Health Administration Program, Faculty of Public Health, Khon Kaen University, for their academic guidance. We thank the administrators of the Provincial Public Health Offices in Health Region 7 for facilitating data collection and all participating medical technologists for their valuable contributions—special thanks to Claude AI for assistance with English language editing.

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Biomechanical analysis of movement time and center of pressure during single-leg and double-leg sit-to-stand in healthy adults

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ARTICLE INFO

Article history:

Received 29 October 2024

Accepted as revised 5 February 2025

Available online 8 February 2025

Keywords:

Sit-to-stand, movement time, center of pressure.

ABSTRACT

Background: The sit-to-stand (STS) task is a fundamental movement integral to daily activities and is widely used as a functional test to evaluate lower limb strength, balance, and postural control across various populations. However, limited literature provides biomechanical analysis comparisons between traditional double-leg STS tasks and single-leg STS tasks.

Objective: This study aimed to compare muscle strength, movement times, and center of pressure (COP) variables across three STS conditions: single-leg STS on the dominant limb, single-leg STS on the non-dominant limb, and double-leg STS.

Materials and methods: Twenty healthy participants (10 males and 10 females; 21.60±1.14 years old) participated in a cross-sectional study. Maximal voluntary isometric contraction of the knee extensors and hip abductors was assessed for both the dominant and non-dominant limbs. Participants performed the three STS testing conditions on the Zebris FDM pressure plate. Movement time and COP outcome variables (sway area, total path length, velocity, and path length in anteroposterior and mediolateral directions) were recorded and analyzed across the three STS tasks. A paired t-test was used to compare the means of the primary outcome variables within groups. A one-way repeated measures ANOVA was conducted to assess outcome differences among the testing conditions, with significance set at $p < 0.05$.

Results: Findings indicated no significant difference in knee extensor or hip abductor muscle strength between the dominant and non-dominant limbs. Movement times for the single-leg STS tasks on both limbs were significantly longer than for the double-leg STS task ($p < 0.001$), with no difference between dominant and non-dominant limbs. Additionally, COP variables (sway area, total path length, mean velocity, and mediolateral path length) were significantly lower in the single-leg STS tasks on both limbs compared to the double-leg STS task (all $p < 0.001$), with no differences observed between dominant and non-dominant limbs.

Conclusion: Compared to double-leg STS tasks, single-leg STS tasks are associated with longer movement times and reduced COP measures. These findings provide preliminary reference values for STS tasks and suggest that the single-leg STS may serve as a potentially useful tool for assessing balance impairments and functional mobility. Further research is required to validate its sensitivity in pathological populations.

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doi: 10.12982/JAMS.2025.045

E-ISSN: 2539-6056

Introduction

The sit-to-stand (STS) task is a fundamental movement integral to daily activities and crucial for maintaining functional independence. This task involves transitioning from a seated to a standing position, requiring coordinated activation of trunk and lower limb muscles

while maintaining balance throughout the movement period.^{1,2} As the STS movement is an essential functional task, it is widely utilized as a functional movement test to evaluate lower limb strength, balance, and postural control. Specifically, the STS task is frequently employed in clinical and rehabilitation settings to evaluate functional mobility and monitor disease progression or treatment effectiveness for lower limb injuries and age-related conditions.³

The STS task is a standard component of various biomechanical studies that provides insights into neuromuscular control and movement efficiency.⁴ This is mainly due to the task's reliance on lower limb strength, particularly the core, hip, and knee extensor muscles, which are critical for ensuring task completion and movement smoothness.⁵ Research indicates that biomechanical outcome variables associated with the STS task, such as movement time, power, and acceleration, are closely linked to the strength of these muscle groups, highlighting the importance of core and lower limb function in performing this task efficiently. A study by Alcazar et al.⁶ found a strong correlation between acceleration parameters measured from the 30-second STS test and bilateral lower limb muscle strength. Stagsted et al.⁷ also reported that lower extremity muscle strength, measured through chair rise and leg press tests, was associated with movement time on the 5-times STS test.

A previous study examined the biomechanical differences between double-leg and single-leg STS tasks, particularly in comparing dominant and non-dominant limbs.⁸ Double-leg STS distributes the load evenly across both legs, resulting in more excellent stability and reduced postural sway.⁹ In contrast, single-leg STS tasks pose more significant challenges to balance, particularly on the non-dominant leg, which often exhibits reduced movement control, longer movement times, and more significant displacement of the center of pressure (COP).¹⁰ These differences highlight variations in muscle strength and coordination between the limbs. Specifically, the muscle strength and power disparity between the dominant and non-dominant legs influences task performance.¹¹ This suggests that functional movements that involve the dominant and non-dominant legs may yield different outcomes, as muscle strength in different muscle groups plays a significant role in movement quality during functional tasks. Thus, it is essential to note that differences in muscle strength between the dominant and non-dominant limbs in sagittal plane movements, such as hip and knee flexion/extension or ankle dorsiflexion, and frontal plane movements, such as hip abduction/adduction, may directly affect the STS task.¹²

Based on recent literature, previous studies demonstrated that strength differences, especially in the lower limb, directly impact movement performance, such as the STS task. However, there is limited evidence comparing the biomechanics of single-leg and double-leg STS tasks using movement time and COP outcomes, a commonly used kinetic variable in biomechanics for analyzing body sway, providing insights into an individual's

ability to control body movement and lower limb function,^{13,14} among young adults. Therefore, this study aimed to compare muscle strength, movement times, and COP variables across three STS conditions: single-leg STS on the dominant limb, single-leg STS on the non-dominant limb, and double-leg STS. It is hypothesized that the non-dominant limb would show longer movement times and greater COP displacement than the dominant limb, with both single-leg tasks performing worse than the double-leg STS.

Materials and methods

Study design and participants

A cross-sectional study design with single-session data collection was employed in this study. Participants were recruited through flyers and online platforms. Data were collected at the Biomechanics Laboratory, Department of Physical Therapy, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand. The sample size was calculated using G*Power version 3.1.9.6 (Universität Kiel, Germany), based on a previous study that analyzed differences between two dependent means of the time required to complete a one-repetition single-leg STS.¹⁵ A total of 20 participants was deemed necessary, considering an expected moderate effect size (0.6), an alpha level of 0.05, and a statistical power of 0.80.

Twenty male and female participants were screened and recruited into the study based on predefined inclusion and exclusion criteria (Table 1). The inclusion criteria required healthy participants aged 18 to 25 years, with a normal body mass index (BMI) of 18.5-22.9 and the ability to independently perform the STS movement for both dominant and non-dominant limbs.¹⁶ Exclusion criteria included participants with vision problems, a history of diagnosed neurological disorders such as stroke or brain injury, or other conditions impairing the function of the vestibular, somatosensory, or visual systems. Participants with conditions affecting their ability to stand, walk, or maintain postural balance during single-leg standing and those experiencing pain or lower limb deformities that limit movement were also excluded. All participants were informed about the testing procedures and provided written informed consent before participation. The study was approved by the Research Ethics Committee of the Institutional Review Board (approval number: AMSEC-67EX-001).

Table 1. Characteristics of participants.

	Mean±SD
N	20
Male: female	10:10
Age (years)	21.60±1.14
Height (m)	1.64±0.06
Body weight (kg)	56.31±5.88
Body mass index (kg/m ²)	20.95±1.42

Procedures

The testing session began with participants providing basic demographic information and filling in the Waterloo Footedness Questionnaire (revised version) to indicate the dominant and non-dominant limbs.¹⁷ Maximal voluntary isometric contraction (MVIC) was then assessed for the hip abductors and knee extensors. Following this, participants completed the STS test under three conditions: single-leg STS on the dominant limb, single-leg STS on the non-dominant limb, and double-leg STS. The order of conditions was randomly performed. Participants were allowed two to three practice trials for each condition before testing. Each condition was conducted in three trials, with a 2-minute rest between trials to prevent neuromuscular fatigue. Before the test, participants were instructed to avoid strenuous exercise, alcohol, caffeine, and any medication that could influence task performance within 24 hours of the session.

A handheld dynamometer (Lafayette, IN, USA) was used to measure the muscle strength of the hip abductors and knee extensors. For the hip abduction test, participants lay supine on a treatment bed, with the dynamometer placed laterally on the knee, 3 centimeters above the knee joint line. Participants performed an MVIC of the hip abductors in both limbs for 5 seconds, with two repetitions and a one-minute rest between trials. For the knee extension test, participants were seated comfortably on a chair, with the dynamometer positioned anteriorly on the shank, 3 centimeters above the ankle joint. Knee extensor MVIC was assessed in both limbs for 5 seconds,

again with two repetitions and a one-minute rest between trials. The average values from each limb were calculated to represent muscle strength. One examiner administered muscle strength measurements for all participants. Test-retest reliability, assessed using the intraclass correlation coefficient ($ICC_{3,1}$) before the actual testing, demonstrated good to excellent reliability (ICC 0.788-0.974) for both muscles across both limbs.

In the single-leg STS test, participants were instructed to sit upright on an adjustable chair without leaning on the backrest, with their arms crossed over the chest. The foot of the testing leg, either dominant or non-dominant, was positioned at the center of the Zebris FDM pressure platform (FDM2, Zebris Medical GmbH, Germany), with data recorded using the Zebris FDM Software Suite. Participants were positioned with approximately 100 degrees of knee flexion, ensuring that the ankle was positioned behind the knee. The non-testing leg was lifted slightly off the ground, ensuring it did not move forward, backward, or touch the floor during the STS task. Participants were then instructed to stand up from the chair on a single leg using their lower limb strength, keeping their body upright while standing, and then sit back down. If the non-testing leg touched the ground or the participant used their arms to assist the movement, the trial was deemed unsuccessful, and they were asked to repeat the task until a successful trial was completed. Participants performed three successful trials on each testing leg (Figure 1).



Figure 1. Single-leg sit-to-stand testing task.

In the double-leg STS test, participants were instructed to sit upright on an adjustable chair without leaning on the backrest, with their arms crossed over the chest. Their feet were positioned shoulder-width apart at the center of the FDM pressure platform. Participants were positioned with approximately 100 degrees of knee flexion, ensuring the ankles were placed behind the

knees. They were then instructed to stand up from the chair using the strength of both lower limbs, maintain an upright posture while standing, and then sit back down. If participants compensated for their movement or used their arms to assist, the trial was deemed unsuccessful, and they were asked to repeat the task until a successful trial was completed (Figure 2).



Figure 2. Double-leg sit-to-stand testing task.

Data reduction

The movement time for each trial was measured by the stopwatch, calculated from the moment the participant's buttocks left the chair until full contact was made again upon sitting. The pressure plate was set to a sampling frequency of 100 Hz. COP variables, including COP sway area, COP total path length, COP velocity, COP path length in the anteroposterior direction, and COP path length in the mediolateral direction, were calculated using the Zebris FDM Software Suite.

Statistical analysis

Descriptive statistics were used to report the mean, standard deviation (SD), and 95% confidence interval (CI) for all outcome variables. Data distribution was assessed using the Shapiro-Wilk test, which confirmed that all data were normally distributed. Accordingly, a paired t-test was employed to compare the means of the primary outcome variables within groups, and a one-way repeated measures ANOVA was conducted to compare the mean values across the three different STS conditions. When the ANOVA identified significant differences, pairwise comparisons using the least significant difference test (LSD) were performed to determine the specific conditions with significant differences. The effect size was estimated using partial eta-squared (η^2_p), where values less than 0.01

indicated a small effect size, values between 0.01 and 0.06 indicated a medium effect size and values greater than 0.06 indicated a large effect size. Statistical analyses were performed using SPSS (version 17, SPSS Inc., USA), with a significance level of $p \leq 0.05$.

Results

The present study included 20 participants. Table 1 presents the demographic characteristics of all participants.

The strength of the knee extensor and hip abductor muscles for both the dominant and non-dominant legs is presented in Table 2. Statistical analysis indicated no significant differences in the strength of the knee extensor and hip abductor muscles between the dominant and non-dominant legs.

The results indicated a significant difference in the duration of the STS task across the three different conditions ($F(2,38) = 14.316$, $p < 0.001$, $\eta^2_p = 0.430$). Specifically, the duration of the single-leg STS on both the dominant and non-dominant limbs was significantly longer than that of the double-leg STS (all $p < 0.001$). However, no significant difference was observed in the duration between the single-leg STS on the dominant and non-dominant limbs (Table 3).

Table 2. Muscle strength of knee extensor and hip abductor muscles.

	Dominant limb (mean±SD)	Non-dominant limb (mean±SD)	t_{19}	p
Knee extensor (N)	192.70±43.74 (173.65-211.75)	199.66±40.21 (182.04-217.28)	-1.414	0.173
Hip abductor (N)	173.09±27.07 (161.23-184.95)	172.21±28.93 (159.53-184.89)	0.306	0.763

Table 3. A comparative analysis of movement time and center of pressure during single- and double-leg sit-to-stand tasks.

	Single-leg STS on dominant limb (Mean±SD)	Single-leg STS on non-dominant limb (Mean±SD)	Double-leg STS (Mean±SD)	F	P	η^2_p
Time (sec)	1.51±0.35 * (1.36-1.66)	1.49±0.38 ** (1.32-1.66)	1.15±0.13 (1.09-1.21)	14.316	<0.001 [#]	0.430
COP sway area (mm ²)	5447.38±1581.75* (4754.16-6140.60)	5340.35±1822.66* (4541.55-6139.15)	16950.43±6933.84 (13911.60-19989.26)	54.180	<0.001 [#]	0.740
COP total path length (mm)	1019.54±234.98* (916.56-1122.52)	999.52±165.11* (927.16-1071.88)	1749.26±468.14 (1544.09-1954.43)	41.097	<0.001 [#]	0.684
COP velocity (mm/sec)	226.20±55.85* (201.72-250.67)	224.67±56.47* (99.92-249.42)	443.11±133.70 (384.51-501.71)	63.253	<0.001 [#]	0.769
COP path length in the mediolateral direction (mm)	38.91±9.65* (34.68-43.14)	38.14±10.65* (33.47-42.81)	111.46±30.21 (98.22-124.70)	15.618	<0.001 [#]	0.251
COP path length the anteroposterior direction (mm)	177.20±23.63 (166.84-187.56)	174.87±27.28 (162.91-186.83)	183.19±35.92 (167.45-198.93)	0.829	0.444	0.042

Note: *significant differences with double-leg STS condition ($p<0.001$), **significant differences with double-leg STS condition ($p<0.01$),

[#]significant differences between the sit-to-stand conditions ($p<0.001$), STS: sit-to-stand, sec: second, mm: millimeter.

There were statistically significant differences in the COP area ($F(2,38)=14.316$, $p<0.001$, $\eta^2_p=0.430$), COP total path length ($F(2,38)=41.097$, $p<0.001$, $\eta^2_p=0.684$), COP average velocity ($F(2,38)=63.253$, $p<0.001$, $\eta^2_p=0.769$), and the length of the COP in the anteroposterior direction ($F(2,38)=15.618$, $p<0.001$, $\eta^2_p=0.251$). Pairwise comparisons indicated that the values for the single-leg STS on both limbs were significantly lower than those recorded during the double-leg STS test ($p<0.001$). In contrast, no significant differences were found in COP area, COP total path length, COP average velocity, or the length of the COP in the anteroposterior direction between the dominant and non-dominant sides (Table 3). Furthermore, there were no differences in the length of the COP in the mediolateral direction between the dominant, non-dominant sides, and double-leg STS.

Discussion

This study compared movement time and COP variables during three STS tests: single-leg STS on the dominant limb, single-leg STS on the non-dominant limb, and double-leg STS. Significant differences were detected between the single-leg STS (for both limbs) and the double-leg STS tasks for all outcomes, except the COP path length in the anteroposterior direction. However, the results did not support our hypothesis, as no significant differences were observed in movement time and COP between the dominant and non-dominant limbs.

Movement time is a commonly used variable to assess overall physical ability in performing STS movements.¹⁸

In this study, we found that the movement time from a seated position in the single-leg STS—both with the dominant and non-dominant legs—was longer than that of the double-leg STS test. This finding is consistent with the study's hypothesis. It aligns with previous research by Thongchoomsin *et al.*, who reported that the time spent raising the body from a chair and returning to a seated position during a 5-repetition double-leg STS test was, on average, 3.36 seconds longer for the single-leg STS.⁸ In the present study, the single-leg STS on the dominant leg took 0.34 seconds longer, while the single-leg STS on the non-dominant leg took 0.36 seconds longer than the double-leg during the one-time STS test. The difference in movement time can be attributed to the larger base of support (BOS) provided by both feet during the double-leg STS, in contrast to the more limited BOS of a single foot during the single-leg STS.¹⁹ Additionally, rising from a seated position using both legs is a familiar and natural daily movement, facilitating a more fluid and smooth motion. From a biomechanical perspective, performing the single-leg STS requires using one leg's muscle strength to counteract the entire body weight. Although the body weight remains constant, only the supporting leg muscles are engaged to generate the necessary force to stand up and return to sitting.^{19,20} This increased demand for a single limb resulted in slower movement and, consequently, a longer completion time for the single-leg STS compared to the double-leg STS.

No statistically significant difference was found between the two conditions when comparing movement

times in the single-leg STS test on the dominant and non-dominant limbs. This lack of difference may be attributed to the finding that the strength of our participants' knee extensor and hip abductor muscles in both legs did not show significant variation. These results are inconsistent with previous research, which identified differences in muscle strength between the dominant and non-dominant limbs regarding the knee extensors and flexors in healthy participants.¹¹ Additionally, the researchers maintained consistent testing postures, including knee and hip angles, across all test formats, resulting in no differences in the angular movement of the trunk and lower limb joints between the two conditions. It was supported by the study by Steingrebe *et al.*, who reported no differences in knee joint moments and loading when rising from a seated position using either the dominant or non-dominant limb, noting that only low chair heights contributed to increased shear forces on the knee.²¹

Regarding the COP outcomes, the COP sway area and total path length serve as indicators of the overall area of the COP and the total distance the COP travels during the STS movement.¹³ These COP variables evolve as the STS test is performed. The path length in the anteroposterior direction (the length of the COP major axis) corresponds to the direction of the body's movement when weight is transferred forward during the standing-up phase. In contrast, the path length in the mediolateral direction (the length of the COP minor axis) assists in maintaining lateral sway balance during the rising motion.^{22,23}

The results of the current study indicate that all COP variables—including COP area, total COP path length, average COP velocity, and COP path length in the mediolateral direction—differed significantly between the double-leg STS and single-leg STS tests, which aligns with the study's hypothesis. This phenomenon can be attributed to the smaller BOS in the single-leg STS compared to the double-leg STS. The restricted BOS inherent to the single-leg STS limits the body's movement range in critical variables, which would otherwise be greater with the larger BOS provided by the double-leg STS. Consequently, if the COP shifts outside of the already limited BOS during the single-leg STS, it will likely result in losing balance control.²⁰ This highlights the increased challenge of maintaining postural stability during single-leg tasks. This factor becomes particularly significant in populations with impaired balance, such as older adults or individuals recovering from lower limb injuries.^{14,24,25}

The COP velocity indicates the speed at which the body's center of mass moves, serving as a crucial indicator of balance control. Effective regulation of movement speed is associated with reduced postural sway.²² In the current study, participants exhibited lower COP velocity during the single-leg STS test, with speeds approximately half of those observed in the double-leg STS test. This reduction in velocity suggests a more controlled and deliberate movement pattern in the single-leg STS, likely due to the increased demand for balance imposed by the smaller BOS. However, when comparing the COP outcomes of the dominant and non-dominant legs, no

significant differences were found across all COP values during the STS test. This lack of difference may stem from the comparable strength of the primary muscles involved in the movement, which allowed for similar movement speeds in the single-leg STS. Specifically, the hip abductor muscles, which play a critical role in maintaining balance in the frontal plane, enabled participants to sustain similar levels of stability during the single-leg STS task, regardless of which leg was tested.²⁰ This finding is further supported by the anteroposterior COP path length, which also showed no significant differences across all conditions between the single-leg and double-leg STS and between the dominant and non-dominant limbs.

The findings from the current study provide preliminary data for the single- and double-leg single-time STS task in healthy adults, which may serve as a basis for future research involving more prominent and more diverse populations. Additionally, the significant differences observed in movement time and COP variables between single-leg and double-leg STS tests suggest potential applications for balance assessment and functional mobility evaluation. Meanwhile, single-leg STS, which involves a small BOS and likely greater demands on muscle power and postural control, may hold promise as a tool for identifying balance impairments and functional deficits. Further research is needed to confirm its sensitivity and utility in clinical populations.

Several limitations should be acknowledged in the current study. First, although the quadriceps and hip abductors are primary muscles for the STS task, other core muscle strength may also impact STS performance. Notably, our study found no significant strength differences between the dominant and non-dominant limbs, which may limit the ability to evaluate the effects of asymmetries on STS performance. Therefore, future studies should consider assessing the role of additional postural muscles and further include participants with known strength asymmetries to explore their influence on postural control during this movement. Additionally, incorporating electromyographic (EMG) assessments could provide valuable information on muscle activation patterns, allowing for a more comprehensive understanding of the coordination and timing of muscle contractions during the STS task. Third, incorporating kinematic data analysis could expand outcome variables by offering detailed insights into joint movement and velocity while also providing a more nuanced understanding of the sub-phases within the STS movement. This approach would facilitate a deeper analysis of the biomechanical and temporal characteristics involved in the STS movement. Lastly, the study limited participants to those aged 18-25 with a normal BMI. This restriction may affect the generalizability of the findings to other populations. Future studies should include individuals with higher BMIs, middle-aged adults, and older adults to understand better how single-leg tasks are performed across a broader range of clinical and demographic groups. Exploring these populations could yield valuable insights for clinicians, particularly in assessing balance and functional mobility in individuals

with varying physical capabilities and health conditions.

Conclusion

This study compared movement time and COP outcomes during single-leg (dominant and non-dominant legs) and double-leg STS tests in healthy participants. The results indicated that the single-leg STS (both limbs) exhibited significantly longer movement time but lower COP sway than the double-leg STS, except for COP path length in the anteroposterior direction. However, no significant differences were found between the dominant and non-dominant legs in any outcome during the single-leg STS. These findings provide preliminary reference values for STS tasks in healthy adults and may serve as a potentially helpful tool for assessing balance impairments and functional mobility. Further research is required to validate its sensitivity in pathological populations.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

The study was approved by the ethical committee of the Institutional Review Board. All participants of this study gave their written informed consent to participate.

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A systematic review of the effect of music therapy compared to speech therapy on social communication skills in preschool children with autism spectrum disorder

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ARTICLE INFO

Article history:

Received 25 November 2024

Accepted as revised 3 February 2025

Available online 13 February 2025

Keywords:

Music therapy; speech therapy; autism; systematic review.

ABSTRACT

Background: Autism spectrum disorder (ASD) is defined by impairment in social communication and the presence of restricted interests and repetitive behaviors. Individuals with ASD receive a variety of interventions tailored to their specific needs, enhancing their social, communication, and adaptive skills. Speech therapy (ST) aims to promote speech and language development and further improve social and communication skills in children diagnosed with ASD. Music therapy (MT) has been recognized as an intervention for individuals with ASD to facilitate social and communication skills. At present, there is no systematic review (SR) of MT compared to ST on social communication skills in preschool children with ASD.

Objective: This SR aimed to (a) investigate the effectiveness of MT compared to ST on social communication skills for preschool children with ASD and (b) investigate which type of MT, compared to ST, affects the social communication skills of preschool children with ASD.

Materials and methods: This SR was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines using five databases: the CINAHL Complete, PubMed, Scopus, ERIC, and Cochrane. The researchers also conducted a hand search for reference lists of identified articles and pertinent reviews for additional studies. We assessed the risk of bias using the Risk of Bias in Non-randomized Studies-of Interventions (ROBINS-I) tool and the Revised Cochrane risk-of-bias tool for randomized trials (RoB 2).

Results: The initial literature search yielded 63 articles. Following the exclusion of non-relevant studies, 2 studies met the inclusion criteria for this review. The results indicated that the differences in overall efficacy between MT and ST were not statistically significant. Both therapies are effective for speech production, including semantics, phonology, pragmatics, and prosody in preschool children with ASD. MT, specifically developmental speech and language training through music (DSLTM), is equally successful as ST in enhancing speech production. Behavioral intervention, applied behavior analysis verbal behavior (ABA-VB), is also effective in improving core symptoms of autism, such as social and communication deficits.

Conclusion: MT has comparable efficacy in enhancing speech production compared to ST, which impacts social communication in preschool children with ASD. Nevertheless, ST remains a cornerstone in addressing communication deficits in children with ASD, focusing on structured speech and language development. MT provides a complementary approach that can enhance speech production and bring more enjoyment to these young children, who frequently struggle with social communication. Therefore, MT could be viewed as a complementary approach to traditional ST. Limitations included research on the use of DSLTM and ABA-VB by music therapists has been sparse, and the number of studies has been small.

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doi: 10.12982/JAMS.2025.046

E-ISSN: 2539-6056

Further study would allow for more precise conclusions regarding the effect of MT compared to ST on social communication skills in preschool children with ASD.

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition that affects children. It is defined by impairment in social communication and the presence of restricted interests and repetitive behaviors.¹ Individuals with ASD receive various interventions tailored to their specific needs, enhancing their social, communication, and adaptive skills. The most effective intervention for ASD remains a subject of controversy. Speech therapy (ST) aims to promote speech and language development and improve social and communication skills in children diagnosed with ASD.² Early intervention with ST is crucial for developing these children's speech, language, and social communication skills.³

Music therapy (MT) has been recognized as an intervention for individuals with ASD to facilitate social and communication skills. Numerous studies have studied approaches that enhance aspects of social communication in preschool and school-aged children with ASD, such as improvisational music therapy,⁴ the Orff method,⁵ family-centered music therapy,⁶ and piano therapy programs,⁷ each with unique benefits and applications. A recent systematic review by Tsigoti and Georgiadi⁸ revealed a wider recognition of MT as a valuable treatment for children with ASD. However, the results of this study were mixed because no conclusions could be drawn regarding the extent of its efficacy compared to the standard treatments that children typically receive.

A few studies have investigated brain regions similarly and differentially involved with listening and covert production of singing relative to speech. It demonstrated the overlap in neural process activations during speech and music tasks. A distinct pattern of differential laterality is observed in the left temporal lobe for both covert production and listening tasks involving speech over singing. Conversely, singing over speech is observed in the right temporal lobe for listening tasks alone.^{9,10}

Research in ASD indicates that while individuals with ASD may exhibit atypical predictive processing in language, their musical prediction abilities can remain intact. This suggests that music could be a bridge to improve language prediction skills, especially when individual differences are considered.¹¹ The shared cognitive and neural mechanisms between music and language, such as pitch, rhythm, and syntax, highlight the potential for music to enhance language skills. However, the precise neural mechanisms remain incompletely understood, necessitating further interdisciplinary research.¹²

So far, there have been a few studies that have compared the effects of MT versus ST on social communication skills in preschool children with ASD. For example, a study by Lim¹³ examined the impact of MT (DSLMT), ST, and no training on the verbal expression of children between the ages of 3 and 5 who have ASD. The findings indicated that individuals who received instruction in MT and ST substantially improved their

verbal production. Both individuals with high and low functioning levels experienced improvements in their speech production. However, participants with low functioning demonstrated a more significant improvement after the MT than the ST. Children with ASD can interpret significant linguistic information from music stimuli, apply pattern perception principles, and produce functional speech.

A study by Lim and Draper examined the impact of MT (ABA-VB), ST (ABA-VB), and no training on the speech production of children between the ages of 3 and 5 who have ASD.¹⁴ The findings indicated that MT and ST effectively produced the four ABA verbal operants. However, there was no statistically significant difference between them. The findings also indicated that MT incorporated with the ABA-VB program was the most successful in enhancing echoic production, whereas ST was the most effective in enhancing tact production. Therefore, the ABA-VB program can incorporate music, and musical stimuli can improve the functional verbal output in children with ASD.

Presently, no SR is comparing the effects of MT versus ST on social communication skills in preschool children with ASD.

The current SR aimed to 1) investigate the effectiveness of MT compared to ST on social communication skills for preschool children with ASD, and 2) investigate which type of MT, compared to ST, affects the social communication skills of preschool children with ASD. The researchers set out to address the following research questions:

- 1) What was the effectiveness of music therapy on the social communication skills of preschool children with ASD compared to speech therapy?
- 2) Which type of music therapy, compared to speech therapy, affects the social communication skills of preschool children with ASD?

Materials and methods

This SR was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.¹⁵

Search strategy

Relevant articles were obtained by searching five databases: CINAHL Complete, PubMed, Scopus, ERIC, and Cochrane. Each database was searched from 2009 to 2024. The researchers also conducted a hand search for reference lists of identified articles and pertinent reviews for additional studies. References from the reviewed articles were screened to find more articles of interest. Only studies in English were included.

The following keywords were used in the search: ("music" OR "music therapy" OR "music intervention") AND ("speech therapy" OR "language intervention") AND ("preschool") AND ("autism" OR "ASD" OR "autism spectrum

disorder" OR "pervasive developmental disorder") AND ("social communication" OR "social behavior" OR "social skills" OR "communication" OR "communication skills"). Each database was searched from 2009 to 2024.

Inclusion and exclusion criteria

The PICOS (participants, interventions, comparisons, outcomes, and study design) framework informed this review's eligibility criteria. Studies that investigated the effect of MT compared to ST on preschool children with ASD were included. The primary outcome of interest was the improvement of social communication skills.

The researchers applied the following inclusion criteria for the selection of articles: 1) preschool children with ASD aged between 2 to 6, 2) music therapy provided by a qualified music therapist or music student, 3) compared to speech therapy, 4) the improvement of communication, social, or social communication skills, 5) experimental (e.g., randomized control trials) or quasi-experimental (e.g., A-B design) designs, 6) studies published between 2009 and 2024, and 7) studies published in English.

The exclusion criteria were as follows: 1) children aged under 2 years or more than 6 years, 2) presence of other neurodevelopmental disorders such as epilepsy, Attention Deficit Hyperactivity Disorder (ADHD), and intellectual disability (ID), 3) outcomes were not reported or not related to social communication, and 4) no experimental studies, case studies, reviews, case reports, commentaries, or letters.

Study selection

After reading titles and abstracts, two researchers (V.B. and N.W.) screened and excluded irrelevant articles. We checked reference lists of retrieved systematic reviews and assessed full texts for eligibility. We attempted to reach a consensus on any disagreements. A third reviewer's opinion was considered to resolve the issue if necessary. However, there was no disagreement.

Quality assessment

Two researchers (V.B. and N.W.) independently assessed the risk of bias according to the criteria of the ROBINS-I tool¹⁶ and the RoB 2.¹⁷

The ROBINS-I tool¹⁶ consists of the following domains: pre-intervention (bias due to confounding, bias in the selection of participants into the study), at intervention (bias in classification of interventions), post-intervention (bias due to deviations from intended interventions, bias due to missing data, bias in measurement of outcomes, bias in selection of the reported result). Each domain is classified into five categories: "Low risk of bias," "Moderate risk of bias," "Serious risk of bias," "Critical risk of bias," or "No information."

The RoB 2 tool¹⁷ consists of the following domains: bias arising from the randomization process, bias due to deviations from intended interventions, bias in missing outcome data, bias in measuring the outcome, and bias in selecting the reported result. Each domain is classified into three categories: "low risk of bias," "some concerns," or "high risk of bias."

Two researchers (V.B. and N.W.) independently assessed the risk of bias in each domain, and the researchers discussed discrepancies until a consensus was reached. A third reviewer's opinion was considered to resolve the issue if necessary. However, there was no disagreement.

Data extraction and analysis

Two researchers (V.B. and N.W.) independently extracted all data using a data collection form. We recorded the following data in a table from each eligible article: 1) the name of the first author, 2) the year of publication, 3) the country, 4) participants, 5) the type of MT intervention, 6) control, 7) duration, 8) measurements, and 9) outcomes.

This review used a descriptive analysis. The researchers (V.B. and N.W.) did not analyze subgroups or subsets or undertake a meta-analysis of the studies.

Results

Figure 1 summarizes the PRISMA flowchart of the study. The initial literature search yielded 63 articles after duplicates were removed. All articles were published from 2009 to 2024. 60 titles and abstracts were examined to remove irrelevant and review articles. After that, 48 studies were excluded after initial screening, leaving 12 full-text articles assessed for eligibility. Then, 10 full-text articles were excluded after applying inclusion and exclusion criteria. A total of 2 articles were included in the current study.

Table 1 summarizes the risk of bias in a non-randomized study using the ROBINS-I scale. The response for an overall risk-of-bias judgment was moderate. Table 2 summarizes the risk of bias in a randomized study using the RoB2 scale. The response for an overall risk-of-bias judgment was some concern.

Table 3 presents the included studies. 2 studies were conducted in the United States of America (USA). Importantly, it should be noted that the same primary investigator conducted these studies. According to Johns Hopkins nursing evidence-based practice model and guidelines, one study was a randomized controlled trial (RCT),¹³ which was high quality scientific (level I evidence),¹⁸ while the other was a non-RCT pre/post evaluation,¹⁴ which was generally good quality scientific (level II evidence).¹⁸

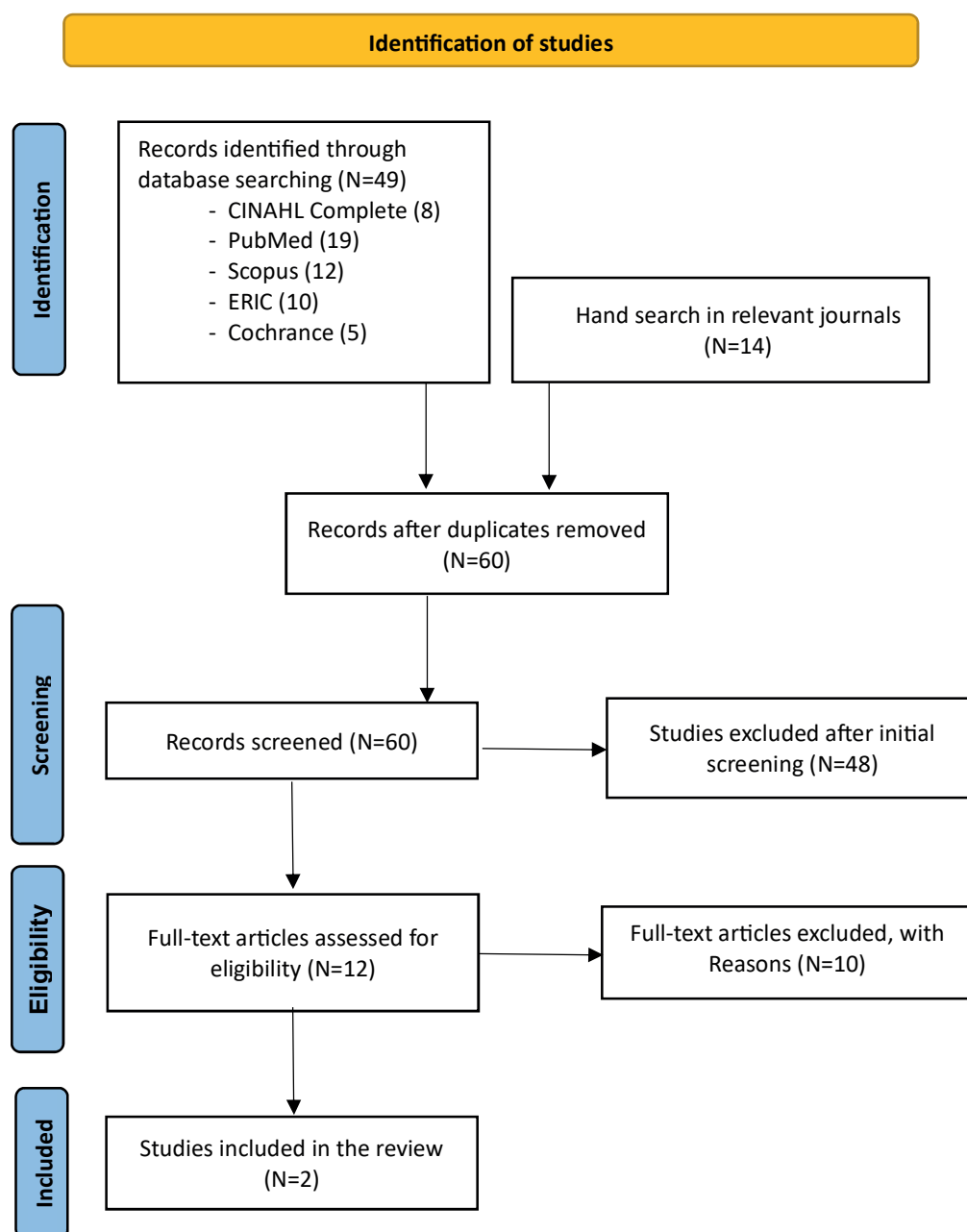


Figure 1. Methodology flowchart.

Table 1. Risk of bias in non-randomized study using the ROBINS-I scale.

Authors	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from the intended intervention	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall bias
Lim and Draper 2011 ¹⁴	Moderate	Moderate	Moderate	Moderate	Low	Low	Low	Moderate

Table 2. Risk of bias in a randomized study using the RoB2 scale.

Authors	Risk of bias arising from the randomization process	Risk of bias due to deviations from the intended intervention	Missing outcome data	Risk of bias in measurement of the outcome	Risk of bias in selection of the reported result	Overall risk of bias
Lim 2010 ¹³	Some concern	Some concern	Some concern	Low	Low	Some concern

Table 3. Included studies.

Study/ Year/ Country	Study design	Participant	Intervention	Control	Duration	Interventionist	Measurements	Outcomes
Lim/ 2010/ USA ¹³	RCT	- Children with ASD aged 3-5 year - N=50, 25 with moderate/severe ASD and 25 with mild ASD	MT, DSLM	- ST - No training	6 sessions 3 days, 2 times/day	Music student and investigator (music therapist)	VPES	- Children in the MT or ST group improved on speech production compared to the no-training group. - No significant difference was observed between the MT group and the ST group on speech production in children with ASD.
Lim&Draper/ 2011/ USA ¹⁴	Non-RCT Pre/post evaluation	- Children with ASD aged 3-5 year - N=22, verbal or preverbal with presence of immediate echolalia	MT, ABA-VB	- ST, ABA-VB - No training	6 sessions 2 weeks, 3 days/week	Certified music therapist	VPES	- Children in the MT or ST group improved on verbal operants production compared to the no-training group. - No significant difference was observed between the MT group and the ST group in speech production in children with ASD.

Studies included children aged from three to five years. In the study by Lim, 25 had moderate/severe ASD, and 25 had mild ASD.¹³ Children who are categorized as “age-appropriate” and “mildly impaired” on the Childhood Autism Rating Scale (CARS) were classified as participants of high functioning level. Children who were identified as “moderately impaired” and “severely impaired” on the CARS were at a low functioning level. On the other hand, in the study by Lim and Darper, 22 children with ASD were verbal or preverbal with the presence of immediate echolalia.¹⁴ This study did not determine the functioning level based on CARS scores.

In the study by Lim, children with ASD in the MT group received DSLM.¹³ The children watched the music video for 9 minutes. All the songs were recorded on videotape and shown to the participants via a television monitor. A female music student sang all six songs, with the investigator accompanying her on guitar. The ST group received the same texts for the six songs used in the music stimuli. Children watched the speech video for 5 minutes and 40 seconds. All the stories were recorded on videotape and shown to the participants via a television monitor. Those in the no-intervention group did not watch the music or speech video. In the MT and ST groups, children with ASD received a total of six training sessions for three days, two times a day. A female music student provided all training sessions.

In the study by Lim and Darper, children with ASD in the MT group received music integrated with the ABA-VB program, which consisted of singing the verbal instructions as part of DSLM.¹⁴ The ST group received speech integrated with the ABA-VB program. Texts for the sentences and directions used were like those in the MT group. Those in the no-intervention group did not receive intervention. In the MT and ST group, children with ASD received a total of six training sessions for two weeks, three days a week. A certified music therapist provided all training sessions.

Lim,¹³ and Lim and Darper¹⁴ used the Verbal Production Evaluation Scale (VPES) to assess the effect of MT on children with ASD by measuring participant production of target words using four speech components: semantics, phonology, pragmatics, and prosody. Semantics provides information on whether the child produced the correct target word. Phonology refers to accurately pronouncing the target word without articulation or phonetic errors in vowels or consonants. Pragmatics is the relationship between language, specific behaviors, and social or linguistic contexts. The child’s verbal response ranged from an immediate response to a delayed response. Prosody refers to the variation of tone used when speaking (i.e., intonation) and vocal stress, which is the relative emphasis given to certain syllables in a word. Prosody also encompasses syntax, turn-taking in conversational interactions, types of utterance, such as question versus statement, and the speaker’s attitudes, intentions, and feelings.

The study by Lim found that children in the MT or ST group improved in speech production compared

to the no-training group.¹³ No significant difference was observed between the MT and ST groups. Children with high-functioning ASD showed more substantial improvement in speech production than those with low-functioning ASD. Echolalia was positively related to high functioning in children with ASD, indicating a correlation between echolalia and speech production. MT enhanced speech production in children with ASD, suggesting a close link between music and language development in early childhood. The study emphasized the importance of level of functioning in indicating speech production performance and improvement, regardless of the training condition.

The study by Lim and Draper found that children in the MT or ST group improved verbal operant production.¹⁴ Children with ASD scored higher on echolalia production than verbal production. Echolalia was positively related to verbal production in children with ASD, indicating a correlation between verbal operant production and echolalia and/or verbal imitation skills. No significant difference was observed between the MT group and the ST group. MT integrated with the ABA-VB program was shown to be the most efficient in echoic production, whereas ST integrated with the ABA-VB program was the most effective intact production. The study emphasized the effectiveness of MT for treating communication problems in children with ASD.

Discussion

The first purpose of this study was to investigate the effectiveness of MT compared to ST on social communication skills in preschool children with ASD. The results indicated that the differences between MT and ST were not statistically significant regarding overall efficacy. Both therapies are effective for speech production, including semantics, phonology, pragmatics, and prosody, in preschoolers with ASD.^{13,14} Furthermore, the results indicated children in the MT group produced a more significant number of acquisitions of functional vocabulary words¹³ and speech production^{13,14} than those in the ST group, even though this was not significant. This aligns with specific theories that suggest shared neural processes between speech and music, potentially enhancing speech production.^{9,10} Therefore, MT holds promise to be an effective intervention, similar to ST, for enhancing speech and language skills in preschool children with ASD.

This study also contributes further knowledge by highlighting that MT was the most effective in echoic production, a verbal operant of vocal imitation.¹⁴ For instance, the antecedent variable in echoic was “I want more, I want more, more. Can you say I want more?” And the target phrase was “I want more.” Consistent with Demaine,²² repetitions in therapy and musical “sing-song” phrases assist children with ASD with better retaining and perceiving information and communicating in a functioning language. Children with ASD who exhibit echolalia remember information similarly to how they remember musical melodies without necessarily associating the information with semantic memory.²³ Whereas ST was

the most successful intact production, a verbal operant of labeling.¹⁴ For instance, the antecedent variable intact was "Can you tell me, What is this?". The target word used was a combined vocabulary with color and animal (e.g., white sheep or pink pig). Tacting is a difficult skill because it requires the child to recognize the correct word and possess the vocal ability to articulate it themselves.²⁴ The use of tact correction techniques is crucial in reducing stereotypic vocalizations and promoting appropriate vocalizations in children with ASD.²⁵ Therefore, it can be seen that the effects of training conditions might be related to factors such as levels of functioning or the ability of children with ASD to communicate using speech or language. Further research is needed.

Moreover, this study revealed that MT might be especially beneficial for low-functioning children with ASD.¹³ It could be that children with ASD interpret significant linguistic information in music stimuli based on pattern perception principles that enhance functional verbal production, consistent with Cooley.¹⁹ Moreover, it could be that children with ASD exhibit restricted social processing abilities and may not interpret human communication in the same manner as typically developing children.²⁰ MT is used to increase verbal expressiveness because it is a leisure activity requiring less demanding interaction with others. Young children with ASD showed a preference for non-speech sounds compared to non-ASD children.²¹ Findings shed light on when choosing interventions for preschool children with ASD. It may be beneficial to consider MT, that music can be particularly enjoyable for these children, who often struggle with social communication.

The second purpose of this study was to investigate which type of MT, compared to ST, affects the social communication skills of preschool children with ASD. The results indicated that both studies used DSLM.^{13,14} The interventionists in the two studies included a music student¹³ and a music therapist.¹⁴

DSLM uses developmentally appropriate musical materials and experiences to improve speech and language skills. This is achieved through activities such as singing, chanting, playing musical instruments, and integrating music, speech, and movement,²⁶ which seems to help improve speech production, including semantics, phonology, pragmatics, and prosody in preschoolers with ASD.^{13,14}

The effectiveness of DSLM may be attributed to its alignment with the Gestalt laws of perception often observed in children with ASD.²⁷ The relationship between gestalt language processing and autism is a complex and multifaceted topic that involves understanding how individuals with ASD perceive and use language. Gestalt language processing refers to the ability to perceive and interpret language as whole units rather than as individual components. This approach to language comprehension can be particularly challenging for individuals with ASD, who often experience difficulties in language and communication. Gestalt language processing can manifest

in echolalia, where children with ASD may repeat phrases or sentences from others.²⁸ The results of this study demonstrated that MT was the most successful in echoic production for young children with ASD.¹⁴ The explanation for this improvement might be that all songs were composed using Gestalt laws of perception, with small pitch intervals that moved in step-by-step patterns. Target words were inserted at the end of phrases, with half and full cadences and sustained note durations. Rhythmic and melodic patterns were employed to help identify target words in both scripts. Songs that utilize repetitive patterns help to maintain attention and improve speech production.

Another noteworthy finding was the efficacy of MT integrated with the ABA-VB program or ST integrated with the ABA-VB program, as investigated by Lim and Draper.¹⁴ ABA is grounded in scientific principles of behavior and aims to improve the fundamental impairments in ASD, namely social and communication deficits.²⁹ While VB imparts comprehensive skills, with a particular emphasis on language skills.³⁰ The verbal behavior approach organizes language teaching based on Skinner's analysis of verbal operants, such as mands (requests) and tacts (labels).³¹ The benefits of this integrated approach, ABA-VB, for preschool children with ASD improve a child's capacity to acquire functional language. The ABA-VB program can tap into individuals' natural motivation for things they want, making communication more meaningful and rewarding. More empirical research is necessary to support the long-term effectiveness of this approach, which has shown promise in language acquisition.³¹ Speech therapists typically provide ST for preschool-aged children with ASD, and speech-language pathology is one of the most commonly accessed services for these children.³² ST is an effective treatment for improving social communication skills,^{2,3} as is MT.⁸ Therefore, music therapists and speech therapists can incorporate the ABA-VB program for children with ASD to improve the core impairments in social and communication, as well as encourage more successful therapy practices.

Limitations and future research

There are some limitations in the current study that need to be addressed in future studies. First, research on using DSLM and ABA-VB by music therapists has been sparse. In practice, ST for preschool children with ASD is usually provided by speech therapists. Evidence-based practice to compare MT and ST is necessary to confirm that the interventions provided by music therapists and speech therapists are similar. Second, the small number of studies in the present study limited the comparison of MT and ST and the type of intervention concerning social communication outcomes. Therefore, a more significant number of studies are needed to support and allow for more precise conclusions regarding the effect and type of MT compared to ST on social communication skills in preschool children with ASD, particularly for use in the field of music and speech therapy.

Conclusion

This present study can corroborate the conclusions of the previously reviewed research that MT, specifically DSLM, has comparable efficacy in enhancing speech production compared to ST, which impacts social communication in preschool children with ASD. Behavioral intervention, ABA-VB, is also effective in improving core symptoms of autism, such as social and communication deficits. Additionally, the unique benefits of DSLM and ABA-VB programs, especially for low-functioning children with ASD, underscore their value as approaches for improving outcomes.

However, ST remains a cornerstone in addressing communication deficits in children with ASD, focusing on structured speech and language development.

MT provides a complementary approach that can enhance speech production and bring more enjoyment to these young children, who frequently struggle with social communication. Therefore, MT could be viewed as a complementary approach to traditional ST.

Conflicts of interest

The author(s) declared no potential conflicts of interest concerning this article's research, authorship, and/or publication.

Acknowledgements

The Faculty of Humanities, Naresuan University, supported this research. The authors appreciate this support.

Ethical approval

The Ethics Committee, Faculty of Associated Medical Sciences, Chiang Mai University (CMU), Thailand (AMSEC-67EM-033) approved the study for exemption review.

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Cause-specific mortality in reproductive-age breast cancer patients: A competing risk approach

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ARTICLE INFO

Article history:

Received 17 August 2024

Accepted as revised 5 February 2025

Available online 17 February 2025

Keywords:

Breast cancer, Competing risk,
Reproductive age, Survival analysis,
SEER database.

ABSTRACT

Background: Breast cancer (BC) is a leading cause of mortality among women, particularly those in the reproductive age (15-49 years).

Objective: This study aims to examine the survival outcomes of female BC patients in reproductive age groups using a competing risk approach, with a focus on identifying significant determinants of BC-specific mortality and mortality due to other causes.

Materials and methods: This study utilized data from the Surveillance, Epidemiology, and End Results (SEER) database. The analysis included women diagnosed with breast cancer within reproductive age groups. The Cumulative Incidence Function (CIF), cause-specific hazard, and Fine-Gray subdistribution hazard models were employed to estimate the impact of potential determinants on BC-specific and other-cause mortality.

Results: A total of 67063 patients were included in the study. The cumulative incidence of breast cancer-specific mortality and other cause mortality at 5 years was 4.8% and 0.95%, respectively. Age at diagnosis, race, tumor stage, and hormone receptor status were significant predictors of breast cancer-specific mortality. The Fine-Gray model revealed that younger age (15-39 years), advanced tumor stage, and negative hormone receptor status were associated with higher hazards for breast cancer mortality ($p < 0.05$).

Conclusion: The study highlights the importance of using a competing risk model to evaluate the cumulative incidence of prognostic factors in breast cancer patients of reproductive age, especially when competing clinical risks are present. These findings can guide personalized treatment strategies based on individual risk profiles.

Introduction

Breast cancer (BC) is the most frequently diagnosed cancer and the leading cause of cancer-related mortality among women globally. According to the GLOBOCAN 2020 report, BC accounts for 24.5% of all cancer cases and 15.5% of cancer deaths among women worldwide.¹ Various risk factors such as early menarche, nulliparity, late age at first pregnancy, use of oral contraceptives, and late menopause have been identified as significant markers for BC development in reproductive-age women.²⁻⁴

Classical survival analysis methods, such as Kaplan-Meier survival analysis, the log-rank test, and the Cox proportional hazards model, are well-established for estimating time-to-event data.^{5,6} However, these methods often fall short in clinical scenarios where multiple endpoints, or competing risks, coexist.⁷ Competing risk analysis extends classical survival analysis by accommodating more than one event

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doi: 10.12982/JAMS.2025.047

E-ISSN: 2539-6056

that affects a patient's survival time.⁸ In the context of BC, the primary outcome is often the time to death due to BC, with death from other causes acting as a competing event.

Competing risk models, such as the cause-specific hazard model and the Fine-Gray subdistribution hazard model, are crucial for accurately identifying predictors of an event and predicting its occurrence.^{9,10} The cause-specific hazard model assesses the effect of covariates on the instantaneous risk of an event. In contrast, the subdistribution hazard model evaluates the impact of covariates on the cumulative incidence function, accounting for competing risks.

Reproductive-age women have unique biological and clinical factors that influence BC prognosis, yet studies focusing on this demographic remain limited. This study aims to address the gap in the existing literature by examining cause-specific mortality among reproductive-age women with BC using these competing risk approaches. By identifying significant predictors of BC-specific and other-cause mortality, this work provides insights that may inform tailored treatment strategies and public health initiatives for this vulnerable group.

Materials and methods

Study design and data source

This retrospective cohort study utilized data from the surveillance, epidemiology, and end results (SEER) database (18-registry, November 2020, 2000-2018), which collects and publishes cancer incidence and survival data from population-based cancer registries covering approximately 34.6% of the U.S. population. The SEER program provides comprehensive information on patient demographics, tumor characteristics, treatment, and survival outcomes.¹¹

Study population

This study focuses on reproductive-age women (i.e., 15-49 years), based on the World Health Organization's reproductive age classification. This range encompasses critical life stages where hormonal influences, fertility concerns, and unique biological responses to treatment can impact cancer progression and outcomes. Compared to older women, reproductive-age women often face more aggressive tumor subtypes and distinct treatment challenges, underscoring the need for focused research on this group.

Patients with missing data on key variables, such as tumor stage and hormone receptor status, were excluded from the analysis to ensure data integrity. The final study population consisted of 67,063 patients diagnosed between 2010 and 2015 and registered in the SEER database.

Variables

The primary outcome of interest was cause-specific mortality, which was classified into breast cancer-specific mortality and mortality due to other causes (Figure 1). Patient status is established by combining SEER cause-specific death classification and SEER other cause-of-death classification variables. Patients' status was categorized as 0=alive, 1=death due to BC (BC mortality), and 2=death due to other causes (other-cause mortality). Covariates involved in the analysis include age (at first diagnosis), race & origin, SEER summary stage, Tumor staging, breast subtypes, Regional node positive, ER status, PR status, HER2 status, and breast subtypes. Age at diagnosis is categorized into three groups, i.e., 15-29 years, 30-39 years, and 40-49 years, respectively. Tumor stage classified according to the American Joint Committee on Cancer (AJCC) staging system.

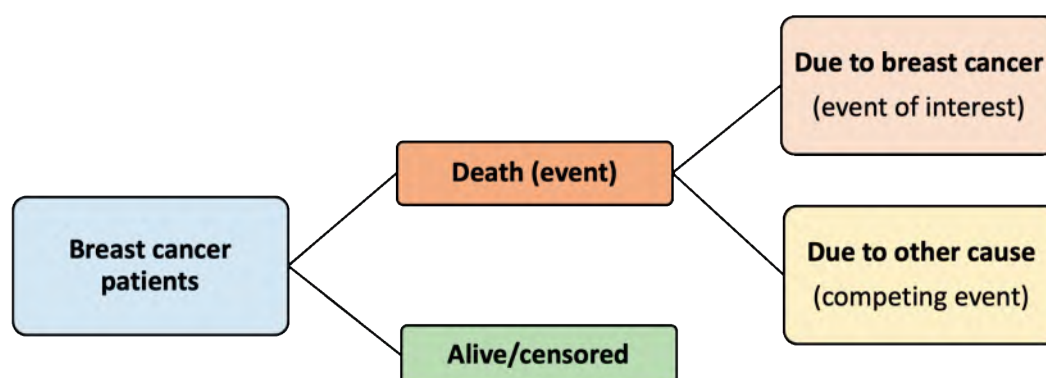


Figure 1. Schematic presentation of competing risks model for breast cancer patients.

Statistical analysis

Internal consistency was assessed in the data. Descriptive statistics such as mean $\pm$ SD and median (interquartile range) are presented for normally distributed data and skewed distribution, respectively. For categorical variables, the proportion is used. Accounting for competing risk factors, two different families of regression models are applied. The Cox model described the effect of covariates on the outcome's cause-specific hazard (CSH),⁹ while the Fine and Gray model¹⁰ dealt with the subdistribution hazard function. Both models consider competing risks, but their interpretations differ (Figure 2). A cause-specific hazard (CSH) model's regression coefficient represented

the relative effect of a certain covariate on the relative change in the rate of occurrence of the event of interest in subjects who were event-free at the time. The impact of covariates on the cumulative incidence function for the event of interest could be estimated using the subdistribution hazard ratio (SHR) model. The details of the mathematical models can be found in Prentice *et al.*⁹ and Fine and Gray.¹⁰ The CSH and SDH models' estimation results are reported as hazard ratios (HR) with a 95% confidence interval (95% CI). Statistical software STATA version 15.1, Stata Corp, Texas, USA, was used for all statistical analysis. The $p < 0.05$ was considered statistically significant.

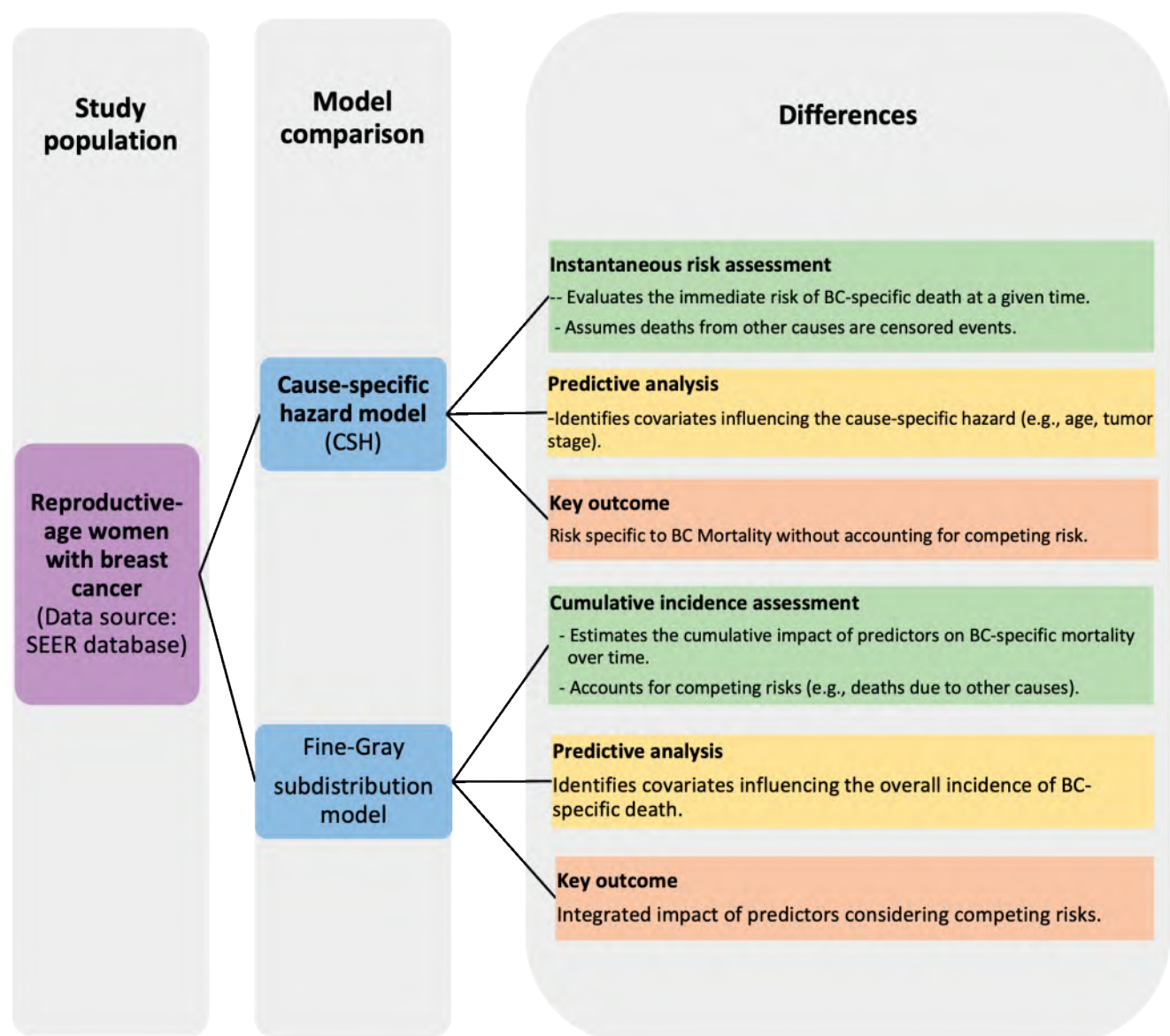


Figure 2. Distinctions between cause-specific hazard and Fine-Gray models in competing risk analysis.

Results

Patient characteristics

A total of 67,063 female BC patients within the reproductive age range were included in the analysis. The majority of patients were diagnosed with stage II breast cancer (42.5%), followed by stage I (37.2%), stage III (15.6%), and stage IV (4.7%). Hormone receptor

status was positive in 79.6% of patients, while 20.4% had negative hormone receptor status. During the follow-up period, there were 7,707 deaths, with 6,813 attributed to BC and 894 to other causes. The mean age of BC patients was 42.7 ± 5.4 years. The overall survival period ranged from 1 to 107 months, with an average survival time of 64. Detailed patient characteristics are summarized in Table 1.

Table 1. Distribution of outcomes concerning demographic and tumor characteristics.

Variables	Categories	Frequency (%)
Year of diagnosis	2010	10,630 (15.85)
	2011	11,210 (16.72)
	2012	11,170 (16.66)
	2013	11,363 (16.94)
	2014	11,257 (16.79)
	2015	11,433 (17.05)
Age (years) [Mean $\pm$ SD]		42.7 $\pm$ 5.4
Age group (years)	40-49	51,357 (76.58)
	30-39	13,938 (20.78)
	15-29	1,768 (2.64)
Race and origin	Hispanic (all races)	11,464 (17.09)
	Non-Hispanic American Indian/Alaska Native	38,208 (56.97)
	Non-Hispanic Asian or Pacific Islander	8,808 (13.13)
	Non-Hispanic Black	433 (0.65)
	Non-Hispanic White	7,871 (11.74)
	Non-Hispanic Unknown Race	279 (0.42)
SEER stage	Local	37,285 (55.6)
	Regional	26,170 (39.02)
	Distant	3,608 (5.38)
AJCC stage	Stage I	24,970 (37.23)
	Stage II	28,478 (42.46)
	Stage III	10,433 (15.56)
	Stage IV	3,182 (4.74)
Breast subtype	HR+/HER2+ (Luminal B)	9,710 (14.48)
	HR+/HER2- (Luminal A)	43,694 (65.15)
	HR-/HER2+ (HER2 enriched)	3,834 (5.72)
	HR-/HER2- (Triple negative)	9,825 (14.65)
ER status	Positive	52,401 (78.14)
	Negative	14,662 (21.86)
PR status	Positive	47,689 (71.11)
	Negative	19,374 (28.89)
Derived HER2	Positive	13,544 (20.2)
	Negative	53,519 (79.8)
Survival months [Mean $\pm$ SD]		64 $\pm$ 24.9
Patient status	Alive	59,356 (88.5)
	BC mortality	6,813 (10.2)
	Other-cause mortality	894 (1.3)

Cumulative incidence of cause-specific mortality

The cumulative incidence of BC mortality at 5 years was 4.8%, and at 9 years was 8.1%. In comparison, the cumulative incidence of other-cause mortality at 5 and 9

years was 0.95%, and 1.7%, respectively. Figure 3 illustrates the cumulative incidence functions (CIF) for both BC and other-cause mortality.

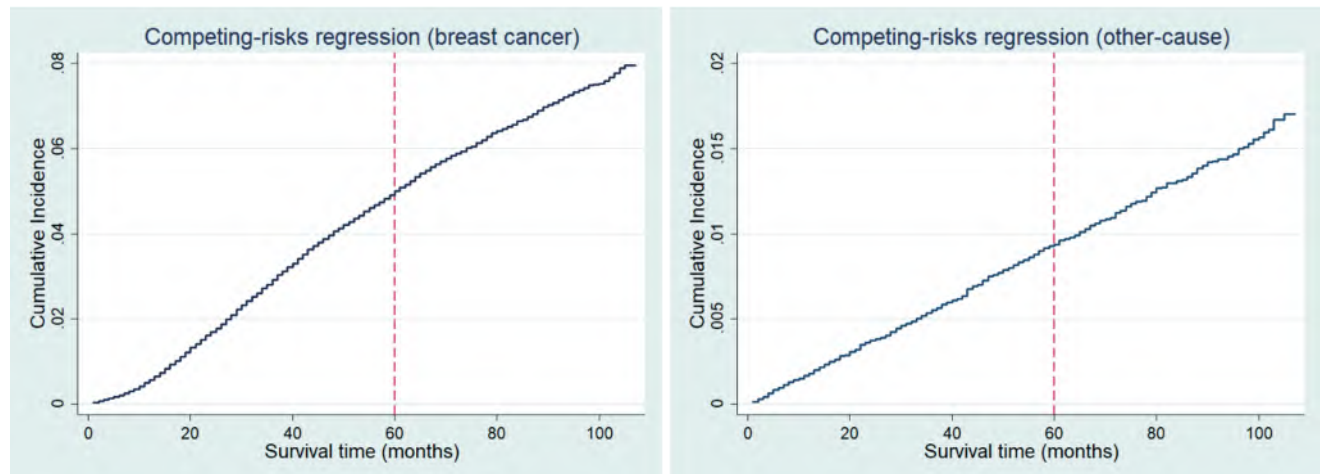


Figure 3. Overall CIF comparison between breast cancer (left) and non-breast cancer (right).

Predictors of breast cancer-specific mortality

In the univariate analysis, several factors were significantly associated with BC mortality, including age at

diagnosis, race, tumor stage, and hormone receptor status (Figure 4 and Figure 5).

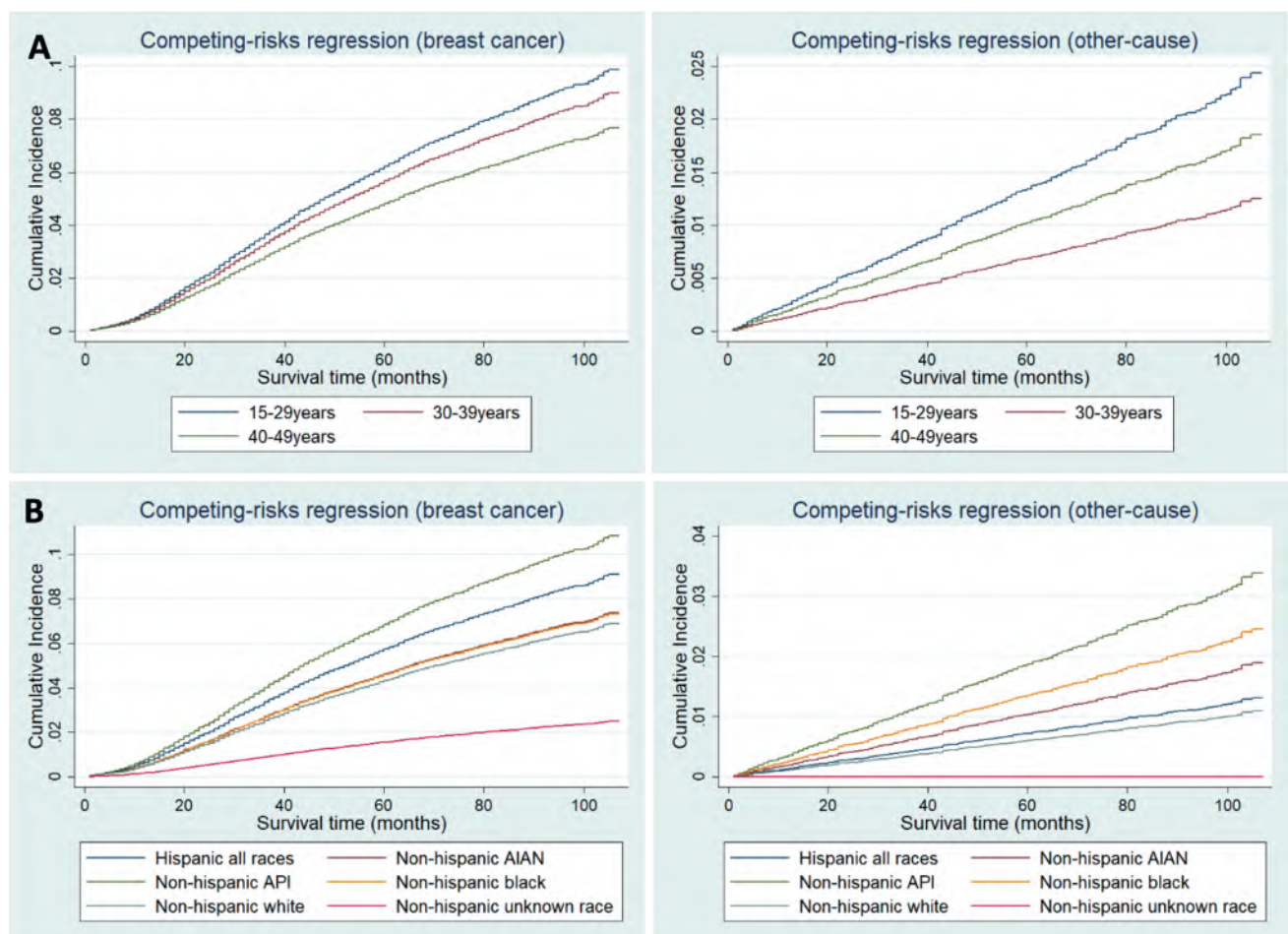


Figure 4. CIF comparison of demographic variables between breast cancer mortality (left) vs other-cause mortality (right). A: age group, B: race and origin.

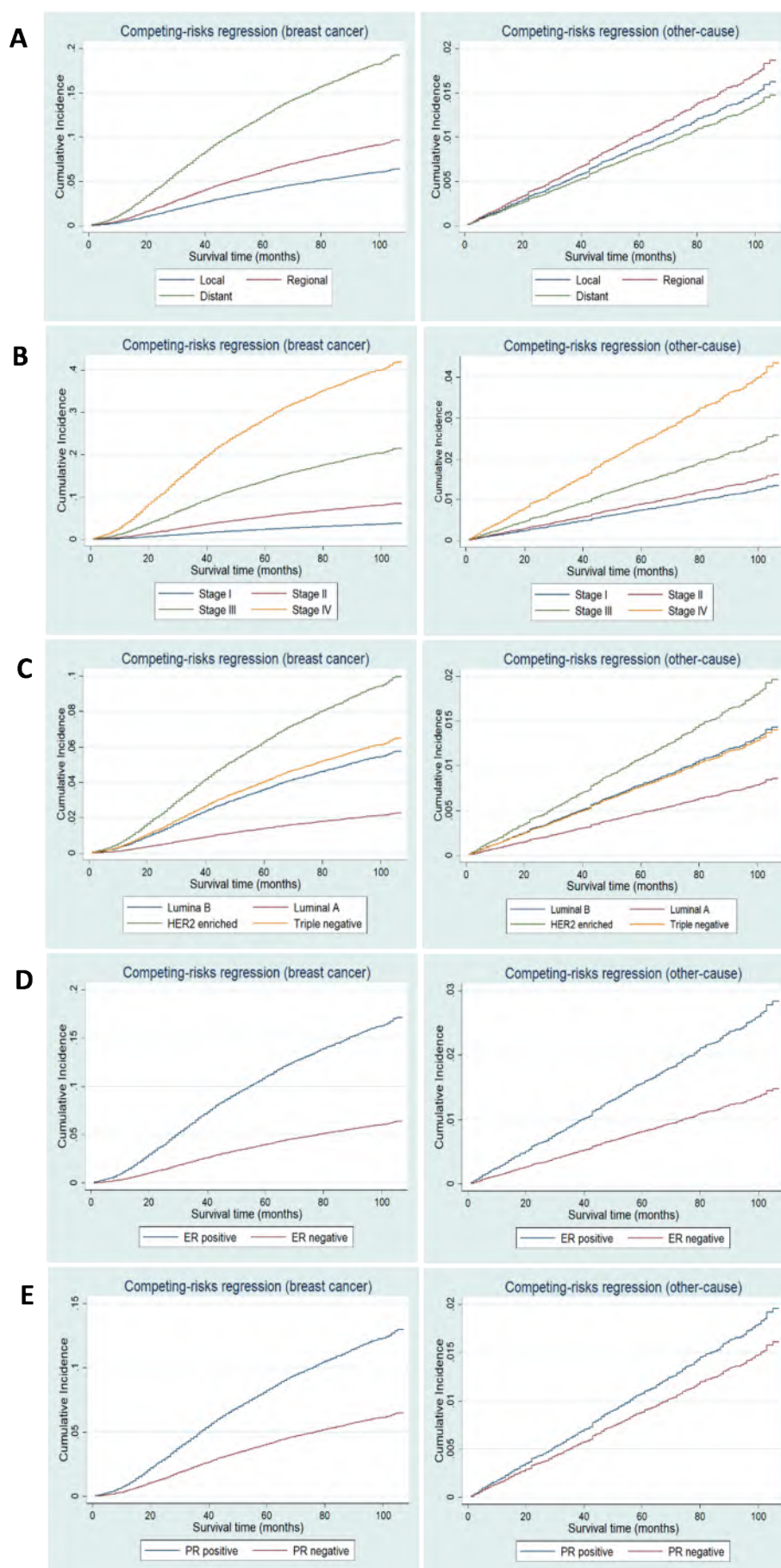


Figure 5. CIF comparison of tumor characteristics between breast cancer mortality (left) vs other-cause mortality (right). A: SEER staging, B: AJCC staging, C: breast subtypes, D: ER status, E: PR status.

Multivariate competing risk analysis

The CSH and SDH models were used to calculate the cumulative incidence of death due to BC and other causes. Because of collinearity, HER2 status, and breast subtype parameters were omitted from the multivariate analysis.

Table 2 shows the hazard ratios for BC and other-cause mortality for each analyzed factor. The findings reveal that while CSH and SDH models give comparable results regarding HR estimates, they differ regarding cause-specific events.

Table 2. Multivariate results under the cause-specific Cox model and subdistribution competing risk model.

Parameters	Cause-specific hazard model CSH (95% CI)		Subdistribution hazard ratio model SHR (95% CI)	
	Breast cancer mortality	Other-cause mortality	Breast cancer mortality	other-cause mortality
Age group (years) (40-49 as Ref.)				
30-39	1.28 (1.20, 1.37)	0.62 (0.50, 0.76)	1.29 (1.20, 1.38)	0.61 (0.49, 0.75)
15-29	1.4 (1.21, 1.63)	1.07 (0.70, 1.64)	1.42 (1.20, 1.64)	1.05 (0.69, 1.61)
Race and origin	(Hispanic all races as Ref.)			
Non-Hispanic American Indian/Alaska Native	0.86 (0.80, 0.94)	1.33 (1.05, 1.69)	0.86 (0.79, 0.93)	1.35 (1.06, 1.71)
Non-Hispanic Asian or Pacific Islander	1.3 (1.19, 1.43)	2.6 (2.00, 3.39)	1.28 (1.16, 1.41)	2.54 (1.95, 3.31)
Non-Hispanic Black	0.82 (0.57, 1.18)	1.75 (0.76, 4.00)	0.82 (0.57, 1.19)	1.77 (0.77, 4.03)
Non-Hispanic White	0.74 (0.66, 0.84)	0.61 (0.41, 0.91)	0.76 (0.66, 0.84)	0.62 (0.41, 0.93)
Non-Hispanic Unknown Race	0.2 (0.07, 0.63)	-	0.2 (0.07, 0.62)	-
SEER stage (local as Ref.)				
Regional	1.61 (1.46, 1.77)	1.23 (0.97, 1.55)	1.6 (1.45, 1.77)	1.21 (0.95, 1.52)
Distant	2.73 (2.15, 3.46)	0.27 (0.04, 1.98)	2.77 (2.12, 3.61)	0.23 (0.03, 1.64)
AJCC stage	(Stage I as Ref.)			
Stage II	2.3 (2.03, 2.61)	1.18 (0.94, 1.48)	2.3 (2.03, 2.62)	1.19 (0.95, 1.49)
Stage III	4.88 (4.20, 5.67)	1.65 (1.19, 2.30)	4.86 (4.17, 5.66)	1.59 (1.15, 2.20)
Stage IV	11.82 (9.00, 15.54)	14.29 (1.89, 38.29)	11.3 (8.37, 15.25)	11.22 (1.48, 45.32)
Positive regional nodes	1.05 (1.01, 1.09)	1 (0.93, 1.09)	1.05 (1.01, 1.09)	0.98 (0.96, 1.03)
ER negative	3.24 (2.67, 3.92)	2.04 (1.24, 3.37)	3.23 (2.65, 3.95)	1.81 (1.10, 2.99)
PR negative	2.5 (2.27, 2.75)	1.31 (1.00, 1.72)	2.47 (2.23, 2.73)	1.21 (0.92, 1.59)

Note: CSH: cause-specific hazard ratio, SHR: subdistribution hazard ratio, CI: confidence interval, ER: estrogen receptor, PR: progesterone receptor. Ref.: reference category.

Breast cancer mortality

Both models show that age group, race & origin, SEER stage, AJCC stage, positive regional node, ER status, PR status, and breast subtypes are statistically significant ($p < 0.05$) for patients who have died due to BC. Patients under 40 years had a significantly higher risk of BC mortality compared to those aged 40-49 years. Non-Hispanic Asian or Pacific Islander patients have a higher risk of BC mortality than Hispanic patients. In contrast, non-Hispanic American Indian/Alaska Native and non-Hispanic White patients have significantly smaller risk of death. Patients with regional and distant-stage disease had 1.6 times and 2.7 times increased risk of BC mortality, respectively, as compared to patients with local stages. Furthermore, patients with stages II, III, and IV have an escalated risk of BC mortality (HR=2.30, HR=4.86, and HR=11.30, respectively) compared to those with stage I. In

addition, patients with ER and PR negative are associated with a greater risk of BC mortality than the patients with ER and PR positive, respectively.

Other cause mortality

Patients aged 30-39 years are found to have significantly less risk of other-cause mortality (HR=0.61) than those aged 40-49 years. Non-Hispanic American Indian/Alaska Native (HR=1.35) and Asian or Pacific islander (HR=2.54) patients have a greater risk of other-cause mortality than Hispanic-origin patients. However, non-Hispanic white race patients have a more negligible risk of other-cause mortality (HR=0.62). Stage III and IV patients also have a significantly higher risk of other-cause mortality. Patients with ER-negative were associated with a substantially greater risk of other-cause mortality than the ER-positive patients (HR=1.81).

Discussion

In this study, we focused on BC mortality among reproductive-age women, considering other-cause mortality as competing events. Recognizing that these competing events can alter the probability of the primary event, we utilized competing risk techniques such as the cumulative incidence function, cause-specific hazard, and subdistribution hazard models for our analysis.

Despite competing risks, our results have significant implications for refining and adjusting absolute risk estimates for BC patients in reproductive age groups. We found that the 9-year cumulative incidence of BC mortality was 8.1%, while other-cause mortality was 1.7%. This study is among the first to provide estimations of BC- and other-cause-specific cumulative incidences of death using competing risks analysis for reproductive-age women.

We identified several established prognostic markers influencing survival or death due to BC in the presence of competing events, including age at diagnosis, race, and origin, staging, ER status, and PR status. These predictors align with published global literature, reinforcing the high risk of BC mortality within the first few years post-diagnosis.¹²⁻¹⁴

Our analysis revealed that younger age groups have an increased risk of BC mortality compared to older age groups, consistent with the conclusions of Keegan *et al.*, who noted higher BC mortality in adolescents and young adults than in patients aged 40-64 years.¹⁵ Interestingly, race plays a critical role; for instance, non-Hispanic American Indian/Alaska Natives exhibited a lower risk of BC mortality but higher hazards for other-cause mortality.¹² Similarly, non-Hispanic Asians or Pacific Islanders faced a greater risk of other-cause mortality.¹³ These observations necessitate further race-specific subgroup analyses to understand the reasons behind these differences.

A notable finding is the higher hazard of BC mortality due to distant-stage cancer compared to a lower risk for other-cause mortality. This is consistent with previous studies, which have shown that the long-term risk of distant metastasis remains substantial in women with non-metastatic breast cancer, affecting survival rates adversely once metastasis is detected.¹⁶ Furthermore, young age at diagnosis (<40 years) significantly impacts prognosis, with worse outcomes observed in particular breast cancer subtypes.¹⁷ These insights emphasize the critical need for continuous monitoring and tailored treatment strategies for patients diagnosed at a distant stage.

Conversely, competing risks of death from other causes remain significant but lower in magnitude. Historical data indicates shifts in the causes of death over time, suggesting improvements in breast cancer treatment and management but highlighting the importance of addressing other health risks that may affect this population.¹⁸

The findings from this study have important clinical implications. The high mortality risk associated with younger age and advanced stage at diagnosis underscores the need for targeted screening and early detection efforts in reproductive-aged women. Healthcare providers should

be vigilant in identifying and managing breast cancer in younger women, considering their unique risk profile and the potential for more aggressive disease. Given the poor prognosis associated with hormone receptor-negative status, there is a critical need for research into novel therapeutic approaches and personalized treatment plans to improve outcomes for this subgroup.

Limitations

While this study provides valuable insights into cause-specific mortality among reproductive-age women with breast cancer, certain limitations should be acknowledged.

First, the retrospective nature of the analysis inherently limits the ability to establish causality. Although we utilized a robust database (SEER) with high-quality data collection standards, the study design is observational and subject to potential biases.

Second, excluding patients with incomplete information on key variables (e.g., tumor stage, hormone receptor status) may have introduced selection bias. While this approach is necessary for ensuring data integrity, it could lead to the underrepresentation of certain subgroups, potentially affecting the generalizability of the findings. Future studies should consider employing imputation or sensitivity analyses to account for missing data.

Lastly, the SEER database lacks detailed information on certain risk factors, such as reproductive history, genetic predispositions, or lifestyle factors, which may influence both breast cancer and competing mortality risks. Including these variables in future research could provide a more comprehensive understanding of mortality predictors.

Despite these limitations, our study demonstrates the utility of competing risk models in understanding mortality patterns in this specific demographic, providing a foundation for further investigation and targeted interventions.

Conclusion

In summary, this study evaluated mortality among reproductive-age women, focusing on deaths attributable to BC in the presence of competing causes of death. Key factors influencing BC mortality include age (with younger women <40 years at higher risk), race and origin, staging, ER-negative status, and PR-negative status. Using a competing risk model provides a more accurate estimate of mortality risks, offering valuable insights for clinical practice. Targeted screening, early detection, and the development of effective treatment strategies are essential for improving survival outcomes in this vulnerable population. Future research should aim to address the limitations of this study and further our understanding of breast cancer prognosis in reproductive-aged women.

Ethical considerations

The SEER database is publicly available and does not contain identifiable patient information. Thus, ethical approval and informed consent were not required for this study.

Data availability statement

All data are available publicly through SEER.

Conflict of interest

There is no conflict of interest.

Acknowledgements

The authors thank the National Cancer Institute for sharing the data of Cancer patients in the SEER database.

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Radiation safety assessment of patient and staff during intracranial aneurysm treatment: A phantom study

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ARTICLE INFO

Article history:

Received 6 October 2024

Accepted as revised 14 December 2024

Available online 17 February 2025

Keywords:

Intracranial aneurysm, coiling embolization, phantom study, staff radiation dose, patient radiation dose.

ABSTRACT

Background: Endovascular treatment using coiling embolization for intracranial aneurysms is common in interventional neuroradiology (INR). However, it is associated with high radiation exposure to both patients and medical staff. The assessment and management of radiation risk have been a concern.

Objective: To evaluate patient and scatter radiation doses at INR staff working position during the coiling embolization procedure in a phantom study.

Materials and methods: An anthropometric phantom from head to pelvis was placed on an angiographic table. To determine the phantom's entrance skin dose (ESD), 19 NanoDot optically stimulated luminescence dosimeters (OSLDs) were placed on the surface of the head phantom. Moreover, 6 NanoDot OSLDs were also placed on the surface of the phantom at 3 critical organs (eyes, thyroid, ovaries). To determine the scatter radiation dose at staff working position, polyvinyl chloride pipes (150 cm height) were placed at the position of all staff, including interventional radiologist (IR), radiology resident (RR), scrubbed nurse (SN), radiologic technologist (RT), circulating nurse (CN), and nurse anesthetists (AN). The InLight® OSLDs were placed on each PVC pipe at 50, 100, and 150 cm height from the floor level. Coiling embolization was simulated following the standard procedure using a bi-plane digital subtraction angiography unit with standard radiation protective equipment. The patient doses from the radiation dose structured report (RDSR) were also recorded.

Results: The highest ESDs were observed in the occipital part ($1,631.56 \pm 36.97$ mGy). These ESD peaks were also observed within the safety limit and produced no skin reaction. The radiation dose at the left eye was observed to be the highest value among critical organs. The highest scatter radiation dose was observed at the position of IR at 100 cm level. Among CN, AN, and RT positions, the highest dose was recorded at the RT position at the level of 150 cm. The RDSR showed that most of the patient radiation dose was received from the 2-dimension angiography.

Conclusion: The distribution of radiation dose varied across different staff positions. As the key person conducting the coiling embolization procedure, the IR position received the highest radiation dose. Thus, close monitoring and development of additional radiation protection methods for this position are essential. Although the radiation dose at the back of the head was elevated, it remained within safety limits. Nevertheless, the monitoring of potential radiation effects should not be neglected.

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doi: 10.12982/JAMS.2025.048

E-ISSN: 2539-6056

Introduction

Endovascular treatment using coiling embolization has become a treatment for intracranial aneurysms due to its minimal invasiveness, high success rates, reduced

risks, and recovery time.¹⁻³ This procedure usually requires prolonged fluoroscopy and multiple angiographic images, causing significant radiation exposure and increasing the risk of developing deterministic and stochastic effects.⁴ It has been reported that early skin reactions such as erythema can occur at doses above 2 Gy, and a severe skin burn might develop if the exposures exceed 5 Gy. Moreover, temporary hair loss, permanent hair loss, and radiation-induced cataracts can be observed at doses around 3, 7, and 0.5 Gy, respectively.⁵ Moreover, the risk of cancer development is estimated to increase by 5% per sievert of effective dose for the general population.⁶ For safety issues, several studies have evaluated patient radiation dose using the radiation dose structured report (RDSR), direct measurement using a dosimeter in clinical practice, and phantom study.⁷⁻¹¹ The report of patient radiation dose is usually presented in terms of dose area product (DAP), reference air kerma, peak skin dose, effective dose, and absorbed dose. Previous studies have revealed that some radiation effects are observed in patients with embolization procedures.^{12,13} Moreover, the eye lens dose has also been reported to exceed the threshold.¹⁰ Monitoring and assessing patient radiation dose during embolization is an essential topic.

In addition to patients, medical staff, including radiologists, radiology residents, scrub nurses, and radiological technologists, are also usually exposed to scattered radiation, which could contribute to their overall radiation burden. It has been reported that using appropriate radiation personal protective equipment and shield techniques such as ceiling-mounted lead shields and lead curtains has been shown to lower radiation dose at staff positions.¹⁴⁻¹⁶ Although several personal protective equipment safety measures were applied to clinical practice, the radiation dose was also detected with high value in a complicated procedure, particularly embolization.¹⁷⁻¹⁹ Due to the complexity and prolonged procedure of coiling embolization, the evaluation of scatter radiation doses received by all staff to ensure that they remain within safe occupational limits should be performed accordingly.

Recently, the number of intracranial aneurysms treated with endovascular techniques has significantly increased,²⁰ which might raise the risk of radiation exposure to medical staff. Furthermore, some staff may still surpass

this threshold due to the inconsistently worn lead glasses for lowering eye lens radiation exposure and the reduction of occupational eye lens dose limit to 20 mSv per year.^{21,22} Although personal dosimeters are regularly used in clinical practice, they cannot sufficiently represent the level of scatter dose distribution in the other important organs. In addition, the variation of equipment, protocol, and complexity of cases in each institute were noted. The assessment of radiation risk for patients and staff at each institute is essential for effective management and minimizing radiation-related hazards. The findings could be utilized to improve safety protocols for medical staff and establish follow-up procedures for patients exposed to high radiation doses.

Therefore, this study aimed to evaluate patient radiation dose and scatter radiation dose in INR staff working positions during the coiling embolization procedure in a phantom study.

Materials and methods

Digital subtraction angiography machine setting

All angiographic procedures were conducted using a digital subtraction angiography (DSA) machine, Artis Zee biplane (Siemens Medical Solutions, Munich, Germany). The DSA system underwent quarterly maintenance by the engineer of Siemens Healthineers Limited (Bangkok, Thailand). In addition, the Department of Medical Sciences and the Ministry of Public Health of Thailand conduct annual quality control checks using the national standard guidelines. During the experiment, the DSA machine was inspected and confirmed to function normally, while annual quality control results met the national standard. The DSA unit consists of two x-ray tubes and two flat-panel detectors. Tube A is located under the couch, while Tube B and Detector B are located laterally, as illustrated in Figure 1A. The system's radiation protection features include a lead curtain with 0.5 mm lead (Pb) equivalent (137x57 cm) positioned alongside the angiographic table and a ceiling-mounted lead glass shield with 0.5 mm Pb equivalent (61 cm x 76 cm). Additionally, lead shield partitions consisting of lead plate thickness of 1.5 mm (120 cm x 100 cm) and lead glass shield with 2.0 mmPb equivalent were provided to enhance radiation protection for nursing staff, as shown in Figure 1B.

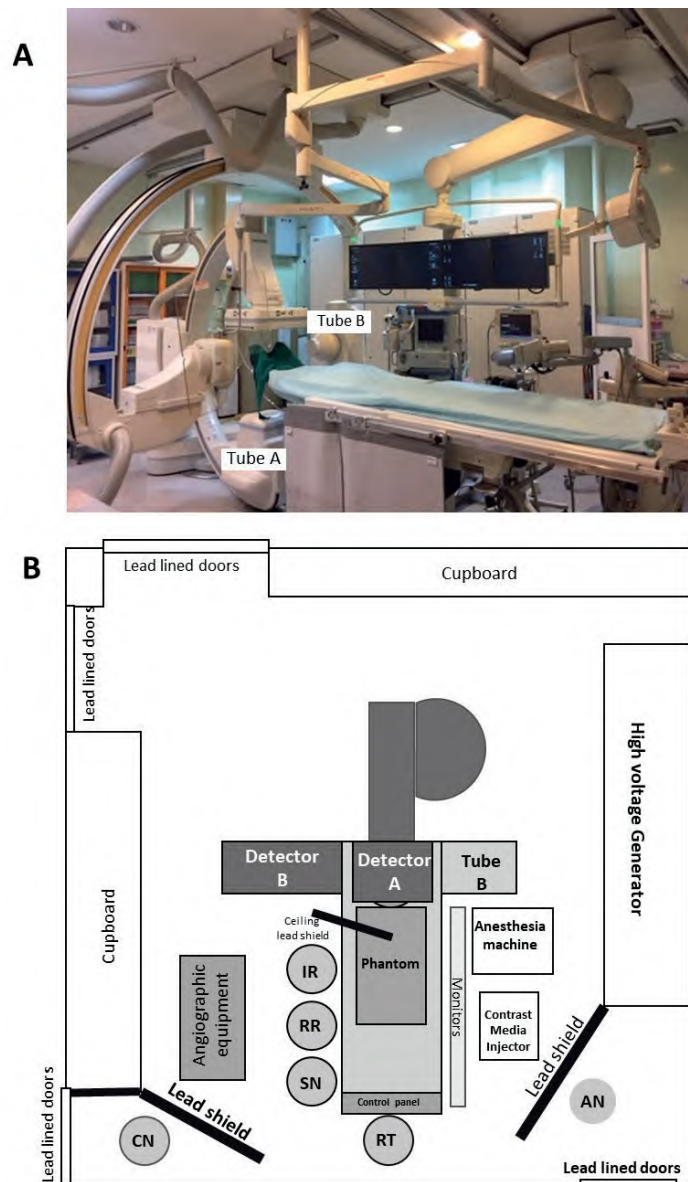


Figure 1. Angiographic suit with bi-plane systems (A) and the top-view perspective illustration demonstrates the position of staff and radiation protective equipment during coiling embolization (B).

The angiographic procedures involved a multi-disciplinary team comprising an interventional radiologist (IR), a radiology resident (RR), a scrub nurse (SN), a radiologic technologist (RT), a circulating nurse (CN), and a nurse anesthetist (AN). The specific working positions of each

staff member are shown in Figure 1B. The angiographic protocol for coiling embolization during the experiments was configured to mirror the clinical settings outlined in Table 1.

Table 1. Coiling embolization protocol.

Setting parameters	Coiling embolization
Fluoroscopy	
Tube angulation of Tube A and B (°)	0°/0°
Pulse fluoroscopy (pulse/second, p/s)	10 p/s
Fluoroscopy time of Tube A and B (min)	23 min/ 12 min
2-Dimensional angiography (2DA)	
Tube angulation of Tube A and B (°)	11°/0°
Frame rate (frame/second, f/s), time (second)*	
Mask image	4 f/s, 1 sec
Arterial phase	4 f/s, 4 sec
Capillary phase	2 f/s, 4 sec
Venous phase*	1 f/s
Number of images	610
3-Dimensional rotational angiography (3DRA)	
Tube rotational angulation (°)	200°
Frame rate and acquisition time	133 f/s, 5 sec
Number of images	532
Digital radiography (DR)	
Tube angulation of Tube A and B (°)	11°/0°
Number of images	2
Source to image distance (cm)	
Fluoroscopy, 2DA and DR (Tube A/ B)	102/94
3DRA (Tube A)	120
Table level (from isocenter point)	
	-15 cm
Field of view (Diagonal length)	
Fluoroscopy and 2DA (detector A/B)	35 cm/25 cm
3DRA (A)	42 cm
DR	42 cm

*Time in the venous phase is not identified, as it depends on the requirement of the radiologist.

Coiling embolization experiments

The coiling embolization procedures were simulated using the parameters detailed in Table 1. The imaging techniques employed in this experiment included fluoroscopy, 2-dimensional angiography (2DA), 3-dimensional rotational angiography (3DRA), and digital radiography (DR). The imaging technique and protocol used in this study were designed specifically for this experiment by the product specialist to ensure the consistency and reproducibility of each experiment. To experiment, fluoroscopy techniques (10 pulses/second) from both planes were performed with fluoroscopy time of 23 minutes (tube A) and 12 minutes (tube B). Then, 2DA was performed using the acquisition as follows: 13 seconds (plane A and B) for 1 acquisition, 8 seconds (plane A and B) for 10 acquisitions, and 8 seconds (plane A) for 1 acquisition. A total of 610 images were

obtained. The 3DRA was performed twice with 4 acquisitions (mask and contrast acquisition) with 532 images. At the end of the experiment, DR images from both planes were obtained. At the same time, reference air kerma, dose area product (DAP) of each radiographic technique, and peak skin dose were recorded from the radiation dose structured report (RDSR). The effective dose was determined by multiplying the DAP value with the conversion coefficient of 0.087 mSv/Gy·cm²,^{9,23} and data was expressed as mSv.

Radiation dose measurement

To determine the scatter radiation dose at staff working positions, 6 polyvinyl chloride (PVC) pipes (150 cm height) were placed at the position of all staff, as shown in Figure 1B. The PVC pipes at IR, RR, and SN locations were placed 15 cm apart from the edge of the angiographic table with

a 50 cm interval between each pipe, while RT, CN, and AN were placed at their respective positions. The InLight® OSLDs (environment type, Ex) were placed on each PVC pipe at three different levels to represent the level of staff organs, including eyes lens (150 cm), hands or arms (100 cm), and legs (50 cm) as displayed in Figure 2. All InLight®

OSLDs Ex were placed facing the radiation source from the X-ray tube. To ensure the experiment's reliability and reproducibility, the locations of PVC pipes for each staff position were marked on the floor with additional marking of the PVC pipes at 50, 100, and 150 cm.

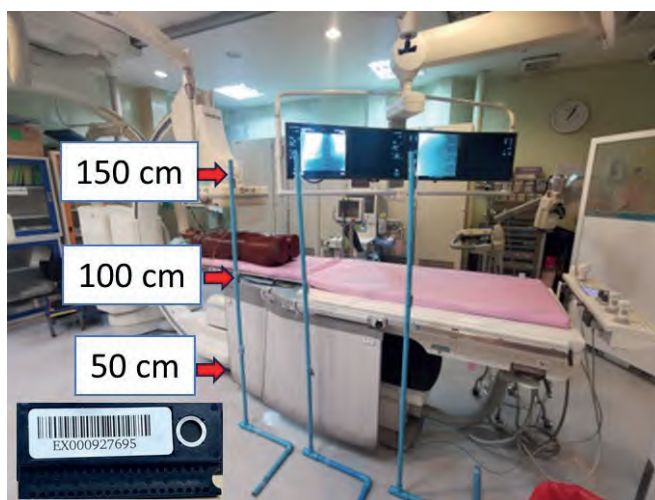


Figure 2. The setting of PVC pipes and level of InLight® OSLDs placement.

The sequence of radiation dose exposure at all staff positions was conducted following the institution's operating procedure. IR, RR, and SN were exposed to radiation during the experiment only during the fluoroscopy procedure and removed from the angiographic suit. In contrast, CN and AN were exposed to radiation during the procedure. Moreover, RT was exposed to radiation during the fluoroscopy at its position and moved to the position of CN during 2DA, 3DRA, and DR procedures.

Since almost all the patients who underwent coiling embolization were critical patients with endotracheal tubes, the direct measurement of entrance skin dose (ESD) in these patients is neither safe nor practical. Therefore, an anthropometric phantom was used instead of patients to replicate radiation dose measurement. To determine the ESD of the phantom, head-to-pelvis anthropometric

phantoms were placed on an angiographic table, as depicted in Figure 3A. NanoDot OSLDs were placed on the surface of the head phantom at 19 points, as displayed in Figure 3B. Moreover, 6 nanoDot OSLDs were also placed on the surface of the phantom at critical organ positions, including left and right eyes (LE, RE), thyroid (LT, RT), and female gonadal organs (LO, RO). To ensure the accuracy of the OSLDs placement in every experiment, these points were marked on the phantom with a permanent marker. The experiment and radiation dose measurements were performed in triplicate. Of these, unexposed OSLDs were used as controls and kept under the same conditions as the exposed OSLDs. The data from these OSLDs were then used for radiation dose calculations. After radiation exposure, all OSLDs were sent to the Personal Dosimetry Laboratory at the Office of Atoms for Peace (OAP) for radiation dose evaluation.

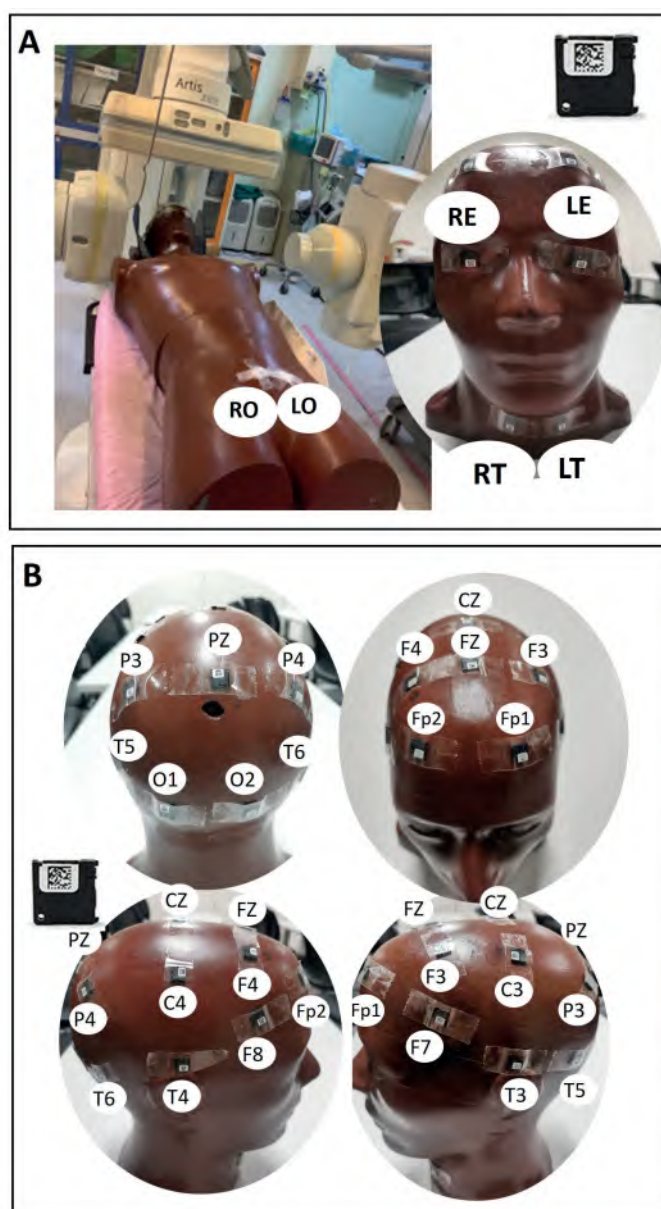


Figure 3. The setting of anthropometric phantom and the nanoDot OSLD placing on phantom skin at 3 critical organs (A). The location of nanoDot OSLD placed on head phantom surface (B).

Radiation dose evaluation

All OSLD radiation doses were evaluated using a MicroStar reader, and data readouts were performed using the MicroStar reader (Landauer Inc., Glenwood, Illinois, USA). The radiation doses were calculated by analyzing the emitted light from the stimulated dosimeters using the commercial software InLight Dose Calculation version 2.0.6.6, developed by Landauer, Inc. (Landauer Inc., Glenwood, Illinois, USA). The software was calibrated using a set of nanoDot dosimeters with known doses exposed to a Reference Quality Radiation 6 or RQR6 beam quality (80 kV) traceable to the National Institute of Standards and Technology (NIST). The most recent calibration was completed in May 2023. Additionally, routine quality control tests are conducted on the MicroStar reader using dosimeters irradiated with different known doses at the Primary Standard Dosimetry Laboratory, Office of Atoms for Peace (OAP) to assure measurement accuracy and reliability.

InLight® OSLDs Ex were read using a configuration that specified the dosimeter type as “environment” and the kind of case default as “Ex”. The dosimeter was placed in an InLight loader, and all elements were read. The results were reported as ambient dose equivalent ($H_p(10)$), which is relevant for whole-body radiation assessment, and directional dose equivalent ($H_p(3)$), which is applicable for skin, extremity, or eye lens.²⁴ Both doses were reported in mSv, the standard unit in radiation dose measurement.

Specific steps were followed during the configuration and reading process to read nanodot OSLDs. The dosimeter type was set to nanoDot, and the display unit was configured to show the dose in mGy. The nanoDot OSLD was fitted with an adapter and inserted into the InLight loader for reading. The results were presented in mGy units.

To ensure objectivity in the analysis, the researcher responsible for OSLDs reading and radiation dose calculation was blinded to the placement positions of each OSLD. To

calculate radiation dose, the residual signal of each OSLD was read after annealing and kept as a baseline. The exposed OSLD was then read twice, and the radiation dose was calculated by subtracting the basis and control values (unexposed OSLD). The radiation dose from all OSLDs of each position was averaged. A phantom skin dose and dose at critical organs were expressed as mGy, while radiation dose scatter radiation dose at staff working position was described as mSv.

Statistical analysis

All data was presented as mean±standard deviation (SD). Radiation dose calculation, the effective dose from

RDSR, mean, and SD were analyzed using the Microsoft Excel.

Results

Radiation dose structured report

The patient radiation dose from the dose report of the DSA modality is presented in Table 2. It was found that most of the radiation dose was received from the 2DA technique (2,665.7±26.2 mGy, 40,255.0±11.3 µGy·m²). The peak skin entrance dose was 2,372±2.8 mGy, and the effective dose from coiling embolization was 55.00±0.01 mSv.

Table 2. Radiation dose coiling embolization form dose report.

Dose report	Value
<i>Reference air kerma (mGy)</i>	
Fluoroscopy	1,130±4.2
2DA	2,665.7±26.2
3DRA	207.0±1.4
DR	3.8±0.1
Total reference air kerma	4,009.5±31.9
<i>DAP (Gy·cm²)</i>	
Fluoroscopy	171.01±0.16
2DA	402.55±0.11
3DRA	57.71±0.04
DR	1.02±0.01
Total DAP	632.28±0.08
<i>Effective dose (mSv)</i>	
Fluoroscopy	14.89±0.01
2DA	35.01±0.01
3DRA	5.02±0.00
DR	0.09±0.00
Total effective dose	55.00±0.01
Peak skin entrance dose (mGy)	2,372.0±2.80

Phantom skin dose

The ESD of the head phantom from coiling embolization is shown in Table 3. The ESD has a varied distribution across the head phantom. The peak ESD was found at O1 ($1,631.56 \pm 36.97$ mGy), followed by O2 ($1,513.49 \pm 88.75$

mGy), located at the back of the head phantom. The point that obtained the lowest radiation dose was Fp2 (40.01 ± 7.83 mGy), which was located at the front of the head phantom. The radiation dose at the left side of all points was higher than that of the right.

Table 3. The skin radiation dose of head phantom from coiling embolization procedure.

Point of measurement		Skin radiation dose (mGy)
1	Fp1 (L)	47.70 ± 15.11
2	Fp2 (R)	40.01 ± 7.83
3	F3 (L)	268.74 ± 13.45
4	F4 (R)	67.12 ± 5.24
5	F7 (L)	346.61 ± 25.30
6	F8 (R)	74.12 ± 5.46
7	Fz (C)	84.01 ± 55.01
8	Cz (C)	121.97 ± 1.11
9	Pz (C)	$1,027.04 \pm 8.53$
10	C3 (L)	477.41 ± 22.59
11	C4 (R)	166.32 ± 9.54
12	P3 (L)	$1,089.60 \pm 143.33$
13	P4 (R)	970.57 ± 80.80
14	T3 (L)	423.07 ± 0.18
15	T4 (R)	277.93 ± 5.48
16	T5 (L)	$1,190.94 \pm 167.99$
17	T6 (R)	$1,082.87 \pm 102.99$
18	O1 (L)	$1,631.56 \pm 36.97$
19	O2 (R)	$1,513.49 \pm 88.75$

Radiation dose of phantom critical organs

Table 4 presents the radiation dose at phantom critical organs. The highest radiation dose at these critical organ points was found at LE (59.97 ± 7.99 mGy), followed by RE

(47.18 ± 3.14 , 9.72 ± 1.27 mGy). The radiation dose on the left side of these points was slightly higher than on the right.

Table 4 The radiation dose to the critical organs of phantom from the coiling embolization procedure.

Location of measurement	Radiation dose (mGy)
LE	59.97 ± 7.99
RE	47.18 ± 3.14
LT	12.38 ± 1.54
RT	9.72 ± 1.27
LO	0.13 ± 0.01
RO	0.13 ± 0.01

Scatter radiation dose at staff working position

The scatter radiation dose at the staff working positions during the coiling embolization procedure is presented in Figure 4. Among the three positions exposed to radiation during fluoroscopy (IR, RR, SN), the IR position at 100 cm received the highest dose (0.193 ± 0.093 mSv), followed by the IR position at 150 cm (0.173 ± 0.020 mSv). The lowest scatter radiation dose was observed at the SN position at 150 cm (0.042 ± 0.018 mSv). At the IR position, the radiation

dose at the level of 50 cm was detected, with the lowest among all three levels. The overall radiation dose at RR and SN was lower than IR's. Although CN, AN, and RT positions were protected by shielding and distance from the radiation source, measurable radiation doses were still detected. The highest dose was recorded at the RT position at 150 cm (0.085 ± 0.007 mSv), while the lowest dose was observed at the CN position at 50 cm (0.043 ± 0.018 mSv).

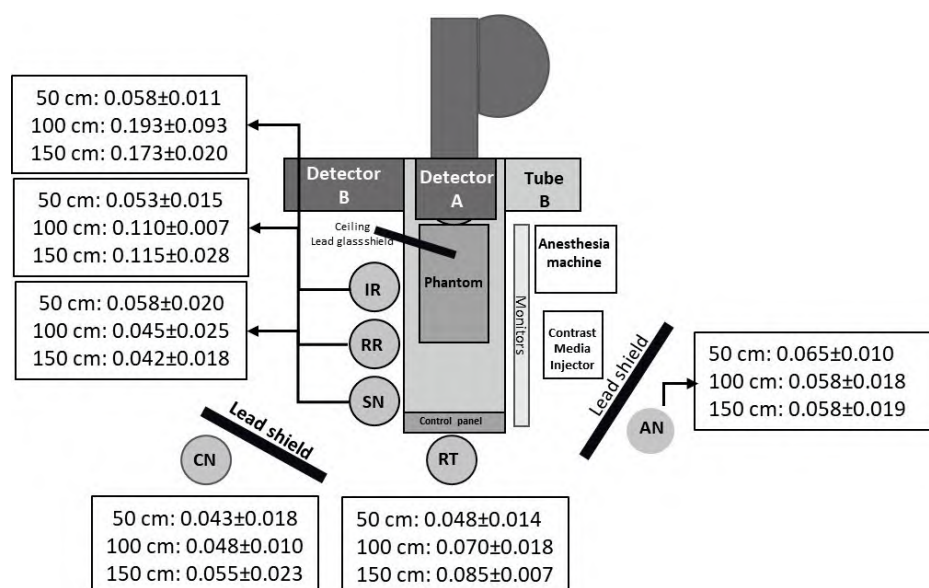


Figure 4. The scatter radiation dose distribution among 6 staffs positions and 3 levels on each position. The radiation dose is expressed as mSv.

Discussion

This study assesses radiation dose during coiling embolization in patients and medical staff using the OSLDs technology. These findings are relevant to our institute and can serve as important information in improving radiation protection strategies for patients and staff in clinical practice. A high dose was recorded at the back of the head (O1, O2 point), which aligned with the previous study's findings.^{8,25} It has been previously reported that the bi-plane DSA system produces a higher radiation dose for plane A DSA.²⁶ The angulation and the magnification of images of plane A DSA play a crucial role in the higher radiation dose of this plane.^{27,28} Since the radiation entered the head phantom from the back (plane A) and left side (plane B) while in a supine position, the left and back of the head phantom received higher radiation doses than the right and front, respectively.

The experimental data from the RDSR revealed that most of the patient's radiation dose exposure during the coiling embolization procedure originated from the 2DA technique, contributing to a significant skin radiation dose.^{25,29,30} The peak skin entrance dose observed ($>2,000$ mGy) suggests a need for optimizing the 2DA technique to minimize patient exposure and decrease the risk of radiation effects, particularly stochastic effects.⁵ This study's total DAP, reference air kerma, effective dose, and fluoroscopy time were similar to the previous report. Still, the peak

skin entrance dose was slightly higher than the reported study.⁹ Interestingly, the peak skin entrance dose from RDSR was higher than the information from nanoDot OSLD. The PSD from RDSR was obtained by multiplying reference air kerma with the conversion factor; hence, these data differed slightly. In addition, the angulation dependence of nanoDot OSLD has been reported,^{31,32} which might contribute an essential role in the difference in radiation dose between RDSR and nanoDot OSLD. When determining a patient's skin dose, an external dosimeter and radiation dose measurement from the RDSR should be considered.

Radiation exposures exceeding the threshold can result in tissue injury, leading to cell death. When the exposure surpasses the threshold, radiation-induced cell death may cause erythema, skin reactions, or hair loss.^{4,5} Several studies have reported that the back of patients' heads usually receives a higher radiation dose during fluoroscopic-guided procedures, leading to radiation effects such as hair loss.^{12,13,33}

A comparison of skin doses with previous reports is shown in Table 5. The skin doses at the back of the head of the phantom in this study were lower than that reported in other studies, where alopecia was observed following embolization of intracranial vascular abnormality. During coiling embolization, particularly in complex procedures, radiation exposure may exceed the threshold, leading to potential radiation consequences. Therefore, the INR unit

must establish a monitoring system to track and manage possible skin reactions in patients exposed to higher radiation levels. Additionally, efforts should focus on identifying and implementing optimization techniques to reduce patient radiation doses.

The critical organs, including eyes, thyroid, and ovaries, were also assessed to determine the safety of these organs. Since the eyes were in the radiation field, they received a higher radiation dose than the others. The threshold for radiation-induced cataracts has been reported as more than 1-2 Gy for acute exposure and 5-8 Gy for chronic exposures.³⁴ This study found that the maximum dose at eye level of the phantom was 59.97 ± 7.99 mGy, which was significantly lower than the threshold dose and remained within safety limits. The left eye and left thyroid received a higher radiation dose than the right side, which conformed with the study of Sánchez and colleagues.¹⁰ On the other hand, lower radiation doses were detected concerning the ovaries far from the radiation field. As reported previously, the angulation of nanoDot OSLD might affect low radiation dose detection.^{32,35}

Numerous studies have demonstrated the occupational dose of staff from interventional radiology units using different dosimeter types in clinical practice and phantom study.^{7,11,18,19,35,36} However, a few recent studies have focused on multidisciplinary staff exposure during neurointerventional procedures. Mussmann and colleagues have demonstrated radiation exposure to staff during INR procedures in clinical practice. Radiologists received the highest radiation dose, ranging from 0.5-218.6 μ Sv, while radiographers received lower doses, ranging from 0.1-51 μ Sv.³⁷ Other studies also reported that the radiologist received eye doses from therapeutic procedures of INR ranging from 13-289 μ Sv.³⁸

Although this was a phantom study, the findings on staff doses were consistent with those of previous research. In addition, this study extended its scope beyond radiologists and radiological technologists to include other staff members.

The difference in radiation dose among the three positions (IR, RR, and SN) across the three levels was attributed to two key radiation protection principles: distance from the radiation source and shielding system.³⁹ At 50 cm level, both IR and RR positions were shielded by a lead curtain placed at the side of the angiographic table. Although a ceiling lead glass shield was also used at the IR position, limitations such as its small size and the gap between the shield and the patient were noted. As a result, the radiation dose at 50 cm level was lower than at 100 and 150 cm levels. In this study, the radiation dose at 150 cm was used to estimate the eye lens exposure for radiologists. During the year of experimentation, coiling embolization was the most common procedure, with 72 cases. Although the calculated annual dose based on the number of cases per year (12.46 mSv/year) did not exceed the dose limit (20 mSv), further exposure from other endovascular treatment procedures may result in a higher eye lens dose than the limit. It has been reported that staff exposed to radiation have a higher risk of developing cataracts compared to those not exposed.^{40,41} Therefore, prioritizing using lead

glasses and improving radiation protection measures for radiologists are essential.

In contrast, at the SN position, the radiation dose was higher at 50 cm levels than at the other levels due to the absence of a lead curtain shield. Among RT, CN, and AN, the highest radiation dose was recorded at the RT position, which was closer to the radiation source. The radiation doses at 50 and 100 cm were used to estimate the skin dose at the leg and hand, respectively. The occupational dose limit for skin exposure is 500 mSv/year.⁴² The estimated annual skin dose at the IR position was 13.90 mSv/year (72 cases/year). Therefore, none of the staff received radiation doses that reached this limit. Nonetheless, developing efficient radiation protection strategies is essential to minimize radiation risk, particularly radiation-induced cancer, among all staff.

Moreover, certain limitations include the following. Although experimental parameters such as tube angulation, fluoroscopy time, number of images, and other conditions were held constant throughout the triplicate experiments, the standard deviation of radiation dose measurements in this study remained relatively high. This variability can be attributed to several factors. Firstly, scatter radiation consists of low-energy X-rays that are less energetic than the primary X-ray beam. Previous studies have demonstrated that low-energy radiation is associated with a higher percentage of measurement errors due to its increased sensitivity to environmental and equipment factors.⁴³ Furthermore, measurement variability is due to each OSLD's unique sensitivity, influenced by manufacturing batches and inherent material properties. To address these challenges, determining the precise energy of scatter radiation and calibrating OSLDs to match the specific radiation energy used could enhance the accuracy and reproducibility of dose measurements. Implementing such calibration techniques might mitigate the effects of sensitivity variability and reduce standard deviations in future investigations. Since the data for simulating coiling embolization were collected retrospectively from a dose report program, substantial details were missing, such as fluoroscopy magnification, tube angulation during coiling procedures, and the number of hand injections of contrast media by interventional radiology.

As a result, this experiment may not fully replicate clinical conditions. Factors influencing OSLDs measurements, such as using a phantom instead of real patients, may not accurately reflect the actual radiation doses patients receive. Additionally, the radiation dose at staff positions was measured using PVC pipes. Because a phantom equivalent was not used, only scatter radiation in the air was estimated, excluding backscatter radiation. Further research is required to address these limitations, such as a prospective study in a clinical setting using different types of dosimeters to monitor radiation doses or a phantom study that simulates the setting as closely as possible to an actual examination, as well as employing a tissue equivalent phantom as staff to include backscatter radiation.

Conclusion

The distribution of radiation dose varied across different staff positions. As the key person conducting the coiling embolization procedure, the IR position received the highest radiation dose. Hence, close monitoring and developing additional radiation protection methods for this position are essential. Although the radiation dose at the back of the head and eye was high, it remained within safety limits. Nevertheless, the monitoring of potential radiation effects should not be neglected. Lastly, the high radiation generated by the 2DA technique should be optimized to enhance its safety and efficiency.

Conflict of interest

The author declared no conflict of interest.

Acknowledgement

This study was granted by Faculty of Medicine Khon Kaen University, Thailand (IN63304).

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MALDI-TOF mass spectrometry in transfusion medicine

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ARTICLE INFO

Article history:

Received 27 January 2025

Accepted as revised 10 February 2025

Available online 17 February 2025

Keywords:

MALDI-TOF MS, SNP, blood group, blood transfusion, molecular genotyping.

ABSTRACT

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a valuable tool in clinical research. In transfusion medicine, DNA-based genotyping is increasingly replacing serological methods for precise blood group determination. MALDI-TOF MS platforms for SNP genotyping have been rigorously developed and validated against serological techniques. MALDI-TOF MS offers high-throughput capacity, exceptional multiplexing, and direct detection, resulting in high accuracy and flexibility. The ability to perform blood group genotyping, bacterial identification via peptide barcoding, and detection of hemoglobinopathies and hemoglobin adducts in both donors and recipients makes MALDI-TOF MS a promising technology for resolving complex blood typing cases and enhancing clinical utility in transfusion medicine.

Introduction

Blood typing by antibody-based methods has been the standard for determining ABO, Rh, and “extended” blood group antigens present on red blood cell membrane.¹ To date, 45 blood group systems containing 362 red cell antigens have been officially nominated by the Red Cell Immunogenetics and Blood Group Terminology of the International Society of Blood Transfusion.² Various antigens were identified in the different blood group systems. The antigens most commonly involved can be classified into the following categories: 1) red blood cell (RBC)-specific antigens; 2) human leukocyte antigens (HLAs), class I (HLA I) are present on all cells and shared by platelets and leukocytes and HLA II antigens are present on leukocytes that are antigen-presenting; 3) granulocyte-specific antigens; and 4) platelet-specific antigens (human platelet antigens [HPAs]).

Blood group genotyping began with the elucidation of the histo-blood group MN and ABO systems by Siebert and Fukuda,³ and Yamamoto *et al.*⁴ A blood group system is defined as a set of antigens controlled by one or more genes located at a single locus or by closely linked homologous genes with minimal or no recombination between them.² Most blood group antigens arise from single nucleotide polymorphisms (SNPs) at critical positions within the respective genes.⁵ However, the accurate identification of many blood group systems requires the consideration of multiple critical SNPs. Developing high-throughput SNP genotyping assays is crucial to effectively integrate molecular methods into transfusion medicine, enabling rapid and cost-effective screening. The emergence of

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doi: 10.12982/JAMS.2025.049

E-ISSN: 2539-6056

advanced genetic diagnostic technologies and the comprehensive characterization of genetic polymorphisms have ushered in a new era in transfusion medicine.⁶⁻⁸

Over the past two decades, Matrix-Assisted Laser Desorption/ionization-time-of-flight Mass Spectrometry (MALDI-TOF MS) has evolved from a research tool to a valuable clinical technology.⁹ Recent advancements demonstrate its successful application in mutation screening and genotyping. High-throughput SNP genotyping using MALDI-TOF MS holds promise as a routine method in both laboratory and clinical settings.^{10,11} This review explores the potential of MALDI-TOF MS as an alternative approach for identifying SNPs associated with blood group antigens.

Principle of MALDI-TOF MS

Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is a powerful analytical technique for analyzing complex samples.^{9,12} It consists of a MALDI ion source, a time-of-flight (TOF) mass analyzer, and a detector. The MALDI source ionizes sample molecules, transferring them to the gas phase. The TOF

analyzer measures these ions' mass-to-charge ratio (m/z), and the detector records ion abundance at each m/z value.

Sample preparation involves mixing the analyte with a suitable matrix and co-crystallizing the mixture on a specialized sample plate. Matrix selection and spotting methods are crucial and depend on factors like target mass range, sample peak characteristics, and preliminary analyses. Optimizing matrix concentration is also critical. Standard MALDI matrices include low molecular weight organic acids such as α -cyano-4-hydroxycinnamic acid, 2,5-dihydroxybenzoic acid, and 3-hydroxypicolinic acid.¹³

The prepared sample plate is then introduced into the mass spectrometer's high-vacuum environment (pressure $<10^{-7}$ Torr). Laser energy absorbed by the matrix causes desorption and ionization of the analytes. These ions are accelerated through an electrostatic field within the flight tube. Smaller ions travel faster than larger ions, separating them based on their m/z ratios. The time of flight (TOF) for each ion to reach the detector is proportional to its mass and charge, generating a unique spectral profile of peaks characteristic of the analyzed species (Figure 1).

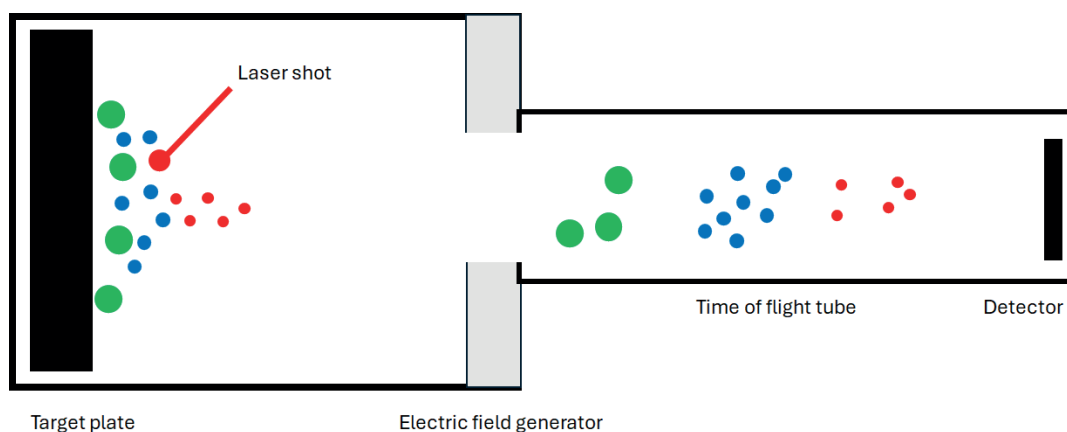


Figure 1. Basic principles of MALDI-TOF MS. A sample is mixed with a suitable matrix compound and ionized by a laser. The resulting ions are accelerated through a flight tube; their time-of-flight is proportional to their mass-to-charge ratio. This allows for precise mass determination and identification of the molecules.

MALDI-TOF MS is a versatile analytical platform that enables rapid data acquisition. The instrument allows for the simultaneous analysis of hundreds of individual samples on a single target plate following offline sample preparation. Moreover, MALDI-TOF MS can be applied directly to complex samples or following sample clean-up/enrichment procedures, enabling the analysis of a wide range of molecular classes, including intact proteins, peptides, lipids, nucleotides, nucleosides, carbohydrates, and small molecules. The user-friendly nature of MALDI-TOF MS instrumentation enables its operation by relatively unskilled personnel, making it well-suited for routine clinical applications. This versatility is further supported by many successful applications in diverse diagnostic settings, as evidenced by previous studies.^{9,13,14}

MALDI-TOF MS-based SNP genotyping

Single-nucleotide polymorphisms (SNPs) are the most common form of human genetic variation, occurring frequently throughout the genome.¹⁵ These variations, defined as having a minor allele frequency of at least 1%, can significantly impact phenotypes by altering gene transcription or protein function. SNPs occur on average every 500-1,000 base pairs. SNPs can result in synonymous or non-synonymous amino acid substitutions depending on their location and the specific nucleotide change. SNPs in non-coding regions can also influence gene expression by affecting regulatory elements. Even synonymous SNPs can impact gene expression by affecting splicing. Therefore, SNP screening in association studies provides valuable insights into disease susceptibility, clinical diagnosis, prognosis, and forensic investigations.

MALDI-TOF MS was first applied to oligonucleotide analysis in 1990.¹⁶ Since then, optimized matrices (e.g., 3-hydroxy-picolinic acid) and matrix additives (e.g., sugars,¹⁷ spermine¹⁸) have been developed to enhance nucleic acid ionization. Sample preparation techniques, such as miniaturizing sample spot sizes, have also improved the homogeneity of matrix-analyte crystals and the reproducibility of mass spectra.¹⁹ Cation exchange resins are used to remove salts from nucleic acid samples, which can interfere with MALDI ionization.²⁰

MALDI-TOF MS-based SNP genotyping offers a distinct advantage through its primer extension and data analysis strategy. Primers are designed to hybridize immediately upstream of the SNP site. Unincorporated nucleotides are removed from PCR products using shrimp

alkaline phosphatase (SAP). A single-base extension reaction is performed, incorporating one base (typically a dideoxynucleotide, ddNTP) at the SNP site.²¹ These ddNTPs have unique masses. MALDI-TOF MS measures the mass of the extended primer, and the mass difference identifies the incorporated ddNTP and, thus, the SNP base. The instrument measures the mass-to-charge ratio of the extended primers, generating a mass spectrum with peaks corresponding to the different SNP alleles.²² High mass accuracy (typically within 5 ppm) is achieved using external calibration and/or internal standards. This technique allows for parallel analysis of thousands of SNPs in a single sample. Integrating PCR and MS enables the detection and characterization of genomic DNA, even from limited sample quantities (Figure 2).

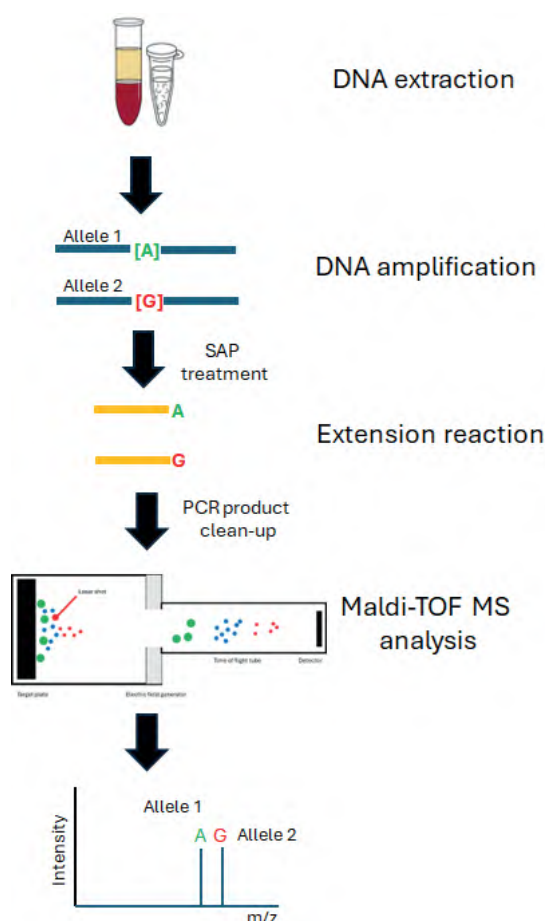


Figure 2. Single nucleotide polymorphism analysis was performed using MALDI-TOF mass spectrometry. Targeted regions were amplified via a multiplex polymerase chain reaction (PCR) assay. Unincorporated nucleotides were neutralized from the PCR products through shrimp alkaline phosphatase (SAP) treatment. A single-base extension reaction was subsequently conducted to extend the PCR fragments at the SNP site. The resulting extended fragments were desalted before mass measurement using MALDI-TOF MS, generating a spectrum of distinct mass peaks.

Specialized software analyzes these spectra, identifying peaks, correlating them to specific masses, and assigning SNP genotypes. MALDI-TOF MS also facilitates high-throughput genotyping through multiplexing, enabling simultaneous analysis of multiple SNPs in a single reaction.^{23,24} Multiplexing is achieved by designing primers with varying lengths or incorporating mass-modifying tags.

Previous studies have demonstrated the utility of MALDI-TOF MS in SNP genotyping for various applications. For example, Nakai *et al.* (2004) investigated ethnic variations in coronary artery disease-associated SNPs.²⁵ Shi *et al.* (2011) screened 50 SNPs in CYP genes within the Chinese Han population, identifying polymorphic SNPs with allele frequency differences compared to Caucasians, highlighting potential forensic applications.²⁶ Xu *et al.* identified SNPs associated with lung cancer patient survival, suggesting potential prognostic value.²⁷ Zhang *et al.* (2025) developed a MALDI-TOF MS-based SNP assay to investigate the genetic basis of poor antidepressant efficacy, aiming to predict individual drug response variations.²⁸

MALDI-TOF MS-based blood group genotyping

Serological methods based on red blood cell agglutination are inexpensive and widely available but can be subjective, time-consuming, and prone to ambiguous results, especially with weak or variant antigen expression.^{29,30} Serology also has limitations in identifying all clinically relevant blood group alleles, particularly in complex genetic backgrounds.^{31,32} Furthermore, the absence of specific antibodies for certain antigens, especially rare ones, prevents their accurate serological detection.³³⁻³⁶

Molecular blood group genotyping advanced significantly with the cloning of *GYPA* (MN blood group system) in 1987,³ followed by the ABO and Rh blood group system genes in 1990 and 1992, respectively.^{4,37} Subsequently, other blood group system genes were cloned. Analysis revealed that single nucleotide polymorphisms (SNPs) are the primary source of allelic variation.⁵ Characteristic SNPs identify specific alleles. Other mechanisms contributing to molecular variation include gene or exon deletions, sequence duplication with nonsense mutations, hybrid gene formation, and exon duplication.

Molecular diagnostic techniques address the limitations of serology. Methods like PCR-SSP, PCR-SBT, BeadChips, MALDI-TOF MS, and next-generation sequencing (NGS) offer more comprehensive and accurate antigen identification.³⁸⁻⁴² Determining the nucleotide state (homozygous or heterozygous) at the SNP position often accurately predicts the phenotype (typically 99%).

PCR-SSP offers improved accuracy and resolution compared to serology, particularly for variant allele identification using sequence-specific primers.^{31,32,43} However, it requires specialized equipment and personnel, and the number of testable alleles is limited. PCR-SBT, using Sanger sequencing (the gold standard for genotyping), provides comprehensive information by identifying a broad range of sequence variations.⁴⁴ Its cost and turnaround time limit high-throughput screening.⁴⁵ BeadChip technology allows simultaneous identification of multiple antigens,³⁸ but is susceptible to false results due to factors like DNA quality and cross-reactivity.⁴⁶ Equipment and assay development costs can also be substantial. NGS enables high-throughput identification of diverse sequence variations, resolving complex serological results, identifying rare alleles, and facilitating high-throughput donor screening.⁴⁷ However, NGS is constrained by high cost, complex data analysis, challenges in detecting large structural variations, and turnaround time.⁴⁸

MALDI-TOF MS-based genotyping, combining PCR amplification and mass spectrometry, facilitates comprehensive blood group profile analysis. This platform detects 261 antigens across 38 blood group systems (including ABO, Rh, Kell, Duffy, and others), as well as multiple human platelet antigens (HPA), human neutrophil antigens (HNA), and human leukocyte antigen (HLA) types.⁴⁹ It offers a balance of accuracy, sensitivity, speed, and throughput, allowing simultaneous analysis of multiple SNPs and identification of a broad range of alleles. While requiring initial capital investment, MALDI-TOF MS can reduce per-sample costs for large-scale studies.^{50,51} Applications range from routine blood typing to research, enhancing transfusion safety and efficacy and furthering our understanding of blood group genetics (Figure 3).

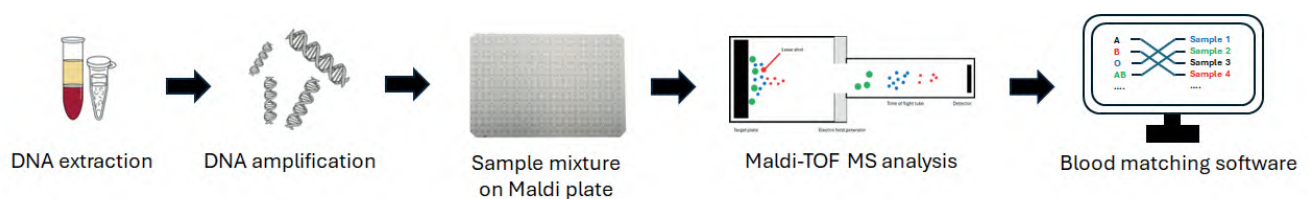


Figure 3. Blood Group Genotyping Workflow by MALDI-TOF MS. MALDI-TOF MS-based genotyping utilizes a streamlined workflow encompassing three crucial steps: multiplex PCR amplification of target regions, allele-specific single-base primer extension, and subsequent mass spectrometric analysis.

Rapid blood group genotyping in transfusion and transplantation medicine using MALDI-TOF MS

Blood transfusions are essential for treating trauma, anemia, surgical procedures, and chronic illnesses like cancer. Accurate and timely blood typing is critical for transfusion safety. Current pre-transfusion testing, primarily ABO/RhD typing and antibody screening, is vital for preventing most fatal hemolytic transfusion reactions (HTRs). However, current methods risk alloimmunization, where recipients develop an immune response to donor antigens,⁵² potentially leading to clinical complications. Identifying and testing for antigens lacking specific serological reagents represents a significant advance, enabling the identification of compatible donor units and potentially saving lives.

MALDI-TOF MS-based blood group genotyping, encompassing red blood cell, human platelet antigen (HPA), human neutrophil antigen (HNA), and human leukocyte antigen (HLA) typing, allows the selection of donor units antigen-matched to recipients beyond the traditional ABO/RhD system. This improves transfusion safety by reducing complications and providing more comprehensive information.⁵³ It is especially important for chronically transfused patients, minimizing alloantibody development and improving outcomes.⁵⁴⁻⁵⁷ MALDI-TOF MS genomic testing is also crucial for donor screening to identify rare Rh variants, ensuring the availability of these essential blood components.^{54,56}

MALDI-TOF MS-based antigen genotyping has applications beyond transfusion medicine. In prenatal care, it aids in assessing fetal and neonatal disease risk and informs Rh-immune globulin prophylaxis decisions. In transplantation, it assists in selecting compatible bone marrow donors, optimizing transfusion support for alloimmunized patients, and confirming A2 status in kidney donors. In hematology, it enables comprehensive typing for patients with chronic transfusion-dependent anemias. Finally, in oncology, it addresses challenges posed by monoclonal antibody therapies that interfere with standard pre-transfusion testing.

Applications of MALDI-TOF mass spectrometry in enhancing transfusion safety and efficacy

While lifesaving, blood transfusions carry a risk of infection transmission, with profound implications for patient health, survival, and healthcare costs.⁵⁸ These infections result from contamination of blood products with bacteria, viruses, or parasites during collection, processing, or storage. Rapid contaminant detection is crucial for transfusion safety. Bacterial contamination is a significant concern, particularly in platelets stored at room temperature. MALDI-TOF MS is a valuable tool for identifying bacterial species in blood components by analyzing peptide profiles (Figure 4).⁵⁹⁻⁶¹ It can also investigate bacterial antibiotic resistance and characterize antimicrobial compounds.^{62,63} This technology facilitates rapid microbial contamination detection, preventing transfusion of contaminated blood and improving safety.

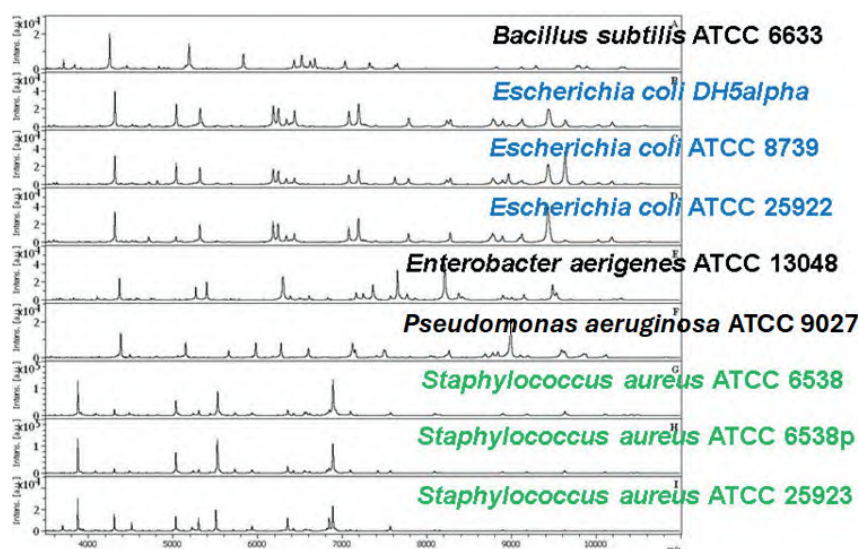


Figure 4. Bacterial identification by MALDI-TOF MS.

MALDI-TOF MS enables simultaneous detection of hemoglobinopathies (including α - and β -thalassemia) and pre-diabetes from a single blood drop.^{64,65} Hemoglobinopathies are identified by the mass difference between α -globin and β -globin peaks ($D\alpha\beta$), with α - and β -thalassemia distinguished by the β -globin to α -globin ratio ($R\beta\alpha$). Pre-diabetes screening is performed by quantifying glycated α -globin (αGlc).⁶⁶ MALDI-TOF MS

also shows potential for detecting other hematological conditions, such as sickle cell disease, blood poisoning, the impact of air pollution, and specific hemoglobinopathy and thalassemia variants.^{64,66,67}

Hemoglobin adducts, formed by hemoglobin binding to molecules like drugs, toxins, or pollutants, can persist for up to 120 days,⁶⁸⁻⁷⁰ making them valuable biomarkers for assessing long-term chemical exposure. These structural

changes may affect hemoglobin stability and oxygen affinity,⁷¹ making high adduct levels relevant to transfusion safety due to potential harm.⁷² MALDI-TOF MS can detect chemically modified hemoglobin.^{64,73,74} Hemoglobin is susceptible to S-glutathionylation, and increased levels of glutathionyl hemoglobin (HbSSG) are observed in various pathological conditions, serving as a biomarker for oxidative stress.^{69,70} MALDI-TOF MS measurement of HbSSG can indicate oxidative stress in blood products,⁷⁵ potentially linked to increased TRALI risk in transfused patients.⁷⁶ Furthermore, hemoglobin glutathionylation increases oxygen affinity, reducing tissue oxygen delivery.

Conclusion

MALDI-TOF MS blood group genotyping offers advantages over traditional serological methods, including high-throughput automation, improved accuracy, and resolution of ambiguous phenotypes. Widespread implementation of rapid, cost-effective MALDI-TOF MS genotyping for clinically relevant blood groups, bacterial identification via peptide barcoding, and detection of hemoglobinopathies and hemoglobin adducts in both donors and recipients could significantly reduce alloimmunization and improve transfusion safety and efficacy.

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Development of dysphagia screening questionnaire for Thai older adults

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ARTICLE INFO

Article history:

Received 4 September 2024

Accepted as revised 17 February 2025

Available online 19 February 2025

Keywords:

Screening tool, oropharyngeal dysphagia, aging, clinimetric properties.

ABSTRACT

Background: Oropharyngeal dysphagia is a common condition in the older person and can lead to serious complications. In Thailand, there is currently no standardized dysphagia screening tool for gathering preliminary information about clients for early evaluation and intervention.

Objective: This study aimed to develop a dysphagia risk screening tool for older persons and investigate its psychometric properties. This study examined the content validity, criterion validity, and test-retest of the Geriatric Dysphagia Risk Screening Tool (GDRST).

Materials and methods: 26 questions were developed from literature review and expert opinion. Five expert occupational therapists examined content validity. The Index of Congruence (IOC) was calculated from five experts, and the items that showed IOC scores of over 0.5 were incorporated into the target screening tool. Thirty older adults who had a risk of dysphagia (Mean $\pm$ SD, 66.13 $\pm$ 4.66 years) participated in the study. The internal consistency was assessed using Cronbach's alpha. An intraclass correlation coefficient (ICC) was used to examine the test-retest reliability of the screening tool. The criterion validity of the GDRST was evaluated by investigating the relationships between a score of the Functional Oral Intake Scale (FOIS) and the Swallowing Quality Assessment Form for Occupational Therapists (SQAOT) using Spearman's correlation coefficients.

Results: Based on the literature review and occupational therapy expert opinions, the screening tool covers three domains, including the cognition domain, oral phase swallowing ability domain, and pharyngeal phase swallowing ability domain. Content validity IOC scores were between 0.6 and 1.0 for all items. The internal consistency of the GDRST was high, with 0.971 Cronbach's alpha for the test. The screening tool had excellent test-retest reliability on all items ($p < 0.05$). The score of the GDRST was significantly correlated with the score of FOIS ($r = -0.468$, $p = 0.009$), and SQAOT ($r = -0.705$, $p = 0.000$).

Conclusion: The GDRST demonstrated excellent reliability and validity against the clinical assessment. It was simple to administer and could be beneficial for screening older people in the community for dysphagia.

Introduction

Thailand has entered an aging society, with the elderly population in 2023 accounting for 20.92% of the total population.¹ Dysphagia occurs mainly in elderly people due to multiple age-related changes in the neural and physiological mechanisms controlling swallowing. As individuals age, there is a decline in body structure and function, including decreased sensory perception of taste, temperature, and food texture, reduced saliva production, tooth loss, impaired coordination of lip and tongue muscles, decreased muscle strength for chewing and swallowing, and reduced strength

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doi: 10.12982/JAMS.2025.050

E-ISSN: 2539-6056

of pharyngeal muscle contractions. Additionally, delayed opening of the upper esophageal sphincter affects the ability to swallow food.²

Oropharyngeal dysphagia is difficulty forming or safely moving a food bolus from the oropharynx to the cervical esophagus due to dysfunction in the oropharyngeal phase of swallowing.³ The prevalence of oropharyngeal dysphagia is between 11.4%-13.7% among older people living independently and 36.5%-50.2% among older people living in care facilities.^{4,5} The occurrence of dysphagia in the elderly can lead to a decreased appetite, fear or anxiety about swallowing, and reduced food intake, resulting in various complications such as malnutrition, weight loss, dehydration, suffocation, and aspiration pneumonia.^{2,6} These complications significantly impact the quality of life of the elderly.

Dysphagia in older individuals often goes undetected until severe symptoms or complications like undernutrition or pneumonia arise. Screening for swallowing difficulties in communities or elderly care facilities is not widely practiced, as these individuals often perceive difficulties in eating due to abnormalities in both the oral and pharyngeal phases as a natural part of aging. Consequently, older adults may choose to endure dysphagia or employ compensatory strategies such as selecting specific foods or reducing food intake, which can lead to malnutrition and increase the risk of complications.^{2,6} Early identification of dysphagia risk allows for early clinical assessment and intervention, which can delay the progression of dysphagia and reduce morbidities.

Instrumental and clinical assessments conducted by professionals specializing in swallowing rehabilitation and using standardized methods can evaluate functional impairments in swallowing-related components and overall swallowing ability.^{7,8} However, due to time and budget constraints, clinical assessments have limitations in community settings, making self-report questionnaires a valuable tool for screening dysphagia in older adults.^{7,9,10} The Eating Assessment Tool-10 (EAT-10) is the dysphagia screening tool widely used in many countries.^{11,12} The EAT-10 has been translated into the Thai version and used as a screening tool for dysphagia. Although the Thai version of EAT-10 has high reliability and validity for screening dysphagia in adult populations who have pathological conditions, its psychometric properties have not been tested for use with the aging population.^{13,14} Specifically, the EAT-10 is designed to evaluate the severity of dysphagia in symptomatic individuals and does not address the factors reportedly contributing to swallowing limitations in this population (e.g., cognitive and physical decline).¹⁵ Therefore, the dysphagia screening tool for older individuals is still limited.

Swallowing problems in the geriatric population coexist and can be undetectable with a variety of degenerative issues in the elderly. The Geriatric Dysphagia Risk Screening Tool (GDRST) in this study is a self-report questionnaire that screens for the elderly's cognitive and oropharyngeal aspects of swallowing performance through questions related to eating behaviors in daily life. The frequency of abnormal behavior in each item is given as a score, with

a higher score showing poor performance. Therefore, the advantage of GDRST is that it is a rapid screening tool that can be done at home or in the community. Each item's score reports the clients' clinical symptoms that provide information to therapists in selecting client-specific clinical assessments, prevention, and intervention in clinical settings. Early recognition of oropharyngeal dysphagia symptoms is highly beneficial in screening for swallowing difficulties in community-dwelling older adults before they become severe. Therefore, this study aims to develop a screening tool for the early identification of dysphagia risk in older persons and test its psychometric properties. It is expected that this tool could be part of an early screening process in the community or even in elderly care facilities to facilitate early assessment and intervention for older adults at risk of oropharyngeal dysphagia.

Materials and methods

This study was divided into two stages. The first stage was the development of the questionnaire based on literature reviews and testing the content validity of the screening tool. The second stage was the study of the internal consistency, test-retest reliability, and criterion validity of the GDRST.

Participants

In the first stage, the research team consisted of three occupational therapists with at least five years of experience working in swallowing rehabilitation, conducting the items of the GDRST questionnaire version-1 based on dysphagia symptoms in older persons. Afterward, five registered occupational therapists with at least five years of experience working in swallowing rehabilitation were invited to participate in testing the questionnaire's content validity. The GDRST was revised and used to examine its psychometric properties in the second stage.

In the second stage, participants were recruited from the community in Bangkok and its surrounding areas through a web-based platform and promotional posters between April 2023 and April 2024. Once interested elderly persons applied to participate in this study, they were screened based on the inclusion and exclusion criteria. Thirty participants who met the criteria participated in the pilot study. Participants were asked to provide informed consent before data collection.¹⁶ The inclusion criteria were an older person aged 60-75 years, at risk of dysphagia as screened by an EAT-10 score >3 and able to understand and respond to the screening tool independently.¹¹ The exclusion criteria were participants having a diagnosed medical condition that acutely affects swallowing ability, such as a stroke or head and neck cancer.

Procedure

First stage: Development of the Geriatric Dysphagia Risk Screening Tool

A literature review was conducted to identify relevant theoretical frameworks related to oropharyngeal dysphagia in older adults. In this population, swallowing undergoes various changes, influenced by cognitive impairments

affecting eating, oral phase swallowing disorders, and pharyngeal phase swallowing disorders. All these factors were considered during item development. The research team developed items based on these factors. The initial version of the screening tool (version 1) consisted of 22 items representing three domains derived from the conceptual framework: 1) cognition (5 items), 2) oral phase swallowing ability (7 items), and 3) pharyngeal phase swallowing ability (10 items). The scoring criteria were based on the frequency of behavior occurrence. Each item had four response options: never=0, rarely=1, sometimes=2, and often=3. Five occupational therapists with over five years of experience in swallowing rehabilitation were asked to assess content validity. The congruence index (IOC) was analyzed, and items with an IOC of 0.5 or higher were accepted. At the same time, those with an IOC below 0.5 were revised based on expert feedback, leading to the refinement of the screening tool.

Second stage: Study the psychometric properties

Thirty older persons participated in the evaluation of internal consistency, test-retest reliability, and criterion validity. The internal consistency reliability was examined to determine the contribution of each item to the overall questionnaire. To evaluate test-retest reliability, the GDRST was completed by the same participants on two occasions, separated by one week. The one-week period was selected because no change in the swallowing process was expected to take place within this period. The intraclass correlation coefficient (ICC) value was calculated for test-retest reliability.

The validity of an outcome measure is the ability of an instrument to predict the participants' swallowing performance. The criterion validity of the GDRST was determined by assessing the correlation between it and clinical assessments, including the Functional Oral Intake Scale (FOIS) and the Swallowing Quality Assessment Form for Occupational Therapists (SQAOT).

The FOIS assesses swallowing ability based on the type or category of food that can be swallowed. It is divided into 7 levels, with a score of 1 indicating no oral intake and a score of 7 indicating total oral intake without restriction. The FOIS is a reliable and valid tool compared with the Videofluoroscopic Swallowing Study and Fiberoptic Endoscopic Evaluation of Swallowing.¹⁷

The SQAOT is a clinical assessment tool occupational therapists use to evaluate swallowing quality.¹⁸ The SQAOT has a maximum total score of 29 points, consisting of assessments in 6 areas: 1) diet/liquid intake (2 points), 2) progression of food intake (5 points), 3) progression of liquid intake (5 points), 4) quantity of oral feeding (2 points), 5) improvement of oro-motor function (9 points), and 6) oral reflex (6 points). A lower score indicates that the patient has less ability in that area.

Statistical analysis

Descriptive statistics were presented to describe demographic data. The Kolmogorov-Smirnov test was used to test the participants' normality of age and score of EAT-10. The internal consistency of each item was measured using Cronbach's alpha. The intraclass correlation coefficient (ICC) model 3,1 was used to examine the test-retest reliability. The Spearman coefficient was used to assess the correlation between the GDRST, FOIS, and the SQAOT to determine criterion validity. The significant level was set as 0.05. All statistical analyses were conducted using SPSS statistical package version 18.

Results

First stage: Development of screening tools

The GDRST version 1 had an IOC score between 0.2 and 1.0. Six items had an IOC score less than 0.5: 4 items in the Cognition domain, 1 item in the Oral phase swallowing ability domain, and 1 item in the Pharyngeal phase swallowing ability domain. Additionally, the experts suggested adding 2 more items to the Cognition domain and 2 more to the Oral phase swallowing ability domain. As a result, the GDRST version-2 comprised 26 items: 7 items in the Cognition domain, 9 in the Oral phase swallowing ability domain, and 10 in the Pharyngeal phase swallowing ability domain. The total score was 78 points. Subsequently, the GDRST version-2 was sent to experts for another round of IOC score review, with scores for all 26 items ranging from 0.6 to 1.0. After a trial with ten older participants, no issues were reported, and all participants could follow the screening instructions. This is the final version of the GDRST, as shown in Table 1.

Table 1. Domain and items of the Geriatric Dysphagia Risk Screening Tool final version and IOC scores.

Domains	Items	Question	Index of congruence
Cognition (7 items)	1	You experience low alertness or drowsiness while eating.	0.6
	2	You lack focus, are easily distracted, or have difficulty paying attention while eating.	0.6
	3	You take bites of food that are too large to swallow completely.	1
	13	You hold food in your mouth and need to be reminded to chew or swallow.	1
	24	You eat less due to difficulties with chewing and swallowing.	1
	25	You need to be reminded when it's mealtime or forget that you've already eaten a meal.	1
	26	You are afraid to swallow food or liquids due to choking or aspiration.	1
Oral phase performance (9 items)	5	Chewed food particles fall out of your mouth while eating.	1
	6	You feel like you cannot chew your food thoroughly.	1
	7	You experience dry mouth and have difficulty chewing and swallowing due to insufficient saliva.	1
	8	You feel like food is stuck in your cheeks, under your tongue, or on the roof of your mouth after swallowing.	1
	9	You have difficulty swallowing food, feel like it's getting stuck, have to exert a lot of effort to swallow, or need to swallow multiple times.	0.6
	10	You have difficulty swallowing solid foods or pills.	1
	11	Liquid comes out of your nose when you drink.	1
	12	You often bite your cheek or tongue while chewing.	1
	14	You have difficulty eating foods with a liquid consistency, such as porridge.	1
Pharyngeal phase performance (10 items)	4	You need to use a neck support device while eating	0.8
	15	You feel like food is stuck in your throat or want to cough after swallowing.	1
	16	You are unable to clear your throat or cough when you need to.	1
	17	You experience difficulty breathing after swallowing.	0.8
	18	You cough or choke while swallowing saliva.	1
	19	You cough or choke while swallowing liquids.	1
	20	You cough or choke while swallowing food.	1
	21	Your voice changes, becomes hoarse, or raspy immediately after swallowing food or liquids.	1
	22	You experience pain in your throat while swallowing.	1
	23	You feel a burning sensation in your chest or upper abdomen during or after eating.	1

Second stage: Study the psychometric properties

The participants consisted of 30 older persons who were at risk of dysphagia (EAT-10 scores >3). The mean age was 66.13±4.66 years, and the EAT-10 scores were 9.76±7.17. GDRST score was 14.67±10.01. The sample group's normality test results show a normal distribution. Table 2 presents the participants' demographic data.

The internal consistency of the GDRST was excellent consistency with a Cronbach's alpha value of 0.971. The ICC value of all items ranged between 0.828 and 1.00 indicated that the instrument is excellent test-retest reliability, as shown in Table 3.

Table 2. Demographic information of participants.

Variables	Participant (N=30)
Sex [N (%)]	
Male	8 (26.67)
Female	22 (73.33)
Age (years) [Mean (SD)]	66.13 (4.66)
Education level [n (%)]	
Elementary Level	20 (66.67)
Secondary Level	4 (13.33)
Bachelor Level	4 (13.33)
Higher than bachelor Level	2 (6.67)
Dental orthosis [n (%)]	
Yes	14 (46.67)
No	16 (53.33)
EAT-10 score [Mean (SD)]	9.76 (7.17)
GDRST score [Mean (SD)]	14.67 (10.01)
FOIS score [Mean (SD)]	6.27 (0.91)
SQAOT score [Mean (SD)]	22.07 (3.94)

Note: N: number, SD=standard deviation, EAT-10: Eating Assessment Tool-10, GDRST: Geriatric Dysphagia Risk Screening Tool, FOIS: Functional Oral Intake Scale, SQAOT: Swallowing Quality Assessment Form for Occupational Therapist.

Table 3. The test-retest reliability of the Geriatric Dysphagia Risk Screening Tool.

Items	Time 1 Mean (SD)	Time 2 Mean (SD)	ICC	95%CI
1	0.20 (0.40)	0.17 (0.38)	0.892	0.786-0.947
2	0.20 (0.48)	0.13 (0.43)	0.848	0.705-0.925
3	0.33 (0.76)	0.30 (0.70)	0.969	0.936-0.985
4	0.00 (0.00)	0.00 (0.00)	1.000	1.000-1.000
5	0.43 (0.72)	0.43 (0.72)	1.000	1.000-1.000
6	1.53 (0.82)	1.77 (0.82)	0.862	0.730-0.932
7	0.73 (0.91)	0.8 (0.99)	0.927	0.852-0.964
8	0.80 (1.06)	0.86 (1.00)	0.938	0.874-0.970
9	0.90 (1.09)	0.90 (1.06)	0.970	0.939-0.986
10	1.20 (1.09)	1.02 (1.06)	0.970	0.939-0.986
11	0.20 (0.48)	0.27 (0.64)	0.900	0.801-0.951
12	0.73 (0.82)	0.83 (0.91)	0.939	0.875-0.970
13	0.10 (0.40)	0.10 (0.40)	1.000	1.000-1.000
14	0.06 (0.25)	0.06 (0.25)	1.000	1.000-1.000
15	0.80 (0.92)	0.80 (0.92)	0.960	0.917-0.981
16	0.17 (0.46)	0.10 (0.40)	0.828	0.670-0.914
17	0.07 (0.25)	0.07 (0.25)	1.000	1.000-1.000
18	0.97 (0.80)	1.03 (0.85)	0.953	0.9.4-0.977
19	1.00 (1.02)	1.03 (0.96)	0.913	0.825-0.958
20	0.70 (0.79)	0.83 (0.87)	0.914	0.828-0.958
21	0.43 (0.81)	0.40 (0.86)	0.927	0.852-0.965
22	0.27 (0.69)	0.23 (0.68)	0.965	0.927-0.983
23	0.73 (0.91)	0.73 (0.94)	0.960	0.917-0.981
24	0.70 (0.87)	0.70 (0.87)	1.000	1.000-1.000
25	0.20 (0.48)	0.20 (0.48)	1.000	1.000-1.000
26	0.33 (0.61)	0.37 (0.67)	0.959	0.916-0.980
Total	13.80 (9.67)	14.33 (9.40)	0.985	0.969-0.993

The results found a significant negative moderate correlation between the GDRST and FOIS scores ($r=-0.468$, $p=0.009$). The scatter plot between the GDRST and the

FOIS scores is shown in Figure 1. Additionally, the GDRST score strongly correlated with the SQAOT score ($r=-0.705$, $p=0.000$), as depicted in the scatter plot in Figure 2.

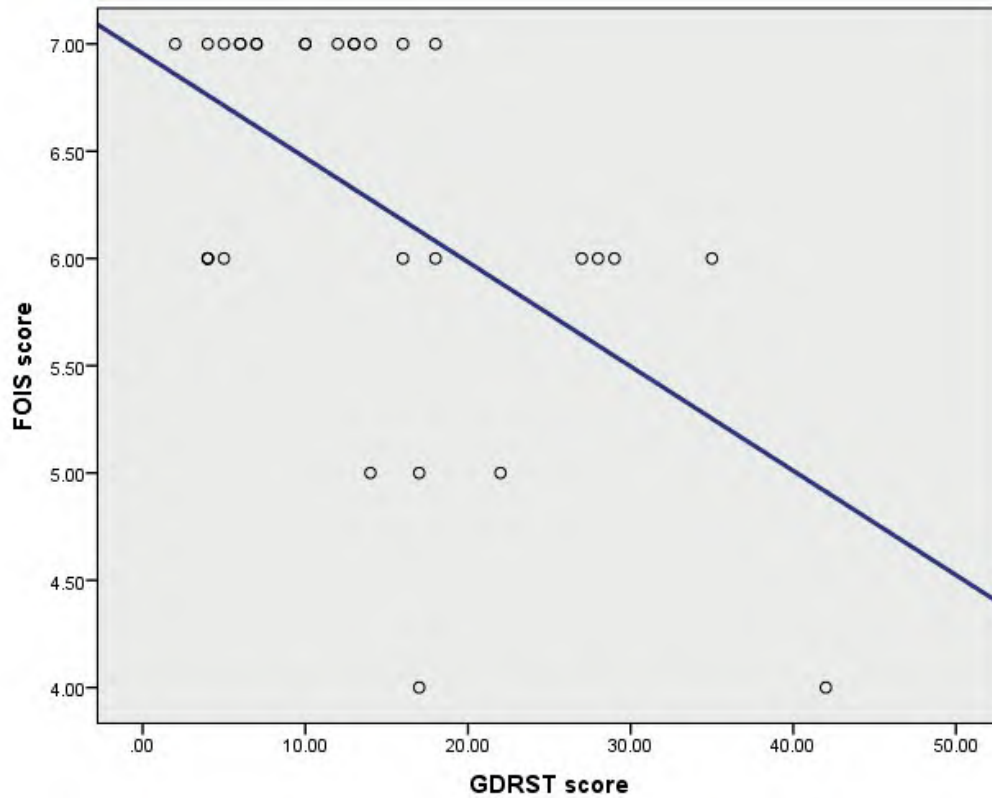


Figure 1. Correlations between GDRST and FOIS score.

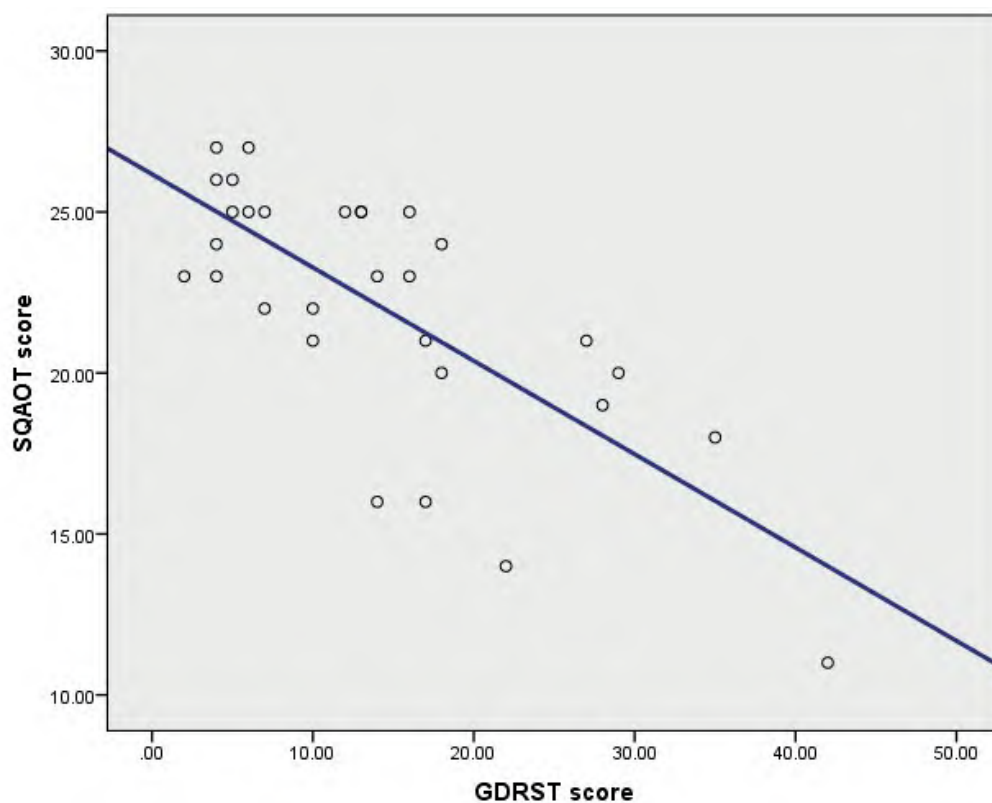


Figure 2. Correlations between GDRST and SQAOT score.

Discussion

The main finding of this study is that the GDRST questionnaire proved to be a helpful screening tool for detecting dysphagia in older adults. Preliminary data and psychometric analyses demonstrated excellent internal consistency and test-retest reproducibility. Furthermore, the GDRST scores correlated with clinical performance as assessed by the FOIS and the SQAOT.

The presence of swallowing dysfunction in older individuals tends to progressively worsen with age and the natural process of physical decline. Unfortunately, these symptoms often go unreported by older adults or remain undetected by therapists until severe symptoms or complications, such as malnutrition and aspiration pneumonia, manifest.^{2,19} Early detection of oropharyngeal dysphagia in older persons is crucial for prevention and reducing the risk of complications. However, standard instrumental approaches and clinical testing require high assessment costs or specialized expertise for definitive diagnosis, making them unsuitable for widespread community screening. Therefore, having a reliable screening tool for identifying older individuals at risk of dysphagia would facilitate appropriate referrals for further specific investigations. The GDRST is a self-report, symptom-specific instrument for oropharyngeal dysphagia. It can be administered quickly and is suitable for older people. The questionnaire addresses factors related to the swallow functional decline of older individuals, including decline in oral function abilities, pharyngeal function abilities, and cognitive ability.²⁰⁻²² The items reflect the pre-clinical symptoms of dysphagia that manifest in both daily life and frequency in the elderly population.

There is limited research on the reliability of screening tools used to identify dysphagia in the elderly population in Thailand. This is because commonly used screening tools from other countries are often in English, which can be challenging for Thai elderly individuals due to language barriers. The EAT-10, although translated into Thai and widely used for dysphagia screening in Thailand, has its limitations. The Thai version of EAT-10 has not been tested for reliability and validity in the elderly population. Additionally, the EAT-10 focuses on the severity of the impact of dysphagia, not specifically on the symptoms of oropharyngeal dysphagia, making it challenging to identify progressive swallowing function changes in older persons.¹³ The 26 questions of the GDRST, which address both oropharyngeal and cognitive functions related to clients' symptoms, differentiate this tool from the EAT-10. This study included older adults at risk of dysphagia, all of whom completed the GDRST independently. The results showed high internal consistency and test-retest reliability, indicating the tool's strong repeatability over time in this population.

Regarding criterion validity, the results showed a correlation between the GDRST and clinical testing using the FOIS and the SQAOT. The FOIS reflects functional oral intake and is associated with dysphagia severity, while the SQAOT reflects oral and swallowing function as assessed by therapists. The correlation suggests that self-reported

symptom information and clinical evaluation based on the clinician's judgment provide complementary information on oropharyngeal dysphagia. The moderate correlation between the GDRST and FOIS might be attributed to the FOIS primarily focusing on oral intake status, while the GDRST focuses on swallowing symptoms of dysphagia. This could also be explained by the distribution of GDRST and FOIS scores (Figure 1), which shows that many participants had the GDRST scores but still had an FOIS score of 7, indicating a ceiling effect. Investigating the relationship between the GDRST and standardized diagnostic tools for swallowing, such as fiber-optic endoscopic evaluation of swallowing (FEES) or videofluoroscopic swallowing study (VFSS), could further enhance the validation of the screening tool.²³

There is a high correlation between the GDRST and the SQAOT. It shows that the screening tool is consistent with the swallowing ability and related components. In addition, the self-report from screening tools can provide complementary information about the dysphagic symptoms that help the therapist select a clinical assessment that is specific to the symptoms that the client experienced. Another interesting aspect is that the participants in this study mostly did not have cognitive problems, which may be present in community-dwelling older adults. As a result, items related to cognitive domains were not selected in this population, potentially affecting the overall assessment scores. Therefore, the results of this study may not be generalizable to older adults with swallowing problems stemming from cognitive impairments.

Limitation

A limitation of this study is that our sample size was relatively small, and most of the sample was older people in the 60-75 age range. It may impact generalizability to the older person in the community, as older age has increased difficulties and functional decline of swallowing function.¹⁹ This study has a small sample size because it tested the swallowing ability of the elderly with the risk of dysphagia outside of a hospital setting without the supervision of a doctor. The Ethics Committee recommends conducting a pilot study to assess whether the testing poses no harm to the participants. This study did not have any adverse events that occurred to the participants. Further research should increase the sample size and examine the validity of this screening tool for late older adults (more than 75 years). Participants in this study are research-interested volunteers who can perform questionnaires by themselves. Some elderly persons in the community may be unable to answer the questionnaire due to cognitive or communication problems. Therefore, receiving information from caregivers or family members can be an alternative method for using this questionnaire. Additionally, the ability of the screening tool to predict outcomes will need to be assessed. Future steps in this research will recommend for inter-rater reliability, concurrent validity, sensitivity, and specificity studies of the screening tool.

Conclusions

The questionnaire created to evaluate the risk of dysphagia for the community older person reflects the complex symptomology and frequency of swallowing difficulties. The questionnaire demonstrates strong content validity, high internal consistency, and test-retest reliability. Furthermore, the questionnaire provides preliminary insight into understanding oropharyngeal dysphagia symptoms before clinical evaluation. There is a correlation between the screening tool and the clinical tests evaluated by the therapist. This study indicates that this questionnaire may be a useful screening tool for early identification and prevention of oropharyngeal dysphagia in aging populations.

Conflict of interest

The authors have no actual or potential conflict of interest to declare that it is relevant to the content of this article.

Ethical approval

Ethical approval for the study was obtained from Mahidol University Central Institutional Review Board for Human Research (MU-CIRB 2023-068.2002). Prior to data collection, all participants provided written informed consent for study participation and for the publication of this study.

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Modified wall-stretching exercises: A practical strategy to promote an immediate effect of shoulder flexibility

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ARTICLE INFO

Article history:

Received 8 April 2024

Accepted as revised 20 February 2025

Available online 24 February 2025

Keywords:

Musculoskeletal disorder, exercise, self-management, rehabilitation, physical therapy.

ABSTRACT

Background: The decreased flexibility could attribute numerous adverse impacts to daily functions and risk of joint injury, especially in the joint with a large range of motion (ROM), such as that of the shoulder. Current management to increase shoulder flexibility mostly face the problems of regular adherence due to the need for expert guidance and the requirement of specific and numerous poses that are difficult to remember and time-consuming.

Objective: To investigate the immediate effects of the modified wall-stretching exercise on shoulder flexibility using a quasi-experimental design. Moreover, the study explored the proportion of arms (dominant and non-dominant) with limited shoulder flexibility.

Materials and methods: Fifty-six participants (38 females, 68%) with limited shoulder flexibility as determined using the back scratch test performed a modified wall-stretching exercise. The method required the participants to place their hands against the wall at the shoulder levels with elbow and wrist extensions while the hands were in three positions: upward, sideward, and downward. Participants moved the upper trunk downward and held at the maximal stretch position for 15 seconds in each hand position. After a sufficient rest period, participants were re-assessed for their shoulder flexibility using the back scratch test. The paired samples t-tests were used to analyze the study findings.

Results: Most participants had limited shoulder flexibility in both arms (N=35, 63%), especially in the non-dominant arm (60%). Immediately after 45 seconds of a modified wall-stretching exercise, participants significantly improved their shoulder flexibility by approximately 3 cm ($p < 0.001$).

Conclusion: The limited shoulder flexibility, particularly on the non-dominant arm, suggests that a sedentary lifestyle, characterized by spending the most time in a flexed posture using social media, may have contributed to reduced muscle strength, particularly in the non-dominant arm when performing overhead activities. The present findings suggest using a modified wall-stretching exercise as an alternative practical, self-administering, and effective method to promote shoulder flexibility that can be applied easily in various settings.

Introduction

Flexibility is an intrinsic property of the body's soft tissues (i.e., muscles and connective tissues) that determines the range of motion achievable without injury at a joint or group of joints.¹ Thus, the ability of a joint or series of joints to move through an unrestricted, pain-free range of motion while executing daily activities is essential.^{2,3} Optimal flexibility also prevents injuries,

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doi: 10.12982/JAMS.2025.051

E-ISSN: 2539-6056

promotes motor performance, and maintains overall physical health.^{4,5} On the contrary, the decreased flexibility, due to several causes (e.g., improper use of the joints in daily activities, sedentary lifestyle inducing muscle weakness, abnormal posture, and system decline due to age advancement), could be attributed adverse impacts to daily functions and risk of joint injury.⁶⁻⁹ This is particularly true for a joint with an extensive range of motion (ROM), such as that of the shoulder, whereby its ROM allows various movements covering overhead reaching, combing hair, and hand behind the back. Consequently, numerous protocols have been proposed to maintain, improve, and manage problems affecting shoulder flexibility.

Current management to increase shoulder flexibility can be done through active and passive stretching exercises, either as a self-management protocol or by an expert, such as static, dynamic, and ballistic stretching protocols.¹⁰⁻¹⁷ However, most of these methods often pose challenges for regular adherence due to the need for expert guidance and the requirement of specific and numerous poses that are difficult to remember and time-consuming.^{13,18,19} Consequently, exploring an alternative practical method to promote shoulder flexibility is needed, especially in a current era of stress, competition, and sedentary lifestyles with spending the most time on a computer and social media.

In general, an exercise protocol to increase joint flexibility can be done by fixing one end and moving the other end of the soft tissues surrounding the joint. Considering the characteristics of muscles and soft tissues surrounding the shoulder joint, they all travel, covering the joint with the attachment between the upper trunk and arms.^{6,20,21} Thus, the techniques to improve shoulder flexibility can be done by moving the arm while fixing the trunk or vice versa with the assistance of the other arm or external force. In addition, shoulder flexibility can be achieved through self-stretching exercises using a wall, such as the so-called wall-stretching exercise for pectoralis muscles. The method is executed by placing both arms at a particular location on a wall to fix the insertion of the pectoralis muscles and moving the trunk (origin) toward the wall to lengthen the muscles.^{5,22,23}

Previously, wall exercises typically utilized a single position and primarily targeted the anterior muscles.²⁴ However, the shoulder muscles are attached at various points⁶, and changing the position of the hands during these exercises could potentially engage other muscles besides the anterior ones. Repetitive movements that are a part of daily life can lead to asymmetries in flexibility between both arms.²⁴ Examining both sides can determine whether there is an imbalance in shoulder flexibility. This information is valuable for modifying training routines to address these imbalances efficiently. Optimizing overall flexibility may involve focusing on balanced motions or applying additional stretching to the tighter side. Evaluating both arms provides a more comprehensive picture of the full range of shoulder motion, as measuring only the dominant hand might underestimate the need for improved flexibility, potentially overlooking limitations on

the non-dominant side.

To develop an easy, self-administering, and practical strategy to promote shoulder flexibility, the researchers proposed a modified wall-stretching exercise from that of a wall-stretching exercise for pectoral muscles. They investigated its immediate effects on shoulder flexibility. In addition, the study secondarily explored the proportion of arms (i.e., dominant and non-dominant arms) with limited shoulder flexibility. Most participants would have different shoulder flexibility between the dominant and non-dominant arms. The researchers believed that the findings would offer an alternative practical strategy that can be applied to promote shoulder flexibility easily in various routine settings.

Material and methods

Study design and participants

This quasi-experimental study was conducted in male and female adults aged 18 years old and over who had limited shoulder flexibility, at least one side, as measured using the back scratch test^{25,26} with a distance less than zero who did not have severe pain (i.e., a visual analog scale <5 out of 10).²⁷ Participants were excluded if they had general laxity and hypermobility of joints as determined using a hyperabduction test^{28,29} or any musculoskeletal or neuromusculoskeletal abnormalities that might interfere with the outcomes of the study, e.g., arthritis, cervical radiculopathy, impingement, fracture, frozen shoulder, and joint dislocation or subluxation.^{11,30} The sample size of this study was derived from a pilot study with a confidence level of 0.05 and power of 86% that recruited 54 participants. The research protocols followed the standards of the University Ethics Committee for Human Research. Eligible participants signed a written informed consent form before participating in this study.

Experimental protocols

Eligible participants were interviewed and assessed for their demographics (including age, body weight, height, and body mass index), musculoskeletal injury history, any form of exercise daily, and dominant and non-dominant arms as determined using the Edinburgh Handedness Inventory.^{31,32} Then, the participants were assessed for their shoulder flexibility using the back scratch test for both arms.^{25,26} Subsequently, they performed a modified wall-stretching exercise, with the repeated back scratch test thereafter. Details of the stretching exercise and testing protocols are as follows.

Stretching protocol: A modified wall-stretching exercise

This technique aims to stretch soft tissues around both shoulders with body-weight assistance. Participants stood facing a wall at arm-length from the wall and placed their hands against the wall at the shoulder levels with elbow and wrist extensions in three positions, including putting the hands upward, sideward, and downward (Figure 1). Participants were instructed to fix their hands on the wall in each hand position, move the upper trunk downward as far as they could without pain, and hold a

maximal stretching position for 15 seconds.^{18,33,34} Then, they repeated the exercise protocol with the other hand positions (Figure 1). Thus, the total stretching duration

was 45 seconds, with a 10-second rest period between the trials as required.



Figure 1. Modified wall-stretching protocols with the different arms placing.
A: starting position, B: position while stretching, C: upward hand position,
D: sideward hand position, E: downward hand position.

Outcome measure: The back scratch test

The study applied a back scratch test to assess soft tissue flexibility before and after the exercise protocols. The intra-rater reliability of the back scratch test, performed by a physiotherapist with seven years of experience, was found to be good (ICC=0.8), indicating high consistency in measurement similar to the previous study that reports It is reliable (ICCs: 0.98, 95 % CI: 0.98 to 0.97) and valid measure for upper limb and upper trunk flexibility.^{25,26} Participants

were in a standing position. Then they attempted to touch both hands behind the back, with one hand reaching over the shoulder and the other behind their back to reach upward as high as possible. The distance between the fingertips was measured. The scores were provided as zero if the fingertips of both hands touched each other and as a negative distance (in cm) if they did not touch (Figure 2). The average outcome over two trials was recorded.^{25,26}



Figure 2. A method of back scratch test (note: participant in this figure is right-handed).
A: measurement for the dominant arm, B: measurement for the non-dominant arm.

Statistical analysis

The Shapiro-Wilk test was used to assess the normality of the data distribution. Descriptive statistics were utilized to explain the demographics and findings of the study. The paired samples t-tests were applied with normal data distribution to analyze the changes before and after the stretching exercise. The independent t-test analyzed the data between dominant and non-dominant arms. The level of significance was set at $p < 0.05$.

Results

Fifty-six participants (38 females, 68%) with an average age of 42.5 ± 16.6 years (ranging from 18 to 75 years old), representing a young to middle-aged adult population, and a body mass index of 25.7 ± 4.8 kg/m², indicating a normal to overweight weight range, were involved in this

study. Most participants had sedentary lifestyles, and 10 participants (18%) had a history of shoulder injury due to strain, falls, and overuse. However, they did not have any obvious pain while moving the shoulders.

Most participants had problems with shoulder flexibility on both arms ($N=35$, 62.5%, Figure 3), while the rest had issues only with one shoulder ($N=21$, 37.5%). Thus, there were 91 shoulders with limited flexibility in this study, whereby most of the problems were found on the non-dominant arm (60.44%, Figure 3). The baseline flexibility of both shoulders, as measured using the back scratch test for both arms, was more significant than -10 cm (Table 1). Immediately after modified wall-stretching, participants significantly improved their flexibility by approximately 3cm ($p < 0.001$, Table 1).

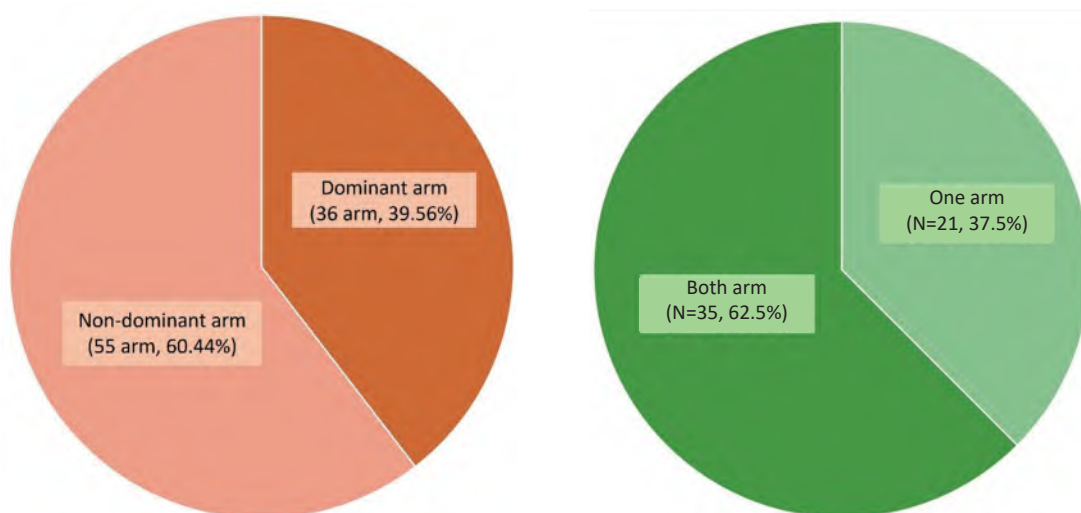


Figure 3. A proportion of participants with limited shoulder flexibility in this study.

Table 1. The change of shoulder flexibility following the modified wall-stretching exercises for the dominant and non-dominant arms.

Variable [‡]	Pre-test (cm)	Post-test (cm)	Mean difference (cm)	p value
Dominant arm (N=36)	-11.13±9.08 (-14.20: -8.06)	-8.02±8.97 (-11.06: -4.98)	-3.10±3.57 (-4.31: -1.89)	<0.001*
Non-dominant arm (N=55)	-13.17±8.26 (-15.40: -10.93)	-10.19±8.52 (-12.50: -7.89)	-2.97±2.95 (-3.77: -2.17)	<0.001*

Note: [‡]There were 91 arms with limited shoulder flexibility from a total of 56 participants.

The data is presented using mean±SD (95%CI) with the p value from the paired t-test, *indicates a significant difference between the pre-test and post-test, cm: centimeter, SD: standard deviation, CI: confidence interval.

Discussion

This study proposed an easy, self-administering, and practical method to improve shoulder flexibility using a modifying wall-stretching exercise and assessed its immediate effects. The findings indicated that participants had limited shoulder flexibility, particularly on the non-dominant arm, i.e., the non-dominant arm on the top and the dominant arm behind their back (Figure 2). After performing a modified wall-stretching exercise, participants immediately improved the outcomes of the back scratch test ($p < 0.001$, Table 1).

The higher proportion of limited shoulder flexibility on the non-dominant arm might be associated with current daily activities. Most participants (65%) had a sedentary lifestyle. They spent most of their time in flexion postures using social media on mobile phones or computers, which could reduce their muscle strength, especially on the non-dominant arm in an overhead activity.³⁵ Moreover, being in a slouching posture over a long time could decrease the flexibility of the anterior compartment of the trunk due to soft tissue tightness that limits the ability to move the arm behind the back.³⁶ Previous studies also found the

shoulder flexibility in the dominant arm exceeding that of the non-dominant arm due to daily use of the dominant limb, including self-grooming, dressing, eating, and lifting objects.²⁴

The modified wall-stretching exercise, a form of static stretching, requires participants to place their hands against a wall and move their upper trunk downward until reaching the position of maximal stretch (Figure 1). This protocol effectively stretches both ends of the soft tissues in the arms and upper trunk, aided by body weight, all within a pain-free range.^{23,37} Previous research has shown that static stretching improves the range of motion and is generally safer than other stretching methods.⁶ However, many methods lack stable surfaces and require expert supervision to ensure proper form. In contrast, using a wall and body weight for stretching provides a stable surface, maximizing the stretch's effectiveness.²⁰ By leveraging body weight, individuals can control the intensity of the stretch, allowing for deeper stretches without overexertion. Muscle spindles and Golgi tendon organs play a crucial role in wall stretching.³⁸ Muscle spindles detect changes in muscle length and initially cause the muscle to contract to prevent overstretching, while Golgi tendon organs sense tension and induce muscle relaxation, enabling deeper stretches.^{9,20,38} Adjusting hand positions—upward, sideward, and downward (Figure 1)—further enhances the stretch by targeting different muscle groups around the shoulders and arms. This helps counteract the effects of rounded shoulders and forward head posture, typical in individuals who spend long periods sitting or using devices. The back scratch test outcomes demonstrated a significant improvement immediately after the exercise ($p < 0.001$, Table 1), with an improvement of approximately 3 cm (Table 1), which is notably more remarkable than the minimum detectable change level of the test (1.41 cm).²⁵ These findings suggest that the modified wall-stretching exercise may offer clinical benefits for enhancing flexibility in the upper limbs and trunk.

Previously, Stanziano *et al.* employed a combination of five stretching positions (back scratch, back arm press, overhead back, overhead side, and cross chest) to target shoulder muscles in older adults aged over 80 years.³⁹ The 9 participants performed these exercises twice a week for eight weeks, actively pulling on a rope looped across the limb. The results showed significant improvements in shoulder flexibility, with gains ranging from 0.83 to 1.76 cm in the back scratch test.³⁹ Similarly, Choi and Sohng investigated the effects of a modified yoga program that included floor-seated exercises on shoulder flexibility in older adults with an average age of 77 years.⁴⁰ After a 12-week exercise regimen, participants improved flexibility in both the dominant and non-dominant shoulders by 2 to 3.29 cm.⁴⁰

In contrast, the present study involved a broader age range, with participants averaging 42.5 ± 16.6 years and ranging from 18 to 75 years. Despite the shorter duration of each exercise—only 15 seconds per position, with three positions and a single repetition—the study demonstrated a similar improvement in shoulder flexibility of approximately

3 cm. This suggests that even brief, focused stretching sessions can be practical across a wider age range. The simplicity and practicality of this method, which does not require specialized equipment or extended supervision, make it easily applicable in various settings. This alternative approach may be particularly beneficial for those with limited time or access to guided exercise programs

Limitations

The study was conducted as a quasi-experimental design without a control group or a follow-up period to assess the long-term effects of the exercise. Participants did not have common shoulder issues such as pain or frozen shoulder, which are often associated with limited flexibility. Future research should use randomized controlled trials involving participants with shoulder flexibility limitations and related conditions (e.g., shoulder pain and frozen shoulder), along with a follow-up period, to evaluate the sustained benefits of the exercise protocol. Additionally, future studies should classify participants by specific age groups or limit the age range to better understand age-specific responses to the modified wall-stretching exercise. This approach will help determine the optimal application of the exercise protocol across different age demographics

Conclusion

This study investigated the immediate effect of modified wall stretching on shoulder flexibility. The results demonstrated a significant improvement in shoulder flexibility immediately after the exercise, particularly addressing limitations in both arms. These findings suggest that the modified wall-stretching exercise offers a practical and accessible method to enhance shoulder flexibility and is suitable for self-administration in various settings.

Conflict of interest

The authors declare no conflict of interest.

Funding

This research is funded by the Young Researcher Development Project of Khon Kaen University Year 2023, Fundamental Fund 2024, and ASEAN-GMS Khon Kaen University research funding scholarship.

Acknowledgements

The authors sincerely thank the Faculty of Associated Medical Sciences, the Improvement of Physical Performance and Quality of Life (IPQ) research group, and all participants in this study for their support and contributions.

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Comparing the effectiveness of lumbar contralateral rotation to unilateral posteroanterior mobilization in the treatment of unilateral low back pain: A single-blinded randomized placebo-controlled trial

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ARTICLE INFO

Article history:

Received 26 August 2024

Accepted as revised 26 February 2025

Available online 3 March 2025

Keywords:

Clinical trial, manual therapy, low back pain, mobilization.

ABSTRACT

Background: The use of manipulative therapy to treat patients with non-specific low back pain (NSLBP) has been proposed based on the patient's clinical presentation.

Objective: To investigate the effects of the lumbar contralateral rotation and unilateral posteroanterior (uniPA) mobilizations on LBP intensity and lumbar range of motion (LROM) in the treatment of unilateral NSLBP patients.

Materials and methods: A single-blinded randomized placebo-controlled trial was conducted. Sixty of unilateral NSLBP were randomly allocated using concealed opaque envelopes into 2 groups either; rotation or uniPA groups and collected the pre-intervention data (O1). All participants were received a detuned short-wave diathermy technique and collected the after-placebo data (O2). Then, the therapist treated the participants according to their group allocation and collected the after-mobilization data (O3). Lastly, the participants were recalled within 2 days to collect the follow-up data (O4). Two-way mixed repeated analysis of variance and Paired t-test was used to investigate the immediate and latent effects, and placebo and flare-up effects, respectively.

Results: The result showed no placebo effect ($p > 0.05$). There was no difference between groups and time effects (both immediate and latent effects) ($p > 0.05$). However, there were statistically significant differences within group of immediate and latent effects on pain reduction and LROM improvement ($p < 0.001$). Moreover, results noted on the flare-up on pain alleviation at rest in the uniPA group ($p < 0.05$).

Conclusion: These findings partially support the recommendation of using mobilization techniques in the treatment of NSLBP patients with unilateral symptoms.

Introduction

Low back pain (LBP) is a prevalent musculoskeletal condition, affecting over 80% of people globally at some point in their lives.^{1,2} Among those, a significant majority experience non-specific or mechanical LBP, characterized by pain without identifiable pathology.³⁻⁷ Clinical guidelines emphasize the importance of non-pharmacologic interventions for managing LBP, particularly exercise therapy and spinal manipulative therapy (SMT). This evidence suggests that SMT can effectively reduce pain and improve function for both acute and chronic NSLBP. Despite its established benefits, particularly in improving lumbar range of motion in the sagittal plane, gaps remain in not only understanding its long-term effectiveness but also comparing the effectiveness of each technique, highlighting the need for further research.⁵

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doi: 10.12982/JAMS.2025.052

E-ISSN: 2539-6056

Briefly, SMT is a kind of passive treatment including manipulation and mobilization, which aim to relieve pain and improve range of motion (ROM).⁸ Spinal mobilization is performed as a low-velocity, non-thrust approach whereas spinal manipulation is performed as a small amplitude high-velocity thrust approach toward the end of range or just beyond a joint movement. As a result spinal mobilization is particularly valued for its safety and gentler application, making it suitable for diverse patient populations.^{6,7} Studies have investigated the effectiveness of the use of spinal mobilization in the treatment of NSLBP patients.⁹⁻¹¹ It has been noted that pressure applied in the posteroanterior (PA) direction to the most symptomatic spinal level resulted in pain relief⁹⁻¹² and improved lumbar range of motion (LROM) in the sagittal plane.^{11,12} Despite studies reporting on the use of SMT, the effectiveness of its use in practice remains unclear.

The use of SMT to treat NSLBP has been proposed based on the patient's clinical presentation or symptom distribution.⁸ For example, for a patient presenting with unilateral NSLBP, lumbar contralateral rotation, and unilateral posteroanterior (uniPA) mobilization are recommended as the first choice for optimal treatment outcomes.⁸ With regards to the lumbar rotation mobilization, the applied force produces a rotation moment from the pelvis to the spinal column. This primarily produces movement in the transverse plane. When the spine is moved toward the end of the joint ROM, it secondly produces movement in the sagittal plane or PA glide of the superior articular facet in relation to the inferior articular facet. With regards to the uniPA mobilization, the force is applied in the vertical direction to the superior articular facet on the inferior articular facet of the lumbar spine. This primarily produces movement in the sagittal plane. When the spine is moved toward the end of the joint ROM, it secondly produces movement in the transverse plane. The difference between these articular movements raises the question of whether the two techniques produce similar effects in the treatment of NSLBP patients.

A few studies investigated the effectiveness of the McKenzie method in the treatment of chronic NSLBP.¹³⁻¹⁶ It has been noted that the McKenzie method had greater improvements in LBP intensity^{13,14} and disability.¹⁵ Briefly, the McKenzie method has been prescribed as repeated exercises in a sustained position.¹⁶ Consequently, the McKenzie method would normally produce movement in sagittal movement similar to the uniPA mobilization. However, there is no study comparing the effectiveness of these two approaches.

Up to present, there is no study comparing these two mobilization techniques against a placebo treatment to identify its true effectiveness. Therefore, this study aimed to investigate (I) the immediate and latent effects of the lumbar mobilization technique on LBP intensity and LROM in the treatment of unilateral NSLBP patients and (II) any continuing effects on LBP intensity and LROM within 2 days after the application of SMT.

Materials and methods

Study design

A single-blinded, randomized, placebo-controlled trial was used to compare the effectiveness of lumbar contralateral rotation to uniPA mobilization in the treatment of unilateral NSLBP patients. This trial was registered with the Thai Clinical Trials Registry (TCTR20181018001). Ethical approval was provided by the Research Ethics Review Committee for Research Involving Human Research Participants, COA No.203/2018.

Participants

NSLBP patients presenting with unilateral symptoms, who had never been treated with physical therapy and first presented for LBP treatment at the Health Science Service Unit, Chulalongkorn University were invited to participate. The patients were screened using the inclusion criteria: 1) aged 20–55 years, 2) unilateral symptoms of NSLBP at rest of more than 25 on a 0-100 mm visual analog scale (VAS), 3) no radicular symptoms in the lower limbs, 4) not taking any medications (including non-steroidal anti-inflammatory drugs, muscle relaxants, and pain relief) within 24 hours, and 5) no history of lumbar spine fracture or any spinal surgery. The patients were excluded if they had any contraindications to mobilization.⁸ The unilateral NSLBP patients who met the inclusion criteria were then explained the details of this study. The patients who agreed to participate were then asked to give written consent. Based on the assumption of normality, a sample of 30 could be assumed to be normally distributed.¹⁷ Therefore, the sample size was set at a total of 60 participants.

Therapists

Two physical therapists enrolled in the Master of Physical Therapy program at the Faculty of Allied Health Sciences, Chulalongkorn University, were involved in this study. The first therapist was trained to manually assess and apply mobilization techniques by an experienced physical therapist with clinical experience in the use of SMT of more than 30 years follow Maitland's concept.⁸ A bathroom scale and a metronome were used to train the application of force range and speed, respectively. The first therapist was responsible for recruiting participants, screening eligible participants, defining appropriate treatment details, treating all participants and preparing concealed allocation envelopes. The concealed opaque envelope was prepared prior to the study using a random function in Microsoft Excel Program (Version 2016). The second therapist, who was blinded to the treatment group, was responsible for establishing the baseline measurements and post-intervention outcomes.

Outcome measurements

LBP intensity

All participants were asked to report their perceived pain intensity, both at rest and on the most painful movement, using the VAS: the left side represented no pain, and the right represented pain as bad as possible.¹⁸ Test-retest reliability has been reported to be high for the

VAS, with an intraclass correlation coefficient [$ICC_{(3,1)}$] of 0.71-0.99.¹⁹ The minimum clinically important difference (MCID) of pain on the VAS has been noted at 20 mm on a 100-mm scale.¹⁸

LROM

The Back Range of Motion™ (BROM II; Performance Attainment Associates, Roseville, Minn., USA) instrument was used to measure the LROM, comprising flexion, extension, left and right lateral flexion, and left and right rotation. All measurements were conducted following a standardized protocol described elsewhere.²⁰ Briefly, all participants received a full explanation of measuring lumbar movements from a 4-minute video clip before the measurement. They were asked to practice all lumbar movements, using consistent verbal instruction.

The intra-rater and inter-rater reliability of LROM procedures using the BROM II have been investigated.²⁰ The intra-rater reliability [$ICC_{(3,1)}$] was good to excellent, ranging from 0.82 to 0.98, except for the LROM in the right rotation, which was moderate to good, ranging from 0.74 to 0.97. The inter-rater reliability [$ICC_{(2,1)}$] was good to excellent, ranging from 0.78 to 0.87. The standard error of measurement and minimal detectable change (MDC) were, respectively, less than 2.0 and 5.6 degrees in flexion, 1.7 and 4.6 degrees in extension, 1.7 and 4.7 degrees in lateral flexion, and 1.0 and 2.8 degrees in rotation.²⁰

Disability

The Thai version of the Functional Rating Index (Thai-FRI) was used to assess physical disability due to LBP.²¹ The Thai-FRI rating was converted into a percentage. The test-retest reliability coefficient was 0.82. The intraclass correlation coefficient [$ICC_{(3,k)}$] was 0.99.²² The MDC and MCID of the Thai-FRI for LBP were 2.5 and 11.5 points, respectively.^{21,23}

Patient satisfaction

The Global Perceived Effect (GPE) scale was used to measure the patients' satisfaction with treatment outcomes.¹⁸ The GPE was noted using a 7-point scale ranging from -3 (worse than ever) to 3 (completely recovered). The MCID of the GPE is "2 (much improved)".¹⁸

Procedures

The first therapist assessed the participants subjectively and objectively. The second therapist, who was blinded to the intervention, was then called to note the baseline data (O1), comprising pain intensity, LROM, and disability. After completion of the O1, the participants were randomly allocated using random function in Excel program to generate a random number. These numbers were then sorted with respect to the group interventions. The assigned group was then put into a concealed opaque envelope and assigned the participant into either rotation or uniPA group. All participants in both groups received a detuned short-wave diathermy technique as a placebo for 10 minutes, either in a side-lying or supine position. Room temperature as well as light of the treatment area

were all controlled. This placebo treatment was used in low back pain placebo-controlled trials to ensure the effectiveness of blinding and minimizing potential bias.¹³ The after-placebo data (O2) were then noted, including pain intensity, LROM, and patient satisfaction.

The first therapist applied the mobilization technique, according to the assigned group in the concealed envelope, as two sets of 1-minute repetitions directly to the symptomatic spinal level. Briefly, the mobilization was applied by rotating the participant's small pelvic oscillatory movements away from the painful side while they lay on the painless side. UniPA mobilization was applied to the superior articular facet of the lumbar spine in the prone position. The details of these techniques and grade of treatment are described in detail elsewhere.⁸ After the application of two sets of mobilization, the participants were asked to rest for 5 minutes. The third outcome measures (O3), including pain intensity, LROM, and patient satisfaction, were then noted. The participants were called into the clinic within 2 days to collect the fourth outcomes of follow-up data (O4), including pain intensity, LROM, patient satisfaction, and disability.

Data analysis

All data were analyzed using SPSS version 22 for Windows. The Kolmogorov-Smirnov test was conducted to ensure that the distributions of the investigated variables met the assumptions of normality for the planned parametric tests. The participants' demographic data and baseline characteristics were compared between groups using the independent *t*-test and Chi-square test. The effects of the placebo treatment were investigated by comparing the pre-intervention data (O1) and after-placebo data (O2) using the paired *t*-test. The mean and standard deviation of the change score, calculated by subtracting the pre-intervention scores from the post-intervention scores, were obtained for each variable except patient satisfaction. The immediate (O2-O3) and latent effects (O2-O4) were investigated by comparing the change scores of LBP and LROM using two-way mixed repeated ANOVA. Flare-up effects were investigated by comparing the pain intensity and LROM from the after-mobilization data (O3) with follow-up data (O4) using the paired *t*-test.

To investigate the effectiveness of the intervention on disability, the paired *t*-test was used to compare the effect of the intervention on the Thai-FRI score. To investigate the effectiveness of the intervention on patient satisfaction, the proportion of participants who reported GPE in each group was calculated.

Results

Sixty-eight unilateral NSLBP patients were recruited. Eight were excluded due to the current intensity of their LBP being less than 25 mm on the VAS. A total of 60 participants (16 men and 44 women) were recruited. The participant selection flow is shown in Figure 1. The Kolmogorov-Smirnov tests indicated the normal distribution of data for all variables with $p > 0.05$. The participants' demographic

data and baseline characteristics are shown in Tables 1 and 2, respectively. No significant difference existed between groups in the participants' demographic data

and baseline characteristics ($p>0.05$), except pain at rest ($p=0.015$).

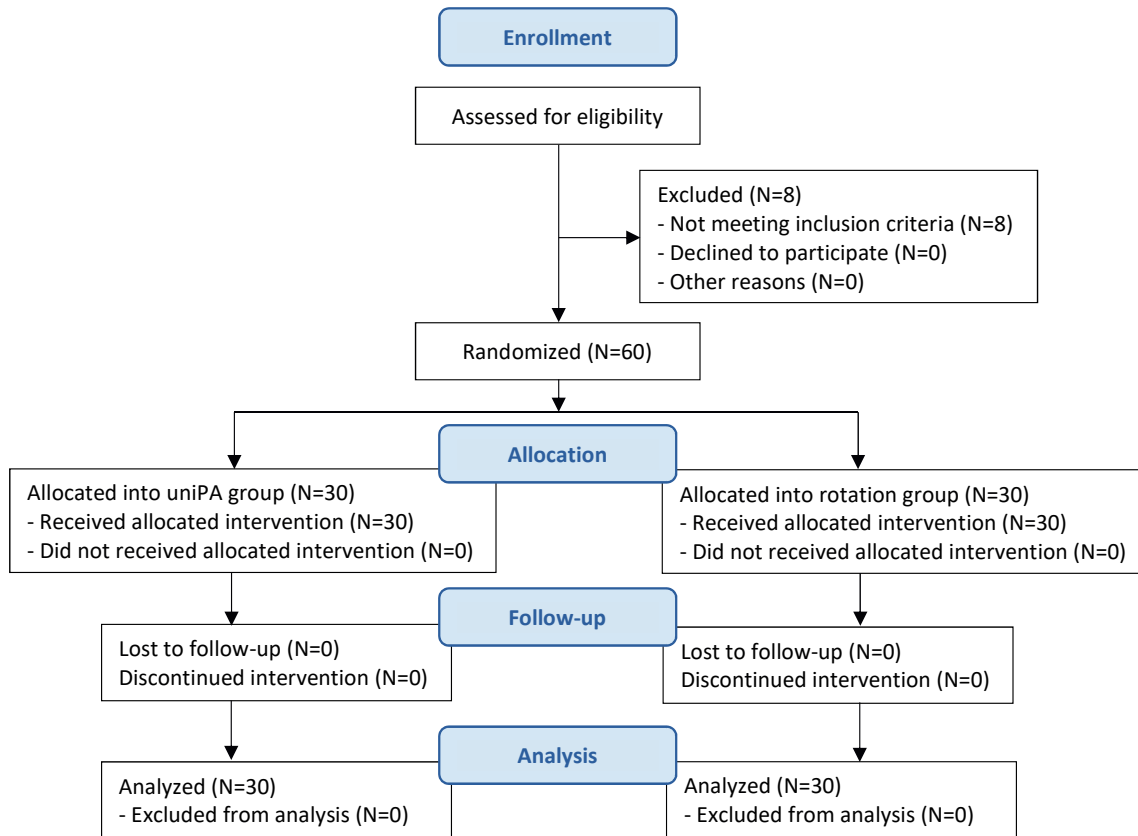


Figure 1. The participant selection flow.

Table 1. Demographic data (Mean±SD).

	Rotation group (N=30)	UniPA group (N=30)	p value
Gender (N): Male/Female	8/22	8/22	1.000 ^b
Age (years)	31.0±10.1	35.8±11.9	0.098 ^a
Height (m)	1.6±0.1	1.6±0.1	0.821 ^a
Weight (kg)	59.8±11.8	61.9±13.2	0.515 ^a
Duration (days)	335.0±593.8	605.6±956.7	0.194 ^a
Symptomatic spinal level (N)			
Upper lumbar/Lower lumbar	5/25	9/21	0.532 ^b
Grade (N): Grade III/Grade IV	1/29	2/28	1.000 ^b
Side (N): Right side/Left side	16/14	16/14	1.000 ^b

Note: ^aanalysis using independent t-test, ^banalysis using Chi-square test.

Table 2. Baseline characteristics (Mean±SD).

	Rotation group	UniPA group	p value ^a
Thai version of Functional rating index (%)	36.6±10.8	42.7±13.9	0.071
Back pain intensity (mm)			
Pain at rest	47.5±14.1	56.9±15.0	0.015*
Pain on worst movement	57.0±17.9	63.4±17.0	0.159
Active lumbar range of motion (degrees)			
Flexion	24.0±5.0	21.1±8.4	0.120
Extension	4.5±4.1	3.1±2.2	0.100
Contralateral lateral flexion	23.6±6.7	21.7±6.3	0.253
Ipsilateral lateral flexion	23.3±7.3	21.5±7.7	0.338
Contralateral rotation	7.8±2.6	7.1±2.3	0.294
Ipsilateral rotation	7.3±3.0	6.6±2.7	0.372
Direction of worst movement (N)			
Sagittal plane	19	17	
Frontal plane	6	9	
Transverse plane	5	4	

Note: * $p < 0.05$, ^aanalysis using independent t-test.

Back pain intensity and LROM

Concerning the placebo effect, no statistically significant differences were found in back pain intensity or LROM between O1 and O2. The results of two-way mixed repeated ANOVA are shown in Tables 3 and 4. No statistically significant difference existed in the interaction between group and time (both immediate and latent effects) on back pain intensity [$F_{(2,116)}$ ranged from 1.79 to 2.08, $p = 0.130$ to 0.171] or LROM [$F_{(2,116)}$ ranged from 0.04 to 1.04, $p = 0.356$ to 0.957 ; Table 4]. However, statistically significant within-group differences existed for immediate and latent effects on back pain intensity [$F_{(2,116)}$ ranged

from 61.62 to 68.01, $p < 0.001$] and LROM [$F_{(2,116)}$ ranged from 31.66 to 48.66, $p < 0.001$] in all directions. As shown in Table 3, both techniques decreased back pain intensity and increased LROM in all directions at the immediate and latent effect intervals compared to the baseline. Additionally, pain at rest in the uniPA group was lower at the latent effect point than the immediate effect point.

Concerning flare-up effect after the lumbar mobilization technique, Table 5 shows the paired t-test results. Statistically significant differences were found between O3 and O4 in pain at rest and lumbar extension in the uniPA group ($p = 0.003$ and 0.026 , respectively).

Table 3. Change scores between data for back pain intensity and LROM (Mean±SD).

Variables	Rotation group		UniPA group	
	Immediate effect [#]	Latent effect [#]	Immediate effect [#]	Latent effect [#]
Back pain intensity (mm)				
Pain at rest	17.5±11.0	19.6±19.7	15.0±10.1	25.5±17.4 ^a
Pain on worst movement	26.0±14.8	22.1±21.1	22.3±15.5	27.6±21.2
LROM (degrees)				
Flexion	-2.9±3.2	-2.5±3.0	-4.2±3.8	-3.6±4.5
Extension	-1.8±1.6	-1.7±2.2	-2.4±1.8	-1.7±1.4
Contralateral lateral flexion	-4.1±3.2	-3.3±4.0	-4.1±3.4	-3.1±3.6
Ipsilateral lateral flexion	-3.8±2.6	-3.6±4.1	-3.0±2.9	-4.0±3.8
Contralateral rotation	-2.0±1.6	-1.6±1.6	-2.4±1.8	-1.9±2.0
Ipsilateral rotation	-2.0±1.7	-1.7±2.2	-2.5±1.5	-1.9±2.1

Note: [#]significant difference when compared to baseline in each group ($p < 0.05$), ^asignificant difference when compared to immediate effect in each group ($p < 0.05$), A negative sign for the change scores represents an increase in LROM.

Table 4. Effects of group, time, and interaction between group and time on back pain intensity and LROM.

Variables	Group effect		Time effect		Interaction effect	
	F _(1,58)	p value	F _(2,116)	p value	F _(2,116)	p value
Back pain intensity (mm)						
Pain at rest	0.29	0.593	61.62	<0.001*	2.08	0.130
Pain on worst movement	0.05	0.824	68.01	<0.001*	1.79	0.171
LROM (degrees)						
Flexion	2.21	0.143	31.66	<0.001*	1.04	0.356
Extension	0.59	0.445	45.80	<0.001*	0.78	0.462
Contralateral lateral flexion	0.05	0.831	41.96	<0.001*	0.04	0.957
Ipsilateral lateral flexion	0.07	0.787	41.61	<0.001*	0.80	0.452
Contralateral rotation	0.78	0.381	48.66	<0.001*	0.38	0.688
Ipsilateral rotation	0.64	0.426	42.76	<0.001*	0.41	0.663

Note: * $p < 0.001$, analysis using two-way mixed repeated ANOVA.

Table 5. The result of follow-up effect within 2 days on back pain intensity and LROM (Mean±SD).

Variables	Rotation group				UniPA group			
	After mobilization	Follow-up within 2 days	t	p value	After mobilization	Follow-up within 2 days	t	p value
Back pain intensity (mm)								
Pain at rest	29.1±16.3	27.0±19.4	0.60	0.551	37.4±19.1	27.0±19.8	3.26	0.003*
Pain on worst movement	28.1±19.4	32.0±20.4	-1.03	0.314	37.9±21.4	32.6±21.2	1.59	0.123
LROM (degrees)								
Flexion	25.8±5.3	25.4±4.9	0.59	0.559	34.4±8.2	23.9±7.5	0.73	0.471
Extension	6.1±4.1	6.0±3.7	0.42	0.679	5.3±2.5	4.7±2.2	2.35	0.026*
Contralateral lateral flexion	26.9±5.8	26.1±5.8	1.24	0.227	25.3±6.6	24.3±7.0	1.35	0.188
Ipsilateral lateral flexion	25.7±7.0	25.5±6.4	0.26	0.794	24.0±6.9	24.9±7.6	-1.53	0.138
Contralateral rotation	9.7±2.2	9.3±2.1	1.10	0.281	9.3±1.7	8.8±2.0	1.55	0.133
Ipsilateral rotation	9.4±2.8	9.1±2.6	0.68	0.502	9.4±1.9	8.8±2.1	1.39	0.174

Note: * $p < 0.05$, analysis using paired t-test.

Thai-FRI and GPE

No significant difference existed in the change in Thai-FRI scores between groups ($p=0.937$). A significant difference was found between pre-intervention and post-intervention scores in both groups ($p < 0.001$; Table 6).

The GPE scores were grouped into three groups. Score 2 to 3, -1 to 1, and -2 to -3 were defined as improved, unchanged and worsened, respectively. The number and proportion of participants with each score on the GPE

post-intervention are shown in Table 7. More than 90% of participants in both groups reported an unchanged GPE at the placebo effect. Approximately 43% of the participants in the rotation group reported an improved GPE at the immediate and follow-up effects. Approximately 93% of participants in the uniPA group reported an unchanged GPE at the immediate effect, and 57% of the participants reported an improved GPE at follow-up.

Table 6. The result of Thai-FRI scores (Mean±SD).

	Pre-intervention	Post-intervention	Change	Within group*		Between group**	
				t	p value	t	p value
Rotation group	36.75±10.83	23.58±13.19	13.17±12.51	5.77	<0.001	-0.80	0.937
UniPA group	42.67±13.91	29.25±13.74	13.42±11.77	6.24	<0.001		

Note: *analysis from pre-intervention and post-intervention using paired t-test, **analysis from change percentage using independent t test.

Table 7. Number and percentage of participants who rated on GPE.

GPE	Rotation group						UniPA group					
	Placebo (N)	%	Immediate (N)	%	Follow-up (N)	%	Placebo (N)	%	Immediate (N)	%	Follow-up (N)	%
3 = Completely recovered	-	3.3	1	43.3	1	43.3	-	6.7	-	0.00	1	56.7
2 = Much improved	1		12		12		2		-		16	
1 = Slightly improved	4	93.3	15	56.7	5	56.7	7	93.3	14	100	11	43.3
0 = No change	22		2		7		19		2		2	
-1 = Slightly worsened	2		-		5		2		14		-	
-2 = Much worsened	1	3.3	-	0.00	-	0.00	-	0.00	-	0.00	-	0.00
-3 = Worse than ever	-		-		-		-		-		-	

Discussion

The statistical analysis reviewed no significant differences between groups in demographic data ($p>0.05$) or baseline characteristics ($p>0.05$), except pain at rest at the baseline ($p=0.015$). The difference between groups in pain at rest at the baseline did not affect the overall results because the data were normalized using the change score between the pre- and post-intervention points.²⁴

The statistical analysis reviewed no significant results with the placebo treatment. This supports the later results, which should not be contaminated by psychological effects. The results of this study demonstrated that both lumbar contralateral rotation and uniPA mobilization techniques are effective in reducing pain and improving LROM in patients with unilateral NSLBP ($p<0.001$). However, no significant differences existed between groups in back pain intensity after receiving mobilization. Based on the sample size on each group ($N=30$), a power of analysis noted power and effect size ranged from 0.99-1.00 and 0.81-0.89, respectively. While a sample size of 60 (between group), a power of analysis noted power and effect size ranged from 0.63-0.71 and 0.21-0.32, respectively. It would be possible that the within group effects noted significant differences in both pain reduction and improving in range of motion, therefore the between groups effect would not be noted in such sample size. Further studies are required to confirm the between group effects in either a larger sample size or various LBP duration.

The hypoalgesic effects noted in within groups effects would be explained by neurophysiological mechanism.²⁵⁻²⁷ These results noted on significant differences on immediate and flare-up effects on pain at rest and pain on worst movement in both groups, supporting the immediate and latent neurophysiological mechanism proposed by Wright.²⁶ Interestingly, significant within-group difference was found in the time effect. LBP intensity was reduced at the latent effect interval by more than 20 mm only in the uniPA group, which is considered clinically relevant.¹⁸ This would be the case that the uniPA mobilization may have been applied directly to the facet joint, which would cause the symptoms resulting in changed mechanical properties of the spine.⁶ This finding is consistent with a study by Latimer *et al.*, which noted an association between LBP and spinal stiffness: spinal stiffness decreased as spinal pain decreased.²⁸

No significant between-group differences existed in LROM. Although the change in LROM was statistically significant within groups, the mean change was less than the MDC (ranging from 2.8 to 5.6 degrees).²⁰ This may imply that the LROM change score is not clinically relevant. Several explanations are possible. For example, most of the participants in this study had chronic symptoms, and the majority had stiffness problems. Due to the chronic stage, the extensibility of the connective tissue might be decreased as it becomes stiffer.⁹ Therefore, a single session of treatment would not be sufficient to effectively increase LROM.⁹

Interestingly, the greatest immediate improvements in LROM in lumbar contralateral lateral flexion and lumbar flexion were noted in the contralateral rotation group and uniPA group, respectively. These findings are consistent with a previous study and can be explained biomechanically.¹² The rotation mobilization produces movements in the transverse plane and then movement in the sagittal plane via PA gliding of the superior articular facet on the inferior articular facet. Consequently, it could promote movement of the lumbar spine in the transverse plane, coupled with movement in the frontal plane, increasing contralateral lateral flexion. UniPA mobilization produces movement on the sagittal plane and then movement in the transverse plane. Consequently, it could promote movement of the lumbar spine in the sagittal plane, resulting in increased flexion.

More than 90% of the participants in both groups reported an unchanged GPE after receiving a detuned SWD technique. More than 40% of the rotation group and none of the uniPA group reported an improved GPE after the intervention. The difference in the contact area may have caused discomfort to participants in the uniPA group but not the rotation group.²⁹ However, the participants in the uniPA group reported improved LBP compared to the rotation group at follow-up within 2 days. This may have resulted from a direct alteration of biomechanical properties during the application of uniPA, which contralateral rotation did not involve.

These results provide useful information to assist novices in precisely choosing a mobilization technique as the first choice of treatment in clinic. The results suggest that both techniques are effective in treating unilateral NSLBP patients. Therapists should firstly choose the lumbar

contralateral rotation technique to treat patients who mostly limit lumbar contralateral lateral flexion, whereas they should choose the uniPA technique to treat patients who mostly limit lumbar flexion. The lumbar contralateral rotation technique may be suitable for patients with high pain intensity at rest to achieve immediate alleviation and patient satisfaction. When the LBP intensity at rest is subsided, the uniPA technique would be selected to alleviate flare-up effects. However, if the use of one technique does not give the desired results, a clinician may apply another technique or approach to achieve their treatment goal.

This study used two-way mixed repeated measure with self-control to prevent bias; therefore, both treatment conditions were equivalent for the research setting.²⁴ However, this study had potential limitations. First, this study did not control daily activities of each participant during the data collection process. It was assumed that individual perception of pain relief would produce the satisfaction of participants after the treatment and thus allow them to perform more load on their daily activities. Difference latent findings might be noted if a further study limit such activities. Second, the participants were treated with only one session of the mobilization. Thus, it is possible that pursuing a course of mobilization might demonstrate different findings. Next, the participants were recruited from the LBP patients with various pain duration. Further studies should add more specific pain duration of the LBP patients that might lead to different findings. Lastly, the participants were treated using mostly grade IV applied to the lower lumbar spinal level, difference findings might be noted in the use of difference grades and treated area.

Conclusion

These results suggest that the selection of mobilization techniques should be based on the symptom distribution in patients with NSLBP. The findings partially support Maitland's recommendation of using mobilization techniques in the treatment of patients with unilateral symptoms.⁸ UniPA mobilization showed flare-up effects within 2 days regarding pain alleviation at rest compared to after the mobilization.

Conflict of interest

The authors declare no conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

The authors thank all participants for participating in this study and all staff at Physical Therapy Clinic, Faculty of Allied Health Sciences and Saladaeng Physiotherapy Clinic for their help during data collection.

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Development, validity, and reliability of harm perception and attitudes questionnaire for smokers of roll-your-own and factory-made cigarettes: A pilot study in Southern Thailand

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ARTICLE INFO

Article history:

Received 28 January 2025

Accepted as revised 6 March 2025

Available online 11 March 2025

Keywords:

Roll-your-own cigarettes, factory-made cigarettes, harm perception, questionnaire survey.

ABSTRACT

Background: Understanding roll-your-own (RYO) and factory-made (FM) cigarette smokers' perceptions of harm and their attitudes toward smoking is essential for identifying the barriers they face in effective smoking cessation counseling. However, there are few tools available for measuring these perceptions and attitudes among smokers of RYO and FM cigarettes.

Objective: This study aimed to develop and subsequently test the validity and reliability of a questionnaire designed to measure harm perception and attitudes among smokers of RYO and FM cigarettes in Thailand.

Materials and methods: The questionnaire was developed based on data from focus groups with eight adult RYO and FM cigarette smokers and in-depth interviews with four medical professionals with experience in caring for smokers. The content validity of the questionnaire was measured by three experts. The Cronbach's alpha coefficient was used to measure the internal consistency and reliability of the questionnaire. Data from 30 adult RYO and FM cigarette smokers were considered for the test-retest of reliability, measured using the intraclass correlation coefficient.

Results: The harm perception and attitudes questionnaire for RYO and FM cigarette smokers demonstrated good content validity. The item content validity index of 21 out of 29 items was higher than 0.79, and the scale content validity index was 0.9. Additionally, Cronbach's alpha coefficient of 0.719 and the intraclass correlation coefficient of 0.975 ($p < 0.001$) indicated the good reliability of the tool.

Conclusion: This study presented a validated questionnaire to assess harm perception and attitudes among smokers of RYO and FM cigarettes. The development and validation process ensured the tool's reliability and effectiveness in elucidating smoking behavior and facilitating targeted cessation interventions.

Introduction

Roll-your-own (RYO) cigarette smoking is the most common smoking habit in Thailand.¹ RYO cigarettes contain approximately 60% loose tobacco and are typically smoked without filters.² Consequently, RYO cigarette smoking may expose users to more cancer risks than factory-made (FM) cigarettes.³ RYO cigarette smoking is associated with a high likelihood of developing esophageal⁴ and laryngeal cancer.⁵ However, the general perception worldwide is that RYO cigarettes are less harmful than FM cigarettes.^{1,6-7} On the other hand, the perception that electronic cigarettes

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doi: 10.12982/JAMS.2025.053

E-ISSN: 2539-6056

(e-cigarettes) were equally as harmful as cigarettes was the most commonly held view among adults who smoke.⁸⁻¹⁰

Tobacco control strategies include higher tobacco tax rates, graphic health warnings on tobacco packages, and the use of social media for education and encouraging smoking cessation.¹¹ Notably, RYO cigarettes have evaded these control strategies. Further, they are a less costly option than FM cigarettes.^{6,12} RYO cigarettes are often locally grown, and smokers worldwide are more likely to roll their own cigarettes. Purchased RYO cigarettes frequently come without packaging, potentially shielding smokers from the government-mandated health warnings. In Thailand, RYO cigarette smokers are typically older, more likely to live in rural areas, have lower levels of education, and lack awareness of the adverse effects associated with smoking.^{1,7} RYO cigarette smokers also appear to be more addicted compared with FM cigarette smokers.¹³

Thailand is grappling with a sizable level of tobacco use among its adult population, with recent data showing that approximately 23.9% of middle-income and 24.2% of upper middle-income adults were currently using tobacco,¹³ representing nearly one-fourth of the adult population and a major public health concern. The government has enacted several policies under the Tobacco Products Control Act B.E. 2560 (2016), effective from July 2017.¹⁴ Despite these comprehensive policies, the reduction in smoking prevalence has been insufficient to meet global health targets. Implementing the World Health Organization Framework Convention on Tobacco Control (WHO FCTC) is ongoing, but challenges remain. The interventions in Thailand have been tailored to the local socio-cultural, economic, and regulatory environment, potentially limiting their generalizability to other regions

without considering local conditions.¹⁵

Harm perception is a combination of hazards and outrage. The hazard component includes not only fear but also perceptions of magnitude and probability, which are components of risk perception. The outrage component encompasses emotional and behavioral responses, allowing for the examination of multifaceted reactions. Thus, harm perception may provide more information for predicting smoking behavior.^{16,17} Assessing the harm perception of and attitudes toward smoking among smokers can help identify the barriers they face in effective smoking cessation counseling.

However, the available tools for measuring harm perception and attitudes among RYO and FM cigarette smokers are limited. We developed and tested the validity and reliability of a questionnaire designed to address the need for a simple tool for assessing harm perception and attitudes that measure the two factors among RYO and FM cigarette smokers.

Materials and methods

This pilot study consisted of two phases: 1) the development of the questionnaire and 2) the validation and reliability testing of the questionnaire. Figure 1 shows the steps that followed for questionnaire development, validation, and reliability testing. The processes conducted with a literature review, focus groups, and in-depth interviews to develop a list of items covering the relevant areas that should inform a questionnaire assessing harm perception and attitudes of RYO and FM cigarette smokers. Interview was performed after the participants gave written informed consent.

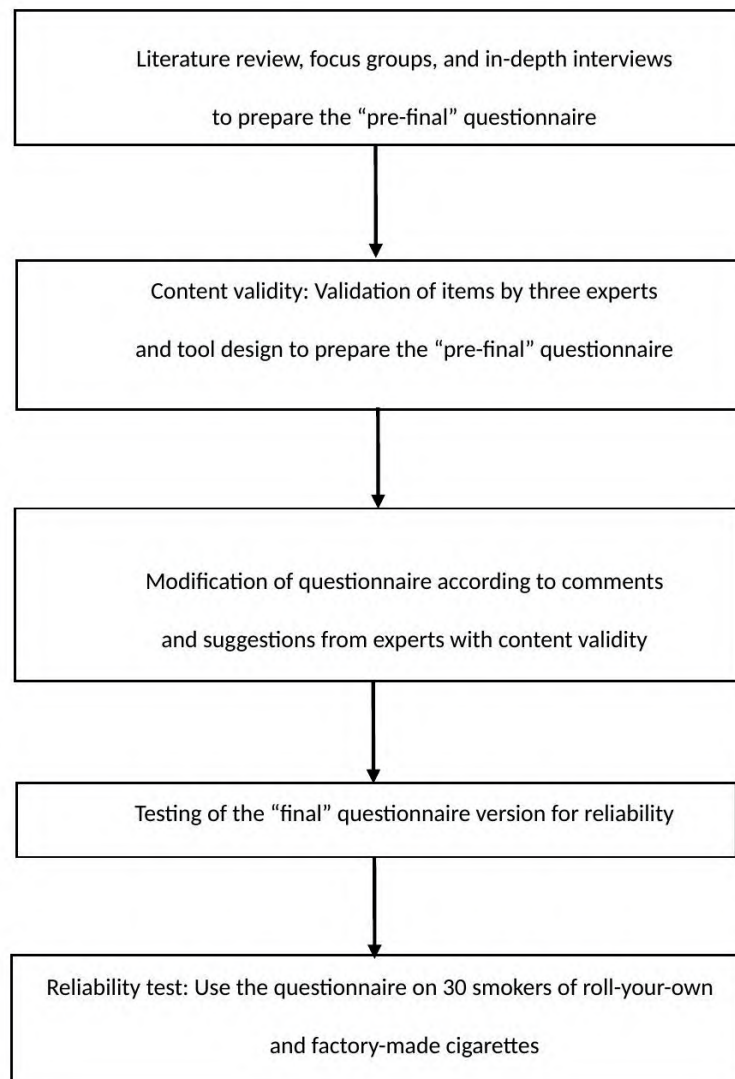


Figure 1. The steps that followed for questionnaire development, validation, and reliability testing.

In Phase 1 (questionnaire development), eight adult smokers of RYO and FM cigarettes from a community in a southern region of Thailand were participated in focus groups. Conducting a focus group entailed defining clear objectives and research questions. As mentioned previously, participants were carefully identified and selected based on relevant criteria. A structured discussion guide was developed to delineate topics and questions pertinent to key areas of interest. The focus group session was then conducted, during which detailed notes were taken to capture key points. Subsequently, the collected data was analyzed in alignment with the study's objectives and questions.

For the in-depth interviews, we extended invitations to two physical therapists, one physician and one nurse, who possessed substantial three years' experience in providing education, screen for tobacco use and incorporate cessation strategies to smokers of RYO and FM cigarettes. In-depth interviews included an open-ended, discovery-oriented approach utilized to acquire comprehensive insights on several key areas, including

perceptions of harm, attitudes toward smoking, factors influencing perceptions of harm and attitudes, and the health impacts associated with smoking. Data were collected from March 2022 to May 2023.

In Phase 2, content validation was evaluated by three experts with at least three years' experience in caring for smokers or conducting research with cigarette smokers, and who had developed or used questionnaires. This panel included two physical therapist instructors and one nurse. The reliability test was performed with 30 adult smokers of RYO and FM cigarettes. Participants in the focus groups and reliability test met the inclusion criteria: aged 18 or older, active smoker of RYO or FM cigarettes, good communication ability, and willingness to cooperate. The exclusion criteria included the inability to communicate in, read, or understand the Thai language and smoking both RYO and FM cigarettes. Data was collected through face-to-face interviews. Adult RYO and FM cigarette smokers were asked the level of agreement questionnaire. The responses were classified according to their harm perception and attitudes toward smoking.

Development of the questionnaire

Literature on harm perception, attitude, factors affecting harm perception and attitude, and the effects of smoking on health were reviewed. The questionnaire included three parts: 1) Harm perceptions, with four items; 2) Attitudes toward smoking, with 20 items; and 3) Attitudes toward smoking cessation, with five items. The focus group among eight smokers of RYO and FM cigarettes to explore their harm perception, attitudes toward smoking, and attitudes toward smoking cessation were conducted. The participants were recruited from communities in a Southern region of Thailand using convenience sampling. We also conducted four in-depth interviews with healthcare experts.

Content validity test

To determine the relevance of the items on harm perception and attitudes, the content validity for scales (S-CVI) and item content validity index (I-CVI) were measured, thereby ensuring consistency with the objective content. The content validation was evaluated by three experts, who rated each item based on its relevance to the objectives for calculating the I-CVI using a 4-point scale: 1 (*not relevant to the measured domain*), 2 (*somewhat relevant to the measured domain*), 3 (*quite relevant to the measured domain*), and 4 (*highly relevant to the measured domain*). The I-CVI index for the three experts must be equal to 1.0 indicating a good content validity.¹⁸ The universal agreement (UA) scores was assessed which obtains the S-CVI/UA, representing the proportion of items achieving a rating of 3 or 4 by all the content experts.¹⁹ An S-CVI/UA index of at least 0.8 is considered acceptable.²⁰

Reliability test

The reliability of the questionnaire was tested with 30 participants that are based on the intra-class correlation test from Cronbach's alpha coefficient result. A sample size of 30 respondents who smoked RYO or FM cigarettes has been proposed sufficiently to assess the reliability of the questionnaire.²¹ The Cronbach's alpha coefficient was classified into four cutoff points: ≤ 0.5 (*low internal consistency reliability*), 0.5-0.7 (*moderate internal consistency reliability*), 0.7-0.9 (*high internal consistency reliability*), and ≥ 0.9 (*excellent internal consistency reliability*).²² Acceptable value for Cronbach's alpha coefficient is typically above 0.7.²³

Next, the test-retest reliability with the intraclass correlation coefficient (ICC) for a two-day interval was evaluated.²⁴ The ICC index ranges from 0 to 1, which is the ICC values of 0.5 and below, 0.5-0.75, 0.75-0.9, and 0.90 and above indicate poor, moderate, good, and excellent reliability, respectively.²⁵ An ICC index of 0.75 or higher is considered acceptable.²⁶

The data were processed and analyzed using IBM SPSS Statistics for Windows, version 26 (SPSS Inc, Chicago, IL). The statistical significance level was set at $p < 0.05$.

Results

Demographic data

The demographic data of 30 respondents to assess the test-retest reliability of the questionnaire is presented in Table 1. Most of participants (63.3%) were FM cigarette smokers.

Table 1. Demographic characteristics of the FM and RYO cigarette smokers (N=30).

Characteristics	N	%
Sex		
Male	30	100.0
Age (years)		
18–35	13	43.3
35–60	16	53.3
>60	1	3.3
Marital status		
Single	15	50.0
Married	11	36.7
Separated	3	10
Divorced	1	3.3
Education level		
Primary school	3	10.0
Secondary school	13	43.3
Diploma school	10	33.3
Bachelor's degree	3	10.0
Non-formal education	1	3.3
Past month income		
<10,000 THB	12	40.0
10,000–30,000 THB	17	56.7
30,001–50,000 THB	1	3.3
Occupation		
Employee	20	66.7
Government official	5	16.7
Others	5	16.7
Comorbidity		
None	25	83.3
Hypertension	5	16.7

Note: 1 THB = 0.03485 USD, as of June, 20

Validity

A total of 21 of the 29 items of the harm perception and attitudes questionnaire showed an I-CVI higher than 0.79, indicating that item content validity was at an acceptable level. The S-CVI index of the harm perception and attitudes questionnaire was 0.9, indicating an acceptable level of content validity (Table 2).

Table 2. Content validity of the harm perception and attitudes questionnaire.

Items of the harm perception and attitudes questionnaire	I-CVI	S-CVI
Harm perception		0.90
Q1: Do you think that RYO cigarettes are less addictive and harmful than FM cigarettes?	1 (>0.79)	
Q2: Are you aware that RYO cigarettes can cause serious diseases, such as cancer, emphysema, and heart disease?	1 (>0.79)	
Q3: Are you aware that FM cigarettes can cause serious diseases, such as cancer, emphysema, and heart disease?	1 (>0.79)	
Q4: Do you think that RYO cigarettes contain less nicotine than FM cigarettes?	0.67 (<0.79)	
Attitudes toward smoking		
Q5: Do you think there are more men who smoke FM cigarettes than those who smoke non-FM cigarettes?	0.67 (<0.79)	
Q6: Do you think there are more men who smoke RYO cigarettes than those who smoke non-RYO cigarettes?	0.67 (<0.79)	
Q7: Do you think there are more women who smoke FM cigarettes than those who smoke non-FM cigarettes?	0.67 (<0.79)	
Q8: Do you think there are more female who smoke RYO cigarettes than those who smoke non-RYO cigarettes?	0.67 (<0.79)	
Q9: Do you think that FM cigarette smoking is more relaxing than non-smoking?	1 (>0.79)	
Q10: Do you think that RYO cigarette smoking is more relaxing than non-smoking?	1 (>0.79)	
Q11: Do you think that men who smoke FM cigarettes are more attractive than those who don't smoke FM cigarettes?	1 (>0.79)	
Q12: Do you think that men who smoke RYO cigarettes are more attractive than those who don't smoke RYO cigarettes?	1 (>0.79)	
Q13: Do you think that women who smoke FM cigarettes are more attractive than those who don't smoke FM cigarettes?	1 (>0.79)	
Q14: Do you think that women who smoke RYO cigarettes are more attractive than those who don't smoke RYO cigarettes?	1 (>0.79)	
Q15: What should you do if a friend or close friend invites you to smoke FM or RYO cigarettes?	1 (>0.79)	
Q16: How difficult would it be for you to reduce or quit smoking?	1 (>0.79)	
Q17: Do you think FM cigarettes are harmful to your health?	1 (>0.79)	
Q18: Do you think RYO cigarettes are harmful to your health?	1 (>0.79)	
Q19: Do you think RYO cigarettes are more or less harmful to your health compared with FM cigarettes?	1 (>0.79)	
Q20: Are you likely to distance yourself from friends who smoke?	0.67 (<0.79)	
Q21: What are your thoughts on men who smoke FM cigarettes?	1 (>0.79)	
Q22: What are your thoughts on men who smoke RYO cigarettes?	1 (>0.79)	
Q23: What are your thoughts on women who smoke FM cigarettes?	1 (>0.79)	
Q24: What are your thoughts on women who smoke RYO cigarettes?	1 (>0.79)	

Table 2. Content validity of the harm perception and attitudes questionnaire (*continued*).

Items of the harm perception and attitudes questionnaire	I-CVI	S-CVI
Attitudes toward smoking cessation		
Q25: Are you thinking of quitting smoking FM or RYO cigarettes from now on?	1 (>0.79)	
Q26: Have you made efforts to quit smoking FM or RYO cigarettes within the past three months?	1 (>0.79)	
Q27: If you want to quit smoking, FM RYO cigarettes can be discontinued?	1 (>0.79)	
Q28: Have you ever received help or given advice on how to quit smoking FM or RYO cigarettes?	0.67 (<0.79)	
Q29: How much time do you think you will need to quit smoking?	0.67 (<0.79)	

Note: FM: factory-made cigarettes, RYO: roll-your-own (RYO) cigarettes, S-CVI: scale level content validity index, I-CVI: item content validity index.

Reliability

Regarding the internal consistency reliability of the harm perception and attitudes questionnaire, the Cronbach's alpha coefficient of 0.719 indicated acceptable internal consistency. The test-retest reliability using ICC between the first and second scores of the questionnaire indicated very high reliability (0.975; $p < 0.001$).

Discussion

The resultant questionnaire, addressing harm perception and smoking attitudes, exhibited favorable content validity and high internal consistency, ensuring its reliability for subsequent research endeavors. A deeper exploration into harm perception, encompassing both cognitive aspects (e.g., fear or hazard) and emotional dimensions (e.g., outrage), becomes pivotal for predicting smoking behavior and designing effective cessation interventions.⁶ The results highlight that RYO smokers found emotional reassurance in product simplicity despite recognizing health risks, while FM smokers associated harm with chemical additives, reflecting a rational risk assessment.^{27,28} Effective interventions must address both components. Unlike previous tools, this survey uniquely measures subtle beliefs, such as RYO naturalness and FM additive suspicion, while distinguishing health perceptions from cost considerations.

Validity and reliability

The content validity of the questionnaire was established through a comprehensive literature review and consultations with medical professionals possessing extensive experience in caring for smokers of RYO and FM cigarettes. The content validity of our questionnaire was established through a meticulous literature review and consultations with experts who have excellent on the education and technology fields. Including items that cover a wide spectrum of relevant topics increased the questionnaire's relevance and comprehensiveness. Furthermore, engagement with focus groups including adult RYO and FM cigarette smokers, alongside in-depth interviews with medical professionals experienced in caring for RYO and FM cigarette smokers, enhanced the questionnaire's clarity and content validity. Regarding

reliability of the harm perception and attitudes questionnaire, the questionnaire exhibited high internal consistency, as indicated by a Cronbach's alpha coefficient of 0.719, signifying an acceptable level of internal consistency among the questionnaire items, affirming their consistent measurement of the underlying constructs.

I- and S-CVI index of the harm perception and attitudes questionnaire were presented to assess construct validity. The CVI revealed a distinct factor structure consisting of three factors—harm perceptions, attitudes toward smoking, and attitudes toward smoking cessation. Each factor exhibited strong factor loadings, confirming that the questionnaire items effectively measured the intended constructs.

Smoking behaviors in Southern Thailand

The use of the questionnaire in Thailand and Asia provides a culturally unique voice to the international debate regarding smoking behavior. For instance, the study established that RYO cigarettes in Thailand are not just an inexpensive alternative but also a rural cultural norm. This type of cultural setting influences harm perception and attitudes differently compared to Western countries where RYO use is specialized and translates to individuality. Through access to these regional variations, the questionnaire provides data crucial to the development of culturally specific public health interventions, particularly for disadvantaged populations like Southeast Asia.

Smoking continues to pose a significant public health concern in Thailand, contributing to over 50,000 deaths annually.²⁹ The smoking prevalence among Thai adults decreased from 32% in 1991 to 20.7% in 2009; however, since 2009, it has remained stable at around 19-20%. Understanding the diverse smoking behaviors requires exploring cultural and socio-economic factors.³⁰ Cultural factors significantly shape smoking behaviors, with varying perceptions influenced by local norms and traditions.²⁹ For instance, in Southern part of Thailand, smoking is often revealed as a rite of passage or a social activity.

Socio-economic status (SES) significantly impacts smoking behaviors. Previous research reported a strong relationship between SES indicators and smoking habits in the Thai population, suggesting that economic factors

like income and education level can influence smoking prevalence.³¹ Thus, lower SES groups might have higher smoking rates due to less access to health education and resources. Consistent with this theory that most participants had low to mid-income levels.

Gender differences are also presented in smoking behaviors in Thailand.²⁹ While men have traditionally been more likely to smoke, recent concerns have been raised about rising smoking rates among women.³² In this study, all participants were men, which may be due to changing societal roles, higher stress, or targeted marketing by tobacco companies. Studies have shown that women's smoking behavior notably differ from men's, especially in terms of place of residence and occupational status,³³ highlighting the need for gender-specific studies and interventions.

Education levels in Thailand significantly may affect smoking habits and prevalence. Previous evidence documented that lower education levels correlated with higher smoking rates and a preference for RYO cigarettes, while higher education levels were associated with reduced smoking rates, especially among men.³³ Therefore, it was consistency with the results in this study that most participants had an education level below a bachelor's degree.

Smoking behaviors in Thailand are influenced by a complex interplay of cultural, socio-economic, and gender-specific factors.³³ Effective tobacco control and cessation interventions must consider these diverse influences to be successful. Recognizing and addressing these varied factors can help tailor public health policies that are culturally sensitive and effective in reducing smoking prevalence across different Thai populations.

A model of harm perception and RYO and FM cigarette attitudes must combine individual, sociocultural, and policy determinants. Harm perception worldwide is influenced by risk perceptions of smoking based on tobacco marketing, packaging, and public health messages.³⁴ RYO cigarettes are commonly perceived as less harmful based on "natural" imagery even though there is proof that it contains increased toxicant levels.¹² The smoking habits in Asia are influenced by norms, customs, and economics, where RYO cigarette price and cultural self-sufficiency are factors that ensure popularity. Thailand's tobacco control measures, including taxation and health warning measures, impact perceptions³⁵, although RYO cigarette smoking remains in poor communities owing to affordability and lower harm perceptions. These are the recommendations that inform targeted interventions in Thailand and Asia.

Limitations and future directions

In the preliminary phases of questionnaire development, only the southern part of Thailand is used for the initial evaluation to identify major issues before expanding to other areas. Despite these challenges, this pilot study provides valuable insights for public health strategies aimed at reducing smoking prevalence and fostering informed decision-making among smokers.

This pilot study's sample size was relatively small, necessitating further validation with a larger and more diverse sample. Conducting a Confirmatory Factor Analysis (CFA) represents a critical next step in this research. This analysis will evaluate the factor structure of the questionnaire, ensuring that the items correspond to the hypothesized dimensions of harm perception and attitudes, thereby strengthening the instrument's validity and alignment with the study's theoretical framework. These limitations underscore the need for future research endeavors to encompass broader participant demographics and delve into multifaceted aspects of smoking behavior and related perceptions.

Recommendations

The research and educational potential of the questionnaire is limited by the lack of harm perception and smoking attitude items. Optimal design necessitates well-defined, validated items that are concordant with study aims. Refinement must involve the addition of detailed, focused items, with pilot testing to determine reliability and validity. The cyclical process is needed for the development of a strong instrument that will provide useful information and make a useful contribution to research and practice in this area. It provides researchers with a reliable and valid tool to examine the ethical considerations and harm perception linked to RYO and FM cigarette smoking in southern Thailand. One noteworthy insight of our study is the heightened levels of addiction observed among RYO and FM cigarette smokers. Our findings highlight the importance of understanding risk perceptions and ethical considerations related to harm perception associated with RYO and FM cigarette smoking in southern Thailand. This newly developed scale provides a valuable tool to healthcare providers and policymakers for assessing and addressing barriers to accessing information and understanding harmful perceptions of cigarette smoking, thereby improving public health outcomes. This situation can be partly attributed to the convenient availability of locally grown tobacco, coupled with the practice of personally RYO cigarettes. This personalized approach to RYO smoking also results in diminished exposure to mandated health warnings, pointing toward a regulatory gap necessitating attention.

Responses to questionnaires on smoking behaviors can vary significantly based on socio-economic status, cultural background, and other demographic factors, which can complicate the generalization of findings and the development of effective public health interventions across different groups.³⁶ While questionnaires are useful for assessing harm perceptions and attitudes towards smoking, their effectiveness is limited by issues such as self-report bias, lack of specificity, predictive power, broad response variability, and social desirability effects.³⁷ A gap in research on localized harm perceptions of tobacco use in Thailand highlights the need for research on these perceptions to inform targeted public health strategies and enhance smoking cessation programs and policies.

Conclusions

Data received through this questionnaire can be used to inform evidence-based targeted public health interventions. For example, the myth that is established that RYO cigarettes are safer because of their perceived naturalness can be utilized to frame the creation of education campaigns against this myth. In addition, understanding economic motivators for the consumption of RYO could provide the foundation for policies like price controls or programs subsidizing quit aids, correcting the economic drivers of smoking behaviors. These applications are in line with the general aim of reducing the prevalence of smoking by directing interventions to the specific attitudes and perceptions revealed in the study. The methodological consistency employed during the development and validation phases instills confidence in the tool's efficacy and reliability, offering a promising avenue for gaining a deeper understanding of smoking behavior and crafting targeted cessation interventions tailored to these distinct smoking cohorts.

Ethic approval

This study was approved by the Human Research Ethics Committee, Faculty of Medicine, Prince of Songkla University (No. 64-489-30-2).

Conflict of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

This study was funded by the Thai Health Promotion Foundation and the National Alliance for Tobacco-Free Thailand. We also extend our gratitude to the participants for their time and contributions.

Funding resources

This study received financial support from the Thai Health Promotion Foundation Fund (PI: Thaworncheep). Grant REC.64-489-30-2.

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The Effect of walking with eyes closed (Makarach) on postural control and the autonomic nervous system (ANS) function: A randomized controlled trial

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ARTICLE INFO

Article history:

Received 6 October 2024

Accepted as revised 6 March 2025

Available online 11 March 2025

Keywords:

Musculoskeletal, postural control, rehabilitation, mindfulness, balance.

ABSTRACT

Background: Postural control (PC) is essential for performing complex movements and often deteriorates over time due to various factors, necessitating more flexible exercise options. Mind-body practices are purported to improve postural control.

Objective: To determine the effect of Makarach, a mind-body practice, on postural control and the autonomic nervous system (ANS) function.

Materials and methods: A randomized controlled trial (RCT) was conducted involving 76 participants, divided into two groups: experimental group (EG, N=38) and control group (CG, N=38). The EG walked forward and backward on two yoga mats with eyes closed while the CG performed tandem walking (TW). Both groups underwent a four-week training program (15 minutes per day, three days per week). The Y Balance Test (YBT) assessed the dynamic postural control (PC) in the anterior (A), posterolateral (PL), and posteromedial (PM) directions. The composite score (CS) was used for overall performance in the YBT. The Balance Error Scoring System (BESS) assessed static PC on firm (FIS) and foam (FOS) surfaces. Heart Rate Variability (HRV) components, including low frequency (LF), high frequency (HF), and low to high frequency (LF/HF) ratio, were used to evaluate the ANS function. All outcomes were measured before and after training. Data analyses were performed using intention-to-treat analysis.

Results: The dynamic and static PC was found to increase in EG only. Significant differences were found in A, PL, PM, and overall dynamic PC (CS) in dominant and non-dominant legs ($p < 0.05$) and ($p < 0.05$), respectively. Significant differences were also found in static PC in FIS ($p < 0.05$), while no significant difference was observed in FOS. There were no significant differences in any HRV components ($p \geq 0.05$). There was significant interaction between groups and time in dynamic PC and FIS, but no significant within-group difference in any CG variables.

Conclusion: Walking with eyes closed (Makarach) was found to significantly increase dynamic and static PC among healthy individuals after four weeks of training in comparison to before training. Additionally, Makarach demonstrated greater effectiveness in improving PC than TW.

Introduction

Postural control (PC) is a fundamental aspect of human motor function, allowing individuals to maintain balance and perform complex movements efficiently. Maintaining PC necessitates the ability of an individual to accurately detect sensory stimuli, interpret the information, and execute adequate responses through the integration of somatosensory, vestibular, and visual

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doi: 10.12982/JAMS.2025.054

E-ISSN: 2539-6056

systems.¹⁻³ The ANS plays an essential role in the interplay of the sensory and motor systems and in maintaining homeostasis during physical activities to sustain PC.^{4,5}

PC decreases over time from the age of 20 years.⁶ Several studies have proposed training programs for improving and maintaining PC, such as TW,⁷ Bosu ball,⁸ Balance Error Scoring System (BESS),⁹ knee position sense, and YBT,¹⁰ highlighting the importance of sensory input in maintaining PC. Sensory deprivation, such as closing the eyes, has been shown to disrupt balance and postural stability, suggesting a significant reliance on visual information for PC.⁶ To balance the body's responses to sensory detection from the numerous stimuli received by the central nervous system, the ANS manages cardiovascular, thermal, and gastrointestinal homeostasis when individuals feel safe or threatened by activating rest and digest/fight or flight.^{11,12}

Makarach is a mindfulness-based walking meditation technique conducted with eyes closed, creating sensory deprivation that enhances reliance on proprioceptive feedback. In contrast to conventional walking meditation, typically performed with eyes open and influenced by visual cues, Makarach focuses on precise foot placement and body movement.¹³⁻¹⁶ The current study uses Tandem walking (TW) as a comparative method since it is commonly

used in clinical and research settings to assess PC. TW requires coordination and balance, providing reliable baseline for evaluating interventions like Makarach.

Therefore, the main purpose of this study is to investigate the effect of walking with eyes closed (Makarach) on PC. The second objective is to investigate the ANS function in Makarach of healthy individuals and compare the outcomes with TW exercises. The hypothesis of the study is that walking with eyes closed (Makarach) is more effective in improving PC than TW.

Materials and methods

Participants

A single-blinded RCT with a pre-test and post-test group was conducted. The sample size, based on the standard deviation of the SEBT in three directions from a previous study,¹⁶ was calculated for 80% power and a 0.05 significance level, resulting in 60 participants. Including a 10% dropout rate, the final sample size was 76 participants (N=38 per group). The CONSORT flow diagram is shown in Figure 1. The study was approved by the Center for Ethics in Human Research, Khon Kaen University, Thailand (HE662196). The study protocol was registered in the RCT registry (TCTRXXXXXX on process)

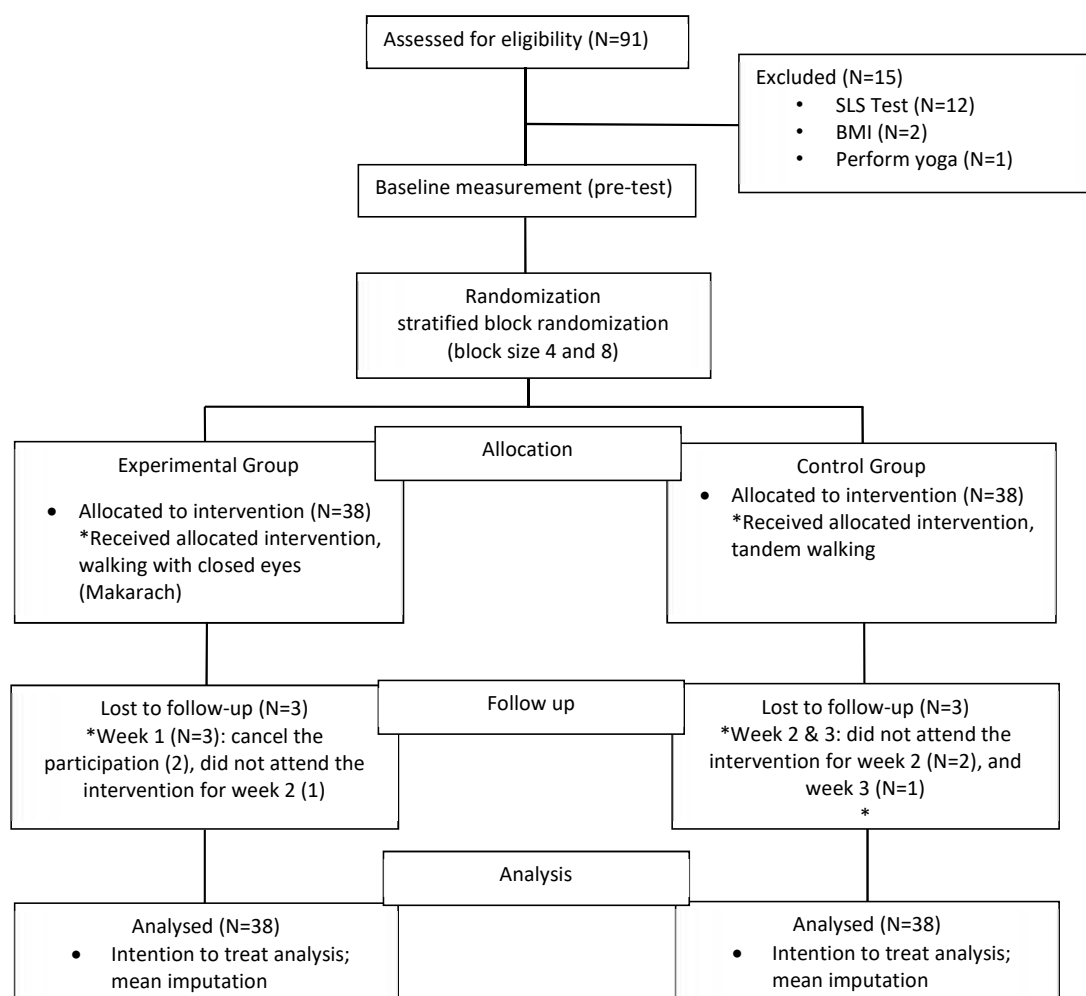


Figure 1. CONSORT flow diagram of participant progression.

Ninety-one participants were recruited, with 76 meeting the inclusion criteria: aged 20-35 years (22.79 ± 2.59) with a BMI of 18.5-25.0 kg/m² (21.18 ± 2.02). Eligibility required a single-leg stance test score with eyes closed of ≤ 13.1 seconds for females and ≤ 10.2 seconds for males on at least one leg. Exclusion criteria included prior walking meditation for a month, neurological or musculoskeletal disorders affecting balance, visual, auditory, and vestibular deficits, and regular smoking. Participants were instructed to avoid meditation activities, including yoga, during the intervention period.

Experimental procedures

At baseline, each participant's balance ability was evaluated using the YBT and BESS. In this study, two assessors measured the YBT and BESS. The interrater reliability was excellent, with an ICC of 0.95-0.99, while the BESS showed good to excellent reliability, with ICC values ranging from 0.90-0.95.

The allocation was blinded to the assessor from the generated random group assignments by a concealed envelope. An internet randomized scheme generator,

which generated blocks of four and eight, was used to allocate the participants into groups for the intervention. The randomization was stratified by groups. The main researcher undertook the randomization and allocation of the participants in the trial.

Walking with eyes closed (Makarach)

Participants in the EG practiced walking meditation with eyes closed for 15 minutes a day, three times a week, for four weeks. Makarach is a walking meditation practice performed by walking forward and backward with eyes closed on the yoga mat. The concept of Makarach in this study was from Mr. Saran Maitreewech, Bangkok, Thailand.¹⁷ Two yoga mats (366 cm) were used (Kojima: each 183 x 61 cm, thickness 10 mm). This intervention focused on leg and foot movements. Participants removed their footwear and stood upright with hands behind their backs. After a three-minute familiarization trial, they started walking backward, closing their eyes, and continued for 15 minutes. The edge of the yoga mat was a benchmark that indicated when to switch directions. The Makarach intervention is shown in Figure 2.

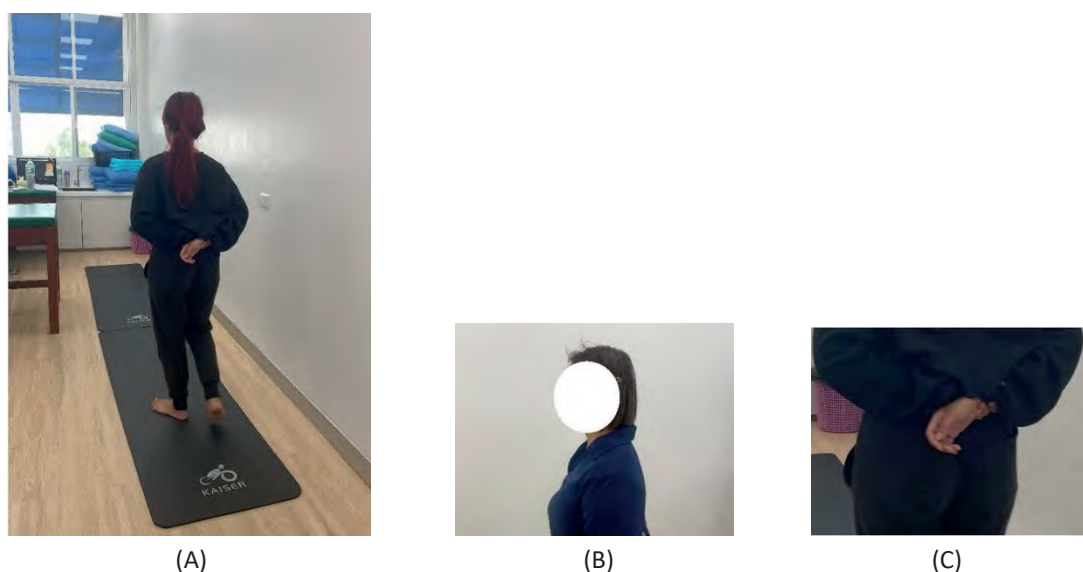


Figure 2. Walking with closed eyes (Makarach): (A) Walking process, (B) Head position, (C) Hand position.

Intervention for the control group (CG)

Participants in CG performed TW in a straight line with their eyes open, placing the heel of one foot in front of the toes of the other along a 3-m, 48-mm-wide line and turning 180 degrees at the end.¹⁸ Each session lasted for 15 minutes, three days per week for four weeks. A spotter stood next to participants in both the experimental and CGs to prevent adverse events, such as loss of balance, and provide verbal cues when the session was up.

Outcome measurements

Y Balance Test (YBT)

The YBT was used for the assessment of dynamic balance based on the guidelines for both dominant and non-dominant legs, respectively. The dominant (DL) was

determined by asking the participants to kick a ball placed on the floor and the kicking legs were recorded as the DL and the other leg as the non-dominant legs (NDL).¹⁹

Prior to the test, participants remove their shoes and perform the test barefoot. Leg length (LL) was measured in a supine position, from the anterior superior iliac spine to the most distal aspect of the medial malleolus. Participants complete four practice trials in three directions: anterior (A), posterolateral (PL), and posteromedial (PM). After a two-minute rest following the practice session, formal testing begins. During the formal testing, participants perform three trials per direction, standing on one leg with their hands on their hips and reaching as far as possible with the other leg while maintaining balance. A 15-second rest is given between trials, switching from one

leg to the other in each direction and a two-minute break is provided between directions to avoid fatigue.

Participants touch the furthest point on the line with the most distal part of their reaching foot, then return to a bilateral stance while maintaining equilibrium. The examiner marks the touch point along the line and measures the distance in centimeters from the center of the grid using a tape measure. The composite score (CS) was measured for the analysis of overall performance. The represented YBT for each direction consisted of the normalized reach distance and the CS of both DL and NDL.^{16,19} Any participant who withdrew for any reason or did not complete all interventions was considered for withdrawal from the study.

Balance Error Scoring System (BESS)

The static balance was measured using the BESS with an AIREX Balance Pad. The BESS includes six trials of 20 seconds each and the assessment of three stances (double leg, single leg, tandem) on two surfaces: firm (FIS) and foam (FOS). The foam surface is unstable. Participants stood still with their hands on iliac crests and eyes closed. Testing began once the proper stance had been assumed.²⁰ For the tandem stance, the non-dominant foot was behind the dominant foot. Errors were recorded for hands lifting off hips, opening eyes, stepping, stumbling, falling, lifting the heel, hip abduction over 30 degrees, or not returning to the test position within five seconds. Each surface had a maximum of 10 errors, and the total errors were summed as BESS scores.

Heart rate variability (HRV)

The HRV measurements were recorded in a quiet environment in the morning using the uBioMacpa version 70 (BioSense Creative, Korea). This reliable device, approved as a medical device (13-262) and certified for manufacturing (No. 3447), is patented (Patent No. 10-0954817). It measured 40-200 BPM with a 2% error rate and paired with the left index finger. The HRV was measured from 8 a.m. to 11 a.m. and maintained a comfortable level.²¹ After five minutes of rest in a sitting position, the participants were asked to lie down on the bed, and the HRV was continuously assessed. The results were displayed on a notebook monitor and included frequency domain metrics: low frequency (LF, ms²), high frequency (HF, ms²), and the LF/HF ratio.

Statistical analysis

Data were expressed as mean±standard deviation (SD). The Shapiro and Wilk tests showed normal distribution. All analyses were performed on an intention-to-treat (ITT) basis. Missing data were handled using mean imputation

of the average post-test scores. Paired sample t-tests were conducted to compare the differences within the groups (pre and post). Independent t-tests were used to compare the two groups at the baseline. The analysis of covariance (ANCOVA) was applied to determine the difference in dependent variables between groups after four weeks of intervention (post-test). The analysis used baseline (pre-test) scores as a covariate. Differences in the PC and HRV between the EG and CG were analyzed at the baseline (pre) and after four weeks of training (post) using two-way ANOVA (group × time). The precision of Makarach was interpreted using partial eta squared (η_p^2), which indicates the effect size in both the ANCOVA and two-way ANOVA analyses. Effect sizes were interpreted as $0.01 < \eta_p^2 < 0.06$ is small, $0.06 \leq \eta_p^2 \leq 0.14$ medium, and $\eta_p^2 > 0.14$ large.²² The significant difference level for all data analysis was set at below 0.05.

Results

A total of 76 individuals participated in this study; 35.5% (N=27) were male, and 64.5% (N=49) were female and randomly assigned to the EG and CG (38 in each), respectively. Throughout the study, adverse events were closely monitored in both EG and CG. One notable event was a tingling sensation reported by participants. This sensation occurred during the first week of the intervention, which involved three sessions per week, each lasting 15 minutes. Specifically, three participants (7.89%) in the EG and five (13.16%) in the CG reported experiencing a tingling sensation. Importantly, the sensation was typically resolved within 15 minutes after each session. These findings suggest that while a transient adverse event occurred, it did not result in any long-term effects on the participant's health, supporting the overall safety profile of the intervention.

The baseline characteristics of participants are shown in Table 1. There was no significant difference in the characteristics between EG and CG. The complete results of the primary and secondary objectives of the study are shown in Tables 2 and 3. The results showed significant differences in EG after four weeks of intervention in all components of dynamic PC ($p < 0.05$). Meanwhile, only one component of static PC (FIS) showed a significant difference ($p < 0.05$). The results showed significant group × time interaction for all components of dynamic PC ($p < 0.05$). After four weeks of intervention, the significant group × time interaction also showed static PC in the FIS ($p < 0.05$). There was no significant difference in all outcomes in the CG ($p > 0.05$). There were no significant differences in the EG and CG at post-intervention for the LF, HF, and LF/HF ratio ($p > 0.05$). There was no significant interaction between group × time interaction in any of the HRV outcomes ($p > 0.05$).

Table 1. Baseline characteristic participants in the experimental group (EC) and control group (CG).

Variable	Experimental Group		Control Group		p value [#]
	mean±SD	min-max	mean±SD	min-max	
Gender, N (%) (male: female)	14 (36.8):24 (63.2)		13 (34.2): 25 (65.8)		0.81
Status, N (%)					0.87
Bachelor	30 (78.9)		30 (78.9)		
Master	3 (7.9)		2 (5.3)		
Employee	5 (13.2)		6 (15.8)		
BMI (kg/m ²)	21.28±2.18	18.6-24.8	21.07±1.87	18.5-24.9	0.65
Age (year)	22.76±2.48	20-32	22.82±2.74	20-33	0.93
Dominant Leg, N (%)					0.40
Right	36 (94.7)		34 (89.5)		
Left	2 (5.3)		4 (10.5)		
Non-Dominant Leg, N (%)					0.40
Right	2 (5.3)		4 (10.5)		
Left	36 (94.7)		34 (89.5)		
Impaired Leg (SLS Positive)					
Right, N (%)	12 (31.6)		7 (18.4)		
Left, N (%)	8 (21.1)		8 (21.1)		
Both, N (%)	18 (47.4)		23 (60.5)		
Performance, sec	5.68±2.41	1.29-10.08	4.51±2.01	1.67-8.32	
Normal Leg (SLS Negative)					
Right, N (%)	8 (21.1)		8 (21.1)		
Left, N (%)	12 (31.6)		7 (18.4)		
None, N (%)	18 (47.4)		23 (60.5)		
Performance, sec	10.44±5.98	1.56-23.99	8.81±6.17	2.27-26.40	
Adverse event, N (%)					
Tingling sensation	3 (7.89)		5 (13.16)		

Note: kg/m²: kilogram per square meter, SLS: single leg stance, sec: seconds. [#]comparison for gender, status, BMI, age, dominant and non-dominant leg by independent t-test.

Table 2. Between- and within-group differences in Y Balance Test (YBT) on dominant and non-dominant legs and Balance Error Scoring System (BESS) in the experimental group (EG) and control group (CG) before and after the 4-week intervention.

Outcomes		EG (N=38)		CG (N=38)		p-value	Two-way ANOVA interaction effect	
		pre	post	pre	post		p-value	effect size
YBT								
Dominant ^{#1}	Anterior	66.30±6.47	69.19±6.31	67.02±5.88	67.07±5.79	<0.001* 0.010 [#]	0.02 [†]	0.07
	Posterolateral	79.71±10.39	87.05±11.51	82.67±12.33	82.60±15.13	<0.001* 0.010 [#]	0.007 [†]	0.10
	Posteromedial	88.00±8.50	94.29±8.49	90.63±6.94	89.67±7.85	<0.001* <0.001 [#]	<0.001 [†]	0.19
	Composite Score	78.10±6.99	83.51±7.20	80.11±6.75	79.81±8.34	<0.001* <0.001 [#]	<0.001 [†]	0.18
YBT non-dominant ^{#1}								
	Anterior	66.75±6.67	69.21±7.09	66.80±5.35	66.87±4.16	<0.001* 0.025 [#]	0.02 [†]	0.07
	Posterolateral	82.78±8.24	89.48±8.09	83.68±11.41	82.07±12.76	<0.001* <0.001 [#]	<0.001 [†]	0.17
	Posteromedial	88.34±7.57	92.64±9.89	90.91±7.51	89.71±10.76	<0.001* 0.017 [#]	0.009 [†]	0.09
	Composite Score	79.29±5.09	83.77±6.52	80.47±6.50	79.55±7.86	<0.001* <0.001 [#]	<0.001 [†]	0.20
BESS ^{#2}								
	Firm	2.79±1.85	1.63±1.50	2.29±1.68	2.24±1.91	<0.001* 0.021 [#]	0.009 [†]	0.09
	Foam	8.16±2.89	7.61±3.34	8.76±3.04	8.24±2.71	0.38 0.47	0.98	0.000

Note: *significantly different within-group (EG), $p < 0.05$. [#]significantly different between group at the post intervention, $p < 0.05$. [†]significantly interaction between group and time, $p < 0.05$. There was no significant difference between-group at the pre-intervention ($p > 0.05$). No significant difference within-group in all outcomes in control group ($p > 0.05$). ^{#1}measured in centimetres (cm), ^{#2}error points (number).

Table 3. Between- and within-group differences in Heart Rate Variability (HRV) components in the experimental group (EG) and control group (CG) before and after the 4-week intervention.

Outcomes ^{#3}		EG (N=38)		CG (N=38)		p-value	Two-way ANOVA	
		pre	post	pre	post		p-value	effect size
HRV	LF	6.42±0.97	6.36±0.99	6.51±0.91	6.60±0.99	0.75 0.36	0.57	0.004
	HF	6.22±0.61	6.13±0.74	6.06±0.71	6.22±0.68	0.39 0.20	0.11	0.035
	LF/HF	1.14±0.66	1.03±0.15	1.08±0.14	1.06±0.14	0.33 0.41	0.43	0.008

Note: There was no significant difference between-group at the pre-intervention ($p > 0.05$). No significant difference within-group in all outcomes in control group ($p > 0.05$). ^{#1}measured in centimetres (cm), ^{#2}error points (number). LF=low frequency, HF=high frequency, LF/HF=low to high frequency. ^{#3}measured in ms^2 .

Discussion

Effect of Makarach on postural control (PC)

The results of this study demonstrated that Makarach significantly enhanced dynamic and static PC, evaluated using YBT and BESS. After training, there was no significant difference in the FOS, LF, HF, and LF/HF ratio, either in the EG or CG, respectively. However, post-training, the EG presented a significant increase in YBT and BESS for the firm surface compared to the baseline.

Makarach is a walking meditation (WM) intervention. The study's findings reveal that Makarach can provide an enhanced dynamic PC. This result is supported by the previous study investigating the effect of WM on balance performance after a four-week intervention in the PL and PM direction.¹⁶ Furthermore, a four-week walking meditation intervention can improve PC compared to gentle walking exercises.^{15,23} One of the key mechanisms underlying Makarach is the enhanced proprioceptive feedback gained through walking meditation. As participants engaged in mindful walking—moving forward and backward with their eyes closed—they likely developed a heightened awareness of their body's positioning in space. Proprioception, the body's ability to sense its position and movement, is crucial for maintaining balance, especially in dynamic tasks.²⁴ This aligns with previous research demonstrating a strong correlation between proprioceptive training and improved balance performance.²⁵

Makarach required participants to rely heavily on proprioceptive feedback from the ankles to maintain stability from the yoga mat surface and led participants to perform a slow walking pattern. This reliance was reflected in improved postural control, as evidenced by significant enhancements in YBT and BESS results. These findings suggest that the Makarach could influence proprioceptive function indirectly, even without direct measurement. According to previous studies, walking at a slow speed requires more coordination control of the lower limb muscles to create a smooth movement.²⁶ Consequently, slow walking, which needs multi-muscular control for smooth movement, resembles the neuromuscular system and proprioceptive sense. An increase in ankle proprioception allows participants to maintain the single-leg stance position for longer when performing both YBT and BESS. As a result, the SLS position can be maintained for longer and the reach distance of the leg becomes greater. These results support those reported in the previous study, namely that balance training with eyes closed leads to an increase in the maximal stance duration up to 60 seconds and reach distance in YBT components.²⁷

Moreover, the meditative aspect of Makarach played a significant role in enhancing participants' mental focus and body awareness. Meditation practices have been linked to enhanced cognitive control and motor skills by promoting a deeper awareness of physical sensations.²⁸ Meditation practice engages specific brain areas, such as the insula. Regular meditation practice has been linked to increased cortical thickness in the right anterior insula, which is crucial for body awareness.²⁹ Body awareness

refers to the conscious perception of one's body and its spatial position. Reduced cortical thickness is associated with cognitive impairment, affecting the ability to sense the body's position and movement. Meditation practice also increases the alpha modulation in the primary sensory cortex, which is involved in sensory perception, including proprioceptive sense.³⁰ Moreover, according to a systematic review and meta-analysis, meditation practice is associated with brain morphological adaptation in some areas (anterior and posterior cingulate cortex, hippocampus, and corpus callosum), responsible for self-control, emotion regulation, and movement control.³¹ Practicing Makarach may promote the perception process of the brain for ankle adjustment and enhance proprioceptive feedback by focusing on leg and ankle movements. This mindful attention to each step trains the proprioceptive system to be more sensitive and responsive.

This study highlights the significant impact of surface type on static PC, measured by the BESS. Notably, improvements in balance (indicated by reduced errors) were observed only on firm surfaces, where participants effectively used proprioceptive feedback from their ankles to maintain their center of mass. This finding aligns with previous research emphasizing the importance of stable surfaces for balance assessment.³²

In contrast, no significant improvements were found on foam surfaces. The instability of the foam disrupted the somatosensory information necessary for PC. The greater thickness of the AIREX Balance foam pad compared to the 10 mm yoga mat likely contributed to this instability, complicating balance maintenance and increasing errors.³³ This scenario reflects real-life conditions, where individuals rarely walk on unstable surfaces. The foam's thickness may increase postural sway and require more active engagement of core muscles to manage shifts in the center of mass.³⁴

Effect of Makarach on the ANS Function

The ANS regulates internal organ functions to support rapid body movements during exercise, adjusting physiological parameters like blood pressure, sweating, respiration, and heart rate through their sympathetic and parasympathetic systems.³⁵ This study used HRV to measure ANS activity with low LF, HF, and LF/HF ratios. The results showed no statistically significant differences in LF, HF, or LF/HF ratios within or between groups.

Several factors may have influenced these results. Individual thermal sensations and comfort levels could have confounded measurements; despite maintaining a comfortable room temperature of 24°C,²¹ some participants felt cold, covering their wrists with towels, potentially impacting measurement accuracy. Stress levels likely also affected the results since many participants were bachelor's degree students undergoing final exams and experiencing sleep deprivation associated with elevated stress. These findings agree with the previous study which reveals that stress indicates low parasympathetic activity, characterized by a decrease in the HF and an increase in the LF.³⁶ Additionally, variability in post-test measurement times

may have introduced further inconsistencies. Although the procedure in this study required HRV post-tests to be conducted up to one day after the final intervention, some occurred two to three days later owing to the participants consuming coffee or alcohol, known to activate the SNS and increase blood pressure, adding variability to the HRV readings. Previous studies have claimed that caffeine consumption increases systolic and diastolic blood pressure, leading to increased SNS.³⁷ Another point to be discussed is the measurement time of HRV values. In prior studies, the HRV values were generally measured for five minutes. However, in this study the subjects were measured for 2.5 minutes based on the minimum amount of time required to measure the HRV according to the previous study.³⁸ Further research is required to examine whether there was no statistical significance in all components of HRV.

Efficacy of Makarach vs tandem walking in improving PC

The findings of this study reveal that Makarach, a form of walking meditation with eyes closed on a foam surface, presents unique and superior benefits for improving balance and sensory integration compared to TW.

The key distinctions between Makarach and TW are rooted in their fundamental mechanisms. Firstly, Makarach involves mindful walking—moving forward and backward with eyes closed—while TW is conducted with eyes open, providing visual cues about the surroundings. In Makarach, the absence of sensory information from visual input requires participants to rely on sensory feedback from their ankles and the vestibular system, heightening proprioceptive awareness to maintain PC. Consequently, the proprioceptive sense in the ankle demands that greater feedback be provided about the position of the feet, increasing sensory awareness and integration. The perturbation of sensory information induces movement adaptation to enhance somatosensory information, thereby assisting task performance.³⁹ This increased reliance enhances feedback on foot positioning and fosters sensory integration. The disruption of sensory information during Makarach prompts movement adjustment, aiding task performance. On the other hand, although TW also poses challenges related to precise foot placement and coordination, the availability of visual input facilitates balance adjustment.

Secondly, sensory input in Makarach is more challenging due to an unstable base of support (BoS), which may play a role in enhancing the proprioceptive sense to maintain PC. The unstable surface in Makarach requires continuous adjustment from the proprioceptive system, particularly in the ankle joints. When visual input is absent, the body relies more heavily on proprioceptive signals in the ankle to maintain balance. This increased demand enhances the sensitivity and responsiveness of the proprioceptive system. This is supported by a previous study, which reveals that balance control mostly involves ankle muscle proprioceptive signals under unstable support.⁴⁰ Another WM study also supports this result, revealing an improvement in ankle proprioception

and balance performance after four weeks of walking meditation intervention in older adults and chronic ankle instability.^{15,16,23} Another possible reason is the tendency to decrease the sway path, potentially contributing to the improvement in ankle proprioception.³³ In contrast, TW provides visual information about the environment, aiding the adjustment of posture and movement for balance. Although TW also appears challenging due to the requirement for precise foot placement and coordination, the presence of visual input aids significantly in integrating environmental cues with movement. While TW offers valuable benefits in terms of precision and coordination, Makarach stands out as a superior method for enhancing overall balance and sensory integration. Its emphasis on proprioceptive training, combined with the mindfulness component, makes Makarach a more effective and comprehensive approach for improving PC and stability.

This study utilized an ITT analysis to ensure that all randomized participants were included in the primary analysis. Missing data were handled using mean imputation, with missing values replaced with the mean value of the observed data for each variable. While mean imputation allows for the inclusion of all participants and maintains the sample size, it has some limitations. Specifically, it may underestimate the variability of the data and potentially attenuate the statistical power of the analysis. However, future studies should consider more sophisticated methods for handling missing data, such as multiple imputation or mixed-effects models, to address these limitations more effectively.

Conclusion

The concepts of Makarach highlight that individuals train their proprioceptive sense in the ankles to allow for the lack of visual input. Not only do they train their physical balance and proprioception, but the key brain regions associated with mindfulness are also strengthened. This holistic approach enhances both mental and physical well-being, leading to improved PC and a more balanced state of mind. Further research may use the concept of Makarach for various applications, including fall prevention and rehabilitation in different populations.

Conflicts of interest

The authors declare no conflict of interest.

Funding

The author discloses receipt of the following financial support for the research: Khon Kaen University with ASEAN-GMS Scholarship and Buranabuddha Foundation.

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Relationship between risk factors at workplace and upper limb work-related musculoskeletal pain among poultry slaughterhouse workers: A cross-sectional study

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ARTICLE INFO

Article history:

Received 15 September 2024

Accepted as revised 12 March 2025

Available online 13 March 2025

Keywords:

Poultry slaughterhouse workers, PSW, upper limb work-related musculoskeletal pain, UL-WMSP, ergonomic risk factors, ergonomic risk assessment, rapid upper limb assessment, RULA.

ABSTRACT

Background: Poultry slaughterhouse workers (PSW) are exposed to various risk factors at work that contributes to upper limb work-related musculoskeletal pain (UL-WMSP) at workplace.

Objective: This study evaluated the relationship between various risk factors at workplace and UL-WMSP among PSW.

Materials and methods: A cross-sectional descriptive study was conducted in Thailand among 98 PSW (52 women and 46 men). A self-reported form was used to collect demographic factors and job task profile among PSW. Rapid upper limb assessment (RULA) was used to evaluate the risk of poor posture at work. The relationship between the risk factors and prevalence of UL-WMSP was evaluated through univariate logistic regression analysis (AMSEC-64EX-112).

Results: About 86.7% (N=85) of PSW reported UL-WMSP over the last 12 months, and 68.4% (N=67) reported UL-WMSP during the period of the last 7 days. Around 91.8% (N=90) of PSW exhibited a poor posture at work with RULA scores at 7 or more indicating a high level of risk for UL-WMSP. Risk factors such as age ($p<0.04$) was significantly related with prevalence of UL-WMSP over the period of the last 7 days. A significant relationship was also found between job tasks such as poultry abdominal slitting task ($p<0.02$), poultry cutting-up task ($p<0.03$), types of tool use ($p<0.01$), poor posture ($p<0.01$) and prevalence of UL-WMSP over the last 12 months.

Conclusion: Risk factors such as age, job tasks and poor posture are related to UL-WMSP among PSW at workplace. There is an essential need to develop musculoskeletal health and wellbeing program for the PSW to reduce UL-WMSP at the workplace and to create a healthy working environment.

Introduction

Work-related musculoskeletal pain (WMSP) is a common occupational disease which involves all parts of the body such as neck, upper limbs, and back region among poultry slaughterhouse workers (PSW).¹ Globally, PSW had high prevalence of upper limb work-related musculoskeletal pain (UL-WMSP) especially in the shoulder, arms, forearms, wrists and hand regions.²⁻⁴ Amid the global competitiveness, the poultry industry in Thailand is rapidly expanding with the poultry exporters vigorously stretching their markets to meet the global demand for chicken products.⁵ The constant drive for higher profit and poultry meat production requirements due to rapid

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doi: 10.12982/JAMS.2025.055

E-ISSN: 2539-6056

expansion of the poultry industry creates adverse factors such as increase in working hours, production line speeds and a decrease in breaking hours for PSW.^{3,4,6,7} Thus, the workplace and job nature of PSW is exposed to several risk factors such as rapid work pace, repetitive movements, heavy lifting, sustained holding, forceful manual exertions and insufficient recovery time, which may contribute to UL-WMSP.^{4,7-9}

The work nature of the PSW involves job tasks which include hanging poultry and head cutting, defeathering and cleaning, abdominal slitting and lastly, cutting-up of the poultry.¹⁰ In addition, the nature of poultry processing tasks involves repetitive movements, excessive use of force, and specific tools such as knives and scissors which can contribute to UL-WMSP among PSW. Various UL-WMSP such as shoulder impingement syndrome, carpal tunnel syndrome and epicondylitis are reported among PSW.^{7, 11-14} Recent evidence from Thailand indicates that 97% of the PSW have reported a high prevalence of WMSP at least in any one body region at a given time.¹⁰ The highest prevalence of WMSP was reported at the shoulder region (61.5%) followed by wrists/hands (60.3%). Approximately 83% of PSW reported disability at upper limbs including shoulder, wrist and hands.¹⁰

Consequently, the UL-WMSP encountered by PSW may lead to reduced work capacity, long-term musculoskeletal illness, sickness absenteeism, and early retirement.¹⁵⁻¹⁸ Despite steps to reduce UL-WMSP at the workplace through implementing several control measures such as occupational safety, engineering controls, use of technology and quality equipment at work, the risk factors that contributes to UL-WMSP needs further investigation for prevention strategies.^{7,8} The Occupational Safety and Health guidelines for poultry processing plants recommends prevention of musculoskeletal disorders and protection of poultry workers health.⁸ The Health and Safety Executive, United Kingdom suggests that adequate evaluation of risk factors is necessary to tackle UL-WMSP and protect the health of the workers.¹⁹

Therefore, there is an immediate need to understand the risk factors behind UL-WMSP among PSW to develop people's health and wellbeing,²⁰ reduce UL-WMSP at the workplace and create a healthy working environment.^{16,21-24} The employers and employees need to develop a basic understanding of the prevention of risk factors that contributes to the UL-WMSP among the PSW. Therefore, the main aim of the study was to investigate the risk factors at workplace that are related to UL-WMSP among PSW. The information obtained from the study would be helpful to design and develop a prevention program to reduce UL-WMSP among PSW at workplace.

Materials and methods

Study design and setting

This was a cross-sectional descriptive study which was conducted among PSW in the northern parts of Thailand. There were three poultry slaughterhouse factories located in this region. A simple random method was used to select one of the three poultry slaughterhouse factories.

This study was conducted between the periods of March-August 2023 and approved by the institutional ethical committee according to the standards of the Declaration of Helsinki (Ethical approval number AMSE64EX-112). The purpose of the study and the study protocol was informed to the department manager, occupational safety officer, and PSW who worked in the factory. The PSW signed a written inform consent prior to their participation in the study.

Participant characteristics

The poultry slaughterhouse factory had 148 workers, including 28 office staff, 109 PSW, 8 storage workers and 3 maids. The inclusion criteria of the study were as follows: 1) males and females who worked in slaughtering tasks, 2) working at a poultry slaughterhouse factory for at least 1 year, 3) working in a standard full-time job for at least 7 hours per working day, and 5) willing to participate in the study voluntarily. Any PSW with a previous history of any musculoskeletal surgeries, a recent history of injury and accidents in the last three months and those workers who started working less than one year of duration in the industry were excluded. There were 109 PSW who worked in slaughtering tasks. About 11 PSW were excluded due to; working at this factory less than 1 year (N=5), had history of back surgery (N=1), recent motorcycle accident (N=2), and did not willing to participated in the study (N=3). A total of 98 participants (52 women and 46 men) who met the inclusion criteria were recruited into the study.

Measures

All participants completed a self-reported form which collected several demographic information related to individual factors such as age, sex, smoking status, alcohol consumption, years of work experience and number of working hours. The information related to the job task profile such as type of job tasks performed at work (hanging poultry and head cutting, defeathering and cleaning, abdominal slitting and lastly, cutting up of the poultry) and types of tools used at the work were also collected. The weight was measured by a mechanical weighing scale (Camry (DT-613), China) and the height was measured by a portable stadiometer (Health-O-Meter Mechanical Beam Scale, United States of America). The body mass index (BMI) of the PSW was calculated by dividing the weight (kg) by height squared (m²).²⁵

The Standardized Nordic Musculoskeletal Questionnaire (SNMQ) was used to collect the presence of UL-WMSP over the last 7 days and 12 months.²⁶ The Rapid Upper Limb Assessment (RULA) was used to evaluate the risk of poor posture at work.²⁷ The RULA score categorizes the levels of risk into three categories such as low risk, medium and high risk and represents the risk of poor posture. The minimum RULA score was 1, and the maximum RULA score was 7. The interpretation of the RULA scores are as follows; Level 1 (1-2 scores): Posture is acceptable, Level 2 (3-4 scores): Low risk, further investigation is needed, and changes may be needed, Level 3 (5-6 scores): Medium risk, investigation and changes are required soon, and

Level 4 (7 scores): High risk, investigation and changes are required immediately. All the data was collected at the factory during the working hours of the PSW by a qualified physiotherapist. The average assessment time was 20 minutes for each participant.

Statistical analysis

The sample size for the study was based on the Krejcie and Morgan Table²⁸ which demonstrated a sample size of 98 PSW were needed for the study. Statistical analyses were performed using the SPSS version 27.0 for Windows (SPSS, Inc., Chicago, IL, USA). The normality of the data distribution was confirmed with the Kolmogorov-Smirnov test. The demographic data were reported in frequency, mean (M), standard deviation (SD), and percentages. The prevalence of WMSP for the past 7 days and 12 months were reported by the descriptive statistics. The association between the risk factors and UL-WMSP for the past 7 days and 12 months was evaluated through the univariate logistic regression analysis. The relationship between prevalence of UL-WMSP for the past 7 days and

12 months and independent variables were shown by the Cox & Snell R square (R^2). The odds ratios (ORs) in the current study were reported by exponential coefficients (β). Statistical significance was set at $p \leq 0.05$.

Results

A total of 98 PSW participated in the study. Around 47% (N=46) were men and 53% (N=52) were women. The mean age of the PSW was 38.12 ± 8.83 years. Their average weight and height were 60.90 ± 12.79 kg and 161.69 ± 10.05 cm, respectively. Among the participants, about 39% (N=38) were smokers, and 65% (N=64) had alcohol consumption. Regarding profiling of job tasks, 7% (N=7) worked in hanging and head cutting task, 20% (N=20) worked in defeathering and cleaning task, 38% (N=37) worked in abdominal slitting task, and 35% (N=34) worked in cutting-up process. The mean work experience and working hours/day were 3.68 ± 3.69 years, and 11.28 ± 0.89 hours, respectively. Approximately 74.5% (N=73) used knives and scissors to perform their tasks. The demographic data and job factors were reported in Table 1.

Table 1. Demographic characteristics of poultry slaughterhouse workers.

Characteristics (N=98)	Mean $\pm$ SD
<i>Gender</i>	
Males, N (%)	46 (46.9%)
Females, N (%)	52 (53.1%)
Age (years)	38.12 $\pm$ 8.83
Weight (kg)	60.90 $\pm$ 12.79
Height (cm)	161.69 $\pm$ 10.05
<i>Body mass index (kg/m²)</i>	23.32 $\pm$ 4.54
Underweight (<18.5), N (%)	10 (10.2%)
Normal (18.5-22.9), N (%)	42 (42.9%)
Overweight (23-24.9), N (%)	17 (17.3%)
Obese ($\geq$ 25.0), N (%)	29 (29.6%)
<i>Previous injury at upper limbs</i>	
No, N (%)	84 (85.7%)
Yes, N (%)	14 (14.3%)
<i>Smoking</i>	
No, N (%)	60 (61.2%)
Yes, N (%)	38 (38.8%)
<i>Alcohol consumption</i>	
No, N (%)	34 (34.7%)
Yes, N (%)	64 (65.3%)
<i>Task section</i>	
Hanging and head cutting, N (%)	7 (7.1%)
Defeathering and cleaning, N (%)	20 (20.4%)
Abdominal slitting - N (%)	37 (37.8%)
Cut-up process, N (%)	34 (34.7%)

Table 1. Demographic characteristics of poultry slaughterhouse workers (*continued*).

Characteristics (N=98)	Mean±SD
Work experience (years)	3.68±3.69
1-5, N (%)	74 (75.5%)
5.1- 10, N (%)	13 (13.3%)
>10, N (%)	11 (11.2%)
Work hours (hours)	11.28±0.89
Tool use (i.e., knives and scissors)	
No, N (%)	25 (25.5%)
Yes, N (%)	73 (74.5%)

The prevalence rate of UL-WMSP reported by the PSW over the period of the last 7 days and the last 12 months were shown in Table 2. The highest prevalence rate of WMSP was reported to be at shoulders region, 38.8% (N=38) over the period of the last 7 days and 78.86% (N=77) over the last 12 months. The prevalence rate of UL-WMSP at wrists and hands was 32.7% (N=32)

over the period of the last 7 days and 59.2% (N=58) over the last 12 months. Moreover, approximately 68.4% (N=67) of participants reported UL-WMSP, i.e., shoulder, elbow/forearm, or hand/wrist during the period of the last 7 days and 86.7% (N=85) reported UL-WMSP over the last 12 months.

Table 2. Prevalence of work-related musculoskeletal pain reported among poultry slaughterhouse workers.

Total participants (N=98)	WMSP in the past 7 days		WMSP in the last 12 months	
	N	Prevalence (95%CI)	N	Prevalence (95%CI)
Neck	23	23.5 (15.3-31.6)	51	52.0 (42.9-61.2)
Shoulders	38	38.8 (29.6-49.0)	77	78.6 (70.4-86.7)
Elbows	3	3.1 (0.1-7.1)	7	7.1 (2.0-13.3)
Wrists and hands	32	32.7 (23.5-42.9)	58	59.2 (49.0-69.4)
Upper back	12	12.2 (6.1-18.4)	21	21.4 (14.3-29.6)

About 8% (N=8) of the PSW were identified to have a poor posture and presented a medium risk of developing UL-WMSP (Table 3). About 91.8% (N=90) of the PSW scored a RULA score of 7, presenting a poor posture and a high risk of developing UL-WMSP (Table 3). Table 4 shows the RULA scores among the PSW classified into 4 different job tasks including handling and head cutting task, defeathering and cleaning task, abdominal slitting task, and finally cutting up task. Table 5 and 6 shows the univariate logistic regression analysis of risk factors related with UL-WMSP for the past 7 days and over the last 12 months, respectively. In the Table 5, age ($R^2=0.043$, $\beta=1.055$, $p=0.045$) showed significant relationship with prevalence of UL-WMSP over the period of the last 7 days.

The finding showed that increasing age was associated with UL-WMSP up to 1.05 times. In the Table 6, abdominal slitting task ($R^2=0.060$, $\beta=8.500$, $p=0.028$) and cutting-up task ($R^2=0.060$, $\beta=7.750$, $p=0.036$), tool use (i.e., knives and scissors) ($R^2=0.055$, $\beta=4.343$, $p=0.017$), and RULA scores ($R^2=0.116$, $\beta=17.083$, $p<0.001$) showed significant relationship with prevalence of UL-WMSP. Abdominal slitting task and cutting-up task showed the relation with UL-WMSP up to 8.50 and 7.75 times more than the other tasks, respectively. Moreover, the tools such as knives and scissors, were associated with UL-WMSP up to 4.34 times more than who no tools. Finally, the high RULA scores had related with UL-WMSP up to approximately 17.08 times.

Table 3. Rapid Upper Limb Assessment scores among the poultry slaughterhouse workers.

RULA scores range	Classification	N (%)
1-2	Posture is acceptable	-
3-4	Low risk, change may be needed	-
5-6	Medium risk, further investigation, change soon	8 (8.2%)
7	High risk, implement change now	90 (91.8%)

Table 4. Rapid upper limb assessment scores among the poultry slaughterhouse workers that classified into four tasks; 1) hanging and head cutting task (N=7), 2) defeathering and cleaning task (N=20), 3) abdominal slitting task (N=37) and 4) cut-up process (N=34).

Classification	Hanging and head cutting task (N=7)	Defeathering and cleaning task (N=20)	Abdominal slitting task (N=37)	Cut-up process (N=34)
1-2: Posture is acceptable	-	-	-	-
3-4: Low risk, change may be needed	-	-	-	-
5-6: Medium risk, further investigation, change soon	3 (42.8%)	-	2 (5.4%)	3 (8.8%)
7: High risk, implement change now	4 (57.2%)	20 (100%)	35 (94.6%)	31 (91.2%)

Table 5. The univariate binary logistic regression analysis of factors related with upper limbs work-related musculoskeletal pain for the past 7 days (N=98).

No pain=31(31.6%), UL-WMSP =67(68.4%)	R ²	Exp(β)	p value
Gender	0.004	1.315	0.529
Age (years)	0.043	1.055	0.045*
Body mass index (kg/m ²)	0.006	1.007	0.890
Previous injury at upper limbs	0.026	3.164	0.149
Smoking	0.031	0.458	0.079
Alcohol consumption	0.001	0.853	0.731
Task section			
Hanging and head cutting			
Defeathering and cleaning	0.002	0.743	0.757
Abdominal slitting	0.002	0.833	0.841
Cut-up process	0.002	0.960	0.965
Work experience (years)			
1-5			
5.1-10	0.023	2.979	0.176
>10	0.023	1.444	0.609
Work hours (hours)	0.001	1.095	0.707
Tool use (i.e., knives and scissors)	0.023	2.082	0.127
RULA scores	0.013	2.333	0.255

Note: *significance difference at $p < 0.05$, R²: Cox & Snell R square, Exp(β): exponential coefficients.

Table 6. The univariate binary logistic regression analysis of factors related with upper limbs work-related musculoskeletal pain for the last 12 months (N=98).

No pain=13(13.3%), UL-WMSP=85(86.7%)	R ²	Exp(β)	p value
Gender	0.000	0.964	0.951
Age (years)	0.020	1.046	0.167
Body mass index (kg/m ²)	0.002	1.028	0.687
Previous injury at upper limbs	0.006	2.167	0.476
Smoking	0.004	1.500	0.527
Alcohol consumption	0.009	1.745	0.355
Task section			
Hanging and head cutting			
Defeathering and cleaning	0.060	3.000	0.246
Abdominal slitting	0.060	8.500	0.028*
Cut-up process	0.060	7.750	0.036*
Work experience (years)			
1-5			
5.1-10	0.006	1.875	0.566
>10	0.006	0.703	0.679
Work hours (hours)	0.014	0.653	0.261
Tool use (i.e., knives and scissors)	0.055	4.343	0.017*
RULA scores	0.116	17.083	< 0.001*

Note: *significance difference at $p < 0.05$, R^2 : Cox & Snell R square, $Exp(\beta)$: exponential coefficients.

Discussion

The study findings showed a high prevalence of UL-WMSP among the PSW with the shoulder region most affected followed by wrist and hand regions. With 78% of PSW reported shoulder pain and 58% reported pain at the wrist and hands over the period of last 12 months, the current study provided insight on the chronic pain condition of UL-WMSP among the PSW. This high prevalence of chronic UL-WMSP reported by the workforce in the poultry industry clearly raises alarm and calls for appropriate prevention and management strategies to tackle UL-WMSP for the health and wellbeing of the workforce in poultry industrial sector. Therefore, an understanding of risk factors among PSW is an important first step towards developing prevention and management strategies to tackle UL-WMSP among the workforce in the poultry sector. Thus, the current study and its findings contributed to a quality of work that has international relevance, significance, originality and rigor for the workforce in the poultry industry. For instance, the poultry industry is expected to see a strong growth across various continents and over 50 countries in the next few years expanding from \$385.37 billion in 2024 to \$494.55 billion in 2028 at a compound annual growth rate (CAGR) of 6.4%. This rapid market expansion will require increased demand and aggravates risk factors at work for PSW globally, hence the current study that focuses on understanding risk factors for supporting health and wellbeing of PSW by prevention of risk factors related to UL-WMSP is of global relevance. The current study is also original and significant as it is the first

study that investigated the risk factors related to UL-WMSP among PSW in an Asian context, however the findings are still transferrable to the global poultry workforce sector. Considering the important focus of the study, several measures were followed to enhance the study rigor. Firstly, all end users such as supervisors, managers, PSW and local stakeholders were engaged and consulted in terms of explaining the benefit and rationale of the study. Secondly, the end users contributed to develop the study methods by sharing their views into study setting, shifts time of the PSW, recruitment strategies, data collection procedures and RULA measurements. Thirdly, the researcher underwent a training to use the RULA tool and conducted some pilot data collection to familiarize with the tool prior to the original data collection. All the said measures contributed to the study rigor.

Increase of age was a significant risk factor for UL-WMSP ($p=0.045$, $ORs=1.05$) in PSW. Previous studies reported that age was associated with WMSP such as rotator cuff syndrome and carpal tunnel syndrome among PSW.^{13,29} Increasing age has been shown to negatively impact muscle function such as muscle power, muscle strength and muscle endurance.^{30,31} These negative changes directly affect muscle performance and function contributing to musculoskeletal stress in both men and women.³⁰ Other risk factors such as specific job tasks performed by the PSW which includes abdominal slitting task and cutting up task ($p=0.028$, $ORs=8.500$, and $p=0.036$, $ORs=7.750$) also showed a significant relationship with UL-WMSP than other tasks (Hanging and head cutting

and Defeathering and cleaning). The nature of abdominal slitting task involves dissecting the abdominal part and remove internal organs, while the arms of the PSW have to pull out the internal organ and repeat motion in the same position.^{7,8} The cutting up task involves repeatedly moving both arms to cut the chicken wings and legs.⁷ Perhaps, if chicken were big sizes, PSW required a lot more force to cut the chicken part before it breaks into pieces.⁷ PSW performed these two tasks always using force during the task and performed the task repetitively. Previous evidence also supported that 87.6% of PSW performed repetitive tasks and 67.2% of PSW felt discomfort in at least one body region, and the symptoms most often reported were pain, fatigue and tingling.³² The body regions most frequently reported were shoulders (62.6%), neck (46.2%), forearms (31.3%), arms (29.2%), wrists (25.6%) and hands (25.6%).^{4,9,32} Therefore, the current study highlights the importance of evaluating job tasks of PSW as part of developing prevention strategies for UL-WMSP.

Evidence suggests that the tools such as knives, saws, scissors, and knife-sharpeners used by the PSW were identified as the risk factors for prevalence of UL-WMSP.^{6,11} Past studies indicated that 40% of PSW used hand tools (e.g., a knife and a knife-sharpener) and 65% of PSW reported bodily discomfort.^{6,11} In the current study, about 75% (N=73) of PSW used a knife or scissors as part of their task. However, about 87% of PSW reported musculoskeletal pain in the upper body region which suggests that the tools used might be a significant risk factor for UL-WMSP ($p=0.017$, ORs=4.343) over the last 12 months than who no tools. Thus, PSW who reported using an equipment (i.e., knives and scissors) had higher prevalence of UL-WMSP. Therefore, addressing the tools used by the PSW could be a potential area for the poultry industry to tackle UL-WMSP. PSW must wear the rubber gloves when working, due to the standard procedure of slaughtering plants.^{7,8} PSW could have difficulty holding an equipment with the rubber gloves because the gloves were quite slippery.⁸ PSW have to grab on to equipment very hard and use excessive of force for gripping and sustained the position for long periods of time especially when the size of the tool is too small which might contribute to the UL-WMSP.⁸ RULA was used in this study to evaluate the risk of poor posture contributing to UL-WMSP among the PSW.^{27,33} The RULA scores indicated approximately 91.8% (N=90) of PSW had high risk of poor posture which was a significant risk factor for UL-WMSP ($p<0.001$, ORs=17.083). This could be because the workstations of PSW were fixed and lacked ergonomic adjustments to the workforce requirements. Evidence suggests that ergonomic risk factors such as awkward posture, static loading, prolonged and forceful mechanical strain contributes to musculoskeletal pain.^{6,11,34,35} Therefore, strategies to tackle musculoskeletal pain may need to consider ergonomic evaluation and posture assessments among the PSW.

Practice and industry implications

Ergonomic risk is multi-dimensional, the magnitude of risk attributable to various factors can be of importance to

therapist and policy makers in designing countermeasures to reduce injury incidence.^{36,37} The findings of the study are useful to suggest some recommendations to tackle UL-WMSP among the workforce in the poultry industry. For instance, with certain tasks such as abdominal cutting tasks increases the risk of PSW developing UL-WMSP. Therefore, the study recommends task rotation for the PSW during a shift as a necessary measure to reduce the risk of developing musculoskeletal pain. This might possibly reduce the higher musculoskeletal strain associated with abdominal slitting task. With age being identified as another risk factor in this study, the task rotation policy at workplace when introduced specially among the experienced and aged workforce might further reduce the risk of UL-WMSP among the PSW. Simple interventions such as providing a rubber glove with enhanced gripping features might reduce the demand on the musculoskeletal force needed to hold the tools while at work. Similarly, making provisions for different sizes of tools with different grips and for different hand sizes might further lessen the musculoskeletal strain while handling the tools in their workplace. Employers should provide a healthy work environment by assessing the risk factors to manage and prevent occupational disease.³⁷⁻³⁹ An induction program incorporating the knowledge on UL-WMSP and risk factors is recommended for the workforce and the industry. In addition, the managers and supervisors should be encouraged to undertake additional training for tackling UL-WMSP through prevention, risk identification and health education strategies. There is an essential need to develop musculoskeletal health and wellbeing program for the PSW to reduce WMSP at the workplace and to create a healthy working environment.^{20,37-39}

Limitations

The study has a few limitations. The data were collected from only one slaughterhouse factory in northern Thailand. It would have been ideal to have the data collected from various slaughterhouse factories. However, the study findings and recommendations are transferrable to a wider group of PSW and poultry industry because of the similar of work conditions. The current study did not investigate environmental factors such as noise, cold temperature, vibration, light, and clothing, hence, future studies should incorporate environmental risk factors related to UL-WMSP. Last but not the least, the study did not measure biomechanical risk factors such as force exertion and organization risk factors such as workplace policies, job support etc., which needs to be studies as UL-WMSP are multidimensional problems. Nevertheless, the study findings contributed to useful practice implications and recommendations for the poultry industry globally.

Conclusion

The PSW reported a high prevalence of UL-WMSP which was contributed by significant risk factors such as age, job tasks such as abdominal slitting task, cutting up tasks, types of tools used and poor posture. The study findings help creating and developing a healthy

work environment to improve working condition for the workforce in the poultry industry.

Conflict of interest

No conflict of interest to declare.

Ethical approval

The study was ethically approved by a university institutional ethics committee (Ethical approval number: AMSEC-64EX-112 as per the Helsinki declarations).

Funding

This study was supported by an internal university research grant from the Faculty of Associated Medical Sciences and the Graduate School, Chiang Mai University, Chiang Mai Province, Thailand.

Acknowledgements

The authors express sincere thanks to the poultry slaughterhouse workers, the supervisors and the managers of the chicken meat factories for their support in the study.

Consent to participate

All the participants gave written consent prior to their participation in the study.

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Development of a self-report questionnaire for evaluating the occupational performance and satisfaction in daily occupations in seven occupational areas of university students with game addiction

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ARTICLE INFO

Article history:

Received 19 January 2025

Accepted as revised 17 March 2025

Available online 26 March 2025

Keywords:

Game addiction, occupational performance, occupational satisfaction, university student.

ABSTRACT

Background: Nowadays, the prevalence of internet usage for university students' game playing is increasing worldwide. However, overabundant gaming may lead to game addiction. Studies have demonstrated that game addiction increases the risks of health problems. Moreover, it also decreases occupational performance via cognitive and psychosocial skill reduction. In occupational therapy, cognitive and psychosocial performance are necessary for daily activities. Furthermore, occupational satisfaction also correlates positively with participation in occupations. Thus, occupational therapists should know the occupational performance and satisfaction in daily occupations of university students with game addiction to explore occupational issues and plan interventions. However, an instrument for evaluating the occupational performance and satisfaction in daily occupations of university students with game addiction has never been investigated.

Objective: This study developed a questionnaire to evaluate the occupational performance and satisfaction in the daily occupation of university students with game addiction.

Materials and methods: The questionnaire, comprising 22 items, assessed the impacts of game addiction on occupational performance and satisfaction in daily occupations of seven occupational areas. Afterwards, the psychometric properties of the questionnaire were investigated in 78 university students with game addiction.

Results: This study showed that the questionnaire had sufficient content and construct validity for evaluating the impacts of game addiction on occupational performance and satisfaction in the daily occupations of university students. Moreover, the internal consistency of the questionnaire in occupational performance is good (Cronbach's alpha coefficient =0.812). Also, the internal consistency of the questionnaire in satisfaction of daily occupation is good (Cronbach's alpha coefficient =0.82).

Conclusion: The questionnaire is valid and reliable for evaluating the occupational performance and satisfaction in daily occupation of university students with game addiction. Screening these components can provide a new occupational therapy tool for such students.

Introduction

Nowadays, the amount of internet usage is increasing in all areas of life¹, and the prevalence of playing games on it is growing among children and adolescents worldwide (the prevalence rate 62.1%)². However, overabundant gaming may lead to game addiction.³ Currently, the World Health Organization (WHO) classifies gaming disorder, or game addiction, as a mental health disease according to the 11th revision of the International Classification of Diseases.⁴ The

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doi: 10.12982/JAMS.2025.056

E-ISSN: 2539-6056

WHO defines game addiction as an impairment of gaming behavior, including uncontrolled gaming, prioritizing game playing over other activities, and continuing game playing despite the negative consequences.⁵ Studies showed that game addiction increases the risks of health problems such as depression, emotional distress, or memory and attention deficits via decreased cognitive and psychosocial skills.⁶⁻⁹ It is established that cognitive and psychosocial skills are necessary for performing daily activities.^{10,11} Thus, a severe level of game addiction possibly impacts the performance of individuals in their everyday lives.

Occupational performance is the ability to choose, organize, and satisfactorily perform meaningful occupations.¹² In occupational therapy, the importance of occupational performance can be described in occupation-based models.¹³ For example, the Person-Environment-Occupation-Performance (PEOP) model describes the importance of the occupational performance of individuals via the interaction between the person, environment, and occupational characteristics.¹⁴ In contrast, the Canadian Model of Occupational Performance and Engagement (CMOP-E) describes the correlation between the person, environment, and occupation, leading to the ability and engagement of individuals to perform occupations. This is consistent with the problems of game addiction, as impairment of gaming behavior correlates negatively with several issues such as impairments of school engagement, cognition, and emotion, possibly leading to poor educational performance in adolescents and young adults.¹⁵⁻¹⁶ For this reason, occupational therapists must know the level of occupational performance in the population with game addiction to explore health problems as well as plan interventions in occupational therapy. In addition, it is known that poor occupational performance correlates with low satisfaction in occupations when performing daily occupation.¹⁷ Occupational satisfaction is contentment with the current engagement, or absence of engagement, in different daily occupations, such as work, leisure, home maintenance, and self-care. Hence, low occupational satisfaction can decrease the participation in individuals' occupations.¹⁸ The population with game addiction may have a low level of satisfaction in performing other occupations, which leads to a lack of occupational participation via decreasing occupational performance. Therefore, occupational therapists must know the level of occupational satisfaction in individuals with game addiction to explore occupational participation and plan interventions for these people.

This study aims to develop an assessment tool in occupational therapy for evaluating occupational performance and satisfaction in daily occupations of individuals with game addiction. This assessment tool was developed based on the concept of the CMOP-E model. It was described that the ability of individuals to perform occupations and occupational engagements occurs from the relation between the person, environment and occupation. The CMOP-E model focuses on the client-centered approach and the ability of clients to perform occupations in three occupational categories: work, self-

care and leisure. Recently, occupational therapists have been able to use the Canadian Occupational Performance Measure (COPM) for evaluating occupational performance and satisfaction in three occupational areas (work, self-care and leisure) via self-scoring by the client.¹² However, the occupational areas in the COPM may not cover the types of occupations affected by game addiction such as education, health management, or social participation. Therefore, the items of a questionnaire in this study were developed based on a broad range of occupations in the fourth edition of the Occupational Therapy Practice Framework: Domain and Process (OTPF-4).¹⁹

Additionally, the question of how severity of game addiction impacts occupational performance is controversial. For example, a study reported that game playing can increase cognitive performance via increasing attention, memory, and planning.²⁰ Therefore, occupational therapists need to know the level of vulnerability in occupational performance and satisfaction in daily occupations among the university students with game addiction, which would lead to an intervention plan in occupational therapy. In the process of COPM tool scoring, the client can self-rate scores in occupational performance and satisfaction in a range from 1 point (poor performance and low satisfaction) to 10 points (good performance and high satisfaction). After that, occupational therapists can use the change in average occupational performance and satisfaction scores between pre- and post-intervention for measuring intervention outcome in occupational therapy.¹² However, no assessment tool evaluates the level of vulnerability in occupational performance and satisfaction in daily occupations of university students with game addiction. Therefore, this study developed the 5-point Likert scale for self-measuring occupational performance ranging from 1 point (unacceptable level) to 5 points (excellent level), and for the levels of self-measuring occupational satisfaction ranging from 1 point (very dissatisfied) to 5 points (very satisfied). Information from a developed questionnaire can help occupational therapists to know the levels of vulnerability in the occupational performance and satisfaction in daily occupations of university students with game addiction, which leads to intervention plans in the future.

In addition, this study focused on designing a self-report questionnaire for young adults, especially university students. It is established that university students move from adolescence into young adulthood at transitional age²¹, which is considered a critical period in human development, characterized by several physical, cognitive, and psychosocial changes. Therefore, skills of adaptation to a new learning environment are challenging at transitional age, especially in university students. This study focused on designing a self-report questionnaire for university students with game addiction. Evidence reported that the prevalence of game addiction (54.3%) is increasing in university students.²² Moreover, game addiction was negatively associated with the psychosocial well-being and school performance of university students.²³ Thus, a self-report questionnaire was developed to evaluate

occupational performance and satisfaction in the daily occupation of university students with game addiction and test its psychometric properties. This information can provide a new occupational therapy tool for evaluating the levels of vulnerability in the occupational performance and satisfaction in daily occupations of university students with game addiction, which leads to the exploration of problem lists and intervention plans in occupational therapy.

Materials and methods

Participants

In this study, the sample size was calculated based on the aims of the study: the construct validity and reliability of internal consistency. The sample size calculation for construct validity was conducted by a known group technique using G*Power software 3.1.9.7, and input parameters consisting of 0.4 effect size, 0.05 alpha, and 0.8 power of test. Of 156 undergraduate students, 78 with game addiction and 78 without taking part in testing known-groups validity. Game addiction was screened by an occupational therapist by using the game addiction self-evaluating test for children and adolescents.²⁴ The value of Cronbach's alpha of game addiction screening test was 0.92. Moreover, the sensitivity and specificity of game addiction screening test for children and adolescents were 68.5 and 89.3, respectively.²⁴

For reliability of internal consistency in the participants with game addiction, the sample size was calculated by Bonett's formula. This study used a single coefficient of Cronbach's alpha test. According to Bonett's formula, the value of Cronbach's alpha at null hypothesis (CA0) and the expected value of Cronbach's alpha (CA1) were set as 0.6 and 0.75, respectively.²⁵ Moreover, the power value of tests, number of items in the questionnaire (k) and probability of type I error (alpha) were set as 0.8, 22, and 0.05, respectively.²⁵ Finally, 77 participants with game addiction were tested for internal consistency.

For the process of data collection, 1st year undergraduate students with and without game addiction were invited to attend research study via online advertisement poster. The interested volunteers can register to participate in the research and choose a convenient date for collecting data with researchers (face to face) via a register link on an advertisement poster. In this study, simple random sampling was used for recruiting the participants. Once enough volunteers had done this, the recruiting participant was stopped immediately. For the inclusion criteria, male and female participants with game addiction screening test scores of less than 24 and 16 points, respectively, were classed as without game addiction, while those with scores of more than 24 and 16 points, respectively, were considered as with game addiction. When recruiting the participant, interested volunteers were informed that they needed to meet the inclusion criteria to ensure equality. The exclusion criteria of this study were the interested volunteers who are not able to read questionnaires and miss scheduled appointments for collecting data.

Research design

Phase 1: Development of the self-report questionnaire for evaluating the occupational performance and satisfaction in daily occupations of university students with game addiction

The items of a self-report questionnaire were developed based on the concept of client-centered practice in the CMOP-E model. The participants can self-evaluate via scoring their own occupational performance and satisfaction in daily occupation. Additionally, all items in the questionnaire were designed in the occupational areas affected by game addiction. For the initiation process, game addiction was evaluated among 30 university students. After that, five university students with game addiction will be chosen by simple random sampling by a lottery method and will receive an invitation by email for participating in a focus group discussion. For the inclusion criteria of 5 participants with game addiction, university students with game addiction test scores of more than 24 points in male and 16 points in female were considered as with game addiction. The date of the focus group will be within 2 weeks after the invitation. An offline focus group was conducted for 2 hours to discuss a given topic for exploring the opinions, perceptions, and concerns of the individuals regarding their health issues and well-being. In addition, they were also interviewed to find out the affected occupations from game addiction as well as their perceptions on performance and satisfaction in daily living activities. This discussion is supervised and monitored by occupational therapists. For specific open-ended question examples are as follows: 1) how often do you play games? and how to play? 2) What leisure activities that you like or are interested in performing besides game playing? 3) What daily activities are affected by your game playing? 4) Do you think playing games affects your ability to perform daily activities? And how? 5) Are you satisfied with your ability to perform daily occupations? And how? After that, the information from focus group discussion was analyzed and designed the components of the questionnaire in two parts including the occupational performance and satisfaction in daily occupation. Moreover, the affected daily occupations from game addiction were categorized, based on a broad range of occupations in the OUTF-4 including activities of daily living (ADLs), instrumental activities of daily living (IADLs), health management, rest and sleep, education, play, work, leisure, and social participation.¹⁹ Finally, the 22 items of the questionnaire focused on the occupational performance and satisfaction in daily occupations of university students with game addiction in seven types of occupations, including activities of daily routine, the daily activities related to environments, activities of health promotion, sleep, recreational and leisure activities, activities relevant to the person's role, and social participation.

In scoring the questionnaire, the participants gave scores of their own performance and satisfaction in each item. The 5-point Likert scale was used for self-measuring

After developing a self-report questionnaire, the content validity was evaluated by five experts using the index of item-objective congruence (IOC). These experts

consisted of a psychiatric physician, two occupational therapists with experience in treating psychiatric patients, and two occupational therapists who had experience in developing assessment tools in occupational therapy. Subsequently, the questionnaire was tested in a pilot group of university students with game addiction to assess their opinion on understanding of language, language integrity, clarity of the question, and volume and response of time in assessment. For the process of recruiting participants in a pilot study, 50 university students were screened for game addiction. After that, we found that 12 university students with game addiction test scores of more than 24 points in male and 16 points in female were considered as with game addiction. Then, 12 university students with game addiction were invited by email to attend a pilot study and to try out the developed questionnaire. Feedback from the pilot study found that one student lacked clarity of the question (items of questionnaire: No. 10 and 11), while two students lacked language understanding and clarity of the question (items of the questionnaire: No. 6, 10, and 11). After revising the feedback, the self-report questionnaire consisted of five Likert rating scales with 22 items to evaluate the occupational performance and satisfaction of university students with game addiction, as shown in Table 1.

Table 1. Items of the questionnaire.

Items	The level of occupational performance					The level of occupational satisfaction					N/A
	5	4	3	2	1	5	4	3	2	1	
Questions: At present, what is the level of your performance and satisfaction in daily occupations when participating in the following activities:											
Daily routine activities											
1. Dressing											
2. Bathing											
3. Activities of personal hygiene (for example: brushing your teeth, washing your face, nail and hand hygiene, etc.)											
4. Toileting											
5. Eating food											
Daily activities related to environments											
6. Caring for others (for example: family members, friends or a lover)											
7. Caring for pets											
8. Home management (for example: cleaning, organizing, laundrying, etc.)											
9. Cooking											
10. Financial management (for ex- ample: cash management, bank transactions, financial reporting, etc.)											
11. Activities of risk management and response to emergency											
12. Shopping											
13. Religious activities											

Table 1. Items of the questionnaire (*continued*).

Items	The level of occupational performance					The level of occupational satisfaction					N/A
	5	4	3	2	1	5	4	3	2	1	
Questions: At present, what is the level of your performance and satisfaction in daily occupations when participating in the following activities:											
Health promotion activities											
14. Healthcare activities											
15. Physical activities											
16. Activities of taking medicine											
17. Activities of nutrition management											
Sleep											
18. Appropriate sleep management											
Activities relevant to the person's role											
19. Education											
20. Work											
Recreational and leisure activities											
21. Recreational and leisure activities											
Social participation											
22. Social activities (for example: joining a party, concert, team sports, etc.)											

Note: Level of occupational performance; 1 = unacceptable (1 point), 2 = needs improvement (2 points), 3 = acceptable (3 points), 4 = good or exceeds expectations (4 points), and 5 = excellent or fully competent performance (5 points), Level of occupational satisfaction; 1 = very dissatisfied (1 point), 2 = dissatisfied (2 points), 3 = neutral (3 points), 4 = satisfied (4 points), and 5 = very satisfied (5 points).

Additionally, the characteristic data of the respondents is designed in the questionnaire. The characteristic data of the respondents consists of the gender, age of the respondents and the time spent playing games per day. It is known that occupational satisfaction is associated with participating in occupations.¹⁸ Hence, the increased level of time spent on game playing possibly decreases occupational satisfaction and leads to occupational imbalance or occupational dysfunction.

1) Construct validity of the questionnaire

The known group technique was used for testing the construct validity of the questionnaire between the participants with and without game addiction. The significant difference (5% margin of error at a 95% confidence level and a 0.05 significance level) between the two groups represented good construct validity by using the independent-t-test.²⁶ For the process of independent t test, the data is checked for normality tests (with a $p < 5\%$). If normality is approved, the independent-t-test was performed to compare means between two groups.

2) Internal consistency of the questionnaire

Cronbach's alpha coefficient was used for testing the reliability of internal consistency of the questionnaire. In this study, the internal consistency of the questionnaire was tested in university students with game addiction. Reliability of internal consistency greater than or equal to 0.7 was considered as the acceptable level.²⁷

Ethic approval

This study was approved by the Ethics Committee at the Faculty of Associated Medical Sciences, Chiang Mai University (Ethic number: AMSEC-66EX-023). The consent form was obtained from all participants before collecting data. The participants in this study comprised 1st year undergraduate students from Chiang Mai University, and they all completed a consent form before the data were collected. In this study, all participants had never been diagnosed with a psychiatric disorder or had physical disabilities. The history of illness was confirmed by interviewing all participants.

Results

Demographic data of the participants

The demographic characteristics of the participants are shown in Table 2. Overall, the percentage number of males and females in the with game addiction group was 41.03 and 58.97, respectively, while that in the without game addiction group was 43.59 and 56.41, respectively.

The average age of the participants in the with and without game addiction group was 18.70 ± 0.73 and 18.51 ± 0.55 years, respectively. In addition, the average time spent on game playing per day in the with and without game addiction group was 4.78 ± 1.60 and 1.07 ± 1.27 hours, respectively.

Table 2. Demographic data of the participants.

Variables	Group with game addiction (N=78)	Group without game addiction (N=78)
Gender		
Male (N, %)	32, 41.03 %	34, 43.59 %
Female (N, %)	46, 58.97 %	44, 56.41 %
Age (years)		
Average	18.70 ± 0.73	18.51 ± 0.55
Time spent on game playing (hour/day)		
Average	4.78 ± 1.60	1.07 ± 1.27

Content validity of the self-report questionnaire for evaluating occupational performance and satisfaction in daily occupations

The content validity of the questionnaire (Table 3) was conducted by experts using IOC values. The IOC scores provided three scales of rating for consistency and congruencies of each item (the IOC score; 1 = consistent,

0 = somewhat consistent, -1 = not consistent). Average scores for each item must have a consistency value equal to or above 0.50. After that, the IOC average score of the questionnaire was calculated and interpreted. The result showed that the developed questionnaire had good content validity (IOC=0.95). Moreover, the content validity of each item also was acceptable level.

Table 3. Content validity of the questionnaire

Questionnaire	The IOC scores of five experts						Interpretation
	Expert No. 1	Expert No. 2	Expert No. 3	Expert No. 4	Expert No. 5	Average score of IOC	
Item 1	1	1	1	1	1	1	usable
Item 2	1	1	1	1	1	1	usable
Item 3	1	1	1	1	1	1	usable
Item 4	1	1	1	1	1	1	usable
Item 5	1	1	1	1	1	1	usable
Item 6	1	1	1	1	1	1	usable
Item 7	1	1	1	1	1	1	usable
Item 8	1	1	1	1	1	1	usable
Item 9	1	1	1	1	1	1	usable
Item 10	1	1	0	1	1	0.8	usable
Item 11	1	0	1	1	1	0.8	usable
Item 12	1	1	1	1	1	1	usable
Item 13	1	0	1	1	1	0.8	usable
Item 14	1	1	1	1	1	1	usable
Item 15	1	1	1	1	1	1	usable
Item 16	1	1	1	1	1	1	usable
Item 17	1	1	1	0	1	0.8	usable
Item 18	1	1	1	1	1	1	usable
Item 19	1	1	1	1	1	1	usable
Item 20	1	1	1	1	1	1	usable
Item 21	1	1	1	1	1	1	usable
Item 22	1	1	1	0	1	0.8	usable
Total items						0.95	usable

Note: IOC score; 1 = consistent, 0 = somewhat consistent, -1 = not consistent

Construct validity of the self-report questionnaire for evaluating occupational performance and satisfaction in daily occupations

The construct validity was tested as shown in Table 4. The average score of all items in the questionnaire was calculated according to the 5-point Likert scale, and it was significantly different between the with and without addiction groups. The results showed that the total items of occupational performance had good construct validity. The construct validity of each item in occupational

performance was seen as also good after testing. Results showed that total items of the questionnaire in occupational satisfaction had good construct validity. However, only 19 items of the questionnaire were found with good construct validity in occupational satisfaction, whereas the average scores of three remaining items (question number 6, 8, and 22) were not significantly different between the with and without game addiction groups.

Table 4. Construct validity of the questionnaire.

Questionnaire	Occupational performance (Mean±SD)			Occupational satisfaction (Mean±SD)		
	Group with game addiction	Group without game addiction	p value	Group with game addiction	Group without game addiction	p value
Total items	77.63±10.66	94.45±10.54	0.00*	83.87±11.91	94.78±10.37	0.00*
Item 1	3.90±0.78	4.42±1.04	0.00*	3.81±0.93	4.42±0.88	0.00*
Item 2	4.05±0.79	4.74±0.61	0.00*	4.08±0.89	4.74±0.57	0.00*
Item 3	3.72±0.87	4.67±0.73	0.00*	4.24±0.84	4.69±0.69	0.00*
Item 4	3.95±0.97	4.65±0.66	0.00*	4.01±1.02	4.61±0.67	0.00*
Item 5	3.91±1.12	4.51±0.77	0.00*	3.87±1.28	4.49±0.80	0.00*
Item 6	3.54±1.04	4.04±1.02	0.00*	3.89±1.10	4.10±1.13	0.21
Item 7	3.49±1.36	4.38±0.79	0.00*	3.87±1.13	4.38±0.77	0.01*
Item 8	3.71±1.05	4.18±0.88	0.01*	3.88±1.13	4.17±0.99	0.08
Item 9	3.32±1.20	4.53±0.60	0.00*	3.87±1.06	4.42±0.59	0.00*
Item 10	3.40±1.05	4.32±1.01	0.00*	3.49±1.20	4.38±1.01	0.00*
Item 11	3.61±0.76	4.34±0.87	0.00*	3.77±0.91	4.38±0.83	0.00*
Item 12	3.88±0.82	4.46±0.79	0.00*	3.94±1.06	4.36±0.88	0.01*
Item 13	2.18±1.17	4.15±1.02	0.00*	3.08±1.30	4.29±0.94	0.00*
Item 14	3.95±0.89	4.35±0.82	0.01*	4.05±1.02	4.35±0.80	0.01*
Item 15	3.63±1.01	4.31±0.79	0.00*	3.91±1.12	4.36±0.78	0.00*
Item 16	3.57±1.23	4.29±0.86	0.00*	3.72±1.16	4.32±0.90	0.00*
Item 17	3.50±1.11	4.35±0.67	0.00*	3.91±1.22	4.50±0.69	0.00*
Item 18	3.25±1.28	4.40±0.74	0.00*	3.71±1.32	4.39±0.78	0.00*
Item 19	3.65±0.94	4.53±0.58	0.00*	3.85±1.11	4.53±0.72	0.00*
Item 20	3.51±1.00	4.39±0.70	0.00*	3.71±0.94	4.29±0.78	0.00*
Item 21	3.59±0.86	4.62±0.63	0.00*	4.09±0.86	4.54±0.64	0.00*
Item 22	3.70±1.17	4.39±0.81	0.00*	4.42±0.86	4.38±0.83	0.82

Note: * $p < 0.05$ represents the significant difference of average questionnaire scores between the two groups.

Reliability of the self-report questionnaire for evaluating occupational performance and satisfaction in daily occupations of university students with game addiction

The reliability of internal consistency of the questionnaire in university students with game addiction is shown in Table 5. Internal consistency of the questionnaire was found by Cronbach's alpha coefficient. Total items of the questionnaire in occupational performance of

university students with game addiction had good internal consistency. Also, the internal consistency of each item was seen as good after testing. In addition, reliability of the self-report questionnaire was tested for evaluating the occupational satisfaction of university students with game addiction, and results showed that total items and each item of the questionnaire had good internal consistency.

Table 5. Internal consistency of the questionnaire in university students with game addiction.

Questionnaire	Cronbach's alpha coefficient	
	Occupational performance	Occupational satisfaction
Total items of questionnaire	0.812	0.820
Item 1	0.819	0.819
Item 2	0.820	0.820
Item 3	0.820	0.818
Item 4	0.819	0.818
Item 5	0.817	0.817
Item 6	0.816	0.818
Item 7	0.818	0.818
Item 8	0.817	0.818
Item 9	0.818	0.817
Item 10	0.815	0.814
Item 11	0.821	0.820
Item 12	0.820	0.818
Item 13	0.823	0.820
Item 14	0.816	0.814
Item 15	0.817	0.819
Item 16	0.816	0.815
Item 17	0.816	0.814
Item 18	0.818	0.816
Item 19	0.818	0.817
Item 20	0.818	0.817
Item 21	0.819	0.817
Item 22	0.818	0.817

Discussion

Recently, many studies showed that game addiction increased the risks of physical and mental health issues, leading to a decrease in school or work performance in adolescents and young adults.^{16,28-29} Interestingly, studies showed that game addiction also decreased the performance of cognitive and psychosocial skills.⁷⁻⁹ It is known in occupational therapy that cognitive and psychosocial skills are necessary for performing daily activities.¹⁰⁻¹¹ Thus, game addiction possibly decreases the efficiency and competence required in occupations.

This study developed a self-report questionnaire for evaluating occupational performance in university students with game addiction. Studies showed that game addiction increases the risks of health problems such as depression, emotional distress, or memory and attention deficits via decreased cognitive and psychosocial skills.⁶⁻⁹ This study

developed a self-report questionnaire for evaluating occupational performance in university students with game addiction. Studies showed that game addiction increases the risks of health problems such as depression, emotional distress, or memory and attention deficits via decreased cognitive and psychosocial skills.⁶⁻⁹ Moreover, a study demonstrated that game addiction correlates negatively with the ability to participate social activity and the college grade point average of university students.¹⁵ For this reason, young adults with game addiction, such as university students, may be unable to complete their daily activities or perform required occupations due to spending too much time on game playing. Thus, it is necessary for occupational therapists to know the level of occupational performance in university students with game addiction and to explore occupational problems as well as plan intervention. In this study, the questionnaire was developed

as Thai language, based on information from interviewing university students with game addiction on the types of occupations affected by game addiction as well as their perception of occupational performance. Then, the items of the questionnaire were categorized as seven types of occupations. Moreover, the 5-point Likert rating scale was used for self-measuring the level of occupational performance in each item. For scoring occupational performance in each item, the individual who had the score of occupational performance as 1 (unacceptable) or 2 (needs improvement) represents as individual who had a low level of occupational performance in that activity. After that, the psychometric properties of a questionnaire were tested and the results showed that the questionnaire had good validity and reliability for assessing occupational performance in game addicts. However, this study did not measure the construct validity and reliability for 7 sub-domains (activities of daily routine, daily activities related to environments, activities of health promotion, sleep, recreational and leisure activities, activities relevant to the person's role, and social activity). In this future, this information needs to be tested. In clinical applications of the questionnaire, a low level of occupational performance can be used as information for exploring health problems, setting intervention goals and planning intervention for university students with game addiction in occupational therapy.

Additionally, this study also developed a self-report questionnaire for evaluating occupational satisfaction in university students with game addiction. It is known that occupational satisfaction is associated with participating in occupations.¹⁸ Occupational satisfaction is defined as contentment with the current engagement, or absence of engagement, in different daily occupations, such as work, leisure, home maintenance, and self-care. Hence, low occupational satisfaction can decrease the participation in occupations of individuals.¹⁸ Therefore, game addiction possibly decreases occupational satisfaction and leads to lack of participation in other occupations. Moreover, poor occupational performance correlates with low satisfaction when performing daily activities (17). Thus, game addiction possibly leads to a low level of occupational satisfaction through reduced occupational performance. Therefore, it is necessary for occupational therapists to know the level of occupational satisfaction in individuals with game addiction when exploring occupational participation and planning future intervention.

In this study, the 5-point Likert scale was used for measuring occupational satisfaction, which ranged from very dissatisfied (1 point), dissatisfied (2 points), neutral (3 points), satisfied (4 points) to very satisfied (5 points). A score of occupational satisfaction, ranging from 1 to 2 for each item, represents a low level of occupational satisfaction. In clinical application of the questionnaire, the low level of occupational satisfaction can be used as information for exploring occupational participation, setting intervention goals and planning intervention in occupational therapy. The results of this study found that the questionnaire had good validity and reliability for

assessing occupational satisfaction. However, when considering construct validity in each item of the questionnaire, it was found that only 19 questions could be used to assess occupational satisfaction in university students with game addiction, while the levels of occupational satisfaction in the three remaining questions were not different between the with and without game addiction groups. It is possible that game addiction may not affect satisfaction in performing three occupations, including taking care of others, home management, and social activity. However, it is still necessary to test this hypothesis.

Overall, this study showed that the questionnaire had good validity for evaluating occupational performance and satisfaction in daily occupations via testing content validity and construct validity in university students with game addiction. Moreover, the questionnaire had an acceptable level of internal consistency for evaluating occupational performance and satisfaction in daily occupations in university students with game addiction. Although COPM is widely used as a tool for assessing occupational performance via self-scoring in range from 1 point (poor performance) to 10 points (good performance), no assessment tool evaluates the level of vulnerability in the occupational performance of university students with game addiction. For this reason, the information from this developed questionnaire can help occupational therapists to know the level of vulnerability in occupational performance (1 point: unacceptable or 2 points: needs improvement) of university students with game addiction, and lead to intervention plans in the future. Moreover, it can provide a new occupational therapy instrument for exploring health problems in people with game addiction, as well as evaluating individual's perceptions on occupational performance and satisfaction in daily occupations. In the future, other psychometric properties of the questionnaire, such as confirmatory factor analysis, need to be tested for ensuring that the instrument represents the underlying construct well.

Conclusion

The questionnaire has validity and reliability for evaluating the occupational performance and satisfaction in daily occupations of university students with game addiction. This information can provide a new occupational therapy tool for evaluating the levels of vulnerability in the occupational performance and satisfaction in daily occupations of university students with game addiction, which leads to intervention plans in the future.

Acknowledgements

This research was funded by the Faculty of Associated Medical Sciences, Chiang Mai University.

Declaration of competing interest

The authors declare no conflict of interests.

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Future of orthopaedic in bone defect by using 3D-printed personalized implants: A systematic review

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ARTICLE INFO

Article history:

Received 8 October 2024

Accepted as revised 10 March 2025

Available online 26 March 2025

Keywords:

Bone loss, 3D-implant, 3D-printer, biomaterials, scaffold.

ABSTRACT

Background: Definitive management and treatment of bone defects caused trauma, infection, inflammation or tumour are still challenging problems in orthopaedics. Three-dimensional (3D) printing is a development strategy that is currently being widely discussed to overcome this problem. This technology has dramatically improved teaching and advanced patient care by giving innovative tools for challenging clinical conditions.

Objective: The aim of this study was to conduct in-depth observations and critical analysis of the current state of 3DPis (3D-printed personalized implants) for bone defects.

Materials and methods: This study's search was performed in PubMed, Science direct, Elsevier, Google Scholar, in October 2023. We included studies involving all bone defects that received 3DPis in surgery and had been followed up for outcomes in several months. There was no date restriction. The recorded outcomes were anatomical outcome, functional outcome, and complication.

Results: We identified 157 studies and 35 studies remained after screening. Finally, 9 studies fulfilled the criteria. These studies consist of 39 bone defects (25 tumours, 12 traumas, 2 infections) which received 3DPis. The result suggested that the patient who received the 3DPis had an excellent condition including minimal anatomical deformity, good functional outcome after several months evaluation (between 6 and 24 months) and minimal complication.

Conclusion: 3DPis show promising outcome as treatment for bone defect with minimal anatomical deformity, good functional outcome and minimal complication. However, as this review was limited by the number of available studies, more studies are required to investigate the definite outcome of such technology.

Introduction

The concept of personalized medicine has gained momentum in recent years, as advancements in technology have allowed for the customization of medical treatments and interventions to suit individual patients. One area where personalized medicine is showing promise is in the field of orthopaedics, specifically in the development of 3D-printed personalized implants for bone defects.¹ With the rapid development of 3D printing technology, personalized medicine has become a reality in the field of orthopaedics.² Rapid prototyping technologies like three-dimensional (3D) printing, generally referred to as additive manufacturing, generate 3D objects by layering printable

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doi: 10.12982/JAMS.2025.057

E-ISSN: 2539-6056

materials on top of digital computer models. The medical community has been introduced to 3D printing in a variety of ways, including the use of medical models for surgical training and medical implants.³

Since layers of a particular material are built up to create the final result, three-dimensional printing is seen as a form of “additive manufacturing”.⁴ This contrasts with the more conventional method of subtractive manufacturing, which involves removing components from a block of material in order to get the desired end result.⁵ With the advancement of technology, 3D printing has become more accessible, affordable, and user-friendly. This has made it easier to apply it in a wider range of industries, including manufacturing, the arts, industry, and medical.

3D-printed personalized implants demonstrate a valuable contribution to the treatment of patients with critical bone defects, offering the potential for improved outcomes due to their accurate anatomical fit and ability to facilitate osseointegration, reduce stiffness at the bone-implant interface, and handle stress-shielding issues. The ability to create highly customizable implants using 3D printing technology allows for a more precise and tailored approach to bone defect reconstruction.^{6,7} Furthermore, 3D printing technology allows for the creation of complex porous structures integrated with the implants, promoting bone ingrowth and improved osseointegration.⁸ Thus, the goal of this study is to address the following questions: What are the current clinical applications of 3DPis in orthopaedic surgery? Does the use of 3DPis in orthopaedic surgery improve complication, anatomical, and functional outcomes?

Materials and methods

Search strategy

This systematic review search was performed in PubMed, Science direct, Elsevier and Google Scholar in October 2023. A combination of 10 keywords were used to obtain all studies that included a 3D-printed personalized implants (3DPis) to treat bone defects. Mendeley was used as a reference manager. We performed a literature search using keywords ‘3-dimensional or 3D’, ‘printing or printer or printed’, ‘implant’, ‘treatment’, ‘biomaterials’, ‘scaffold’ and ‘bone defect’. In the retrieval process, we performed manual search to identify additional literatures. The registered id number of the PROSPERO is CRD42024511657.

Study selection

A rigorous search for all relevant case reports and series was performed, based on specific inclusion and exclusion criteria. The inclusion criteria were relevance (papers were required to report first-hand case report, case series, retrospective cohort or prospective cohort using 3DPis as treatment method), outcome (papers were required to report anatomical outcome, functional outcome, and complication), and language (only papers written in English were included in this review). The exclusion criteria were reviews, commentaries and in vitro studies, and articles not in English. The PRISMA flow diagram is shown in Figure 1.

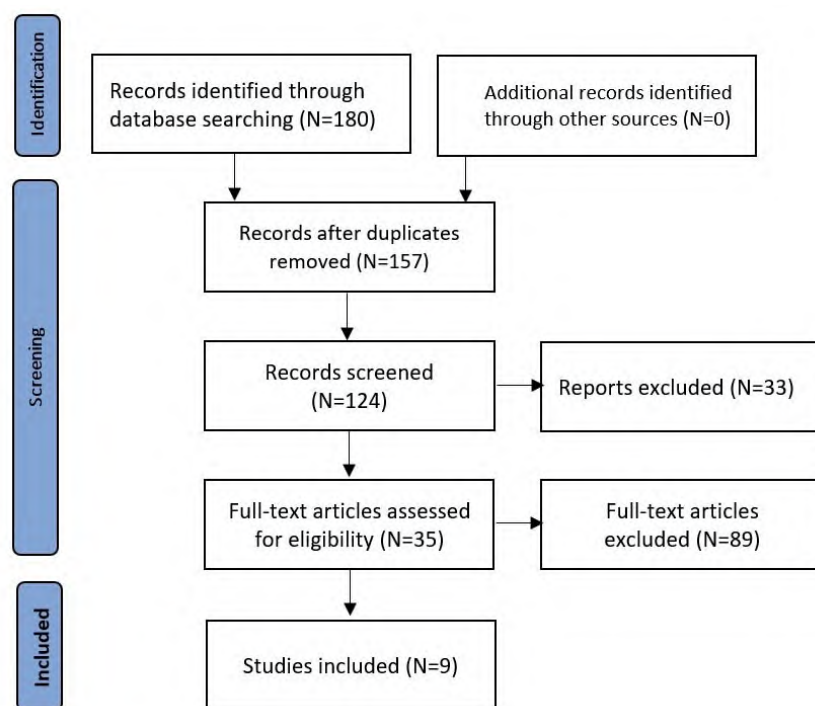


Figure 1. PRISMA flow diagram.

Quality assessment

The studies were critically appraised with ROBINS-I using Robvis (robvis (shinyapps.io)) by 2 reviewers. Two independent reviewers gathered data from each study and structured it into a standard table (Figure 2). We define each criteria as follow; D1 (bias due to confounding) evaluates whether the study adequately controlled for confounding factors (variables that can influence both the intervention and outcomes); D2 (bias due to selection of participants) assesses whether study participants were selected in a manner that introduces bias; D3 (bias in

classification of interventions) checks if the interventions were clearly and correctly categorized; D4 (bias due to deviations from intended interventions) evaluates if participants adhered to the assigned interventions and if deviations were handled appropriately; D5 (bias due to missing data) determines if missing outcome data could bias results; D6 (bias in measurement of outcomes) assesses if outcomes were measured appropriately and objectively, and D7 (bias in selection of the reported result) looks at whether all prespecified outcomes were reported, or only favourable ones.

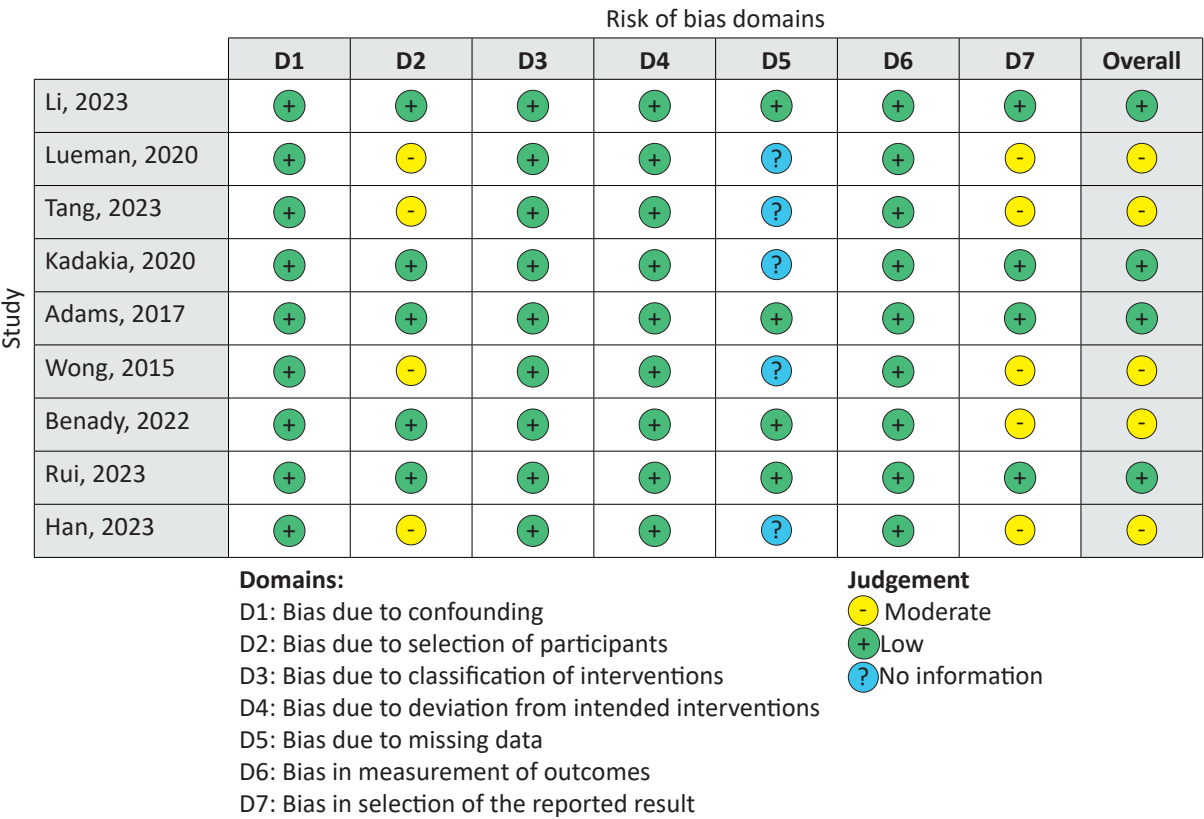


Figure 2. Risk of bias assessment.

Results

We identified 157 studies. Screening was carried out and 35 studies were remained. Finally, 9 studies fulfilled the criteria. These studies consist of 39 bone defects (25 tumours, 12 traumas, 2 infections) which received 3DPs (Table 1). The available data suggested the patient who

received the 3DPs ended up with an excellent condition including minimal anatomical deformity, good functional outcome after several months evaluation (the follow-up period ranged between 6 and 24 months), and minimal complication.⁹⁻¹¹

Table 1. Characteristics of all studies included in this literature review.

Author	Year	Case	Bone, joint or region	Sample(s)	Study type	Mean of age	Material	Complication	Anatomical Outcome	Functional Outcome
Li, 2023	2023	Tumor	Pelvis	7	Case series	43	Titanium	Delayed wound healing (1)	Good	Good
Luenam	2020	Trauma	Humerus	1	Case report	25	Titanium	-	Good	Good
Tang	2023	Infection	Tibia	1	Case report	25	Titanium	-	Good	Good
Kadokia	2020	Trauma	Ankle, talus, navicular, tibia	4	Case report	45,75	Titanium	-	Good	Good
Adams	2017	Trauma	Foot and ankle	7	Case series	54,6	Titanium	Deep infections (1)	Good	Good
Wong	2015	Tumor	Pelvis	1	Case report	65	Titanium	-	Good	Good
Benady	2022	Tumor	Pelvis	11	Case series	14,6	ULTEM™ 1010	Superficial infection (3)	Good	Good
Lv	2023	Tumor	Sacrum	6	Case series	40,1	Not mentioned	Delayed wound healing (3)	Good	Good
Wang	2022	Infection	Shoulder	1	Case report	Not mentioned	Titanium	-	Good	Good

Functional outcome

3DPs has enable us to achieve good clinical results and reasonable functional outcome.⁹ It also provides satisfactory functional result in treatment of severe fracture.¹⁰ Kevin Tang et al. reported a successful limb salvage of infected tibial shaft non-union using 3D-printed titanium mesh cage.¹¹ Orthopaedic surgeons can employ 3D printing technology in foot and ankle surgery to handle complex deformities and cases of substantial bone loss.¹¹ Personalized prosthesis reduces stress and improves patient outcomes.¹¹ 3D printing technology allows for the creation of a tailored, biomechanically assessed implant for proper reconstruction following a pelvic tumor removal in a single step.¹⁰

For the functional outcome, we couldn't perform an accurate analysis due to the difference scoring being used in the studies [MSTS (Musculo-Skeletal Tumour Society Scoring), Mayo Elbow Performance Index, Patient-Based Disabilities of The Arm, Shoulder and Hand Score, VAS (Visual Analog Scale), FAAM (Foot and Ankle Ability Measure) and AOFAS (American Orthopaedic Foot and Ankle Score)]. However, all authors reported a good functional outcome based on those scoring that was taken pre-surgery and post-surgery.

Li reported an MSTS (Musculoskeletal Tumour Society) score of 24,4 (range 25-27) on the last follow up (45 months post-surgery) after wide resection with 3DPs on pelvic GCT patients.⁹ Lv reported a patient with sacral giant cell tumour who received reconstruction with

3DPs with MSTS score of 24,1 post-surgery with good ambulatory functions.¹⁰ Benady reported MSTS score of 20,5 post-surgery on patients with pelvic sarcoma who underwent resection and reconstruction with 3DPs.¹⁰ Luenam reported good functional outcome of patient with open distal humeral fracture with severe articular destruction of capitellum and lateral trochlear ridge who received reconstruction with 3DPI with Mayo Elbow Performance Index of 85 and Patient-Based Disabilities of The Arm, Shoulder and Hand Score of 22,5.¹⁰

Tang reported a patient with infected tibial shaft non-union who received reconstruction with 3DPs and bone graft.¹¹ On one-month post-surgery, the patient was able to ambulating independently and her mental status gradually improved, and on one-year post-surgery, there was no pain and the patient had improvement in strength and sensation of her extremity.¹¹

Anatomical outcome

We found that 3DPs can be used in a wide variety of bones, joints and regions. This is because 3DPs are highly customizable due to its nature as an additive manufacturing that can be formed into various complex form and size. All authors reported a good anatomical outcome. Li reported that the 3DPI matched the shape and size of the bone defect.⁹ Luenam reported good congruity of the elbow joint during the range of motion.¹⁰ Kadokia, Adams and Han reported good alignment of bone and 3DPI.^{3,12}

Complication

There were minimal complications, but those complications were not related to the 3DPis. Li reported a complication of delayed wound healing in 1 patients.⁹ Adams reported a complication deep infections in 1 patients.³ Benady reported a complication of superficial infection in 3 patients.¹³ Lv reported a complication of delayed wound healing in 3 patients.³ Complications related to the implant such as implant loosening etc were not observed on all studies. Those studies showed that 3DPI could minimize the implant related complications due to its nature of being close-to-perfect size and form to fill the defect.

Discussion

Anatomical outcome

3DPis have demonstrated promising results in improving anatomical outcomes in orthopaedic surgery. 3D-printed patient-specific anatomical models have been shown in studies to provide surgeons with correct analysis of complicated anatomical structures, enabling for better surgical planning, training, and simulation.¹⁴ These models have proven to be very useful in orthopaedic trauma surgery, oncological surgery, and bone and joint reconstructions.⁷ The 3DPis have also been successful in resolving orthopaedic difficulties that traditional implants

cannot handle, offering good anatomical and functional results with fewer complications.

Complex internal mesh designs and porous structures for medical implants allow bone to grow into the implant for better integration, as well as the ability to adjust the density to more precisely match the stiffness of the surrounding bone. Figure 3 depicts design procedure profile of PSBCG (patient-specific bone-cutting guide) and 3DPI for hip reconstruction following wide resection due to pelvic giant cell tumor. Figure 4 depicts a prototype additively built tibial and femoral stem knee implant displaying the complex internal structure and porous surface feasible with additive printing, as well as an x-ray of a knee implant in position to demonstrate how they might be used.⁵ Three-dimensional printing has played a significant role in the development of patient-specific implants, with increasing evidence of therapeutic efficacy. Figure 5 depicts an example of patient receiving a custom 3D-printed implant for a major tibia bone defect experienced in a car accident.¹² 3DPis provide satisfactory result in treatment of severe bone defect. 3D printing technology can be utilized to improve preoperative planning, modify implants and tools, and enhance surgeon education and training. Its strength is on the accuracy and complexity of the size and form of the implant.

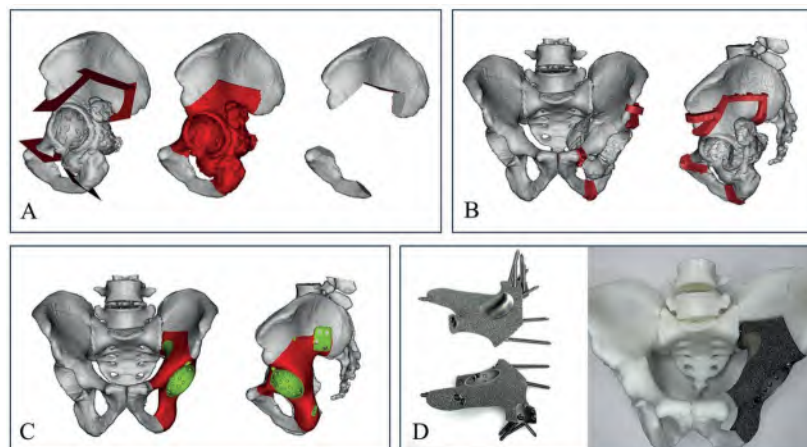


Figure 3. Design procedure profile for PSBCG (patient-specific bone-cutting guide) and 3DPI. **A:** multiplanar osteotomy is proposed, **B:** 3D models of the PSBCG, **C:** 3D models of the 3DPI, **D:** physical images of the 3DPI.⁹

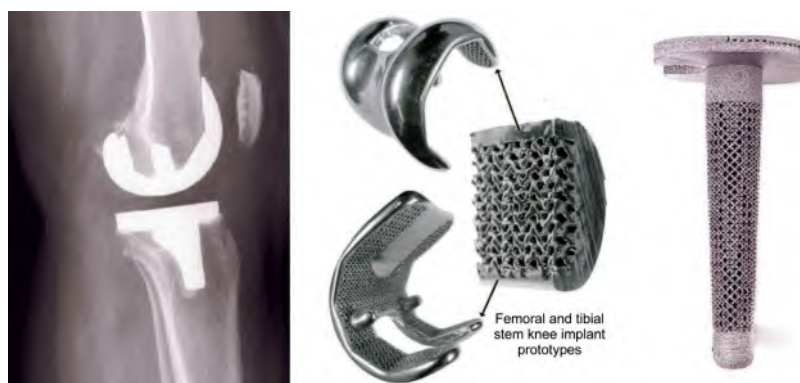


Figure 4. Complex Internal Structure of a Knee Implant.⁵

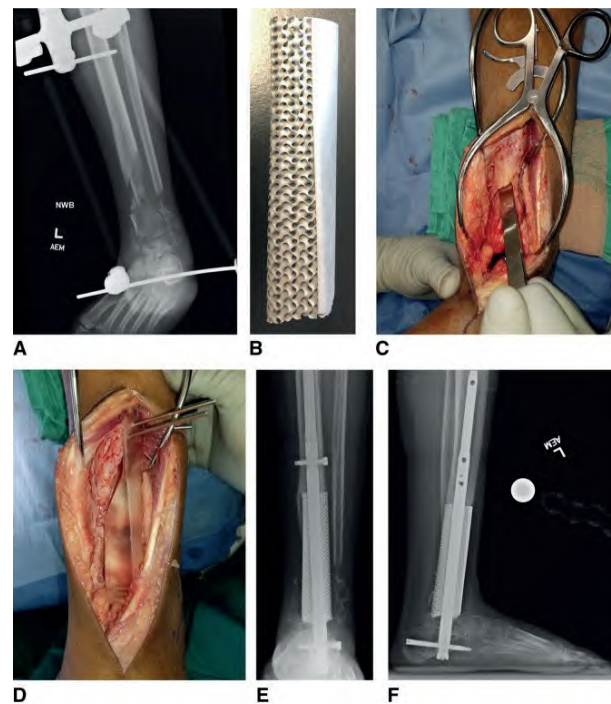


Figure 5. A 22-year-old woman's left leg after she was injured in a car accident. She broke both her tibia and fibula open. A: anterior-posterior (AP) radiograph A huge bony defect resulted from the loss of bone at the location, B: a three-dimensional (3D) printed implant built specifically to fill the bone deficiency, C: an intraoperative picture of a distal tibia fracture with bone loss, D: 3D printed anatomic spacer block for determining alignment and length as well as performing intramedullary reaming, E: AP radiograph demonstrating successful 3D-printed implant insertion, F: lateral radiograph demonstrating effective 3D-printed implant insertion.¹⁶

Functional outcome

Three-dimensional (3D) printed orthopaedic implants have demonstrated excellent functional outcomes. Custom 3D-printed implants with dual-mobility bearings resulted in outstanding functional improvement, implant survival, and osseointegration in an acetabular reconstruction study.¹² Another study on personalized 3D printed prosthesis found satisfactory anatomical and functional results, a shorter surgical time, and a high level of patient satisfaction.⁷ In challenging cases of failed hip arthroplasty and acetabular bone loss, a series of custom 3D printed titanium acetabular implants exhibited high overall and implant-specific survival at up to five years follow-up.¹² Furthermore, 3D printing technology has been employed in traumatology to improve pre-operative planning, hence optimizing surgical procedures and outcomes. These findings show that 3D-printed orthopedic implants may improve functional outcomes.

Complication

In other studies, we found that complications associated with 3D-printed orthopaedic implants include implant dislocation, leg-compartment syndrome, limited range of motion, and local recurrence.¹⁴ Complications were found in 31.3% of cases with custom 3D printed titanium acetabular implants used in revision total hip arthroplasty, with four cases necessitating revision surgeries.¹¹ At two and five years, however, total operation

survival was 88.7% and implant-specific survival was 98%. These issues underscore the need for additional research and large, long-term trials to properly understand the value and future of 3D printing in orthopaedic surgery.

Material

Titanium is used for 3D-printed personalized implants in orthopaedic surgery because it offers suitable mechanical properties and is biocompatible.¹⁵ Titanium implants used in orthopaedic surgery have crucial mechanical and biological features that affect their performance. The mechanical properties of titanium implants, such as elastic modulus and compressive yield strength, can be modified by the implant structure, which includes lattice structures and fiber-based biomaterials.^{15,16} These features can improve implant longevity and osseointegration by reducing stress shielding and mimicking the mechanical characteristics of bone.¹⁷ Furthermore, the use of additive printing techniques enables the creation of patient-specific implants with functionally graded porosity, which improves mechanical properties and tissue integration.¹⁸ To promote bioactivity and prevent cell death around the implant, titanium implants can be coated with bioactive materials such as hydroxyapatite.¹⁹ According to several opinion, additive printing also allows for the use of alternative materials for parts, which may result in superior performance than identical parts created using traditional materials, such as lower weight and greater strength.

Manufacturing process

3D-printed personalized implants are created depending on: i) the geometries of the bone and implant; ii) the material characteristics of the bone; and iii) the loading condition relative to the patient's height and weight.²⁰ The shape of the 3D-printed personalized implants was created with the surgical approach and surrounding soft tissues in mind. Geometric data is obtained from taking 2D data from CT scans or MRI. This 2D data will then be processed into 3D images with an FDA-approved image-processing software application (Mimics, Materialise, N.V. Leuven[®] as an STL file into an FDA-approved CAD software program (3-matic, Materialise N.V.) The materials used for making 3DPis use materials that are taken into account in terms of the bioactivity of the body's reaction to implants, material toxicity, and osteoinduction.²⁰ Mostly used materials for implants are titanium and biocompatible high-strength and thermal-resistant materials (ULTEM).²⁰ After the surgeon approved the pre-surgical plan and design, 3DPis were produced. To ensure complete installation, each 3DPI was designed with a unique footprint based on the patient's bone morphology, height, and weight.

Cost

The cost of 3D-printed orthopaedic implants varies based on the process and type of implant used. In the case of glenohumeral arthrodesis, adopting 3D printing technology to construct patient-specific implants saved around 38.8 minutes of intra-operative time, resulting in a \$2586 cost savings per case.²¹ The use of 3D printed guidance for screw placement in the surgical treatment of adolescent idiopathic scoliosis resulted in significantly higher implant prices compared to traditional freehand approaches, but also reduced intraoperative blood loss.²² A 3D-printed implant was found to be more cost-effective than its nearest competitor on the market for acetabular revision total hip arthroplasty, with a potential annual cost reduction of €20500 in Belgium.²³ Using open-source software and cloud 3D printing services in the manufacture of patient-specific orthopaedic implants resulted in implants being made for as little as £32, which is approximately 1/100th of the traditional cost of implant fabrication.²⁴

However, according to a recent report, the market for medical 3D printing is valued at roughly \$1.25 billion, with potential growth of up to \$6 billion over the next decade.²⁵ 3D printers currently range in price from \$200 to well over \$100,000.²⁵

While the cost of 3D-printed orthopaedic implants in Indonesia is not specified, those studies indicate that 3D printing technology has the potential to cut costs in orthopaedic implant production. To ascertain the actual cost of 3D-printed orthopaedic implants in Indonesia, more research or local studies would be required.

Mechanical properties of 3DPI

A variety of factors influence the mechanical qualities of 3D-printed titanium implants used in orthopaedic surgery. The implant's tension and strain are determined

by the additive manufacturing process parameters.²⁶ The strength of the implant can be increased by choosing the right printing parameters and heat treatment methods.²⁷ The implant's tear and wear resistance can be improved by adding nanospikes to the surface, which also can kill germs and prevent infections.²⁸ Finite element modelling can be utilized to forecast the overall stiffness and local strain distribution of the implant, taking into consideration the complicated material behaviour at different scale.²⁹ The 3D-Ti plates show comparable biocompatibility and stability for stiff internal fixation to Synthes-Ti plates, with reduced roughness (106.44 ± 78.35 nm), stronger mechanical strength (370.78 ± 1.25 HV10), greater ultimate stress (2153.13 MPa), higher elasticity modulus (747.81 GPa) and a higher bone-plate contact rate (96.9%).²⁹

Conclusion

3DPis have shown promising results in correcting bone deformities. The design of these implants is based on 3D imaging or surface scans of the patient's defect. 3DPis have reduced the likelihood of complications while improving anatomical and functional outcomes. However, because this analysis was limited by the number of accessible papers, more research is needed to determine the definitive effect of such technology, especially in the future of orthopaedics in bone defect repair by using 3D-printed personalized implants.

Ethical approval

This study was a secondary analysis based on the currently existing data and did not directly involve with human participants or experimental animals. Therefore, the ethics approval was not required in this paper.

Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Conflict of Interest

The authors declare that there are no relevant conflicts of interest.

Funding

This study received no particular grants from public, commercial, or non-profit funding entities.

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The innovative development of the aphasia speech application for Thai adults with aphasia

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ARTICLE INFO

Article history:

Received 16 October 2024

Accepted as revised 20 March 2025

Available online 26 March 2025

Keywords:

Aphasia speech, mobile application, stroke, rehabilitation.

ABSTRACT

Background: The growing trend of internet usage among the Thai population, both in terms of user numbers and time spent online, has led to a growing demand for self-training applications. This trend presents opportunities to enhance access to healthcare services, especially in speech rehabilitation for aphasic patients. However, Thailand faces a shortage of speech-language pathologists (SLPs), making mobile applications a viable solution to expand access to treatment and improve patient outcomes.

Objective: This study aimed to develop a digital tool for speech training in adult aphasic patients under the supervision of SLPs and a multidisciplinary team. It also sought to evaluate the tool's effectiveness in improving speech outcomes for aphasic patients and assess user satisfaction among healthcare professionals, healthy participants, aphasic patients, and their caregivers.

Materials and methods: This developmental research was conducted in four phases, starting with a literature review, developing the speech training application, expert validation of the application's content, and tool implementation. The application was tested with 15 SLPs, six physicians, six nurses, six occupational therapists, six physical therapists, six healthy participants, 15 aphasic patients, and 15 caregivers. Based on the feedback from these diverse groups, the application was further refined to address suggestions and improve its usability. The application's efficiency was measured through satisfaction surveys, while its effectiveness was evaluated using Thai adaptation of the Western Aphasia Battery test (WAB-Thai). Pre- and post-training scores were compared following 12 traditional speech therapy sessions with the Aphasia Speech application, each lasting 60 minutes over three months.

Results: Satisfaction levels among healthcare professionals and healthy participants ranged from 4.0 to 4.2, whereas satisfaction levels among patients and caregivers were 3.9 to 4.6, indicating high satisfaction. The WAB-Thai scores showed statistically significant improvements in all areas, including fluency, comprehension, repetition, naming, reading, and the Aphasia Quotient (AQ) scores, with $p < 0.05$.

Conclusion: The developed application significantly enhanced the effectiveness of speech training, as evidenced by improved language and speech outcomes across all metrics. High satisfaction levels from users supported its implementation as a valuable tool for continuing speech therapy at home. Further refinements are necessary to optimize its usability, with plans for broader dissemination among healthcare providers to address the shortage of SLPs in underserved regions.

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doi: 10.12982/JAMS.2025.058

E-ISSN: 2539-6056

Introduction

According to the 2019 Burden of Disease study in Thailand, stroke was the leading cause of disability-adjusted life years (DALYs) lost among individuals aged

60 and older, accounting for 11.8% of DALYs lost in males and 12.9% in females. Overall, stroke ranked as the second most significant cause of DALYs lost, following road traffic accidents for males and diabetes for females.¹

The prevalence of stroke is 1.88% among adults aged 45 and older, with higher prevalence in men than women, and the mean age of stroke onset is 65 years.² Approximately 25–50% of strokes result in aphasia,³⁻⁸ an acquired language disorder resulting from brain injury, typically in the left hemisphere. It affects spoken and written expression, comprehension, and reading.⁹ Aphasia significantly diminishes patients' quality of life, with 32% of patients reporting limitations in daily activities, 23% experiencing worsened depression, and 24% facing difficulties communicating with others.¹⁰

Aphasia rehabilitation includes individual therapy, group therapy, and tele-speech therapy, which utilizes technology such as video conferencing tools to provide remote care. Tele-speech therapy improves accessibility, reduces costs, and addresses the shortage of speech-language pathologists (SLPs) in Thailand.¹¹⁻¹⁴

As reported by the Electronic Transactions Development Agency (ETDA) in the Thailand Internet User Behavior 2019 report, internet usage has risen to 70% of the Thai population. Adults aged 55-73 spent 10 hours online daily, while those aged 39-54 spent 9 hours and 49 minutes online daily.¹⁵ This significant engagement with digital platforms among older adults underscores the potential for tele-speech therapy solutions in aphasia rehabilitation. By leveraging the widespread internet access, it is feasible to enhance the reach and effectiveness of rehabilitation services, catering especially to the needs of those with communication disorders.

Thailand had 280 SLPs for a population of 65,951,210, resulting in a ratio of 0.42 SLPs per 100,000 individuals.^{16,17} Furthermore, the distribution of SLPs presents an issue, as 70% of them worked in Bangkok and the metropolitan area.¹⁸ In response to the shortage of SLPs in Thailand, this study aimed to develop a digital tool for speech training in stroke-induced aphasia, guided by SLPs and a multidisciplinary team, and to evaluate its effectiveness in improving language and speech outcomes and user satisfaction among healthcare professionals, healthy participants, aphasic patients, and caregivers. The application would be specifically tailored for Thai patients, with the goal of increasing accessibility and motivation for training. By integrating technology, patients can achieve comprehensive rehabilitation, leading to improved language and speech skills and an enhanced quality of life.

Materials and methods

The research comprised four phases aimed at the systematic development and validation of the application, as detailed in the 'Development and Validation Process of the Aphasia Speech Application' flowchart (Figure 1). Each phase of the study involved iterative refinement based on empirical feedback, ensuring the application was rigorously tested and adapted to meet the needs of its diverse user groups. In the first phase, the content was developed using exercises from Supawatjariyakul *et al.*,¹⁹ and Sirindhorn National Medical Rehabilitation Institute,²⁰ which includes comprehension training, phonation exercises, repetition practice, and naming pictures. These features were then provided to programmers for application development, incorporating a scoring system, adjustable difficulty levels, and auditory and visual cues with images and spoken instructions. The application was designed to be compatible with devices running both Android and iOS operating systems. Please see Figure 1 for a visual overview of the application's design and features. In the second phase, the application was validated by five experts using Item-Objective Congruence (IOC) ratings, with revisions made based on their suggestions. The third phase involved testing with six healthy participants and 39 healthcare providers, including six rehabilitation physicians, six nurses, six physical therapists, six occupational therapists, and 15 SLPs. Participants aged 20-60 were required to be Thai, have no neurological impairments, and be able to use mobile applications. Satisfaction was then assessed, and feedback led to further revisions. In the final phase, the application was tested on 15 aphasic patients and their caregivers. The sample size calculation was based on 4,576 stroke survivors in Nonthaburi province.²¹⁻²⁴ Patients were native Thai speakers without dialects, aged 20 or older, with at least a Year 4 of primary school education, and diagnosed with stroke-induced aphasia. They completed 12 one-hour speech therapy sessions using the application, conducted once per week under the supervision of SLPs. The SLPs guided both patients and their caregivers on how to use the application effectively. They underwent a baseline and post-training assessment using Thai adaptation of the Western Aphasia Battery Test (WAB-Thai).²⁵ Pre- and post-training scores were compared using a paired t-test, and patient and caregiver satisfaction levels were also evaluated.

The research was conducted from November 2021 to September 2024. The study took place at the Speech Therapy Department of Sirindhorn National Medical Rehabilitation Institute and the Faculty of Information Communication Technology (ICT) at Mahidol University.

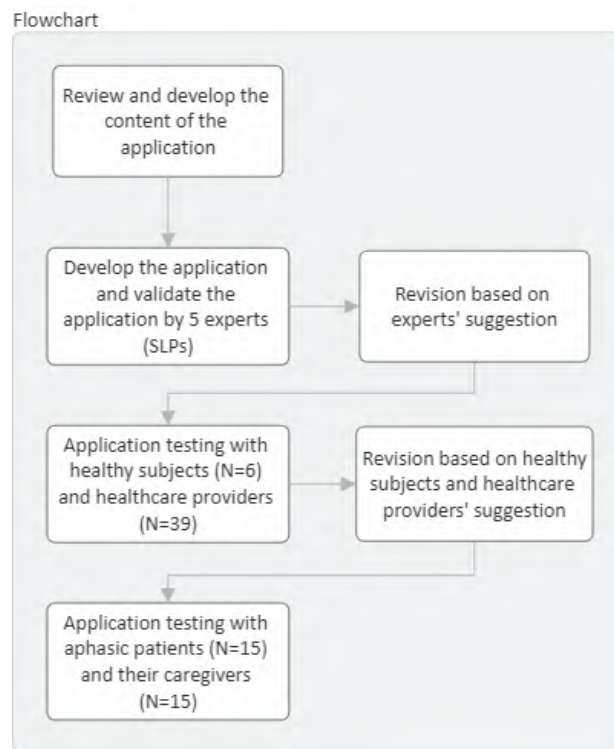


Figure 1. A Flow chart depicting the development and validation process of the aphasia speech application.

Results

Results of Phase 1 and 2: Development and validation of the application

Five expert SLPs evaluated the content validity, resulting in IOC scores of 0.6 for listening comprehension, 0.8 for speech production, 0.7 for repetition, 0.7 for naming, and 0.1 for design. Design aspects requiring revision included font clarity (0.4), appropriateness of illustrations (0.0), aesthetic quality and clarity of illustrations (0.2), appropriateness of accompanying sound (0.0), ability for independent use (-0.4), operational smoothness (-0.8), and the ability to record patient data (-0.4). Based on

this feedback, revisions were implemented. For example, users reported slow response times, requested a profile deletion feature, and experienced recording issues on some devices. The application had difficulty recognizing soft-spoken words and similar consonants, such as /p/ and /b/ or /t/ and /d/, while certain images were unclear and instructions lacked variety. Additionally, some questions had multiple correct answers, and unfamiliar vocabulary posed challenges. Figure 2 illustrates the final design and features of the application, including the Home Screen and Main Features.

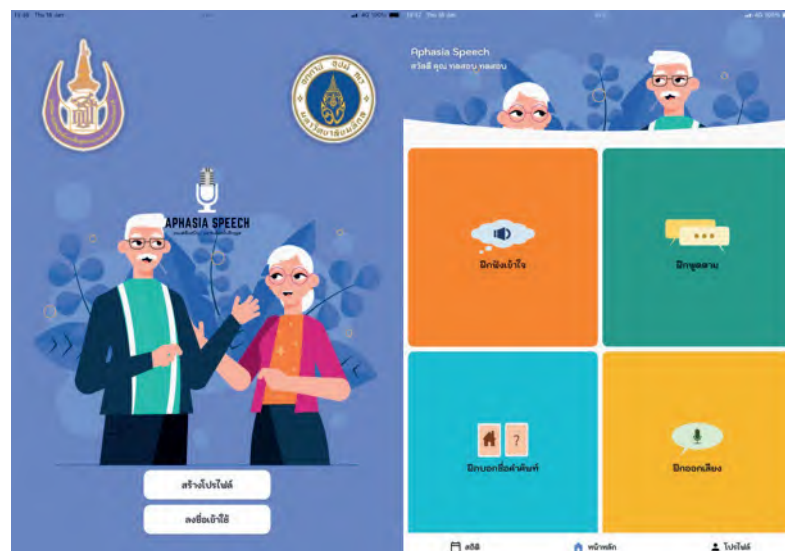


Figure 2. Application's home screen and main features.

Results of Phase 3 and 4: Satisfaction levels

The sample group for Phase 3 included 45 individuals: 15 SLPs, six healthy participants, six occupational therapists, six physical therapists, six rehabilitation physicians, and six rehabilitation nurses. The average age was 35.5 years (SD=8.4), ranging from 23 to 52 years, with five males and 40 females. Of these, 33 participants held a bachelor's degree, and 12 had a master's or doctoral degree.

Satisfaction levels were categorized as 0.0-1.4 (very low), 1.5-2.4 (low), 2.5-3.4 (moderate), 3.5-4.4 (high), and 4.5-5.0 (very high). As shown in Table 1, the overall satisfaction score for healthy participants and healthcare providers averaged 4.0, indicating a high level of satisfaction. Perceived ease of use, design, and perceived value were scored 4.1, 4.0, and 4.2, respectively, all indicating high satisfaction. Recommendations included requests for larger buttons, clearer images, automatic progression after correct answers, improvements in voice recognition, and the ability for caregivers or trainers to

score speech production exercises based on the patient's ability.

In Phase 4, caregivers reported very high overall satisfaction (4.5). Perceived ease of use was scored 4.6 (very high), with 4.4 (high) and 4.5 (very high) in design and perceived value, respectively. Aphasic patients' overall satisfaction was 4.2, with ease of use at 4.3, design at 3.9, and perceived value at 4.3, all indicating a high level of satisfaction.

Feedback from Phase 4 included suggestions for improving images to be larger and clearer, especially in sections with multiple choices, improving voice output consistency, improving the accuracy of voice recognition, expanding vocabulary in repetition exercises, incorporating images or videos to support pronunciation, and improving button placement, as the repeat button was difficult to press on small devices. Additionally, it was suggested that the level of difficulty in every training session be elevated to increase the challenges for users.

Table 1. User satisfaction with the aphasia speech application among healthy subjects, healthcare providers, caregivers, and aphasic patients.

User satisfaction with the aphasia speech application	Phase 3	Phase 4	
	Healthy subjects & healthcare providers (N=45)	Caregivers (N=15)	Aphasic patients (N=15)
Perceived ease of use			4.3
1. The application menu is easy to use	4.2	4.6	
2. Categories are clearly organized and easily accessible	4.4	4.7	
3. Can be used independently	4.1	4.7	
4. Smooth and continuous operation	3.5	4.5	
Design			3.9
5. The font is clear and easy to read	4.2	4.7	
6. Illustrations are beautiful, clear, and appropriate	3.8	4.4	
7. Buttons are well-arranged and easy to use	3.8	4.6	
8. Accompanying sound is appropriate	3.7	4.2	
9. The language used is clear and easy to understand	4.1	4.4	
10. The level of difficulty is appropriate	4.2	4.2	
Perceived value			4.3
11. Saves on costs for hospital-based speech therapy	4.4	4.5	
12. Enables more frequent practice	4.4	4.7	
13. Overall satisfaction with the application	3.8	4.5	
Overall Average	4.0	4.5	4.2

Results of Phase 4

In Phase 4, aphasic patients and their caregivers tested the application. Patients completed a baseline WAB-Thai assessment, followed by 12 one-hour sessions combining traditional therapy and the application. A post-training WAB-Thai assessment was conducted three months later. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess data normality, with a p-value less than 0.05 indicating a non-normal distribution. The Shapiro-Wilk test was preferred for its higher sensitivity with small sample sizes, while the Kolmogorov-Smirnov test was used for larger datasets.²⁶ Variables found to deviate from normality included time since stroke onset ($p=0.018$), caregiver's educational level ($p=0.045$, 0.015), pre-training naming score ($p=0.019$), and pre-training

reading score ($p=0.044$). Due to non-normal distributions, non-parametric analyses were applied, with the Wilcoxon Signed Ranks Test used instead of the Paired t-test, although paired t-tests were also conducted for confirmation.

Table 2 presents demographic data for aphasic patients (N=15) and caregivers (N=15). Patients had an average age of 50.5 years (SD=12.2) and an average education of 15.2 years (SD=3.0). The average time since stroke onset was 20.5 months (SD=21.3). Among caregivers, the average age was 46.0 years (SD=14.3), with 15.4 years of education (SD=2.5). Caregivers were predominantly female (73%) and were mainly spouses (47%), followed by children (33%), other relatives (13%), and parents (7%).

Table 2. Demographic information of participants in phase 4.

Characteristics	Aphasic patients (N=15)	Caregivers (N=15)
Gender		
- Males	7 (47%)	4 (27%)
- Females	8 (53%)	11 (73%)
Age (years)	50.5 (12.2)	46.0 (14.3)
Education level (years)	15.2 (3.0)	15.4 (2.5)
Stroke onset (Months)	20.5 (21.3)	-
Relationship with patient	-	
- Parent	-	1 (7%)
- Spouse	-	7 (47%)
- Child	-	5 (33%)
- Relative	-	2 (13%)

Note: Values are presented as mean (SD).

Regarding application usage, 13% of participants used it once a week, 13% used it 2-3 times weekly, 67% used it 4-5 times weekly, and 7% used it more than 5 times weekly. For session duration, 40% used the application for under 15 minutes, 53% for 15-30 minutes, and 7% for 31-45 minutes, with no sessions exceeding 45 minutes. The most practiced activity was naming (67%), followed by repetition (53%), phonation (47%), and comprehension (27%).

Table 3 presents the differences between baseline and week 12 scores across various speech and language

measures, which were analyzed using both the Paired t-test and the Wilcoxon Signed Ranks Test. Significant improvements were observed across all measures, with p-values less than 0.05, indicating statistical significance. The results from the Paired t-test closely aligned with those from the non-parametric Wilcoxon Signed Ranks Test, supporting the robustness of the findings. The largest improvement was observed in the Aphasia Quotient (AQ) scores, with a mean increase of 10.2 (SD=8.3) and a p-value of less than 0.001.

Table 3. Difference between baseline and week 12 scores using paired T-test and Wilcoxon Signed Ranks Test.

	Mean (SD) baseline (N=15)	Mean (SD) week 12 (N=15)	Paired t-test			Wilcoxon Signed Ranks Test	
			Mean Diff. (SD)	95 % CI	Sig. (2-tailed)	Z	Asymp. Sig. (2-tailed)
Fluency	4.3 (2.6)	5.0 (2.9)	0.7 (0.9)	0.2-1.2	0.012*	-2.46 ^b	0.014*
Comprehension	4.9 (3.0)	6.2 (2.8)	1.3 (1.5)	0.5-2.1	0.004*	-2.73 ^b	0.006*
Repetition	4.6 (3.4)	5.6 (3.7)	1.0 (1.5)	0.2-1.8	0.015*	-2.75 ^b	0.006*
Naming	4.0 (3.7)	5.2 (3.6)	1.2 (1.3)	0.5-1.9	0.004*	-2.94 ^b	0.003*
Reading	5.7 (3.3)	6.9 (3.1)	1.2 (1.0)	0.6-1.8	0.001*	-3.19 ^b	0.001*
AQ	44.3 (28.3)	54.5 (30.6)	10.2 (8.3)	5.7-14.8	<0.001*	-3.29 ^b	<0.001*

Table 4 shows the comparison of the distribution of aphasia types among participants at baseline and week 12. Notably, the number of participants with global aphasia decreased from 6 (40%) at baseline to 3 (20%) at week 12, while the number of participants with anomic aphasia increased from 4 (27%) to 6 (40%). Minimal changes were observed in the distribution of other aphasia types.

Table 5 presents the results of the subgroup analysis comparing the mean score differences in each domain between participants aged 50 years or younger and those aged over 50 years. The independent t-test and Mann-Whitney U test results indicated no statistically significant differences in the Pre- and post-training mean scores for fluency, comprehension, repetition, naming, reading,

and AQ scores between the two age groups. The p-values ranged from 0.187 to 0.972, all of which exceeded the significance threshold of 0.05.

Table 6 presents the results of the subgroup analysis comparing the mean score differences between participants with post-stroke durations of 1 year or less and those with durations of more than 1 year. The independent t-test and Mann-Whitney U test results indicated no statistically significant differences in the pre- and post-training mean scores for fluency, comprehension, repetition, naming, reading, and AQ scores between the two groups. The p values ranged from 0.731 to 0.954, all of which exceeded the significance threshold of 0.05.

Table 4. Comparison of types of aphasia at baseline and week 12.

Types of Aphasia	Baseline (N=15)	Week 12 (N=15)
Global	6 (40%)	3 (20%)
Isolation	1 (7%)	1 (7%)
Wernicke's	2 (13%)	2 (13%)
Broca's	1 (7%)	2 (13%)
Transcortical motor	1 (7%)	1 (7%)
Anomic	4 (27%)	6 (40%)

Table 5. Comparison of mean differences in speech and language scores between participants aged 50 years or younger and those aged over 50 years.

	Mean differences (SD)		p value ^a	Asymp. Sig. ^b
	Age ≤50 years (N=7)	Age >50 years (N=8)		
Fluency	0.9 (1.1)	0.5 (0.8)	0.464	0.481
Comprehension	1.5 (1.0)	1.2 (1.8)	0.710	0.728
Repetition	0.5 (0.6)	1.5 (1.8)	0.187	0.522
Naming	1.2 (1.6)	1.2 (1.1)	0.928	0.728
Reading	1.1 (0.5)	1.0 (1.4)	0.972	0.294
AQ	10.1 (5.1)	10.4 (10.7)	0.949	0.728

Note: ^aIndependent t-test, equal variances assumed, two-sided p value, ^bMann-Whitney U Test, 2-tailed.

Table 6. Subgroup analysis of mean score differences in each domain between participants with post-stroke duration of 1 year or less and those with post-stroke duration of more than 1 year.

	Mean differences (SD)		p value ^a	Asymp. Sig. ^b
	Post onset 0-12 months (N=8)	Post onset >12 months (N=7)		
Fluency	0.6 (0.7)	0.7 (1.1)	0.856	0.898
Comprehension	1.2 (1.9)	1.4 (0.9)	0.787	0.908
Repetition	1.1 (1.4)	1.0 (1.6)	0.952	0.907
Naming	1.2 (1.3)	1.2 (1.5)	0.952	0.954
AQ	10.4 (10.5)	10.0 (5.6)	0.930	0.728
Reading	1.1 (1.4)	0.9 (0.5)	0.731	0.771

Note: ^aIndependent t-test, equal variances assumed, two-sided p value, ^bMann-Whitney U Test, 2-tailed.

Discussion

This study aimed to develop a digital tool for speech training in adult aphasic patients supervised by SLPs and a multidisciplinary team. Additionally, the study sought to evaluate the effectiveness of the tool in enhancing speech outcomes for aphasic patients and assess user satisfaction.

The application was designed based on a literature review and speech therapy exercises.^{19,20} Content validity was assessed by five expert SLPs, yielding an IOC score of 0.6. Design revisions were made according to the experts' recommendations. The application was further evaluated by a multidisciplinary group of 45 individuals, including rehabilitation professionals and healthy participants. Overall satisfaction ranged from 4.0 to 4.2, indicating high satisfaction. Feedback from the group was incorporated into the application, which was then tested on patients and their caregivers. Reported satisfaction ranged from 3.9 to 4.6, indicating strong approval from both patients and caregivers.

When combined with traditional therapy, the Aphasia Speech application was tested on 15 stroke patients with aphasia, who completed 12 one-hour sessions over 3 months. The WAB-Thai test showed significant improvements in fluency, comprehension, repetition, naming, reading, and AQ scores. Both the Paired t-test and Wilcoxon Signed Ranks Test indicated statistically significant improvements between pre- and post-training scores, confirming the effectiveness of the training.

The statistically significant improvement in patients' average language ability scores can be attributed to the frequency of speech training they received. Patients attended speech therapy sessions for one hour per week, and the majority (67%) continued practicing at home 4-5 times per week. Of these, 53% practiced for 15-30 minutes per session, while 40% practiced for less than 15 minutes per session. This consistent practice contributed to the improvement in language and speech abilities across all areas.

These findings aligned with the study by Ngamsuriyaroj et al., which demonstrated that game-based aphasia training applications significantly improved patients' abilities when practiced for 10-15 minutes per day, once per week, over eight consecutive weeks. Pre- and post-treatment comparisons showed improvements in all abilities. Additionally, the use of the application increased the frequency of home practice.²⁷ Similarly, Garcia found that the use of the 'Theraphasia' application for 10-15 minutes per day, once a week, for eight consecutive weeks resulted in improvements in all subtests of the Quick Aphasia Battery (QAB), including alertness, speech ability, language comprehension, picture naming, repetition, and reading.²⁸

Considering the severity levels of aphasia,^{25,29} post-training results showed a decrease in the number of patients with the most severe type, global aphasia, from six individuals (40%) to three individuals (20%). Meanwhile, the number of patients with the least severe type, anomic aphasia, increased from four individuals (27%) to six individuals (40%). Survey results indicated

that the most frequently practiced activity was word-naming exercises, with 67% of participants engaging in this activity. This is consistent with the types of aphasia present in this study, as all aphasic patients experienced word-finding difficulties, making word-naming exercises essential for their training.²⁸⁻³⁰

The average age of the participants in this study was 50.5 years, with a standard deviation of 12.2 years (95% CI: 43.1, 57.8). A subgroup analysis found no statistically significant differences in the improvement of scores between participants younger than 50 years (N=7) and those older than 50 years (N=8). This finding aligned with the results of a systematic review by Ellis,³¹ which analyzed 40 studies with a total sample of 14,795 participants. While stroke is more prevalent in older adults, age appears to influence only the risk of developing aphasia and the type of aphasia. Stroke patients with aphasia tend to be older than those without aphasia, and there is a correlation between age and aphasia type, with younger patients more likely to experience non-fluent aphasia, such as Broca's aphasia. However, age does not show a clear relationship with aphasia recovery or clinical outcomes.³¹

In addition to the age subgroup analysis, the researchers also conducted a subgroup analysis based on post-stroke duration. The average time since stroke onset in this sample was 20.5 months, with a standard deviation of 21.3 months (95% CI: 7.6, 33.3), ranging from 1 to 65 months. When comparing the subgroup with post-stroke duration of 1 year or less (N=8) and the subgroup with a duration of more than 1 year (N=7), no statistically significant differences were found in the improvement of scores between these two groups. These results suggested that the length of time since the stroke may not be a significant factor in the recovery or improvement of language and communication abilities in aphasic patients. This finding is consistent with previous studies, such as Nakagawa et al., which noted that many patients continued to recover even long after the stroke.³² Similarly, Kertesz and McCabe found that some aphasic patients experienced gradual recovery over several years after their stroke onset.²⁹ In line with these findings, Naeser et al. observed improvements in naming scores and word length within five years after a stroke³³, while Fitzpatrick et al. reported that aphasic patients continued to show improvement in picture naming between five and fifteen years post-stroke.³⁴

Furthermore, the lack of statistically significant differences between groups with varying post-stroke durations may suggest that recovery ability could depend on factors beyond age or time since stroke onset, such as the frequency and quality of speech therapy, family support, or access to continuous care and rehabilitation. This finding was consistent with the study by Harvey et al., which found that some aphasic patients continued to make language improvements years after a stroke, indicating that long-term therapy may benefit this population.³⁵ Therefore, providing appropriate information and therapy for individuals with chronic aphasia could enhance their chances of continued language development.

The use of the application in this study had similar limitations to those identified in the study by Ngamsuriyaroj *et al.*, which found several factors that could affect usability. These included the ease of use for both patients and caregivers, the accuracy of visual and audio output, language, user perception, family support, service conditions, and training in the application's use.²⁷ In this study, patient selection criteria specified that participants must be able to use the application. Patients should be able to look at the interface and understand how to interact with it. While some individuals may access the application independently, others might receive assistance from relatives to launch the application or navigate through its sections. This may have excluded individuals with more severe impairments or less familiar with technology. As a result, the findings may not be fully generalizable to all aphasic patients, particularly those with more significant cognitive or physical impairments. Additionally, the interface design took into account screen and item sizes, resulting in a satisfaction score of 4.7 from participants for the clarity and readability of the font size. However, feedback from caregivers and patients indicated that further improvements were needed, including adjustments to image size and clarity, as well as enhancements to the accompanying audio. There were also suggestions to increase the number of exercises and introduce more difficulty levels.

Limitations

1. The application required further revisions and additional funding before it could be made available for self-download on iOS and Android operating systems.
2. Some patients were unable to use the application independently due to limitations in the accurate verification of speech. Specifically, activities involving speech production, repetition, and naming still necessitated assistance from others, such as SLPs or caregivers, to ensure proper pronunciation.
3. The patients' and caregivers' proficiency with mobile applications may have influenced their satisfaction levels. Those with more experience or confidence in using mobile applications may have found the tool easier to use, which could have positively impacted their overall satisfaction. This factor should be considered when interpreting the results.

Recommendations

1. A randomized controlled trial (RCT) should be conducted to compare the effectiveness of traditional speech therapy alone and traditional therapy combined with the application, using a larger sample size.
2. The application should be enhanced and refined to improve its functionality for future studies.

Conclusion

In summary, the findings of this study indicated that the developed application effectively enhanced

the training process, with users reporting high levels of satisfaction. The application significantly improved the effectiveness of speech therapy services, as evidenced by increased language and speech scores across all areas, including fluency, comprehension, repetition, naming, reading, and AQ scores.

The application has the potential to increase the frequency of practice and improve accessibility for patients. Moreover, it could serve as a valuable tool for other healthcare professionals and public health workers in regions where there is a shortage of SLPs. By providing initial support to aphasic patients under the supervision of an SLP, the application could facilitate preliminary care before patients are referred for comprehensive speech therapy with a certified practicing SLP.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethics approval

This research was approved by the Human Research Ethics Committee of Sirindhorn National Medical Rehabilitation Institute under approval number 64004 from March 25, 2021, to September 30, 2024.

Funding

This research was funded by the Academic Support Fund of Department of Medical Services, Ministry of Public Health, for the fiscal years 2021-2024.

Acknowledgments

The research team gratefully acknowledges the speech-language pathologists at Sirindhorn National Medical Rehabilitation Institute, as well as the participating patients, caregivers, and families. We also thank the multidisciplinary team, healthy volunteers, and ICT students from Mahidol University for their contributions to the application development, as well as the external experts who validated the content. Lastly, we extend our appreciation to the Director, Deputy Director, and staff of the Institute, and the Academic Support Fund, Department of Medical Services, for their support.

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The reference intervals for biochemistry analytes obtained through the indirect method indicate the metabolic shifts in the Thai adult population

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ARTICLE INFO

Article history:

Received 15 January 2025

Accepted as revised 16 March 2025

Available online 27 March 2025

Keywords:

Biochemistry analyte, laboratory database, reference interval, indirect method.

ABSTRACT

Background: Reference intervals (RIs) of biochemistry analytes are required to make medical decisions. According to the standards, medical laboratories shall validate and/or verify RI for their own uses because there are biological and laboratory variations.

Objective: To determine RIs of 14 biochemistry analytes, including fasting blood glucose, total cholesterol, HDL cholesterol, triglyceride, total protein, albumin, BUN, creatinine, uric acid, AST, ALT, ALP, amylase, and lipase in Thai adults.

Materials and methods: RIs were determined by using the indirect method. Test results from 18 years old or older out-patient department patients were collected from the hospital laboratory database from January 2019 to December 2021. The results from subjects with a high likelihood of disease were excluded, outliers were removed, data distribution was tested, and RIs were determined as either mean $\pm$ 2SD or the 2.5th to 97.5th percentile for normal and non-normal distribution data, respectively.

Results: There were 197,897 test results available for RI determination. Most of the determined RIs were comparable to those from the manufacturer, except uric acid. All 14 RIs passed verification criteria in the population served. Interestingly, our RIs for blood total cholesterol, triglyceride, and uric acid were higher than those established in the previous reports in Thai population.

Conclusion: The RIs were established and verified in Thai adults; therefore, they could be implemented for the population. In addition, our RIs of total cholesterol, triglyceride, and uric acid reflect metabolic changes of the studied population.

Introduction

Biochemistry analysis is laboratory tests used to assess metabolic abnormalities and organ pathology. Routine biochemical analytes include fasting blood glucose (FBG), total cholesterol (TC), HDL cholesterol (HDL-c), triglyceride, total protein, albumin, blood urea nitrogen (BUN), creatinine, uric acid, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), amylase, and lipase. Medical laboratories must report the results with reference intervals (RIs). Therefore, they are interpreted and provide information for medical decisions, including diagnosis and treatment plan. RI is the statistical intervals of analyte levels, representing the 95% central of the test result data from reference population,^{1,2} which are determined as either mean $\pm$ 2SD or the 2.5th to 97.5th percentile ($P_{2.5}$ - $P_{97.5}$), depending on the data distribution.

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doi: 10.12982/JAMS.2025.059

E-ISSN: 2539-6056

According to standards and regulations for medical laboratory quality, including the Clinical & Laboratory Standards Institute (CLSI) guideline C28-A3c, medical laboratories shall validate and/or verify RI for their own uses because there are biological and laboratory variations.^{2,3} The biological variations include age, sex, ethnicity or genetic background, diets, lifestyle and environmental conditions,⁴ while the laboratory variations include pre-analytical and analytical system, especially method principles being used.^{3,5} Therefore, RIs may not be transferable if there is incompatibility of the measurement system or the subject population.³ Consequently, determining RIs for the subject population served is crucial for interpretation of test results.

There are two methods for determination of RI, direct and indirect methods.⁵⁻⁸ The former is done by collecting specimens of interest from at least 120 presumably healthy subjects, measuring the analytes of interest, gathering laboratory test results, and establish the RI. To select presumably healthy subjects, the inclusion and exclusion criteria must be cautiously designed. Altogether, the direct method is costly and time-consuming. Alternatively, medical laboratories can conduct the indirect method by using their own database.^{1,5,6} With the present technology, medical laboratories with limited resources can achieve the method. Data collection and analysis can be done by designing selection criteria and analyzing the data for RI determination by using data processing software.

The indirect method is costly and time-consuming; therefore, it is not a practical task in most of medical laboratories in Thailand. Moreover, RIs provided by the manufacturers, which is usually done in foreign population, may not be transferable because of incompatibility in

population, and measurement system.^{3,6} Therefore, this study aimed to determine RIs for analytes in routine blood tests, including FBG, TC, HDL-c, triglyceride, total protein, albumin, BUN, creatinine, uric acid, AST, ALT, ALP, amylase, and lipase, in Thai adults by using the indirect method.

Materials and methods

This study was exempt from review by the Human Research Ethics Thammasat University (project no. 113/2565), and data collection was done under the approval by Saraburi Hospital Human Ethics committee (project no. SRBR65-033).

Biochemical analytes

Fourteen routine biochemistry analytes, FBG, TC, HDL-c, triglyceride, total protein, albumin, BUN, creatinine, uric acid, AST, ALT, ALP, amylase, and lipase, were studied. All test results were done by using the chemical analyzer, model DxC700AU (Beckman Colter, Inc., California, USA), based on method principles shown in Table 1. The calibrators were purchased from Beckman coulter Ireland Inc. (Co. Clare, Ireland). The MAS ChemTRAK Controls were derived from Thermo Fisher Scientific Inc. (California, USA) and used as internal quality control (IQC) materials. Three levels of IQC materials were done twice a day. External quality assurance (EQA) was done monthly, and laboratory performance was reported at either good ($1 < |z| \leq 2$) or excellent ($|z| < 1$) levels from two EQA providers, the External Quality Assessment Schemes in Clinical Laboratory, Faculty of Medical Technology, Mahidol University (EQAS MUMT) and Division of Proficiency Testing Department of Medical Sciences, Ministry of Public Health (DMSc PT).

Table 1. Method principles used for analysis of biochemical substances in blood.

Analytes	Principle of analysis	Method principle
Glucose	Enzymatic UV	Hexokinase / glucose-6-phosphate dehydrogenase
Total cholesterol	Enzymatic colorimetric	Cholesterol oxidase/peroxidase
Triglyceride	Enzymatic colorimetric	Lipase/ glycerol kinase/glycerol phosphate oxidase/peroxidase
HDL cholesterol	Enzymatic colorimetric	Anti human- β -lipoprotein antibody/cholesterol esterase/cholesterol oxidase/peroxidase
Total protein	Chemical colorimetric	Biuret reaction
Albumin	Chemical colorimetric	Bromocresol green
BUN	Enzymatic UV	Urease/glutamate dehydrogenase
Creatinine	Enzymatic colorimetric	Creatininase/creatinase/sarcosine oxidase/ peroxidase
Uric acid	Enzymatic	Uricase/peroxidase
AST	Kinetic UV	IFCC without pyridoxal phosphate activation
ALT	Kinetic UV	IFCC without pyridoxal phosphate activation
ALP	Kinetic colorimetric	DGKC optimized standard method
Amylase	Kinetic colorimetric	2-chloro-4-nitrophenol
Lipase	Kinetic colorimetric	monoglyceride lipase/glycerol kinase/ glycerol phosphate oxidase/ peroxidase.

Data collection and Data selection

Laboratory results and baseline characteristics were collected from the laboratory database of Saraburi Hospital from January 2019 to December 2021. Data selection was done by using the inclusion and exclusion criteria. The inclusion criteria were test results collected from check-ups and out-patient department (OPD) patients who were 18 years old or older, and the results with a high probability of disease were excluded by using the exclusion criteria. The following data were excluded: the FBG data from individuals with abnormal amylase and lipase results; the lipid profile (TC, HDL-c, and triglyceride), AST, ALT, ALP, BUN, total protein, and albumin data from individuals with positive hepatitis B screening test (chemiluminescent immunoassay, the ARCHITECT i2000SR immunoassay analyzer, Abbott, USA) and abnormal liver function tests (total protein, albumin, total and direct bilirubin, AST, ALT, and ALP); the BUN, creatinine, and uric acid data from individuals with abnormal non-protein nitrogenous (NPN) compounds (BUN, creatinine, and uric acid) and UACR; the total protein and albumin data requested from oncology or nephrology clinics; the amylase and lipase data from individual with abnormalities of either amylase or lipase; and the ALP data requested from antenatal care and orthopedics clinics.

Determination of reference intervals and statistical analysis

The selected data were analyzed by Microsoft Excel, Version 2408 Build 16.0.17928.20114. Determination of reference intervals was done as previously described.^{5,9} First, the outliers were removed by using Turkey method.⁸ The outliers were identified by interquartile range (IQR), quartile (Q)1, and Q3. The lower limit and the upper limit were calculated as $Q1 - 1.5IQR$ and $Q3 + 1.5IQR$, respectively. The data value outside the limit were specified as outliers and removed from the dataset. Next, data distribution was assessed by using skewness, kurtosis, and normal distribution was tested by the Jarque-Bera

(JB) and chi square tests. After that, RIs were determined based on their distribution. If the dataset is normally distributed, mean and SD were used for RI determination as $\text{mean} \pm 2SD$. However, if the dataset is not normally distributed, data transformation was performed, based on Tukeys' ladder of power.¹⁰ The datasets with positive skewness were transformed by using log10 or square root, and the negative skewness datasets were transformed by using square or cube. After data transformation, RIs were determined, according to data distribution, $\text{mean} \pm 2SD$ for normal distribution data and $P_{2.5} - P_{97.5}$ for non-normal distribution. In addition, difference between male and female data was tested by the student's *t* test, and if there is significant difference between sex ($p < 0.05$), RIs for males and females were done separately.

Data verification

The determined RIs were verified as previously described.^{2,11} Briefly, twenty laboratory results were collected from assumed normal individuals and compared with the corresponding RIs. The percentage of the result value in the interval was calculated. If ninety percents of the laboratory results ($N=18$) are in the interval, the RI is acceptable for clinical use.

Results

Baseline characteristics of biochemical analytes

RIs were determined for 14 routine blood tests, including FBG, TC, HDL-c, triglyceride, total protein, albumin, BUN, creatinine, uric acid, AST, ALT, ALP, amylase, and lipase, by the indirect method. There were 205,485 test results collected by using the inclusion and exclusion criteria. Amounts of males and females in the datasets were shown in Table 2. The data with a high likelihood of disease were further removed as outliers, resulting in 197,897 data for determination of RIs. The average age in the datasets were between 47 and 64 years old, and the average age for males and females for each dataset was comparable (Table 2).

Table 2. Characteristics of the datasets used for determination of reference intervals.

Tests	Total		Males		Females	
	N	Age*	N (%)	Age*	N (%)	Age*
FBG	4,112	47±9	1,108 (26.9)	47±10	3,004 (73.1)	47±8
Total cholesterol	4,470	47±9	1,207 (27.0)	47 ± 11	3,263 (73.0)	47±9
Triglyceride	3,817	48±9	881 (23.1)	48 ± 11	2,936 (76.9)	47±9
HDL cholesterol	3,365	47±9	715 (21.2)	44 ± 11	2,650 (78.8)	47±9
Total protein	32,716	61±19	17,108 (52.3)	59 ± 19	15,608 (47.7)	63±18
Albumin	44,680	62±17	22,930 (51.3)	60 ± 17	21,750 (48.7)	64±18
BUN	2,402	51±14	961 (40.0)	52±14	1,441 (60.00)	51±13
Creatinine	5,115	48±12	1,398 (27.3)	49 ± 13	3,717 (72.7)	48±11
Uric acid	1,741	49±10	724 (41.6)	49 ± 10	1,071 (58.4)	48±9
AST	4,604	49±12	1,184 (25.7)	51 ± 12	3,420 (74.3)	49±11
ALT	4,499	49±12	1,197 (26.6)	52 ± 13	3,302 (73.4)	49±11
ALP	74,889	58±17	34,701 (46.3)	57 ± 17	40,188 (53.7)	58±17
Amylase	5,949	50±18	3,476 (58.4)	49 ± 18	2,473 (41.6)	52±19
Lipase	5,538	50±18	3,182 (57.5)	49± 18	2,356 (42.5)	52±19

Note: *data are presented as mean±SD, FBG: fasting blood glucose.

RIIs of biochemical analytes derived by the indirect method

There were six datasets becoming normal distributions after outlier removal, including uric acid (males and females), AST (males and females) and ALT (male and females) datasets, and RIIs could be then determined as mean±2SD; 4.1-9.9, 3.1-7.4 mg/dL, 14-39, 12-31, 4-47, and 4-29 IU/L, respectively (Table 3). For other test results with non-normal distributions, the datasets were transformed to adjust the values to become normal distributions. After data transformation, two

datasets become normally distributed, FBG and BUN, and the RIIs were established as mean±2SD: 75-111 and 7.1-19.7 mg/dL, respectively (Table 3). For the rest of the datasets, TC, HDL-c, triglyceride, total protein, albumin, creatinine (males and females), ALP, amylase, and lipase, the distributions were still non-normal after data transformation and their RIIs were determined as $P_{2.5} - P_{97.5}$; 135-287, 41-216, 34-87 mg/dL, 5.3-8.6, 2.2-4.6 g/dL, 0.65-1.25, 0.50-0.90 mg/dL, 38-127, 35-121, 22-128, and 5-59 IU/L, respectively (Table 3).

Table 3. Comparison of RIs derived from the present study, the manufacturer, and previous studies in Thai population.

Analytes [#]	The present study	Manufacturer	Viriyayudhakorn ¹³	Chamnanpai ¹⁴	Yotsakullert ¹⁵	Chatchawal ¹⁶
Method	Indirect	Direct	Direct	Direct	Direct	Indirect
FBG	75-111	74-106	69-100	74-106	NA	71-103
Total cholesterol	135-287	<200*	120-246	150-220	NA	145-299
Triglyceride	41-216	<150*	NA	26-143	NA	37-212
HDL cholesterol	34-87	>40*	NA	41-91	41-85	32-77
Total protein	5.3-8.6	6.0-8.0	6.7-8.3	NA	NA	NA
Albumin	2.2-4.6	3.5-5.0	NA	NA	NA	NA
BUN	7.1-19.7	6-20	NA	6-19	NA	8-19
Creatinine						
Males	0.65-1.25	0.70-1.20	0.8-1.4	0.5-1.1	0.64-1.29	0.5-1.3
Females	0.50-0.90	0.60-1.00	0.6-1.1		0.51-0.91	
Uric acid						
Males	4.1-9.9	3.5-7.2	2.5-7.7	2.5-7.0	NA	NA
Females	3.1-7.4	2.6-6.0	2.0-5.6			
AST						
Males	14-39	0-50	NA	13-30	16-35	16-35
Females	12-31	0-35	NA		15-33	
ALT						
Males	4-47	0-50	NA	10-38	12-32	9-36
Females	4-29	0-35	NA		12-31	
ALP						
Males	38-127	30-120	NA	28-93	40-113	30-86
Females	35-121	30-120				
Amylase	22-128	25-160	NA	NA	NA	NA
Lipase	5-59	0-60	NA	NA	NA	NA

Note: FBG: fasting blood glucose, [#]concentration units: mg/dL for FBG, total cholesterol, triglyceride, HDL cholesterol, BUN, creatinine, and uric acid, mg/dL for total protein and albumin (g/dL), IU/L for AST, ALT, ALP, amylase, and lipase, NA: not available, *based on NCEP-ATPIII.¹²

Data verification of RIs

After RIs have been established, they were verified by using laboratory results analyzed in the laboratory. It was shown that over 90 percent of the results were in the

RI of FBG, TC, HDL-c, triglyceride, total protein, albumin, BUN, creatinine, uric acid, AST, ALT, ALP, amylase, and lipase (Table 4).

Table 4. Verification results of reference intervals (RI) determined by using the indirect method.

Analytes	Results within RI* (%)	Analytes	Results within RI* (%)
Glucose	95	Albumin	90
TC	95	AST (males)	90
Triglyceride	100	AST (females)	90
HDL-c	100	ALT (males)	90
BUN	100	ALT (females)	90
Creatinine (males)	100	Amylase	95
Creatinine (females)	100	Lipase	95
Uric acid (males)	100	ALP (males)	95
Uric acid (females)	100	ALP (females)	100
Total protein	90		

Note: *percent of test results from 20 assumed normal individuals lining within the corresponding RI.

Discussion

This study established and verified RIs for 14 routine tests, FBG, TC, HDL-c, triglyceride, total protein, albumin, BUN, creatinine, uric acid, AST, ALT, ALP, amylase, and lipase, by using the indirect method (Table 3). The protocol is not costly and less complicated than the direct method, but it requires skills for data analysis and data processing software. In this study, Microsoft Excel was chosen as a data processing software for data analysis because of its availability in medical laboratories in Thailand. In method prospective, the RI determination process could be divided into three patterns. The first pattern was done for uric acid, AST, and ALT. Their RIs were defined as mean $\pm$ 2SD by using the datasets which were normally distributed after outlier removal. The second pattern was FBG and BUN. The datasets were normally distributed after outlier removal and data transformation; therefore, their RIs were defined as mean $\pm$ 2SD. The third pattern was done for TC, HDL-c, triglyceride, creatinine, total protein, albumin, ALP, amylase, and lipase. Their RIs were determined as $P_{2.5} - P_{97.5}$ since the datasets were not normally distributed after outlier removal and data transformation.

When compared to RIs provided by the manufacturer (using NCEP-ATPIII as RIs of TC, HDL-c and triglyceride),¹² the lower reference limits (LRLs) and the upper reference limits (URLs) of FBG, TC, HDL-c, triglyceride, BUN, creatinine (males), AST (females), ALT (males and females), ALP (males and females), amylase, and lipase determined in the present study were comparable. However, both LRL and URL of RI of uric acid for male and female were higher than those from the manufacturer with % difference of 17.1% (4.1 vs 3.5 mg/dL), 19.2% (3.1 vs 2.6 mg/dL), 37.5% (9.9 vs 7.2 mg/dL), and 23.3% (7.4 vs 6.0 mg/dL), respectively. Therefore, RI of uric acid from manufacturers might not be transferable. The explanations include variations of the measurement system and the subject population.³ It is possible that Thai population have different genetic background, diets, lifestyle and environmental conditions, affecting non-diseased levels of the analytes.⁴

When we compared our RIs with previous studies in Thai population, it was found that the RIs for FBG, HDL-c, BUN, creatinine, AST, ALT, and ALP were comparable.^{1,13-16} However, our RIs of TC, triglyceride and uric acid were increased when compared with previous studies published a few decades ago by Viriyayudhakorn, *et al.*,¹² and Chamnanpai, *et al.*¹⁴ Conversely, our RIs for TC and triglyceride were comparable to more recent study by Chatchawal, *et al.*¹⁶ Unfortunately, these two recent studies did not report RI of uric acid. Nonetheless, the increased trend of TC, triglyceride and uric acid RIs may reflect changes in diets and lifestyle, affecting metabolism and blood levels.¹⁷⁻¹⁹ It has been shown that animal-based diet, red and processed meat is associated with hyperlipidemia and plant-based diet is conversely associated with hyperuricemia.^{20,21} Nowadays, there are alterations in health behaviors among Thai people, including imbalance diets and less exercise. It is possible that foods rich in purine, such as bamboo shoot, vegetables with high purine

content, and organ meat, alcohol, fructose, and fructose-sweetened beverages are the reason for change in blood uric acid.²² Moreover, hyperlipidemia is a part of metabolic syndrome, and hyperuricemia is now considered as a risk factor of metabolic syndrome.²³⁻²⁶ Altogether, elevations of TC, triglyceride, and uric acid reflect metabolic change, leading to metabolic syndrome and other non-communicable diseases, including gout, diabetes, and cardiovascular diseases. Therefore, our study revealed not only the RIs of the routine tests, but also increasing trends in blood lipids and uric acid among Thai people.

Limitations

The number of the data in the study was large enough for RI determination by the indirect method with the minimum of 724 for uric acid (males) and the maximum of 40,188 for ALP (males).²⁷ However, it is possible that the data were not represent Thai population because it is derived from a single database. Moreover, the datasets in the present study may contain sub-clinical diseased subjects, affecting validity of RIs, which is a limitation of RI determination by using the indirect method. Moreover, medical information in the database was limited; therefore, eliminations of test results from diseased subjects may not be completed, subsequently interfering with the LRL and URL of RIs. Therefore, further research in multicenter setting and complete set of medical information will make RIs transferable to other laboratories.

Conclusion

In conclusion, the present study established and verified RIs of 14 routine tests, FBG, TC, HDL-c, triglyceride, total protein, albumin, BUN, creatinine, uric acid, AST, ALT, ALP, amylase, and lipase through the indirect method. They can be implemented in medical laboratories with verification. In addition, our results indicated increasing trends in blood lipids (TC, triglyceride) and uric acid among Thai adult population. This observation indicates metabolic change among Thai adult population and should be further investigated.

Conflict of interest

The authors state no conflict of interest.

Ethical approval

This study was exempt from review by the Human Research Ethics Thammasat University (project no. 113/2565), and data collection was done under the approval by Saraburi Hospital Human Ethics Committee (project no. SRBR65-033) during January 2019 to December 2021.

Acknowledgements

We would like to express our appreciation to Associate Professor Narisa Kengtrong Bordeerat and Assistant Professor Somsak Fongsupa for their valuable suggestions in the research.

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A survey of foodborne bacterial contamination in Chiang Mai Province, Thailand: A pilot study focused on *Aeromonas* and *Vibrio*

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ARTICLE INFO

Article history:

Received 7 February 2025

Accepted as revised 16 March 2025

Available online 27 March 2025

Keywords:

Aeromonas, *Vibrio*, seafood, salmon sushi.

ABSTRACT

Background: The consumption of raw or undercooked food contaminated with microbial pathogens is a major cause of foodborne illness. *Vibrio* and *Aeromonas* are significant pathogenic bacteria found in raw seafood. Currently, the consumption of raw and undercooked seafood, including salmon, is becoming popular in Thailand. Nevertheless, studies focusing on the prevalence of human pathogenic *Vibrio* and *Aeromonas* in seafood and salmon in Thailand are scarce.

Objective: This study aimed to detect *Vibrio* and *Aeromonas* in undercooked foods from Chiang Mai Province, Thailand.

Materials and methods: Ten food samples (five spicy mixed seafood salads and five salmon sushi) individually collected in Chiang Mai, Thailand, from August to October 2024. Samples were processed as previously described and bacteria were identified by 16S rRNA gene amplification by PCR and Sanger sequencing.

Results: The results indicated that three of ten (30%) were contaminated with human pathogenic *Vibrio*, *V. parahaemolyticus*, *V. cholerae*, and *V. vulnificus*. Moreover, *Aeromonas* species regarded as potential human pathogens, *A. caviae*, *A. dhakensis*, *A. veronii*, and *A. hydrophila*, were isolated from eight food samples (80%).

Conclusion: Our findings suggest that undercooked seafood in Chiang Mai, Northern Thailand, frequently contains pathogenic bacteria, posing significant food safety risks.

Introduction

Foodborne diseases are major public health concern worldwide. The primary cause of human illness is the consumption of raw or undercooked food contaminated with microbial pathogens. The World Health Organization (WHO) reported that approximately 600 million people suffer from foodborne illnesses, and 420,000 die each year.¹

Vibrio and *Aeromonas* are gram-negative bacteria commonly found in aquatic environments, including estuaries, marine coastal water, sediments, and aquaculture settings. *Vibrio* belongs to the *Vibrionaceae* family, with 147 individual species currently identified.² Approximately 10 species, such as *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, cause human diseases. In addition, *V. parahaemolyticus* has been reported as the leading cause of foodborne infections in Asia, and is almost always associated with the consumption of raw

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doi: 10.12982/JAMS.2025.060

E-ISSN: 2539-6056

fish and shellfish.³ *Aeromonas* consists of 36 species, is currently being identified, 19 of which are potentially human pathogens including *A. caviae*, *A. dhakensis*, *A. veronii*, and *A. hydrophila*.⁴

Chiang Mai Province, a landlocked region in Northern Thailand, is located over 900 km from the coast. Salmon is imported from various salmon-producing countries. Both seafood and salmon are at risk of microbial contamination during transportation. Thus, evaluating the microbial safety of seafood and salmon in the supply chain is essential. Over the past decade, consumption of raw and undercooked seafood, including salmon, has become increasingly popular in Thailand. However, studies focusing on the prevalence of *Vibrio* and *Aeromonas* in seafood and salmon in Thailand are scarce.

The present study aimed to isolate human pathogenic *Vibrio* and *Aeromonas* from two undercooked foods; spicy mixed seafood salad and salmon sushi. Undercooked seafoods carrying potential pathogens are a concern for food safety.

Material and methods

Sample collection

A total of 10 food samples were collected from individual restaurants in urban Chiang Mai, Thailand, between August and October 2024. These included five spicy mixed seafood salads (*Litopenaeus*

vannamei, *Anadara granosa*, *Crasostrea gigas*, and *Loligo duvauceli*) and five salmon sushi.

Laboratory processing of food and bacterial isolation

Sample processing followed previous established protocols.⁵ Briefly, 25 gm of material was cut with sterile scissors, transferred into a sterile blending bag containing 25 mL of alkaline peptone water (APW), and blended using a stomacher (AES MIX2 Lab blender, France) for 2 min. The solution was then aseptically transferred to a 500-ml bottle containing 200 ml of APW and incubated at 35 ± 2 °C for 6 hrs. After incubation, 100 µl of each solution collected from the surface pellicle, was streaked on thiosulfate citrate bile salt sucrose (TCBS) agar (Himedia, USA) for *Vibrio* and blood agar supplemented with ampicillin (BASA) for *Aeromonas*.⁶ Plates were incubated under suitable conditions at 35 ± 2 °C for 24 hrs. The example of bacterial colonies that grow on TCBS and BASA plate were shown in figure 1. Colonies on TCBS plates and BASA were selected and, subsequently cultured in biochemical test media, triple sugar iron (TSI) agar slant, and motile-indole-lysine (MIL), and further incubated at 35 ± 2 °C for 24 hrs. Suspected *Vibrio* and *Aeromonas* colonies on the TSI slant were suspended in 500 µl sterile distilled water, heated at 100 °C for 10 min, and centrifuged at 14,000 rpm for 10 min. The supernatant containing genomic DNA was stored at -20 °C until PCR amplification.

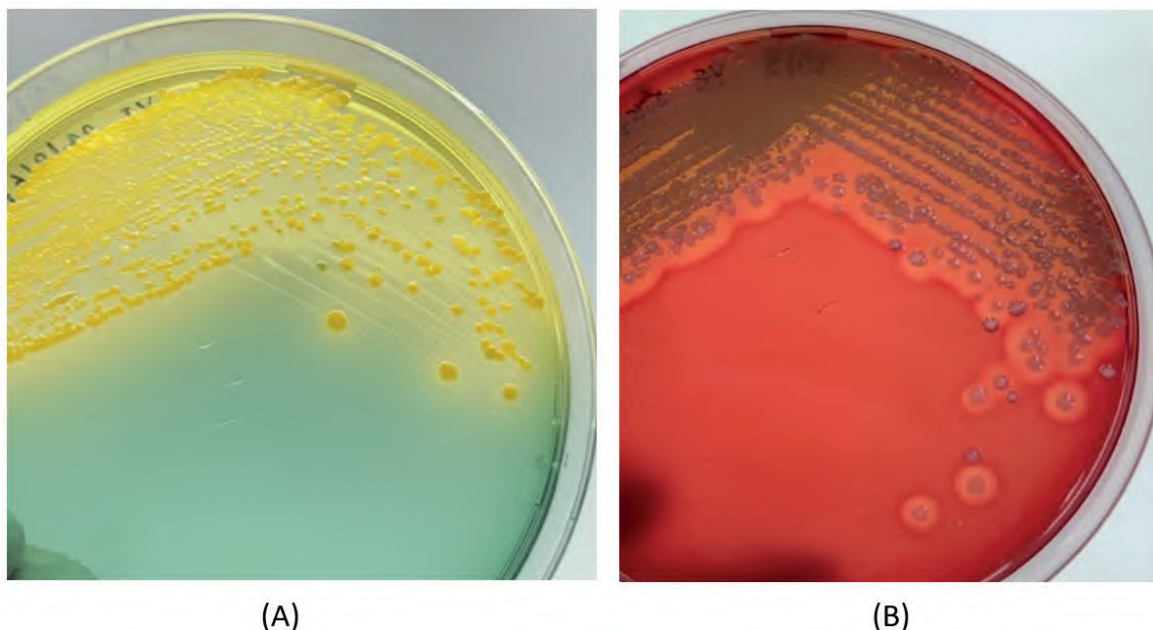


Figure 1. The example of bacterial colonies that grow. A: on TCBS, B: BASA plate.

PCR amplification and Sanger sequencing

The combination of 16S rRNA gene amplification by conventional PCR and Sanger sequencing were used for bacterial identification in this study. The 27F (5'-AGA GTT TGA TCM TGG CTC AG -3') and 800R (5'-GAC TAC CAG GGT ATC TAA TCC-3') specific for the V1-V4 region of the 16S rRNA gene were used as universal primers for amplification.⁷ The 25-µL PCR reaction contained 2× Quick Taq HS dye master mix (Toyobo, Japan), 1 pmol of

27F and 800R primers and 2 µl (approximately 100 ng) of DNA template. PCR was performed in a thermal cycler (Labcycler gradient SensoQuest, Germany) and the amplification profiles were as follows: pre-denaturation at 94 °C for 3 min, 30 cycles of 94 °C for 30 sec, and 68 °C for 60 sec, with a final extension at 68 °C for 5 min. The expected PCR product was approximately 800 bp. PCR products were electrophoresed on a 1.5% agarose gel stained with RedSafe nucleic acid staining solution

(iNtRON Biotechnology, Inc., Korea) and visualized using a UV transilluminator (G:BOXchemi XRQ gel doc system, UK). The PCR product was purified using the QIAquick PCR Purification Kit (Qiagen, Germany). Nucleotide sequences were obtained using Sanger sequencing that provided by the company (BigDye Terminator v3.1 Cycle sequencing kit, Bionics, Korea).

Identification of bacteria

Bacterial identification in this study was performed based on sequence comparison with public database. Each sequencing result was aligned with public sequences in the GenBank database using the BLASTN program (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The parameters

were automatically provided by the program. The highest percentage identity was selected for bacterial identification. Moreover, the evolutionary relationship among microorganisms found in this study and retrieved from the database was demonstrated using the phylogenetic tree public software (iTOL interactive, <http://itol.embl.de/index.shtml>).

Results

Ten food samples were included and 63 colonies suspected *Vibrio* and *Aeromonas* were selected for this study. After PCR and agarose gel electrophoresis, the results indicated that the amplified products of approximately 800-bp in size were obtained (Figure 2).

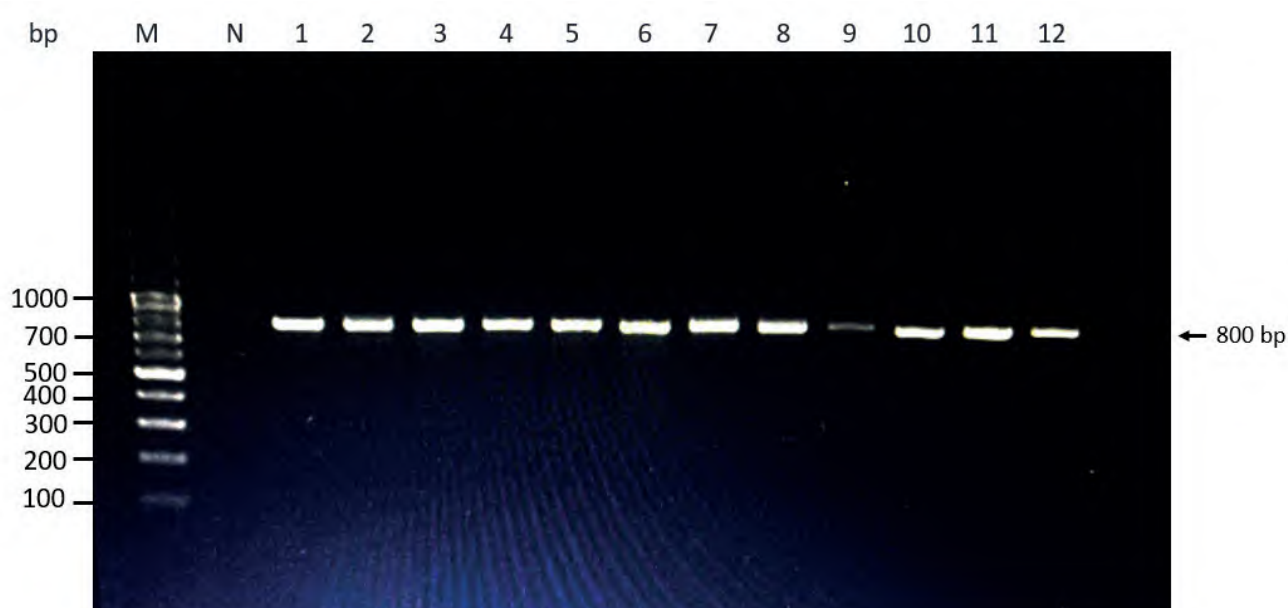


Figure 2. The agarose gel electrophoresis of 16S rRNA gene amplification by PCR. Bacterial genomic DNA collected by the heat lysis method was used a target for 16S rRNA gene amplification by PCR and analyzed by 1.5% agarose gel electrophoresis. Approximately 800-bp amplified products were purified and sequenced using Sanger sequencing. Lane M: standard 100 bp DNA marker, lane N: negative control, lane 1-12: sample No. 34-45, respectively.

After PCR product purification and sequencing, each bacterial species was identified using the BLASTN public program with the highest percentage identity (99-100%). The results showed that *V. parahaemolyticus*, *V. cholerae*, and *V. vulnificus*, were found in three out of ten (30%) seafood samples. A mixture of *V. parahaemolyticus* and *V. vulnificus* was found in one sample. Additionally, *A. caviae*, *A. dhakensis*, *A. veronii*, and *A. hydrophila*, were isolated from eight food samples (80%). A mixture of *A. hydrophila* and *A. caviae* was found in a single sushi sample. A summary of the human pathogenic *Vibrio* and *Aeromonas* strains isolated from food is shown in Table 1. The phylogenetic analysis representing the evolutionary relationship of

the 16S rRNA gene among the six- sequencing results derived from bacterial colonies in this study compared to the 23 sequences of *Aeromonas* spp. and *Vibrio* spp. retrieved from the GenBank database is illustrated in Figure 3. The 16S rRNA partial sequences of 12 isolates of *A. caviae* (isolate No. 1), *A. salmonicida* (isolate No. 63), *A. veronii* (isolate No. 49), *E. cloacae* (isolate No. 24), *E. hormaechei* (isolate No. 31), *K. pneumoniae* (isolate No. 23), *P. mirabilis* (isolate No. 6), *P. rettgeri* (isolate No. 20), *V. harveyi* (isolate No. 10), *V. mediterranei* (isolate No. 56), *V. parahaemolyticus* (isolate No. 46) and *V. vulnificus* (isolate No. 43) were submitted to GenBank under accession No. PQ855910-PQ855921, respectively.

Table 1. Summary of human pathogenic *Vibrio* and *Aeromonas* isolated from food in this study.

Microorganisms	Mixed seafood salad (N=5)	Salmon sushi (N=5)	Total (N=10)
<i>Vibrio</i> spp.	3*	0	3
<i>V. parahaemolyticus</i>	2		
<i>V. cholerae</i>	1		
<i>V. vulnificus</i>	1		
<i>Aeromonas</i> spp.	4	4 [#]	8
<i>A. caviae</i>	1	2	
<i>A. hydrophila</i>	2	1	
<i>A. veronii</i>	1	1	
<i>A. dhakensis</i>		1	

Note: *Both of *V. parahaemolyticus* and *V. vulnificus* were isolated in one sample, [#]Both of *A. hydrophila* and *A. caviae* were isolated in one sample.

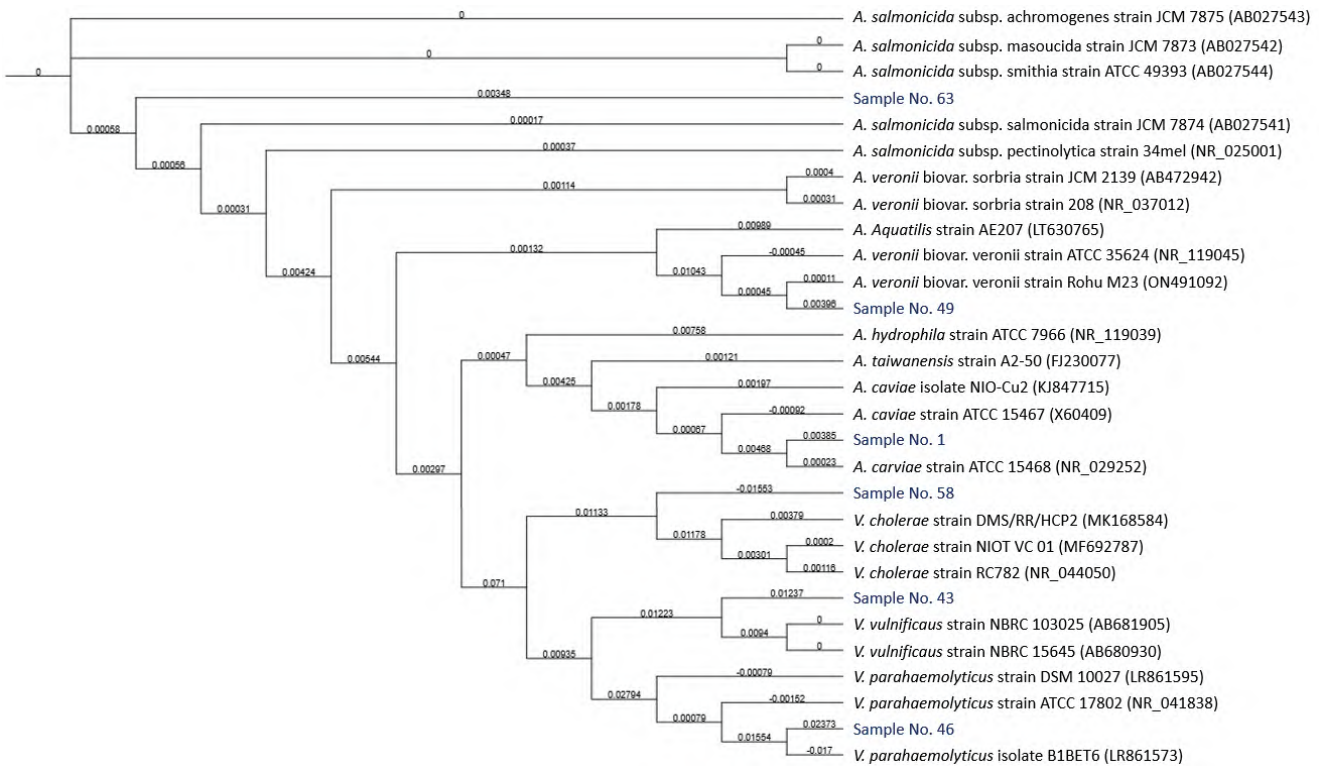


Figure 3. Phylogenetic analysis representing the relationship of the 16S rRNA gene among 6 sequencing results. The 16S rRNA gene of each bacterium was sequenced and identified using the BLASTN program with the highest percentage identity (99-100%). A phylogenetic tree was constructed using iTOL public software (<http://itol.embl.de/itol.cgi>). Samples 1, 43, 46, 49, 58, and 63 were identified as *A. caviae* (Accession No. PQ855910), *V. vulnificus* (Accession No. PQ855921), *V. parahaemolyticus* (accession no. PQ855920), *A. veronii* (accession no. PQ855912), *V. cholerae* (accession no. MW165382), and *A. salmonicida* (accession no. PQ855911), respectively. The accession numbers of the sequences retrieved from the GenBank database are shown in the blankets.

Discussions

Foodborne illnesses remain a persistent public health challenge, often linked to the consumption of raw or undercooked food contaminated with pathogenic bacteria. In seafood, *V. parahaemolyticus*, *V. cholerae*, and *V. vulnificus* are the three most common pathogens associated with human infection. *Vibrio parahaemolyticus* is recognized as a common cause of acute gastroenteritis worldwide,⁸ and accounted for approximately 20-30% of bacterial foodborne cases.⁹ *Vibrio cholerae* is a pathogen that causes cholera. The disease remains a serious problem in the developing world, including in Asian and African countries. *Vibrio vulnificus* is an opportunistic human pathogen that causes severe wound infections, gastroenteritis, and septicemia. A part of *Vibrio*, the *Aeromonas* is another causative agent of variety of human diseases such as gastroenteritis, hepatobiliary infection, wound infection and septicemia.¹⁰ In particular, gastrointestinal infection is the most prevalent form, ranging in severity from mild self-limiting watery diarrhea to severe chronic infection, and antibiotics for treatment are required.¹¹ Lobatón et al. revealed that up to 40% of *Aeromonas*-induced gastrointestinal infections require antibiotics for treatment.¹² At least six mesophilic species have been established as human pathogens, including *A. hydrophila*, *A. caviae*, *A. veronii* biotype veronii, *A. veronii* biotype sobria, *A. schubertii*, and *A. jandaei*.¹³ Recently, *A. dhakensis* has been recognized as an important human pathogen. Although the role of gastroenteritis remains controversial, *A. dhakensis* bacteremia is more lethal than bacteremia caused by other *Aeromonas* species.¹⁴

Of the food samples, three *Vibrio* spp., *V. parahaemolyticus*, *V. cholerae*, and *V. vulnificus*, which are strongly associated with human diseases were detected in three of ten seafood samples (Table 1). *Vibrio parahaemolyticus* and *V. cholerae* were identified in two (20%) and one (10%) food samples, respectively. Similarly, the prevalence of *V. parahaemolyticus* in raw seafood isolated from local wet markets and supermarkets in Phayao Province, Northern Thailand, was studied, and approximately 22% were positive.¹⁵ In addition, the prevalence of *V. cholerae* in seafood collected from retail markets in Yala Province, Southern Thailand, has been reported to be 34%.¹⁶ *Vibrio mimicus* was detected in one seafood sample in this study. *Vibrio mimicus* has previously been isolated from ornamental fish in various countries, including Singapore, China, and Thailand.¹⁷ Although rare, this microorganism has been reported to produce cholera-like toxin and can infect humans, leading to sporadic cases of diarrhea and gastroenteritis.¹⁸

Aeromonas caviae, *A. dhakensis*, *A. veronii*, *A. hydrophila*, and *Aeromonas* spp. associated with human pathogens, were isolated from eight food samples (80%) in this study. The detection of human pathogenic *Aeromonas* spp. in edible raw salmon has never been reported in Thailand. Uncooked seafood samples from Bangkok were previously examined and approximately 27% consisting of *A. caviae*, *A. veronii* biovar sobria, and *A. hydrophila* had detectable.³ Although pathogenic *Vibrio* spp. was

not detected in salmon sushi in this study, up to 12% of salmon meat collected from supermarkets and restaurants in China was positive for *V. parahaemolyticus*.¹⁹

Other *Vibrio* spp. was detected in the present study, including *V. harveyi*, *V. hyugaensis*, and *V. mediterranei*. These are generally caused by vibriosis in marine animals such as bivalve mollusks and sea cucumber. *Aeromonas salmonicida* was observed in one sample of salmon sushi. Currently, there are five subspecies, namely *salmonicida*, *smithia*, *achromogenes*, *masoucida*, and *pectinolytica*. Phylogenetic tree analysis indicated that *A. salmonicida* isolated here (No. 63) was closely related to these five subspecies (Figure 3). The bacterium is classified as a psychrophilic bacterium and has been recognized as the causative agent of furunculosis and septicemia in the salmonid family.²⁰ Due to global warming, the abundance of bacteria in coastal waters, including *A. salmonicida*, has adapted to grow at elevated temperatures. The number of clinical cases infected with mesophilic *A. salmonicida* infection has significantly increased in recent years. Mesophilic *A. salmonicida* isolated from human blood, skin, and eyes has been reported.²¹⁻²³ Although the pathogenesis is currently unclear, human diseases, including acute gastroenteritis and foot cellulitis, caused by *A. salmonicida* have been ascribed.²⁴ According to climate change, infection with mesophilic *A. salmonicida* and uncommon *Aeromonas* and *Vibrio* should be raised in health awareness in the future.

Previous studies have reported challenges in accurately identifying *Aeromonas* species using conventional biochemical methods. Genetic analysis using DNA-DNA hybridization is highly accurate and is considered the gold standard method for *Aeromonas* identification. However, they are rarely used in clinical laboratories because of the high cost and long time required for analysis.²⁵ Identification of *Aeromonas* based on 16S rRNA gene sequences and some other housekeeping genes such as *gyrB*, *dnaJ* and *rpoB* have successfully been reported.²⁶ To ensure reliable bacterial identification, combined bacterial culture and identification using 16S rRNA gene amplification and sequencing was conducted for both *Vibrio* and *Aeromonas* species identification in this study.

Finally, our data implied that undercooked seafoods from spicy mixed seafood salads and salmon sushi in Chiang Mai province, Northern Thailand, frequently harbored pathogenic bacteria caused by foodborne illness. Therefore, food safety is a concern for customers.

Conclusion

Foodborne diseases are a major public health concern worldwide. In this study, the human pathogens *Vibrio* and *Aeromonas* were often detected in spicy mixed seafood salad and salmon sushi. These findings highlight the potential food safety risks associated with seafood and salmon transportation to Chiang Mai Province, Thailand.

Limitations

This study has several limitations that should be acknowledged. First, the small sample size of only 10

food samples (five spicy mixed seafood salads and five salmon sushi) limits the generalizability of the findings. A larger sample size, collected across different seasons and locations, would provide a more comprehensive understanding of bacterial contamination patterns. Second, the study was geographically restricted to Chiang Mai, a landlocked province in Thailand. The microbial contamination levels in this region may differ from those in coastal or seafood-producing areas, highlighting the need for future studies in diverse geographic settings. Third, while bacterial species were identified using 16S rRNA sequencing, the study did not assess antibiotic susceptibility patterns, which are crucial for understanding potential antimicrobial resistance (AMR) risks. Conducting antibiotic susceptibility testing in future studies would help determine whether these foodborne pathogens pose a public health threat due to drug resistance. Additionally, the study did not differentiate whether contamination originated from the seafood itself, improper food handling, or storage conditions. Future research incorporating environmental sampling, microbial source tracking, and supply chain analysis would help identify the exact sources of contamination. Another limitation is that virulence gene and toxin detection were not performed, meaning the study could not determine whether the isolated *Vibrio* and *Aeromonas* strains could cause severe disease. Finally, while PCR-based identification is a widely used method, it has limitations in distinguishing closely related bacterial species. Complementary approaches, such as whole-genome sequencing (WGS) or multilocus sequence typing (MLST), would provide higher-resolution bacterial characterization in future research. To address these limitations, future research should focus on expanding sample sizes across multiple geographic regions, incorporating seasonal variations to better assess contamination trends. Additionally, studies should include antibiotic susceptibility testing to evaluate potential antimicrobial resistance among foodborne pathogens. Investigating the sources of contamination through environmental monitoring, supply chain assessment, and microbial source tracking will also be crucial in developing targeted food safety interventions. Furthermore, the application of advanced molecular techniques, such as WGS and MLST, will enhance the accuracy of bacterial identification and provide insights into the genetic diversity and pathogenic potential of these microorganisms. These efforts will contribute to a more comprehensive understanding of foodborne bacterial risks and support the development of improved food safety policies in Thailand and beyond. Despite these limitations, the study provides valuable preliminary data on bacterial contamination in undercooked seafood in Northern Thailand, emphasizing the importance of food safety measures.

Acknowledgements

The authors would like to thank the Division of Clinical Microbiology, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, for kindly providing chemicals and culture media.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This study did not involve in human subjects and laboratory animals.

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Supplement data

Table S1. 16S rRNA sequence alignment of amplified products of bacterial colonies isolated from spicy mixed seafood salad.

Restaurant	Colony No.	BLASTN results (bacterial identification)	% gene identity	Accession No.
A	BASA 1	<i>Aeromonas caviae</i> strain DSILV26 (16S ribosomal RNA gene, partial sequence)	100	MN581908
	BASA 2	<i>Aeromonas</i> sp. strain JF8 (16S ribosomal RNA gene, partial sequence)	100	MN865804
	BASA 3	<i>Enterobacter cloacae</i> strain Uyi_28 (16S ribosomal RNA gene, partial sequence)	100	MT507221
	TCBS Y 4	<i>Enterobacter</i> sp. strain XQ53 (16S ribosomal RNA gene, partial sequence)	99	OP326122
	TCBS G 5	<i>Enterobacter</i> sp. strain P2 (16S ribosomal RNA gene, partial sequence)	99	MN400353
	TCBS G 6	<i>Proteus mirabilis</i> strain S74-1 (++)-2 (chromosome, complete genome)	100	CP053614
B	BASA 10	<i>Vibrio harveyi</i> strain B14-1 (16S ribosomal RNA gene, partial sequence)	100	MK102612
	BASA 12	<i>Enterobacter</i> sp. strain P2 (16S ribosomal RNA gene, partial sequence)	99	MN400353
	BASA 13	<i>Vibrio</i> sp. strain LQ2 (16S ribosomal RNA gene, partial sequence)	100	MN420866
	BASA 14	<i>Pantoea agglomerans</i> strain 600 (16S ribosomal RNA gene, partial sequence)	100	MT585395
	TCBS Y 15	<i>Vibrio hyugaensis</i> strain DS1807-13 (16S ribosomal RNA gene, partial sequence)	99	MT269595
	TCBS G 16	<i>Vibrio parahaemolyticus</i> strain TY-47 (16S ribosomal RNA gene, partial sequence)	100	MT505701
C	BASA 23	<i>Klebsiella pneumoniae</i> strain AP-1 (16S ribosomal RNA gene, partial sequence)	99	HQ199598
	BASA 24	<i>Enterobacter cloacae</i> strain YT58 (16S ribosomal RNA gene, partial sequence)	100	MN416258
	BASA 25	<i>Aeromonas</i> sp. strain M7 (16S ribosomal RNA gene, partial sequence)	100	OM617799
	BASA 28	<i>Aeromonas veronii</i> strain NK04 (16S ribosomal RNA gene, partial sequence)	100	KP899501
	TCBS Y 31	<i>Enterobacter hormaechei</i> subsp. <i>xiangfangensis</i> strain M1E (16S ribosomal RNA gene)	100	OP492057
	TCBS Y 32	<i>Pseudocitrobacter</i> sp. strain Cco1-MG-4 (16S ribosomal RNA gene, partial sequence)	100	OP393786
	TCBS Y 33	<i>Enterobacter cancerogenus</i> strain KNUC5011 (16S ribosomal RNA gene, partial sequence)	99	JQ682639
D	BASA 42	<i>Aeromonas hydrophila</i> strain CH-GX-NN-SC-3-2021 (16S ribosomal RNA gene, partial sequence)	99	ON203021
	BASA 43	<i>Vibrio vulnificus</i> strain TSG003 (16S ribosomal RNA gene, partial sequence)	100	KC748469
	BASA 46	<i>Vibrio parahaemolyticus</i> strain H3 (16S ribosomal RNA gene, partial sequence)	100	PP389745
	TCBS G 48	<i>Vibrio parahaemolyticus</i> strain H3 (16S ribosomal RNA gene, partial sequence)	100	PP389745
E	BASA 55	<i>Aeromonas hydrophila</i> strain A9 (16S ribosomal RNA gene, partial sequence)	100	OP109737
	TCBS Y 58	<i>Vibrio cholerae</i> strain LTW-F1 (16S ribosomal RNA gene, partial sequence)	100	MW165382
	TCBS Y56	<i>Vibrio mediterranei</i> strain PD104 (16S ribosomal RNA gene, partial sequence)	100	PP967835
	TCBS G 60	<i>Vibrio mimicus</i> strain NIOT VM 06 (16S ribosomal RNA gene, partial sequence)	100	MW578858

Table S2. 16S rRNA sequence alignment of amplified products of bacterial colonies isolated from salmon sushi.

Restaurant	Colony No.	BLASTN results (bacterial identification)	% gene identity	Accession No.
F	BASA 7	<i>Pseudomonas</i> sp. strain RL104 (16S ribosomal RNA gene, partial sequence)	100	MN234077
	BASA 8	<i>Aeromonas hydrophila</i> strain AH10 (16S ribosomal RNA gene, partial sequence)	100	MT605959
	BASA 9	<i>Aeromonas caviae</i> strain DSILV (16S ribosomal RNA gene, partial sequence)	100	MN581908
G	BASA 17	<i>Pseudomonas</i> sp. strain H374 (16S ribosomal RNA gene, partial sequence)	100	MH669304
	BASA 18	<i>Aeromonas dhakensis</i> strain NR_042155.1 (16S ribosomal RNA gene, partial sequence)	100	MH071515
	BASA 19	<i>Aeromonas</i> sp. strain MK_J3 (16S ribosomal RNA gene, partial sequence)	100	MK426653
	TCBS G20	<i>Providencia rettgeri</i> strain EGAPR (16S ribosomal RNA gene, partial sequence)	100	MH368158
H	BASA 34	<i>Aeromonas caviae</i> strain CH-GX-LZ-QJ-2-1-2021 (16S ribosomal RNA gene, partial sequence)	99	ON202885
	BASA 38	<i>Enterobacter huaxiensis</i> strain GP-SG-2 (16S ribosomal RNA gene, partial sequence)	100	MW406864
	BASA 39	<i>Pseudomonas monteilii</i> strain BFPB95 (16S ribosomal RNA gene, partial sequence)	99	EF600893
	BASA 41	<i>Acinetobacter pittii</i> strain L50 (16S ribosomal RNA gene, partial sequence)	100	OQ405779
I	BASA 49	<i>Aeromonas veronii</i> strain NK04 (16S ribosomal RNA gene, partial sequence)	100	KP899501
	BASA 50	<i>Aeromonas</i> sp. strain M7 (16S ribosomal RNA gene, partial sequence)	100	OM617799
	BASA 51	<i>Klebsiella michiganensis</i> strain 5-274 (16S ribosomal RNA gene, partial sequence)	99	PP512815
	BASA 52	<i>Enterobacter</i> sp. strain NCCP-281 gene for 16S rRNA, partial sequence	100	AB641890
	BASA 53	<i>Pseudomonas lundensis</i> strain SeaQual_P_B794 (16S ribosomal RNA gene, partial sequence)	99	MT626771
J	BASA 63	<i>Aeromonas salmonicida</i> strain 349 (16S ribosomal RNA gene, partial sequence)	100	OM837138

***In vitro* anti-leukemia activity of *Gynostemma pentaphyllum* (Thunb.) on HL-60 leukemia cell line**

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ARTICLE INFO

Article history:

Received 23 January 2025

Accepted as revised 27 March 2025

Available online 9 April 2025

Keywords:

Gynostemma pentaphyllum, extract, (FMS)-like tyrosine kinase 3, Wilms' tumor 1, Acute myeloid leukemia (AML)

ABSTRACT

Objective: This study aimed to investigate the *in vitro* anti-leukemia activities of the active extract derived from the leaves of *Gynostemma pentaphyllum*, focusing on its cytotoxic properties, cell cycle regulation, and impact on key protein markers involved in leukemia cell proliferation.

Materials and methods: *G. pentaphyllum* fractional extracts were prepared through sequential extraction using hexane, ethyl acetate, and ethanol solvents through maceration. The cytotoxicity of these extracts was determined against HL-60 leukemia cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The most cytotoxic extract was further fractionated through column chromatography. The anti-leukemia activities of the active extract and its active fraction were evaluated through cell cycle analysis using flow cytometry. Western blotting was performed to assess the expression levels of key proliferation markers, Feline McDonough Sarcoma (FMS)-like tyrosine kinase 3 (FLT3) and Wilms' tumor 1 (WT1) proteins.

Results: The intermediate-polar ethyl acetate fractional extract (F-EtOAc) was found to be highly cytotoxic against HL-60 leukemia cells, with an IC₅₀ value of 43.41±2.75 µg/mL. Among the isolated fractions of F-EtOAc, fraction F10 was identified as the most active. It was shown that F-EtOAc and F10 significantly induced S-phase cell cycle arrest and reduced the expression levels of FLT3 and WT1 proteins in HL-60 cells in a dose-dependent manner (*p* < 0.001), suggesting that anti-proliferative molecular pathways contribute to their anti-leukemia potential.

Conclusion: This study's findings indicate that the ethyl acetate extract of *G. pentaphyllum* contains bioactive compounds with anti-leukemia potential, supporting further investigation into its underlying molecular mechanisms.

Introduction

Leukemias are hematopoietic malignancies characterized by uncontrolled growth and clonal expansion of immature lymphoid or myeloid precursor cells. These abnormalities disrupt normal bone marrow function and hinder cellular differentiation.¹ Acute myeloid leukemia (AML) is the most prevalent form of acute leukemia in adults, accounts for approximately 33% of all blood-related cancers.² The disease arises from impaired differentiation of myeloid, monocytic, erythroid or megakaryocytic lineage precursors.^{1,2} Acute promyelocytic leukemia (APL) was defined as one of the most aggressive form of AML.³ Severe bleeding tendencies, chloroma, fever, anemia, fatigue, and

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doi: 10.12982/JAMS.2025.061

E-ISSN: 2539-6056

frequent infections are the symptoms or complications mostly found in APL patients. FLT3, frequently mutated and overexpressed in AML, plays a critical role in disease progression by driving cell proliferation and survival, making it an attractive therapeutic target.⁴ Similarly, WT1, a transcriptional regulator expressed at elevated levels in leukemic cells compared to normal hematopoietic cells, is involved in leukemia progression and serves as a marker of cell proliferation.⁵ Although cytotoxic chemotherapy induces complete remission in 50-70% of younger AML patients, disease relapse is common.⁶ Moreover, the high toxicity of standard chemotherapy is generally unsuitable for elderly patients who represents the majority of AML cases. The genetic complexity of AML plays a significant role in chemotherapy resistance^{1,4} and this drives ongoing research to explore alternative therapeutic strategies.

Medicinal plant extracts have long been recognized as sources of pharmacologically active compounds for treating wide range of diseases. Plant-derived extracts, which often contain multiple active components, can interact with various targets within tumor cells and their microenvironment, affecting critical biochemical and molecular pathways involved in disease progression.⁷ In Thailand, renowned for its rich biodiversity and geographical location, approximately 9,097 plant species have been documented.⁸ Among these, *Gynostemma pentaphyllum* (Thunb.), commonly known as Jiaogulan, has been widely used in Asian traditional medicine since the nineteenth century for its diverse therapeutic properties, including the ability to regulate inflammation, blood sugar and lipid levels.⁹⁻¹¹ A member of the *Gynostemma* genus, *G. pentaphyllum* is native to South China, Japan, Korea, India, Nepal, and other regions due to its strong environmental adaptability.¹² The plant is a perennial vine characterized by creeping rhizomes and climbing stem with leaves.⁹⁻¹³ Compared to ginseng, *G. pentaphyllum* has a shorter growth cycle, faster growth rate, and higher concentrations of bioactive flavonoids and terpenoids.¹³ These compounds primarily concentrated in the leaves,¹³ and exhibit potent antioxidant, anti-inflammatory, and anti-tumor activities against various cancers, including oral, breast, lung, and liver cancers, as demonstrated in both *in vitro* and *in vivo* studies.^{9,11} Notably, the saponin-rich extract of *G. pentaphyllum*, abundant in gypenosides compound, has shown significant anti-leukemia activities against HL-60 cells *in vitro* and *in vivo* xenograft mouse models, as reported by Lin et al.¹⁴

This study aims to evaluate the cytotoxic activity of three fractional extracts of *G. pentaphyllum* against HL-60 leukemia cell line. The most potent extract will undergo further fractionated using column chromatography, and the anti-leukemia properties will be investigated by evaluating cell cycle progression and the effect on key protein markers involved in leukemia cell proliferation.

Material and Methods

Materials

Analytical-grade solvents, including n-hexane, ethyl acetate, ethanol, and dimethyl sulfoxide (DMSO) were

purchased from Labscan (Dublin, Ireland). The biochemical assay reagents, such as MTT and Folin-Ciocalteu reagents, were procured from Sigma-Aldrich (St. Louis, MO, USA). Roswell Park Memorial Institute (RPMI)-1640 culture medium, penicillin-streptomycin, L-glutamine, and 0.4% trypan blue solution, were purchased from Gibco, Thermo Fisher Scientific, Inc. Fetal bovine serum (FBS) was obtained from Capricorn Scientific (Hesse, Germany). Chromatographic analysis used Silica gel 60 (40-63 µm) for column chromatography and Silica gel 60 F254 plates for thin-layer chromatography (TLC), both manufactured by Merck (Darmstadt, Germany).

Preparation and fractionation of *G. pentaphyllum* active extract

Gynostemma pentaphyllum plant was taxonomically identified by a botanist at the Chiang Mai University Herbarium, Faculty of Pharmacy, Thailand, and a voucher specimen (reference no. 0023392) was deposited for reference. Fresh air-dried leaves were ground into powder and extracted sequentially with n-hexane, ethyl acetate and ethanol. Each solvent extraction was conducted over 72 hrs, divided into three cycles of 24 hrs. Then, the extracts were pooled, filtered, concentrated using a rotary evaporator, and dried in a hot air oven at 40 °C. The dried extracts were weighed and stored at -20 °C for further experiments. Cytotoxicity of three fractional extracts was evaluated against HL-60 leukemia cell model, and the most active extract was selected for further fractionation using normal silica column chromatography (NSCC) with a hexane/ethyl acetate solvent gradient. Collected fractions were monitored using TLC and tested for cytotoxicity. The most active fraction was selected for further study. The extracts and fractions were dissolved in DMSO to obtain the working concentration of 12.5 mg/mL and stored at -20 °C for further use.

Cell line and culture conditions

The acute promyelocytic leukemia cell model (HL-60) was cultured in RPMI-1640 medium containing 1 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin, supplemented with 10% FBS. The cell line was maintained at 37 °C in a humidified incubator with 5% CO₂.

Cytotoxicity evaluation by MTT assay

The cytotoxicity of the extracts and isolated fractions from the active extract was assessed using the MTT assay. HL-60 cells at 1.0×10⁴ cells/well were treated with sample concentrations ranging from 0 to 100 µg/mL for 48 hrs. A vehicle control (VC) containing 0.8% DMSO was included as a control. Dose-response curves were plotted based on the mean percentage of cell survival for each concentration from triplicate experiments. The percentage (%) of cell viability was calculated using the following formula:

$$\% \text{Cell viability} = \left[\frac{OD_s}{OD_c} \right] \times 100$$

where OD_s is the mean absorbance of cells treated

with the extract and OD_c is the mean absorbance of cells treated with the VC. The 50% and 20% inhibitory concentrations (IC_{50} and IC_{20}) were determined as previously described.¹⁵

Cell cycle analysis

Cell cycle progression was assessed using propidium iodide (PI) staining to determine the nuclear DNA content after treatment. The leukemic cell model was treated with non-cytotoxic concentrations (IC_{10} , IC_{15} , IC_{20}) of the active extract and its active fraction for 48 hrs. VC was included for comparison. Cells were fixed in 70% ethanol, stained with a PI solution (0.02 mg/mL PI, 2 mM EDTA, 0.1% Triton X-100, 8 μ g/mL RNase A, in PBS pH 7.4), and analyzed using a DxFLX Flow Cytometer (Beckman Coulter) and FlowJo software V10 (Ashland, OR, USA).

Western blot analysis

For protein expression analysis, leukemia cells were treated with non-cytotoxic concentrations (IC_{10} , IC_{15} , IC_{20}) of the active extract and its active fraction for 48 hrs, with VC included as control. Cells were lysed using RIPA buffer containing protease and phosphatase inhibitors. Protein concentrations were measured with the Pierce™ BCA Protein Assay Kit (ThermoFisher Scientific, IL, USA). Lysates were separated by SDS-PAGE (7.5% or 12% gels) depending on target protein size, transferred to PVDF membrane, and probed with antibodies against FLT3 (Abcam; ab245116) and WT1 (Cell signaling Technology; 83535). HRP-conjugated Anti-rabbit IgG (Abcam; ab205718) was used as the secondary antibody. GAPDH (glyceraldehyde 3-phosphate dehydrogenase) (Abcam; ab9485) was used as a loading control. Bands were visualized using chemiluminescence and quantified with Quantity One 1-D software (Bio-Rad, USA).

Trypan blue exclusion test

This test aimed to assess the cytotoxicity of the test samples. After leukemia cells were treated with non-cytotoxic concentrations (IC_{10} , IC_{15} , IC_{20}) of the active extract and its active fraction for 48 hrs, cells were washed with PBS, pH 7.4, and resuspended. The cell suspension

was then diluted with PBS, pH 7.4 and mixed with 0.2% trypan blue solution. Viable and non-viable cells were counted with a hemocytometer under a light microscope and the total cell number of each was calculated.

Statistical analysis

Data were analyzed using SPSS software V22 (SPSS Inc., USA). Statistical significance was determined using Student's t-tests for two-group comparisons and one-way ANOVA for comparisons of three or more groups. A $p < 0.05$ was considered statistically significant (* $p < 0.05$).

Results

Cytotoxic effects of *G. pentaphyllum* fractional extracts on leukemic cell viability

The maceration of *G. pentaphyllum* dried leaf samples yielded three distinct crude fractional extracts: F-Hex (non-polar hexane extract), F-EtOAc (intermediate polar ethyl acetate extract), and F-EtOH (polar ethanol extract). Each extract appeared as a pasty greenish semi-solid mass, with F-EtOH yielding the highest amount ($2.70 \pm 0.06\%$), followed by F-EtOAc ($1.60 \pm 0.35\%$) and F-Hex ($0.58 \pm 0.04\%$). The cytotoxic effects of extracts on HL-60 cells were examined using the MTT assay, which measure cell viability (%). The half-maximal inhibitory concentration (IC_{50}) was calculated to determine the potency of each extract in reducing cell viability. The results showed differences in cytotoxic activity among the extracts against HL-60 cells (Figure 1). Among the three extracts, F-EtOAc exhibited the highest cytotoxic activity, with an IC_{50} value of 43.41 ± 2.75 μ g/mL, whereas F-Hex and F-EtOH showed IC_{50} values exceeding 100 μ g/mL, indicating lower cytotoxicity. The National Cancer Institute (NCI) categorizes crude extracts based on their cytotoxicity levels; extracts with $IC_{50} > 50$ μ g/mL are classified as weakly cytotoxic, those with IC_{50} between 20-50 μ g/mL as moderately cytotoxic, and those with $IC_{50} < 10$ μ g/mL as highly cytotoxic.¹⁶ In addition, treatment with F-EtOAc at 50 and 100 μ g/mL significantly increased the cytotoxicity to the HL-60 cells when compared with F-Hex and F-EtOH ($p < 0.001$). Based on this classification, F-EtOAc demonstrated moderate cytotoxicity in HL-60 cells.

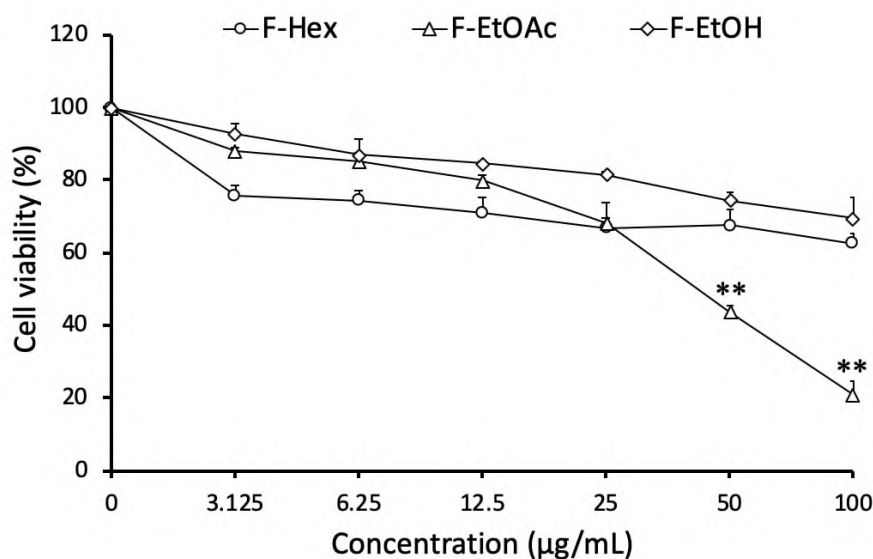


Figure 1. Cytotoxicity of *G. pentaphyllum* hexane fractional extract (F-Hex), ethyl acetate fractional extract (F-EtOAc), and ethanol fractional extract (F-EtOH) on HL-60 leukemia cell line after 48 hrs of treatment. Data are presented as mean±SD from three independent experiments, each performed in triplicate. Asterisks (**) indicate significant differences of % cell viability of HL-60 after treatment with F-EtOAc compared to those of F-Hex and F-EtOH; (** $p < 0.001$, one-way ANOVA).

Cytotoxic effects of F-EtOAc isolated fractions on HL-60 cell viability

The F-EtOAc extract was fractionated using NSCC with Silica gel 60 N (neutral) as a stationary phase and a gradient solvent system of hexane and ethyl acetate. Samples were collected and initially screened by TLC. Fractions with similar TLC profiles were pooled into ten fractions. Subsequently, the dried fractions were used to determine their cytotoxic effects against HL-60 leukemic

cells using the MTT assay. Among ten fractions, fraction F10 showed the most potent cytotoxic activity after 48 hrs, with an IC_{50} value of 27.40 ± 0.20 µg/mL. This fraction had a dry weigh of 96 mg per 4 g of F-EtOAc (w/w), yielding 2.4%. The other fractions showed minimal cytotoxic effects (Table 1). These findings indicate that the F-EtOAc extract and fraction F10 possess strong anti-proliferative potential against HL-60 cells and were examined further.

Table 1. Cytotoxicity of F-EtOAc isolated fractions on HL-60 leukemic cell line performed by MTT assay.

F-EtOAc fractions	IC_{50} value (µg/mL)
F1	>100
F2	>100
F3	>100
F4	>100
F5	>100
F6	>100
F7	85.26 ± 4.31
F8	77.32 ± 2.71
F9	47.76 ± 0.98
F10	27.40 ± 0.23

Note: IC_{50} value are presented as mean±SD from three independent experiments, each performed in triplicate.

Effect of F-EtOAc and F10 on HL-60 cell cycle progression

HL-60 cells were treated with three non-cytotoxic concentrations (IC_{10} , IC_{15} , IC_{20}) of F-EtOAc and F10 for 48 hrs, followed by PI staining to analyze DNA content. The results presented in Figure 2 showed that treatment with both F-EtOAc and F10 caused a significant dose-dependent increase in the number of cells in S-phase, suggesting

an arrest in S-phase progression. At IC_{20} concentration of F-EtOAc (12 $\mu\text{g/mL}$) and F10 (6 $\mu\text{g/mL}$), a significantly higher percentage of HL-60 cells were in the S-phase compared to the VC group ($p < 0.001$, Figure 2B). These findings demonstrate that the anti-proliferative activities of F-EtOAc and F10 on HL-60 cells are mediated, at least in part, by inducing cell cycle arrest at the S-phase.

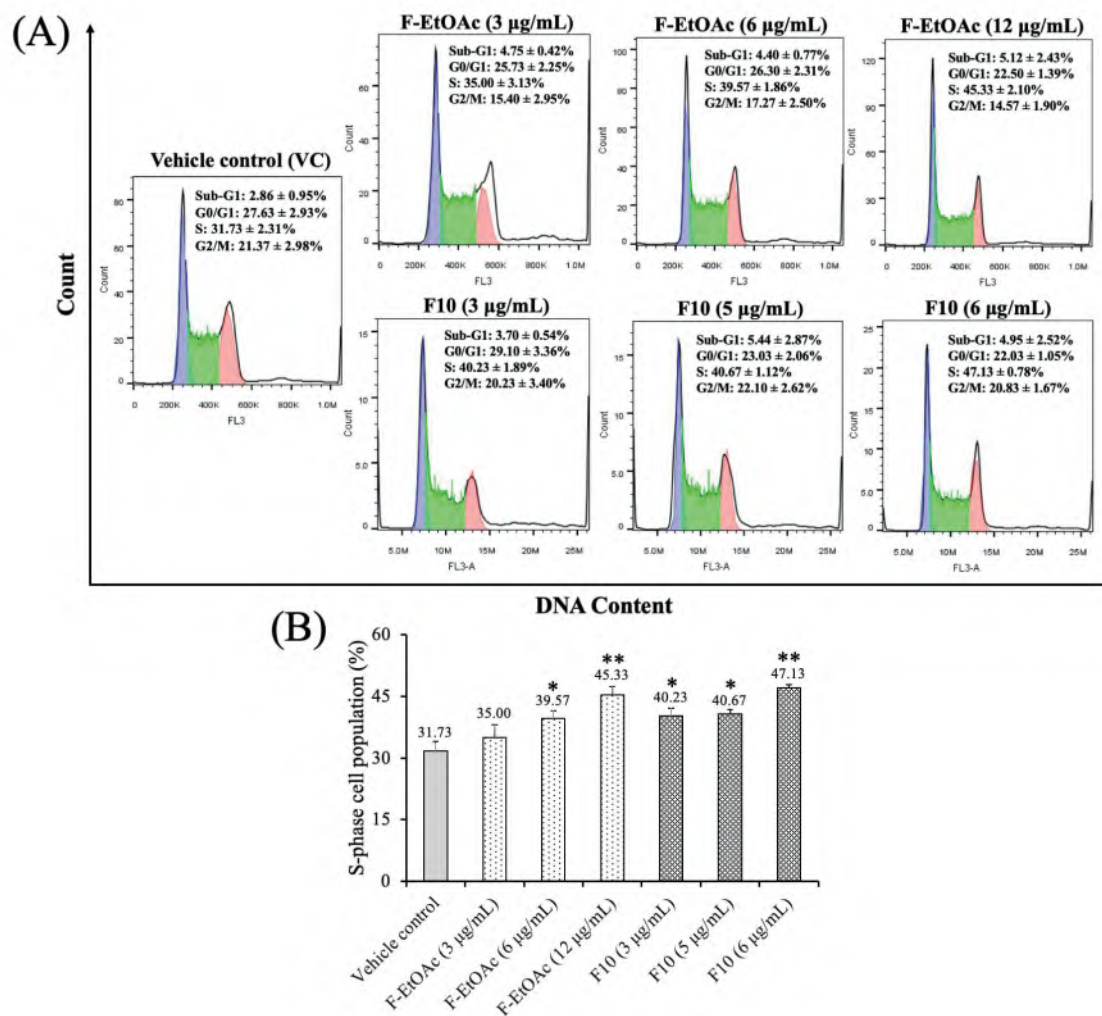


Figure 2. Cell cycle distribution of HL-60 cells. A: after treated with vehicle control (VC) and IC_{10} , IC_{15} , IC_{20} concentrations of F-EtOAc and F10 for 48 hrs, B: S-phase cell population (%) of HL-60 cells after treated with F-EtOAc, and F10 for 48 hrs in comparison with VC. The bars represent the mean $\pm$ SD from three independent experiments. Significant differences (* $p < 0.05$, ** $p < 0.001$) were determined by One-way ANOVA compared to the vehicle control (VC).

Effect of F-EtOAc and F10 on FLT3 and WT1 expression in HL-60 cells

The expression levels of FLT3 and WT1 proteins, which are important markers associated with leukemia cell proliferation, were evaluated using Western blot analysis. HL-60 cells were treated with three non-cytotoxic concentrations (IC_{10} , IC_{15} , IC_{20}) of F-EtOAc and F10 to investigate their effects on FLT3 and WT1 expression. The

results showed that both F-EtOAc and F10 significantly reduced FLT3 and WT1 protein levels in a dose-dependent manner, thereby influencing the proliferation of HL-60 cells (Figure 3). A significant decrease in FLT3 (Figures 3A) and WT1 (Figures 3B) expression was observed at the IC_{20} concentration, demonstrating pronounced inhibitory effects compared to the VC after 48 hrs of treatment.

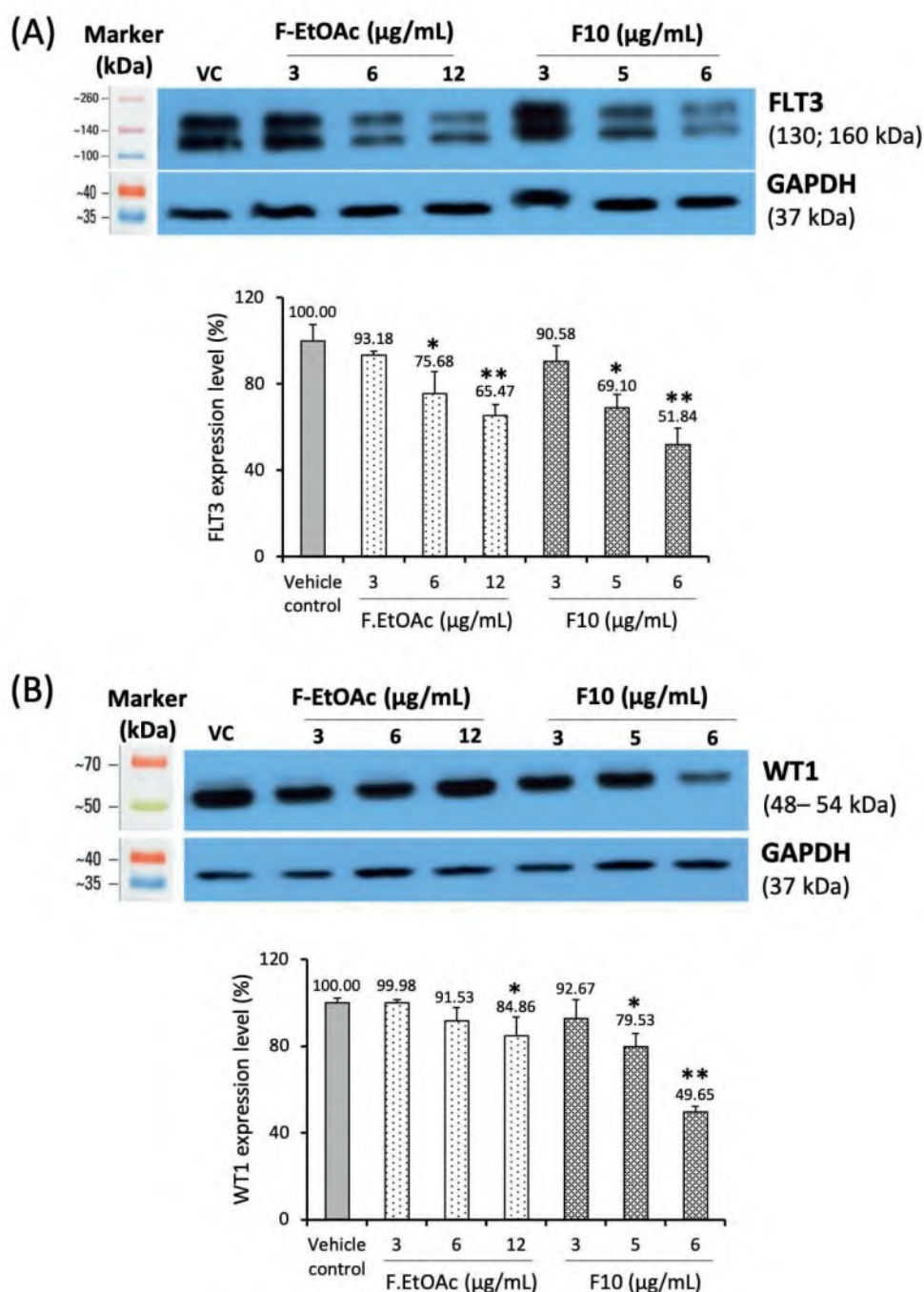


Figure 3. Expression levels of FLT3 and WT1 protein in HL-60 cells treated with the vehicle control (VC) and IC_{10} , IC_{15} , and IC_{20} concentrations of F-EtOAc and F10 for 48 hrs. A: FLT3, B: WT1 protein. GAPDH (37 kDa) was used as the loading control. Histograms represent the mean (%) protein expression $\pm$ SD from three independent experiments. Significant differences (* p <0.05, ** p <0.001) were determined by Student's *t*-test compared to the vehicle control (VC).

These findings were consistent with the reduction in total viable cell numbers, as confirmed by the trypan blue exclusion assay (Figure 4). Taken together, the results

suggest that downregulation of FLT3 and WT1 protein expression contributes to the anti-proliferative activities of F-EtOAc and F10 in HL-60 cells.

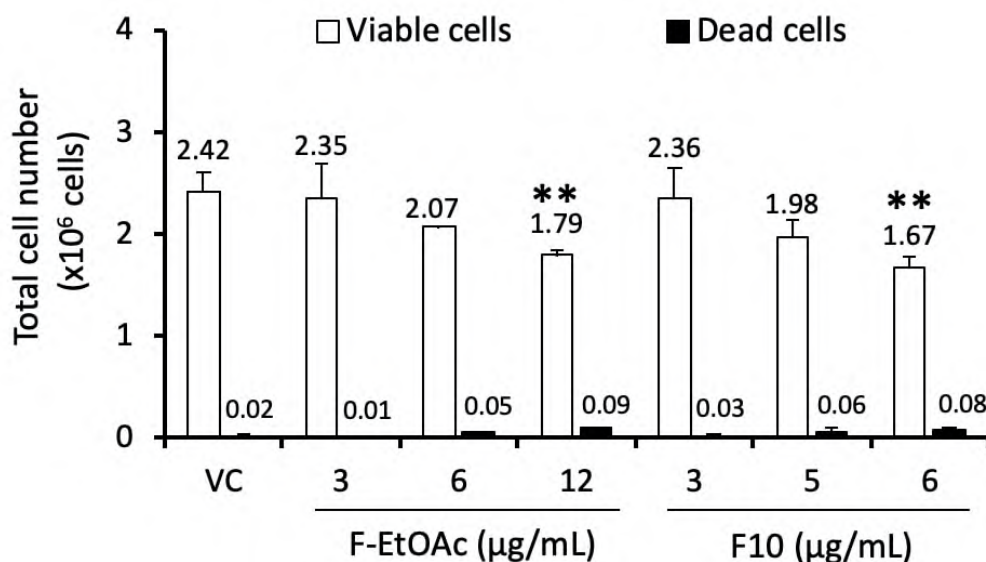


Figure 4. The effects of F-EtOAc and F10 on total cell numbers in HL-60 leukemic cells are determined using trypan blue exclusion test. Histograms show the mean±SD from three independent experiments. Significant differences (** $p < 0.001$) were determined by Student's *t*-test compared to the vehicle control (VC).

Discussion

In this study, we investigated the effects of dried leaf fractional extracts from *Gynostemma pentaphyllum*, collected from a farm in Chiang Mai Province, Thailand, on the cell viability, cell cycle progression, and expression of proliferation-associated proteins in HL-60 AML cells *in vitro*. Previous studies demonstrated that extracts from *G. pentaphyllum* herbs can inhibit the growth and induce apoptosis in various solid cancer cell lines.^{9,11,17} Among the limited studies on leukemia cells, Lin *et al.*¹⁴ and Hsu *et al.*¹⁰ showed that gypenosides-enriched *G. pentaphyllum* saponin extracts possess cytotoxic activity, modulating proliferation and apoptosis in HL-60 and WEHI-3 cells (mouse myelomonocytic leukemia cell model) both *in vitro* and *in vivo*. In our study, crude plant extracts were prepared using the maceration method, known for its high efficiency and suitability for extracting active ingredients from medicinal plants.¹⁸ Among the extracts, ethanol (F-EtOH) yielded the highest extraction percentage (2.70±0.06%), followed by ethyl acetate (F-EtOAc) and hexane (F-Hex). This result indicates that polar protic solvents are more effective in getting higher extraction yields. The superior yield with alcohol-based solvents can be attributed to their ability to dissolve both polar and non-polar compounds.¹⁹ Specifically, ethanol is particularly effective at extracting polar components, such as polysaccharides and proteins, which are likely the predominant constituents in the *G. pentaphyllum* leaf samples used in this study.

Among the three fractional extracts tested, only the ethyl acetate fractional extract (F-EtOAc) strongly inhibited cell growth in HL-60 cells. This high cytotoxic activity is likely due to the presence of specific bioactive

phytochemicals in the ethyl acetate fraction, supporting the potential of *G. pentaphyllum* as source of anti-cancer compounds. F-EtOAc showed the strongest activity against HL-60 cells, with an IC₅₀ value of 43.41±2.75 µg/mL. On the other hand, F-Hex and F-EtOH fractions did not significantly affect the viability of leukemic cell lines, as the percentage of viable cells remained above 50% (Figure 1). This suggests the need for further investigation into the cellular mechanisms underlying the sensitivity of AML cells to F-EtOAc and its active components. Similar findings reported by Wang *et al.*²⁰ observed higher anti-cancer activity of the ethyl acetate fraction from *G. pentaphyllum* against A549 lung cancer cells compared to the ethanol extract, highlighting enrichment of potent compounds in the ethyl acetate fraction.

Using column chromatography, the crude F-EtOAc extract was separated into distinct fractions containing compounds based on the polarity and molecular size. Of the ten fractions derived from F-EtOAc, fraction F10 exhibited the strongest cytotoxic activity, with IC₅₀ value of 27.40±0.20 µg/mL against HL-60 cells (Table 1). Fraction F10 was obtained using hexane/ethyl acetate solvent system with a ratio of 55:45, indicating the presence of semi-polar character to the compounds in the extract. This finding contrasts with one previous study that identified triterpenoid saponin compounds isolated from 75% ethanol extract as moderately cytotoxic against HL-60 cells with IC₅₀ value of 17.44 µg/mL.²¹ Consistent with earlier research, nearly all cytotoxic compounds and extracts derived from *G. pentaphyllum* have shown anti-proliferative activity, with IC₅₀ values ranging from 0.05 to 74.3 µg/mL.⁹ Since plant crude extracts are composed

of both active and inactive phytochemicals, their overall effectiveness is generally lower compared to purified fractions. Our findings suggest that ethyl acetate fraction of *G. pentaphyllum* may serve as interesting source of bioactive compounds that are yet to be explored for its characterization and biological activities.

In this study, cell cycle arrest was investigated as a mechanism of action for natural cytotoxic compounds, as several natural compounds inhibit cancer cell proliferation by suppressing the cell cycle progression.⁹ Molecules that can block uncontrolled proliferation or induce cell death are increasingly recognized as promising candidate for therapeutic applications in cancer treatment. The effects of the most potent extract F-EtOAc and fraction F10 on the cell-cycle progression of HL-60 cells were analyzed using flow cytometry. Recent studies suggest that the inhibitory effects of medicinal plant-derived extracts on cell proliferation may be mediated through arresting cells at the G0/G1, S, or G2/M phases or by promoting apoptosis.^{22,23} In line with this, treatment of HL-60 cells with the IC₂₀ concentration of F-EtOAc and F10 significantly arrest the cell cycle at S-phase ($p < 0.001$, Figure 2). Previous research studies reported that gypenosides from *G. pentaphyllum* inhibit cancer cell growth by inducing G0/G1-phase arrest and promoting apoptosis in various cancer cell lines, including A549,²⁴ Colo 205,²⁵ HL-60,¹⁴ and WEHI-3 cells.¹⁰ In another study, flavonoids and saponins isolated from *G. pentaphyllum* have been shown to cause cell cycle arrest at the S and G2/M phases in PC-3 prostate cancer cells by modulating the expression of cyclins.^{26,27} To further explore the molecular mechanisms underlying these effects, in our study, we examined the expression of cell proliferation-associated markers in HL-60 cells. To the best of our knowledge, no prior studies have reported the effects of *G. pentaphyllum* compounds on FLT3 and WT1 protein expression in leukemic cells. Western blot analysis showed that treatment with F-EtOAc (12 µg/mL; $p < 0.05$) and F10 (6 µg/mL; $p < 0.001$) for 48 hrs significantly reduced FLT3 and WT1 expression levels in HL-60 cells (Figure 3). This reduction in protein expression was consistent with a decreased total cell count observed using trypan blue exclusion (Figure 4), indicating a suppression of cell proliferation.²⁸ These findings suggest that F-EtOAc and F10 reduce the viability of AML cells through mechanisms involving FLT3 and WT1 inhibition. The precise molecular pathways by which *G. pentaphyllum* down-regulates FLT3 and WT1 expression, leading to S-phase cell cycle arrest, awaits further studies.

Conclusion

The present research highlights the potential anti-proliferative properties of *Gynostemma pentaphyllum* leaves against HL-60 leukemia cells, providing evidence for further investigation. The study demonstrates that ethyl acetate extract (F-EtOAc) and its active fraction F10 exhibit significant anti-leukemia activities by inducing S-phase cell cycle arrest and reducing expression of FLT3 and WT1. Overall, these findings suggest that ethyl acetate

extract of *G. pentaphyllum* contains physiologically active compounds targeting FLT3 and WT1, making it a promising source of anti-leukemic biomolecules. Further research should focus on isolation and characterization of the specific compounds responsible for the anti-proliferative effects, as well as their mechanisms of action.

Acknowledgements

The authors sincerely thank Ms. Lapamas Rueankham (Faculty of Associated Medical Sciences, Chiang Mai University) for the valuable experimental assistance. Support from the PhD Degree Program in Biomedical Sciences, Faculty of Associated Medical Sciences, Chiang Mai University, through the CMU Presidential Scholarship, is gratefully appreciated.

Conflict of interest

The authors declare no conflict of interest.

Funding sources

This study was supported by the Office of the Permanent Secretary, Ministry of Higher Education, Science, Research and Innovation (OPS MHESI), Thailand Science Research and Innovation (TSRI) and Chiang Mai University under Grant No. RGNS 65-074.

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The state of occupational therapy practice in the community settings in Thailand: A scoping review

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ARTICLE INFO

Article history:

Received 12 February 2025

Accepted as revised 27 March 2025

Available online 11 April 2025

Keywords:

Community-based rehabilitation, village health volunteers, occupational therapy, stroke recovery, mental health, community reintegration.

ABSTRACT

Background: Community-based occupational therapy (CBOT) extends beyond traditional institutional care to address social, cultural, and environmental factors that influence health and participation. In Thailand, demographic shifts, the rise of non-communicable diseases, and gaps in the healthcare system underscore the potential of CBOT for meeting community needs. However, the extent to which occupational therapy (OT) practice in Thailand is implemented within community settings remains unclear. A clearer understanding of existing practices, key interventions, and emerging trends is therefore needed to guide policy, inform practice, and expand CBOT effectively.

Objective: This scoping review aims to map current OT practice in Thai community settings. Specifically, it explores areas of practice, key interventions, and emerging trends, examining how they align with CBOT principles. By identifying gaps and opportunities, this review seeks to inform future research, practice, and policy development toward evidence-based CBOT.

Materials and methods: Following Arksey and O'Malley's scoping review framework, a systematic search of PubMed, Scopus, and the Thai-Journal Citation Index Center was conducted. Articles published in English or Thai describing OT in Thai community settings were included, yielding 14 studies. Data extraction followed a structured template, and a content analysis was performed to identify thematic patterns.

Results: Two overarching categories emerged: areas of practice and implementation strategies. The areas of practice primarily emphasized physical rehabilitation, particularly for stroke survivors, focusing on functional recovery and activities of daily living. Fewer studies addressed mental health, but those that did underscored the importance of social participation and caregiver education. Implementation strategies included improving functional recovery, collaborating with village health volunteers, integrating technology, and developing CBOT models.

Conclusion: Occupational therapy in Thai community settings demonstrates promising practices, such as integrating village health volunteers and employing technology in rehabilitation. However, limited attention to mental health, underutilization of OT-specific skills, and uneven geographical distribution highlight areas for further development. Expanding community-based services to underserved regions and strengthening the evidence base-especially in mental health and policy engagement-will be crucial for maximizing CBOT's impact. Future research should explore diverse populations, assess model sustainability, and align current practices more firmly with evidence-based strategies.

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doi: 10.12982/JAMS.2025.062

E-ISSN: 2539-6056

Introduction

Occupational therapy (OT) is a client-centered profession that enhances health and well-being by enabling participation

in meaningful activities, known as occupations, across various populations.¹ Traditionally rooted in institutional settings such as hospitals and rehabilitation centers, OT has gradually expanded into community-based occupational therapy (CBOT) practices.² While community care is not a novel concept in healthcare, OT's involvement in this area has been limited, partly due to workforce shortages and systemic challenges.³ This shift toward CBOT reflects a growing recognition of the limitations of the biomedical model, which often focuses on treating impairments while neglecting broader social, cultural, and environmental factors that influence health and participation.^{4,5}

CBOT adopts a holistic, strengths-based approach to address occupational needs, foster social inclusion, and improve the quality of life for individuals and communities.⁶ Its key principles—client-centeredness, capacity-building, and empowerment—highlight the dynamic relationship between people, their occupations, and their environments.⁷ This approach aligns with global priorities, including community-based rehabilitation (CBR) and health promotion, to address the diverse needs of populations.

CBOT is especially relevant in Thailand due to the country's distinctive demographic, epidemiological, and sociocultural challenges. Rapid population aging poses significant healthcare challenges, with nearly one-third of the population projected to be 60 or older by 2040.⁸ This trend underscores the need for innovative strategies to support aging in place, manage chronic diseases, and promote participation in meaningful occupations within communities.⁹ Additionally, the rising prevalence of non-communicable diseases (NCDs), including cancer (CA), coronary artery disease (CAD), chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM), hypertension (HTN), and stroke, which require long-term care,¹⁰ highlights the need for comprehensive community-based rehabilitation and prevention strategies.¹¹

Healthcare reforms in Thailand, including the Universal Coverage Scheme (UCS), have improved access to primary care but exposed gaps in community-level rehabilitation and disability services—particularly a shortage of occupational therapists in Thailand.^{12,13} CBOT offers an opportunity to bridge these gaps by providing accessible, culturally responsive interventions tailored to the needs of diverse communities.¹⁴ Thailand's rich cultural diversity further emphasizes the need for community-driven, culturally sensitive approaches that encourage occupational engagement while respecting local traditions and values.⁴

Although community-based occupational therapy (CBOT) has gained global recognition, its development and implementation in Thailand remain limited and underexplored¹⁵. This scoping review aims to map current occupational therapy (OT) practice in community settings in Thailand, providing insights into areas of practice, key interventions, and emerging trends. It examines how these practices align with CBOT principles and identifies gaps and opportunities in the field. By synthesizing the existing literature, this review seeks to inform future

research, practice, and policy development, supporting the advancement of evidence-based CBOT in Thailand.

Materials and methods

This research was conducted following the scoping review methodological framework proposed by Arksey and O'Malley.¹⁶ A scoping review is a research method that provides an overview of the available written evidence on a particular topic, typically without assessing the methodological quality of the existing evidence¹⁷. A scoping review method is appropriate for both research questions related to investigating the implementation of community-based occupational therapy in Thailand, as it provides a comprehensive approach to mapping the existing literature on this topic, identifying gaps in knowledge, and informing future research and practice in this area.

Search strategies

The literature search was conducted in February 2024 using PubMed, Scopus, and the Thai-Journal Citation Index Center. In addition, citation and reference tracking were performed as part of hand searching. The search strategy incorporated Boolean operators (AND, OR) to combine key concepts and included truncation symbols where applicable to capture variations in terminology. The final search string used was: ("Occupational therapist" OR "Occupational therapy" OR "Rehabilitation") AND (Community OR "Community-based rehabilitation" OR "Community services") AND (Thai OR Thailand) AND ("Health services" OR "Social support" OR "Healthcare access" OR "Inclusive care"). Truncation symbols (e.g., *) were not applied in databases. The search strategy aimed to capture a comprehensive range of studies related to OT practice in community settings in Thailand while ensuring specificity and relevance.

Eligibility criteria

Before conducting the literature search, inclusion and exclusion criteria were established to identify studies relevant to occupational therapy practice in community settings. Eligible articles had to: 1) Be published in English or Thai from inception through February 2024, 2) Report on original research (quantitative, qualitative, or mixed methods), and 3) Specifically describe the role of occupational therapy professionals in community contexts. Excluded were conference presentations, expert opinions, as well as studies that did not clearly distinguish occupational therapy perspectives from those of other professionals.

Study selection

The study selection process involves 5 phases. Phase 1: 339 studies were identified through databases and hand searches. Phase 2: duplicated articles were removed, and there were 331 articles to review. Phase 3: articles were screened at title and abstract levels. Following the screening, 32 articles remained and were further rigorously assessed for eligibility. Phase 4: articles were assessed at

the full-text level. In this phase, 18 articles were excluded after the full-text review due to articles not relevant to the OT role. Phase 5: 14 articles were included in the final review. The PRISMA diagram indicating the overall

review process is shown in Figure 1. However, no quality assessment regarding the research design or methodology has been conducted.

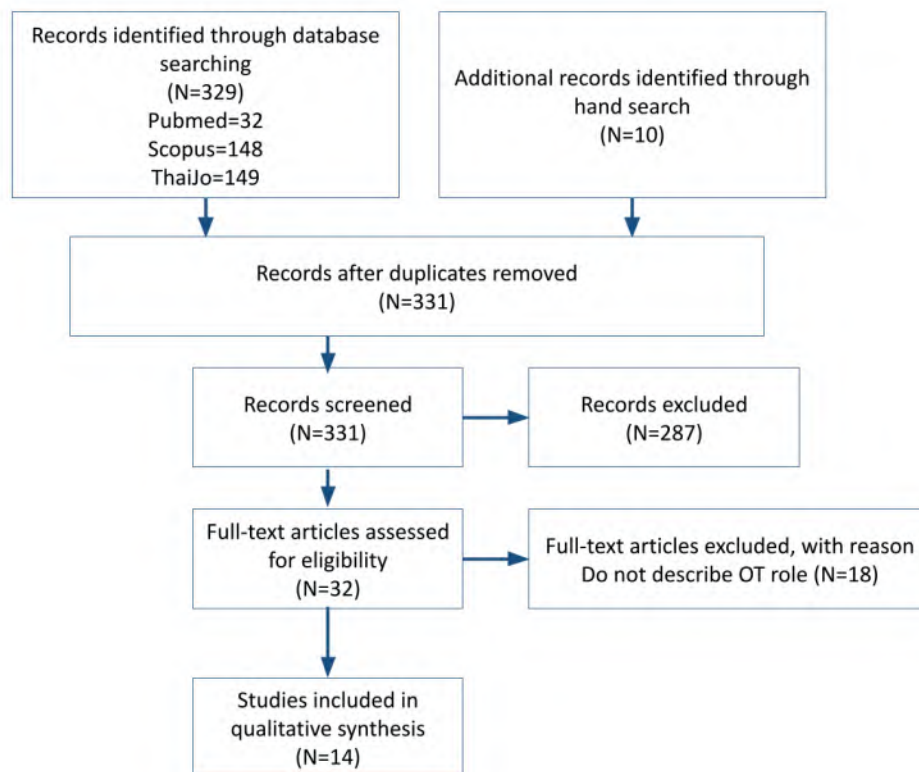


Figure 1. The PRISMA diagram indicating the overall review process.

Study procedure

Descriptive tables were created to organize study characteristics, summarize the selected activities and activity choices, and highlight key findings. Two researchers (CT and SP) extracted information from each article, which was then cross-checked by the research team. A custom Excel spreadsheet was used for data extraction. If an article contained information irrelevant to the purpose of this review, it was excluded from the data table.

Content analysis was used to examine the data from the primary finding.¹⁸ Two researchers carefully examined the included articles and used a spreadsheet application to cluster common features according to the review purpose. An inductive coding approach was applied, allowing themes to emerge from the data without predefined categories. This analysis was verified by the research team to ensure its reliability and validity. All authors reviewed the descriptions for clarity and relevance, and some topics

were merged after the initial review to improve readability and reduce redundancy.

Results

Our scoping review identified 14 studies that met the inclusion criteria. These studies encompass a range of research designs, populations, and focus areas within community-based occupational therapy practice in Thailand. Of the 14 studies, 10 employed quantitative methods, including quasi-experimental designs (4 studies) and single-group pre-post-test designs (6 studies). Two studies used qualitative methods, specifically grounded theory methodology. The remaining two studies utilized mixed methods approaches. Following synthesis, two main categories emerged: area of practice and implementation strategies (Table 1). A summary of the final 14 articles is presented in Table 2

Table 1. Categories of included study that arose from the analysis.

Categories	Sub-categories
Area of practice	- Physical rehabilitation - Mental health
Implementation strategies	- Improving functional recovery - Collaborating with village health volunteers - Integrating technology - Developing community-based occupational therapy models

Table 2. Summary of included articles.

Study/ Year	OT field	Research purpose	Study design	Participants	Key results	Role of Occupational Therapists
Chinchai et al. ¹⁹	Physical Dysfunction	To investigate the effects of home health care and rehabilitation programs provided for caregivers on QOL of stroke survivors	Quasi-experimental design	Stroke client and caregiver Experimental group (N=30 dyads) Control group (N=30 dyad)	There was no pre-intervention QOL difference between groups, but the experimental group showed significant improvements in physical (F(1, 29)=41.05; $p<0.05$), psychological (F(1, 29)=37.96; $p<0.05$), and environmental domains (F(1, 29)=9.58; $p<0.05$).	Provide education programs for home healthcare and rehabilitation
Chinchai et al. ²⁰	Physical Dysfunction	To investigate rehabilitation education provided for VHV toward ADL performance of stroke participants.	Single group, pre-test-post-test	VHV (N=7) Caregivers (N=27) Stroke client (N=27)	ADL performance improved significantly from 85.59±13.02 (pretest) to 89.63±8.95 (posttest, $p<0.05$).	Provide stroke rehabilitation education for the VHV
Chinchai et al. ²¹	Physical Dysfunction	To investigate the functional abilities of stroke survivors who received services provided by trained VHV for 3 months at their community rehabilitation center.	Single group, pre-test-post-test	Stroke client (N=25)	Stroke participants showed significant improvement in functional abilities (90.60±13.36 vs 85.24±18.88, $p<0.05$), with greater gains in self-care (33.40±5.10 vs 30.60±8.55) and social skills (4.16±1.80 vs. 3.08±1.61, $p<0.05$).	Provide stroke rehabilitation education for the VHV
Chinchai et al. ²²	Physical Dysfunction	To investigate the effectiveness of rehabilitation services provided by trained VHV on community integration and QOL for stroke patients in Thailand	Single group, pre-test-post-test	Stroke client (N=25)	Community integration scores significantly improved from 9.80±3.96 (pre-test) to 11.44±4.68 (post-test, $p<0.05$). QOL scores also significantly increased from 71.44±8.38 (pre-test) to 84.88±12.07 (post-test, $p<0.05$).	Provide stroke rehabilitation education for the VHV
Chinchai et al. ²³	Physical Dysfunction	To investigate rehabilitation outcomes in areas of QOL and community integration of stroke participants before and 8 weeks after receiving rehabilitation from the trained VHV	Single group, pre-test-post-test	Stroke client (N=11)	QOL (overall, physical, and environmental domains) and community/home integration scores significantly increased from the pretest to the post-test ($p<0.05$)	Provide stroke rehabilitation education for the VHV
Chinchai et al. ²⁴	Physical Dysfunction	To investigate rehabilitation outcomes in ADL and QOL of stroke participants who received rehabilitation services from trained VHV	Quasi-experimental research	Stroke client (N=10)	BADL, IADL, and total ADL scores significantly increased post-rehabilitation ($p<0.05$), except for sexual expression. QOL scores also significantly improved after intervention ($p<0.05$).	Provide stroke rehabilitation education for the VHV

Table 2. Summary of included articles (continued).

Study/ Year	OT field	Research purpose	Study design	Participants	Key results	Role of Occupational Therapists
Chinchai and Khamwong ²⁵	Physical Dysfunction	To investigate the effect that rehabilitation education provided to VHV would have on walking speed and upper extremity function in stroke survivors who stay at home in Thailand.	Single group, pre-test-post-test	Stroke client (N=27)	Walking time significantly decreased from 34.73±8.48 (pre-test) to 32.18±9.32 (post-test, $p<0.05$). However, UE function scores showed no significant change (36.81±9.59 pre-test vs 37.26±9.67 post-test).	Provide stroke rehabilitation education for the VHV
Chinchai and Kongsawasdi ²⁶	Physical Dysfunction	To investigate the effectiveness of rehabilitation services provided by trained VHV in activities of daily living performance for stroke patients	Single group, pre-test-post-test	Stroke client (N=11)	Basic ADL performance ($z=-2.223$, $p<0.05$) and IADL scores ($z=-2.805$, $p<0.05$) both showed significant improvements from pre-intervention to post-test.	Provide stroke rehabilitation education for the VHV
Pakdee and Chinchai ²⁷	Physical Dysfunction	To investigate the effects of a home visit program on functional abilities and quality of life of persons with disabilities resulting from stroke.	Quasi-experimental research	Stroke client Experimental group (N=18) Control group (N=18)	Both groups showed significant improvements in ADL and quality of life scores ($p<0.05$). However, the experimental group had a significantly higher post-intervention QOL score (89.11±10.84) compared to the control group (80.61±8.75, $p<0.05$).	Home visits and provided rehabilitation education to participants
Phadsri et al. ²⁸	Mental Health	Understanding the process of how OTs work to redevelop the social participation of community-dwelling Thai adults with depression	Grounded theory	OTs' experience providing mental health care in community services (N=14)	The proactive community occupational therapy service for depression (PCOTS-D) consists of three concepts: integrating depression care into community occupational therapy services (COTS), supporting meaningful participation, and forming collaborative networks.	PCOTS-D facilitates social participation by transitioning from proactive depression care to COTS, supporting meaningful participation for self-management, and building collaborative networks with inter- and intra-professional teams.
Sirisatayawong et al. ²⁹	Mental Health	To investigate the effect of the rehabilitation education program given to their caregivers on QOL of persons with severe mental disorders.	Quasi-experimental research	Persons with severe mental disorder Experimental group(N=13) Control group(N=14)	The experimental group showed significant improvements in quality of life across physical, psychological, social, environmental, and overall health ($p<0.05$), while the control group showed significant declines in physical health, environmental factors, and overall health ($p<0.05$).	Provide educational programs for rehabilitation for the caregivers

Table 2. Summary of included articles (continued).

Study/ Year	OT field	Research purpose	Study design	Participants	Key results	Role of Occupational Therapists
Putthinoin et al. ³⁰	General	To develop an information system for supporting OT services in the community.	Mixed methods research	OTs working in the community Qualitative phase (N=9) Quantitative phase (N=30)	Participants requested two components: 1) Occupational Therapist data and 2) a referral system. The system allowed active data input for referrals, with a high usability rating (mean±SD=4.27±0.07).	Develop the information system
Lersilp et al. ³¹	General	To develop a prototype for a simplified power wheelchair and develop and evaluate a training program that has the potential to encourage evaluation and modification of the wheelchair for the elderly and their caregivers in the community.	Mixed methods designed	Older adults (N=12) Caregiver (N=9) Older adults who have caregiver role (N=3)	Participants with no prior knowledge of wheelchair modification learned to assist wheelchair users. The evaluation revealed two themes: “benefits from the training” and “future improvements.” Key concepts included sufficient knowledge, fieldwork value, team and organizational support, expanded contexts, and continued services post-training.	Develop AT prototype and provide a training program
Phadsri et al. ³²	General	To explore key issues in community OT services and to develop a model to guide this practice	Grounded theory	Ots (N=17)	The proactive approach highlights key issues in community occupational therapy and provides a framework for Thai therapists, helping them work more confidently with clients and strengthen occupational therapy services.	The proactive approach was elaborated in terms of proactive policy, collaboration and networking, integrating local context and OT knowledge, and family-community participation.

Note: ADL: activity of daily living, OT: occupational therapy, OTs: occupational therapist, QOL: quality of life, VHV: village health volunteer.

Physical rehabilitation

Most studies (10 out of 14) focused on physical rehabilitation, particularly for stroke survivors. These studies investigated various aspects of rehabilitation, including functional abilities, activities of daily living (ADL) performance, quality of life (QOL), and community integration. Chinchai and colleagues conducted a series of studies examining different aspects of stroke rehabilitation in community settings.¹⁹⁻²⁶ The first study employed a quasi-experimental design to investigate the effects of home health care and rehabilitation programs on the QOL of stroke survivors.¹⁹ Subsequent studies focused on the effectiveness of rehabilitation services provided by trained Village Health Volunteers (VHVs), examining outcomes such as ADL performance,²⁰ functional abilities,²¹ community integration and QOL,^{22,23} and overall rehabilitation outcomes.²⁴ Chinchai and Khamwong specifically investigated the effect of rehabilitation education provided to VHVs on walking speed and upper extremity function in stroke survivors.²⁵ Chinchai and Kongsawasdi further explored the effectiveness of VHV-provided rehabilitation services on ADL performance for stroke patients.²⁶ Pakdee and Chinchai conducted a quasi-experimental study to investigate the effects of an occupational therapist's home-visitation program on ADL abilities and QOL for persons with disabilities resulting from stroke.²⁷

Mental Health interventions

Two studies addressed mental health concerns, indicating an emerging focus in this area. Phadsri *et al.* applied a grounded theory approach to understand how occupational therapists support the social participation of community-dwelling Thai adults with depression.²⁸ Their analysis involved identifying core processes and strategies used by occupational therapists, such as fostering collaborative networks and adapting interventions to local contexts. Sirisatayawong *et al.* conducted a quasi-experimental study examining how a rehabilitation education program for caregivers influenced the QOL of individuals with severe mental disorders.²⁹ The intervention included structured education, skill-building exercises, and ongoing support, and the authors reported improvements in quality-of-life measures among participants who received the program.

Implementation strategies

Improving functional recovery

Several studies examined aspects of functional recovery in community-based occupational therapy contexts. Chinchai *et al.* investigated ADL performance and functional abilities in stroke survivors who received services from trained VHVs.^{20,21} Chinchai and Khamwong²⁵ explored walking speed and upper extremity function in a similar population, while Pakdee and Chinchai assessed ADL abilities alongside QOL measures.²⁷ Each of these studies focused on quantifiable indicators of recovery, such as performance in daily tasks and mobility. In a related investigation, Sirisatayawong *et al.* evaluated the effects of caregiver education on the QOL of individuals with severe

mental disorders, reporting changes in psychosocial and functional outcomes within community settings.²⁹

Collaborating with VHVs

Seven studies evaluated the role of VHVs in providing community-level rehabilitation for stroke survivors. These studies often involved educational programs delivered by occupational and physical therapists to equip VHVs with the necessary skills to support clients in their communities. Reported outcomes included functional abilities,²¹ ADL performance,^{20,26} walking speed and upper extremity function,²⁵ as well as QOL and community integration.²²⁻²⁴ The authors documented how trained VHVs contributed to rehabilitation efforts after formal hospital-based services ended, focusing on the utilization of local resources to continue care.

Integrating technology

Two studies focused on integrating technology into occupational therapy practice in community settings. Putthinoi *et al.*³⁰ used a mixed-methods approach to develop an information system aimed at supporting and streamlining occupational therapy services in community settings, reporting improvements in data collection, client information organization, and provider communication. Lersilp *et al.*³¹ introduced a simplified power wheelchair and a corresponding training program for older adults and their caregivers, documenting increased user mobility and caregiver confidence. Both studies reported positive outcomes from technology-based interventions within community settings.

Developing CBOT models

Phadsri *et al.*³² introduced a "Proactive Approach" as a core category for developing CBOT services in Thailand, describing four key components: policy development, collaboration and networking, integration of local context with occupational therapy knowledge, and family-community participation. The authors reported that these elements provided a structured method for occupational therapists to engage stakeholders, adapt interventions to community needs, and align service delivery with existing resources. In a separate study, Phadsri *et al.*²⁸ examined a model of CBOT for adults with depression, detailing the integration of meaningful participation and collaborative networks within local contexts. Both studies described context-sensitive strategies aimed at addressing diverse community needs in occupational therapy practice.

Discussion

This scoping review highlights the unique characteristics of OT practice in community settings in Thailand, underscoring its evolving nature within a holistic framework. Thai OT practice in community settings reflects a broader global shift towards health promotion, prevention, and community integration. The integration of village health volunteers (VHVs) in community-based OT services is a key component of service delivery in Thailand. While VHVs provide essential support in delivering

rehabilitation and community interventions, their role should be guided by occupational therapists (OTs), who provide training, supervision, and expertise to ensure effective implementation. However, there is significant potential for OTs to take on greater leadership roles in designing and expanding holistic, community-centered approaches, ensuring that interventions are occupation-focused and aligned with evidence-based practices.⁷

Currently, the primary focus of OT practice in community settings in Thailand is stroke rehabilitation, with VHV delivering basic interventions under OT supervision. While this model has resulted in positive outcomes for functional recovery and quality of life, it highlights an underutilization of OTs' specialized skills. Strengthening OTs' leadership in stroke rehabilitation may improve both physical recovery and psychosocial reintegration by enabling more comprehensive, client-centered care.³³ Research suggests that OTs possess the advanced knowledge necessary to address the broader spectrum of client needs, including mental health and social reintegration, which are critical for sustainable rehabilitation.³⁴

A critical challenge for Thai OT practice in community settings is the shortage of trained OTs, which has led to increased reliance on VHV for essential care. The reliance on VHV highlights the workforce gap, emphasizing the need for systemic strategies to expand OT capacity. Initiatives such as targeted education programs, scholarships, and rural practice incentives could help address this gap.³⁵ Additionally, policy advocacy is crucial to integrate OTs into primary care and community health initiatives. By leading capacity-building programs for VHV, OTs can ensure high standards of care while promoting a sustainable partnership model.^{35,36}

Mental health is an emerging focus within Thai OT practice in community settings, and OTs are well-positioned to lead in this area. Globally, OTs have successfully integrated mental health services into community-based care. For example, in Japan, community mental health centers run by OTs provide therapy and reintegration support.³⁷ While international models provide valuable insights, a key area for development is strengthening policy frameworks and workforce capacity to support OT-led mental health services in Thai communities. Incorporating mental health interventions into existing OT community models could address unmet needs and reinforce the profession's role in holistic, person-centered care.³⁸

Technology plays an increasingly important role in enhancing OT services worldwide, including in community settings. In developed countries, telehealth platforms,³⁹ monitoring technology,⁴⁰ and smart assistive technologies have been widely integrated into community and home-based rehabilitation programs, improving service coordination and accessibility. In Thailand, research on technology use in community OT remains limited, with existing studies focusing on information systems for service management³⁰ and the development of assistive devices.³¹ While these innovations suggest that technology can expand access to rehabilitation, particularly in underserved

areas, more evidence is needed to understand their long-term impact. Future research should assess the feasibility of telehealth applications, culturally adapted assistive technologies, and scalable digital interventions in Thai community OT settings.

The "Proactive Approach" model proposed by Phadsri *et al.*³² represents a unique conceptualization of OT practice in community settings in Thailand, emphasizing proactive policy, collaboration, integration of cultural contexts, and family-community participation. While this model aligns with CBR principles outlined by the World Health Organization,⁴¹ challenges such as limited policy support, workforce constraints, and inconsistent implementation strategies hinder its full integration. Although similar holistic approaches, such as the Collaborative CBOT model,⁴³ have been described in other contexts, persistent workforce shortages, policy limitations, and implementation challenges highlight the need for further development.

Despite the increasing recognition of community-based models, challenges remain in their implementation. Although Thai OT has evolved over the past 45 years, many CBOT frameworks still face barriers to full integration into healthcare systems. Existing limitations may be influenced by workforce shortages, service delivery challenges, or misalignment with broader policy frameworks. Further research is needed to clarify these barriers and explore strategies to enhance OT service effectiveness in community settings. Understanding the factors that have prevented community OT models from achieving widespread acceptance in Thai communities is essential for developing sustainable, Thai-driven OT frameworks that are both effective and culturally relevant.

Despite its potential, OT practice in Thai community settings faces systemic challenges, including limited funding and resource constraints. Advocacy for sustainable funding and resource mobilization is essential. Collaborative models that engage VHV as supportive partners while maintaining OTs in leadership roles could strengthen community rehabilitation services. Building effective partnerships with community organizations, healthcare providers, and policymakers will be critical to ensuring the long-term sustainability of OT initiatives.⁴⁴

OT practice in Thai community settings demonstrates innovative approaches, particularly in stroke rehabilitation and technology integration. However, its alignment with community-based occupational therapy (CBOT) principles remains limited. CBOT emphasizes a holistic, client-centered, and community-driven approach,⁶ yet much of Thai community OT remains rehabilitation-focused, prioritizing functional recovery over occupation-based, participatory models. The reliance on VHV enhances community integration but limits OTs' roles in program leadership and capacity-building. Strengthening OT involvement in service design could enhance alignment with CBOT by ensuring interventions are occupation-focused, collaborative, and culturally responsive.

Future research should explore how OT practice in Thai community settings can shift beyond impairment-

based rehabilitation toward a more integrated CBOT framework. Greater incorporation of occupation-based and participatory approaches is needed, particularly in mental health, aging, and social inclusion. While technology is used in rehabilitation, its potential to enhance community participation and accessibility remains underexplored. Evaluating the Proactive Approach model across different settings could inform strategies to better integrate CBOT principles. Longitudinal studies on sustainability and cost-effectiveness are also necessary to move Thai community OT toward a more holistic, empowerment-driven model. By addressing these gaps, OT practice in Thailand can better align with CBOT principles and more effectively serve diverse community needs.

Limitation and future research

This review is limited by the small number of included studies, which may not fully capture OT practice in Thai community settings. Future research should include diverse populations, as evidence on OT-led community services for aging populations is limited. Studies focusing on supporting aging in place, managing chronic diseases, and promoting participation in meaningful occupations within communities are essential. Additionally, most research focuses on ADL, quality of life, and social participation, with lacks the focus on other areas of occupations. Expanding these domains would provide a more comprehensive view of OT's role in community services. Many studies used single-group pre-post designs, limiting causal inferences. Future research should employ RCTs and comparative effectiveness studies for stronger evidence. Furthermore, studies on cost-effectiveness are needed to inform resource allocation and policy, as recommended by the WHO's CBR guidelines. Addressing these gaps will help develop sustainable, evidence-based OT services for Thai communities.

Conclusion

This scoping review highlights the evolving role of OT practice in community settings in Thailand, with a strong focus on physical rehabilitation, the integration of VHV, and emerging interests in mental health and technology adaptation. While the current model has demonstrated positive outcomes, the underutilization of occupational therapists in leadership roles limits the profession's potential to address broader needs, such as psychosocial reintegration, mental health, and geriatric care. To advance CBOT in Thailand, systemic strategies are needed to expand OT capacity, promote policy advocacy, and integrate culturally responsive, proactive approaches tailored to local contexts. Future research should explore diverse populations, long-term outcomes, and cost-effectiveness to strengthen the evidence base and ensure sustainable, equitable CBOT practices that enhance participation and QOL for Thai communities.

Conflict of interest

The authors declare no conflict of interest.

Funding

None

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Assessment of colistin resistance in *Enterobacteriaceae*: Comparison of the rapid polymyxin NP test, MicroScan system, and broth microdilution in Thailand

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ARTICLE INFO

Article history:

Received 16 January 2025

Accepted as revised 27 March 2025

Available online 12 April 2025

Keywords:

Colistin, *Enterobacteriaceae*, rapid polymyxin NP test, MicroScan system.

ABSTRACT

Background: Antimicrobial resistance is a global health crisis, with colistin serving as a last-resort antibiotic for the treatment of multidrug-resistant *Enterobacteriaceae*. However, colistin resistance is becoming increasingly prevalent, necessitating rapid and accurate susceptibility testing.

Objective: This study evaluated the rapid polymyxin NP (RPNP) test and the MicroScan antimicrobial susceptibility system compared to the reference broth microdilution (rBMD) method for detecting colistin resistance in colistin-resistant *Enterobacteriaceae* (CRE) isolates from clinical setting in Thailand.

Materials and methods: A total of 158 non-repetitive *Enterobacteriaceae* isolates (85 *Escherichia coli* and 73 *Klebsiella pneumoniae*) were collected from a tertiary hospital in Bangkok between August 2021 and January 2023. Colistin susceptibility was assessed using the RPNP, MicroScan system, and rBMD method following the Clinical and Laboratory Standards Institute (CLSI) guidelines. The categorical agreement (CA), very major errors (VME), and major errors (ME) of colistin resistance test results from the RPNP and MicroScan methods were determined by comparison with the rBMD method.

Results: Among the 158 *Enterobacteriaceae* isolates, 46.2% were intermediate and 38.6% were colistin-resistant by rBMD. The sensitivity of RPNP was 98.4% and the specificity was 95.9%, compared to 95.1% and 97.9% for MicroScan. Both RPNP and MicroScan achieved high CA (96.8%) with rBMD. Discrepancies, primarily in isolates with borderline MICs (1-4 mg/L), accounted for 4 of the 8 discordant strains. In addition, *mobilized colistin resistance-1 (mcr-1)* gene were detected in 5 colistin-resistant *E. coli* isolates with MIC of 8 mg/L.

Conclusion: Both the RPNP and MicroScan systems demonstrated high categorical agreement with the rBMD method, underscoring their reliability as practical tools for routine colistin susceptibility testing in clinical microbiology laboratories where rBMD may not be feasible.

Introduction

Antimicrobial resistance in bacteria is a critical and continuously increasing global health concern. Regarding many of the available antimicrobial agents are no longer effective in treating infections, leading to over 700,000 deaths annually from drug-resistant infections worldwide, a number that is projected to rise to 10 million deaths per year by 2050.¹ In Thailand, antimicrobial-resistant infections cause approximately 20,000-38,000 deaths

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doi: 10.12982/JAMS.2025.063

E-ISSN: 2539-6056

per year, resulting in an economic loss of up to 1.3 billion dollars annually.²

The resurgence of colistin as a therapeutic option is driven by rising carbapenem resistance, particularly in infections caused by multidrug-resistant (MDR) gram-negative bacteria.³ Colistin, or polymyxin E, is a cationic cyclic decapeptide that binds to the negatively charged lipid A of the lipopolysaccharide (LPS) in the outer membrane of gram-negative bacteria, disrupting the membrane and leading to bacterial cell lysis.⁴ Despite its renewed clinical relevance, colistin resistance is increasingly reported. Chromosomal mutations in genes such as *pmrA*/*pmrB* and *mcrB* can mediate colistin resistance by modifying lipid A, thereby reducing colistin's binding affinity.⁵ Moreover, efflux pumps contribute by expelling the drug from bacterial cells, lowering its intracellular concentration and effectiveness. Importantly, the 2015 discovery of the *mcr-1*, which enables plasmid-mediated horizontal transfer of colistin resistance, has heightened concerns regarding its global dissemination.⁵ This increasing resistance complicates treatment, and it is critical for clinical microbiology laboratories to accurately and promptly report colistin susceptibility to ensure appropriate patient management.

Antimicrobial susceptibility testing for colistin poses challenges, as colistin's large molecular size limits its diffusion in solid media, making simple methods like disk diffusion unsuitable for testing.⁶ Gradient diffusion strips are convenient for determining colistin MIC. However, their reliability is still questioned.^{7,8} The rBMD method recommended by the CLSI is considered the gold standard for colistin susceptibility testing.⁹ Still, it is labor-intensive and impractical for routine laboratory use. To address these limitations, faster colistin susceptibility tests have been developed, such as the RPNP test, which detects pH changes from glucose metabolism in bacteria resistant to colistin. This method provides results within 2-4 hrs and demonstrates high sensitivity and specificity.¹⁰ Therefore, the RPNP test is well-suited as a screening method, particularly in regions experiencing endemic resistance, such as those with widespread carbapenemase producers.¹⁰⁻¹⁴ However, there are some limitations to this method. It requires careful interpretation, as false positives can occur due to minor pH changes, and it may not perform as well with certain bacterial species or strains.

Many automated drug susceptibility testing systems have been implemented, such as MicroScan, VITEK, and Phoenix systems. These platforms provide efficient and standardized methods for determining bacterial susceptibility to antimicrobial agents, enabling rapid and reliable results to support clinical decision-making. In our country, the MicroScan system is widely used in hospital settings for antimicrobial susceptibility testing. Testing was conducted in a 96-well microplate, with two wells specifically designated for colistin testing at concentrations of 2 and 4 mg/L, while in the remaining wells were allocated for testing other antibiotics, enabling comprehensive susceptibility profiling in a single run. However, few studies have investigated the performance of

the MicroScan system in colistin susceptibility testing.^{15,16} In this study, we evaluated the efficacy of the RPNP test and MicroScan system in *Enterobacteriaceae* clinical isolates from Thailand by comparing them with the rBMD method.

Material and methods

Sample collection

A total of 158 non-repetitive *Enterobacteriaceae* isolates were included in this study, comprising 85 and 73 isolates of *E. coli* and *K. pneumoniae*. These isolates were collected from various clinical specimens at a tertiary hospital located at Bangkok Metropolitan between August 2021 and January 2023. The bacterial isolates were initially identified through standard microbiological and conventional biochemical methods, with further confirmation performed using the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) system (Bruker, Germany). The obtained *Enterobacteriaceae* isolates were stored at -80 °C in tryptic soy broth with 30% glycerol for further study.

Reference broth microdilution (rBMD)

Broth microdilution (BMD) was performed according to the CLSI guidelines for antimicrobial susceptibility testing of aerobic bacteria.¹⁷ Cation-adjusted Mueller Hinton broth (CAMHB) was prepared by adding the appropriate salts to achieve final concentrations of 20-25 mg/L of calcium and 10-12.5 mg/L of magnesium. The powdered colistin sulfate (Sigma-Aldrich, USA) was used for this study. Bacterial suspensions in sterile 0.85% saline were adjusted to a McFarland standard No. 0.5 and then diluted 100-fold in CAMHB to achieve a bacterial concentration of $1-2 \times 10^6$ CFU/mL. Subsequently, 50 μ L of the bacterial suspension was added to each well of a 96-well plate containing 50 μ L of CAMHB with colistin, prepared at concentrations ranging from 32 to 1 mg/L through 2-fold serial dilutions. This resulted in a final bacterial concentration of 5×10^5 CFU/mL in each well, with colistin concentrations ranging from 16 to 0.5 mg/L. The plates were incubated at 37 °C for 18-20 hrs. The MIC was determined as the lowest concentration of colistin at which the medium remained clear, indicating bacterial inhibition. Growth control (0 mg/L colistin) and sterile control (0.85% sterile saline) were included in the testing. The antimicrobial susceptibility was interpreted according to the CLSI guidelines, which define colistin breakpoints as resistant at MIC ≥ 4 mg/L and intermediate at MIC ≤ 2 mg/L.⁹

Rapid Polymyxin NP (RPNP)

RPNP was performed in accordance with the protocol described by Nordmann *et al.*¹⁰ The rapid polymyxin NP solution was prepared with 0.005% phenol red and 1% D(+)-glucose in CAMHB. For testing, the solution was supplemented with colistin sulfate at a 5 mg/L concentration. Bacterial suspensions were adjusted to McFarland standard No. 3, and 50 μ L of the suspension was transferred into wells of a 96-well plate containing

150 μ L of the rapid polymyxin NP solution, both with and without colistin (5 mg/L). The final bacterial concentration was 10^8 CFU/mL per well, and the colistin concentration in the test wells was 3.75 mg/L. Sterile 0.85% normal saline was used as a control, whereas *E. coli* ATCC 25922 and *E. coli* NCTC 13846 served as negative and positive controls, respectively. The plates were incubated at 37 °C and monitored for color changes every hour for up to 4 hrs. A shift in color from orange to yellow within 4 hrs indicated resistance to colistin, while an unchanged orange color after 4 hrs indicated susceptibility.

MicroScan system

The antimicrobial susceptibilities of *Enterobacteriaceae* tested were determined using the MicroScan antimicrobial susceptibility system with the NM EN47 panel (Beckman Coulter Diagnostics, USA). The wells were prepared with water containing PLURONIC, a non-ionic surfactant. Bacterial suspensions were prepared using the Prompt Inoculation System and inoculated using the RENOK inoculator system. The plates were then incubated for 16 to 20 hrs. Results were read automatically using WalkAway plus system. The antimicrobial susceptibility was interpreted following the CLSI guidelines.⁹

Quality control

E. coli ATCC 25922, *P. aeruginosa* ATCC 27853, and *E. coli* NCTC 13846 were utilized as quality control strains. The control strains were required to produce MIC values within the specified ranges. The expected MIC ranges were 0.25-2 mg/L for *E. coli* ATCC 25922, 0.5-4 mg/L for *P. aeruginosa* ATCC 27853,⁹ and 2-8 mg/L for *E. coli* NCTC 13846.¹⁸

Detection of *mcr-1* gene in colistin-resistant *E. coli* and *K. pneumoniae*

The presence of *mcr-1* gene in isolates displaying MIC ≥ 2 mg/L by rBMD was detected by PCR. Bacterial crude DNA was extracted by boiling methods. A pair of specific primers, CLR-F (5'CGGTCAGTCCGTTTGTTTC'3)

and CLR-R (5'CTTGGTCGGTCTGTAGGG'3) were used for amplification.⁴ The PCR reaction was performed in a total volume of 25 μ L, consisting of 1 $\times$ DreamTaq Green PCR Master Mix (Thermo Scientific™, USA), each primer at a concentration of 1 μ M, and 1 μ L of the boiled DNA template. The PCR conditions included an initial denaturation at 95 °C for 20 sec, denaturation at 95 °C for 10 sec, annealing at 64 °C for 30 sec, extension at 72 °C for 30 sec, repeated for 30 cycles, followed by a final extension at 72 °C for 5 min. The PCR products were analyzed using agarose gel electrophoresis with a 1.0% (w/v) gel. In this assay, crude DNA derived from *E. coli* NCTC 13846 was used as the positive control, and distilled water served as the negative control.

Statistical analysis

The colistin resistance test results from the RPNP and MicroScan methods for *E. coli* and *K. pneumoniae* isolates were analyzed and compared with the rBMD method. VME were defined as the percentage of instances where the rBMD method indicated resistance, but the RPNP/MicroScan systems showed intermediate results. ME referred to the percentage of isolates tested where the rBMD method indicated intermediate resistance, but the RPNP/MicroScan system reported resistance. CA were defined as the percentage of test results that match between the rBMD method and the RPNP/MicroScan methods. A high CA indicates that the test methods produce results consistent with the reference method, demonstrating their reliability.¹⁹

Results

By using rBMD, 97 isolates (61.4%) were identified as intermediately susceptible to colistin, and 61 isolates (38.6%) were classified as colistin-resistant. Among these, 13 *E. coli* and 48 *K. pneumoniae* isolates were found to be colistin-resistant (Figure 1). Among the isolates with MICs ≥ 2 mg/L, five colistin-resistant *E. coli* isolates (MIC=8 mg/L) were positive for *mcr-1*, whereas none of the *K. pneumoniae* isolates tested positive.

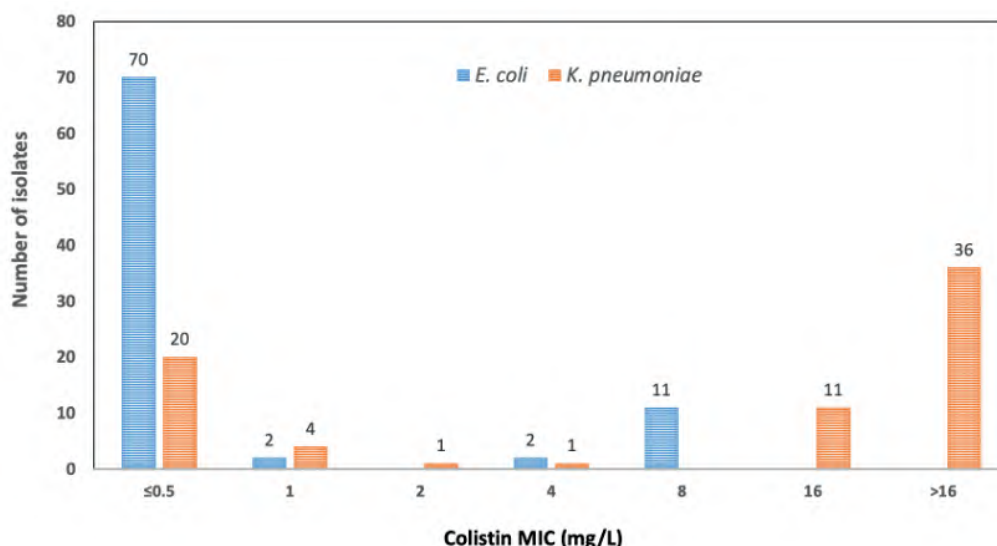


Figure 1. Colistin MIC for 158 isolates of *E. coli* and *K. pneumoniae* determined by rBMD.

The RPNP yielded negative results in 94 isolates (59.5%) and positive results in 64 isolates (40.5%), with 12 *E. coli* isolates and 52 *K. pneumoniae* isolates testing positive. Meanwhile, the MicroScan system detected colistin intermediate in 98 samples (62.0%) and colistin resistance in 60 samples (38.0%), including 12 *E. coli* isolates and 48 *K. pneumoniae* isolates (Table 1).

When comparing the colistin susceptibility results and MIC values between the RPNP and the rBMD, RPNP demonstrated agreement with rBMD in 153 isolates, achieving a CA of 96.8% (Tables 1 and 2). Of the tested isolates, 60 were categorized as colistin-resistant and 93 as colistin-intermediate. However, discrepancies were observed in four *K. pneumoniae* isolates, where rBMD classified them as intermediate (MIC ≤ 0.5 -2 mg/L), but the RPNP test yielded resistant results, suggesting potential overestimation of resistance by the RPNP method (Table 3).

The MicroScan system also showed a CA of 96.8%. The concordant results included 58 isolates as colistin-resistant and 95 as colistin-intermediate by both MicroScan and rBMD. However, discrepancies were noted in four *K. pneumoniae* isolates where colistin susceptibility results from MicroScan systems were inconsistent with those from the rBMD method (Table 3). In two of these isolates, colistin resistance was detected by the MicroScan system (MIC >4 mg/L), but rBMD was intermediate (MIC ≤ 0.5 mg/L). Conversely, in two other *K. pneumoniae* isolates, the rBMD method identified them as resistant (MIC ≥ 16 mg/L), while the MicroScan system yielded intermediate results (MIC ≤ 2 mg/L).

Additionally, in one *E. coli* isolate, colistin resistance was detected using the rBMD method (MIC=4 mg/L), but both the RPNP and MicroScan systems returned negative results.

Table 1. Results of RPNP and MicroScan system for the detection of susceptibility to colistin among *Enterobacteriaceae*.

rBMD Colistin MIC (mg/L)	N		RPNP		MicroScan system		
			Pos	Neg	>4	4	≤ 2
>16	36	Resistance	36	0	35	0	1
16	11		11	0	10	0	1
8	11		11	0	9	2	0
4	3		2	1	1	1	1
2	1	Intermediate	1	0	0	0	1
1	6		2	4	0	0	6
≤ 0.5	90		1	89	2	0	88

Note: MIC: minimum inhibitory concentration, RPNP: rapid polymyxin NP test, rBMD, reference broth microdilution, Pos: positive, Neg: negative.

Table 2. Performance of RPNP and MicroScan system to detect colistin resistance among *Enterobacteriaceae* (N=158) compared to rBMD.

Method	Genera	Performance (%)						
		Sensitivity	Specificity	PPV	NPV	CA	ME	VME
RPNP	Total	98.4	95.9	93.8	98.9	96.8	4.1	1.6
	<i>E. coli</i>	92.3	100.0	100.0	98.6	98.8	0.0	7.7
	<i>K. pneumoniae</i>	100.0	84.0	92.3	100.0	94.5	16.0	0.0
MicroScan	Total	95.1	97.9	96.7	96.9	96.8	2.1	4.9
	<i>E. coli</i>	92.3	100.0	100.0	98.6	98.8	0.0	7.7
	<i>K. pneumoniae</i>	95.8	92.0	95.8	92.0	94.5	8.0	4.2

Note: RPNP: rapid polymyxin NP test, CA: categorical agreement, PPV: positive predictive value, NPV: negative predictive value, ME: major error, VME: very major error.

Table 3. Discrepancies observed among *Enterobacteriaceae* with RPNP and MicroScan system compared to rBMD.

Species	rBMD		RPNP	MicroScan (mg/L)	Category error
	MIC (mg/L)	Result			
<i>K. pneumoniae</i> K4	≤0.5	I	Neg	>4	Major error
<i>K. pneumoniae</i> K47	≤0.5	I	Pos	>4	
<i>K. pneumoniae</i> K40	1	I	Pos	≤2	
<i>K. pneumoniae</i> K44	1	I	Pos	≤2	
<i>K. pneumoniae</i> K60	2	I	Pos	≤2	Very major error
<i>E. coli</i> E21	4	R	Neg	≤2	
<i>K. pneumoniae</i> K26	16	R	Pos	≤2	
<i>K. pneumoniae</i> K19	>16	R	Pos	≤2	

Note: MIC: minimum inhibitory concentration, RPNP: rapid polymyxin NP test, rBMD: reference broth microdilution, Pos: positive, Neg: negative, I: intermediate, R: resistance.

Discussion

Detecting colistin resistance in *Enterobacteriaceae* poses significant challenges due to the variability in resistance mechanisms, such as the presence of *mcr* genes and modifications of lipid A in the bacterial outer membrane. These factors complicate the accuracy and reliability of phenotypic testing methods, thereby impacting treatment decisions and patient outcomes. This study aimed to evaluate the performance of the RPNP test and MicroScan system for detecting colistin susceptibility in *Enterobacteriaceae* isolates, using rBMD as the gold standard. The findings revealed notable levels of CA for both methods, with RPNP and MicroScan systems achieving a CA of 96.8%. This agreement underscores the reliability of these methods for colistin susceptibility testing in clinical settings.

RPNP quickly detects colistin resistance by identifying the pH changes due to glucose metabolism. Resistant bacteria metabolize glucose in the presence of colistin, causing a color change from orange to yellow, with results available within 2-4 hrs.¹⁰ In this study, RPNP demonstrated high sensitivity (98.4%), with a positive predictive value (PPV) of 93.8% and a negative predictive value (NPV) of 98.9%. However, it exhibited an error rate of 4.1%, particularly in strains with MIC values in the borderline range (0.5-4 mg/L). Several possible explanations for the error observed in the RPNP method include difficulty in interpreting color changes, especially when they are subtle. This can lead to the misclassification of results, particularly near MIC breakpoints. The inoculum effect is another factor, as variations in the bacterial concentration can affect the pH change and subsequent color shift, leading to false positives or negatives. Additionally, borderline MIC values near the interpretive thresholds (such as MICs of 0.5-4 mg/L, as found in our results) can cause discrepancies and contribute to the observed error. Previous studies have highlighted similar limitations of in-house and commercial RPNP tests, suggesting that these challenges are consistent across different testing settings.²⁰

The MicroScan antimicrobial susceptibility testing system is an automated platform designed to identify bacteria and determine their antimicrobial susceptibility

profiles. Broth microdilution technology provides MIC values by detecting bacterial growth in the tested panel. In this study, the MicroScan system exhibited VME and ME rates of 4.9% and 2.1%, respectively. Comparatively, Jayol *et al.* reported a higher accuracy for MicroScan in detecting colistin resistance in *Enterobacteriaceae*, with no VME and an ME rate of 3.1%, suggesting possible variability in performance due to differences in study populations or methodologies.¹⁶ The differences in results between our study and previous studies could be attributed to several factors. First, variations in sample size, source, and isolate composition may have played a role, with previous studies potentially including fewer strains with borderline MIC values. Second, differences in the prevalence of resistance mechanisms between studies could impact the ability of the system to detect resistance with consistent accuracy. A notable limitation of the MicroScan panel is its restricted testing range for colistin concentrations, which is confined to 2 and 4 mg/L. This narrow range makes it difficult to accurately determine the MIC values for isolates with MICs outside this range, potentially leading to under- or overestimation of resistance. As such, the MicroScan system may be more suitable as a screening tool rather than for comprehensive MIC determination. These limitations reduce the panel's utility for monitoring resistance trends or validating results in clinical settings where precise MIC data are critical for guiding treatment decisions. To improve the accuracy of MicroScan, laboratories may consider using confirmatory testing such as rBMD and colistin broth disk elution (CBDE) with a broader concentration range. Expanding the MIC dilution range in automated systems may also strengthen the reliability of colistin susceptibility reporting.

We performed PCR to detect the *mcr-1* in isolates with MIC ≥2 mg/L, focusing on those with phenotypic evidence of colistin resistance or reduced susceptibility. In this study, *mcr-1* gene was detected in five *E. coli* isolates but was absent in *K. pneumoniae*. This finding aligns with previous studies in Thailand, which reported a higher prevalence of *mcr-1* in *E. coli* (29.7 %) compared to *K. pneumoniae* (1.4 %), suggesting that plasmid-mediated colistin resistance may be more common in *E. coli* in Thailand.²¹ All five *mcr-1*-harboring

E. coli isolates in our study exhibited colistin MIC values of 8 mg/L as determined by rBMD and were identified as colistin-resistant by both the RPNP and MicroScan methods. This finding aligns with most studies reporting MIC values for colistin in *mcr-1*-positive *E. coli* typically ranging from 4 or 8 mg/L.^{5,22,23} Moreover, *mcr-1*-positive *E. coli* can also display low or no phenotypic resistance.²⁴ While the clinical relevance of polymyxin therapy for *mcr*-positive isolates remains uncertain, it is important to recognize that *mcr-1* may facilitate the selection of chromosomal mutations, potentially leading to high-level colistin resistance (HLCR).²⁵ This study focused on *mcr-1* due to its predominant prevalence in *Enterobacteriaceae*. However, other *mcr* variants (e.g., *mcr-2*, 3, 8 and 9) are acknowledged in Thailand.^{26,27} These variants may confer low- to moderate-level colistin resistance, often with MIC values ranging from 2 to 8 mg/L. However, the phenotypic expression associated with these genes can be variable, resulting in minimal or no detectable resistance. Thus, reliance on phenotypic detection alone may be insufficient. Enhancing laboratory protocols to include targeted testing of isolates with borderline MIC values (e.g., 2 mg/L) using complementary methods or molecular detection of *mcr* genes is crucial for accurate and comprehensive resistance monitoring.

The limitations of this study include the relatively small sample size and collection of isolates from only one hospital. Moreover, the molecular mechanisms underlying colistin resistance have not yet been determined. This might limit the application of our findings to other geographical areas because of the differences in the mechanism of antimicrobial resistance. Another notable limitation of this study was the limited number of isolates with MIC values at borderline interpretive thresholds (e.g., 1-2 mg/L). This could affect the robustness of the data in evaluating the performance of the RPNP and MicroScan systems in accurately detecting resistance within these critical ranges. Future studies with a greater representation of borderline MIC values would help to provide a more comprehensive understanding of the reliability of each method under these conditions.

Conclusion

This study provides critical insights into the performance of different methods for detecting colistin resistance in *Enterobacteriaceae* isolates, including RPNP test, MicroScan, and rBMD methods. The RPNP and MicroScan systems both demonstrated high CA (96.8%) with the rBMD method, underscoring their reliability as practical tools for routine colistin susceptibility testing in clinical microbiology laboratories. However, with 4.12% ME for RPNP and 4.92% VME for MicroScan, this study highlights the challenges of relying solely on these methods. Discrepancies frequently arise when MIC values are near the interpretive threshold, and the limited testing range of the MicroScan system further complicates accurate resistance detection. Therefore, while these methods are suitable for initial screening, confirmation with rBMD or expanded testing protocols is crucial for

borderline isolates (2-4 mg/L). Nevertheless, the small sample size, particularly with borderline MIC values, and the lack of a detailed investigation into the resistance mechanisms are key limitations of this study, highlighting the need for further study.

Conflict of interest

The authors declare that there is no conflict.

Ethical approval

This study was conducted in accordance with the ethical guidelines approved by The Human Research Ethics Committee of Thammasat University No.3 Sciences (COE 017/2565).

Acknowledgments

This study was supported by the Thammasat University Research Fund, Contract No. TUFT0007/2566 and Thammasat University Research Unit in Medical Technology and Precision Medicine Innovation.

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Variation of sputum expectoration within day in bronchiectasis: Preliminary observational descriptive study

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ARTICLE INFO

Article history:

Received 25 October 2024

Accepted as revised 9 April 2025

Available online 12 April 2025

Keywords:

Sputum expectoration, sputum weight, airway clearance, bronchiectasis, physical therapy.

ABSTRACT

Background: Effective prescription of physical therapy airway clearance (ACT) is crucial for bronchiectasis patients, yet the optimal timing and frequency remain undefined. Understanding sputum expectoration patterns may determine the most suitable times for patients to undergo ACT.

Objective: This study aimed to investigate the daily sputum expectoration patterns in patients with bronchiectasis.

Materials and methods: Sputum was collected from ten bronchiectasis patients at home over three days, during morning (6 to 9 am), daytime (9 am to 6 pm), and evening (6 to 9 pm) periods. Collected sputum was centrifuged to remove saliva, weighed, and then converted to grams per hour for analysis.

Results: A total of 90 sputum containers were collected over three days from 10 patients. Most patients (N=8) expectorated a median (IQR) of 14.34 (7.96, 23.67) gm, showing a higher trend in the morning (2.07 (1.16, 4.03) gm/hr), followed by evening (1.18 (1.86, 1.92) g/hr) with the least during daytime (0.41 (0.32, 0.82) gm/hr). A minority of patients (N=2) with massive sputum production (72 and 63.98 for participant 1 and participant 2 respectively) exhibited the highest rate of expectoration during daytime (5.71 /hr), followed by the evening (3.83 /hr) and morning (1.69 /hr) on average over three days.

Conclusion: Most bronchiectasis patients exhibited higher rates of sputum expectoration in the morning and evening. Therefore, it is recommended to administer physical therapy for airway clearance twice daily, in the morning and evening, to prevent accumulation and enhance clearance.

Introduction

Bronchiectasis is an uncommon disease characterized by permanent distortion of the bronchi.^{1,2} This structural abnormality promotes the stasis of mucus in the airways and lungs.^{2,3} Moreover, mechanism such as inflammation and oxidative stress are often remarked in this disease which corresponding the underlying mechanism for mucus hypersecretion.^{4,5} Mucus stasis, coupled with mucus hypersecretion in bronchiectasis, creates a favorable environment for bacterial colonization, promoting persistent infection, exacerbating the progression of lung disease,

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doi: 10.12982/JAMS.2025.064

E-ISSN: 2539-6056

and perpetuating the vicious cycle.^{2,6} Therefore, acute exacerbation is common, finally lead to in further deterioration in lung function and decline in quality of life.

To solve this problem, physical therapy intervention emphasizes on secretion elimination. Numerous studies have investigated the benefits of secretion clearance through various airway clearance techniques, often using sputum expectoration weight or volume as indicators of treatment efficacy.⁷⁻¹⁰ However, these studies did not specify intervention times. In addition, there are no clear guidelines recommending the physical therapists on the optimal time and frequency to focus airway clearance intervention on patients with bronchiectasis. Moreover, physical therapy airway clearance is usually prescribing at the time of convenience with no specific time and frequency of ACT in daily basis in clinical practice.

Airway clearance may depend on the time of day, influenced by factors such as diurnal variations in lung function, airway inflammation, ciliary activity, ventilation, cough reflex, airway pressure on cilia cells, and vagal tone.¹¹⁻¹⁸ There could be a pattern of sputum expectoration throughout the day in patients with bronchiectasis. Understanding this pattern may help optimize the timing and frequency of airway clearance interventions. We hypothesized that sputum expectoration in bronchiectasis patients follows a distinct daily pattern, which could guide physical therapists in effectively managing secretion clearance. Accordingly, this study aims to determine the nature and variation of sputum expectoration at three different times of the day in relation to responsibilities of physical therapists managing in this patient population.

Materials and methods

Design and participant

This was descriptive study finding out within day variation of sputum production in relation to ACT prescription in this patient with bronchiectasis. The participants were recruited from Khon Kaen Hospital, Srinagarind Hospital, and Nam Phong Hospitals, Thailand. Participants with bronchiectasis (X-rays, CT chest by chest physician) who were age >18 years, sputum production >2 teaspoons, good cooperation & communication and stable vital sign were included in this study. History of pulmonary embolism, empyema, pleural disease, pulmonary fibrosis, hemoptysis, SpO₂ <90%, active pulmonary tuberculosis, exacerbation within 3 months, any cancer, cardiac dysfunction & hemodynamic instability, history of brain aneurism or retinal detachment, recent facial, oral, or skull surgery, abdominal aneurism, neurological diseases such as stroke and pregnancy were excluded. All participants were asked to sign an informed consent before start data collection. The researcher also asked about lifestyle of every participant before beginning of the study.

Sputum collection procedure

Patients were asked to collect sputum for three consecutive days at home during three-time intervals: morning (6 to 9 am), daytime (9 am to 6 pm), and evening (6 to 9 pm). Containers with labeled lids specifying the time interval and date were provided. Patients were instructed to expectorate sputum directly into the containers and tightly seal the lids after each expectoration. They were also asked to bring the sputum containers with them when going out. After each collection period, participants were instructed to immediately store the containers in the freezer. Patients were also advised not to swallow any sputum. During the sputum collection period, mucolytic medications were suspended, while other prescribed medications were continued. Additionally, participants were given an estimated minimum water intake based on their body surface area according to Gaspar with a minimum of 1500 ml/day recommended for those with a very low body surface area.¹⁹

The sputum samples collected were centrifuged at 4,700 rpm at 4 °C for 20 mins. After that the supernatant saliva were removed and were weighed (using the METTLER TOLEDO PL202S). To calculate rate per hour of sputum expectoration, the weight of the sputum without saliva from each interval was divided by the collection hours of that interval.

Statistical analysis

The Shapiro-Wilk test was applied to assess the normality of the data distribution. Differences in sputum expectoration rates per hour across collection intervals were analyzed using the Friedman test, followed by post hoc Dunn's test for pairwise comparisons. A $p < 0.05$ was considered significant. Descriptive statistics, including numbers, medians, and interquartile ranges, were used to summarize the data and inform the discussion. Based on the participants' sputum expectoration rates in the morning, daytime, and evening, the possible patterns were categorized as follows: Finding I indicated that the highest expectoration rate occurred in the morning over the three-day average, whereas Finding II showed that the highest expectoration rate occurred during the daytime over the three-day average. Finding III was expected to show that the highest expectoration rate occurred during the evening.

Results

Ten participants with bronchiectasis were enrolled between September 2022 and October 2024. The characteristics of participants were shown in Table 1. The mean age was 65.0 ± 10.3 years. Five participants were male and the other five participants were female. Moreover, 3 participants affected ≤ 2 lobes and 7 participants affected > 2 lobes. The average total CAT score was 13.50 ± 6.86 .

Table 1. Participants' characteristics.

Characteristics	Value
Gender (N=10) ^a	
Male	5 (50)
Female	5 (50)
Age (year) (N=10) ^c	65.0±10.3
Body mass index (kg/m ²) (N=10) ^c	19.00±3.16
Lobes affected ^a	
≤ 2 lobes	3 (30)
>2 lobes	7 (70)
Lung's function (N=6) ^c	
IC (L)	1.23±0.18
IC %pred	72.70±22.14
SVC (L)	1.63±0.24
SVC%pred	66.08±10.36
FEV1 (L)	0.93±0.29
FEV1%pred	52.17±20.20
FVC (L)	1.44±0.19
FVC%pred	65.33±17.29
FEV1/FVC	64.80±19.81
PEF (L/min)	206.80±77.40
PEF%pred	64.83±31.08
Comorbid (N=10) ^a	
COPD	4 (40)
Diabetes	2 (20)
Hypertension	1 (10)
Medications (N=10) ^a	
Doxofylline	1 (10)
Ipratropium bromide + Fenoterol hydrobromide	3 (30)
Tiotropium / olodaterol	4 (40)
Fluticasone propionate /Salmeterol xinafoate	1 (10)
Salbutamol	3 (30)
Tiotropium bromide	2 (20)
Budesonide/ formoterol fumarate	1 (10)
Modified Medical Research Council Dyspnea Scale (N=10) ^a	
Grade 1	7 (70)
Grade 2	2 (20)
Grade 3	1 (10)

Table 1. Participants' characteristics (*continued*).

Characteristics	Value
COPD assessment test score (N=10)	
Cough ^b	3 (1.75, 4)
Phlegm in chest ^b	3 (2, 4.25)
Chest tightness ^b	0 (0, 0.5)
Breathless on walk up a hill or one flight of stairs ^b	2 (0, 2)
Limitation of activities at home ^b	0.5 (0, 1)
Confidence of leaving home ^b	1 (0, 3)
Sleep soundly ^b	1.5 (0, 3.25)
Energy ^b	1.5 (0, 3.25)
Total score ^c	13.50±6.86
Leicester Cough Questionnaire (N=10) ^c	
Physical	4.5±0.8
Psychological	4.5±0.9
Social	4.6±1.2
Total	13.6±2.7

Note: BMI: body mass index, IC: inspiratory capacity, %pred: % predicted, SVC: slow vital capacity, PEF: peak expiratory flow. The data of lung's function for 4 participants were not available due to spontaneous cough during the spirometry procedure. Data are presented as a: number (%), b: median (Q1,Q3), c: mean±SD.

Figure 1 showed the total sputum wet weight in day 1, day 2, day 3 (N=10). The median (IQR) weights for day1, day2 and day3 were 20.17 (9.73, 40.61) gm, 18.77 (8.50, 39.79) gm and 15.02 (8.17, 45.35) gm, respectively. There

were no significant different between day1, day 2 and day 3. As for average 3 days of sputum wet weight, median (IQR) weight of 9.07 (8.27, 48.55) gm was observed.

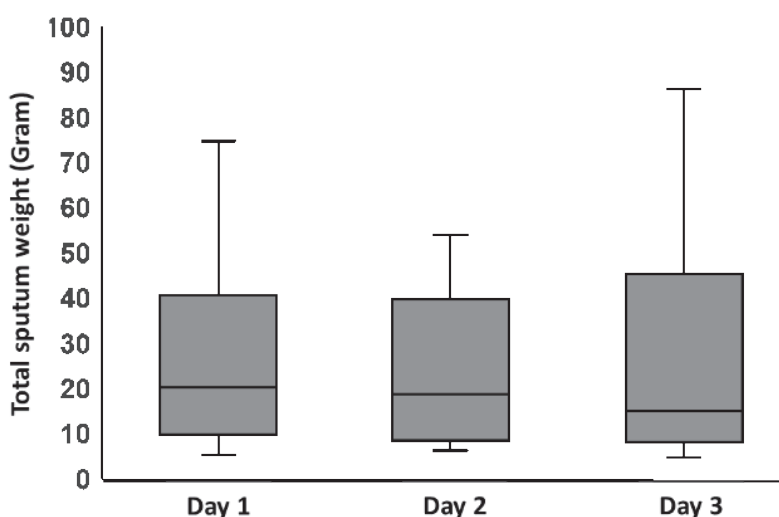
**Figure 1.** Sputum wet weight in three different days (N=10).

Figure 2 displayed the rate of sputum expectoration per hour for three distinct periods of finding I (N=8). The median (IQR) of total amount of sputum expectoration was 14.34 (7.96, 23.67) gm among finding I participants. For the morning, daytime, and evening periods, the median (IQR) rates per hour were 2.07 (1.16, 4.03) gm/hr, 0.41 (0.32, 0.82) gm/hr, and 1.18 (1.86, 1.92) gm/hr. There was statistically significant difference between morning and daytime for an average of 3 days ($p=0.01$). No statistically significant difference was found between morning and evening ($p=0.24$) as well as daytime and evening ($p=0.24$).

The rate per hour of sputum expectoration among in finding II (N=2) was shown in Figure 3. The amount of

total sputum expectoration were 72 gm and 63.98 gm for participant 1 and participant 2 respectively in this pattern. The median of sputum expectoration rate per hour was 1.69, 5.71 and 3.83 gm/hr for morning, daytime, and evening period for 3 days average.

In the interview response of participants' daily activities, all participants in finding I (N=8) reported no special activities and stated that they spent most of their time at home, including during the daytime period. In finding II, both participants (N=2) worked during the daytime period, managing their tasks, which included frequent walking and driving.

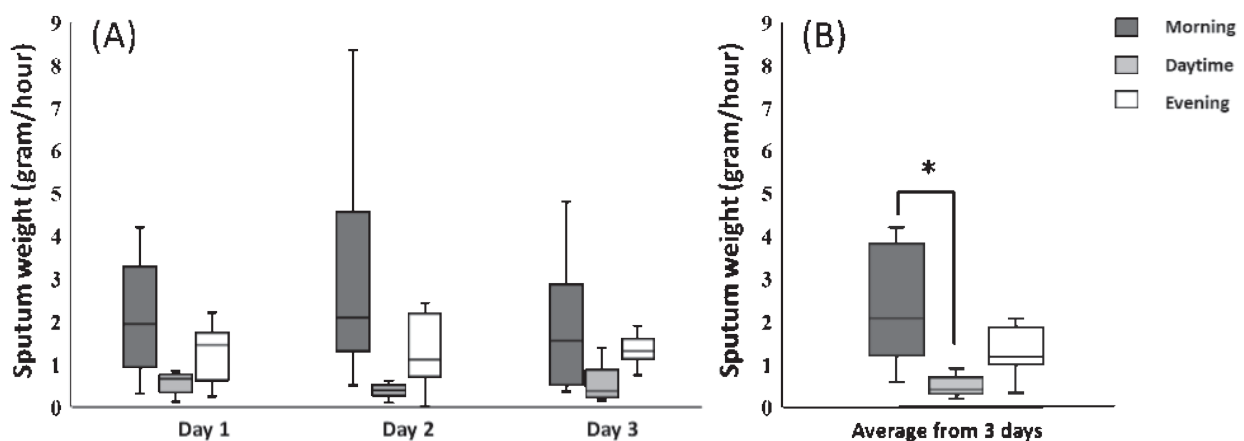


Figure 2. Sputum changes in three different days of finding I. A: sputum rate per hour (N=8), B: sputum rate per hour in average of three days (N=8). Significant between morning and daytime was found ($p=0.01$, the Friedman test, followed by post hoc Dunn's test for pairwise comparisons)

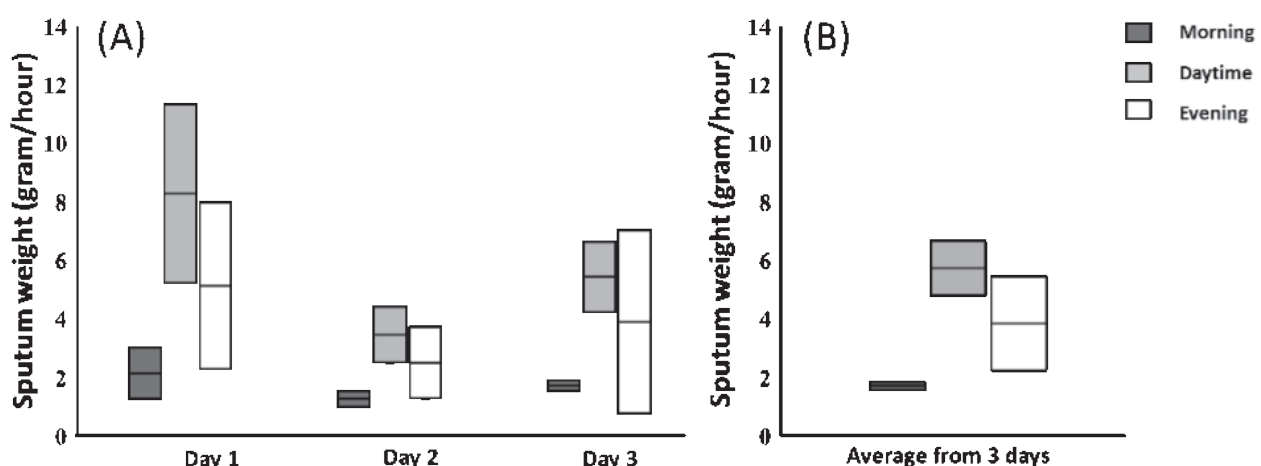


Figure 3. Sputum changes in three different days of finding II.

A: sputum rate per hour in three different days (N=2), B: sputum rate per hour in average of three days (N=2).

Discussion

This study demonstrated the nature of sputum production in ten stable bronchiectasis with the mean age of 65.0 ± 10.3 years. Our data showed that there was medium impact on health-related quality of life (CAT score 13.50 ± 6.86) in our stable bronchiectasis patients.

Concerning with the sputum wet weight, the present study done the collection of sputum from 6 am to 9 pm for three consecutive days. Median sputum wet weight per day for average three days were 20.17 (IQR, 9.73- 40.61) gm. Additionally, we observed no significant differences in the total sputum expectoration across day 1, day 2, and day 3. This suggests that sputum production is consistent in stable patients diagnosed with bronchiectasis.

The natural respiratory secretion clearance relies on two mechanisms. The first is mucociliary clearance (MCC), where airway cilia act like an escalator, propelling mucus in the cephalad direction. Once the mucus reaches the throat, it is either swallowed and delivered to the gastrointestinal tract for degradation or, in cases of excess, expelled through coughing as sputum. The second mechanism is airflow-dependent clearance, particularly through coughing, serving as a backup mechanism for secretion clearance when MCC is insufficient.²⁰

In the present study, the variation of sputum expectoration revealed 2 findings. Most of the patients in this study was observed as finding I where the trend of highest sputum production rate in the morning, followed by evening, and the lowest in the daytime across all three collection days. Importantly, there was a significant difference in the rates per hour of expectoration between morning and daytime in average of three days.

The elevated morning sputum expectoration in finding I could be attributed to the accumulation of sputum overnight. One explanation is that respiratory secretion production increases during sleep due to vagal stimulation.¹⁵ Additionally, the cough reflex is diminished during sleep, making lung clearance primarily reliant on mucociliary activity.¹⁷ Recent studies have indicated potential correlations between tidal breathing and mucociliary clearance rates. This is because the regulation of airway surface nucleotides and nucleosides occurs through the release of ATP, which is stimulated by the mechanical stress induced by tidal breathing.^{12,13} Yet, during sleep, ventilation is notably reduced due to decreased tidal volume sleeping.¹⁶ All these factors could contribute to mucus retention overnight. As a result, when individuals get up, a shift occurs with intensified breathing, potentially aiding in the expulsion of the accumulated secretions. Therefore, physical therapist should emphasize on airway clearance techniques during morning period to reinforce the clearance of accumulated secretions.

The daytime sputum expectoration in finding I was the least compared to the other times of a day. The possible explanation is that it might be related to the positioning change and physical activities during daytime. The benefits of physical exercise on secretion clearance have been demonstrated in other respiratory disease such as cystic fibrosis.²¹⁻²³ The possible mechanism through

which active physical activity aids in secretion clearance is the release of exercise-induced catecholamines, which stimulate cilia activity and trunk oscillation as well as increased ventilation exercise might exert mechanical stress on the airways, leading to the release of ATP. All of these might enhance the MCC.^{12,21-23} Therefore, the enhancing MCC might be sufficient to carry the newly produced secretion immediately. Since removing of secretion by MCC at that moment is efficiently and steady with not massive amount, secretion reaching to the throat might deliver to gastrointestinal tract. For this reason, it might be explained why daytime sputum expectoration the least was in finding I.

Regarding the evening period of finding I, the expectation was higher than during the daytime but lower than the morning period. This may be due to the same reason as the higher sputum expectoration rate in the morning period in this finding, as the evening time might also have less transition and fewer activities compared to the daytime; therefore, the accumulation of mucus might take place. This accumulated mucus could then trigger coughing, resulting in expectoration. Therefore, if physical therapy airway clearance is performed during the evening period, it might reduce sputum retention in the nighttime, allowing patients to rest more comfortably.

As for finding II, a minority of patients in this study (only 2 patients), the finding of sputum expectoration was different from finding I, where the rate of expectoration was greater during the daytime. When comparing the daily sputum expectoration, finding II participants showed a much larger amount of sputum production (72 gm and 63.98 gm for participant 1 and participant 2 respectively) than finding I (median (IQR) 14.34 (7.96, 23.67) gm, indicating that finding II participants had a greater secretion production from the lung compared to participants in finding I. Although greater activities and changes in positioning might enhance MCC in finding II participants during the daytime as in finding I, the massive amount of sputum production in these participants might result in a significant clearance of secretions by MCC and could also induce airflow-dependent clearance. For this reason, it might be explained why the greater rate of expectoration during the daytime in finding II. Another possible explanation is that these two participants engaged in more physical activity, such as frequent walking, during the daytime (from interviewing the participants), which may have contributed to improved secretion clearance. However, given the small sample size of only two participants and no quantitative data of physical activity, it is difficult to draw firm conclusions from this finding.

This study has several strengths. It is the first to identify the natural pattern of sputum expectoration in bronchiectasis patients without the use of mucolytics. However, the usual bronchodilator of the participants was allowed to be taken, which might affect the secretion clearance. Furthermore, by collecting sputum over three consecutive days, our research offers a more accurate portrayal of expectoration patterns. We are also aware limitations in our study. We collected sputum at three

distinct times of the day, with notably broad intervals: morning (6 to 9 am), daytime (9 am to 6 pm), and night (6 to 9 pm). A more detailed understanding of sputum nature and production in bronchiectasis might have been achieved with shorter collection intervals, such as hourly. However, we chose these three different intervals intentionally to accommodate the convenience of our participants' daily living because many participants faced difficulties in storing multiple containers in the freezer compartment of their refrigerators, and some had daily schedules that made it burdensome to carry several containers, especially during the daytime. Our study was limited to ten participants primarily because bronchiectasis patients with daily sputum production are uncommon in our region. Over a two-year screening period, 66 bronchiectasis patients were assessed, of whom 48 (72%) reported no or insignificant daily sputum production. Consequently, only 13 (18%) met the inclusion criteria, and 10 ultimately participated in the study. Interestingly, according to King *et al.*, 2006, most bronchiectasis patients exhibit daily sputum production.²⁴ This discrepancy might be attributed to regional differences, which could influence the underlying etiology. As for determining the physical activities of participants, we only interview the participants about their daily routine such as gardening, walking, sedentary tasks, and exercise. The counting of the steps per day is a popular method to determine physical activities. However, it cannot represent the different time activities and positioning change, one factor that impact the clearance. Moreover, we were unable to classify the severity of bronchiectasis or discuss its relationship with the variation in sputum expectoration throughout the day. The FACED score and the Bronchiectasis Severity Index are the most widely accepted classification systems for bronchiectasis severity, both of which require FEV1 measurements and the presence or absence of *Pseudomonas aeruginosa* in sputum cultures.²⁵

However, in our study, some participants could not complete the lung function test due to spontaneous coughing during the forced vital capacity maneuver. Additionally, sputum bacterial culture results were unavailable for some participants, preventing us from effectively using these classification systems. As a result, only four participants could be classified by severity in our study. Therefore, further study should be carried out with large samples of bronchiectasis with subgroup pattern of specific lung functions and severity.

Conclusion

Most patients exhibited a higher trend in expectoration during the morning, which could be attributed to nocturnal accumulation. Additionally, there was a higher trend in expectoration during the evening period. However, daytime expectoration was the least in most patients, possibly due to greater mucociliary clearance related to activities and positional changes during this period compared to others. Therefore, to prevent accumulation and enhance clearance, physical

therapy ACT should be performed at least twice a day—once in the morning and once in the evening.

Ethical approval

Approval of this study was obtained by Center of Ethics in Human Research, Khon Kaen University, Thailand (HE651328).

Conflict of interests

We declare no conflict of interest in this study.

Acknowledgements

This research was supported by dissertation, thesis, and independent study grants from the Faculty of Associated Medical Sciences, Khon Kaen University. We also appreciate funding from the Postdoctoral Training Type 1 Grant, Faculty of Associated Medical Sciences, Khon Kaen University. Additionally, this study received support from the Khon Kaen University Scholarship for the Fundamental Fund (2023) and the National Science Research and Innovation Fund (NSRF) Research Grant for New Scholars (2022).

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The effects of computer-based cognitive training on attention and working memory in Thai older adults with mild cognitive impairment

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ARTICLE INFO

Article history:

Received 5 November 2024

Accepted as revised 3 April 2025

Available online 12 April 2025

Keywords:

Attention, working memory, mild cognitive impairment, computer-based training, older adult.

ABSTRACT

Background: Mild cognitive impairment (MCI) is an intermediate state occurring between healthy aging and dementia among the elderly which result in cognitive, especially regarding attention and working memory. However, the declined state can be abated if training or stimulations are promptly promoted by a computer-based cognitive training program.

Objective: The aim of this study was to explore the effects of the Neofect Cognition as a computer-based cognitive training program on attention and working memory in Thai older adults with MCI.

Materials and methods: Twenty-three Thai older adults with MCI were divided into two groups; the experimental group (N=13) receiving the Neofect Cognition program for 6 executive weeks, 3 times per week, 45 minutes per session, and the control group (N=10) receiving education about MCI. Data was collected by assessing before and after participating in the program using the Trail Making Test (TMT) and the Memory subtest of the Thai Cognitive-Perceptual Test (Thai-CPT).

Results: The results showed that the experimental group had a statistically significant difference of attention (TMT-B) and working memory scores, while the control group had no statistically significant difference for both attention and working memory. Furthermore, there was a significant difference between the groups in attention (TMT-B) and working memory.

Conclusion: From the results, it can be stated that the computer-based training program (Neofect Cognition) might improve attention and working memory in Thai older adults with MCI.

Introduction

Mild cognitive impairment (MCI) is a condition in which individuals show cognitive impairment with minimal impairment of daily living, is in an intermediate state between healthy aging and dementia.¹ MCI is considered to be a 6-30 % risk for developing dementia.² Clearly, MCI is a risk factor for dementia.³⁻⁵ A review of the prevalence of MCI worldwide was published in 2020-2023, which showed it arising at a rate of 12 to 71.4 % and that high-risk factors associated with MCI were age and educational level.⁶⁻⁹ MCI causes cognitive deterioration and distress in family, caregivers, and the economy of a country.¹⁰ However, the declined state can be abated if training or stimulations known as “cognitive interventions” are promptly promoted and utilized.^{1,11}

Cognitive interventions aim at improving or maintaining cognitive abilities¹² and are divided into 2 types:

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doi: 10.12982/JAMS.2025.065

E-ISSN: 2539-6056

pharmacological and nonpharmacological cognitive intervention.^{13,14} The pharmacological cognitive interventions have potential agents for slowing or reversing cognitive decline but have limitations in the efficacy of treatment, and evidence suggests that these do not improve cognitive function.¹⁴⁻¹⁶ This is the key reason for paying particular attention to nonpharmacological treatment.¹⁴ Previous studies have shown that older adults with MCI improved cognitive function after receiving nonpharmacological treatment.^{14,17-19} Examples of non-pharmacological interventions are physical exercise, music therapy, behavior therapy, education and group training, and technology-based training.

Nowadays, technology-based training is widely used for improving cognitive function. It is not only useful for improving cognitive function but also has several advantages over traditional intervention.^{15,18,20-23} For example, it can motivate patients and is enjoyable; it is flexible and can be personalized; and it provides immediate feedback using a game format. Moreover, the computer-based training also has positive effects on psychosocial function in older adults with MCI.²¹ Based on the benefits of the computer-based training program, the rapid growth in the number of older adults in Thailand,²⁴ and the problems resulting from MCI, we employed a computer-based cognitive training program having Thai cultural aspects that can be tailored to target specific cognitive domains affected by MCI.²⁵

The Neofect Cognition is a computer-based game used for improving cognitive functions.²⁵ It can be used to stimulate 4 domains of cognition, including attention, memory, executive function, and visuospatial. The Neofect Cognition consist of 10 games in each domain. For example, 1) catching chameleon; game in attention domain; challenges patients to pay selective attention and divided attention, 2) making phone call; game in memory domain; challenges patients to pay working memory, 3) catching mole; game in executive function domain; challenges patients to use organizational skills and problem solving and 4) separate image; game in visuospatial domain. Previous studies in Asian countries involving various groups of participants showed that the Neofect Cognition has beneficial effects on cognition for stroke patients,²⁶ brain injury patients,²⁷ Alzheimer's patients,²⁸ ADHD children,²⁹ and high-functioning autism spectrum disorder.³⁰ Nevertheless, applying computer-based cognitive training with older adults with MCI in Thailand has been limited, especially in occupational therapy profession. Although the Neofect Cognition was developed in Asia, there is a lack of evidence, which has investigated the effectiveness and feasibility of the Neofect Cognition for Thai older adults with MCI.

The aim of this study was to investigate the effect of computer-based cognitive training using the Neofect Cognition on attention and working memory in Thai older adults with MCI.

Materials and methods

A quasi-experimental design, controlled study with pre-test and post-test was conducted with 23 Thai older adults having mild cognitive impairment (MCI) in Chiangmai, Thailand, from December 2021 to October 2023. This study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University (Study Code: AMSEC-64FB-006). The participants were divided into two groups with purposive sampling method: 1) the control group, which received education about MCI; 2) the experimental group, which received education about MCI and a computer-based training program using the Neofect Cognition for 6 executive weeks, 3 times per week, 45 minutes per session. The diagnosis used for MCI was based on the DSM-5, conducted by the geriatric psychiatrist through a clinical interview.

Inclusion and Exclusion criteria: This study included the participants as the following criteria: 1) age 60-79 years; 2) elementary education level 4 (Prathom 4 in Thai education system) or upper; 3) none of stroke and brain injury history; 4) capability with normal arm movement; 5) none of visual and auditory problems; 6) The Barthel index for activity of daily living scores equal to 12 or greater; 7) MSET-10 scores over 17; 8) MoCA-B scores below 25; 9) Thai version of the Geriatric Depression scores below 6; 10) no dementia; and 11) the consent obtained to the participation in computer-based intervention. Furthermore, the withdrawal criteria were obtained as the followings: 1) participants stating a possibility of quitting; 2) participants experiencing limited movement or brain injury while participating in the program. Additionally, the participants who were absent more than 3 times and/or those who currently used other cognitive training programs, would be excluded from the study.

The sample size estimation was derived from a related conducted by Kim and co-authors.³¹ The G*Power program (effect size=0.77, power of test=0.95, α =0.05). The calculation produced 21 people in each group, and we added 20% in case of dropout. Finally, 50 older adults with MCI were recruited. One hundred and forty people were invited to participate in the study; 101 were excluded due to unwillingness to participate, and 12 were excluded because they did not meet the criteria for the MCI. Finally, 27 participants were included in this study; 15 were in the experimental group and 12 in the control group. However, during the intervention program periods, 2 in experimental group were excluded due to unwillingness and absent to received program more than 3 times. Moreover 2 in control group were excluded due to accident. Finally, leaving 23 participants for the final analysis (Figure 1).

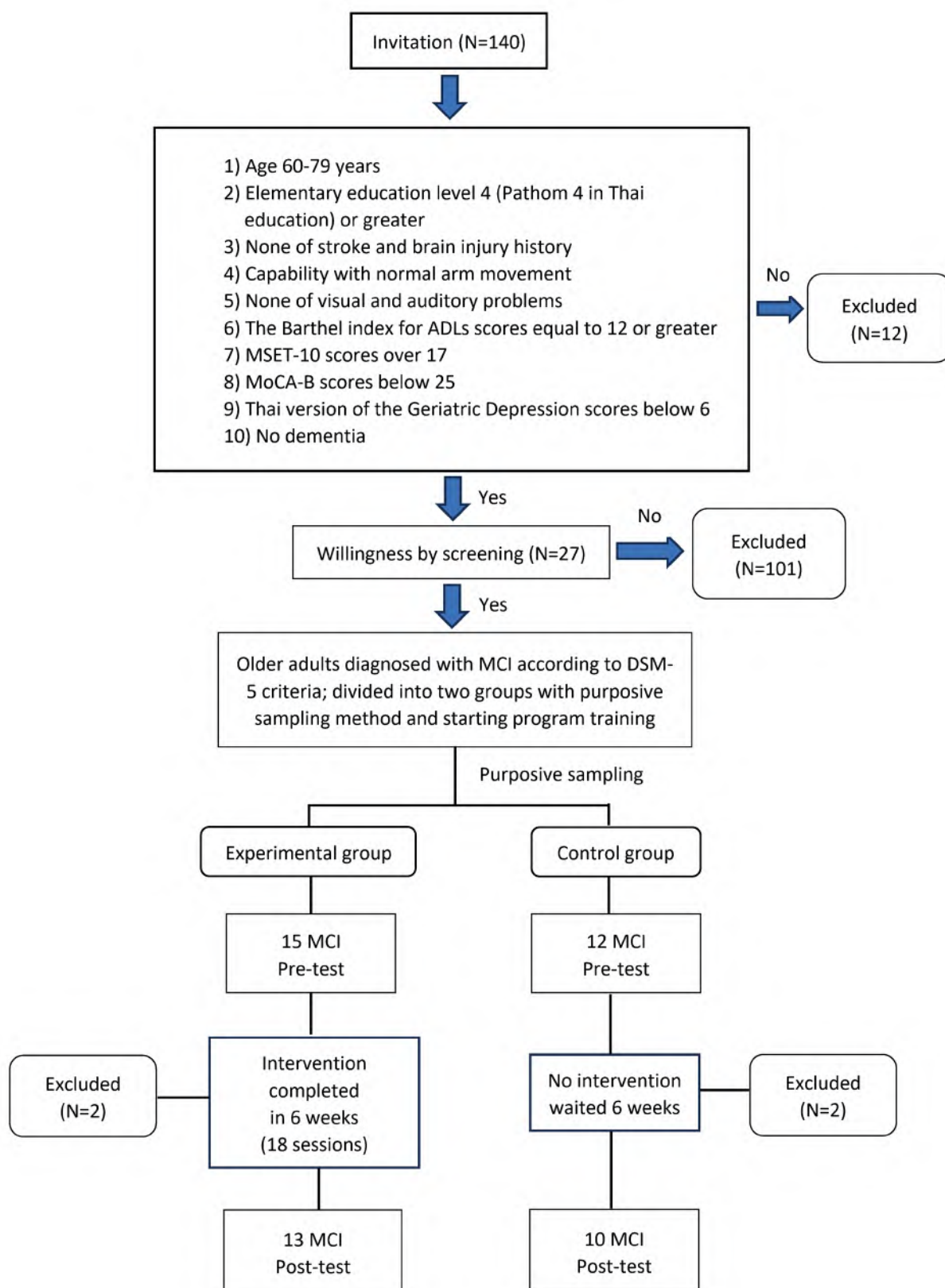


Figure 1. Flowchart of this study.

Measurements

Screening Tools

Barthel ADL index

Barthel ADL index is used to assessed physical performance in activities of daily living (ADL). Thai version of Barthel ADL index used in patients with low-energy femoral neck fracture has good interobserver and intra-observer reliabilities (0.71 and 0.96, respectively). Additionally, the internal consistency was acceptable (Cronbach's $\alpha=0.69$).³²

Mental status examination Thai 10 (MSET-10)

Mental Status Examination Thai 10 (MSET-10) is a screening test for dementia. It has excellent sensitivity and specificity. Additionally, it had been suggested that MSET-10 is appropriated for using in illiterate adults. It was adapted from Mini-Mental state exam-Thai 2002 (MMSE-Thai 2002) by Department of Psychiatry, Faculty of Medical of Chiang Mai University.³³

Montreal cognitive assessment-basic (MoCA-B)

Montreal Cognitive Assessment-Basic (MoCA-B) is a screening test for Mild Cognitive Impairment in Thai illiterate older adults.³⁴ It was developed as a collaboration among the MoCA Clinic and Institute in Canada, the Prince Mahidol Award Foundation, and Faculty of Medical of Chulalongkorn University in Thailand. MoCA-B evaluates 6 cognitive domains: visual perception, executive function, language, attention, memory, and orientation. It has good to excellent sensitivity, specificity, test-retest reliability, and internal consistency (0.81, 0.86, $r=0.91$, and $\alpha=0.82$, respectively)

Geriatric depression scale-15 (GDS-15)

Geriatric Depression Scale-15 (GDS-15) is a test of screening for depressive symptoms in older adults.³⁵ It has good reliability and validity. The Thai version of the Geriatric Depression Scale (TGDS) has good psychometric properties,³⁶ good internal consistency ($\alpha=0.85$).

Outcome measurement

The outcome was measured within 1 week before and after the intervention. The assessments were conducted by the trained therapists, 1 clinical psychologist and 1 occupational therapist, who blinded the group allocation.

Trail making test (TMT)

Trail Making Test (TMT), i.e., TMT-A and TMT-B, is a neuropsychological test of visual attention and task switching. It consists of 2 parts. In part A, it requires participants to connect the circles in a sequence as quickly as possible using a single continuous line. In part B, it requires participants to draw a line connecting several alternating letters in sequence. It has average to good test-retest reliability in parts A and B ($r=0.36-0.79$ and $0.44-0.89$, respectively).^{37,38} In this study it was used for assessing attention by a clinical psychologist.

The Thai cognitive-perceptual test (Thai-CPT)

The Thai Cognitive-Perceptual Test (Thai-CPT) is a perceptual-cognitive outcome measure for patients with brain injuries in Thailand. The Thai-CPT consists of 6 subtests which includes Visual Perception, Body Scheme Perception, Praxis, Memory, Matching and Categorization, and Problem Solving. It has good to excellent test-retest reliability (ICC=0.83-0.97), excellent inter-rater reliability (ICC=0.91-1.00), and acceptable internal consistency ($\alpha=0.78-0.96$).³⁹ This study used a Memory subtest for working memory with assessment by an occupational therapist.

The assessors were blinded from the participants in both groups.

Statistical analysis

Descriptive statistics included frequency and percentage. Cognitive outcomes in attention and working memory were assessed using the Wilcoxon signed-rank test to compare the effects of the intervention program between pre-test and post-test, the Mann-Whitney U-test to compare the effect of the intervention program on post-test in both groups, and the Chi-square to compare age and levels of educational between group.

Results

A total of 23 participants were included in this study, with 10 assigned to the control group and 13 to the experimental group. Participants in both groups were carefully matched based on key demographic variables including sex, age, and educational level, to ensure comparability. The demographic characteristics are summarized in Table 1.

Table 1. Characteristic of the participants in this study.

Variables		Control group (N=10)	Experimental group (N=13)	p value
Gender	Female	10 (100%)	11 (84.62%)	1.47
	Male	0 (0%)	2 (15.38%)	
Age range (years) Mean±SD 67.47±3.92	60-69	8 (80%)	9 (69.24%)	1.05
	70-79	2 (20%)	4 (30.76%)	
Status	Single	2 (20%)	2 (15.38%)	0.41
	Married	7 (70%)	9 (69.24%)	
	Divorce	1 (10%)	2 (15.38%)	
Levels of education	Level 4 (Prathom 4 in Thai education system)	10 (100%)	12 (92.30%)	1
	More than level 4	0 (0%)	1 (7.70%)	

The control group had a mean age of 66.50±3.84 years, while the experimental group had a mean age of 68.23±3.98 years. The Chi-square test indicated no significant difference in age between the two groups. In terms of educational background, all participants in the control group (100%) had attained an educational level of grade four, while in the experimental group, 12 participants (92.30%) had completed grade four, with one participant (7.70%) having a higher level of education. Overall, the demographic data revealed no statistically significant differences between the control and experimental groups across any of the measured variables, ensuring that both groups were comparable in terms of baseline cognitive ability before participating in the intervention.

The cognitive scores comparing the control and experimental groups after the intervention are presented in Table 2. The Mann-Whitney U test identified statistically significant differences between the groups in terms of performance on the TMT-B and working memory, with the experimental group demonstrating a higher mean rank compared to the control group in working memory and lower mean rank in TMT-B. And range of working memory scores of the control and experimental groups after the intervention are 20-32 and 26-32, respectively. These findings suggest that participants in the experimental group who underwent the Neofect Cognition intervention showed improved cognitive abilities, particularly in areas related to task-switching and working memory, as assessed by TMT-B and Thai-CPT.

Table 2. Post-intervention assessment between groups.

Test	Control group			Experimental group			Mann-Whitney U test	
	Mean (SD)	Mean rank	Sum of ranks	Mean (SD)	Mean rank	Sum of ranks	Z	p value
TMT-A*	-0.5 (33.88)	12.40	124	1 (26.57)	11.69	152	-0.314	0.39
TMT-B*	10.6 (39.41)	15.20	152	-11.92 (18.43)	9.54	124	-1.960	0.02
Thai-CPT**	-1.6 (1.50)	5.9	59	3.53 (3.30)	16.69	217	3.049	0.01

Note: *used for assessing attention, unit of performance is "seconds"; **used for assessing working memory, unit of performance is "score"; The scores range in Thai-CPT (memory subtest) is 0-34.

In contrast, no significant difference was observed between the groups in the TMT-A times, indicating that the intervention may not have had a notable effect on simpler attention tasks that primarily involve visual scanning and processing speed. The results imply that Neofect Cognition could be especially effective in enhancing alternating attention, as evidenced by the superior performance in TMT-B, as well as improving working memory among the participants in the experimental group. These findings provide promising evidence for the potential application of Neofect Cognition as a computer-based cognitive training tool aimed at improving specific cognitive functions in populations with similar needs.

Tables 3 and 4 present the statistical analysis and comparisons of cognitive scores within the control and experimental groups, respectively. In the control group, the comparison of pre- and post-test scores using the Wilcoxon Signed-Rank test revealed no statistically significant differences in TMT-A, TMT-B, and working memory, as

presented in Table 3. This suggests that the control group did not exhibit notable cognitive improvements across these measures over the course of the study. Conversely, in the experimental group, significant differences were observed in TMT-B and working memory scores, indicating that participants in this group experienced meaningful cognitive enhancements in these areas. However, TMT-A times in the experimental group did not show a statistically significant difference (Table 4).

When examining the mean rank scores, the experimental group consistently outperformed the control group across all cognitive measures. These results suggest that the experimental group demonstrated improvements in both attention and working memory following participation in the intervention program. The findings imply that the Neofect Cognition program may be particularly effective in enhancing alternating attention, as required by the TMT-B, and in improving working memory.

Table 3. Pre- and post-intervention assessment of control group (N=10).

Test	Control group (N=10)					
	Pre-test	Post-test	Wilcoxon Signed-Rank Test			
	Mean (SD)	Mean (SD)	Mean rank	Sum of ranks	Z	p value
TMT-A	121 (53.91)	120.5 (54.35)	4.67	28	-0.015	0.50
TMT-B	117.7 (28.37)	128.3 (52.27)	4.30	21.5	-0.612	0.74
Thai-CPT	28 (3.09)	26.4 (3.50)	0	0	-2.55	0.99

Table 4. Pre- and post-intervention assessment of experimental group (N=13).

Test	Experimental group (N=13)					
	Pre-test	Post-test	Wilcoxon Signed-Rank test			
	Mean (SD)	Mean (SD)	Mean rank	Sum of ranks	Z	p value
TMT-A	79.08 (29.04)	80.07 (24.79)	5.38	43	-0.314	0.39
TMT-B	104.69 (33.84)	92.76 (25.65)	8.17	73.5	-1.960	0.02
Thai-CPT	25.38 (3.70)	28.92 (1.97)	7.42	89	3.049	0.001

Discussion

The results demonstrated that the Neofect Cognition might help older adults who are suffering from MCI with attention and working memory impairment. After the experiment, the experimental group showed that the Neofect Cognition provided more positive results than in the control group on attention and working memory both within (Tables 3 and 4) and between groups (Table 2). These results were obtained after assessment using the TMT-B and the Thai-CPT. The results suggest that computer-based cognitive training using the Neofect Cognition may be effective for improving attention and working memory

in the experimental group. This confirms previous studies that support the beneficial effects of computer-based cognitive training on attention and working memory.^{12,40-43} It is likely that the Neofect Cognition is preferable due to its ability to specifically and reliably address attention and working memory. In addition, the games in the Neofect Cognition were assessed for content validity in Thai context by five experts who determined its suitability before the experimental group used these games.

Moreover, despite the small sample size, these results are like the results of previous studies, indicating that the computer-based cognitive training program affect cognitive

ability. Based on the results of previous studies of the characteristics of Alzheimer's dementia patients conducted by Hwang and a co-researcher,²⁸ it was shown that the computer-based cognitive training is effective in improving memories of Alzheimer's dementia patients. Kim and co-researcher,³¹ and Finn and McDonald⁴⁰ reported that computer-based cognitive training program increased cognitive ability, as in our study.

However, from the results of the TMT-A portion, there was no significant difference within groups (Tables 3 and 4) or between groups (Table 2). Likewise, Yang and a co-researcher found that attention times in the experimental group did not improve after experimentation.¹⁰ It is reasonable to assume that insufficient attentive intervention in TMT-A is related to inferior visual scanning and sustained attention compared to TMT-B's alternate attention and divided attention formats. To clarify, in cases where the experimental group had received the attention training based on a computer-based cognitive training program for a longer period, the attention times would likely decrease.

Additionally, when considering the baseline attention of both groups before the experiment, the cognitive function of the TMT-A was significantly different before the experiment ($p=0.02$) and would notably influence the attention times in the only slightly different times obtained between groups. Moreover, when we consider the results of the TMT-B and the TMT-A, it was found that differences had a large mean and standard deviation. This could most likely be the consequence of the purposive sampling method being chosen, which led to the differentiation among the samplings in terms of attention, and of failure to abort specific criteria for sampling participants. In the meantime, the control group having not received the computer-based cognitive training, was prone to be derive a declination in the cognitive ability as the result of pathological effects of disease; in the other word, MCI is a cognitive deterioration.^{1,44,45} Among the participants, as a matter of fact, there are personal diseases such as hypertension and diabetes mellitus confounding factored to the cognitive ability.^{5,41}

This study had some limitations due to the small sample size which may have affected the ability to examine the absolute effects of computer-based cognitive training on MCI. Furthermore, due to restricted conditions along with little variation in educational levels, the results could not be generalized for other populations. Lastly, since the data was collected in a specific area, being a community in Chiangmai, there are clear limitations. For the further studies, it is recommended the data be gathered in more diverse populations residing in other areas. Moreover, using higher measurement to accurately represent the brain area responding to each domain of cognition while using the Neofect Cognition would be benefit for the further study.

Conclusion

The result from this study showed that the Neofect Cognition might help improve attention and working memory in older adults with MCI. These findings confirmed the

effectiveness of computer-based cognitive training which could be helpful for older adults with MCI to improve attention and working memory and might prevent the onset of dementia when stimulating activities are regularly and sequentially provided.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical approval

This study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences (study code AMSEC-64FB-006; approval date July 8, 2021).

Funding

This research received no external funding.

Acknowledgements

We are thankful to all older adults who participated in this study.

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The applications of three-dimensional computed tomography images of the greater sciatic notch for sex determination in forensic anthropology aspect in Thai population

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ARTICLE INFO

Article history:

Received 16 January 2025

Accepted as revised 10 April 2025

Available online 17 April 2025

Keywords:

Greater sciatic notch, pelvis, postmortem computed tomography (PMCT), sex determination, Thai population.

ABSTRACT

Background: The identification of highly decomposed corpses or skeletons remains a significant challenge for forensic medical examiners. The pelvic bone, recognized for its reliability in sex determination, is frequently utilized through visual assessment as an initial step in such cases. However, the subjectivity of this method has prompted research into more accurate and objective approaches than traditional anatomical morphological evaluations.

Objective: The research was aimed to identify practical osteometric parameters of the greater sciatic notch, measured using a postmortem computed tomographic (PMCT) program, that have significant differences between male and female adults and provide high accuracy for sex determination in the Thai population. In addition, equation with high accuracy for sex determination using discriminant function analysis (DFA) was also developed.

Materials and methods: This study analyzed 385 Thai adults aged over 20, whose postmortem computed tomography (PMCT) scans included the hip region with an intact greater sciatic notch. The sample comprised 129 females and 256 males. The analyzed 6 osteometric parameters include *two distances*: PIIS-IS; the distance between the posterior inferior iliac spine (PIIS) and the ischial spine (IS), PIIS-SP; the distance between PIIS and Separating point (SP), a ratio of PIIS-SP to PIIS-IS (PIIS- SP/PIIS-IS), and *three angles*: Angle between planes passing PIIS and IS, and PIIS and DP (PIISA); Angle between planes passing PIIS and DP, and IS and DP (GSNA) and Posterior part when the GSNA is divided by DP-SP plane (GNSPA). Discriminant function analysis was used for data evaluation.

Results: Three-dimensional measurements of the greater sciatic notch demonstrated high reliability, with intra- and inter-observer ICC values exceeding 0.8 ($p < 0.05$). All five measurements and the ratio achieved over 92% accuracy in multivariate analysis for cross-validated cases. DFA confirmed the suitability of all parameters for inclusion in the discriminant equation, which provided thresholds for sex determination: $D < -1.03$ indicated male, $D > 2.04$ indicated female, and intermediate values were indeterminate, accounting for 67.73% of population variance.

Conclusion: This study demonstrates that all the selected measurements of the greater sciatic notch can effectively differentiate between male and female adults through the application of discriminant function with an overall accuracy of 92%.

Introduction

In forensic autopsy, as outlined in Section 154 of the Criminal Procedure Code,¹ forensic physician must determine the deceased's identity, the timing and location of the incident, and the cause and manner of death. While a complete dissection is not always necessary if sufficient information is available, identification becomes challenging in cases involving decomposed corpses or skeletal remains. The Central Institute of Forensic Science

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doi: 10.12982/JAMS.2025.066

E-ISSN: 2539-6056

in Thailand reported 1,420 unidentified corpses between 2014 and 2023, with 102 unidentifiable due to decomposition.² In 2023, approximately 13.3% of corpses could not have their sex determined.

Several methods have been applied in past decades using different parts of the bony structure to find the most reliable and practical method for sex determination. While anatomical morphology is commonly used for convenience, it is subjective and affected by postmortem changes.³ Metric measurements offer more accuracy but can be challenging to maintain consistency in correcting the position and still can be affected by postmortem changes. Geometric analyses using 3D imaging are the most precise method and allow for data preservation without alteration; however, they require advanced technology, limiting their accessibility in some contexts.

Computed tomography (CT) is an imaging modality generating detailed 3D images from consecutive two-dimensional axial slices for medical diagnosis. It is increasingly utilized due to its efficiency, durable data storage, rapid results, and non-invasive autopsy capabilities. Since its introduction in Thailand in 2016, PMCT has enhanced forensic investigations by enabling comparisons with antemortem data and providing detailed imaging of structures that are difficult to assess through traditional autopsies.³

Accurate sex estimation relies on population-specific data, which becomes more reliable after puberty (ages 15-18) when skeletal features have been fully developed. By age 20, the skeleton is typically fully fused, allowing for nearly 100% accuracy using the entire skeleton, 98% with the skull and pelvis, 95% with the pelvis alone, and 92% with the skull alone.^{4,11}

Although the pelvic bone is claimed to be the most accurate sex-determination tool, owing to its parturition function, the pubis tends to be fragile and poorly preserved.⁵ However, the greater sciatic notch (GSN) remains a reliable indicator as anatomically, the greater sciatic notch is deep and narrow in males and wider in females. Also, due to its robust cortical bone, the GSN remains highly resistant to damage or degradation, achieving an accuracy of 80-83% in sex classification using visual, angular, and geometric morphometric assessment across various studies.^{6,7,8}

The accuracy increases when considering the deepest point of the greater sciatic notch.⁹ Knecht's study⁹ demonstrates that the Diagnose Sexuelle Probabiliste (DSP) method, using ten measurements from a global dataset,^{10,12,16} achieves an accuracy ranging from 86% to 99% in different ethnic groups, with the lowest accuracy in the African group. Furthermore, using the greater sciatic notch alone, accuracy ranges from 70% to 93%.

In a 2006 study in France by Christophe Boulay and colleagues, pelvic symmetry was examined using 3D measurements from 12 common anatomical reference points. The study revealed that the right and left greater sciatic notches have nearly identical anatomical dimensions, making them suitable for use as standard parameters in our study.¹³

There was also a study conducted in Thailand that assessed three parameters—greater sciatic notch depth, width, and posterior segment distance—using a digital Vernier caliper on 100 dry adult bone samples for sex determination.¹⁴ Using descriptive statistics and discriminant analysis, the measures showed high accuracy, ranging from 92% to 96% with the maximum width (MW), width-to-depth ratio (MW/MD), and width-to-posterior segment ratio (MW/PS).

In Korea, 2018, the visual scoring method developed by Walker,¹⁵ struggled with accuracy due to age and ethnic overlaps, making it difficult to distinguish between males and females in the intermediate zone. Kim Dong-Ho and colleagues employed a metric method on 3D models derived from radiographic images, ensuring reproducible and stable measurement of the greater sciatic notch with over 85% accuracy.¹⁵ Measurements related to the posterior part of the greater sciatic notch showed high accuracy (93.1%).

This study aims to establish a practical method for sex determination using pelvic measurements from PMCT, focusing on Thai population data. Six osteometric parameters are identified: three distances (PIIS-IS, PIIS-SP, PIIS-SP/PIIS-IS ratio) with 78.70%-90.10% accuracy and three angles (PIISA, GSNA, GNSPA) with 90.10%-93.10% accuracy. These parameters were selected for their high accuracy from the study of Kim *et al.*¹⁵ The goal is to find convenient PMCT parameters for effective sex determination that avoid time-consuming procedures like tissue removal.

Materials and methods

Materials

Study design and target population

This retrospective analytic study examined deceased Thai adults aged 20 years or older who underwent forensic autopsies at the Department of Forensic Medicine, Chulalongkorn University, in 2023. Sex and age were verified using national identity cards. Cases with pelvic bone abnormalities, such as defects, trauma, or metallic objects that could affect PMCT image interpretation, were excluded from the analysis.

Sample size acquisition

The sample size, calculated using Cochran's formula, comprised 385 cases.¹⁷ To reduce the imbalance in the female-to-male ratio from the total of 622 cases, all 129 eligible female cases meeting the inclusion-exclusion criteria were included. From the remaining 480 male cases, 256 were randomly selected using the random function in Microsoft Excel 2019 to ensure unbiased sampling.¹⁶

The Cochran's formula used for sample size calculation: n =sample size, p =proportion of the population (0.5), Z =confidence level (1.96), e =allowable error (0.05).¹⁷

$$n = \frac{Z^2 p(1-p)}{e^2}$$

Methods

Informed consent process

This retrospective study analyzed PMCT images routinely collected at the Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University. The identity and specific information of the deceased were kept confidential and cannot be used to identify further personal information, so informed consent from the deceased relatives was not obtained in this study.

The study was approved by the Research Ethics Committee of the Faculty of Medicine, Chulalongkorn University, receiving an exemption from ethics consideration with Institutional Review Board (IRB) number 665/67 and Certificate of Exemption (COE) number 051/2024.

Ethical consideration

During the procedures, only certain information such as sex, age, nationality, and the pelvic PMCT images was utilized. Other personal details, including the deceased's

name, were kept confidential, with data access restricted to the Forensic Department at Chulalongkorn University.

Standard plane and anatomical landmark creation

PMCT scans were performed using a GE Revolution EVO CT machine with standardized parameters (1x64x0.625 mm collimation, 120 kV, 200 mA, 0.8 s rotation time). The AW server 3.2 ext. 4.0 SW AND DOCS for Virtual AW server (GE Healthcare, France) was used to generate and evaluate the 3D PMCT images. To standardize measurements, the pelvic bone was aligned in an anterior view, with the pubic symphysis positioned along the midline of the lumbar and sacral spine. Then rotated horizontally 60° counterclockwise and visualized to maximize the visibility of the greater sciatic notch and ensure consistency in osteometric measurements (Figure 1). Key anatomical landmarks—Posterior Inferior Iliac Spine (PIIS), Deepest Point (DP), Ischial Spine (IS), and Sciatic Process (SP)—were identified after removing the sacral region for clarity (Figure 2 and 3). The abbreviation of each anatomical landmark and measurement was shown below (Table 1).

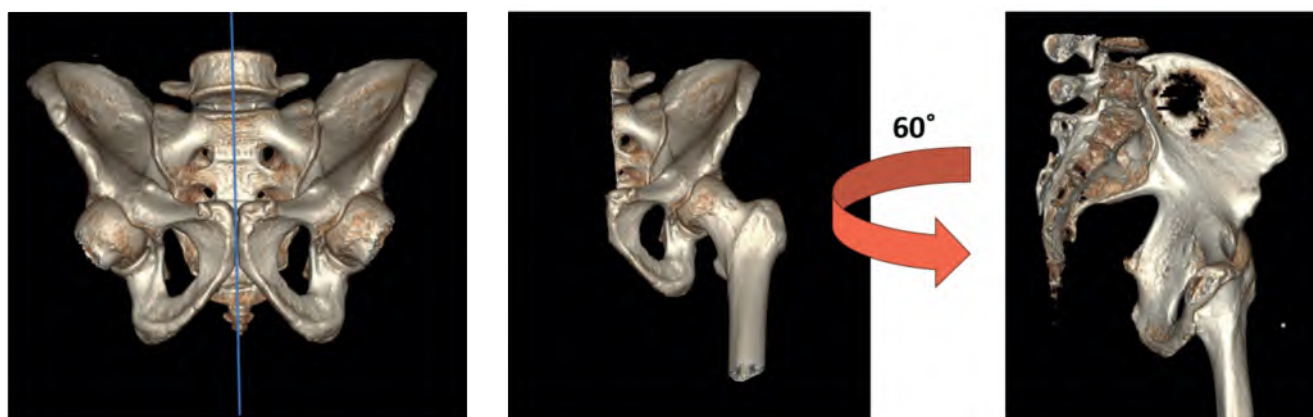


Figure 1. Demonstrates the adjustment axis and rotates it to the optimal angle of the left greater sciatic notch.

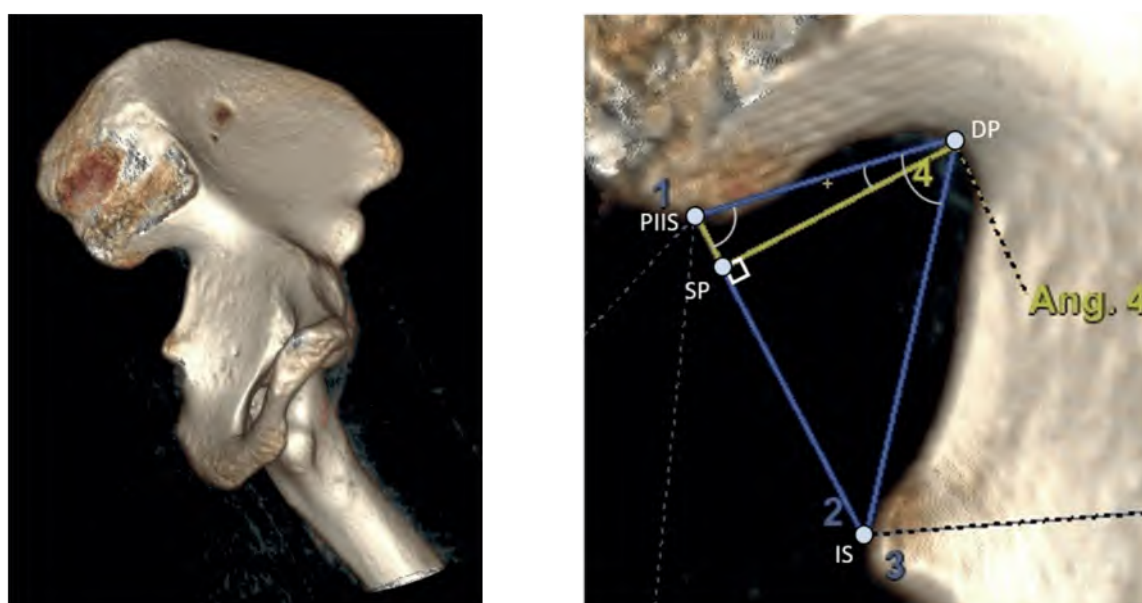


Figure 2. Clear margin of the left greater sciatic notch after removing the sacral part and marks the anatomical points before the osteometric measurements were done.



Figure 3. Compares the same procedures with the other side, the right greater sciatic notch.

Table 1. Definition of anatomical landmarks and measurements.

Landmarks	
PIIS	Posterior inferior iliac spine
DP	The deepest point of the greater sciatic notch
IS	Ischial spine
Sp	Separating point: the point where perpendicular line from DP meets the PIIS-IS line
Distances	
PIIS-IS	Distance between PIIS and IS.
PIIS-SP	Distance between PIIS and SP.
Angles	
PIISA	The angle between planes passing PIIS and IS, and PIIS and DP. The angle between planes
GSNA	passing PIIS and DP; and IS and DP.
gsnpa	The posterior part where the GSNA is divided by the DP-SP plane.

Comparative Analysis

Randomly selected 20 cases, using the random function in Microsoft Excel 2019,¹⁶ were used to assess measurement differences between the left and right hip bones through a paired t-test. To evaluate inter-observer and intra-observer reliability, the Intra-class Correlation Coefficient (ICC) was employed. Intra-observer error was assessed by a single author (AS), who measured 20 randomly selected samples, comprising 10 females and 10 males, twice with six months apart. For inter-observer error, we compared the results of two authors — the principal investigator, a third-year forensic resident (AS), and a forensic staff in the Department of Forensics, Chulalongkorn University (AC).

Statistical analysis

Statistical analyses were performed using SPSS software (version 29.0.2.0, SPSS, Thailand). Discriminant function analysis (DFA) calculated each parameter using

a weight matrix, with Canonical discrimination functions assessed through Eigenvalue and Canonical correlation values to determine the strength of the correlation between the derived equation and population-specific data.

Results

A comparative analysis was performed using 20 randomly selected cases to assess measurement differences between the left and right hip bones. Paired t-tests showed no statistically significant differences across all parameters (*p* values: 0.928, 0.937, 0.885, 0.802, 0.519, and 0.689) (Table2). Consequently, the left hip bone was selected as the reference side for further measurements. This selection aligns with anthropological standards favoring the non-dominant side, typically the left, to minimize the impact of degenerative changes from overuse, ensuring greater measurement reliability.⁷

Table 2. Comparison of all parameters between left and right greater sciatic notch.

Left and right comparison	Paired differences					p value
	Mean	SD	SE mean	95% CI of the difference		
				Lower	Upper	
PIIS-IS	0.0060	0.29396	0.06573	-0.13158	0.14358	0.928
PIIS-SP	-0.0065	0.36325	0.08123	-0.17651	0.16351	0.937
PIIS-SP/PIIS-IS ratio	0.0030	0.09125	0.02041	-0.03971	0.04571	0.885
PIISA	0.3350	5.89355	1.31784	-2.42327	3.09327	0.802
GSNA	-0.8050	5.47929	1.22521	-3.36939	1.75939	0.519
GSNPA	-0.3950	4.34093	0.97066	-2.42662	1.63662	0.689

The results of intra- and inter-observer error assessments, detailed in Table 3 ($p < 0.05$), indicate that 3D measurements of the greater sciatic notch demonstrate good to excellent reliability, with ICC values exceeding 0.8 for repeated measurements. Accuracy estimation of all measurements was conducted through multivariate analysis, revealing that all five measurements and one

ratio achieved high accuracy. Discriminant function analysis showed that the absolute values of the structure matrix for all parameters exceeded 0.3, confirming their suitability for inclusion in the discriminant equation. Among these, the PIIS-SP parameter was identified as the best predictor for sex determination (Table 4).

Table 3. Intra- and Inter-observer error of direct measurements using ICC value.

	PIIS-IS	PIIS-SP	PIIS-SP / PIIS-IS ratio	PIISA	GSNA	GSNPA
Intra-rater ICC	0.978	0.992	0.987	0.980	0.984	0.988
Inter-rater ICC	0.946	0.919	0.861	0.937	0.991	0.944

Table 4. Discriminant function analysis of all parameters.

Measurements	Total		Male		Female		Structure matrix	Standardized canonical discriminant function coefficients
	Mean	SD	Mean	SD	Mean	SD		
Distance								
PIIS-IS	4.33	2.04	4.10	2.44	4.81	0.53	0.559	-0.394
PIIS-SP	0.84	0.71	0.52	0.55	1.47	0.57	0.553	1.231
Ratio								
PIIS-SP / PIIS-IS	0.19	0.15	0.13	0.14	0.30	0.10	0.455	-1.697
Angle								
PIISA	74.61	13.05	81.14	9.69	61.64	8.31	-0.620	-0.131
GSNA	64.90	13.02	57.44	7.38	79.69	8.27	0.944	0.809
GSNPA	15.44	13.07	8.89	9.63	28.49	8.32	0.695	0.673

Using discriminant function analysis (DFA) from the weight matrix, Canonical discrimination functions were used in the study with the Eigenvalue of 2.106 and Canonical correlation value of 0.823, which implied that the obtained equation could correlate with 67.73% of this population due to the R-square value of 0.6773. The obtained discriminant equation (D) is as shown below. The average D value for the male group is -1.028, while for the female group is 2.039, which indicates that if the D value is lower than -1.03, the predicted sex is male, and if the D value is greater than 2.04, the expected sex is female. When the D value falls between -1.03 and 2.04, the predicted sex is uncertain.

$$D = 2.205(PIIS - SP) + 0.105(GSNA) + 0.073(GSNPA) \\ - 0.013(PIISA) - 0.788(PIIS - IS) \\ - 13.657(PIIS - SP / PIIS - IS \text{ ratio}) - 2.970$$

The discriminant function equation. The information obtained from the graph's discriminant function distribution offers additional evidence to support greater sexual differences. The average D value is -1.028 in males and 2.039 in females (Figure 4).

According to the validation process, accuracy is determined using a discriminant function derived from all cases except the one being classified. The predicted group membership achieves an accuracy of 92.5% in cross-validated grouped cases.

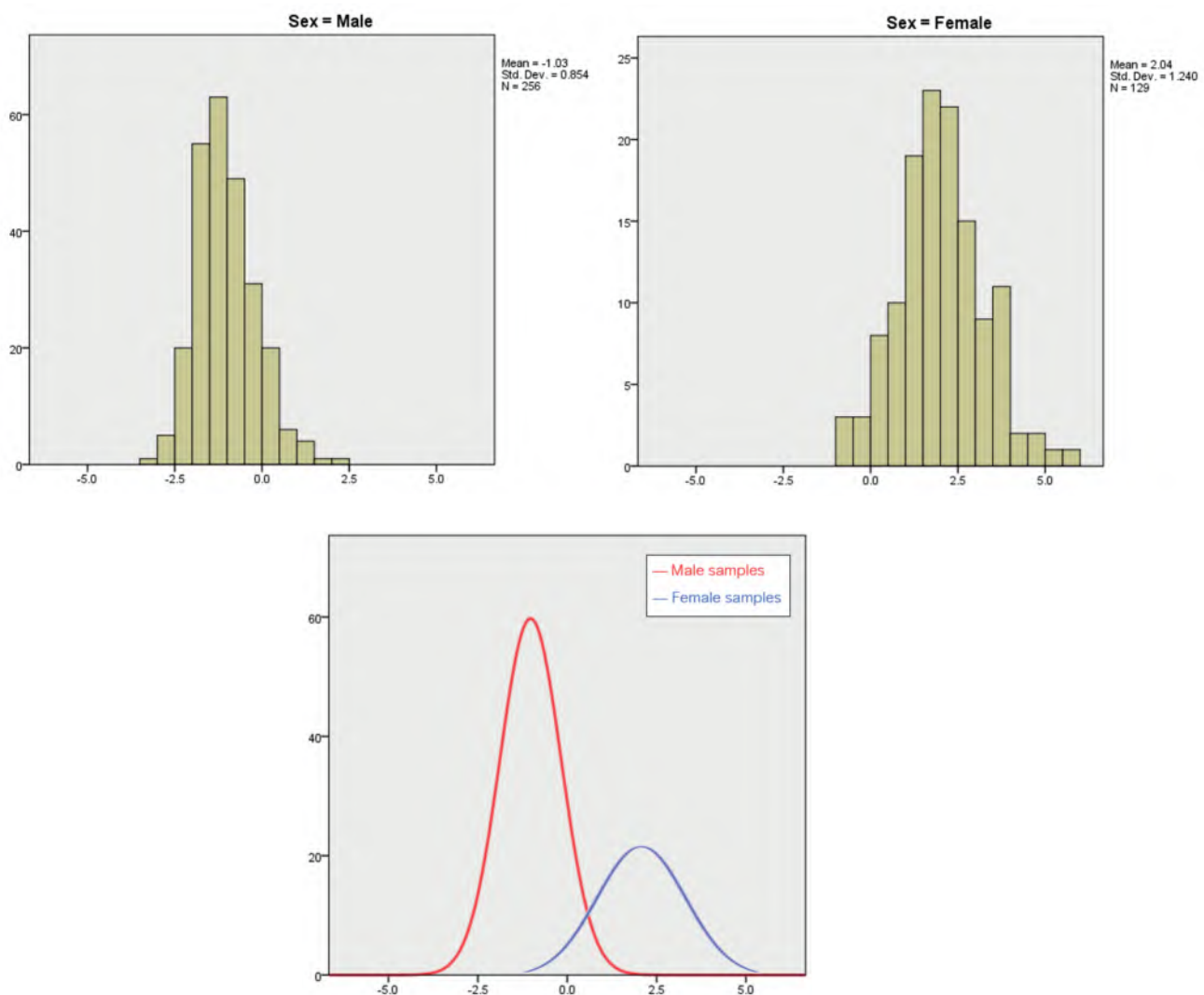


Figure 4. The discriminant function graphs comparing male and female cases with the average D value is -1.028 in males and 2.039 in females.

Discussion

Analysis of the greater sciatic notch parameters (PIIS-IS, PIIS-SP, the ratio, PIISA, GSNA, and GSNPA) using PMCT images achieved an overall accuracy of 92.5% for sex determination via the discriminant function equation. The results reflect population-specific characteristics while aligning fundamental anthropological principles and findings from other population studies.

In the sample size acquisition process to decrease the selection bias by using the Mersenne Twister algorithm, the random function in Microsoft Excel 2019 (RAND) is considered a sufficient and reliable random number generator.¹⁶ Furthermore, to ensure accuracy and consistency, 3D modeling was employed to verify anatomical landmarks and establish reference planes, addressing challenges in landmark identification and enhancing measurement reliability. This method provides a guide for researchers to accurately identify landmarks and perform precise measurements based on these reference planes verified through computational tools.

Reproducibility was confirmed with high intra- and inter-observer reliability, yielding ICC values exceeding 0.98 and 0.86, respectively (Table 3). The findings for the Thai population’s greater sciatic notch are consistent with data from other populations, supporting the applicability of prediction equations across diverse groups.

As shown in Table 5, this study achieved the highest accuracy among PMCT-based sex differentiation methods while using the fewest parameters. Its accuracy, exceeding 90%, is comparable to studies using dry bone measurements, supporting its practicality due to ease of

data collection. Additionally, since the results for the Thai population align with findings from other ethnic groups in previous studies, it can be concluded that this approach is applicable to other populations as well.

Radiological imaging, used for data collection, offers a non-invasive method and facilitates the convenient archiving of skeletal data. The subsequent application of 3D modeling permits reproducible measurements and enables the exploration of a wider range of measurement techniques. However, this method has inherent limitations, such as the need for a CT imaging system and the necessary training to operate it. Despite these challenges, the accessibility of such facilities greatly enhances the utility of skeletal collections, providing researchers with expanded opportunities for data analysis through 3D measurements.

Based on the findings of this study, the greater sciatic notch is a highly reliable tool for sex determination. Future research should investigate the applicability of this parameter across different ethnicities to evaluate its effectiveness and generalizability.

Limitations

This study is limited by the operator-dependent measurement procedures, particularly in assessing distances and angles within the greater sciatic notch, which may introduce inconsistencies and affect accuracy. Despite standardized protocols, some subjectivity persists. Additionally, anatomical variations in pelvic morphology, influenced by genetic and environmental factors, may lead to overlapping measurements between sexes, reducing the reliability.

Table 5. Comparison of the accuracy of sex determination with other studies.

Study	Population	Material	Variables	N	accuracy
This study	Thai	PMCT 3D images	GSN (6 parameters)	385	92.5%
Kim <i>et al.</i> ¹⁵	Korean	PMCT 3D images	GSN (17 parameters)	202	78.7-93.1%
Soltani <i>et al.</i> ¹⁸	Iran	PMCT 3D images	GSN (10 parameters)	237	89.5-90.3%
Gomez-Valdes <i>et al.</i> ⁶	Mexican	Dry bones images: Visual scoring Geometric morphometric measurement	GSN (2 landmarks and 30 semi-landmarks)	130	68.5% 82.3%
Bruzek <i>et al.</i> ¹⁹	Multiethnic (American, African, Asian, European)	Dry bones geometric measurement	Whole pelvis 10 parameters 8 parameters 4 parameters	2,040	99.65% 99.65% 99.53%
Knecht <i>et al.</i> ⁹	French	Dry bones geometric measurement	GSN (16 parameters)	60	92-97%
Boonsonti S. ¹⁴	Thai	Dry bones geometric measurement	GSN (5 parameters)	200	83-96%

Conclusion

This study offers a comprehensive analysis of the greater sciatic notch through 3D measurement, providing valuable population-specific data. Our findings demonstrate that 3D measurement techniques yield results that are applicable to samples from the same

population, offering a more objective evaluation compared to traditional anatomical assessments based on visual methods for sex determination. This supports the broader use of 3D measurement in anthropological research and when examining dry bone specimens, thereby reducing bias.

Additionally, we confirmed the presence of sexual dimorphism in the greater sciatic notch within the Thai population, which aligns with findings from other populations. The results of this study aim to serve as a foundation for future research that compares different populations and enhances our understanding of population-specific skeletal characteristics.

Conflict of interest statement

The authors declare no conflict of interest.

Funding

This research received no specific grant from public, commercial, or non-profit funding agencies.

Acknowledgements

We would like to thank the forensic radiologic specialized staff in the Forensic Department of Chulalongkorn University, the computed tomographic technician, and the statistician from the research affairs in the Faculty of Medicine Chulalongkorn University. Language assistance for this research was provided by ChatGPT, a language model developed by OpenAI.

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Established in 1968

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