JOURNAL OF ASSOCIATED MEDICAL SCIENCES

JAMS

Journal of Associated Medical Sciences

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2. Radiologic Technology
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6. Other related fields

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Journal of Associated Medical Sciences publishes 3 issues a year
- Issue 1: January-April
- Issue 2: May-August
- Issue 3: September-December

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Content

Content

Energy resolution, peak to total ratio, peak to Compton ratio of cerium bromide crystal scintillator

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ARTICLE INFO

ABSTRACT

Background: $CeBr_3$ Inorganic scintillator is a favorite scintillator in various fields, especially in medical imaging and X-rays/gamma rays detectors. One is important before being applied in any application is the fundamental properties that it has to measure such as energy resolution, PTR, PCR, and light yield.

Objectives: In this research, a $CeBr_3$ crystal was carried out trial for calculating the energy resolution, peak to total (PTR), and peak to Compton (PCR) values.

Materials and methods: When high photon energy undergoes with the $CeBr_3$ crystal, the crystal will occur to interact with photon energy by energy's absorptions via the interaction ternary as the photoelectric absorption, Compton scattering, and pair production. The nuclear instrument module (NIM) was used in this experiment. The crystal will generate light and come into a photomultiplier tube (PMT 9256 KB) for amplifying the light signal via the radiation sources as Ba-133, Na-22, Cs-137, and Co-60, which generated the energies at 0.356 MeV, 0.511 MeV, 0.662 MeV, 1.173 MeV, and 1.332 MeV, respectively.

Results: The result found that the energy resolution of $CeBr_3$ showed the energy resolution of $CeBr_3$ crystal showed a linear pattern of inverse the square root of the energy and found the energy resolution values were increased when the photon energy decreased. The PTR and PCR values decreased with increasing energy ranges via experiment of each full energy peak of radiation sources.

Conclusion: From the fundamental properties of $CeBr_3$ crystal showed a good energy resolution and is a possible candidate to apply for any application such as radiation detection and high-energy physics. Anywise, other properties should be also considered, for example, light yield, decay time, and nonproportionality of light yield.

Introduction

Advantages of high photon energies were widely used in fields such as medicine, academic and scientific, industrial application, nuclear power plant, and so on.[1] Medically, A medical tool that takes advantage of radiation for the patient radiotherapy is an important to appropriate consideration. Positron Emission Tomography (PET) utilizes isotopes that emit positrons that annihilate

to produce two 511 keV gamma rays emitted in the back-to-back opposite direction and can be detected quickly by the scintillation detectors.[2] The detector's design is tied to the PET's equipment. The detectors should be designed so that the maximal annihilation photons may be traced along the decay line by specifying the two interaction channels.[3]

Scintillation detectors are the most widely used detectors for determining gamma rays[4] and constitute the foundation of virtually all PET scanners. Other detectors, such as a solid-state photodetector, block detector, continuous gamma camera detector, position-sensitive multichannel photomultiplier tube, depth-encoding detector, and avalanche photodiode, are also applied in PET. Normally, scintillation detectors consist of crystal material that responds with gamma rays and photons of high energy, and properties of these scintillator materials should have a high resolution for identification of full energy peaks. After absorbing energy, they release visible light. After detecting this light, a visible photon detector turns it into an electrical current and occurs imaging in the next process of the PET scan mechanism.[1-3,5,6] The $CeBr_3$ scintillator is one of the most popular to use in various applications because of its very high density effective atomic number and high light output. These properties would be possible to develop for a high-energy resolution scintillation detector, which shows the ability of a detector to identify particles of different energies. Anyway, the basic properties should be considered first such as the peak to total ratio (PTR) which is related to the photopeak area, Compton continuum area, and peak to Compton ratio (PCR) which shows the height of the photopeak and Compton continuum by including energy resolution to keep as a basic physics database.

In this work, the radiation source Ba-133, Na-22, Ce-137, and Co-60 at energies 0.356 MeV, 0.511MeV, 0.662 MeV, 1.173 MeV, and 1.332 MeV were used to experiment for calculating the peak to a total ratio (PTR), and peak to Compton ratio (PCR) of $CeBr_3$ crystal and included the energy resolution.

Materials and methods
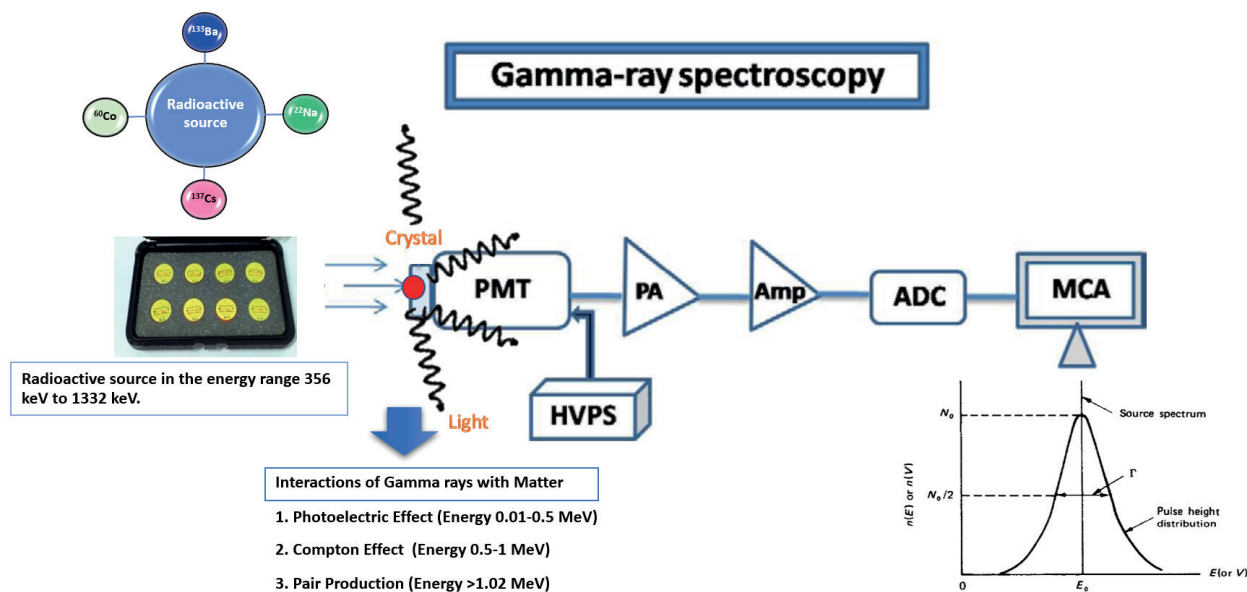
Figure 1 Measurement process of a $CeBr_3$ crystal.[6]

The $CeBr_3$ sized 10x10x10 mm³ from (Kinheng Crystal Co.LTd) is connected with a photomultiplier tube (PMT) by using silicone grease on the top surface of the PMT, and wrapped with black Teflon for protecting against external light. When high photon energy undergoes with the $CeBr_3$ crystal, the crystal will occur to interact with photon energy by energy's absorptions via the interaction ternary as the photoelectric absorption, Compton scattering, and pair production. The crystal will generate light and come into a photomultiplier tube (PMT 9256 KB) for amplifying the light signal, which is an analog signal change to be a digital signal into a multichannel analyzer (MCA). Then, the energy resolution, peak to total ratio, and peak to Compton ratio have experimented via the radiation source as Ba-133, Na-22, Ce-137, and Co-60 at energies 0.356 MeV, 0.511 MeV, 0.662 MeV, 1.173 MeV, and 1.332 MeV, respectively. The energy resolution can be calculated from equation (1), which is the detector's ability to identify particles of energy by E_0 is the energy peak centroid. The full width at half maximum (FWHM) was designated by[6,7]

$$\%R = \frac{FWHM}{E_0} \times 100\% \qquad (1)$$

Peak to total ratio (PTR) is the ratio between the area of the photopeak and the entire area of the spectrum, which may be calculated using the equation: (2).[6,7]

$$PTR = area\ of\ photopeak\ (N_p)/total\ area\ in\ Compton\ continuum\ (N_t) \qquad (2)$$

Peak to Compton ratio (PCR), which may be calculated using the equation, is the ratio of the height of the photopeak to that of the Compton continuum (3).[6,7]

$$PCR = Heigh\ of\ photopeak\ (C_p)/average\ heigh\ of\ Compton\ continuum\ (C_c) \qquad (3)$$

Results and discussion

The energy resolution of CeBr$_3$ experimented at 0.356 MeV, 0.511 MeV, 0.662 MeV, 1.173 MeV, and 1.332 MeV at full energy peak of the Ba[133], Na[22], Cs[137], and Co[60] via the equation 1. Figure 2 shows the energy resolution of the CeBr$_3$ scintillation detector in the photon energy range of 0.356-1.332 MeV. The results found that the energy resolution of the crystals was responsible for a linear pattern of inverse the square root of the photon energy and found the energy resolution values were increased when the photon energy decreased. The energy resolution result of CeBr$_3$ was reported at 6.7%, 6.4%, 5.6%, 5.4%, and 5.3%, respectively (Figure 3). The peak to a total ratio (PTR) and peak to Compton ratio (PCR) values of CeBr$_3$ crystal at 0.511 MeV to 1.332 MeV of the energies can calculate from peak area on the spectrum of Na[22], Cs[137], and Co[60] of full energy peaks by using the equation 2 and 3 (Figure 3). The experimental results of both PTR and PCR values were shown in Table 1. The results illustrate the PTR and PCR values of the crystal decreased with increasing energy due to increasing photon energy affected by the reduction of the photoelectric absorption when compared with the Compton scattering interaction (Figure 4 and Figure 5). As a result, the highest points that showed the absorption peak ratio of each energy are reduced. However, due to the Ba[133] generated low energy, photoelectric is dominant which appeals to the Compton scattering ratio is very less and affected to the PTR and PCR values of the crystal at 0.356 MeV.

Equation	y = a + b*x
R-Square (COD)	0.92358
Adj. R-Square	0.8981
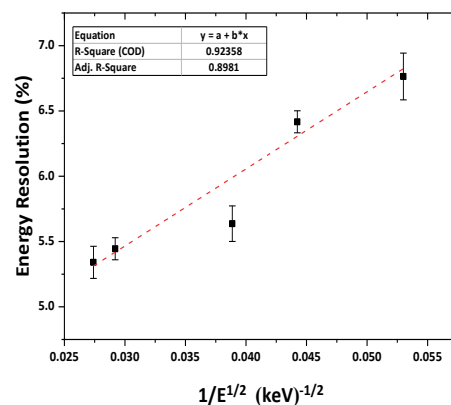
Figure 2 *Energy resolution of CeBr$_3$ crystal measured with Ba-133, Na-22, Cs-137, and Co-60 by the photomultiplier tube 9256 KB.*
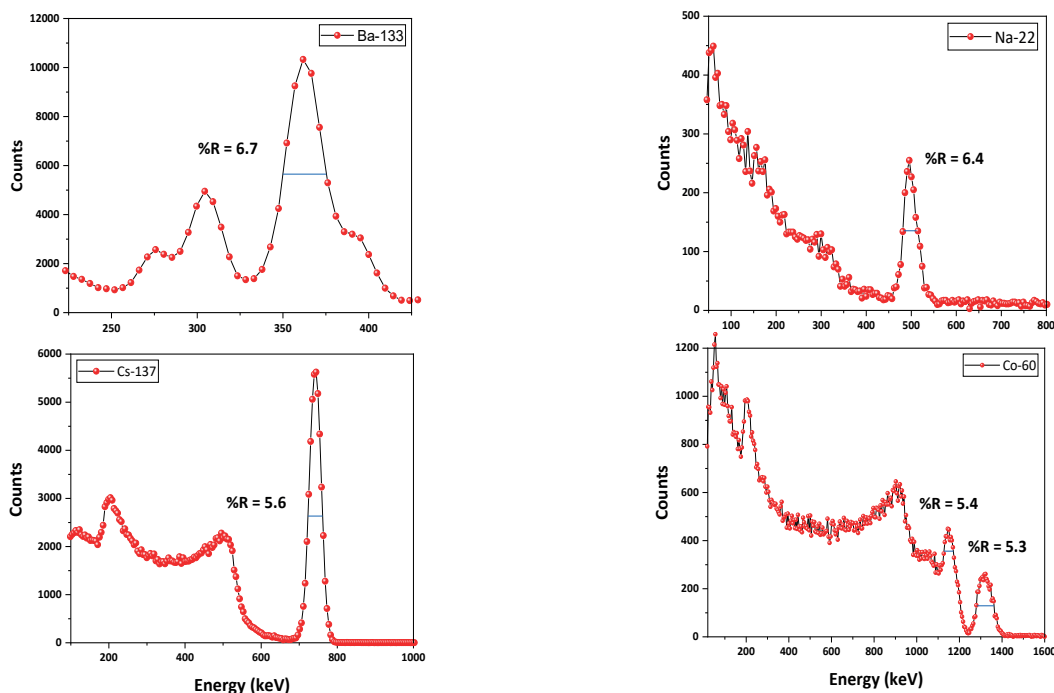
Figure 3 *Spectrum of CeBr$_3$ crystal measured with Ba-133, Na-22, Cs-137, and Co-60 by the photomultiplier tube 9256 KB.*

Table 1 PTR and PCR values of CeBr$_3$ crystal at the energy 0.511 MeV to 1.332 MeV.

Energy (MeV)	PTR	PCR
0.511	0.15	2.53
0.662	0.12	2.00
1.173	0.07	0.90
1.332	0.05	0.53

Equation	y = a + b*x
R-Square (COD)	0.98664
Adj. R-Square	0.97996

Equation	y = a + b*x
R-Square (COD)	0.99559
Adj. R-Square	0.99339
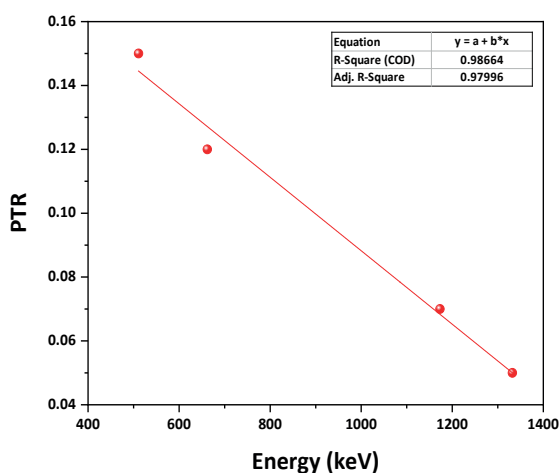
Figure 4 *Peak to total ratio of CeBr$_3$ crystals at 0.511 MeV to 1.332 MeV.*
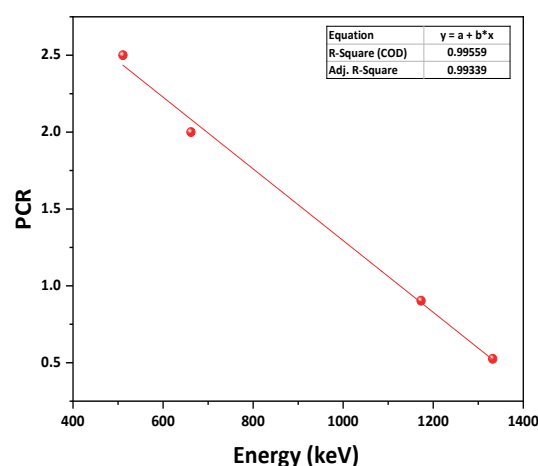
Figure 5 *Peak to Compton ratio of CeBr$_3$ crystals at 0.511 MeV to 1.332 MeV of the energies.*

Conclusion

Successfully of this experimental with CeBr$_3$ scintillation crystal, the radiation source Na-22, Ce-137, and Co-60 was used to experiment for calculating the energy resolution, PTR, and PCR values at 0.356 MeV to 1.332 MeV of the photon energies. The energy resolution of CeBr$_3$ crystal showed a linear pattern of inverse the square root of the energy and found the energy resolution values were increased when the photon energy decreased. The PTR and PCR values showed to decrease with increasing energy ranges. However, it is the investigation of primary scintillation properties, which are fundamental data of scintillation material for manufacturing advanced medical equipment applications such as PET scans in the future.

Acknowledgements

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Development of occupational therapy screening tool for clients with stroke

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ARTICLE INFO

ABSTRACT

Background: The number of new stroke cases in the Thai population increases every year. Occupational therapy has a crucial role in stroke rehabilitation. In Thailand, there is currently no standardized occupational therapy screening tool for gathering clients' impairments to facilitate further evaluation and intervention.

Objectives: To develop an occupational therapy screening tool for clients with stroke and investigate its psychometric properties.

Materials and methods: The development of the screening tool was based on a literature review and opinions of registered occupational therapists (OTs) who have had at least five years of experience working with stroke clients. Five experts examined content validity. The Index of Congruence (IOC) was calculated, and the items that showed IOC scores of over 0.5 were incorporated into the target screening tool. Afterward, two types of reliability were measured, including test-retest and inter-rater reliability. Using purposive sampling, 44 participants with chronic stroke were selected to test each type of reliability. Intraclass correlation coefficient (ICC) models 3, 1, and 2, k were used to determine test-retest and inter-rater reliability, respectively.

Results: Based on the literature review and 128 OTs' opinions, the screening tool covers four domains: sensation, voluntary movement control of upper extremity and hand function, perception, and cognition. Content validity IOC scores were between 0.8 and 1.0 for all items. The screening tool had excellent test-retest reliability on all items, except memory. And the ICC between the two raters ranged from 0.91 to 1.00, which showed excellent inter-rater reliability.

Conclusion: The occupational therapy screening tool for clients with stroke has proven valid and reliable as a screening tool in clinical settings. Further research is recommended for concurrent, sensitivity, and specificity studies.

Introduction

A stroke is a sudden onset of abnormalities in the functioning of the nervous system. It is caused by bleeding or blocked blood vessels in the brain. It is now considered a common disease in the world population.[1] In Thailand, there are more than 250,000 new stroke cases each year, and the trend is increasing.[2] Stroke is a significant problem in the public health sector. It can cause death or disabilities which vary from person to person, depending on the severity of the stroke and the area of the brain affected. Typically, stroke clients are admitted to the hospital for the first few weeks to stabilize conditions and for observation. The most improvement is seen in the first three months,

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but recovery could continue for many years after a stroke.[3] Some never fully recover. The long-term sequelae of stroke have a negative impact on the health of stroke clients and their ability to work.[1] It also has a considerable impact on the family, as well as society in general.[4] Therefore, continuing rehabilitation for a long period of time is required to decrease the impact of stroke which could occur at the inpatient and outpatient clinics, community rehabilitation centers as well as stroke clients' dwellings.

An occupational therapist has a key role in stroke rehabilitation to improve the functions of stroke survivors. In the occupational therapy process, assessment is the first step that occupational therapists use to understand their clients.[5] Even though occupational therapy has a grounded philosophical and theoretical base that supports a holistic, functional-based approach to assessment, performance deficits are viewed as support for an occupational focus for all occupational therapy evaluations and interventions.[6] Both functional and impairment-level assessments enable the occupational therapist to understand the impacts of the stroke, set goals, and design appropriate interventions for the stroke clients.[7,8] Therefore, an appropriate assessment tool needs to be used. It is recommended that an appropriate assessment tool should be simple, practical, and brief. However, it should be a standardized tool with clear procedures and scoring.[7,9] Assessment can be performed either through screening tools or comprehensive assessment tools. Whilst the comprehensive assessment tool usually considers multiple domains of functioning, the screening tool is a preliminary assessment that is quick to administer and score.[5,10] The screening tool enables occupational therapists to learn rudimentary information about their client's abilities and deficits. It is used before performing a comprehensive assessment.[10]

Based on the literature review, multidisciplinary screening tools for stroke clients have been developed. One example is the Post-Stroke Checklist (PSC). The PSC is designed to be dichotomous questions for identifying long-term problems in stroke survivors and facilitating appropriate referrals for treatment.[11] However, as it consists of 'yes/no' questions, therapists will not have subscale information about the stroke clients' performance and challenges. In addition, no study of psychometric properties has been reported.[11] Other screening tools are designed for assessing specific impairments, such as the Occupational Therapy Adult Perceptual Screening Test (OT-APST) and the Language Screening Test (LAST).[12,13] An assessment tool developed in the Thai context is called the Activities of Daily Living Assessment Tool (ADL-AT).[14] It is developed specifically for assessing basic and instrumental daily activities in Thai stroke clients. It was examined to be reliable and valid and recommended to be used in the services system.[14] However, screening tools covering the various aspects of impairment have not been developed.

Regarding the current practice of occupational therapists in Thailand, stroke clients have been evaluated for impairment via clinical assessment. Even though they are licensed occupational therapists, differences in clinical experience might affect how to gather information from stroke clients. Standardized screening tools for assessing impairments should be used for consistency and to reduce errors due to differences in clinical experience. In addition, using standardized screening tools is regarded as the basis for an evidence-based assessment. It enables occupational therapists to communicate and collaborate with other professionals effectively. Therefore, this study aims to develop an occupational therapy screening tool specifically for performance components within the Thai context and test its psychometric properties. It is expected that the occupational therapy screening tool could be used in clinical settings as a screening tool for Thai stroke clients while rehabilitation is necessary for stroke clients for a long period of time.

Materials and methods

This study was divided into two stages. The first stage was the development of a screening tool based on literature reviews and occupational therapists' opinions. The second stage was the study of the psychometric properties of the screening tool, which includes content validity, test-retest reliability, and inter-rater reliability.

Ethical approval for the study was obtained from the ethical review committee for research in humans, Faculty of Associated Medical Sciences, Chiang Mai University (Ref. number 399/2562).

Participants

In the first stage, registered occupational therapists with at least five years of experience working with stroke patients were invited to participate in the study. They were asked to complete a questionnaire about the characteristics of the occupational therapy screening tool for clients with stroke who still need rehabilitation.

In the second stage, stroke participants were recruited from four rehabilitation centers. The sample size was calculated by using the G*Power 3.1 computational program ($\alpha=0.05$, power test=0.95). The result was 36 samples, with the 20 percent dropout rate considered. Forty-four stroke patients were selected for test-retest and inter-rater reliability tests. The inclusion criteria are: stroke occurred at least three months prior, age range between 20 and 80 years old, able to read and write Thai, and no visual or hearing impairments.

Procedure
First stage: Development of the screening tools

The literature review was carried out to retrieve relevant theoretical frameworks related to stroke rehabilitation, the occupational therapy assessment process, and published instruments for cerebrovascular diseases. Then, the researcher developed a questionnaire to survey occupational therapists on the characteristics of their ideal screening tool, including what it should screen for. Registered occupational therapists of The Occupational Therapist Association of Thailand database with at least five years of experience were emailed the questionnaire. Based on the literature review and the occupational therapists' opinions, the screening

tool version 1 was developed by the research team.

Second stage: Study the psychometric properties

To measure content validity, five occupational therapists with more than five years of experience working with stroke clients were asked to examine the extent to which the screening tool version 1 represented all facets of a given construct. The index of congruence (IOC) was analyzed. The items with IOC equal to or higher than 0.5 were accepted whilst items with IOC less than 0.5 were revised according to the experts' comments. From this, the occupational therapy screening tool version 2 was subsequently developed. Then, it was used to assess ten stroke clients to test the screening tool. If the clients need clarification about the commands or testing process, the instruction was explained once to make the test process clearer.

For the reliability test, two independent raters (rater A and rater B) were invited to join the study. They were trained on how to use the occupational therapy screening tool version 2. Rater A was asked to screen 44 participants using the occupational therapy screening tool. Videos were recorded throughout the entire process of screening for rater B to assess. The results of the two raters' assessments were blinded. For test-retest, participants were asked to live as usual between the first and second assessments. The participants were asked not to keep a record in the first assessment to prevent them from copying the prior answer. Also, rater A performed the second assessment after a 14-day interval to prevent participants from recalling the screening procedure. If participants did not return for the second assessment, the researcher would perform the first and second assessments on new participants.

Statistical analysis

Descriptive statistics were presented to describe demographic data. Test-retest and inter-rater reliability were calculated using intraclass correlation coefficients (ICC 3,1 and ICC 2,k, respectively). The reliability would be excellent, good, moderate, or poor when ICC values were ≥0.75, between 0.60 and 0.74, between 0.40 and 0.59, or <0.40, respectively.[15] All statistical analyses were conducted using SPSS statistical package.

Results
First stage: Development of screening tools

One hundred and twenty-eight occupational therapists returned the questionnaire, giving their opinions on a screening tool for stroke clients. The results showed that they required a screening tool that was less time-consuming to conduct (no longer than 20 minutes). It should consist of an interview and a test. It should measure the clients' performance components with a test score. Based on the occupational therapists' questionnaire results, combined with insight from the literature review, the occupational therapy screening tool for stroke clients-version 1 was developed. It consists of four domains and 15 items. Each item has its own score, as shown in Table 1.

Second stage: Study the psychometric properties
Content valid

The occupational therapy screening tool version 1 had an IOC score between 0.4 and 1.0. It was suggested that the perception domain should be modified which resulted in changing of scoring criteria. The 'ideomotor apraxia' and 'ideational apraxia' items were merged to be 'apraxia' items with a total score of 8. The result was the

Table 1 Domains and items of the occupational therapy screening tools for clients with stroke version 1.

Domains	Items	Total scores
Sensation	Light touch	6
	Superficial pain	6
	Proprioceptive	6
Voluntary movement control	Upper extremity	18
	Hand function	12
Perception	Autotopagnosia	4
	Unilateral spatial neglect	4
	Visual object agnosia	4
	Figure ground	4
	Spatial relation	4
	Ideomotor apraxia	4
	Ideational apraxia	4
Cognition	Memory	3
	Attention	3
	Orientation	4

development of the occupational therapy screening tool version 2. It includes four domains and 14 items with IOC scores between 0.8 and 1.0, as shown in Table 2.

After trial use with ten stroke clients, no problem was reported. All stroke clients could follow the screening instructions. The screening process could be completed within 20 minutes. The time varied according to the individual client's abilities and the therapist's expertise.

Test-retest and Inter-rater reliability

Participants' demographic data are presented in Table 3. For the test-retest, the participants consisted of 44 stroke clients. Most were males (72.73%) with an average age of 55.86±11.17. Most also had primary education. More than half of the participants (54.55%) have had a stroke for over 1 year. Participants for the inter-rater reliability test also had a similar demographic except 52.27% of participants have had a stroke for less than 1 year.

Table 2 Domains and items of the occupational therapy screening tools for clients with stroke version-2 and IOC scores.

Domains	Items	Index of congruence (IOC)	
		Procedure	Scoring
Sensation	Light touch	1	1
	Superficial pain	1	1
	Proprioceptive	1	1
Voluntary movement control	Upper extremity	1	1
	Hand function	1	1
Perception	Autotopagnosia	1	1
	Unilateral spatial neglect	0.8	0.8
	Visual object agnosia	1	1
	Figure ground	1	1
	Spatial relation	1	1
	Apraxia	1	1
Cognition	Memory	1	1
	Attention	1	1
	Orientation	1	1

Table 3 Demographic data of the stroke participants (N=44 for each test).

Demographics	Test-retest reliability No. of person (%)	Inter-rater reliability No. of person (%)
Gender		
Male	32 (72.73)	32 (72.73)
Female	12 (27.27)	12 (27.27)
Age (year)		
20-40	2 (4.54)	1 (2.27)
41-60	25 (56.82)	25 (56.82)
61-80	17 (38.64)	18 (40.91)
Mean (SD)	55.86 (11.17)	57.41 (11.83)
Education		
Elementary level	15 (34.09)	17 (38.64)
Secondary level	12 (27.27)	12 (27.27)
Diploma level	3 (6.82)	1 (2.27)
Bachelor level or higher	14 (31.82)	14 (31.82)
Time after the onset		
3-6 months	12 (27.27)	10 (22.72)
>6 months-1 year	8 (18.18)	13 (29.55)
>1 year-2 years	13 (29.55)	13 (29.55)
>2 years-3 years	5 (11.36)	3 (6.82)
>3 years	6 (13.64)	5 (11.36)

Test-retest reliability was calculated with the intra-class correlation coefficient (ICC 3,1) with 95% confidence. Most items from the occupational therapy screening tool version 2 had ICC values between 0.80 and 1.00, indicating excellent reliability. Only the score for memory fell outside this range. Nevertheless, it was 0.73, indicating good reliability. Similarly, inter-rater reliability was calculated using the intra-class correlation coefficient (ICC 2,k) with 95% confidence. All items showed excellent reliability, as shown in Table 4. Moreover, the average time for screening each participant was 15.17 minutes.

Table 4 Test-retest reliability and inter-rater reliability with intra-class correlation coefficient (N=44 for each test).

Item	Test-retest reliability				Inter-rater reliability			
	Time 1 (Mean) SD	Time 2 (Mean) SD	Intraclass correlation coefficient		Rater 1 (Mean) SD	Rater 2 (Mean) SD	Intraclass correlation coefficient	
			ICC (3,1)	95%CI			ICC (2,k)	95%CI
Sensation								
Light touch	2.45 (1.82)	2.50 (1.82)	0.99	0.994-0.998	2.18 (1.88)	2.16 (1.90)	0.99	0.979-0.994
Superficial pain	2.57 (1.58)	2.86 (1.50)	0.92	0.837-0.955	2.30 (1.75)	2.27 (1.78)	0.98	0.962-0.989
Proprioceptive	2.50 (1.69)	2.43 (1.72)	0.99	0.975-0.992	2.34 (1.80)	2.34 (1.84)	0.98	0.961-0.988
Voluntary movement control								
Upper extremity	6.52 (4.79)	6.82 (4.94)	0.99	0.980-0.994	6.09 (4.81)	6.16 (5.30)	0.96	0.928-0.979
Hand function	4.66 (3.31)	4.77 (3.40)	0.99	0.982-0.995	4.14 (3.74)	3.91 (3.77)	0.97	0.939-0.982
Perception								
Autotopagnosia	3.91 (0.60)	3.91 (0.60)	1.00	1.000-1.000	3.89 (0.49)	3.89 (0.49)	1.00	1.000-1.000
Unilateral spatial neglect	0.82 (0.39)	0.75 (0.44)	0.80	0.643-0.892	0.73 (0.45)	0.70 (0.46)	0.91	0.834-0.950
Visual object agnosia	3.98 (0.15)	3.98 (0.15)	1.00	1.000-1.000	3.93 (0.33)	3.91 (0.36)	0.95	0.910-0.973
Figure ground	3.91 (0.36)	3.89 (0.39)	0.95	0.923-0.977	3.91 (0.36)	3.91 (0.36)	1.00	1.000-1.000
Spatial relation	3.77 (0.52)	3.82 (0.54)	0.91	0.840-0.952	3.80 (0.55)	3.84 (0.43)	0.95	0.913-0.974
Apraxia	7.75 (1.24)	7.82 (1.21)	0.98	0.965-0.989	7.80 (0.67)	7.80 (0.67)	1.00	1.000-1.000
Cognition								
Memory	1.11 (1.10)	1.70 (1.25)	0.73	0.407-0.867	1.27 (1.21)	1.36 (1.22)	0.98	0.958-0.888
Attention	2.32 (0.80)	2.45 (0.76)	0.80	0.637-0.891	2.36 (0.75)	2.41 (0.76)	0.98	0.963-0.989
Orientation	3.59 (1.09)	3.61 (0.95)	0.95	0.905-0.972	3.55 (0.95)	3.57 (0.97)	0.97	0.941-0.982

Discussion

The occupational therapy screening tools for clients with stroke were developed based on the opinions of occupational therapists in Thailand and literature reviews. To operate the developed screening tool, two procedures were required including an interview and a test such as performing specific tasks such as moving parts of the body as instructed. In general, a screening tool should focus on elementary information and take minimal operation time. In this study, the developed screening tool took around 15 minutes to assess basic performance components in various aspects, including sensation, voluntary movement control, perception, and cognition. For example, in the part of the cognitive domain, there were only three basic cognitions including orientation, attention, and memory that were prerequisite aspects for performing daily life. This was consistent with the item of other screening tools that have three or more elements of cognitive function that also emphasize basic cognition.[11,16,17] The data obtained from the screening tool is quantitative, which allows the occupational therapist to objectively gauge the level of the patient's performance. Thus, the developed screening tool is consistent with what was found in the literature review, namely that screening tools should be a preliminary assessment that uses less time to assess clients' ability through a performance test or observing the client's behavior.[5,18] In addition, screening tools that can provide quantitative data have the advantage of allowing therapists to interpret the results quickly, which would make the screening process more convenient.[19]

Regarding the psychometric properties of the occupational therapy screening tool, its content validity was found to be at an acceptable level (IOC scores between 0.8 and 1.0).[20] This might be a result of the development process of the screening tool, which followed the instrument development process including reviewing the literature relevant to assessment theory, taking into account the role of occupational therapists in the rehabilitation of stroke clients, existing assessment tools,[1,21] as well as the opinions of Thai occupational therapists involved in the development process who have had experienced working with stroke clients. Thus, appropriate items and methods of screening were obtained. This was in line with the development processes of other assessments in using experienced professionals' opinions to make the assessment appropriate, in other words, the content is valid.[11] In addition, the occupational therapy screening tool consists of a manual guideline and

detailed interpretation. Also, the items had been adjusted following expert recommendations and re-examined, which resulted in a high level of IOC.[22,23]

In the test-retest reliability study, the study conducted two assessments on 44 stroke clients with an interval of two weeks between the first and second assessments. This was consistent with the test-retest studies of other assessment tools where the re-test interval was approximately two weeks or 15 days, and their reliability was at the same high level.[24,25] ICC scores in this study were between 0.80 and 1.00 which are considered excellent, indicating that the rater could understand the method and interpret the screening results accurately. Only memory had good, rather than excellent, reliability (an ICC score of 0.73). As the memory questions were short and uncomplicated, the participants got higher scores in the second screening compared to the first screening due to the learning effect.[26,27] In addition, clients having a stroke for less than a year memory function could recover through spontaneous rehabilitation during this time.[28-30] These factors might affect the change in memory and make the test-retest reliability good.

The study of inter-rater reliability showed excellent reliability on all items. This meant that all procedures of the developed screening tool were clear and appropriate for both raters. They could understand the assessment process, how to give instructions, and interpret the results of the screening. This might be attributable to the fact that the screening tool has had its methods and interpretation criteria adjusted according to the advice of experts.[31] In addition, both raters were trained until they were familiar with the screening tool. The training process is recommended to enable practitioners to obtain knowledge, understanding, and skills to perform the screening accurately and effectively.[32] In this study, inter-rater reliability was performed by using the videos from the first rater's assessment to score the participants' performance. Therefore, the scores were accurate because it was a one-time assessment of the participant's performance, and the scores were reached independently by both raters. This methodology was consistent with those in the literature review in which all raters scored participants' performance from recorded videos. The inter-rater reliability range is categorized as excellent.[25,33,34]

Limitation

These results should be considered within the context of the study's limitations. The data was collected only in clinical settings. It may not represent all stroke clients in the community. In addition, the screening tool has only been proven content valid, and test-retest and inter-rater reliability for clinical use. Therefore, all of these factors should be considered when occupational therapists select what assessment tool should be used. Further research is recommended for concurrent validity, factor analysis, sensitivity, and specificity studies.

Conclusions

The occupational therapy screening tools for clients with stroke have been developed and their psychometric properties tested, including content validity, test-retest, and inter-rater reliability. It could be used as a screening tool for Thai stroke clients in four areas: sensation, voluntary movement of UE, perception, and cognition performance. Occupational therapists could use the data derived from the developed screening tool to identify impairment and determine the need for further evaluation and treatment. It is unnecessary for occupational therapists to be trained in how to use it; however, the manual guidelines providing information about the developing screening tool and how to interpret testing results should be studied.

Conflict of interest

The authors declare that there is no conflict of interest.

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A survey of standardized tests for language-delayed children used by speech-language pathologists in Thailand

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ARTICLE INFO

ABSTRACT

Objectives: The objective of this study was to investigate how speech-language pathologists in Thailand utilized standardized assessments to evaluate children with language development disorders and to discover the perspectives of Thai speech-language pathologists regarding standardized tests.

Materials and methods: This survey sample included 80 Thai speech-language pathologists who were at least 21 years old and had worked for at least one year. The research instrument consisted of a questionnaire of the speech-language pathologists' use of the standardized test to evaluate children on their language development problems. The standardized tests utilized in this study were based on the research of graduate students at the Faculty of Medicine Ramathibodi Hospital in the field of communication problems, as recommended by the Audiology and Speech-Language Pathology professional standards in Thailand.

Results: A questionnaire survey found that 92.50% of speech-language pathologists have assessed patients by using standardized tests in combination with informal tests. The most common reason for speech-language pathologists in choosing the standardized test was to summarize the patient's abilities during training. The other reasons, used in the early stages of receiving patients, were to refer patients, to assess a patient's language proficiency and progress, and to prepare for the discharge of patients.

Conclusion: The majority of speech-language pathologists suggested that standardized tests should be improved to match the current applications, cover patients, and allow easy interpretation of the test results.

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  doi: 10.12982/JAMS.2023.049
  E-ISSN: 2539-6056

Introduction

In 2020, the Department of Health, Ministry of Public Health, Thailand reported a survey for children aged 0-5 years in which 31.82% of children with delayed receptive language development and 31.30% of children with delayed expressive language development were found.[1] In general, assessment has been an important tool for diagnosing language and speech problems. Prior to a training process, a language and speech examination is basically taken with patients in order to determine their main issues and assess their capabilities. Also, assessment is used to measure the progress after an intervention.[2] In addition, the assessment data is used to summarize patient information for referrals to specialists of different fields who choose the approach of speech and language stimulation.[3] The evaluation process can be carried out in

various ways, including monitoring behaviors, gathering histories, or administering standardized tests.[4] Standardized test is a formal evaluation devoid of context with an impartial and reliable standard approach. This is because the test was developed based on the standardization in a relatively large sample and the provided instructions on how to administer a particular exam. Therefore, standardized tests can be used with children of the same age to compare the patients' abilities.[3]

In 2009, Caesar and Kohler surveyed 409 school-based speech-language pathologists (SLPs) in Michigan about their methods of evaluating children's language skills.[5] The study found that SLPs evaluated the language of children using a combination of formal and informal tests. This study revealed that, despite the existence of at least 37 standardized tests, only six of them were used more frequently.[5] They were Clinical Evaluation of Language Functions (CELF), Peabody Picture Vocabulary Test (PPVT), Preschool Language Scale (PLS), Expressive One-Word Picture Vocabulary Test [EOWPVT], Test of Language Development, and Receptive One-Word Picture Vocabulary Test, all of which were evaluated through informal testing as opposed to standardized testing. This demonstrated that SLPs used informal assessments, such as parent-teacher interviews, Language sampling, informal observations, classroom observations, and dynamic assessment, in addition to the formal ones to improve the assessment in a number of ways.[5]

The research conducted by Khoja investigated the use of formal and informal assessments by 122 SLPs in Saudi Arabia.[6] According to the research, SLPs developed and enhanced standardized tests for their patients. Although some Arabic standardized tests were created, they have not been distributed widely. This may be due to the fact that some authentically developed Arabic tests have been unpublished, standardized on a population that speaks a different dialect of Arabic, or inadequately publicized[6]. It can be partially concluded that the use of assessment tools is closely concerned with the local language and culture if they are designed to be easily accessible. The language used in the test will help enhance the usefulness of the assessment for SLPs.[6]

Since 1987, standardized tests have been used in Thailand to evaluate children with language and speech developmental delays. These tests are listed in the professional standards of Audiology and Speech-Language Pathology in Thailand.[7] However, because they were covered by the Graduate School's copyright, numerous tests created over a lengthy period of time by graduate students in the major of communication disorders at the Faculty of Medicine Ramathibodi Hospital were not easily available.

Therefore, the purpose of this study was to investigate how SLPs in Thailand used standardized assessments to evaluate children with language development deficits and to determine the perspectives of Thai SLPs on standardized tests.

Materials and methods

The population of this study consisted of 173 Thai SLPs who were at least 21 years old and had worked for a minimum of one year. The names and addresses of the population were taken from the all of Speech-Language Pathologist's directory of the Thai Speech-Language and Hearing Association. The research instrument was a questionnaire regarding the use of a standardized test by SLPs in Thailand to evaluate children with language development problems and speech delays. The standardized tests used in this study were based on the research of graduate students at the Faculty of Medicine Ramathibodi Hospital in the field of communication disorders, as indicated in the professional standards of Audiology and Speech-Language Pathology in Thailand (Table 1).[7] The questionnaire had three sections attached with some open-ended and closed-ended questions. Part 1 had a group of questions for SLPs to provide their general information, such as gender, age, education, current work experience, and workplace. Part 2 comprised questions regarding the use of a standardized test to evaluate children with language developmental delays. And part 3 contained the questions that required additional recommendations for evaluating language and speech using standardized tests.

According to the research instrument, the researchers completed a questionnaire using Google Forms and then distributed it via E-mail and public relations via the Thai Speech-Language and Hearing Association's official Facebook page. After gathering the information for over two months, from September to November 2021, a total of 80 SLPs replies were analyzed and summarized. The low response rate might be because the researchers did not have access to the current email addresses of all Thai SLPs. For the process of analysis, the data were entered into a Microsoft Excel spreadsheet. Descriptive statistics were used to analyze the data collected. Also, the free-text responses were analyzed, and the frequencies were calculated for the proportion of respondents.

Results

The questionnaire link was sent to 173 SLPs in total in Thailand. There was a total of 80 respondents, representing 46% of the population. The participants' general information was summarized in Table 2.

A total of 92.50% of the sample, or 74 SLPs, examined the children with language delays using both standardized and informal testing. Meanwhile, 6.25% of SLPs assessed patients using only the informal test, and 1.25% used only the standard test. The outcomes were displayed in Figure 1.

According to Figure 2, 75 individuals provided information on the standardized tests that they had previously taken. It was discovered that the Auditory Comprehension of Language in Thai Children Test was the most popular standardized test, with a score of 85.33%. This was followed by the Ability of Auditory Comprehension of Basic Vocabularies Test, which had a score of 74.67%. Both assessments are standardized tests relating to language comprehension. Respectively, the Thai Semantic Development Test, which was a standardized assessment for evaluating receptive and expressive language skills, came in third place with a score of 72%.

Table 1 Standardized assessments for children with language difficulties utilized in this study.

Standardized tests	Age range	Year of development	Number of samples
Auditory comprehension of language in Thai children[8]	5 years to 6 years 11 months	1987	200
Auditory comprehension of language in Thai children[9]	3 years to 4 years 11 months	1987	200
The auditory comprehension of some adjectives and prepositions in Thai Language[10]	3 years 6 months to 4 years 11 months	1989	150
The auditory comprehension ability of some adjectives in the comparative and superlative degree among Thai children [11]	3 years to 4 years 11 months	1989	400
The psycholinguistic abilities of children[12]	6 years to 9 years 11 months	1993	128
The ability of auditory comprehension of numeral classifiers in Thai children[13]	3 years to 7 years 11 months	1995	100
The production ability of verbs in Thai children[14]	3 years to 4 years 11 months	1996	320
The ability of auditory comprehension of basic vocabularies in Thai children[15]	4 years to 6 years 11 months	1996	300
The verbal production of sentences in normal children by picture arrangement[16]	4 years to 6 years 11 months	1996	210
The production ability of adjective and preposition antonym pairs in Thai children[17]	4 years to 6 years 11 months	1998	360
Thai syntactic development test for children[18]	3 years to 7 years 11 months	2000	500
Thai semantic development test for children[19]	3 years to 7 years 11 months	2000	500
Thai adaptation of the receptive-expressive emergent language test (reel-3)[20]	birth to 36 months	2010	600

Table 2 Summary of the participants' general information.

General participant information		N=80	
		N	%
Age	20-29 years old	39	48.75
	30-39 years old	25	31.25
	40-49 years old	2	2.50
	50-59 years old	8	10.00
	60 years older	6	7.50
Work experiences	less than 5 years	29	36.25
	5-9 years	19	23.75
	10-14 years	16	20.00
	over 15 years	16	20.00

Figure 3 demonstrated that SLPs who utilized standardized tests justified their use during five distinct time periods. Firstly, the purpose of receiving the highest score of 89.33% was to evaluate the training performance of patients. Secondly, 82.67% were assessed when first receiving the patient. Thirdly, 28% were evaluated upon patient referral. Fourthly, 20% were assessed for the patients' language proficiency and progress. Lastly, the purpose with the lowest score of 1.33% was for pre-discharge evaluations.
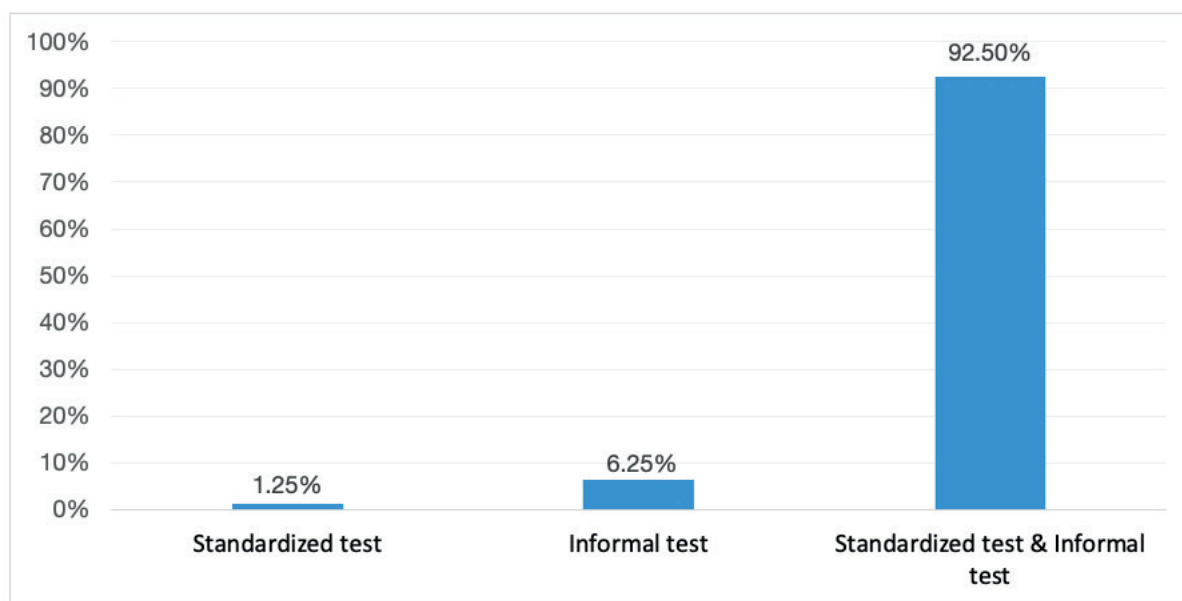
Figure 1. *Assessment methods used by Speech-Language Pathologists in Thailand.*
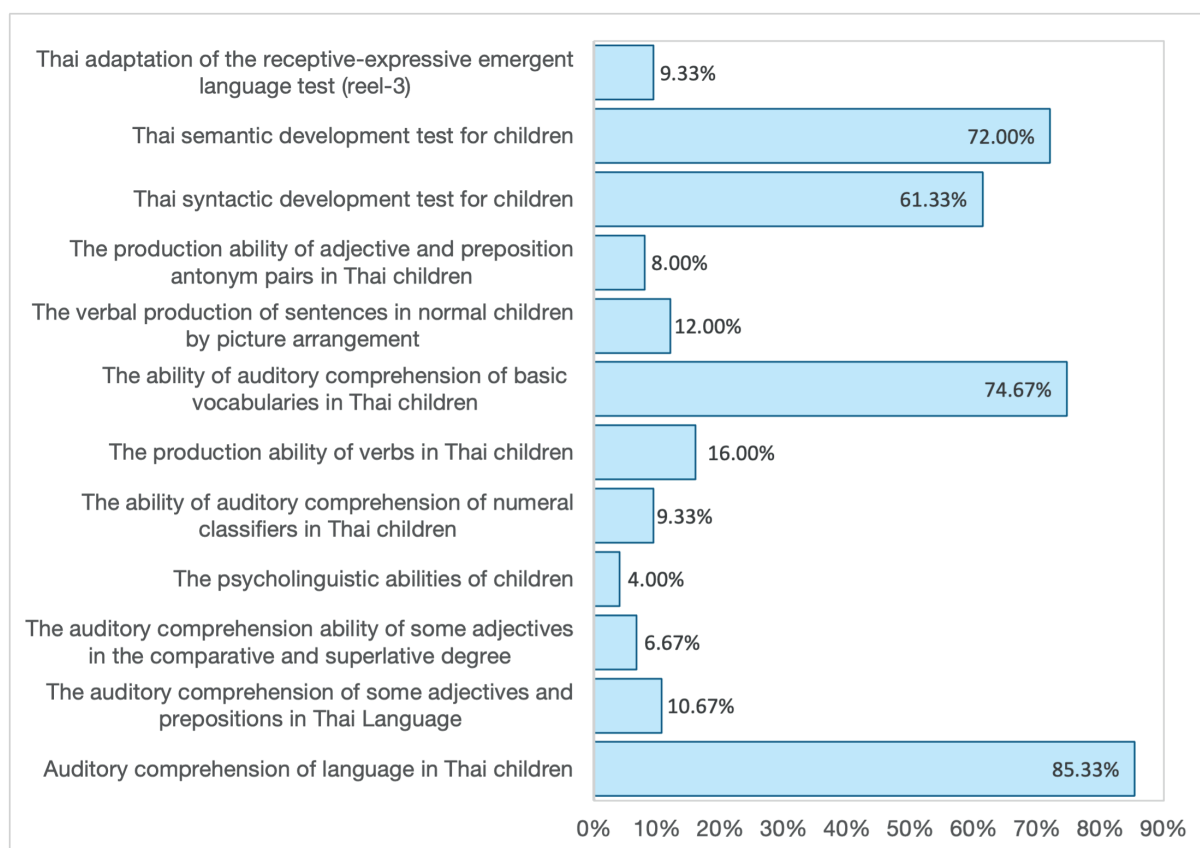
Figure 2. *The number of speech-language pathologists in Thailand using each standardized test.*
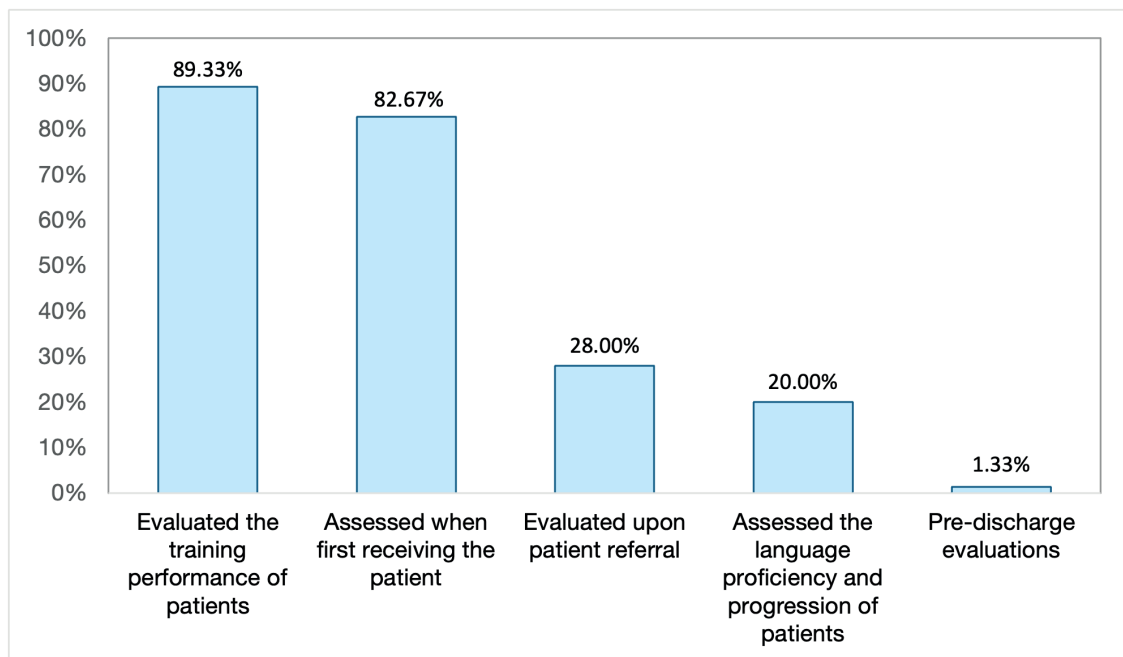
Figure 3. *The purposes for which speech-language pathologists select standardized tests.*
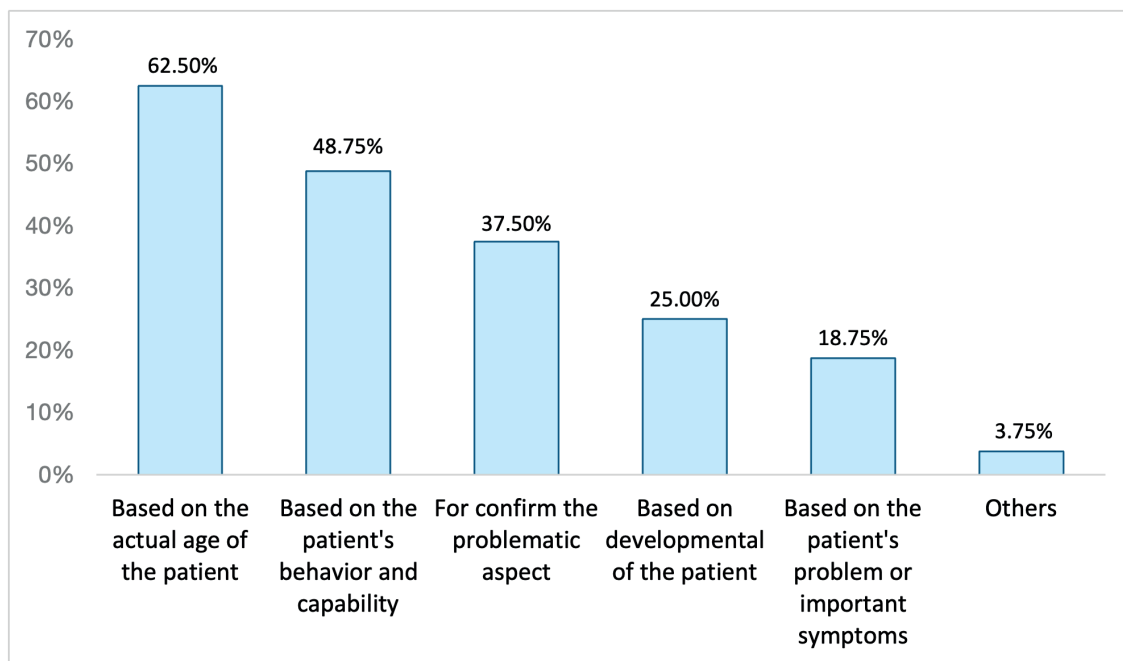
Figure 4. *Factors influencing the decision of speech-language pathologists when selecting a standardized test.*

Figure 4 showed that, among 80 participants, SLPs deemed the standardized test to be appropriate for all patients for the following six factors. First, 62.5% of SLPs selected the standardized test based on the actual age of the patient. The factors secondly chosen by 48.75% of SLPs was the patient's behavior and capability and thirdly by 37.5% of SLPs was to confirm the problematic aspect respectively. The development of the child was the basis for the fourthly selected factor by 25%. 18.75% of SLPS chose the test according to the patient's problem or important symptoms as the fifth factor. Finally, as least number as 3.75% of SLPs chose the standardized test based on other considerations, such as the test's reliability and validity, how it was interpreted, or the length of time.

In the exploration of the tests used by SLPs when standardized tests were unused, the obtained data was clearly shown in Figure 5. Informal assessment through play, language milestone screening, and observation of social communication became the top three informal assessments, accounting for 43.04%, 36.7%, and 34.18%, respectively.
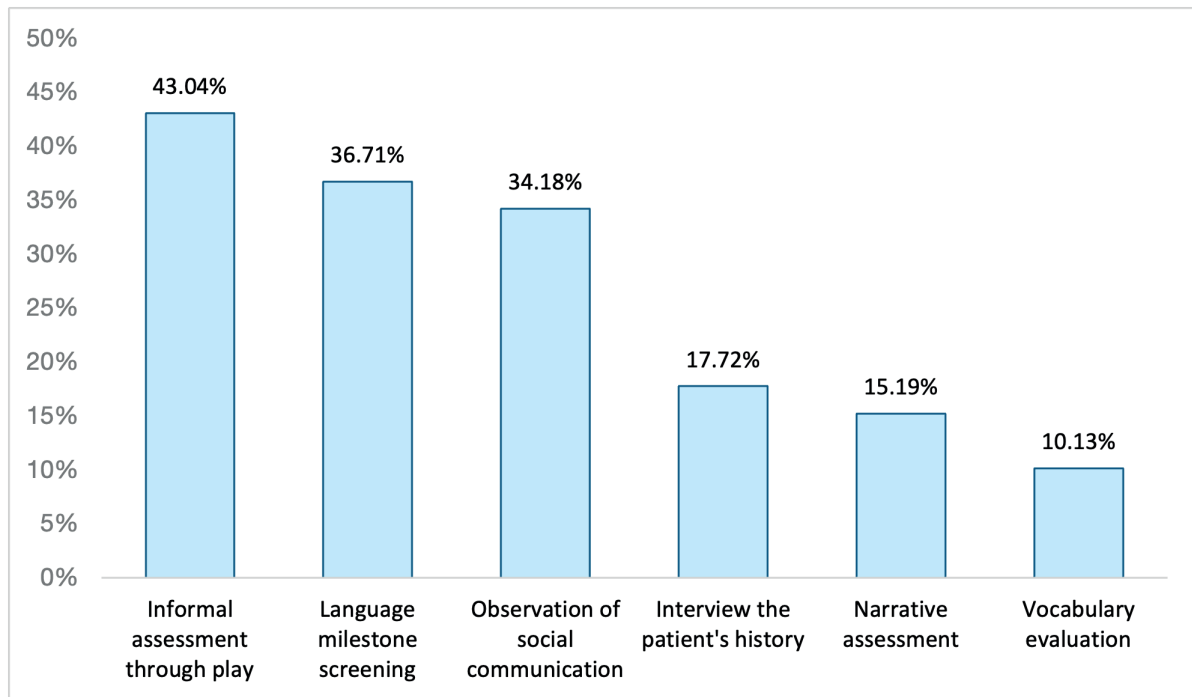
Figure 5. *Other assessments are chosen by speech-language pathologists when not utilizing standardized tests.*
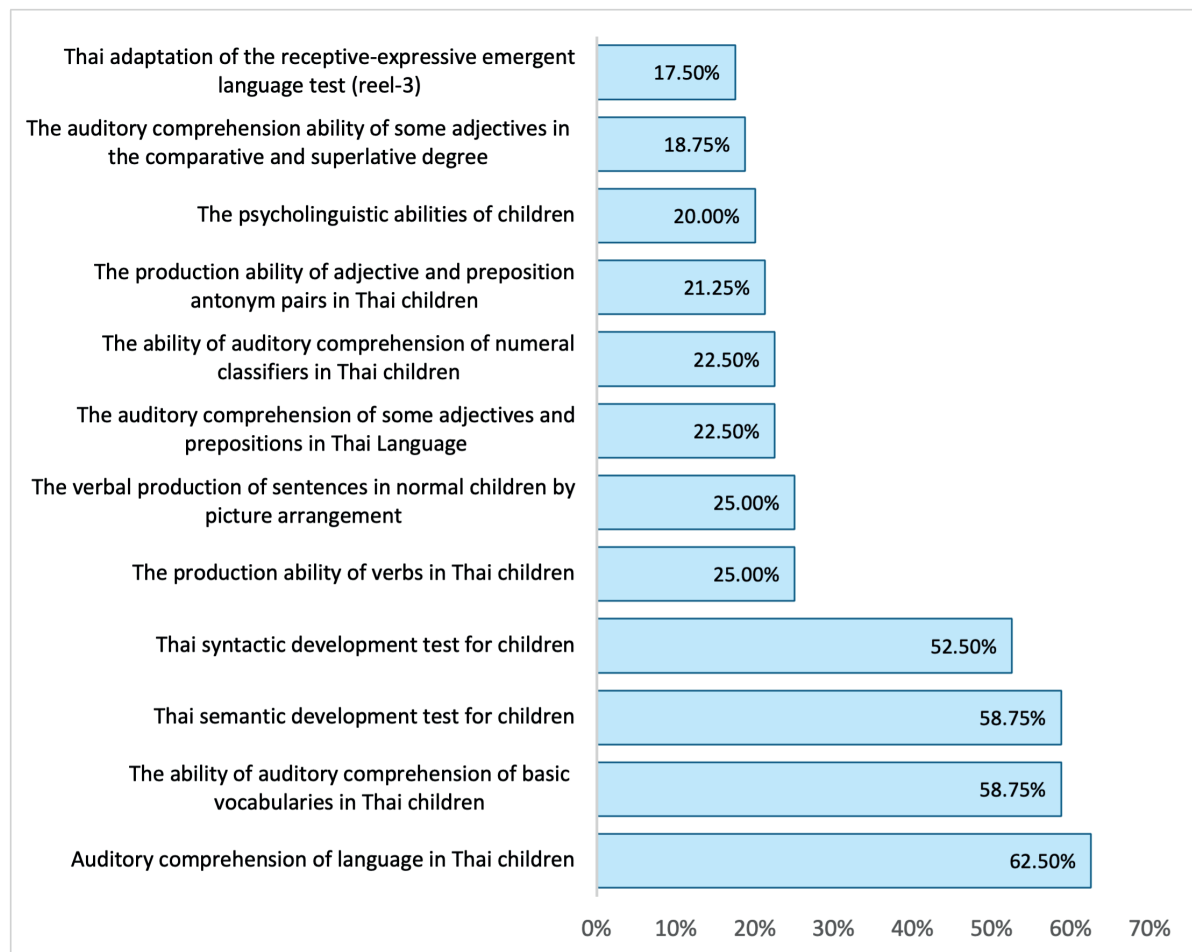
Figure 6. *Standardized tests that speech-language pathologists considered to be improved.*
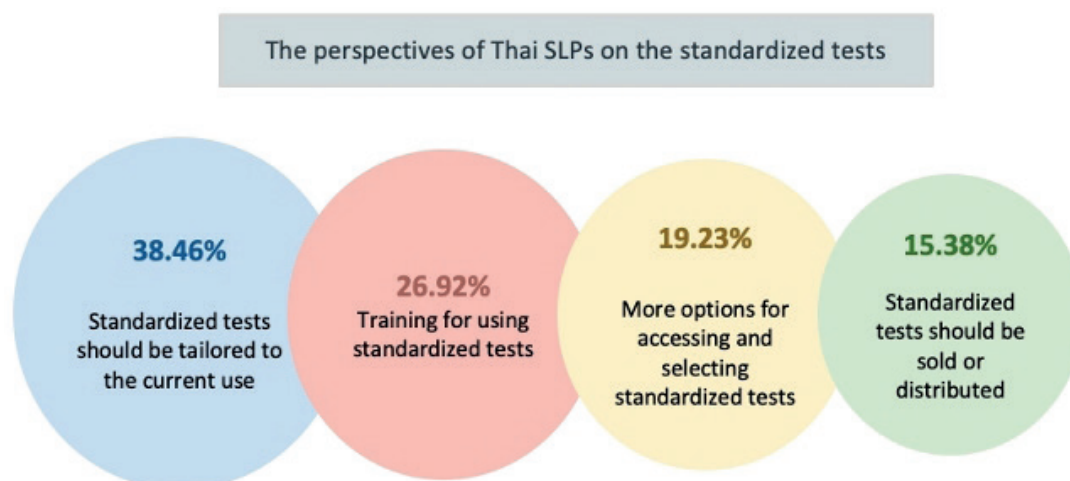
Figure 7. The recommendations of Thai SLPs on the standardized tests.

According to Figure 6, the Auditory Comprehension of Language in Thai Children Test (62.5%) was the standard test that SLPs in Thailand needed the most improvement. The Ability of Auditory Comprehension of Basic Vocabularies in Thai Children Test and the Thai Semantic Development Test came in second place with 58.75% each, followed by the Thai Syntactic Development Test in third place with 52.5% respectively.

Based on the results of the survey, SLPs suggested that the standard test should be concerned and revised in the following points: the words and images should be updated; the sample group has evolved and changed over time; the testing time was too long; the results were difficult to be interpreted; and the questions were unclear or with least possible answers.

In Figure 7, regarding the standardized test, SLPs in Thailand made a number of suggestions that could be divided into four main recommendations. First, 38.46% of SLPs recommended that standardized exams should be tailored to the present use in terms of language and test format. The tests should be provided in electronic form with more concise and varied tests so that they can cover a wider range of patient types. Second, 26.92% of respondents indicated that a training program should be arranged to facilitate the speech-language pathologists whose knowledge of the tests was different. Third, 19.23% of respondents suggested that SLPs should have more options for accessing and selecting standardized tests. Finally, 15.38% of those who provided the feedback indicated that the standardized test should be sold or distributed.

Discussion

The purpose of this study was to explore the use of standardized tests for children with speech and language development delays among SLPs in Thailand. The sample consisted of 80 SLPs with at least one year of work experience. The questionnaire survey found that 92.5% of participants assessed their patients by using standardized tests in combination with informal tests. This result was consistent with Caesar and Kohler[5] who demonstrated that the school-based SLPs in Michigan, United States frequently evaluated children using a combination of a standard test and an informal test. This was also consistent with the findings of Khoja who discovered that SLPs in Saudi Arabia evaluated the patients using a combination of standardized and informal tests.[6]

According to this report, the Auditory Comprehension of Language in Thai Children Test was the most popular standardized test, followed by the Ability of Auditory Comprehension of Basic Vocabularies Test. Both tests were assessments of receptive language ability. The Thai Semantic Development Test was ranked in third place and was a standard receptive and expressive language examination. The findings were in accordance with those of Caesar and Kohler who discovered that the SLPs in Michigan most frequently used the United States the Clinical Evaluation of Language Functions (CELF), followed by the Peabody Picture Vocabulary Test (PPVT), and the Preschool Language Scale (PLS) as their standardized tests.[5] The CELF and PLS were the tests of receptive and expressive language respectively, whereas the PPVT was the test of language comprehension. However, some different areas were found in the findings of Khoja.[6] The Preschool Language Scale (PLS), Receptive-Expressive Emergent Language (REEL), and Clinical Evaluation of Language Functions (CELF) were the formal test most frequently used by the SLPs in Saudi Arabia. All these three tests were standardized receptive and expressive language examinations. Nevertheless, these investigations demonstrated that SLPs were accountable for determining a child's language using standardized tests.

Thai SLPs administered standardized tests, taking their application throughout five distinct time periods into consideration. The training performance of patients was evaluated using standardized examinations. It was discovered during the patient's initial visit, transfer to another hospital, examination of the patient's linguistic ability and progress, and the time prior to the patient's discharge. This study concurred with Owen who claimed that SLPs utilized assessments to diagnose language skills, summarize the patient's level of competence and progress, identify the

need for therapy, forecast the duration of treatment, select treatment options, and summarize patient data for specialist referrals.[2] The findings of this study were also consistent with Shipley's who stated that a good assessment should be based on the patient's skills and abilities. In addition, it must be appropriate for the patient's condition including gender, age, skill level, and cultural background.[3]

When SLPs did not use standardized tests, they employed informal assessment through playing, language milestone screening, and social communication observation. The SLPs chose non-standard assessments because children's abilities were not compatible with standardized tests. They had expected to evaluate the children's basic abilities prior to administering standardized tests, and such assessments required less time to administer. The informal assessment used by SLPs as a descriptive-approached assessment was consistent with the findings of Caesar and Kohler which found that SLPs in the United States had other assessments when the standardized test was not in consideration for use.[5] The top three forms of informal evaluation were parent-teacher interviews, language sampling, and informal observation.

Thai SLPs identified the Auditory Comprehension of Language in Thai Children Test as the instrument requiring the most improvement. Meanwhile, the Ability of Auditory Comprehension of Basic Vocabularies in Thai Children Test and the Thai Semantic Development Test ranked in second and third place, respectively. It could be seen that the standardized test that the SLPs needed to be revised corresponded to the three most popular and well-known tests. This also showed that if a standardized test was utilized frequently, it would reveal problems or opportunities for improvement. Changes should be made to enhance and/or modernize psychological and neuro-psychological assessment instruments if the test content and normative data have become outdated.[21]

Conclusion

This study aimed to investigate the use of standardized testing for children with speech and language development deficits among Thai SLPs. Most SLPs conducted patient evaluations using a combination of standardized and informal tests. The most popular assessment was a standardized test of language comprehension and language expression. This indicated that SLPs used standardized tests in order to compare children's language abilities. SLPs confirmed language and speech problems by using standardized tests based on the child's age, behavior, and abilities. In addition, the majority of SLPs recommended that standardized exams should be redesigned to match the current language and test format, as well as to include a wider range of patient types. In conclusion, the findings of this study can be utilized as a guide for improving the standardized tests for children with delayed language and speech development in Thailand.

Limitations of the research and suggestions for future study :

The limitation of this study was that the researchers collected the data only from the standardized test which was based on the research of the graduate students of the Faculty of Medicine Ramathibodi Hospital in the field of communication disorders. Besides, the researcher did not send the questionnaire to SLPs by letter, using only the online sample collection. Suggestions for future research include that the survey should inquire about other standardized assessments established by SLPs. In addition, questionnaires should be distributed via mail and online to collect data in multiple ways. The validity and reliability of the questionnaire should be determined in future studies.

Conflicts of interest

The authors declare no conflicts of interest.

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Effect of the cognitive strategy training protocol on task mastery and cognitive performance during the instrumental activity of daily living in stroke patients

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ARTICLE INFO

ABSTRACT

Background: Stroke leads to decrease in physical, emotional, social function, and activities in daily living. Cognitive functions, either basic or higher-level, are fundamental factors in performing tasks and functional activities in daily life. Therefore, cognitive training to enhance functions in stroke patients is necessary. This study developed a cognitive strategy training protocol using the Perceive, Recall, Plan & Perform (PRPP) System of Intervention and the Multicontext Approach to improve cognitive function during performance in instrumental activities of daily living (IADL) tasks.

Objectives: This study aimed to examine the task mastery of stroke patients during the performance of IADL tasks and to examine the effects of the cognitive strategy training protocol on cognitive performance in stroke patients.

Materials and methods: The study was a quasi-experimental, one-group, pretest-posttest research design. Eight stroke patients with cognitive impairment aged between 18-70 years old participated in the study. The intervention called "cognitive strategy training protocol" included 12 sessions, 3 times a week across 4 consecutive weeks. Each session took approximately 60 minutes. An outcome measure was the PRPP System of Task Analysis-Thai version. Descriptive data obtained from Stage One of the PRPP System-Thai version were analyzed to determine for task mastery, and pre-and post-scores of Stage Two of the PRPP System-Thai version were computed using the Wilcoxon signed-rank test.

Results: After the cognitive strategy training protocol, all participants had higher percentage scores obtained from Stage One of the PRPP System-Thai version for at least 1 activity. Five out of eight participants showed higher percentage scores in both activities. Comparing the pre- and post-test scores from Stage Two of the PRPP System-Thai version using the Wilcoxon signed-rank test revealed no significant difference in all activities ($p>0.05$). However, the descriptive results of the posttest percentage scores of Stage Two of the PRPP System-Thai version showed that 4 out of 8 participants had higher scores for at least 1 activity and 2 participants showed higher scores in both activities.

Conclusion: The cognitive strategy training protocol was likely to be possible to improve task mastery and cognitive performance during the IADLs tasks in the participants despite no statistically significant difference.

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Introduction

Over 30 percent of the 15 million stroke patients in the world have remaining disabilities.[1] The term stroke is used to explain dysfunction or brain damage that causes an interruption in the blood flow to the brain, and a stroke

can occur in any area of the brain such as the cerebral hemispheres, the brainstem, or the cerebellum.[2] The effects of stroke can lead to decreasing in physical, social, emotional function, and occupational performance in daily living. Regarding cognitive impairments, both basic cognitive and higher-level cognitive functions such as memory, attention, and executive functions are affected as a consequence of stroke.[3] Cognitive functions are fundamental for performing activities in daily living. Therefore, many stroke patients may have difficulty in inhibiting automatic responses and choosing tasks while monitoring alternative plans of action while performing other functional tasks.[4] Stroke patients in any level of severity of cognitive impairment may have trouble performing a variety of life activities related to person, communities, and engagement, including basic activities of daily living (BADL), IADL, work, leisure, rest and sleep, education, play, and social participation.[3,5]

In general, there are two main approaches for cognitive rehabilitation: one is the bottom-up approach and the other one is the top-down approach. The bottom-up approach is impairment-based and focuses on performance components and cognitive and perceptual skills.[6] Conversely, the top-down approaches focuses on occupational performance despite cognitive impairment.[7] In the past, occupational therapists implemented bottom-up approach to improve abilities by retraining particular cognitive-perceptual functions through paper and pencil tasks, tabletop activities, computer-based tasks, and virtual reality. However, a limitation of the bottom-up approach is the generalization or transfer of learning skills in the training session to a person's context or environment. The results of interventions utilizing a bottom-up approach did not show how specific skills are related to task performance and cognitive processing.[8] Moreover, paper and pencil tasks, tabletop activities, computer-based tasks, and virtual reality were not considered occupation-based practices and thereby did not focus on facilitating meaningful occupations based on clients' desires. On the other hand, the top-down approaches focus on an individual's participation in occupation rather than improving function or performance components by providing internal and external strategies or adaptation of the activity demands or environment in order to use the patient's cognitive abilities to replace their limitation of cognitive function.[9]

There are models, intervention approaches, and techniques that contain information processing strategy training such as the Multicontext Approach[8], the Cognitive Orientation to Daily Occupational Performance (CO-OP) approach[10], and the PRPP System of Intervention.[11] The implementation of the top-down approach with acquired brain injury (ABI) tends to increase, especially in the PRPP System and the Multicontext Approach. Nagelkop *et al*.[12] were applied the Multicontext Approach with a 41-year-old ischemic stroke to increase online awareness of performance, strategy use, and functional performance. Recently, Jaywant *et al*.[13] used the Multicontext Approach in a single case series with ABI which provided guided

questioning and self-generation of strategies practiced across everyday functional cognitive tasks. The results of these two studies showed a positive change in self-awareness and cognitive strategy uses.[12-13] Similarly, the study of White *et al*.[14] used the PRPP System of Task Analysis with stroke patients to measure occupational performance and figure out strengths and difficulties in cognitive strategy uses. In addition, Lindstad *et al*.[15] applied nine sessions of the PRPP System of intervention for application in community-based rehabilitation with older adults with cognitive impairment following stroke to increase performance in everyday tasks. Presently, Smith *et al*.[16] applied the PRPP assessment to examine the effectiveness and relevance of this measurement with two Aboriginal Australian people in the Northern Territory of Australia following ABI. The finding of this study presented the PRPP assessment assessing change in cognitive strategy application over a 6-month period.[16] In Thailand, there is a research limitation about applying top-down approach to enhance functions in stroke patients with cognitive impairments. Juntorn *et al*.[17] were perceived as the first study that combined the intervention between the PRPP System of Intervention and the Four-Quadrant Model (4QM) of Facilitated Learning to examine the effect of information strategy training for children with learning disabilities. However, no research or evidence applied the combination of the PRPP System and the Multicontext Approach to provide cognitive strategy training for stroke patients in Thailand and foreign countries.

In this study, the researchers intend to combine two top-down approaches namely, the Multicontext Approach and the PRPP System of Intervention, to promote the performance of stroke patients who have cognitive impairment. The Multicontext Approach is based on the Dynamic Interactional Model which emerged from cognitive and educational psychology in 1992 by Toglia. This approach is a metacognitive strategy-based intervention that focused on practicing a specific cognitive strategy in multiple contexts, with a variety of meaningful activities, and with several techniques.[8] The PRPP System of Intervention and the PRPP System of Task Analysis were based on the Occupational Performance Model (Australia) (OPMA) which was developed by Chapparo and Ranka in 1997. The intervention programs in the PRPP System of Intervention provided a sequence of processing strategies "Stop/Attend, Sense, Think, Do" which are provided through physical, verbal, and visual prompts.[11] In this study, the intervention is based on a cognitive strategy training protocol that focused on using cognitive strategy training and self-awareness training in various contexts while the participants perform IADL tasks on their needs and desires. From this viewpoint, this study emphasized on observation difficulties in performing task and strategy usage in information processing systems through information processing theory and explored task mastery of the participants through observable information processing behaviors while performing the tasks. In this study defined task mastery is a person's skill that is measured against what is expected of a person within the

environment where the performance usually occurs. However, the cognitive skills in cognitive strategy training protocol, combining the PRPP System of Intervention and the Multicontext Approach, was based on the information processing theory[11]. The PRPP System views the skills of cognitive function through the descriptors in each sub-quadrant of the PRPP System of Task Analysis[11], descriptors are also called cognitive behaviors as depicted in Figure 1. While the Multicontext Approach views the skills of cognitive function were metacognitive skills which are also

called higher-level cognitive skills including self-awareness and executive function. In addition, the cognitive function of the processing strategy in this approach consists of attention, visual processing, memory, organization, and problem solving skills.[8]

This study had two purposes. The first purpose was to examine the task mastery of stroke patients during the performance of the IADL tasks, and the second purpose was to examine the effects of the cognitive strategy training protocol on cognitive performance in stroke patients.
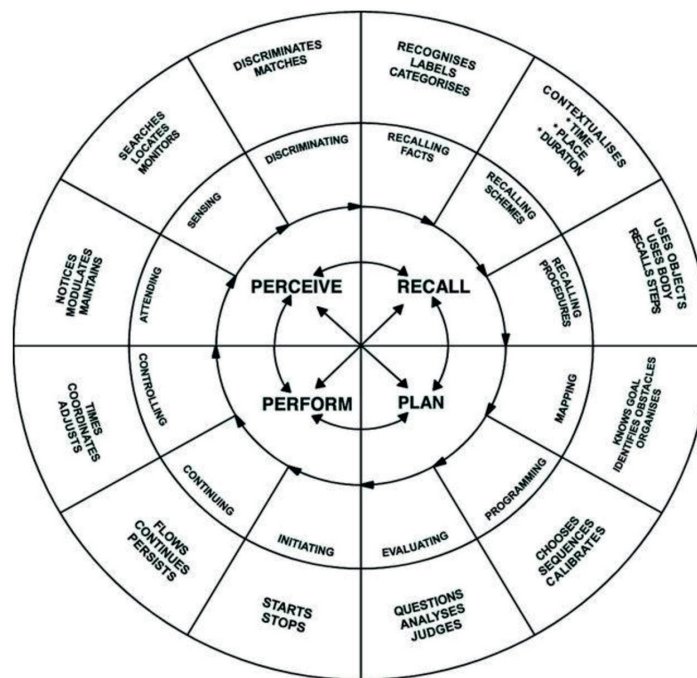
Figure 1. *PRPP System of Task Analysis: a conceptual model of information processing behaviors[11]*

Materials and methods

Participants

Eight stroke patients with cognitive impairment who received occupational therapy services in hospitals, institutes, or rehabilitation settings in Chiang Mai and Bangkok participated in this study. The sample size was calculated using the G*power program (version 3.1.9.4)[18] based on the study of Nott, Chapparo, and Heard,[6] with effect size=0.92, α error probably=0.05, power (1-β error probably)=0.8, and correlation among repeated measures =0.001. The inclusion criteria were stroke patients with cognitive impairment between 6-24 months after the first onset of stroke, 18-70 years old, had cognitive impairment (MoCA or MoCA-B score ≤24), had zero to mild depression conditions as measured by the Patient Health Questionnaire (PHQ-9)-Thai version, did not have aphasia (motor, sensory, and global aphasia), and did not have diagnostic psychotic disorders.

Instrumentation

The Montreal Cognitive Assessment (MoCA)-Thai version and the Montreal Cognitive Assessment Basic (MoCA-B)-Thai version were used to screen for cognitive

impairment. The cut-off score of MoCA-Thai version was below 25[19] and 1 point was added for individuals having ≤ 6 years of education. The sensitivity and specificity of the MoCA-Thai version in Thai elderly were 0.80 and 0.80, respectively, for a mild cognitive impairment.[20]

The MoCA-B-Thai version was developed from MoCA to detect mild cognitive impairments for participants who had low educational attainment. Scoring in this instrument included adding 1 point for individual had ≤4 years of education, and adding 1 point with low educational attainment. The optimal cut-off score is 24 out of 25. This test presented a test-retest reliability of 0.91 (*p*<0.001) and had an internal consistency of 0.82.[21]

The PHQ-9-Thai version was used to screen for depression of the participants. It is a self-reported screening diagnostic instrument used for making criteria-based diagnoses of depressive and other mental disorders. The PHQ-9-Thai version showed internal consistency, a Cronbach's alpha=0.79, and displayed moderate convergent validity with the Hamilton Rating Scale for Depression (HAM-D)-Thai version, r=0.56; *p*<0.001. The cut-off score of this instrument was ≥9.[22]

The Canadian Occupational Performance Measure (COPM) 4th Edition was also used to select IADL activities in this study. It is a semi-structured interview, asking the participant to identify problem areas (self-care, productivity, and leisure) in daily function and scoring for satisfaction and performance.[23] The 4th Edition of COPM examined the test-retest reliability in 30 Thai stroke survivors and their family caregivers. The Spearman's rho correlation coefficient of the test-retest performance and satisfaction scores for the stroke survivors were 0.88 ($p<0.001$) and 0.956 ($p<0.001$). The Spearman's rho correlation coefficient of the test-retest performance and satisfaction scores for the family caregivers were 0.99 ($p<0.001$) and 0.992 ($p<0.001$).[24]

The outcome measurement in this study is the PRPP System-Thai version which was developed by Munkhetvit[25] to be used in the Thai context. It applies procedural task analysis to break down occupational performance into measurable elements and considers related criteria for evaluating one's abilities of occupational performance through systematical-process observation. This outcome measurement is separated into two stages. Stage One is the procedural task analysis that is used to indicate an error during the performance. The therapist must break down the unit of activity task into steps to be performed and record the participant's errors. Four error types have been divided including errors of accuracy, repetition, omission, and timing. The interpretation of each score will be indicated as a percentage score of the actual task performance which is termed the level of task mastery. Stage Two focused on observable processing strategies (in this instrument called "descriptors") considered regarding processing behaviors in which the participant has difficulty performing the task in each of the four quadrants of the PRPP System as illustrated in Figure 1. Task performance is broken down into processing strategies in Stage Two, while these descriptors become the criterion for which cognitive elements of task performance are to be considered. The therapists analyzed the behaviors and errors of participants during the performance through videotaped performances by using the Stages One and then using the Stage Two, to identify and accurately record more of the details of descriptor performance. The PRPP System-Thai version has been examined for psychometric properties in Thai clients who suffer from an ABI. It showed high inter-rater and test-retest reliability.[25] Stage one was analyzed for task mastery, and Stage Two was analyzed to examine the effect of the cognitive strategy training protocol.

Intervention

The intervention of this study, called a cognitive strategy training protocol as presented in Figure 2, was the combination of the PRPP System of Intervention and the Multicontext Approach. It was implemented in stroke patients with a cognitive impairment through performing IADL tasks which the participants had the opportunity to perform both in the hospital and in their environments. The intervention protocol included 12 sessions. Each session was administered for 60 minutes per day, three times a week, for a period of 4 consecutive weeks. The participants were asked to complete and prioritize five activities or tasks according to their needs and desires by the COPM. These first two IADL tasks were used for training with the cognitive strategy training protocol. Each session of the protocol consisted of 3 phases including the beginning phase (10-15 minutes), the intervention phase (30-35 minutes), and the ending phase (10-15 minutes). Starting with the beginning phase, the participant was asked to anticipate any problem that might occur before the intervention phase and rate the levels of difficulties or challenges. Afterward, the researcher provided the opportunity for the participant to generate strategies or to guide strategy choices when needed. The intervention phase was divided into three parts including error detection, strategy training, and relearning of strategy uses. The participant must self-evaluate to find an error in specific tasks while the researcher asked a guided question to facilitate self-assessment and strategic thinking and
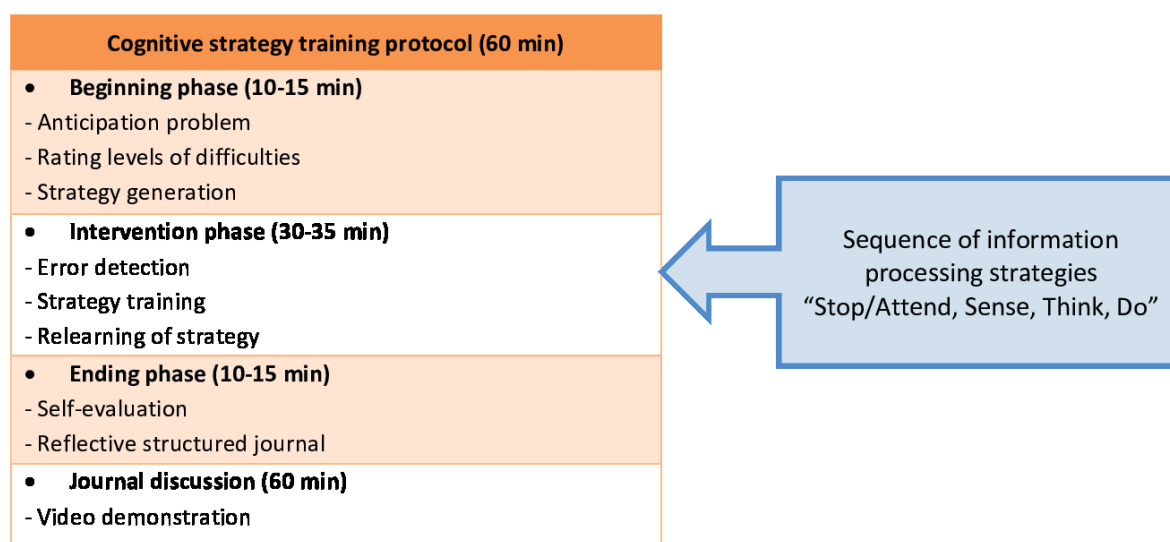
Figure 2. Cognitive strategy training protocol.

generation. When the participant encountered a challenge while performing the task, the researcher provided the sequence of information processing strategies "Stop, Sense, Think, Do" of the PRPP System of Intervention. Moreover, the participant learned the effective strategy used and was linked to the previous activity experiences in the relearning of strategy uses part. The ending phase included two parts: self-evaluation and a reflective structured journal. The self-evaluation required the participant to indicate and rate the level of difficulties and strategies used and experienced during the intervention phase. Using a reflective structured journal facilitated to the production of self-monitoring, connecting the strategy to other tasks, and establishing a goal for the next session interventions. Each activity session of intervention was included in the three main phases listed above except sessions 6, 9, and 12. The journal discussion involved a video demonstration in session 6, 9, and 12. The journal discussion helped the participant learn through another's experience and they could discuss their errors and strategies used by watching the video demonstration of the tasks being performed. Examples of activities included meal preparation, personal device caring, clean-up, making a telephone call, making a planner, arranging pills in a pill organizer, and creating a weekday schedule. In order to decrease the limitation of motor problems of the participant, the researcher did provide adaptive/assistive devices or equipment to facilitate the participants who could not use two hands properly in task performance. For example, providing a larger handle for optional choices in clean-up tasks, and preparing a meal.

Procedures

The participants who met the criteria were asked to accept and sign an informed consent form and were asked to complete and prioritize five activities or tasks by the COPM. The researchers asked the participants to perform the first two tasks from their COPM's list. The participants were videotaped while they were performing the tasks. The video clips of the participants were sent to the occupational therapist who did not involve in the intervention to score using the PRPP System-Thai version. Scores obtained from this stage were the pre-test scores. After that, all participants received the intervention three times a week for 4 consecutive weeks, approximately 60 minutes per day. After the intervention period was over, the participants were asked to perform the two tasks again and were videotaped while they were performing the tasks. The video clips were sent to the same therapist for scoring using the PRPP System-Thai version. Scores obtained from this stage were the post-test scores. Scores from Stage One Analysis of the PRPP System-Thai version were analyzed to determine the task mastery and were presented as the mean, standard deviation, and percentiles. Scores obtained from Stage Two Analysis of the PRPP System-Thai version were computed to compare the disparity between pre-and post-intervention using the Wilcoxon signed-rank test (see Figure 3 for the details). Ethical approval was obtained by the review board. All participants signed a consent form verbal and written explanation of the study was provided.
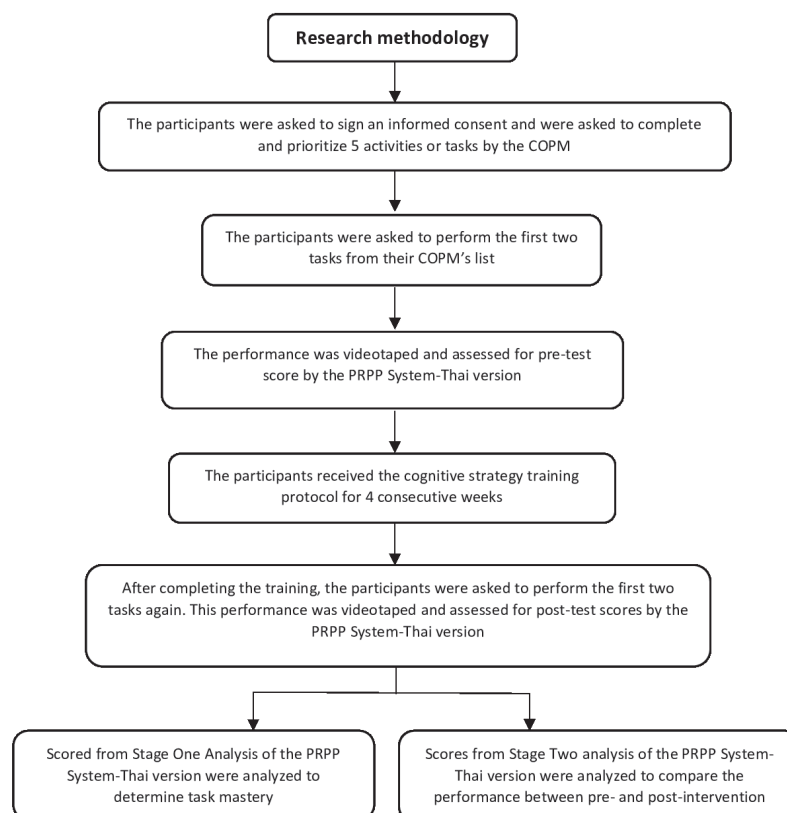
Figure 3. Summary of research methodology.

Results

The demographic characteristics of the participants are divided into two parts. The first part is characteristic of the participants who met the inclusion criteria is presented in Table 1. The other part is the scores from the screening tests which are depicted in Table 2.

From Table 1, there were 8 stroke patients, 4 males, and 4 females, participated in this study. The average age was 63±8 years old. The average level of education was 7±5 years. The average onset of the first stroke was within 6±7 months. Most of them (75.00%) were married, 62.50% were retired, and 87.50% were diagnosed with ischemic stroke. All participants were right-handed dominant. The number of brain-side lesions was equal, in that there were 4 left-sided strokes and 4 right-sided strokes.

From Table 2, most of the participants were at the Brunnstrom stage of motor recovery in stage 6 (62.50%) while there was only one participant who was in stages 1, 3, and 4 (12.50%). The average MoCA and MoCA-B scores were 13.50 and 11.33 respectively. The average PHQ-9 score was 2.25.

Table 1 Demographic characteristics of the participants (N=8)

Characteristic	Min-Max	$\bar{x}$ ±SD	N	Percentage
Age (year)	46-70	63.12±8.03	8	-
Year of education	3-16	7.00±4.63	8	-
Onset (month)	6-24	6.25±7.38	8	-
Gender				
Male	-	-	4	50.00
Female	-	-	4	50.00
Marriage status				
Single	-	-	2	25.00
Married	-	-	6	75.00
Disability				
Left hemiparesis	-	-	4	50.00
Right hemiparesis	-	-	4	50.00
Diagnosis				
Hemorrhage stroke	-	-	1	12.50
Ischemic stroke	-	-	7	87.50
Hand dominance				
Left side	-	-	0	0
Right side	-	-	8	100.00
Job				
Merchant	-	-	1	12.50
Private business	-	-	1	12.50
Secretary	-	-	1	12.50
Retire	-	-	5	62.50

Table 2 Scores obtained from screening tests (N=8)

Characteristic	Min-Max	$\bar{x}$ ±SD	N	Percentage
Brunnstrom stage of motor recovery				
Stage 1	-	-	1	12.50
Stage 3	-	-	1	12.50
Stage 4	-	-	1	12.50
Stage 6	-	-	5	62.50
MoCA scores	12-15	13.5±2.12	2	-
MoCA-B scores	9-14	11.33±2.25	6	-
PHQ-9 scores	0-5	2.25±1.75	8	-

Task mastery

The percentage of task mastery in all tasks is analyzed from Stage One of the PRPP System-Thai version. All performances were estimated against a pre-determined criterion of 100%. However, in this study, each participant was asked to select only two tasks and perform them. The percentage scores of task mastery from Stage One of the PRPP System is presented in Figure 4. The findings show that before the intervention, all participants had scores lower than the criterion of 100% and showed below the expected task mastery. After the intervention, every participant had higher scores of the post-test percentage scores of Stage One of the PRPP System for at least 1 activity even though some still had scores below 100%. Most of them (5 out of 8) showed higher scores in both activities but some participants indicated lower scores after the intervention in one out of two activities, and one participant showed equal pre-test and post-test scores.
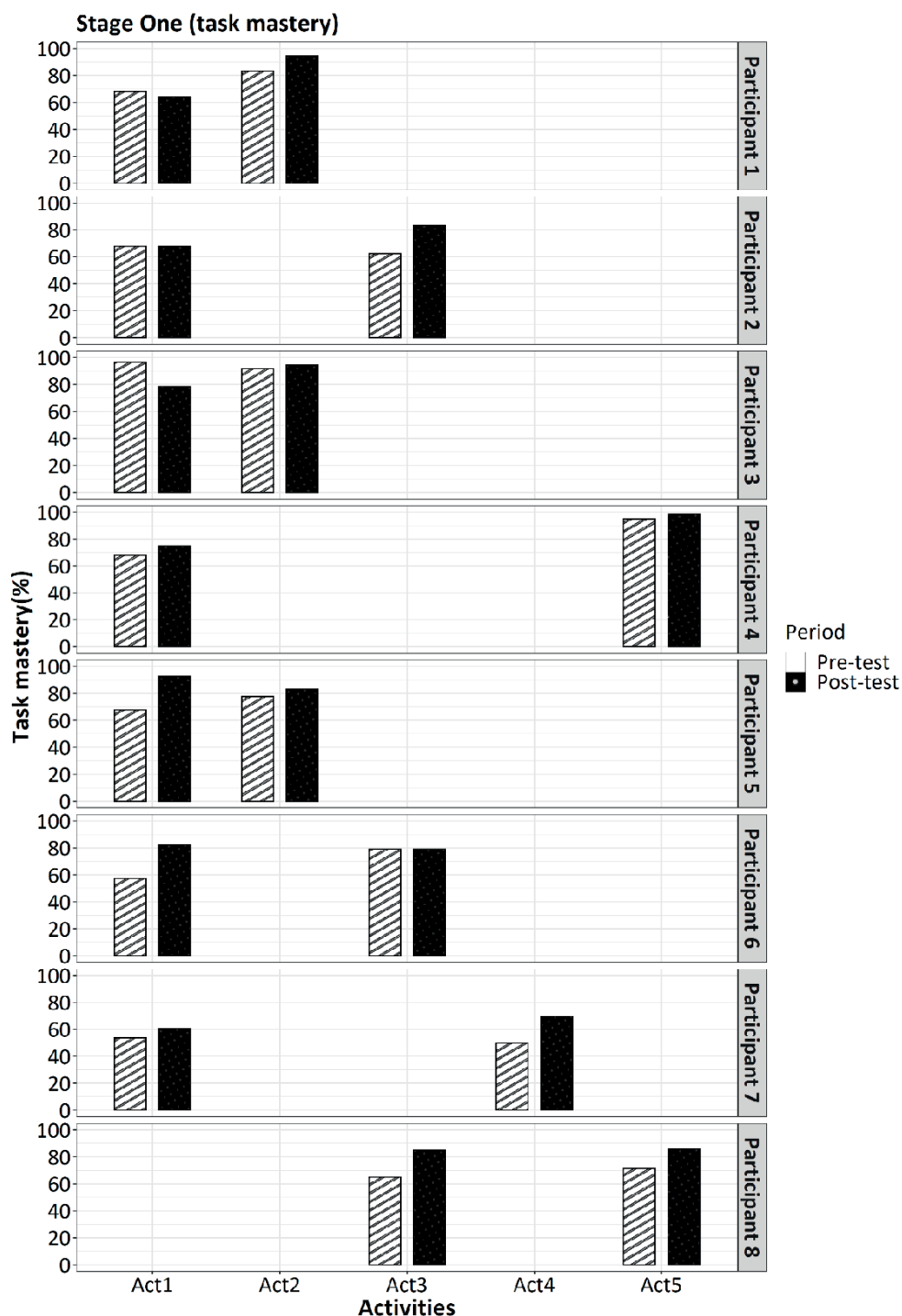
Figure 4. *Percentage scores of Stage One of the PRPP System-Thai version in the two tasks of each participant.*

The cognitive performance

After receiving the cognitive strategy training protocol, the cognitive performance of the participants was obtained from the scores in Stage Two of the PRPP System-Thai version that were computed using the Wilcoxon signed-rank test and was presented in Table 3. The analysis results of activity 1 show positive ranks for 3 persons, negative ranks for one person, and ties of scores occurred in 3 participants. In activities 2 and 3, only one person received a positive rank, 2 people had ties of scores, and there were no negative ranks. Although activity 5 presents all positive ranks for all participants, activity 4 shows ties occurring in only one person. Notwithstanding, the overall tendency of the results seem to be positive, as there were no differences of statistical significance within the cognitive strategy training protocol ($p>0.05$). However, the descriptive-analytic results of the post-test percentage scores of Stage Two of the PRPP System as depicted in Figure 5, 4 out of 8 participants had higher scores for at least 1 activity even though still had scores below 100%. Two participants presented higher scores in both activities. Nonetheless, one participant showed lower scores after the intervention in one out of two activities, and some participants also indicated equal pre-test and post-test scores. The tendency of enhanced performance of the participants after the intervention despite the significant difference was found.

Table 3 Change in ability for each activity of the participants (N=8).

Wilcoxon Signed Ranks Test					
Stage Two (Posttest-Pretest scores)		**N**	**Mean rank**	**Sum of ranks**	***p* value**
Activity 1	Negative ranks	1	2.00	2.00	0.273
	Positive ranks	3	2.67	8.00	
	Ties	3			
	Total	**7**			
Activity 2	Negative ranks	0	0.00	0.00	0.317
	Positive ranks	1	1.00	1.00	
	Ties	2			
	Total	**3**			
Activity 3	Negative ranks	0	0.00	0.00	0.317
	Positive ranks	1	1.00	1.00	
	Ties	2			
	Total	**3**			
Activity 4	Negative ranks	0	0.00	0.00	-
	Positive ranks	0	0.00	0.00	
	Ties	1			
	Total	**1**			
Activity 5	Negative ranks	0	0.00	0.00	0.180
	Positive ranks	2	1.50	3.00	
	Ties	0			
	Total	**2**			
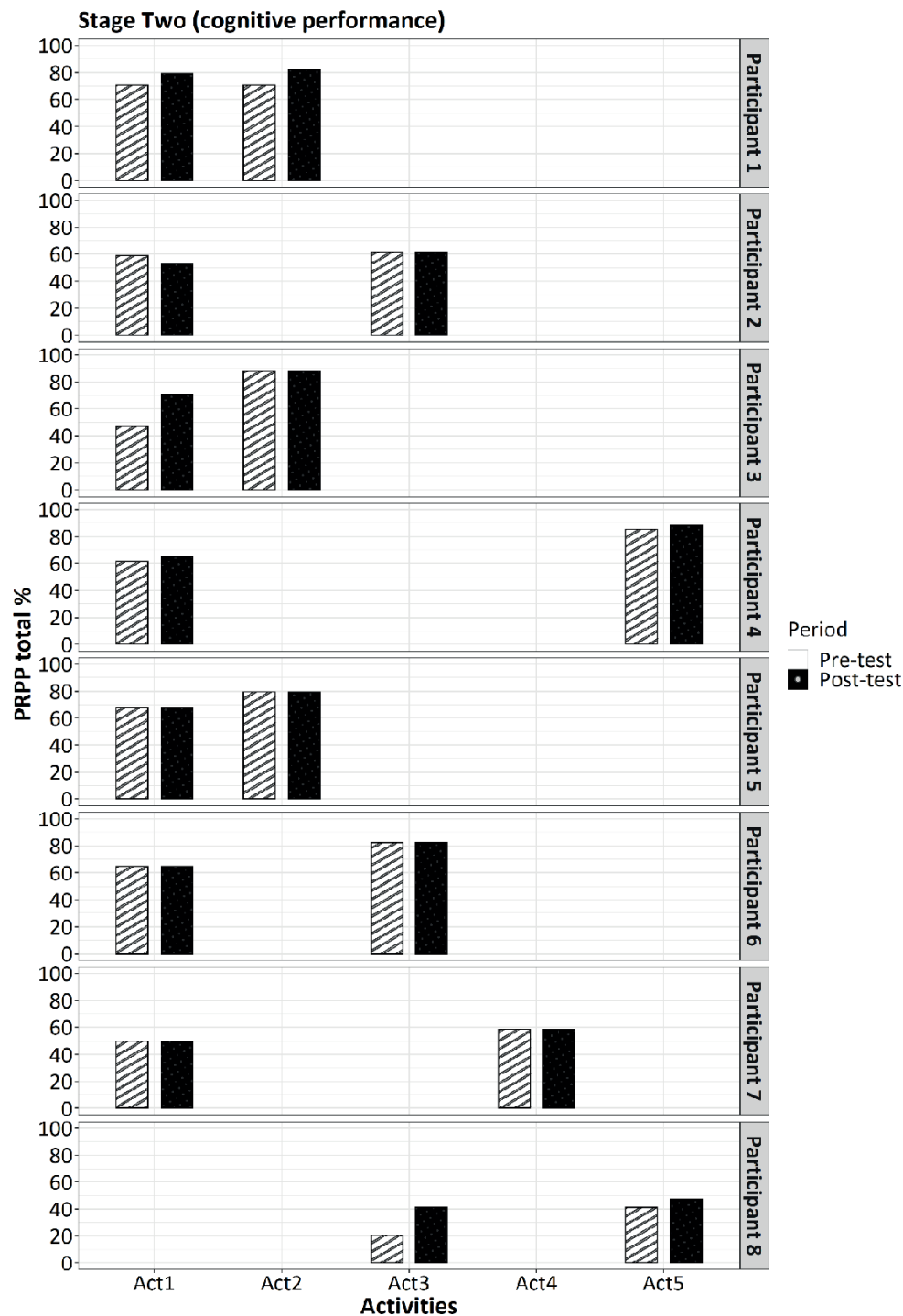
Figure 5. *Percentage scores of Stage Two of the PRPP System-Thai version in the two tasks of each participant.*

Discussion

This study aimed to examine the task mastery of stroke patients during the performance of the IADL tasks and to examine the effects of the cognitive strategy training protocol on cognitive performance in stroke patients. According to the definition of the term in this study, task mastery is an individual's skill that is measured against what is expected of a person where the performance usually occurs within the environment. In this study, activities in the protocol were divided into two groups. The first group performed easier tasks that

included 7 steps, which required 3 to 4 pieces of tools and materials per task; involved the orientation to time, place, and person in daily living; and included activity 3 (making a calendar) and activity 1 (making a weekly planner). These activities facilitated to the improvement of self-awareness, recognizing present events, recalling the past, predicting the future in daily living situations, and helping the participants organize their thoughts, plans, decision-making, and usage strategies while performing. The other group performed more difficult activities that required more complex skills, including 10-14-step tasks,

which required 4-9 tools and materials per task, and performing the task with another person. This group performed activity 5 (making a telephone call), activity 2 (arranging pills in a pill organizer), and Activity 4 (hot drink preparation).

From the results of Stage One of the PRPP System, the participants tended to improve their task mastery after the cognitive strategy training protocol, especially the participant who performed the more difficult activities (Activities 2, 4, and 5). These activities provided an opportunity for the participants to use multi-strategies while performing the challenging tasks. Multi-strategy training may help the participants by providing more choices of strategies to apply to their performance when needed.[26] The helpful concept of the Multicontext Approach involves practicing specific strategies and many techniques by asking and guiding the participants before, during, and after the task to facilitate participants to anticipate problems, generate their strategies, discover their errors, and evaluate their performances when completing the tasks. In addition, the combination of the Multicontext Approach and the PRPP System of Intervention in this study also probably increased the positive task mastery of the participants. The sequence of information processing strategies termed "Stop/Attend, Sense, Think, and Do" of the PRPP System of Intervention focuses on using specific strategies to guide and address the errors for each participant. However, the participants who performed the easy task group, especially Activity 1 (making a weekly planner), showed no change in scores, and some participants had decreasing scores when compared with pre-test scores because the activity may have been too easy and did not challenge their cognitive abilities.

Interestingly, the three phases of the cognitive strategy training protocol also enhanced to improve task mastery among the participants. Following the beginning phase, the anticipation of problems and self-strategy generation probably showed more changes in self-awareness and level of task mastery by observation than by self-rating levels of difficulties. Likewise, the intervention phase and ending phase of the cognitive strategy training protocol seemed to be effective too. Particularly, the reflective structured journal of the ending phase may contribute to the participant rechecking their performance, figuring out problems, learning from experience, identifying strengths and weaknesses, and creating plans for future performance.[27] Moreover, video demonstrations presented during discussion sessions may help the participants to learn through another's experience and the participant can train to clarify the happening errors and assess the strategic behaviors of another person by watching them performing tasks.[28] Furthermore, this study applied the two IADLs for each participant which were obtained following their needs and desires as measured by the COPM. Because using meaningful and relevant activities impacts participants to compensate or adapt by using new cognitive strategies.[29] Applying predictable and familiar tasks can increase the

motivation of the participants to intently perform the tasks leading to more active engagement in performance.[26,30]

The second purpose of this study was to examine the effects of the cognitive strategy training protocol on cognitive performance. The findings presented that after the cognitive strategy training protocol, the pre-test and post-test scores showed no significant difference ($p > 0.05$) despite the tendency to increase performance both from Stage One Analysis and analysis of the raw scores in Stage Two. Many factors might be considered as affecting cognitive performance change. Firstly, the characteristics of the participants include age, years of education, jobs, and levels of cognitive performance. Most of the participants (7 out of 8) in this study were aged over 60 years, possessed undergraduate degrees, and had low cognitive scores on the MoCA/MoCA-B; 13.50 and 11.33 respectively. A review of risk factors for cognitive impairment in stroke survivors found that one of the major predictors for cognitive impairment was increased age and a lower level of education.[31] By its very nature, stroke can expedite cognitive decline in older adults, influence novel learning, and can lead to slow response to cognitive stimulation. Similarly, an individual having a higher education level as a stroke victim probably does not influence cognitive performance very much since they have a larger brain storage capacity allowing for compensation for the damaged brain.[32] Moreover, patients with a low level of cognitive performance tend to gradually respond to strategy training.[33] Secondly, the characteristic of the intervention protocol might be another factor that results in little or no cognitive performance changes discovered in this study as well. Recently, systematic review and meta-analysis of the effectiveness of intervention protocols to improve ADL performance in neurological adults indicated mixed results. In forty-one studies, the intervention time ranked from 30-120 minutes/session. The duration time of intervention ranged from 1 to 16 weeks, and the frequency was between 1 and 5 days per week.[34] This study provided a duration time of about 60 minutes per day, 3 times a week for 4 consecutive weeks. The duration and frequency of this study might not be sufficient and intense enough for patients with severe cognitive impairment or those having low levels of education to show a cognitive change. Furthermore, the cognitive strategy training protocol might not fit low levels of cognitive function. IADL tasks done by low-level cognitive functioning participants are too hard to complete without guidance and as such they do not show an obvious change in their performance outcomes. Similarly, the level of difficulty of the activities in this study might be one of the influencing factors as well. According to the Dynamic Interactional Model perspective, more complicated tasks demand more time and effort to process and may be related to the use of multiple strategies.[29] Even if IADL tasks in this study were obtained based on the needs and desires of the participants expressed by the COPM, these selected tasks appeared to be too easy and were apparently not very challenging activities. Finally, this study is a preliminary study, combining the PRPP System of Intervention and the Multicontext Approach,

was applied the top-down, occupation-based assessment, and intervention in Thai clients with stroke. The finding of this study might affirm the effectiveness of cognitive strategy training protocol to improve task mastery and cognitive performance during the instrumental activity of daily living in stroke patients in Thailand.

Limitations

There are some limitations of this study that need to be considered. The results from this study might not be generalized to the overall population due to the small sample size. likewise, there was no control group to compare with conventional interventions or to eliminate the effect of simultaneous recovery. Consequently, future research with a larger sample size having higher levels of cognitive function with a younger group of participants, having higher levels of education along with a control group is recommended. Similarly, having various levels of difficulty among the activities and having a variety of tasks within several environments and contexts are also suggestions for further study. In addition, increasing the frequency and duration of time intervention might show better cognitive performance outcomes.

Conclusion

It was found that most of the participant's post-test scores in both Stage One and Two of the PRPP System-Thai version showed higher than pre-test scores in both activities. It is possible that task mastery and cognitive performance can be improved while performing the IADLs tasks despite no statistically significant difference (p>0.05) was found. Therefore, future research with a larger sample size having higher levels of cognitive function with a younger group of participants having higher levels of education along with a control group is recommended.

Conflicts of Interest

The author declares no conflict of interest regarding the publication of this paper.

Acknowledgements

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Determination of the *JAK2* V617F mutation in thrombosis patients

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ARTICLE INFO

ABSTRACT

Background: Janus kinase 2 (*JAK2*) gene mutation causes uncontrolled myeloproliferation independent of cytokines and abnormal formation of the endogenous erythroid colony. *JAK2* mutations were frequently observed in myeloproliferative neoplasms (MPNs), especially in polycythemia vera (PV) and essential thrombocythemia (ET). MPNs represent a risk factor for the development of thrombosis that is a significant cause of morbidity and mortality in patients.

Materials and methods: We aimed to study the correlation between *JAK2* mutation and thrombosis. Thirty-nine patients with a clinical diagnosis of thrombocytosis and Budd-Chiari syndrome were collected to determine the *JAK2* V617F mutation. Genomic DNA from all specimens was amplified and detected the presence of *JAK2* V617F mutation by AS-PCR.

Results: We demonstrated *JAK2* V617F mutation in both patients with a clinical diagnosis of thrombocytosis and Budd-Chiari syndrome. We found that 11 of 37 (29.7%) thrombocytosis patients had a *JAK2* V617F mutation. Moreover, one of two patients who represented as Budd-Chiari syndrome was positive for *JAK2* V617F mutation. In addition, *JAK2* V617F mutation was associated with thrombosis. However, further study in large series is needed to support this finding.

Conclusion: Determination of the *JAK2* V617F mutation may be helpful for screening latent or occult MPNs patients who have an occurrence of thrombosis to adjust the appropriate treatment for good patient outcomes.

Introduction

Human Janus kinase 2 (*JAK2*) is located on the short arm of chromosome 9 (9p24.1) and encodes JAK2 tyrosine kinase protein, which is critical for several cellular signaling pathways. During the past three decades, mutation on exon encoding Janus kinase 2 (*JAK2*) has been associated with the development of myeloproliferative neoplasms (MPNs). These include the *JAK2* V617F (exon 14) mutation, which is predominantly identified in the majority of polycythemia vera (PV) and essential thrombocythemia (ET).[1,2] Recently, our group demonstrated that *JAK2* V617F is positive in 95% of PV, 75% of ET, and 25% of primary myelofibrosis (PMF)[3]. Additionally, the mutation load (tumor burden) of *JAK2* V617F has been investigated and reported to be correlated with phenotypic differences among a subgroup of classical MPNs.[3-5] Moreover, several studies reported that mutation in *JAK2* genes is involved in leukemogenesis. These include translocation involving *JAK2* (e.g., *ETV6*::*JAK2* and *PAX5*::*JAK2*) in Philadelphia-

liked acute leukemia.[6-8] These data highlighted the role of *JAK2* in homeostasis and the development of several blood cancers.

The common causes of mortality in several Philadelphia negative MPNs are recognized as arterial and venous thrombosis. These include portal vein thrombosis (PVT) and Budd-Chiari syndrome (BCS) (with hepatic vein thrombosis). Alteration of *JAK2* results in several physiological changes, including hypercellularity inducing blood viscosity, alteration of coagulation cascades and vessel wall, and disruption of cell-cell interaction/adhesion.[9] Recent reports demonstrated that polycythemia vera with *JAK2* mutation are associated with increased risks to thromboembolic events.[10-14] Additionally, there was evidence indicating that nearly half of BCS are diagnosed as chronic MPNs.[15-19] In this work, we aimed to investigate the prevalence of *JAK2* V617F mutation in patients with a clinical diagnosis of thrombocytosis and BCS.

Materials and methods
Patients and samples
Thirty-nine genomic DNA (gDNA) samples isolated from peripheral blood and bone marrow of patients who had a clinical diagnosis of thrombocytosis and Budd-Chiari syndrome were included in this study. gDNA was extracted by using QIAamp DNA Blood mini kit (Qiagen, Germany) and subsequently quantified by using Nanodrop 2000 spectrophotometer (Thermo Scientific, USA). All samples were stored at -20 °C refrigerator prior *JAK2* V617F genotyping. This work was approved by the ethic committee on human Rights related to research involving human subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand, and performed according to the principles of the Declaration of Helsinki (ID 03-58-51).

JAK2 V617F genotyping
Allele-specific polymerase chain reaction (AS-PCR) was performed according to the previously published protocol [1] to determine the *JAK2* V617F mutation statuses in all tested samples. Briefly, optimal PCR conditions were comprised of an initial denaturing at 95°C for 10 minutes, 30 cycles of 94 °C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. PCR was detained at 4° C until continuing to further steps. Specific nucleotide primer details are following; Forward outer (FO) 5'TCCTCAGAACGTTGATGGCAG3', Reverse outer (RO) 5' ATTGCTTTCCTTTTTCACAAGAT3', Forward wild type specific (Fwt) 5'GCATTTGGTTTTAAATTATGG AGTATAT<u>G</u>3', Reverse mutant specific (Rmt) 5'GTTTTAC TTACTCTCGTCTCCACAAA<u>A</u>3', respectively. PCR products were subsequently analyzed and detected by using QIAxcel Advanced system (QIAGEN, Germany) system, which

technology was based on capillary gel electrophoresis. The sample was automatically loaded into a gel matrix with dye-filled capillary. Then, the staining dye nucleic acid was detected by a photomultiplier detector and converted to a gel image and electropherogram by the QIAxcel ScreenGel Software. Positive samples later confirmed the *JAK2* V617F mutation status by using direct PCR sequencing (Sanger sequencing) as described by Baxter *et al*.[1]

Statistical analysis
The statistical analysis of obtained data was performed using the SPSS version 16.0 (SPSS Inc, Chicago, IL, U.S.A.) software. Chi-squared test was used for the comparison of categorical variables. A *p* value of less than 0.05 was considered to indicate statistical significance.

Results
Allele specific-PCR was performed to analyze *JAK2* V617F mutation in genomic DNA samples derived from 37 thrombosis and 2 Budd-Chiari syndrome patients. We found that *JAK2* V617F was positive in 11 of 37 (29.7%) patients with thrombocytosis. As expected, most ET with thrombosis (10 of 12, 83.3%) harbored *JAK2* V617F mutation. Interestingly, *JAK2* V617F mutation was positive in one out of two patients with Budd-Chiari syndrome (50%). To confirm *JAK2* V617F mutation, samples positive for *JAK2* V617F were subsequently analyzed by using the direct PCR sequencing technique (Figure 1). The baseline characteristics of patients and *JAK2* V617F mutation status are demonstrated in Table 1 and Table 2. While our data revealed that there was no statistic significant in *JAK2* V617F mutation status between patients with ET and BCS (p=0.325), BCS and other diseases (anemia, pure red cell aplasia (PRCA), middle cerebral artery (MCA) stroke, spinal stenosis, lung cancer, breast cancer, branch retinal vein occlusion (BRVO), portal vein thrombosis, cirrhosis, sinus venous thrombosis, and superior mesenteric vein (SMA) thrombosis) with thrombocytosis (p=0.526), *JAK2* V617F mutation was predominantly identified in ET compared with those who were characterized as other disorders with thrombocytosis (p<0.05) (Figure 2). Although limited in the sample size of BCS group, we observed that the platelet count in patients with thrombocytosis was not statistically significant to patients with BCS (p=0.0952) (Figure 3).

In summary, we demonstrated the beneficial use of *JAK2* V617F genotyping for the molecular classification of patients with thrombocytosis. *JAK2* V617F mutation was predominantly identified in patients with essential thrombocytosis. Moreover, half of the patient with BCS was positive for *JAK2* V617F. Our data further highlight the practical use of *JAK2* V617F mutation analysis for the classification of MPNs and differential diagnosis of MPNs with BCS from BCS with other diseases/complications.
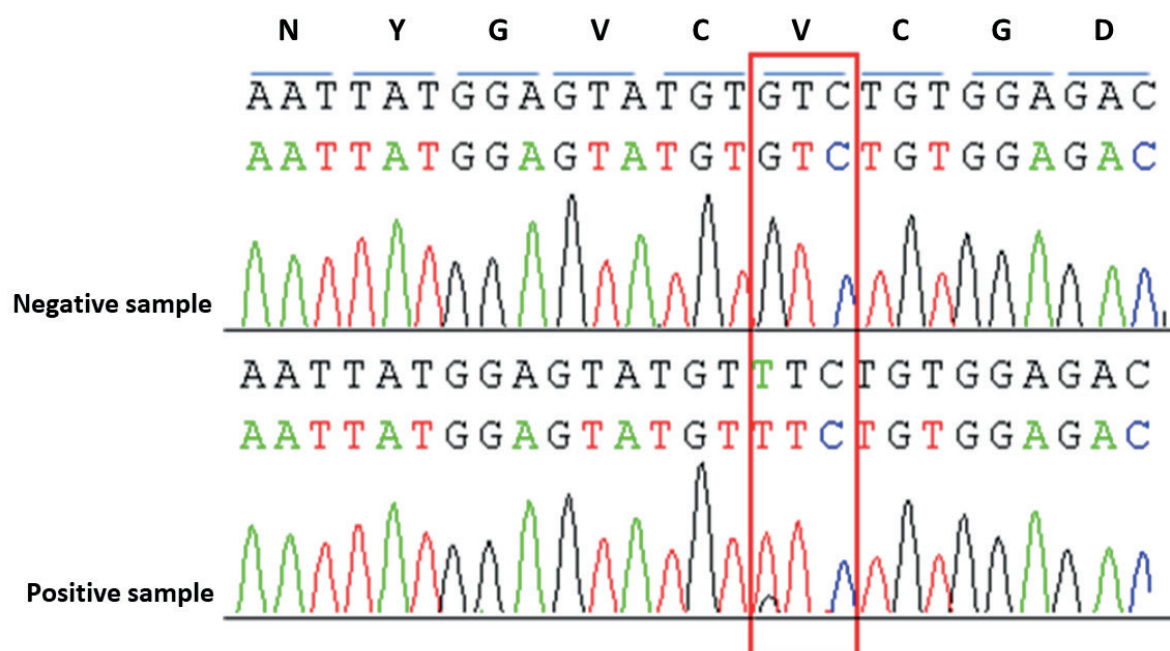
Figure 1. *Electropherogram of DNA sequencing data on JAK2 exon 14 from samples negative for JAK2 V617F (upper panel) and positive for JAK2 V617F (lower panel).*

Table 1 Baseline characteristics of the patients and *JAK2* V617F mutation status.

Diagnosis, Thrombocytosis	37/39 (94.8%)
ET with thrombocytosis	12/39 (30.7%)
Other disorders with thrombocytosis	25/39 (64.1%)
Budd-Chiari syndrome	2/39 (5%)
Sex (male/female)	11/28 (1:2.5)
Median age at thrombocytosis	57
Male	57
Female	53
JAK2 V617F positive	12/39 (30.7%)
ET with thrombocytosis	10/12 (83.3%)
Other disorders with thrombocytosis	1/25 (4.0%)
Budd-Chiari syndrome	1/2 (50.0%)

Table 2 *JAK2* V617F mutation status and platelet count in 37 thrombocytosis and 2 Budd-Chiari syndrome.

Patient	Diagnosis	*JAK2* V617F status	PLT x10³/uL
HGL001	Thrombocytosis	Negative	ND
HGL002	Thrombocytosis	Negative	932
HGL003	ET with Thrombocytosis	Positive	360
HGL004	Thrombocytosis	Negative	569
HGL005	ET with Thrombocytosis	Positive	727
HGL006	Thrombocytosis	Positive	624
HGL007	ET with Thrombocytosis	Positive	519
HGL008	Thrombocytosis	Negative	483
HGL009	Thrombocytosis	Negative	1027
HGL010	ET with Thrombocytosis	Positive	1598
HGL011	Thrombocytosis	Negative	236
HGL012	ET with Thrombocytosis	Positive	745
HGL013	ET with Thrombocytosis	Positive	636
HGL014	ET with Thrombocytosis	Positive	2647
HGL015	ET with Thrombocytosis	Positive	492
HGL016	ET with Thrombocytosis	Positive	739
HGL017	Thrombocytosis	Negative	1103
HGL018	Thrombocytosis	Negative	586
HGL019	Thrombocytosis	Negative	ND
HGL020	Thrombocytosis	Negative	282
HGL021	Thrombocytosis	Negative	491
HGL022	Thrombocytosis	Negative	795
HGL023	Thrombocytosis	Negative	862
HGL024	Thrombocytosis	Negative	1046
HGL025	Thrombocytosis	Negative	1158
HGL026	Thrombocytosis	Negative	628
HGL027	Thrombocytosis	Negative	ND
HGL028	ET with Thrombocytosis	Negative	794
HGL029	Thrombocytosis	Negative	258
HGL030	Thrombocytosis	Negative	1240
HGL031	Thrombocytosis	Negative	1377
HGL032	Thrombocytosis	Negative	50
HGL033	Thrombocytosis	Negative	209
HGL034	Thrombocytosis	Negative	300
HGL035	Thrombocytosis	Negative	223
HGL036	ET with Thrombocytosis	Negative	900
HGL037	ET with Thrombocytosis	Positive	1079
HGL038	BCS	Negative	382
HGL039	BCS	Positive	80

Note: ET: essential thrombocythemia, BCS: Budd-Chiari syndrome, PLT: platelet, ND: no data.

Comparing Ratio of *JAK2* V617F Positive
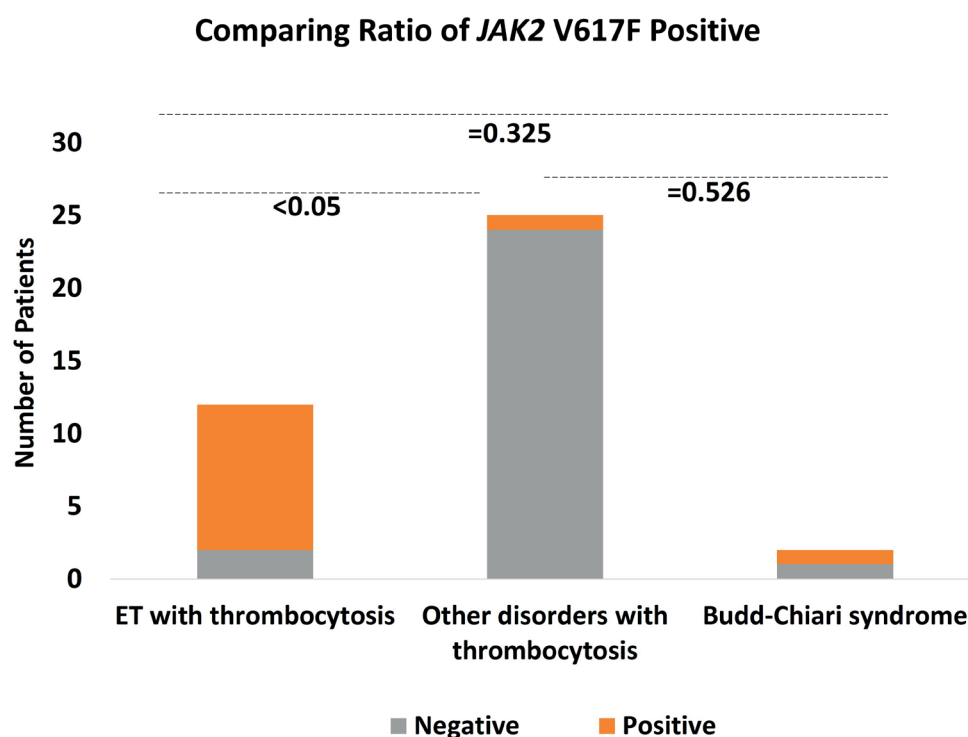
Figure 2. Comparison of JAK2 V617F mutation status in a different group of patients with thrombocytosis.
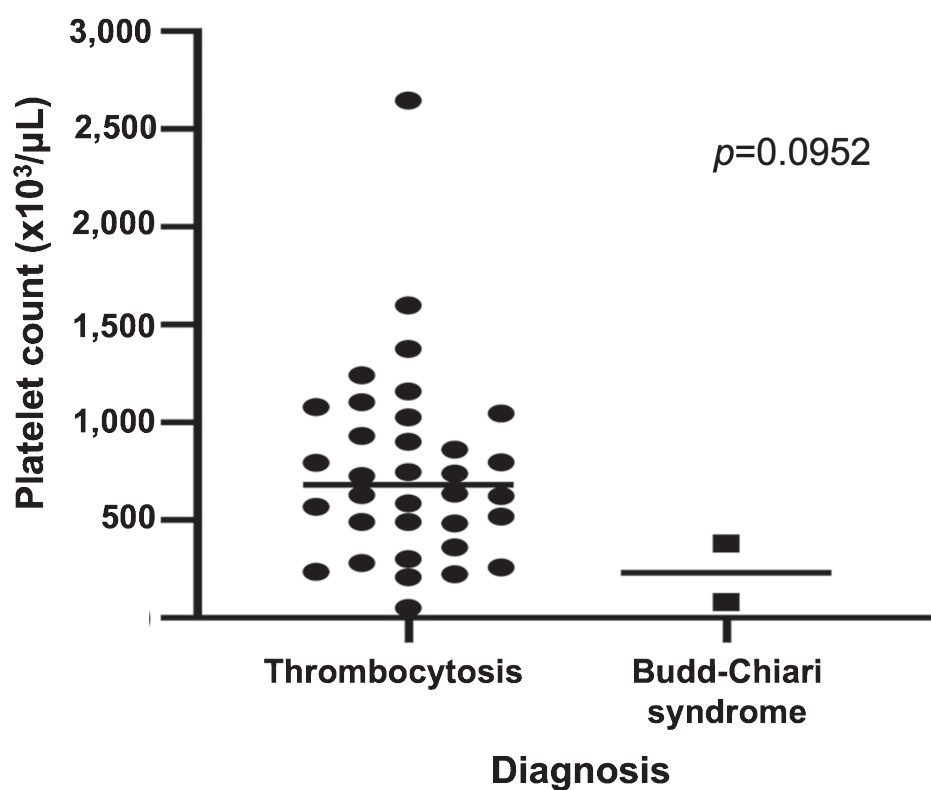
Figure 3. Comparison of platelet number in patients with thrombocytosis and BCS.

Discussion

Allele-specific PCR (AS-PCR) was applied as described previously for qualitative analysis of *JAK2* V617F mutation.[1] This assay has been widely used to detect *JAK2* V617F, which is predominantly positive in patients with myeloproliferative neoplasms (MPNs). Additionally, this assay has been expandable and used for the risk assessment of Budd-Chiari syndrome overt myeloproliferative disorders.[20,21] In this work, we used automatic capillary gel electrophoresis, QIAxcel (Qiagen), to replace traditional labor-intensive gel electrophoresis. This machine could perform genotyping analysis with highly sensitive sizing accuracy and is friendly used when compared with conventional slab-gel electrophoresis (Figure 4).

This data further confirmed the beneficial use of *JAK2* V617F as a genetic biomarker for the differential diagnosis of ET from other disorders with thrombocytosis.[22-24] Additionally, evidence indicates that the blood count of patients with MPNs is closely normal at BCS present.[25] Thus, the determination of *JAK2* V617F and other biomarkers for MPNs, such as calreticulin (*CALR*) and *MPL* are critical for the differential diagnosis of primary BCS and MPNs with BCS.

At present, there is less clarity about the incident of BCS in Thailand and worldwide. While the frequency of BCS among ethnic groups is consistent, the disease's observed frequency in Asian countries varies, which is a markedly high prevalence in Kathmandu, Nepal[26]. Perspective on the distribution of *JAK2* V617F in unselected patients with BCS, we compared our data to other groups working on the impact of *JAK2* V617F in BCS (Table 3). Similar to Yonal, Pinarbasi *et al*.[17] and Amarapurkar, Punamiya *et al*. 2008.[27], half of BCS patients were positive for *JAK2* V617F. In contrast, lower incidences of *JAK2* V617F mutation were reported in some studies which were conducted in a larger number of BCS samples.[21,28-30] In European countries, several studies indicated that *JAK2* V617F mutation was predominantly identified in BCS with underlying MPNs (nearly half of all BCS and about 90% of those with MPNs).[16,31-33] For a clearer view of the incidence of BCS in Thailand and the beneficial use of *JAK2* V617F for
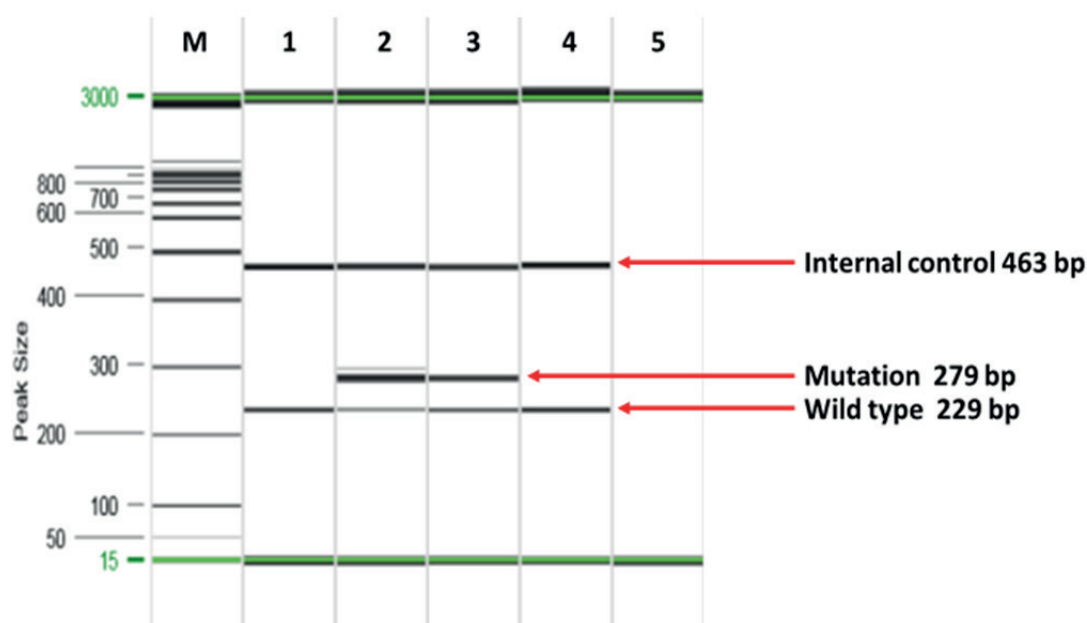
Figure 4. *Genotyping analysis of JAK2 V617F using automatic capillary gel electrophoresis. Lane M: size marker, lane 1: sample with a negative for JAK2 V617F, lane 2: sample with a positive for JAK2 V617F, lane 3: positive control, lane 4: negative control, lane 5: no template control, respectively.*

Table 3 Distribution of *JAK2* V617F in patients with unselected Budd-Chiari syndrome among different ethnic groups.

Study	Observed frequency (number of BCS samples)	Country
This study	50% (2)	Thailand
Qi[30]	5.2% (77)	China
Shetty[28,29]	8.8% (137)	India
Sakr[29]	19.1% (94)	Egypt
Karakose[21]	20.5% (31)	Turkey
Yonale[17]	50.0% (26)	Turkey
Amarapurkar[27]	47.8% (23)	India

categorizing the disease, more tested samples should be considered for improvement.

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Journal of Associated Medical Sciences

Detection of weak A antigen associated with *Proteus vulgaris* infection

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ABSTRACT

Background: A person with weak A antigen expression is presented.

Objectives: The study aimed to reveal weak A antigen by different methods applied in laboratories.

Materials and methods: The reaction of adsorption, agglutination, and inhibition of agglutination were performed at 4 °C, 10 °C, 13 °C, 20 °C, and 37 °C for 1-13 hrs and one-day incubation. The complement of the guinea pig was added to the heated serum to reveal hemolysis.

Results: A person's red blood cells reacted with commercial anti-B sera. Whereas anti-A and anti-B (reactive at 37 °C) antibodies were found in the serum. The person demonstrated anti-A antibody adsorbing ability at 4 °C. The plasma of the person inhibited agglutination of A and B RBCs by polyclonal sera. The blood culture was positive for *Proteus vulgaris* infection.

Conclusion: The presence of weak A antigen was detected the best by the adsorption method at low temperatures with polyclonal sera from the O blood group.

Introduction

Weak A subgroups (A$_3$, A$_x$, A$_{end}$, A$_m$, A$_y$, and A$_{el}$) are usually detected as O groups, which may lead to transfusion complications. The cases of weak A antigen are presented by researchers. The patient's RBCs were not agglutinated by anti-B antibodies and showed mixed field agglutination of agglutination with anti-A and 1[+] with anti-A$_1$ antibodies, the serum showed the presence of anti-B antibodies. The presence of a weak A subgroup with Type II discrepancy was stated.[1]

Weak A antigen variants may be revealed by various methods.[2,3] Special techniques (adsorption-elution, glycosyltransferase estimation, and genotyping) are performed to confirm these blood groups.[4] ABO discrepancy was detected when in forward typing O blood type was revealed and reverse typing showed the presence of anti-B antibodies. No agglutination of the RBCs with anti-A and anti-AB antibodies was obtained, A$_m$, A$_y$ or A$_{el}$ phenotype was suggested. The RBCs were investigated in adsorption-elution methods with polyclonal anti-A antibody and eluate agglutinated A RBCs pointing to the presence of A antigen on the red cells. The eluate showed agglutination with two different A RBCs, did not agglutinate O RBCs and the final wash solution did

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not agglutinate A, B, AB, and O RBCs. Blood groups of the husband and son appeared to be of O type. The person was a secretor and showed A and H substances in saliva. A_y is considered to be similar to Am and adsorption-elution tests often fail to differentiate these variants. Since the eluted anti-A antibody agglutinated weakly A RBCs (1^+ by tube technique) A_y phenotype was reported. In the case of Am phenotype, the agglutination strength is much stronger; thus, a person of weak A subgroup was typed.[4]

A blood group person showing weak B antigen associated with *Proteus vulgaris* bacteremia and anti-B antibody in the serum has been described. The antigen was associated with *Proteus vulgaris* bacteremia. The acquired B antigen reacted with a fraction of the anti-B antibody missing in the person's anti-B serum.[2] The relevance of antigen acquisition through bacteria has been widely discussed. Microorganism's effects on RBCs are various: causes of autoimmune diseases, effect *in vivo* and *in vitro* on RBCs, blood group specificity, and stimulation of anti-A and anti-B antibody production.[3]

Acquired B antigen is well described in the literature.[5-8] Marsh demonstrated an association *E. coli* with acquired B antigen. Springer showed 137 gram-negative with a blood group activity (20% of them showed high *in vitro* activity).[9] *E. coli* O88 had the most B activity, as well as *Proteus vulgaris*. The mechanisms of acquired B antigen are the adherence of bacterial antigen to the erythrocyte membrane and enzymatic alteration of the erythrocyte membrane.[9] The bacterial antigen may be adsorbed on the RBCs membrane and be the basis of bacterial hemagglutination tests. The lipopolysaccharide from *E. coli* O86 was found to be similar to the B blood group antigen and eluted back off the RBCs by washing them at 58 °C.[10]

The lipopolysaccharide of *Proteus vulgaris* OX-19 was found to have receptors for anti-B antibodies and antisera to *Proteus* OX-19 agglutinated B RBCs.[11] Platelet-activating ureases produced by *Proteus mirabilis* were described. The cytotoxic and pro-inflammatory activities of enzymes in *Proteus* were detected.[11] The mechanisms involving endogenous or extraneous enzymes that transform A-specific antigen into B antigen were discussed. Marsh showed, the B antigen may be acquired through an enzymatic reaction, since the B-producing mechanism was destroyed by heat, and the B substance was absent in the filtrate (the filtrate did not inhibit the reaction of anti-B serum with normal B RBCs).[9] A fraction of anti-B antibody against acquired B antigen, neutralized by the bacterial polysaccharide was demonstrated. The patient's cells were not agglutinated by antisera against *Proteus*. A factor, enzymatic in nature, associated with polyagglutinable cells in rabbits with enteritis, was to have an ability to persist in the circulation and transfer from cell to cell. The interesting feature of the acquired B is the presence of anti-B antibody reacting with B RBCs except with own cells with anti-B antibody. The researchers showed, when anti-B serum is adsorbed with the patient's cells all activity for the acquired B antigen is removed, however, a strong agglutinin for usual B RBCs is present.[9] The importance

of anti-A and anti-B antibodies reacting with acquired A and B antigen show a defensive mechanism and a person lacking this anti-B antibody may develop an infection with bacteria (*Proteus*), that has a B-like antigen on the membrane. Hyperimmune anti-B sera are able to kill *E.coli* 086 in the presence of complement.[1]

Thus, microorganisms cause immune responses within the ABO blood group system, that may influence blood group specificity. Platelet-activating ureases produced by *Proteus mirabilis* were described. The cytotoxic and pro-inflammatory activities of enzymes in *Proteus* were detected.[4] Weak A subgroups are reported to require advanced methods such as adsorption and elution. Molecular tests are required for confirmation and typing of the subgroups. Hemagglutination-based methods are used to detect weak subgroups. However, variation in reagents and techniques leads to the missing of weak phenotypes, often mistyped as the O group. Meanwhile, weak subgroups of A may cause transfusion reactions, if the donor mistyped as O is transfused to O blood type recipient, causing intravascular hemolysis. Therefore, it is necessary to reveal weak subgroups since the accurate determination of ABO type would help in better managing transfusion practice.

The objective of this study was to reveal weak A antigen by different special methods.

Material and methods

The person with B blood group and anti-A antibody adsorbing ability was examined at Sytenko Institute of Spine and Joint Pathology because of skin infection. The blood was drawn into an EDTA tube. On common blood analysis: white cells: 7.8×10^9, lymphocytes: 18.6%, monocytes: 5.7%, granulocytes: 75.7%, platelets: 210×10^9, MCV 96 fL, RBCs: 4.94×10^{12}/L, Hb: 161 gm/L, Hematocrit 0.46. The patient's RBCs reacted with the anti-B antibody, the indirect antiglobulin test of the patient's cells, and the anti-A antibody was negative when tested with a broad-spectrum antiglobulin reagent. The patient's cells reacted with commercial anti-B sera, and 10 group A donor sera. The results obtained with the group A donors varied from weak microscopic agglutination to strong macroscopic positives. The serum contained an antibody, that reacted with B cells at 37 °C at a low titer 1:2 at 37 °C. No family was available for genetic studies. The person's RBCs could adsorb anti-A antibodies at 4°C.

The RBCs and sera were taken from 25 healthy volunteers aged 62.4±2.20 years, among those were 11 men and 14 women. Washed RBCs of O, A, B, AB, and O blood groups were used for the study.

The RBCs' diameter and their quantity were fixed by microscope with a digital camera Sigeta DCM-900, 9.1 Mp, recorded on the hard disk, quantity and diameter of each erythrocyte were measured (in μM). Antibody titration was performed by diluting 100 μL of the serum with normal saline in 1:2-1:32 titer. Titrations were carried out by making doubling dilutions of the test sera in saline. Two drops of serum or diluted serum were mixed with one drop of a suspension of cells in saline and

incubated. The RBCs were washed three times with saline and centrifugated at 1,000 g for 10 min. The reaction of adsorption, agglutination, and inhibition of agglutination was performed at 4 °C, 10 °C, 13 °C, 20 °C, 37 °C for 1-13 hrs and one-day incubation. The guinea pig complement was added to the heated serum to reveal hemolysis. To obtain IgG antibodies the sera were heated for 30 mins at 60 °C. Fifty µL of RBCs were added to 100 µL of the serum in different dilutions and incubated at 20 °C. One hundred µL of the guinea pig complement was added to the reaction for revealing immune antibodies. Agglutination reactions were performed by standard tube methods and checked microscopically. Antiglobulin test was performed with a broad-spectrum antiglobulin reagent following incubation at 37 °C for 30 min.

Results

The RBCs of the studied person in cell grouping were agglutinated by anti-B commercial sera and 10 polyclonal anti-B sera. Anti-A serum agglutinated washed RBCs after 20 hrs of incubation at 13 °C contrary to the incubation with unwashed RBCs (decreased quantity) and contact at 20°C. Two samples adsorbed by B RBCs anti-A,B serum contacted with the studied RBCs at 37 °C for 1 hr did not cause agglutination. The contact of anti-A serum with unwashed RBCs for one day led to the agglutination (RBCs diameter 5.96±0.43 µM), more expressed than after the contact with washed RBCs (diameter 5.83±0.86 µM) (Table 1). However, anti-A serum for one day incubation at 37°C with the studied RBCs did not cause agglutination (diameter 5.65±0.45 µM).

Table 1. The studied RBCs s diameter after contact with anti-A heated serum and complement.

Dilution	without complement			with complement		
	Agglutination	Diameter (µM)	N	Agglutination	Diameter (µM)	N
1:2	3$^+$	6.08±0.2	29	2$^+$	4.52±0.94	18
1:4	2$^+$	4.93±0.77	15	1$^+$	4.46±0.66	10
1:32	1$^+$	5.64±0.94	8	w$^+$	4.02±0.06	8
1:2	3$^+$	5.83±0.5	24	2$^+$	4.74±0.38	22
1:4	2$^+$	6.55±0.39	21	1$^+$	6.42±0.49	17
1:8	1$^+$	6.33±0.43	10	w$^+$	5.85±0.62	10

Note: w: weak agglutination.

One of the two samples adsorbed by B RBCs anti-A,B serum after one-hour incubation at 20 °C showed the tendency to agglutination of the studied RBCs, as well as after the use of antiglobulin serum (AGS) with unwashed incubated RBCs. Adsorbed by B RBCs anti-A,B serum after one-hour incubation at 37 °C did not cause agglutination of the studied RBCs, use of AGS decreased the quantity of RBCs with the tendency to agglutination. Adsorbed by A and B RBCs anti-A,B serum increased the diameter of the studied RBCs at 37 °C. At 37 °C, hemolysis of the studied RBCs in the presence of anti-A serum combined with complement was noted.

The RBCs of the studied person demonstrated an irregular shape with a diameter 4.9±0.89 µM. The contact of the studied RBCs with anti-A serum led to the increased diameter, decreased quantity, and appearance of RBCs shadows. Shadows of objects were detected after the same reaction in a centrifuge tube. However, anti-A serum did not modify the studied RBCs being loaded with serum from an AB person, although decreased their quantity.

The complement use did not induce agglutination of the studied RBCs, however, increased their diameter from 4.0±0.1 µM to 4.38±0.16 µM, from 3.98±0.17 µM to 4.2±0.4 µM, from 4.14±0.38 µM to 4.43±0.28 µM (4.2±0.24 µM initially), as well as diameter of A RBCs s (4.16±0.4 µM to 4.6±0.21 µM, from 4.0±0.2 µM to 4.27±0.1 µM, initially 4.44±0.31 µM) and O RBCs (from 4.03±0.08 µM to 4.24±0.38 µM, from 3.9±0.17 µM to 4.22±0.32 µM,

initially 4.6±0.4 µM). The quantity and diameter of the studied RBCs after the contact with anti-A,B serum were modified after the use of Lincomycin (from 3.87±0.9 µM to 3.9±0.18 µM), nystatin (4.16±0.06 µM), Fenbendazole (increased RBCs diameter: 4.13±0.43 µM, decreased titer). Interestingly, the incubation of the studied RBCs with fenbendazole increased the diameter of the studied RBCs to 6.0±0.1 µM contrary to the incubation with Nistatin (5.06±0.21 µM). Incubation of the studied RBCs with normal saline at 37 °C for 13 hrs led to the increased movement of irregularly shaped objects contrary to the incubation of normal B RBCs without anti-A antibody adsorbing ability. The use of the Lanta apparatus led to the agglutination of the moving objects. The diameter of the studied RBCs incubated with Fenbendazole was increased to 6.0±0.34 µM, with Nystatin: 5.06±0.21 µM, with Reosorbilact: 4.7±0.83 µM. The contact of anti-A serum with unwashed studied RBCs for 23 hours at 4 °C led to the rouleaux formation and RBCs diameter 5.0±0.5 µM, with serum from AB blood group: rouleaux formation with diameter 5.37±0.94 µM.

Anti-A heated serum did not agglutinate the studied RBCs at 13 C for 2.5 hrs contact, although increased the diameter of the studied washed RBCs from 4.18±0.38 µM in 1:16 titer to 6.08±0.2 µM in 1:2 titer on the contrary to anti-A heated serum combined with complement (4.02±0.06 µM in 1:32 titer to 4.52±0.94 µM in 1:2 titer, decreased quantity) (Table 2). For the comparison: anti-A,B

Table 2. Incubation of the studied RBCs with various sera.

Contact of the serum	with the studied RBCs		
	Agglutination	Diameter (μM)	N
Anti-A heated 2.5 hrs at 13 °C	neg	↑	
Anti-A,B after B RBCs + AGS 13 hrs at 13 °C	neg		normal
Anti-A		↑	↓
Anti-B	3⁺	5.9 ±0.5	
Indirect Coombs' test: citrate anti-A 1 hr at 37 °C	neg		
Serum from AB	Rouleaux formation	5.37±0.94	
Anti-A,B after B RBCs + AGS	neg		
Plasma from BA+ 13 hrs at 37 °C		↑ 4.54±0.36-6.48±0.46	
Anti-A,B after B RBCs 1 hr at 37°C water bath 1 hr at 37 °C	neg		
Anti-A + Complement		↑4.0±0.1-4.38±0.16	
Anti-A 1 day	2⁺	5.83±0.86	
Anti-A 1 hr at 13 °C	Tendency to w⁺ʰⁱ		
Anti-A,B after B RBCs at 20 °C	Tendency to w⁺		
Anti-A at 37 °C 1 day	neg	5.65±0.45	
Anti-A,B after A,B RBCs at 37 °C		↑	
Anti-A,B + Lincomycin		3.87±0.9-3.9±0.18	
Anti-A,B + Nystatin		4.16±0.06	
Anti-A,B + Fenbendazole		4.13±0.43	

Note: d: diameter, N: quantity of RBCs, AGS: antiglobulin serum, neg: negative

serum increased the diameter of A RBCs from 5.16±0.5 μM in 1:32 titer to 6.33±0.61 μM in 1:2 titer, as well as the heated one: from 5.56±0.7 μM in 1:32 titer to 5.83±0.86 μM in 1:2 titer. Anti-A serum after 1 hr contact with the studied RBCs at 13 °C showed a slight tendency to agglutination contrary to the contact at 20 °C.

Study of the person's plasma.

The plasma agglutinated A RBCs at 4 °C and 37 °C and B RBCs at 37 °C. Fungi growth was detected. However, the heated to 60 °C serum for 30 mins did not agglutinate B RBCs at 37 °C. EDTA plasma from the studied person after incubation with his own RBCs at 37 °C for 13 hrs led to the increased diameter of RBCs from 4.54±0.36 μM to 6.48±0.46 μM with shades of the objects on the contrary to the contact with normal B RBCs (diameter 4.94±0.13 μM) (Table 3). The plasma from the studied person agglutinated A RBCs in 1:16 titer and increased their diameter (from 4.3±0.52 μM to 5.93±0.49 μM), as well as the heated plasma (at 60 °C for 30 mins) (from 5.51±0.28 μM to 5.86±0.43 μM).

The studied plasma did not increase the diameter of O RBCs after the contact at 20°C for 3 hrs (4.28±0.66 μM-4.02±0.07 μM) and A RBCs (4.0±0.1 μM-3.96±0.22 μM). The studied plasma agglutinated A RBCs weaker at 20 °C as compared to 13 °C. The plasma did not agglutinate B RBCs, however increased the diameter of the one-third of RBCs (from 4.05±0.26 μM to 4.86±1.0 μM).

Three-hour incubation of the studied plasma did not induce agglutination of B RBCs at 20 °C, however, increased their diameter (4.05±0.26 μM to 4.86±1.05 μM). The studied plasma weaker agglutinated A RBCs and decreased their diameter after the use of the complement at 20°C contrary to B RBCs. The plasma from the studied person agglutinated B RBCs at 37 °C in 1:2 titer after 7 hrs of incubation at 20 °C contrary to incubation at 10 °C. The studied plasma strongly agglutinated A RBCs (the highest titer) at 4 °C and weakly at 20 °C (the lowest titer). On the contrary, at 4 °C the studied plasma showed the absence of anti-B antibody activity, which was revealed at 37 °C in 1:2 titer. The studied plasma did not agglutinate B RBCs at 13 °C for 2 hrs contact, as well as the heated one, and did not increase the diameter of B RBCs on contrary to the heated plasma (5.75±0.7 μM in 1:32 titer to 6.02±0.4 μM in 1:2 titer).

Adsorption tests

Anti-A,B serum after adsorption by the studied RBCs for 13 hrs at 15 °C increased the diameter of A RBCs after the contact for 4 hrs (from 4.47±0.19 μM in 1:64 titer to 4.81±0.45 μM in 1:16 titer), however in a higher concentration the serum decreased A RBCs diameter (4.28±0.31 μM in 1:2 titer, 4.32±0.37 μM in 1:4 titer) (Table 4). Moreover, the serum did not lose the ability to agglutinate RBCs. Anti-A,B serum after adsorption by B RBCs increased the RBCs diameter as well (from 4.51±0.35

Table 3. Incubation of the studied plasma with A and B RBCs.

The contact of	Cells A		Cells B	
	Agglutination	Diameter (µM)	Agglutination	Diameter (µM)
Plasma from BA⁺ at 4 °C	+ (highest titer)		neg	
Plasma from BA⁺ at 20 °C 3 hrs	+ (lowest titer)	4.0±0.1-3.96±0.22	neg	↑4.05±0.26-4.86±1.05
Plasma from BA⁺ at 37 °C			+ (1:2)	
Plasma from BA⁺ 2 hrs at 13 °C			neg	
Anti-A+ plasma from BA⁺ Complement at 20 °C'	↓			
Anti-A + plasma from BA⁺	w⁺		neg	
Plasma from BA⁺ at 10 °C			neg	
Plasma from BA⁺ 7 hrs at 20 °C			+	
Plasma from BA⁺ 3 hrs at 20 °C		↑1:16 4.3±0.52-5.93±0.49		Normal 4.28±0.66-4.02±0.07
Heated plasma from BA⁺ 2 hrs at 13 °C		5.51±0.28-5.86±0.43	neg	↑ 5.75±0.7-6.02 ±0.4 (1:32-1:2)
Anti-A,B after BA⁺	↓ (1:32-1:16)			

Note: BA⁺: the studied RBCs, w: weak, neg: negative

Table 4. Incubation of A RBCs with sera after adsorption by the studied RBCs.

The contact of the serum	Cells A	
	Agglutination	Diameter (µm)
Anti-A,B after BA⁺RBCs 13 hrs at 15 °C		↑ 4.47±0.19-4.81±0.45 (1:64-1:16)
Citrate anti-A after unwashed BA⁺RBCs	↓	↑ 5.03±0.81-5.5±0.86 (1:32-1:4)
Anti-A,B after unwashed BA⁺RBCs 13 hrs at 10 °C	↓ (1:32-1:16)	↑ 4.34±0.23-4.48±0.59 (1:16-1:2)
Anti-A,B after unwashed BA⁺RBCs13 hrs at 20 °C	↓↓ (1:16)	
Anti-A after BA⁺RBCs 13 hrs at 20 °C	↑	4.14±0.24-4.2±0.21 (1:32-1:2)
Anti-A after BA⁺RBCs+ Fenbendazole	↓ (1:8)	
Anti-A,B after BA⁺RBCs at 37 °C	↓ (1:16)	
Anti-A,B after BA⁺RBCs 13 hrs at 10 °C	↓ (1:32-1:16)	↑ 4.4 ±0.32-4.51±0.45
Anti-A,B after BA⁺RBCs 13 hrs at 20 °C	↓ (1:16)	4.13±0.24-4.45±0.35 (1:16)
Anti-A after BA⁺RBCs loaded by AB serum 5 hrs at 10 °C	Neg (1:2 initially)	
Anti-A after BA⁺RBCs loaded by AB serum 5 hrs at 20 °C	neg	
Anti-A,B after BA⁺RBCs1 day at 10 °C	↓ from 1:16-1:8	
Anti-A,B after BA⁺RBCs + Fenbendazole	↑ from 1:16-1:32	
Anti-A,B after BA⁺RBCs + Lincomycin	↓ from 1:16-1:8	
Anti-A after BA⁺RBCs 14 hrs at 4 °C	neg	
Anti-A,B after BA⁺RBCs at 37 °C	↓ (1:16)	
Anti-A,B after BA⁺RBCs 1 day	↓ (1:16-1:8)	
Anti-A,B after BA⁺RBCs + Fenbendazole	+(1:8)	↑
Anti-A,B after BA⁺RBCs	↓ from 1:8-1:4	
Anti-A,B after BA⁺RBCs + Nystatin	↓ (1:4)	
Anti-A 1:2 after 2 volumes of BA⁺ RBCs 14 hrs at 4 °C	↓ (1:8-1:4)	
Anti-A,B after unwashed BA⁺RBCs	Normal	
Anti-A,B after unwashed BA⁺RBCs+ Fenbendazole	↓ (1:8)	
Anti-A,B after unwashed BA⁺RBCs + Nystatin	+(1:8)	
Anti-A after BA⁺RBCs(6:1) 23 hrs	↓	4.38±0.2-4.05±0.2 ↓ (1:32-1:16)

Note: BA⁺: the studied RBCs.

µM in 1:64 titer to 4.62±0.42 µM in 1:2 titer), as well as without adsorption (4.53±0.25 µM in 1:64 titer to 4.65±0.3 µM in 1:2 titer).

After adsorption of citrate anti-A plasma by unwashed studied RBCs light decrease of agglutination of A citrated RBCs was noticed in 1:32 titer with increased RBCs diameter (5.03±0.81 µM in 1:32 titer to 5.5±0.86 µM in 1:4 titer). This effect was not observed with nonadsorbed citrate anti-A plasma (4.76±0.85 µM in 1:32 titer and 5.1±0.78 µM in 1:2 titer). Anti-A,B serum after adsorption by the studied unwashed RBCs for 13 hrs at 10 °C revealed the titer of agglutination of A citrated RBCs from 1:32 to 1:16 and increased their diameter (4.23±0.45 µM in 1:16 titer to 4.48±0.59 µM in 1:2 titer after 5 hr incubation at 10 °C) as well as without adsorption (4.16±0.25 µM in 1:16 titer to 4.42±0.5 µM in 1:2 titer). Anti-A,B serum after adsorption by unwashed studied RBCs for 13 hrs at 20 °C significantly lost the agglutinating ability of A RBCs: weak agglutination was detected in 1:16 titer. An interesting effect was noticed: RBCs diameter 4.01±0.18 µM in 1:16 titer and 4.3±0.48 µM in 1:2 titer.

Two samples of anti-A,B sera after adsorption by washed studied RBCs for 13 hrs at 10 °C decreased the titer of agglutination of A RBCs from 1:32 to 1:16 and increased RBCs diameter (4.4±0.32 µM in 1:32 titer and 4.51±0.45 µM in 1:2 titer, 4.58±0.41 µM in 1:32 titer and 4.67±0.34 µM in 1:4 titer, 4.4±0.56 µM in 1:2 titer).

Anti-A,B serum after adsorption by the studied RBCs for 13 hrs at 20 °C significantly decreased the titer of agglutination of A RBCs: less expression of agglutination in 1:16 titer with increased RBCs diameter (from 4.13±0.24 µM in 1:16 titer to 4.45±0.35 µM in 1:4 titer, 4.28±0.24 µM in 1:2 titer). Anti-A serum after adsorption by the studied RBCs at 7 °C for 5 hrs, loaded previously with serum from AB person (1:3 ratio), stopped agglutinating A RBCs (initial titer 1:2) as well as after adsorption at 20 °C. Anti-A,B serum after adsorption by the studied RBCs at 37 °C decreased the expression of agglutination of A RBCs in 1:16 titer. Anti-A,B serum after adsorption by B RBCs contacted with the studied RBCs for 13 hrs at 37 °C, and antiglobulin serum did not lead to agglutination. Anti-A,B serum did not decrease the titer of agglutination of A RBCs after adsorption by unwashed studied RBCs (1:16 and 1:32), however, decreased the titer to 1:8 after adsorption by the studied RBCs with fenbendazole and nystatin. Adsorption of anti-A,B serum with the studied RBCs for one day at 10 °C the titer of A RBCs decreased from 1:16 to 1:8. Interestingly, from two anti-A sera after adsorption by the studied RBCs for 23 hrs (6:1 ratio of the sera and RBCs) only one serum decreased the titer of agglutination of A RBCs (from 1:32 to 1:16 titer) and decreased the diameter of A RBCs (4.38±0.2 µM in 1:32 titer to 4.05±0.4 µM in 1:2 titer). The nonadsorbed serum increased the diameter of A RBCs (3.96±0.1 µM in 1:32 titer to 4.33±0.68 µM in 1:2 titer).

Adsorption by washed and unwashed RBCs anti-A,B serum decreased the titer of agglutination of A RBCs from 1:32 to 1:16 titer. Adsorbed by washed and unwashed RBCs with saline in 2:1 ratio anti-A,B serum decreased the titer

of agglutination of A RBCs. Adsorption by washed RBCs appeared more successful as compared to adsorption by unwashed RBCs. The most optimal adsorption appeared to be at 4 °C for 13 hrs with anti-A,B serum. Thus, anti-A,B serum after adsorption by the studied RBCs as well as RBCs combined with Nystatin decreased the expression of agglutination of A RBCs contrary to the adsorption by the studied RBCs combined with Fenbendazole.

The adsorption of the studied RBCs by anti-A serum was better at 4 °C as compared to 37 °C. Anti-A,B serum increased the diameter of A RBCs s from 5.08±0.6 µM in 1:32 titer to 6.5±0.4 µM in 1:4 titer. However, did not increase the diameter of A RBCs after adsorption by the studied RBCs and e RBCs combined with fenbendazole, however, increased the RBCs diameter after adsorption by RBCs combined with nystatin (from 5.33±0.81 µM in 1:32 titer to 5.47±0.33 µM in 1:8 titer).

Anti-A serum continued agglutinating A RBCs at 1:8 titer after adsorption by the studied RBCs without previous loading with serum from AB person and increased A RBCs diameter (4.16±0.41 µM in 1:64 titer to 4.6±0.28 µM in 1:2 titer). The anti-A,B serum decreased the titer of agglutination of A RBCs s from 1:16 to 1:8 after adsorption by the studied RBCs for one day contrary to adsorption by the studied RBCs with fenbendazole (1:32 titer), however, the titer of the other anti-A,B serum also decreased after adsorption by the studied RBCs with Lincomycin (from 1:16 to 1:8).

The supernatant after washing the studied RBCs in 1:2 and 1:4 titer decreased the expression of agglutination of A RBCs by anti-A,B serum. Anti-A,B serum after adsorption by the studied RBCs decreased the titer of agglutination of A RBCs from 1:32 to 1:16 contrary to the serum after adsorption by the studied RBCs with Ofloxacine: the titer 1:32 (sensitive to *Proteus vulgaris* of the person's blood culture of the person appeared to be sensitive to Ofloxacine).

Anti-A serum did not decrease the titer of agglutination of A RBCs after adsorption by washed studied RBCs for 13 hrs at 20 °C and increased the RBCs diameter (4.14±0.4 µM in 1:32 titer and 4.2±0.33 µM in 1:2 titer) as well as nonadsorbed serum (4.32±0.37 µM in 1:32 titer and 4.42±0.43 µM in 1:2 titer). However, anti-A,B sera with a higher quantity of IgG antibodies showed inhibition of agglutination after adsorption with the studied RBCs. After the contact with Fenbendazole the adsorption of anti-A antibodies by the studied RBCs was increased: the titer of agglutination of A RBCs was decreased to 1:8 (with smaller RBCs diameter 4.01±0.33 µM in 1:2 titer and increased RBCs diameter in 1:4 titer: from 4.02±0.18 µM in 1:16 titer to 4.24±0.29 µM). One sample of anti-A,B serum showed the absence of agglutination of A RBCs after adsorption by the studied RBCs with fenbendazole (1:32 initial titer).

The supernatant from washed studied RBCs did not decrease agglutination of B RBCs by citrate anti-B plasma and somewhat increased the expression of agglutination as compared with the same concentrations of anti-A citrate plasma (control) and increased RBCs diameter (from 4.96±0.95 µM in 1:16 titer and 6.05±0.49 µM in 1:2

titer). The serum decreased agglutination of A RBCs in 1:16 titer after adsorption by unwashed studied e RBCs with fenbendazole, as well as after adsorption by the studied RBCs with nystatin. Anti-A,B serum increased the diameter of A RBCs (from 4.62±0.29 µM in 1:32 titer to 4.95±0.57 µM in 1:2 titer, and somewhat less, increased the diameter of A RBCs after adsorption by the studied RBCs s (from 4.32±0.28 µM in 1:32 titer to 4.65±0.21 µM in 1:2 titer, after adsorption by the studied RBCs with Nystatin: from 4.06±0.11 µM in 1:32 titer to 4.9±0.65 µM in 1:2 titer). Anti-A,B serum after adsorption by the studied RBCs with fenbendazole decreased the titer of agglutination of A RBCs from 1:32 to 1:2. Anti-A,B serum after adsorption by the studied RBCs decreased the titer of agglutination of A RBCs from 1:8 to 1:4 (the diameter was decreased with decreasing concentration of the serum) on the contrary to the adsorption with fenbendazole (the titer appeared 1:8) and the RBCs diameter increased with decreased concentration of the serum. The adsorption of the serum by the studied RBCs combined with nystatin decreased the titer of agglutination of A RBCs to 1:4 (as well as after adsorption by the studied RBCs without nystatin). After adsorption of the studied RBCs with fenbendazole the titer of anti-A,B serum with A RBCs was not decreased (1:16 and 1:32). After adsorption by the studied RBCs with Lincomycin the titer of anti-A,B serum decreased from 1:16 to 1:8.

Indirect Coombs' test

Indirect Coombs' test with anti-A serum at 37 °C did not show agglutination of the studied RBCs. Citrate anti-A plasma was incubated with the studied RBCs for 1 hr at 37 °C, the supernatant was removed and antiglobulin serum (AGS) was added, however, agglutination was not determined, as well as with anti-A,B serum after adsorption by B RBCs. Anti-A,B serum without anti-A and anti-B antibodies (being adsorbed by A and B RBCs) in a water bath with AGS and incubation at 20°C for 13 hours did not decrease the quantity of the studied RBCs contrary to the sample adsorbed only by B RBCs with the presence of anti-A antibodies, that led to the significant decrease of the quantity of the RBCs with a tendency to agglutination.

The study of the transfer of weak A antigen expression from the person's plasma to normal B RBCs

Anti-B serum containing A transferase for one day contact with B RBCs at 4 °C induced anti-A antibody adsorbing ability of B RBCs. Thus, the titer of agglutination of A RBCs by anti-A,B serum adsorbed by decreased from 1:32 to 1:8 after adsorption by B RBCs previously incubated with anti-B serum the on the contrary to the titer of anti-A,B serum adsorbed by usual B RBCs.

To reveal the activity of A and B transferase in the studied plasma A and B RBCs were contacted with the studied plasma for two days at 37 °C. Thus, A RBCs contacted with the studied plasma showed anti-B antibody adsorbing ability: the titer of agglutination of B RBCs by anti-A,B serum was decreased from 1:4 to 1:2. Similarly, the titer of agglutination of A RBCs by anti-A,B serum

adsorbed by B RBCs after contact with the studied plasma was decreased from 1:32 to 1:16.

Discussion

Weak subgroups are detected, if there is a discrepancy between RBCs and serum grouping and are often found when unexpected anti-A or anti-B activity of the serum is determined.[12,13] Weak subgroups of A blood type are reported by the researchers. Thus, a patient with a discrepancy in forward and reverse blood typing was studied by adsorption elution tests and saliva tests. The RBCs were similar to A_3 subtype. The person appeared to be a secretor (A and H substances were detected in the saliva). Anti-A_1 antibody was not revealed in the serum. The weak A antigen showed serological characteristics of A_3.[14]

Weak A antigen may be differentiated by various methods. These weak antigen appear due to weak expression of weak allele at the ABO loci. The subgroups are weakly agglutinated by anti-A sera (A_3, A_x, and A_{end}) or do not react (A_m, A_y, and A_{el}). Saliva hemagglutination inhibition test and adsorption-elution procedures are used to confirm the presence of weak antigen. While saliva investigation was reported to reveal specific substances, adsorption-elution showed A antigen specificity in different of A_m, A_y, and A_mB antigen.[15] A antigen subgroups have been classified into two main classes. Approximately, 20% of individuals having A antigen in blood belonging to A_2 antigen were identified in 20% of persons with A blood type thus, forming A_2 or A_2B subgroups. Weaker than A_2 subgroups are seldom detected. A_1 and A_2 antigen differ by the reaction with lectin: anti-A_1 (a cold agglutinin) agglutinates A_1 RBCs. A_2 and A_2B subgroups show the presence of anti-A_1 antibodies reacting at 37 °C.[16,17]

The distinction between A_1 and A_2 was made by testing red cells with the lectin from Dolichos biflorus. The A_2 gene has two nucleotides different from A_1 gene and has diminished enzymatic activity.[13,18,19] Scanning immune electron microscopy with monoclonal anti-A antibodies showed A antigen on less than 5% of A_m and A_{el} cells with strong labeling, therefore Am and A_{el} cells are able to adsorb significant amounts of anti-A antibodies without visible agglutination. Thus, the method of adsorption, but not agglutination is preferred for revealing weak A antigen, that showed effectiveness in the conducted study.

A_3 is considered as a heterogenous subgroup, A antigen were found on 82 and 58% of the v of A_3 persons. A receptor was detected on 75% of A_x RBCs. Weak A antigen are thought to be better detected if anti-A antibodies react with other A oligosaccharide chains than type 2 is used. From hyperimmune pregnancy sera A_x, A_m, and A_{el} cells adsorbed antibodies that had other characteristics than antibodies adsorbed by A_2 RBCs. We conclude that weak subgroups of A may deviate from A_2 both by the number of RBCs expressing A antigen and the biochemical nature of the antigen.[20]

The discrepancy between forward and reverse blood typing often occurs due to the low expression of antigens on RBCs. Neutralization agglutination is used for

the detection of group antigens in saliva. Surface plasmon resonance imaging is also used for antigen identification on RBCs and in saliva. Antigen are detected by anti-A, anti-B, and anti-H antibodies, the array is immobilized on the sensor surface. RBCs and saliva specimens are analyzed by passing them over the antibody array, where the secretor status and blood group may be identified.[19]

ABO discrepancy cases were described when RBCs from blood group A persons come into contact with certain gram-negative bacteria of colonic origin. These bacteria carry an enzyme, that cleaves a part of A antigen (acetyl group from the last sugar on A chain, N-acetylgalactosamine), leaving galactosamine, that resembles the B antigen. Acquisition of A and B antigen are widely discussed, especially in ABO-incompatible hematopoietic stem cell transplantation (HSCT). The studies investigated the weak blood group A or B antigen expression by donor-derived group O RBCs observed following transfusion or minor ABO-incompatible HSCT.[21]

The persons typed as A, B, and AB and transfused from group O donors show the presence of A antigen on donor-derived RBCs (A- A_x - profiles). After group O donor RBCs were incubated with group A/B secretor/non-secretor donor plasma or RBCs adsorption of A/B antigen-bearing glycolipids from secretor plasma pointed to a secretor-independent mechanism for A/B antigen acquisition. Conversion of donor-derived blood group O RBCs to ABO subgroup-similar RBCs *in vivo* after transfusion or minor ABO-incompatible HSCT shows the necessity to reveal and control weak antigen expression.[21]

Some reports of A subgroup with acquired B antigen were described in urinary tract infections. The RBCs showed mixed field agglutination with anti-A, anti-AB and microscopic agglutination with anti-B antibodies and anti-B antibodies on serum grouping. There was a strong reaction with A_1 Lectin and anti-H, a positive auto-control, and direct antiglobulin. A subgroup blood transfusion was considered to be avoided since the patient demonstrated the presence of autoantibodies.[22]

The cases of weak A antigen appear rather often. Thus, the RBCs of type O persons with *Proteus mirabilis* infection were agglutinated by an adsorbed anti-A antibody. The reaction with anti-A antibody was inhibited by blood group A substance. Acquisition of A-like antigen was considered to be induced by *Proteus* infection but, the changes persisted after the bacterial infection was cured. Various abnormalities of the RBCs membrane were noticed. Therefore, the presented study confirms the data of the association of anti-A antibody adsorbing ability with *Proteus* infection. Other researchers report the person with acquired A antigen. The patient's RBCs were agglutinated by anti-A antibodies and less strongly by anti-B antibodies with mixed field agglutination and serum anti-A and anti-B antibodies were found in serum. The antiglobulin test was positive. The person's serum contained hemolytic anti-A antibodies and saline agglutinating anti-B antibodies. The person's serum agglutinated but never hemolyzed his own cells. Neither freezing in liquid nitrogen nor in glycerol had any effect on the acquired A-like antigen of the person's

cells. Eluates made on several occasions displayed weak antibody activity. The eluate agglutinated A_1 RBCs. The weak positive direct antiglobulin test was seen in the same specimen in which the patient's serum lacked auto-agglutinating anti-A. Adsorption with a person's cells did not significantly lower the titer of anti-A antibodies. A substance caused total inhibition of anti-A agglutination while B and H substances had no effect. When total neutralization of the anti-A antibody was achieved, as evidenced by its failure to agglutinate normal A_1 and A_2 cells, some neutralized sera still reacted weakly with the person's cells. The reactions between five different commercial anti-A or anti-A_1 reagents and a person's cells were totally inhibited by A substance. An activity of RBCs was absent after ficin treatment. The enzyme activity of the supernatant was destroyed by boiling. Bacterial or viral infections seem likely to initiate the erythrocyte changes, the Ogata phenomenon was also reported. Lack of a particular fraction of anti-A and anti-B antibodies may be the factor, that allows an individual to acquire A and B antigen.

In a person with acquired A antigen a difference between A antigen on RBCs and antigen on polyagglutinable cells is that the person's anti-A antibody could be inhibited by A substance while anti-A reaction could be inhibited only with N-acetyl-D-galactosamine. Further evidence of bacterial association is provided by the numerous studies that have convincingly demonstrated the presence of both A- and B-like substances in *Escherichia* and *Proteus* organisms. A marked difference between acquired A and B antigen in recently described cases is that the acquired A antigen of the person survived freezing in glycerol and liquid nitrogen while the acquired B antigen in patients with polyagglutinable cells did not. The mechanism of A antigen acquisition is discussed. RBCs with decreased sialic acids have occurred due to the inherited variance or an acquired alteration. Thus, if a portion of the glycolipid and/or glycoprotein chain is removed, an A-like antigen is exposed, possibly following the reorientation of the remaining portions of the chains. Acquisition of an A-like antigen might be independent of the *Proteus* infection, since the acquired A antigen persisted for six months after the disappearance of *Proteus* infection. The *Proteus* infection may have initiated, an alteration in the antigenic expression of RBCs.

Revealing of A antigen in person with B blood group was also reported. The B(A) phenotype showed the presence of anti-A antibodies in the serum and since A antigen on the RBCs were presented few, thus no hemolysis occurred. The cell grouping showed A weak B, while the serum grouping demonstrated B. A set of anti-A antibodies was showing weak agglutination. Thus, the case highlighted the serological characteristics of a B(A) phenotype.[23]

A blood type subgroups are known to secrete fewer antigen in saliva. They may adsorb naturally occurring anti-A_1 without hemolytic complications (cold antibodies), however, may develop anti-A_1 antibodies when exposed to A_1 antigen (reacting at 37°C), since they are of IgG

nature.[24,25] This explains the observed peculiarities in optimal temperatures for anti-A and anti-B antibodies of the studied person's plasma. The mechanisms of acquisition of A and B antigen deal with the glycosylation of complementary domains.[26] Thus, further studies on the mechanisms of A and B antigen acquisition are needed. This is the second case where we report a person with B blood group, weak A antigen expression, and anti-B antibodies reactive at 37 °C.[27]

Thus, a person's B blood group RBCs were agglutinated by anti-B antibody, and the agglutination was 1[+] at 37 °C. However, the RBCs did not adsorb anti-B but anti-A antibodies. The RBCs were agglutinated by anti-A at 37 °C with signs of hemolysis in the presence of complement. Meanwhile, the serum agglutinated A RBCs at room temperature with less activity at 37 °C and B RBCs at 37 °C. The RBCs were agglutinated at 37 °C by polyclonal serum, whereas serum weakly agglutinated A RBCs at 37 °C. The absence of anti-B antibody absorbance by the person's RBCs was accompanied by the presence of anti-B antibodies, active at 37 °C. The incubation of the person's serum with O RBCs induced the ability of RBCs to adsorb anti-A antibodies and to be hemolyzed by anti-A in the presence of complement. Adsorption and agglutination at room temperature and 37 °C by heated serum with the use of complement helped to reveal weak A antigen. However, the person was not studied bacteriologically.

The present study is in accordance with data of other researchers pointing to the possible associations between anti-A antibody adsorbing ability and *Proteus vulgaris* infection. Whether *Proteus vulgaris* infection induces A antigen expression or the presence of weak A antigen predisposes the development of *Proteus vulgaris* infection needs to be elucidated. Acquisition of A antigen was found long ago and reports of new cases stimulate the discussion and study of this phenomenon.[28,29] Thus, not only weak B antigen has been found to be associated with bacterial infection but A antigen acquisition as well.[30]

Conclusions

Subgroups are usually wrongly typed as O, which may develop into a hemolytic transfusion reaction. Adsorption tests of the subgroups could be helpful in confirming weak antigen. The presented case emphasizes the important role of cell and serum grouping in revealing such discrepancies, that may lead to complications during transfusion recipient, if not resolved. Any should be examined microscopically. The presented case emphasizes the important role of cell and serum grouping in revealing such discrepancies. If ABO discrepancy in forward and reverse typing is found, a high probability of weak antigen expression exists to reduce the incidence of transfusion-related reactions. The presence of weak antigen in the blood group should be accompanied by a thorough bacteriological investigation of the person.

Ethical approvals

The study was approved by the Kharkiv National Medical University ethics committee (protocol 4).

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Decelerate amyloid fibrillation by the alkaloids extracted from *Stephania venosa*

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ARTICLE INFO

ABSTRACT

Background: Naturally occurring phytochemical compounds have received considerable attention as alternative candidates for anti-amyloidogenic agents.

Objectives: This study, utilizing human insulin and amyloid-β peptide as an *in vitro* model, determined the anti-amyloid effects of alkaloids extracted derived from *Stephania venosa*.

Materials and methods: Alkaloids extracts including crebanine, *O*-methylbulbocapnine, tetrahydropalmatine and *N*-methyltetrahydropalmatine were used. The Inhibition of amyloid protein aggregation was studied by fluorescence spectroscopy.

Results: Most alkaloids, except *N*-methyltetrahydropalmatine, exhibited inhibitory properties against amyloid fibrillation either insulin or amyloid-beta peptide. Among the alkaloids group, crebanine and tetrahydropalmatine showed potent properties of anti-amyloidogenesis.

Conclusion: These results suggest that alkaloids could be used as a natural compound for the development of drugs against amyloid protein aggregation for the treatment of amyloid-related diseases.

Introduction

Suppression of amyloid protein aggregation is considered a promising therapeutic approach to prevent or treat amyloidosis-related disorders. One of the current strategies aimed at finding the therapeutic compound against amyloidogenic activity is to inhibit the toxic amyloid formation and stabilize its native monomeric form or destabilize the fibrillated misfold form.[1] Knowing that protein aggregation is a shared property of all proteins, model proteins can be used to study this process. Various peptides and proteins can undergo self-aggregation that leads to the formation of amyloid fibrils. Human insulin is one protein that was chosen and widely used as a model protein for the study of amyloid formation *in vitro*. Recently, much attention has been paid to find out an inhibitor of insulin amyloid fibrils.

Natural products are a major class of amyloid inhibitors, and natural product-based amyloid inhibitors have been identified and characterized in recent years.[1] Several natural polyphenolic compounds have been well-studied as amyloid inhibitors such as epigallocatechin gallate (EGCG), curcumin, and resveratrol. Quinones

show inhibitory effects to different extents on insulin oligomerization, especially for 1,4-benzoquinone and 1,4-naphthoquinone.[2] Quercetin dose-dependently inhibited the amyloid formation of insulin via destabilizing the preformed insulin fibrils and transforming the fibrils into amorphous aggregates.[3]

Alkaloids, a class of nitrogen-containing compounds, are found primarily in plants, especially in flowering plants.[4] Alkaloids have a broad spectrum of pharmacological effects including analgesic, antiasthmatic, antiarrhythmic, anticancer, etc.[4,5] Galantamine, the isoquinoline alkaloid family, inhibits Aβ-aggregation and cytotoxicity.[4,6] However, galantamine is associated with side effects, the most common being nausea, vomiting, diarrhea, and anorexia.[7] The *in vitro* and *in vivo* studies showed that Aβ and Aβ-induced neurotoxicity was reduced by the effect of caffeine.[8] In addition, caffeine also reduces levels of Aβ in neuroblastoma-2a cells stably expressing human Swedish mutant APP and protecting cerebellar granule neurons and basal forebrain neurons from neurotoxicity caused by Aβ.[8,9] Furthermore, it should find out an additional alkaloid, either novel compounds or an old one, that could be used for inhibiting Aβ-aggregation.

Stephania venosa (Blume) Spreng., belongs to the Menispermaceae family, and has been traditionally used as a tonic drug and treatment of various diseases in South East Asian countries.[10] It was found that alkaloids are the main phytochemical compound of this genus.[11] Their biological activities have been reported including anti-cancer activity chemosensitizer and acetylcholinesterase inhibition.[12-15] Crebanine, a major component of *S. venosa*, exerts anti-proliferative and anti-invasive effects on human cancer cells through the induction of cell cycle arrest at the G1 phases, induce apoptosis in K562, K562/adr, GLC4 and reduce the expression of MMP-2, MMP-9, uPA, and MT1-MMP.[13,16-18] In addition, there are several natural alkaloids, including *O*-methylbulbocapnine, tetrahydropalmatine, and *N*-methyl tetrahydropalmatine have been found in the tubes of *S. vernosa*.[12,13] Furthermore, tetrahydropalmatine inhibits LPS-induced IL-8 secretion by blocking MAPKs signaling pathway.[19] However, there is no report about the anti-amyloid effects of these alkaloids.

In the present study, we measured protein aggregation of the model amyloid-forming protein using human insulin and amyloid beta peptide in the presence and absence of alkaloids. Human insulin fibril formation was generated by incubating at high temperatures and acid environment (pH 2.5 and 80 °C) and the effect of alkaloids on this fibrillation was investigated by intrinsic Tyrosine fluorescence assay and Thioflavin T assay. We reported here the capacity of alkaloids to interact with the insulins and thereby prevent their conversion to amyloid fibrils, indicating a therapeutic potential of the alkaloids in protein aggregation diseases.

Materials and methods
Chemical reagents
Recombinant human insulin was purchased from Gibco, Life Technology. Before the experiments, the insulin solution was diluted in 0.025 M HCl, 0.1 M NaCl pH 1.6. Amyloid β peptides, i.e. $A\beta_{40}$ and $A\beta_{42}$, were purchased from EZBiolab Laboratories and were initially solubilized, in 1,1,1,3,3,3-hexafluoro-2-propanol or hexafluoroisopropanol (HFIP) (Fluka). Stock solution 1 mM in HFIP was aliquoted in 20 µL in each microtube and dried under N_2 gas atmosphere to undergo dried films, then stored at -20 °C. Before performing the experiments, aliquots were resuspended at a final concentration of 5 mM in DMSO (Sigma), sonicated using a bath sonicator for 10 min, and diluted to 100 µM with a phosphate buffer solution (PBS) plus 0.05 % sodium dodecyl sulfate (SDS) (Sigma). Thioflavin T (ThT) (Sigma) was dissolved in PBS pH 7.4 and filtered through a 0.2 µm syringe filter. The concentration of ThT was determined using UV absorbance at 412 nm and calculated by using the extinction coefficient of 36,000 $M^{-1}cm^{-1}$. Four alkaloids including crebanine (A1), *O*-methylbulbocapnine (A2), tetrahydropalmatine (A3) and *N*-methyltetrahydropalmatine (A4) were kindly provided by Associated Professor Dr. Wilart Pompimon, Department of Chemistry, Faculty of Science, Lampang Rajabhat University, Thailand[13] These alkaloids were extracted from the tuber of *S. venosa*. Tetrahydropalmation and crebanine are productions from ethyl acetate extraction. While *O*-methylbulbocapnine and *N*-methyltetrahydropalmatine were extracted by acetone. The extraction method was clearly described by Nantapap.[13] All alkaloid molecules were dissolved in DMSO (Figure 1). Other chemical reagents include MEM, HAM/F12 medium with L-glutamine (Caisson, USA), DMEM with high-glucose and L-glutamine (Caisson, USA), Penicillin-Streptomycin (Caisson, USA), Fetal bovine serum (Gibco®, Invitrogen, USA), Dimethyl sulfoxide (DMSO).

Kinetics of insulin fibrillation by intrinsic Tyrosine fluorescence
The fluorescence intensity of tyrosine (Tyr) was used to investigate insulin fibrillation. The insulin fibrillation was performed by using the thermal-induced fibrillation method.[20] The experiment was assigned by incubating 2 mL of insulin (0.02 mg/mL) or insulin with alkaloids (0.002 mg/mL) at 80 °C for 24 hrs. The emission spectra of Tyr were recorded from 280 to 500 nm in a 1cm quartz cell by exciting at 276 nm. The fluorescence intensity of Tyr at 306 nm was plotted against the time of incubation. The efficiency of alkaloids to inhibit insulin fibrillation was assessed by two terms; 1) the half-time value of insulin fibrillation ($t_{0.5}^{ins}$) and 2) the altered fluorescence intensity of tyrosine (ΔF_{tyr}). The half-time value of insulin fibrillation ($t_{0.5}^{ins}$) is defined as the time when the signal has reached 50% of the amplitude of the transition ($A/2=(F_i-F_n)/2$) that as shown in Figure 2a. Where F_i and F_n are the fluorescence intensities at the initial reaction and steady state, respectively, and A is the amplitude of the reaction. The altered fluorescence intensity (ΔF_{tyr}) signified the amount of insulin fibril formation and defined the percentage of the diminution of fluorescence intensity (A/F_i*100). (Figure 2a)
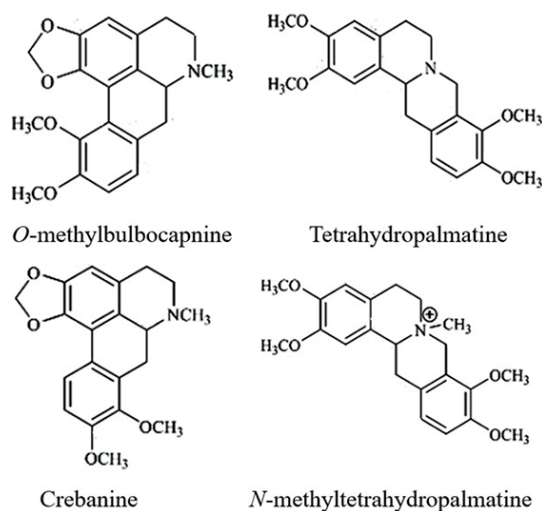
Figure 1 *Chemical structure of four alkaloids derived from Stephania venosa (Blume) Spreng.*
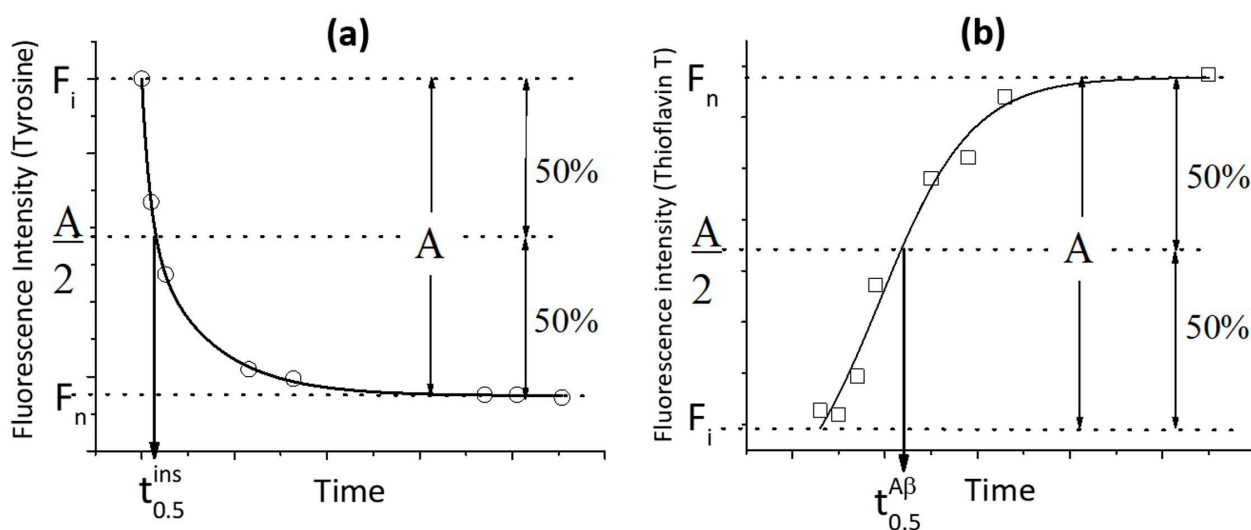
Figure 2 *Kinetic of insulin fibrillation monitoring by the fluorescence intensity of tyrosine (a). and the kinetic of amyloid beta fibrillation monitoring by the fluorescence intensity of Thioflavin T(b).*

Effect of alkaloids on insulin fibrillation

Various concentrations of alkaloids (0.002-0.01 mg/mL) were added into insulin solutions (0.02 mg/mL) prior to warming at 80 °C for 24 hrs. After incubation, 20 μM ThT was added, and the fluorescence emission spectra (excitation wavelength at 420 nm) were obtained. Relative ThT fluorescence values were calculated from the ratio of ThT fluorescence intensity of insulin in the presence of alkaloids and insulin control.

Kinetic analysis of amyloid fibrillation

The Aβ fibrillation was performed in 1 μM $A\beta_{40}$, 1 μM $A\beta_{42}$, and a combination of $A\beta_{40}$:$A\beta_{42}$ (0.2 μM:0.8 μM). All samples were added in 250 μL of PBS buffer pH 7.4 with 0.05% SDS containing 10 μM of ThT, and then incubated at 40 °C. The fluorescence intensity of ThT was taken using a spectrofluorometer (Perkin Elmer LS55) with an emission wavelength of 488 nm excitation wavelength of 420 nm.

Effect of alkaloids on Aβ fibrillation

The experiments were performed by co-incubating of $A\beta_{40}$ (1 μM), $A\beta_{42}$ (1 μM), or $A\beta_{40}$:$A\beta_{42}$ (0.2:0.8 μM) with alkaloids (0.002 mg/mL) at 40 °C and used 10 μM of ThT for fibrillation analysis. The fluorescence intensity of ThT was measured at 488 nm when excitation was at 420 nm using a spectrofluorometer. The kinetics of Aβ fibrillization could be described as sigmoid curves and the aggregation parameters were determined by fitting the plot of fluorescence intensity versus time as indicated in Figure 2b. The fibrillation rate presented in the half-time value ($t_{0.5}^{A\beta}$) was used for data analysis. The efficiency of alkaloids to inhibit the Aβ fibrillation was assessed by two terms; 1) the half-time value ($t_{0.5}^{A\beta}$) defined as the time when the signal has reached 50% of the amplitude (A) of the transition ($A/2 = (F_n - F_i)/2$). Where F_i and F_n are the fluorescence intensities at the initial reaction and at steady state, respectively, and A is the amplitude of the reaction. (Figure 2b)

Statistical analysis

All data are expressed as mean±SD. Statistical significance was determined using Student's t-test between the groups treated and the control. A probability *p*<0.05 was considered statistically significant.

Results

Alkaloids extracts inhibited the kinetic of insulin aggregation.

To determine whether alkaloids extract inhibited insulin fibrillation, tyrosine emission spectra of insulin (0.02 mg/mL) in the presence of alkaloids (0.002 mg/mL) were observed for 24 hrs. The results showed a decrease in Tyr fluorescence intensity during insulin fibrillation. This becomes apparent on plotting the emission intensity at 306 nm against time with the half-time ($t_{0.5}^{ins}$) of insulin equal to 0.55±0.11 hrs. However, the addition of A1, A2, A3, and A4 to the insulin did not decrease in intensity of the emission at 306 nm over the time of incubation. Therefore, the half-time ($t_{0.5}^{ins}$) of A1, A2, and A4 significantly increased except A3.

Alkaloids extracts inhibited insulin fibril formation.

To confirm the presence of insulin fibril formation. ThT fluorescence assay was performed. It was found that ThT binds specifically to the cross-β sheet structure of amyloid fibers and gives more intense once bound. In this experiment, after incubation for 24 hrs, 20 mM ThT was added and measured the fluorescence intensity at 488 nm after exciting with 420 nm. The fluorescence intensity of ThT of insulin incubated with A1, A2, and A3 after incubation was lower than that of insulin control with the relative ThT fluorescence equal to 0.44±0.06, 0.59±0.05 and 0.57±0.07, respectively (Table 1). Interestingly, the ThT fluorescence intensity of A4 incubated with insulin did not change when compared with insulin control. Therefore, all alkaloid extracts, except A4, inhibited insulin fibril formation.

Table 1 Effect of alkaloids on insulin fibrillation detected by Tyr fluorescence and Thioflavin T versus insulin control.

Alkaloids	Tyrosine fluorescence		Relative ThT fluorescence
	Half time ($t_{0.5}^{ins}$), h	ΔF_{tyr}	
Insulin	0.55±0.11	88.0±4.7	1.00
Insulin +A1	0.81±0.01*	25.2±4.6*	0.44±0.06*
Insulin +A2	0.89±0.09*	16.9±4.6*	0.59±0.05*
Insulin +A3	0.69±0.07	61.5±10.6*	0.57±0.07*
Insulin +A4	0.79±0.09*	33.5±4.5*	1.29±0.27

*Note: *p<0.05*

Alkaloids extracts inhibited the insulin fibril formation in a dose-dependent manner.

The previous data demonstrated that most alkaloids, except A4, had the potential to be an inhibitor of insulin fibrillation. We performed a further experiment to determine whether alkaloid extracts affect the insulin fibrils formation in a dose-dependent manner, different concentrations of A1-A4 (0.002-0.01 mg/mL) were added into insulin (0.02 mg/mL) before warming them to 80°C for 24 hrs. After incubation, 20 µM ThT was added and the fluorescence intensity at 488 nm (excitation wavelength at 420 nm) was obtained. Relative ThT fluorescence values were calculated and derived from the ratio of ThT fluorescence intensity of insulin in the presence of alkaloids and the ThT fluorescence intensity of fibrils insulin control. Increased concentration of alkaloid extracts was found that A1, A2, and A3 potently inhibited insulin fibril formation in a dose-dependent manner, while A4 did not. Among alkaloid molecules (at 0.002 mg/mL), A1 might be the most inhibitor of insulin fibrillation. (Figure 3)

Alkaloids extracts inhibited the Aβ fibrillation.

To characterize the process of Aβ fibrillation, the kinetic fibrillization process of Aβ peptide with different mixing ratios of $A\beta_{40}$ to $A\beta_{42}$ was performed. Our model is based on the finding that there are two main Aβ peptides of different lengths involved in Alzheimer's disease, $A\beta_{40}$, and $A\beta_{42}$ residues. It was found that the mixing of $A\beta_{40}$ and $A\beta_{42}$ enhanced toxicity in the early onset of some familial Alzheimer's diseases.[21] Otherwise, our previous study found that the ratio of $A\beta_{40}$: $A\beta_{42}$ (1:4) increased the toxicity in a neuroblastoma cell line, SK-N-SH, higher than treated with $A\beta_{40}$ or $A\beta_{42}$ alone (data not shown). To mimic the pathology of AD, three Aβ peptides were prepared in 1 µM $A\beta_{40}$, 1 µM $A\beta_{42}$, and a combination of $A\beta_{40}$:$A\beta_{42}$ (0.2 µM:0.8 µM). The fibrillization of Aβ was observed by an increase in ThT fluorescence due to the binding of the dye to the fibrils. The representative fibrillization curves were shown in Figure 4. Our results showed that $A\beta_{40}$, $A\beta_{42}$, and $A\beta_{40}$:$A\beta_{42}$ demonstrated similar fibrillization kinetics which exhibit a sigmoidal appearance. It seems to be that the fibril growth rate which is represented by $t_{0.5}^{A\beta}$ for $A\beta_{42}$ was shorter than that of $A\beta_{40}$, indicating $A\beta_{42}$ exhibited a fast fibrillation rate than $A\beta_{40}$. Mixing of $A\beta_{40}$ to $A\beta_{42}$ seems to decrease the fibril growth rate of $A\beta_{42}$ compared with $A\beta_{42}$ alone (Figure 4d).

In the presence of alkaloid extracts (0.002mg/mL), the results showed that different alkaloid extracts showed the different effects on the kinetic of amyloid beta fibrillation as indicated by half-time ($t_{0.5}^{ins}$) and the relative ThT fluorescence value is shown in Table 2. Increased half-time and decreased relative ThT fluorescence value represent the decreased amyloid fibrillation formation. The half-time of amyloid formation for $A\beta_{40}$ was increased from
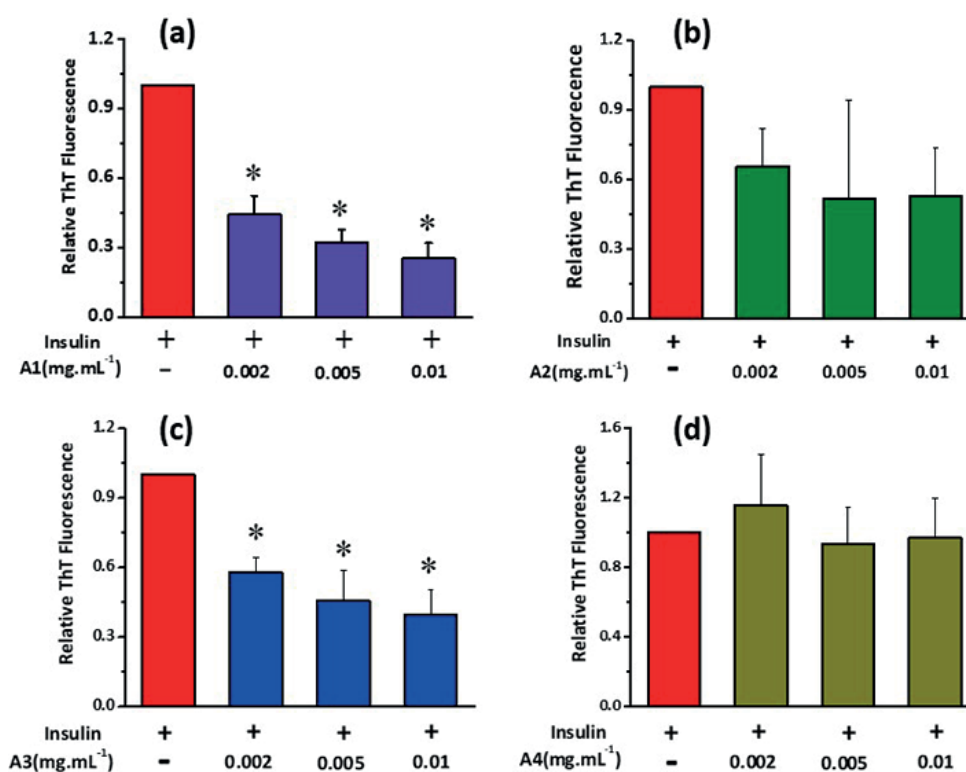
Figure 3 *Effect of various concentrations of alkaloid extracts, crebanine (a), O-methylbulbocapnine (b), tetrahydropalmatine (c) and N-methyltetrahydropalmatine (d), on insulin fibrillation, detected by thioflavin T. The data were presented as mean±S.D. (N=3), *p<0.05 versus insulin control.*
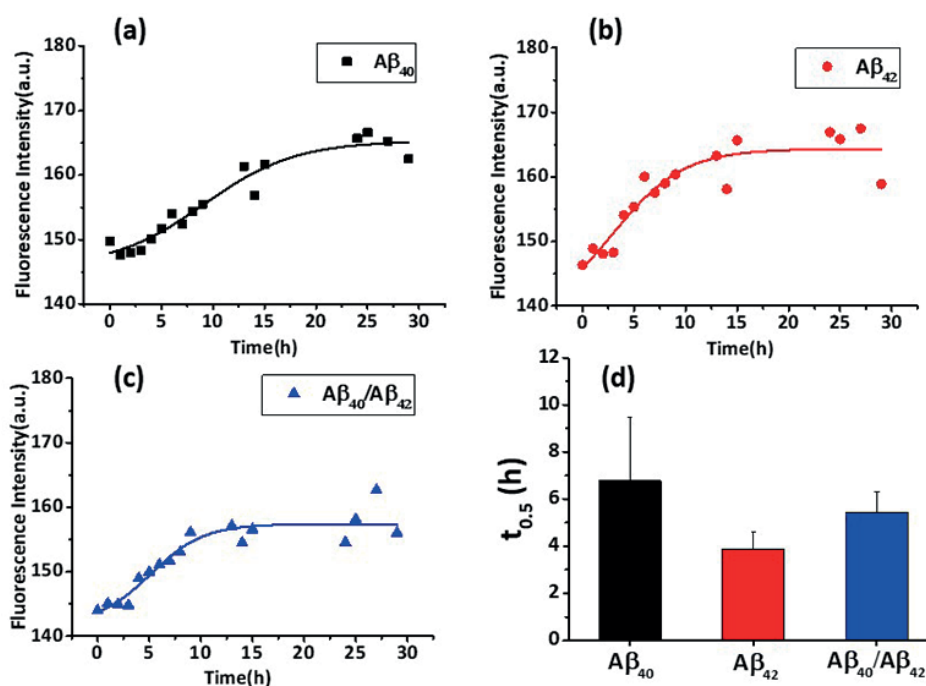
Figure 4 *Kinetic of amyloid-β fibrillation detected by Thioflavin T. ThT fluorescence emission at 488 nm was monitored upon excitation at 420 nm. The Aβ concentration was performed in 1 μM Aβ$_{40}$ (a), 1 μM Aβ$_{42}$(b), and a combination of Aβ$_{40}$:Aβ$_{42}$ (0.2 μM:0.8 μM)(c). All samples were added to 250 μL of PBS buffer pH 7.4 with 0.05% SDS containing 10 μM of ThT, and then incubated at 40 °C.*

Table 2 Effect of alkaloids (0.002 mg/mL) on Aβ fibrillation detected by Thioflavin T assay versus insulin control.

Phytochemicals	Thioflavin T assay					
	Half time ($t_{0.5}^{A\beta}$), hr.			Relative ThT fluorescence		
	$A\beta_{40}$	$A\beta_{42}$	$A\beta_{40:}$ $A\beta_{42}$	$A\beta_{40}$	$A\beta_{42}$	$A\beta_{40:}$ $A\beta_{42}$
Aβ Control	6.8±2.7	3.9±0.7	5.5±0.9	1.00	1.00	1.00
Aβ+A1	8.8±1.9	3.9±1.2	5.0±1.8	0.20±0.03*	0.70±0.05	1.02±0.11
Aβ+A2	6.4±2.7	8.4±8.0	6.3±1.2	0.55±0.12*	0.93±0.38	1.03±0.18
Aβ+A3	7.7±3.3	5.2±0.9	9.3±2.7	0.67±0.07*	1.01±0.06	1.54±0.74
Aβ+A4	9.0±0.6	4.4±3.4	4.3±1.2	1.25±0.05	1.35±0.25	1.58±0.04

*Note: *p<0.05*

6.8 hrs to 8.8 hrs and 7.7 hrs in the presence of A1 and A3 respectively. Accordingly, with half-time value, the relative ThT fluorescence values were decreased in the presence of A1 and A3 compared with Aβ control. Therefore, A1 and A3 inhibited the amyloid fibrillation for $A\beta_{40}$. It was also found that amyloid formation for $A\beta_{42}$ was inhibited by A2. However, it seems to be that A2 inhibited amyloid formation for $A\beta_{40}$ and A1 inhibited amyloid formation for $A\beta_{42}$ as indicated by the decreased relative ThT fluorescence value compared with its control.

Discussion

The previous study in our laboratory found that at concentrations ranging from 0.002 to 0.01 mg/mL of crebanine, O-methylbulbocapnine, tetrahydropalmatine, and *N*-methyltetrahydropalmatine exhibited a cytotoxic effect of less than 10% on the neuronal cell lines (SK-NSH and SH-SY5Y). However, it's important to note that this data has not been published. The incidence of amyloid-related diseases has been growing continuously. Finding an effective treatment became more important. Amyloid fibrillated protein has been the focus of research for many years.[22] An ever-growing incidence of amyloid-related diseases has led researchers and clinicians to discover a cure. Hence, the purpose of drugs that prevented amyloid accumulation may be a potential treatment. Recent research has focused on natural products to avoid the side effect of clinical use.[23] Natural products such as flavonoids, alkaloids, and curcuminoids have been extensively researched regarding reducing the amyloid-associated toxicity of Aβ.[24,25] In this study, we have proposed alkaloids as an *in-situ* inhibitor for amyloid protein fibrillation. Four alkaloids derived from *Stephania venosa* including crebanine, O-methylbulbocapnine, tetrahydropalmatine, and *N*-methyltetrahydropalmatine were used as interested molecules.

We first studied the interaction of alkaloids with human insulin. The reasons why human insulin was chosen as the model protein in this study are as follows; 1) insulin and Aβ protein share a common characteristic. 2) Under appropriate conditions, they both aggregate into amyloid fibrils. 3) Although the proteins do not share sequence homology, they exhibit similar insoluble filaments and fibrillation responses.[26,27] According to the process of insulin aggregation, it proceeds through the dissociation of oligomeric states into monomers, which then undergo conformational changes and make themselves into a stable state by forming fibrous amyloid aggregates rich in β-sheets.[28] In the present work, the aggregation kinetics of human insulin was studied at low pH and high temperatures. Decrease in Tyr fluorescence intensity, an intrinsic fluorophore was monitored during insulin aggregation that accompanies insulin fibrillation using Thioflavin T. Our results clearly found that most alkaloids inhibited insulin aggregation and fibril formation in as dose-dependent manner. *O*-methylbulbocapnine is isomeric with crebanine with different positions of the two methoxyl.[13] Both alkaloid molecules exhibited similar properties of anti-insulin fibrillation. Therefore, different positions of the two methoxyl did not affect to the anti-amyloidogenic properties. Interestingly, it was found the different properties of anti-insulin fibrillation between *N*-methyltetrahydropalmatine and tetrahydropalmatine. *N*-methyltetrahydropalmatine is an analogue of tetrahydropalmatine. We found that the methyl group on the nitrogen atom of *N*-methyltetrahydropalmatine decreases the capacity of insulin fibril formations. Therefore, the nitrogen atom on tetrahydropalmatine seems presumably to play a role as an active site for an inhibitor of amyloid fibril formation.

We successfully demonstrated the ability of alkaloids to inhibit the kinetics of insulin aggregation. We postulated that a similar strategy could be used to study amyloid β peptide. According to the evidence of the major form of the Aβ peptide found in amyloid plaque that showed $A\beta_{40}$ and $A\beta_{42}$ form mixed aggregates.[29] It attempted to investigate the influence of each Aβ peptide on their aggregation kinetics behavior. The kinetic analysis found that $A\beta_{42}$ exhibited a fast fibrillation rate than $A\beta_{40}$. However, the mixing of $A\beta_{40}$ to $A\beta_{42}$ seems to slow down the fibril growth rate of $A\beta_{42}$ when compared with $A\beta_{42}$ alone. The study from Pauwels *et al*. used the NMR experiments for visualizing the spontaneous aggregation of mixing $A\beta_{40}$ to $A\beta_{42}$. It was shown that $A\beta_{40}$ slows down the aggregation kinetics of $A\beta_{42}$.[30]

Conclusion

In conclusion, amyloid fibrillation could be monitored by using intrinsic Tyrosine fluorescence to accompany with Thioflavin T assay. Alkaloids have

shown some promise against amyloid fibrils both in insulin and amyloid beta peptide. Most alkaloids group, except *N*-methyltetrahydropalmatine, exhibited potent properties of anti-amyloidogenesis. These results suggest alkaloids can be used as the natural compound for the development of drugs against amyloid protein aggregation for the treatment of Alzheimer's disease.

Conflict of interest
The authors declare no conflict of interest.

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A review of literature on the therapeutic use of music with military populations experiencing post-traumatic stress disorder

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ARTICLE INFO

ABSTRACT

Background: Post-traumatic stress disorder (PTSD) is frequent among military populations. Symptoms range from physical to behavioural, and emotional difficulties. Loneliness and feeling misunderstood are common among military personnel, particularly veterans. Service members returning from duty often experience a lack of support and this predisposes them to an increased risk for suicide. Music can be therapeutically used with military populations through a range of methods, especially in mental health settings. Participating in music can be a meaningful occupation for many but there is a scarcity of literature on the therapeutic use of music in occupational therapy.

Objectives: The purpose of this literature review was to synthesize current qualitative evidence on the perceptions and experiences of the military population who engage in music therapy.

Materials and methods: A scoping review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis extension for Scoping Reviews (PRISMA-ScR) checklist guidelines. Varied databases were searched to retrieve qualitative papers on the lived experiences of the therapeutic use of music among people in the military published between January 2012 and December 2022. Following the quality appraisal, data from the included papers were collated in a data abstraction form before being synthesized thematically.

Results: Seven studies met the inclusion criteria and were included in this scoping review. Three themes were identified: emotional regulation, engaging with others, and mental health stigma. Therapeutic use of music was found to be beneficial and service members expressed that they have managed to overcome mental health challenges, find new connections, and engage with others. However ethical challenges and the delivery of therapeutic use of music must be carefully monitored.

Conclusion: Overall, the therapeutic use of music within military populations with PTSD is perceived to be beneficial and worth continuing with however, careful monitoring during treatments should be a priority to prevent outbursts, relapse, or aggressive behaviours. Combining occupational therapy and music therapy could maximize the benefits of music to people with mental health conditions.

Introduction:

Post-traumatic stress disorder (PTSD) is frequent among military populations.[1] This stress-related disorder develops after a traumatic event usually in a combat zone. It involves a combination of symptoms that are unique to the individual. Symptoms range from physical to behavioural, and emotional difficulties.[2] Executive functions can also be impacted resulting in communication difficulties and memory problems.[3] Without effective interventions the

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military population can experience a loss of identity and struggle to find meaning in daily activities. Sustaining a family role can be challenging.[3] Loneliness and feeling misunderstood are common amongst military personnel, particularly veterans. Veterans often need further support outside of interventions targeted at military populations.[4] Female military personnel are 4% more likely to develop PTSD compared to their male peers. One in five women reported PTSD caused by military sexual trauma, these numbers are thought to be higher, however, the true statistics are not known as some women did not access the Departments of Veterans Affairs for support due to fear of stigma.[5]

Lack of support for service members returning from duty has been reported to put them at increased risk of suicide.[4] This emphasizes the need for the military population to be offered a unique variety of meaningful opportunities that can help express their emotions and needs successfully.[1] Publication on the therapeutic value of music began in the 18th century.[6] Since then, music therapy has been delivered across military populations by a range of different methods which involve song writing, musical instruments, and video technology.[7]

Occupational Therapists have knowledge in neurological science, habituation, rehabilitation, and mental health as well as fulfillment of well-being through meaningful occupation. Limited studies have been conducted on the use of music therapy from an occupational therapy perspective.[8] A better understanding of how occupational therapists can use music therapy combined with their expertise to support people with symptoms of PTSD in the military is lacking. Likewise, the delivery of music therapy sessions and their environment play a key part in how successful the treatment can be.[7] Music therapy can be delivered through telecommunication or face-to-face. Therapy can be conducted in a group or one-to-one depending on the individual needs. The use of music in therapy consists of song writing, playing musical instruments, and singing.[9] This review aimed to synthesize literature on the perceptions and experiences of the therapeutic use of music with military populations with PTSD.

Materials and methods

A scoping review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Extension for Scoping Reviews (PRISMA-ScR) checklist.[10,11] A wide range of databases were searched including APA PsycInfo, Academic Search Ultimate, MEDLINE Complete, CINAHL Complete, APA PsycArticle, Art & Architecture Complete, SocINDEX covering the period from January 2012 to December 2022.

The search terms used included: *military OR veterans OR soldiers OR armed forces OR service members OR army OR navy OR air force OR marine Corp OR active duty AND music therapy OR music intervention OR musical therapy OR music-based intervention OR therapeutic music OR music OR singing AND PTSD OR post-traumatic stress disorder OR posttraumatic stress disorder OR post-*

traumatic symptoms OR mental illness.

The inclusion criteria included qualitative papers published in English on the lived experiences of the therapeutic use of music among people in the military. The search strategy yielded 180 papers, duplicates were excluded and further screening by title, abstract, and full text was conducted. Finally, seven papers were judged to be addressing the research question and they scored highly in the quality assessment process using Critical Appraisal Skills Programme (CASP) tools.[12]

A data extraction table was developed to capture details such as the study aims, design, participants, type of musical intervention, and key findings from the included papers. Thematic analysis was then used to analyze the collated data to identify codes. The codes were then synthesized based on similarities into descriptive sub-themes. Further synthesis of the sub-themes yielded three main themes.

Results

All seven studies were appropriate qualitative methodology studies providing evidence of the perceptions and lived experiences of the participants who were involved. Figure 1 (adapted from Page *et al*[10]) shows the PRISMA diagram and how the retrieved papers were systematically screened. The study aimed to focus on the thoughts and perspectives of military veterans, therefore, a qualitative approach was the best method for this study.[2] Due to scarcity of literature, all included studies were from Western countries with most being from the USA, one from Denmark, one from Canada, and one a collaboration between participants from USA and Uganda. The included studies had more male than female service members or veterans as study participants.

The studies focused not only on musical performance but also included song writing and listening. The approach of having songs created by military service members was reported and this demonstrates meaningful occupation beyond just singing.[3,7, 9,13,14] Themes that came from the data synthesis were emotional regulation, engaging with others, and mental health stigma. Table 1 presents the summarised data extraction table showing the key findings that informed the themes, followed by a description of each theme.

Emotional regulation

Findings reveal that therapeutic use of music provided veteran service users the ability to understand and regulate their emotions. This was associated with reducing destructive behaviours and negative emotions.[2,5,7,9] An understanding of emotions enabled clients to describe feelings in a better way, control their choices and express the struggles that they have felt or are feeling.[2,5,7] This regulation is especially important when breaking down fronts or "warrior" culture and being true to oneself.[15] Songs facilitated the expression of the inner struggles and hope for the future among veterans which enabled the improvement of their emotional well-being.[3] In addition, other participants found music to have a calming effect
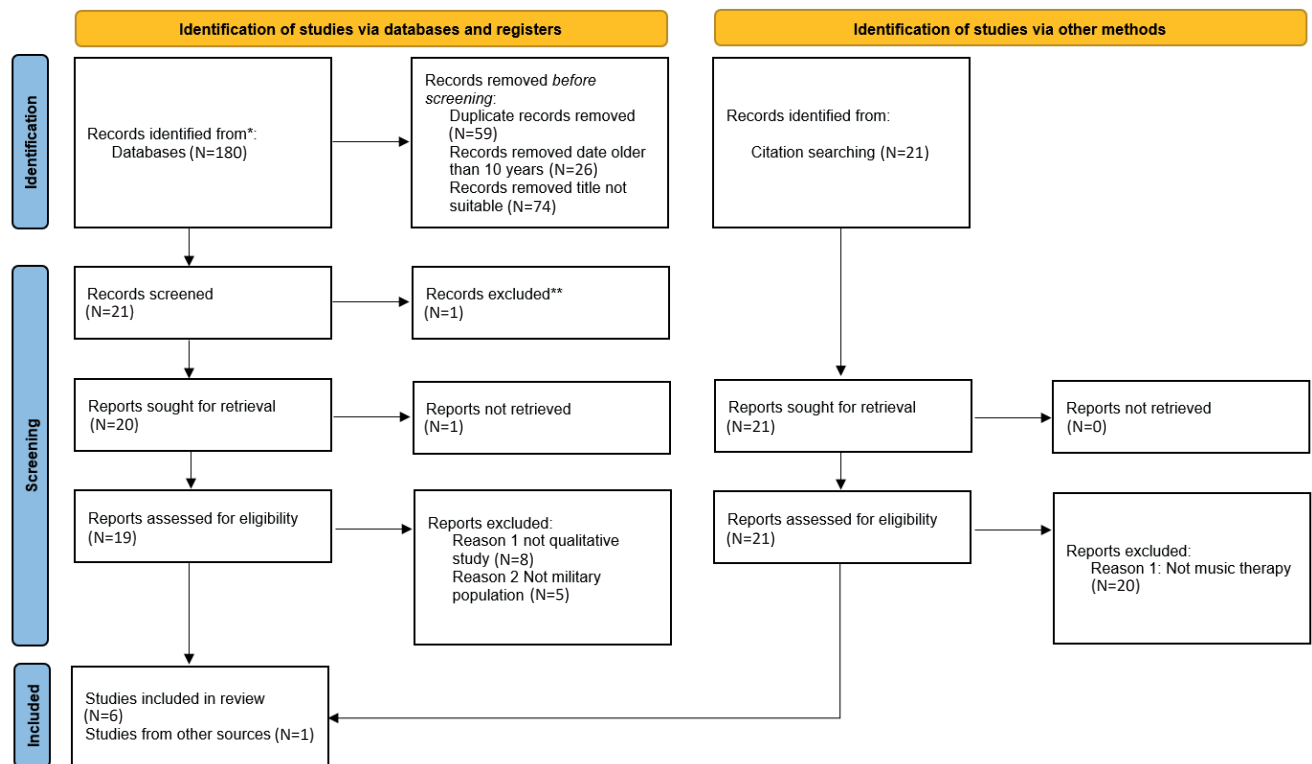
Figure 1 *PRISMA diagram.*

Table 1 Data extraction table.

Study	Aims	Design	Participants	Type of intervention	Key Findings
Story, and Beck, 2017 (Denmark)	Female veterans' experiences with guided music and imagery	Qualitative design-semi structured interviews and focus group	5 Female veterans	Guided imagery and music therapy	• Increased self-awareness through recognizing feelings and reactions to music was a learning process. • Understanding music choices was in their control. • Lack of reporting of mental health issues due to fear and shame • Music helpful to regulate feelings. • Music as a means to connect with others. • Addressing PTSD symptoms and expression of repressed feelings • Therapeutic guiding was considered to be empowering
Lauber, D. E., Estes, S., & Sherr, M. 2022 (USA)	To explore the experience of a song writing program for military veterans with posttraumatic stress disorder (PTSD).	Qualitative design- semi structured interview	6 Veterans (5 male, 1 female)	Song writing programme	• Catalyst to invoke change and expression. • Comfortable atmosphere to talk about things otherwise repressed. • Peer conversation and engagement helped them make sense of the experience more so than in clinical settings. • Involvement of empathic and compassionate professional song writers helped participants to confidently tell their story. • Enabled participants to share the stories with their families and others. • A sense of belonging and emotional catharsis through listening to each other's songs. • Motivator for quitting smoking and mood stabilizing medication.

Study	Aims	Design	Participants	Type of intervention	Key Findings
Vaudreuil *et al*. 2019 (USA and Uganda)	To explore the therapeutic use of music via telehealth	Qualitative evaluation of 3 case studies	Case examples of 3 projects conducted with veterans	Example 1: MUSIC health pilot project for sustained social engagement through music Example 2: Individual and group therapy programme to ensure continuity of care and morale building Example 3: Integration of music therapy into telehealth	• Answers a need to deliver services remotely to patients who have been discharged or live remotely. • Music Therapy promotes creative processes that break down the warrior culture myth and assist with disclosure and sustained social engagement. • Decreased perception of symptoms - pain, depression, and anxiety • Music therapy telehealth, the patients conveyed positive responses to clinical services, endorsed that telehealth was not a deterrent to continued engagement in music therapy and convenient as it allows them to integrate therapy into their daily routines. • Patients requested continued music therapy via telehealth and reported that they would recommend music therapy via telehealth to their peers.
Lightstone *et al*. 2015 (Canada)	To review and report the experience of a military veteran with PTSD's receiving remote music therapy intervention.	Qualitative retrospective case study	1 Male military veteran	Remote music therapy	• Gained greater insight into emotional states. • Expanded limited emotional vocabulary Identified that therapies and treatment team were his only source of social and emotional support. • Was able to use the music interventions to express rage.
Vetro-Kalseth *et al*. 2021 (USA)	Experiences of active-duty service members in a phased music treatment programme during transitioning to veteran status	Qualitative case study report	3 Male service members transitioning to veteran status	Phased group music therapy	• Phased group Music Therapy can build meaningful relationships and peer support. • Finding self-acceptance, coping, and finding happiness again. • Performance increasing community understanding of veterans. • Symptom management, social connections, military transition, and emotional support improved from music therapy.
Bradt *et al*. 2019 (USA)	Thematic analysis of songs written during music therapy	Qualitative retrospective analysis	11 Active-duty service members with PTSD. (10 male, 1 female)	Individual music therapy sessions	• Songs facilitate expression of struggles with injuries and invisible wounds of war. • Lyrics reflect resilience and love for family and friends. • Songs include motivating messages aimed at providing hope for other service members. • Lowering threshold for seeking mental health services in military. • Improves emotional wellness. • Mental health stigma and difficulties with interpersonal relationships.
Liebowitz *et al*. 2015 (USA)	Exploring the sense of engagement experienced by veterans participating in group music therapy	Qualitative semi-structured interviews	6 Veterans (4 male, 2 female)	Group music therapy	• Opportunities to connect with others through shared interests contributing to a sense of engagement. • Feeling anxious at first, being resilient and then enjoying participation. • Calming or mood elevating effect. • Diversion from pressing concerns.

on their mood; allowing diversion from their stressors and increasing their emotional vocabulary.[7,13] The ethical challenge in the use of music to regulate emotions is for professionals to know the implications of using music to enable veterans to express rage and not pose a risk of harm.[7]

Engaging with others

Veterans and military populations often experience difficulties with feelings of loneliness, isolation, loss of identity, and being misunderstood.[2] The use of music in therapy showed an ability to enable the patients to improve the way they communicated and participated. Participating in musical therapy with loved ones was found to be beneficial in the reviewed papers.[9] Music enabled participants to show love for their loved ones and improved understanding with each other thereby strengthening the relationships. In group music therapy clients built meaningful relationships and peer support and forged connections through increased recognition associated with performances and shared interests.[9,12] Through music performance and creation participants were able to increase the community's understanding of veterans and veterans utilized their voices to motivate other service members to participate in music as a meaningful occupation.[2,9]

Mental health and stigma

Lack of support puts military populations at high risk of suicide, the warrior culture within the military is a significant factor found to be preventing service members from asking for support.[2,16] Furthermore, findings indicate that the military population finds it difficult to seek support.[4] This was further backed up by woman members stating that they do not want to speak out due to fear, stigma, and shame[5]. Reviewed papers show that song writing gave a strong way for veterans to describe those feelings in a psychotherapeutic way.[3,7, 9,13] This gave an alternative route to recovery, rather than traditional mental health services that are often stigmatized.[5] Participants reported experiencing a decrease in symptoms of pain, depression, and anxiety following engagement in music sessions.[15] However, the stigma associated with mental health hindered interpersonal relationships and the process of finding happiness again.[3,9]

Discussion and conclusion

Occupational therapists, through their clinical reasoning, and understanding of the ethical of challenges can support mental health service users through recovery using expert knowledge of occupational identity, engagement, participation, and understanding of the individual's life experiences and how these can impact behaviour.[8,14] In a case report the participant made significant therapeutic progress with music therapy compared to the previous eight years of standard treatment. Combining occupational therapy and music therapy could maximize the benefits of music to people with mental health conditions.[16] All the papers included in this review did not discuss the role of occupational therapy in supporting mental health in the military. However, the participants' involvement in music as a meaningful occupation indicates the potential and value that occupational therapy can bring to this service user group.

PTSD survivors tend to look to the future or be stuck in the past inducing a sense of loss of control which causes further distress and panic. Therapeutic use of music has a way of grounding individuals in the present moment creating either calm or catharsis to release negative emotions.[4,14] However, the therapist needs to have the ethical skills to be able to understand that in certain situations re-traumatization can occur through the use of music. Having the appropriate skill set to navigate through these challenges is essential.[15,17] Song writing has been proven to develop a sense of mastery through improving confidence, self-esteem, and sense of self. Being able to tell their story in a written way is more beneficial for some individuals depending on their personality and the trauma experienced.[2,14,18] Giving service members more opportunities to express their needs and feelings can support some of the common barriers to recovery. The therapeutic use of music can encourage military populations to move forward, find value, remain productive, and achieve goals. For example, through music, some participants with a history of drug and alcohol use reported discussing substance abuse less and looked forward to their music treatment sessions and healthy relationships.[9,12]

Further research is required to gain a deeper insight into the perspectives of occupational therapists delivering music therapy to a military population and how this can support those with PTSD. The authors acknowledge the limitations that a few studies were available for inclusion in this review and that the sample sizes used in the studies were small and mainly focused on men. All the studies included in this paper focused on Western countries except one which limits the transferability of findings. Nevertheless, the therapeutic use of music within military populations appears beneficial and worth continuing with however, careful monitoring during treatments should be a priority to prevent outbursts, relapse, or aggressive behaviour.

Conflict of interest

The authors declare no conflict of interest and the review did not require ethical clearance as it was a review of published literature.

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Evaluation of conventional methods for species identification of *Staphylococcus aureus* using MALDI-TOF MS, protein identification and quantification

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ARTICLE INFO

ABSTRACT

Objectives: This study aimed to evaluate conventional methods for species identification of Staphylococcus aureus by MALDI-TOF MS. Additionally, the representatives of different species were used to analyze protein expression.

Materials and methods: A total of 185 non-duplicated clinical *S. aureus* identified using the conventional method (colony morphology, Gram's stain, slide coagulase test, tube coagulase test, catalase test, and mannitol fermentation) was confirmed the identification by MALDI-TOF MS and analyzed by Mass spectral profiling (MSP) and Principal component analysis (PCA). The representatives of different species reported by both methods were confirmed using *16SrRNA* sequence and analyzed proteins expression by timsTOF MS.

Results: All *S. aureus* suspected isolates could discriminate among species by MALDI-TOF MS including *S. aureus* (N=151, 81.6%), *S. argenteus* (N=32, 17.3%), *S. hominis* (N=1, 0.5%), and S. *haemolyticus* (N=1, 0.5%). Using *16S rRNA* gene-based analysis, *S. aureus* and *S. argenteus* could not differentiate from each other. Protein expression of *S. aureus* was similar to *S. argenteus.* These genes including *rpsT, Huti, pyrF, atpD.1, cpfC, SAUA300_0786, atl.1,* and *MW2416* showed higher expression in *S. aureus* (MS076) than *S. argenteus* (MS060), *S. haemolyticus* (MS095) and *S. hominis* (MS060).

Conclusion: MALDI-TOF MS provides an excellent tool for accurately species identification of staphylococci. *S. aureus* expressed protein analyzed higher than the other 3 species. The highest protein expression in *S. aureus* implies the most virulence of this strain.

Introduction

Staphylococci including *Staphylococcus aureus* constitute the microbiota of humans and animals but are the critical cause of hospital-acquired infection, particularly methicillin-resistant *S. aureus* (MRSA) in intensive care units.[1] The high pathogenicity is associated with multidrug resistance and their adaptation to a variety of environmental conditions.[2] *S. aureus*, one of the most important clinical pathogens, causes various diseases such as skin and soft tissue, bacteremia, pneumonia, endocarditis, or osteomyelitis which conducts an increasing number of morbidity and mortality in the world.[3] The conventional culture method had a limitation to differentiate some species of Staphylococci. For the species identification of staphylococci, slide coagulase,

tube coagulase, and mannitol salt agar have been used for presumptive isolation among *S. aureus* and other staphylococci, however, these biochemical tests are not sufficient to distinguish between *S. aureus* and other staphylococci.[4]

Molecular analysis for the *Staphylococcus* identification into species level has been performed manually with electrophoresis, taking hours to obtain the result and resulting in a heavy workload for technicians. Several methods have been developed to be a rapid and effective tool to profile bacteria at the genus and species level with high reproducibility such as more recently the matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS).[5] MALDI-TOF MS with library-based and/or bioinformatics-enabled methods has been successfully applied in many areas. For example, in clinical microbiology, MALDI-TOF MS has been used to identify pathogens, which can be rapidly reported by comparing spectra to a database and this technique has been used to accurately identify various microorganisms including *S. aureus*.[6] Kim *et al.* identified 100% *of S. aureus* 89.5% of *Staphylococcus* spp. other than *S. aureus* isolated from food samples by MALDI-TOF MS. PCA and MSP-based dendrogram of each species of *Staphylococcus* isolates were clearly clustered.[7] This study aimed to evaluate conventional methods for species identification of *Staphylococcus aureus* by MALDI-TOF MS. Additionally, the representatives of different species were used to analyze protein expression.

Materials and methods
Study design, Sample Collection, and Ethical Approval
The study was designed as a cross-sectional study; 185 non-duplicated clinical samples *S. aureus* were collected from Prapokklao Hospital, a tertiary healthcare setting, during the period 2018-2020. The bacterial isolates were frozen at -80 °C until use. This study was approved by the Ethics Committee of Prapokklao Hospital, Chanthaburi (Approval Number: CTIREC 048).

Bacterial Isolation and Identification
A total of 185 non-duplicated clinical *S. aureus* suspected isolates were recovered and identified using conventional biochemical tests. The isolates underwent colony morphology, Gram staining, catalase test, coagulase test, and mannitol salt fermentation.

MALDI-TOF MS identification
The bacterial colony was extracted using the formic acid extraction method. Briefly, the bacterial isolates were grown on Mueller-Hinton agar (Becton, Dickinson and Company, USA) plates overnight at 35±2 °C. Fresh bacterial cells 4-5 colonies were transferred into 300 µL of deionized water, stir and mix with 900 µL of ethanol thoroughly until the material was completely in suspension. Sediment was obtained after centrifugation 13,000xg for 2 min and repeated this step until the supernatant was completely removed. Air-dry the pellet for at least 15 min at room temperature. Added 50 µL 70% aqueous formic acid and

50 µL 100% acetonitrile and mix with a vortex mixer for 1 min. Centrifuged the mixture 13,000xg for 2 min. One µL supernatant was directly spotted on a MALDI polish steel target plate (Bruker Daltonics). The preparation was overlaid with 1 µL of 4-hydroxy-α-cyanocinnamic acid (HCCA) in Standard solvent (2.5% trifluoroacetic acid/50% acetonitrile). The colony was then air-dried at room temperature to allow co-crystallization with the experimental sample. MALDI-TOF MS was performed on an autoflex maX™ TOF/TOF mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany) equipped with smartbeam-II laser with FlexControl™ software 3.4 (Bruker Daltonics) for automatic acquisition of mass spectra in the linear positive mode within a range of 2 to 20 kD. Each spectrum was acquired with 2,000 laser impulses at a frequency of 200 Hz. The sample was triplicate collecting the spectra. The mass spectrometer was periodically calibrated using a Bacterial Test Standard (BTS) (*Escherichia coli* ATCC 25922). All isolates were confirmed species by comparing to the MBT compass explorer, library version 4.1 (9,999 entries; Bruker Daltonics) at the species level with a score value of >2.0. For this library version could identify 45 species of staphylococci *eg*. *S. aureus, S. argenteus, S. schweitzeri, S. hominis, S. haemolyticus*, *etc*.

Analysis of mass spectrometry data
BioTyper required summarization of mass spectra of biological and/or technical replicates of bacteria. The resulting composite mass spectra are called main spectral profiles (MSPs) Dendrogram. With regard to the strain-level characterization, BioTyper has been suggested to be useful in characterizing bacteria at the strain level, but typically with the assistance of additional software, such as Clin-ProTools™.[5] Analysis of the entire spectrum with rigorous analytical tools is often required to obtain reliable strain identification. The ClinProTools™ provides a Principal component analysis (PCA) that also can differentiate between closely related samples, recalibrate to generate the necessary alignment, normalize each peak, and then can contribute equally to model generation.[8] PCA is a widely used mathematical technique designed to extract, display, and rank the variance within a data set.[9]

16S rRNA gene sequence
Representative different species were investigated using *16SrRNA* gene sequence. Briefly, the bacterial isolates were grown on Mueller-Hinton agar (Becton, Dickinson and Company, USA) plates overnight at 35±2 °C. The single bacterial 3-5 colonies were re-suspended in 1x TAE buffer (sterile)200 µL and heated in the chamber at 95 °C for 15 min. After centrifugation at 12,000xg for 5 min, the supernatant was used as a DNA template for PCR. The total PCR reaction volume was 20 µL containing 10x PCR buffer minus $MgCl_2$, 2 µL of DNA template, 0.2 U of Taq DNA polymerase, 0.05 µM of each primer (forward primer: 5'AGAGTTTGATCCTGGCTCAG 3' and reverse primer: 5'GC-GTGGACTACCAGGATATC 3'), 200 µM of each dNTP per and 2 mM of $MgCl_2$.[10] Afterwards, amplification was performed in a PCR Thermo Cycler (BioRad) under the following

conditions: 94 °C for 5 min, followed by 30-cycles using parameters: denaturing at 94 °C for 30 sec, annealing at 53 °C for 30 sec, and extension at 72 °C for 30 sec, followed by a final extension at 72 °C for 5 min. The PCR products were 802 bp analyzed using 1% (w/v) agarose gel stained with loading dye. The PCR product was purified using QIAquick gel extraction kit (Qiagen, Germany). Fifteen µL of purified product was sent to U2Bio company, Thailand for sequencing. Thailand for sequencing. The obtained sequences were analyzed using nucleotide BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) and aligned to the reference sequences deposited in the GenBank (v. 253.0 ; Jan 12, 2023)

Protein identification and quantification

Representative different species were identified proteins by timsTOF MS. Bacterial pellets were first resuspended in a lysis buffer that contained 2 M thiourea, 7M urea, 4% CHAPS, and 1% protease inhibitors cocktail. The bacterial cells were disrupted using a sonicator with an amplitude of 60 and 0.5 cycles, while keeping the samples on ice. The resulting lysate was then centrifuged at 14,000 rpm for 30 minutes at 4 °C. This centrifugation step would have caused the lysate to separate into different components based on their density. The protein concentration of the resulting pellet was measured using Bradford's method. The protein samples were then collected and stored at -80 °C for later use in downstream applications.[11]

The first step involved conducting acetone precipitation to transition from the lysis buffer to 8 M urea. To prepare each sample, 100 µg of protein was reduced with 100 mM dithiothreitol in 100 mM TEAB at room temperature for 30 min. Next, an alkylating buffer containing 100 mM iodoacetamide in 100 mM TEAB was added, and the sample was incubated in the dark at room temperature for 30 min. After quenching with reduction buffer for 15 min, the sample was treated with ice-cold acetone and incubated overnight at -20 °C. The pellet was collected by centrifugation, resuspended in 8 M urea in 100 mM TEAB, and digested using Trypsin, Gold (mass spectrometry grade; Promega, USA) for 16 hrs at 37 °C. The resulting sample was dried using a CentriVap DNA Concentrator (Labconco Co., Kansas City, Missouri, USA), cleaned up with a C18 Zip tip, and then dried again in the CentriVap before storage at -80 °C until further processing. Finally, the peptide concentration was measured using NanoDrop 1000 (Thermo Fisher Scientific, Bremen, Germany) after resuspending the sample in 0.1% formic acid.[12]

A nanoElute nano-flow chromatography system was coupled online to a hybrid trapped ion mobility spectrometry - quadrupole time of flight mass spectrometer (timsTOF Pro flex, Bruker Daltonics, Bremen, Germany) with a modified nano-electrospray ion source (CaptiveSpray, Bruker Daltonics). Liquid chromatography was performed at 50 °C and with a constant flow of 400 nL/min on a reversed-phase column (15 cm × 75 µm i.d.) packed with 1.9 µm C18-coated porous silica beads. Mobile phases A and B were 98/2/0.1 water/ACN/formic acid (v/v/v) and 99.9/0.1% ACN/formic acid (v/v/v), respectively. In 42-min experiments, peptides were separated with a linear gradient from 2 to 32% B within 30 min followed by a washing step at 95% B for 12 min, and re-equilibration.

In the timsTOF Pro Flex mass spectrometer ions are generated in a captive spray source and 20 µm tapered emitter, transferred into the vacuum system through a glass capillary, and then deflected by 90° into the TIMS device where ions are accumulated and released from the device based on their size-to-charge ratio. The quadrupole switches mass position extremely quickly in sync with the elution time of the precursor ion packages from the TIMS device, isolating the precursors for subsequent fragmentation in the collision cell. This PASEF method significantly increases the sequencing speed up to >120 Hz and therefore is ideally suited to short gradient measurements.[13,14] Data were collected over an m/z range of 100 to 1,700 for MS and MS/MS on the timsTOF Pro instrument using an accumulation time and ramp time of 100 msec. The data acquisition cycle times of 1.1 s were used.

Data processing and analysis of Protein identification and quantification

The protein sequence database was the combination of unreviewed *Staphylococcus* genus proteins (accessed 01/31/2023 from UniProt; 13,591 entries), with decoy sequences and contaminants added. IonQuant (version 1.8.10) was used to perform quantitative analysis. MS-Fragger (version 3.7) coupled with FragPipe (version 19.1) and Philosopher (4.6.0) was used to perform a closed search.[14,15] FragPipe-Analyst (http://fragpipe-analyst.nesvilab.org) was employed for data visualization.

Results
The Identification of S. aureus suspected isolates

Using MALDI-TOF MS, 185 isolates were correctly identified as *S. aureus* (N=151, 81.6%), *S. argenteus* (N=32, 17.3%), *S. hominis* (N=1, 0.5%), and S. haemolyticus (N=1, 0.5%) at the species level with a score>2.0. With ≥99% average nucleotide identity, *16S rRNA* gene sequence could classify *S. haemolyticus* and *S. hominis* from *S. aureus* whereas *S. argenteus* isolates were misidentified as *S. aureus* (data not shown).

PCA and MSPs Dendrogram analysis

The Biotyper program (version 4.1) was used to create the dendrogram based on the main spectrum profiles (MSPs). The dendrogram of 185 *Staphylococcal* isolates showed that *S. aureus, S. argenteus, S. haemolyticus,* and *S. hominis* were completely separated from each other (Figure 1). Using ClinPro Tool program, the distribution of the isolates was plotted according to the peak intensity of m/z 6905 (x-axis) and *m/z* 6889 (y- axis) (Figure 2). The peaks at m/z 6905 and m/z 6889 were the top two characteristic peaks among staphylococcal isolates. The peak at m/z 6905 was found in *S. argenteus* S060 whereas *m/z* 6889-6895 was *S. aureus* S076 and *S. haemolyticus* S096. Both of these peaks were not found in *S. hominis* S080. In addition, *m/z* 2305 in *S. aureus* and *S. argenteus* could be used to separate from *S. haemolyticus* and *S. hominis* (Figure 3).
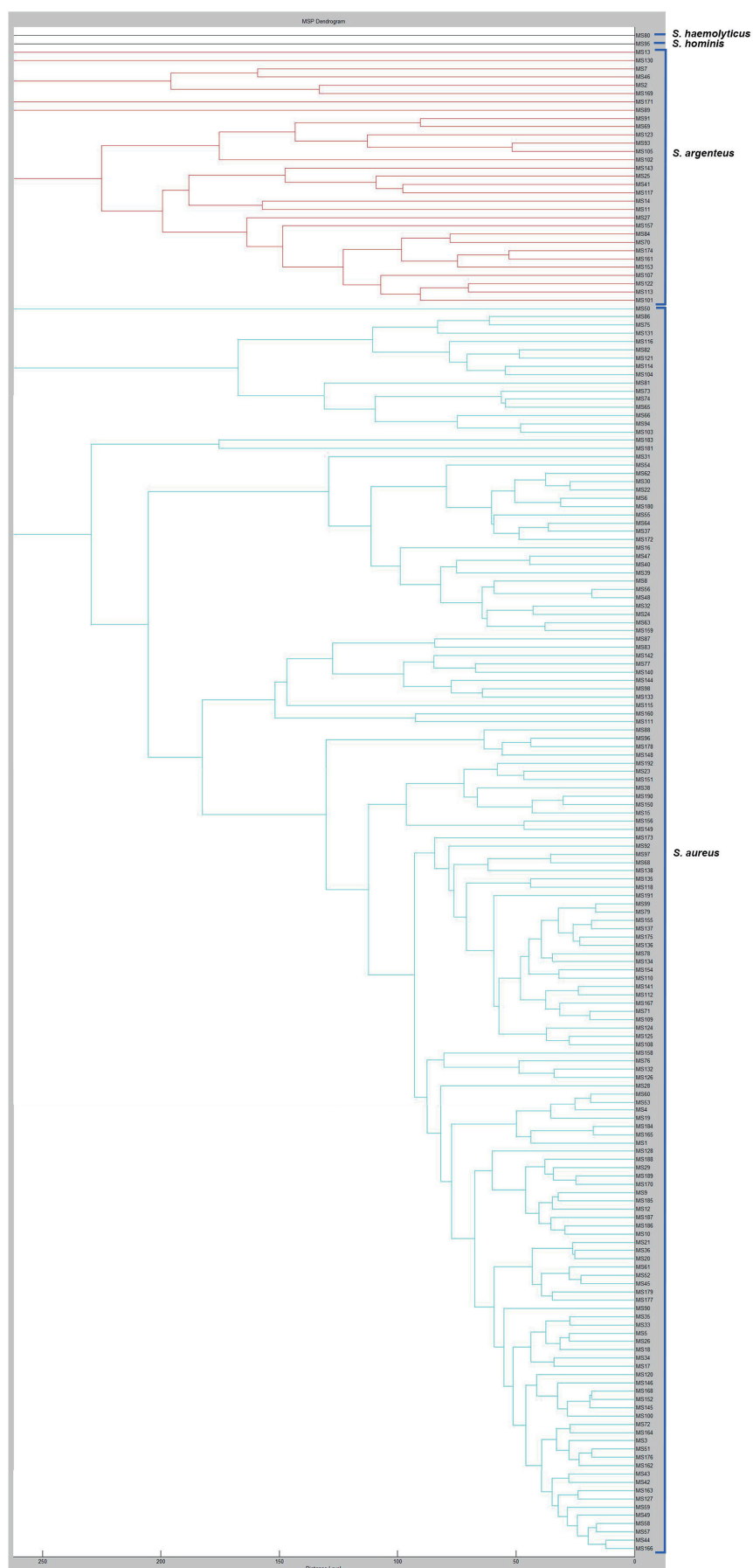
Figure 1 MALDI-TOF MS-based main spectrum profile (MSP) dendrogram showing the relatedness of 185 staphylococcal isolates. Top: *S. haemolyticus*) followed by *S. hominis*, Red lines: *S. argenteus isolates*, Blue lines: *S. aureus isolates*.
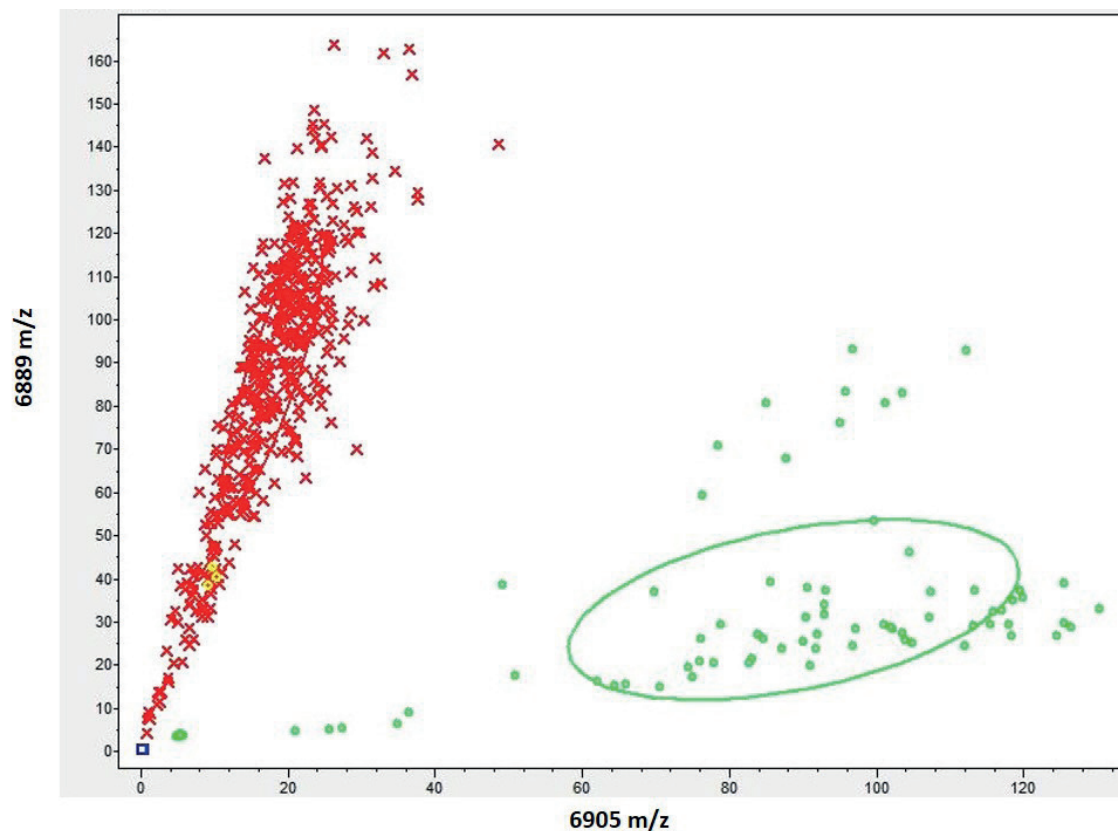
Figure 2 Scatter plot of various Staphylococcal isolates. The peaks at m/z 6905 and m/z 6889 served as the X- and Y- axis, respectively. The intensities of the characteristic peaks were expressed in arbitrary intensity units. The ellipses represent the 95% confidence intervals of peak intensities for each isolate. In the preliminary analysis, *S. hominis* (blue squares) could not be separated based only on these two characteristic peaks. The peak at or near *m/z* 6889 was expressed in *S. aureus* (red crosses) and *S. haemolyticus* (yellow diamonds) whereas the peak at or near *m/z* 6905 were expressed in *S. argenteus* (green diamond).
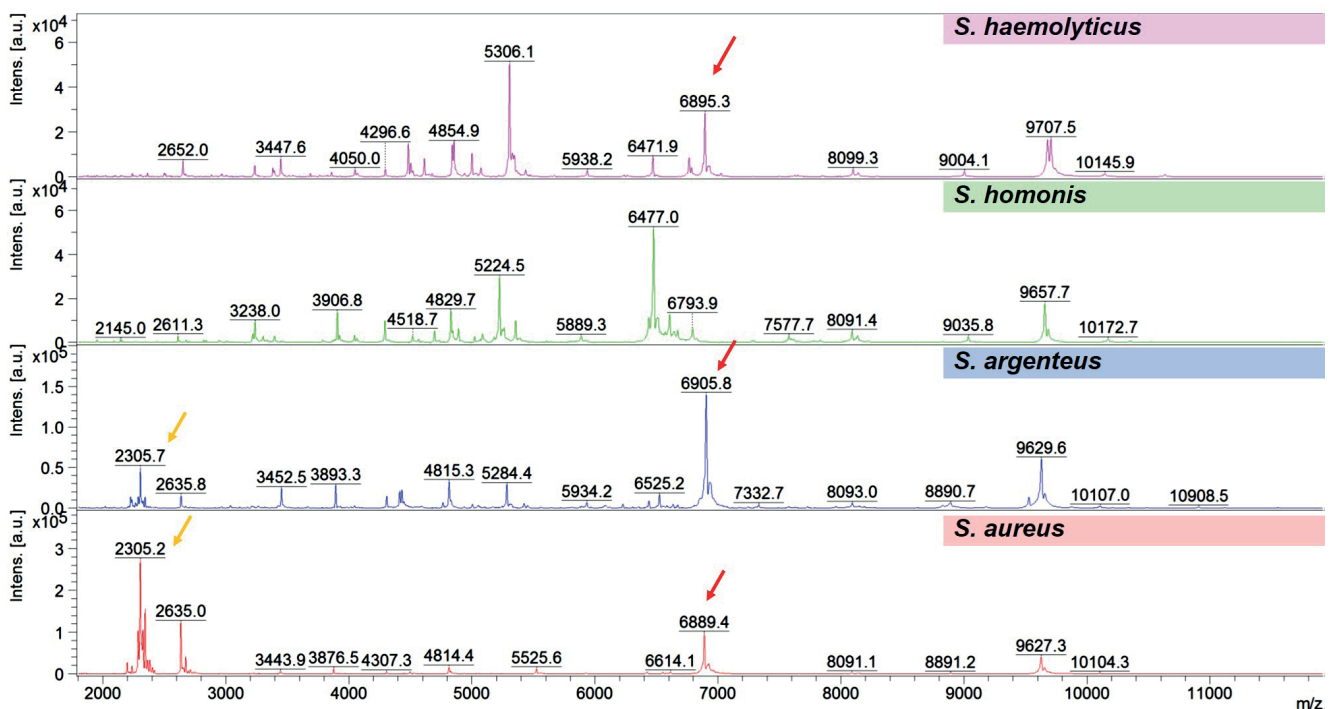
Figure 3 Comparison of MALDI-TOF MS representative spectra of 4 species of staphylococci. *m/z* 6889 -6895 (red arrow) were presented in *S. aureus* and *S. haemolyticus*. m/z 6905 were presented in *S. argenteus.* m/z 2305 (yellow arrow) was presented *S. aureus* and *S. argenteus*.

Principal component analysis (PCA) was conducted on the selected features to illustrate the distribution of various staphylococcal isolates. Each spot referred to a protein spectrum and the different colors illustrate the considered cluster involvement in which each spot is considered as one measured protein spectrum profile. The results of the PCA cluster views of the staphylococcal protein mass spectrum peaks showed that most of the peaks completely separated the species of staphylococci from each other (Figure 4). In the 3D scatter profile, each species group was located to each other in the same species (Figure 4).

The comparison of protein expression among Staphylococcus argenteus **MS06·***, Staphylococcus aureus* **MS076,** *Staphylococcus haemolyticus* **MS09ヿ** *and Staphylococcus hominis* **MS080**

Staphylococcus aureus MS076 has fewer proteins than the other 3 species (Figure 5). In the univariate analysis (fold-change >2, *p*<0.01), the volcano plot identified eight genes including *rpsT, Huti, pyrF, atpD.1, cpfC, SAUA300_0786, atl.1,* MW2416 to be of higher expression in *S. aureus* MS076 than *S. argenteus* MS060, *S. haemolyticus* MS096, and *S. hominis* MS080 (Figure 6). The corresponding proteins of *rpsT, Huti, pyrF, atpD.1, cpfC, SAUA300_0786, atl.1, MW2416* genes were 30S ribosomal protein S20, imidazolonepropionase, orotidine 5'-phosphate decarboxylase, ATP synthase subunit beta, coproporphyrin iii ferrochelatase, organic hydroperoxide resistance protein-like, Bifunctional autolysin, and putative surface protein MW2416, respectively.

Discussion

S. aureus is a major cause of hospital and community-acquired infection that can result in worse clinical outcomes, although it is possible to increase morbidity and mortality due to MRSA infections. In this investigation, the clinical specimens were collected and processed based on the standard microbiological procedure. It takes a long time consuming, labored intensity, and defective reproduction to identify *S. aureus* from a cultured colony using conventional and PCR methods, whereas MALDI-TOF MS enables a few minutes of identification from a colony.

For the conventional method, the isolates underwent colony formation and its morphological analysis, gram staining, catalase test, coagulase tube test, mannitol salt fermentation. The PCR assay was performed using a single set of primers for the amplification of *16S rRNA* gene. When comparing the conventional method with MALDI-TOF MS (species level identification) and PCR for *16S rRNA* gene, the results showed 34 of 185 strains (18.4%) were mistakenly identified as *S. haemolyticus, S. hominis* and *S. argenteus.* Previous studies report misidentified *S. haemolyticus* and *S. hominis* as *S. aureus* resulted from an intermediate result in the test for the utilization of D-mannose [16]. Being closely related species, *S. aureus* and *S. argenteus* resembled each other as they were reported previously in northeast Thailand between 2010 and 2013, 19% were infected with *S. argenteus* and 81% with *S. aureus* [17] because *S. argenteus* shares a pathogenicity island, bacteriophages, virulence genes, and resistance genes with *S. aureus* [18]. The nearly
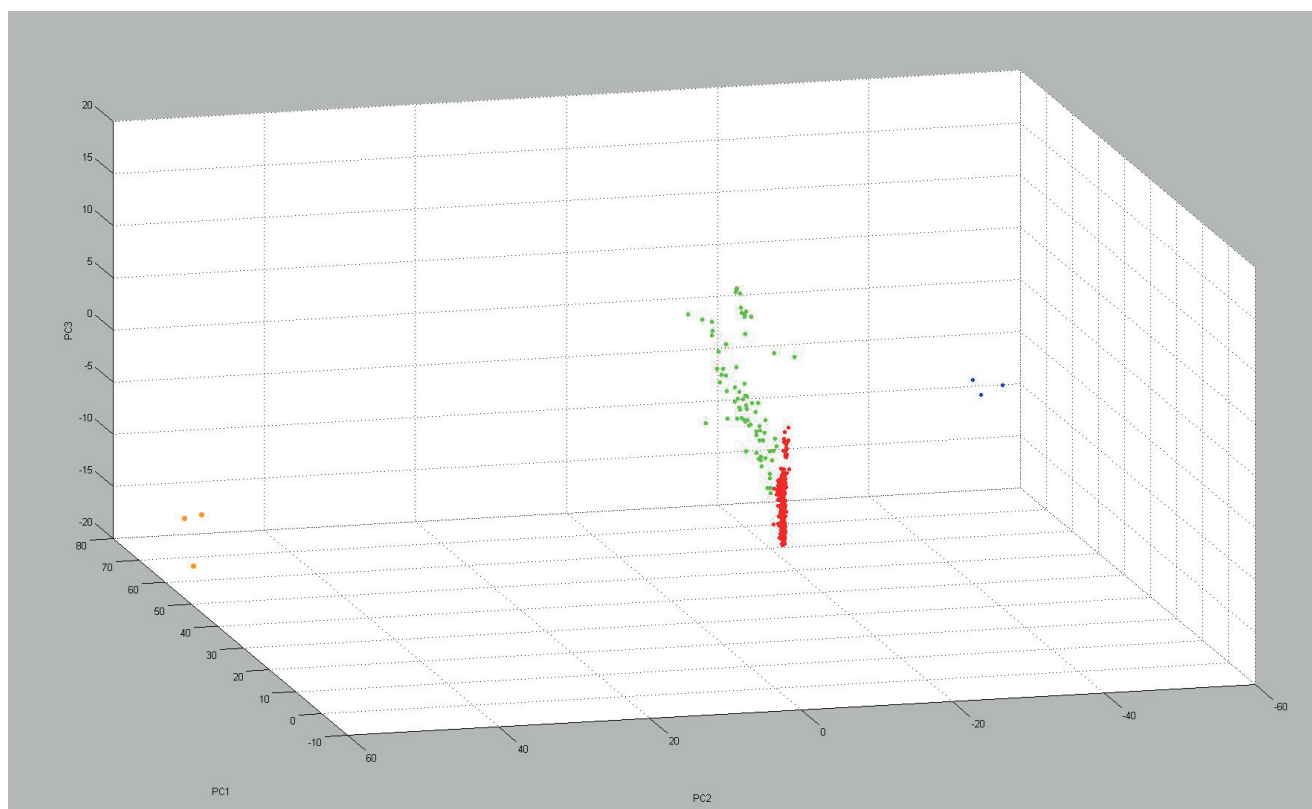
Figure 4 3D scatter profile of 185 isolates of Staphylococci. The spot represents one spectrum, and the plots were generated by PCA. *S. aureus*: red spots, *S. argenteus*: green spots, *S. haemolyticus*: blue spots, and *S. hominis*: yellow spots.
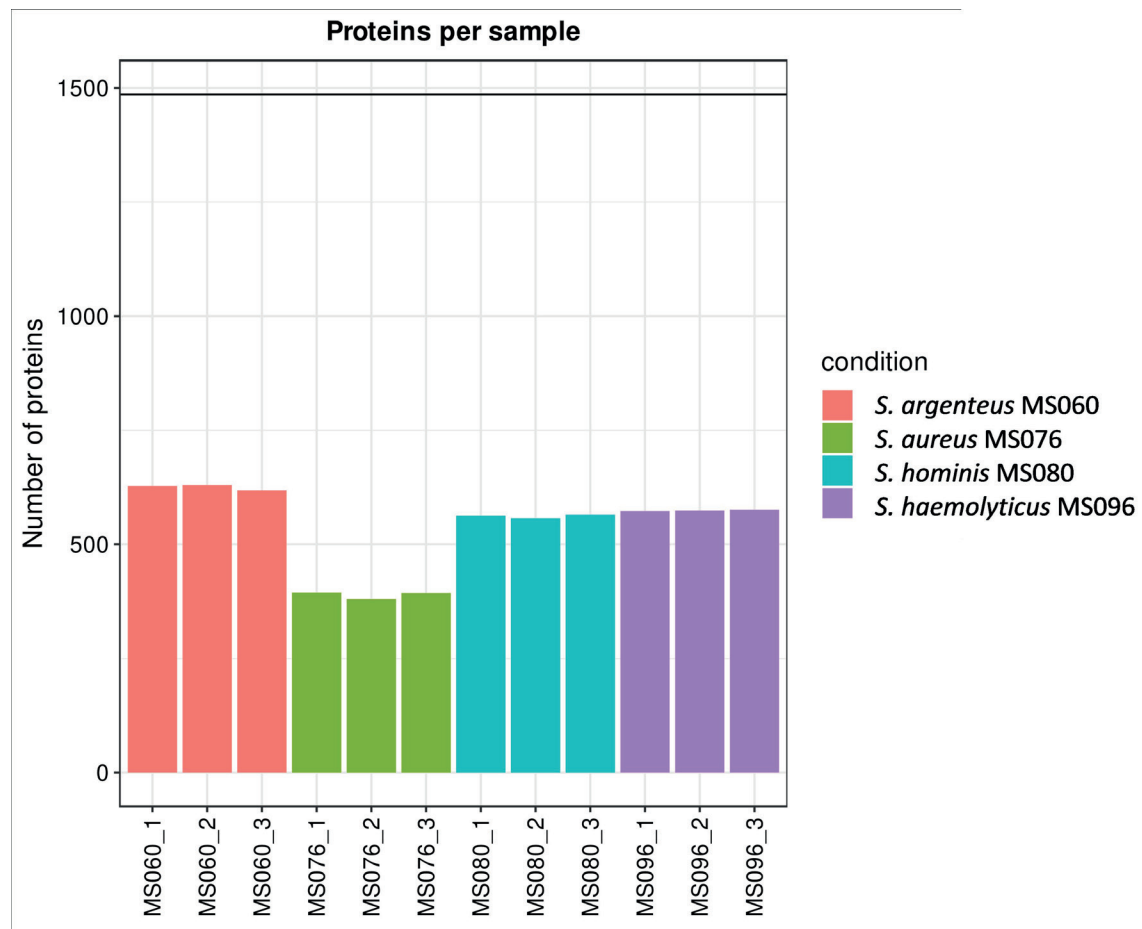
Figure 5 Protein Groups for each group after pre-processing.
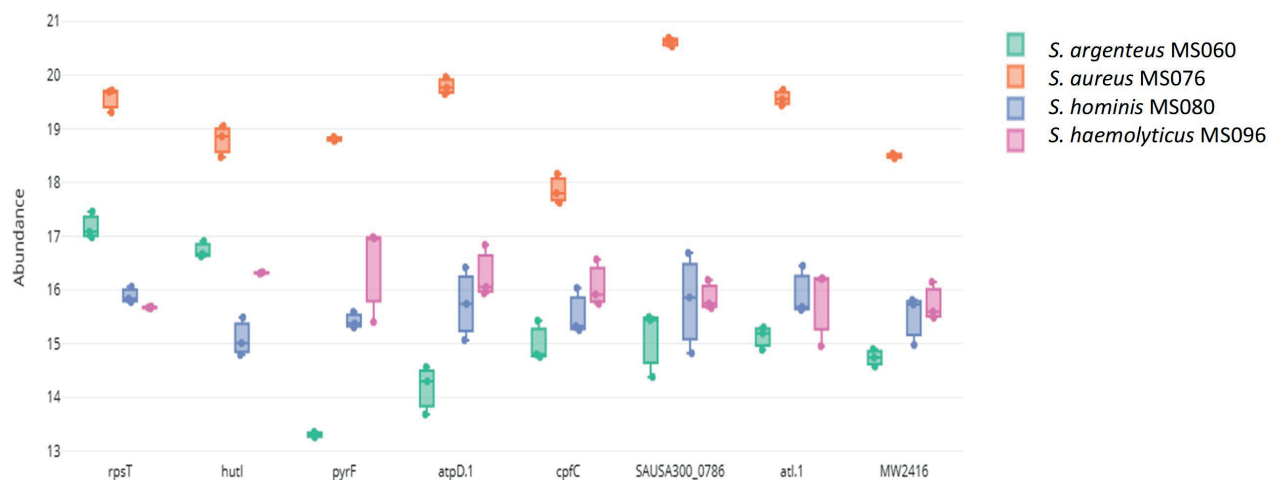
Figure 6 **Box plot for protein expression**. The vertical axis indicates abundance on the $\log_2$ scale. The horizontal axis indicates protein expressed group. Numbers in the plot indicate the MS experiment in which the protein was detected. Orange box: *S. aureus* MS076, Green box: *S. argenteus* MS060, Pink box: *S. haemolyticus* MS096, Blue box: *S. hominis* MS080, *rpsT*: 30S ribosomal protein S20, *hutI*: imidazolonepropionase, *pyrF*: orotidine 5'-phosphate decarboxylase, *atpD.1*: ATP synthase subunit beta, *cpfC*: coproporphyrin iii ferrochelatase, *SAUSA300_0786*: organic hydroperoxide resistance protein-like, *atl.1*: Bifunctional autolysin, *MW2416*: putative surface protein MW2416.

identical *16S rRNA* gene sequences could not classify *S. argenteus* from *S. aureus* whereas the protein mass spectral profile on a database of MALDI-TOF MS could successfully differentiate *S. argenteus* from *S. aureus*. Kosecka-Strojek reported that sanger sequencing of the *16S rRNA* gene could identify 27 (54%) species level of Staphylococci.[19] The strains of *S. aureus* and *S. argenteus* indistinguishable by sanger sequencing of *16S rRNA* gene could be identified with sanger sequencing of *rpoB*, *sodA*, or *tuf* genes and NGS of the *16S-23S rRNA* gene region.[19] S. *argenteus* formerly reported as *S. aureus* clonal complex 75 could differentiate from S. aureus using MALDI-TOF MS.[20] In addition, MALDI-TOF MS technique was used to identify *Staphylococcus* species isolated from food samples at the highest performance by formic acid extraction method (83.3-89.5% accuracy) which *S. aureus, S. haemolyticus, S. hominis* were 100% accurate identification at the species level.[7] Our study observed that using MALDI-TOF MS, mass spectral peak m/z 6905 was used to differentiate *S. argenteus* from *S. aureus*, *S. haemolyticus* and *S. hominis*. When the proteins expression among *S. aureus, S. argenteus, S. haemolyticus* and *S. hominis* was analyzed, *S. aureus* was similar to *S. argenteus* containing fewer proteins group than S. *argenteus, S. haemolyticus* and *S. hominis*. PCA analysis can clearly separate each group from each other (Figure 4).   *S. aureus* expressed protein analyzed higher than the other 3 species. The 5 types of protein expressed (Figure 6) including organic hydroperoxide resistance protein-like (SAUSA300_0786), beta subunit of ATP synthase (*atpD.1*), bifunctional autolysin (*atl.1*), putative surface protein MW2416 (MW2416) and imidazolonepropionase (*huti*) are virulence factors of staphylococci which are the highest expression in *S. aureus*. In the reports of the organic hydroperoxide resistance (*ohr*) gene, it might be associated with multiple mechanisms such as the expression control of virulence genes in *Vibrio cholerae* and *Burkholderia thailandensis*, the regulation of bactericides production in *Streptomyces avermitilis*.[21-23] The *atpD* gene encodes beta subunit of the ATP synthase catalytic core which *atpA* is upstream of *atpD* and *atpG*. The *atpA* mutant (Δ*atpA*) biofilm extracts analysis decreased levels of ATP synthase subunit alpha (*atpA*), beta (*atpD*), gamma (*atpG*) and Δ*atpA* biofilm supernatant analysis reduced many virulence factors and toxin including serine protease, alpha-hemolysin and leucocidin-like proteins and biofilm growth.[24] Moreover, the major autolysin (encoded by *atl* gene) is a cell surface-associated peptidoglycan hydrolase with amidase and glucosamininidase domains involved in *Staphylococcus* pathogenesis during colonization and infection such as adherence with multiple host cellular components (fibronectin, gelatin, and heparin), biofilm formation and bacterial cell wall degradation and cell separation during cell division.[25] The putative surface protein or cell wall surface anchor family protein MW 2416 encoded with *sasG* gene was correlated with biofilm formation.[26,27] The *huti* (encoding imidazolonepropionase) and *hutU* (encoding urocanate hydralase) involved in the synthetic pathway of L-glutamate from L-histidine were found upregulate of *gltS* gene which played an important role in biofilm

formation in MRSA.[28] Two types of protein expression including coproporphyrin iii ferrochelatase (encoded by *cpfC*) and orotidine 5'-phosphate decarboxylase (encoded by *pyrF*) played important role in the survival of *S. aureus* during infection.[29,30] In addition, Ribosomal protein S20 (encoded by *rpsT* gene) regulates the translation initiation of ribosomal protein synthesis. Mutations of *rpsT* were defective in many steps of translation initiation and ribosomal assembly.[31] In this study, the infections from *S. aureus.* imply more virulence than those from *S. argenteus, S. hominis* and *S. haemolyticus.*

Based on the Eshaghi study, the recommendation for a clinical report of *S. argenteus* accurately using MALDI-TOF MS because of its potential severity of the infection and incorrect species reports resulting in incorrect breakpoint for cefoxitin susceptibility results.[32,33] MALDI-TOF MS provides an excellent tool for the accurate and rapid identification of staphylococci and can potentially supplant labor-intensive and high-complexity molecular testing, thus increasing in-house capabilities and allowing information to be provided to physicians in a timely manner.[34]

Conclusions

MALDI-TOF MS provides an excellent tool for the accurate and rapid identification of staphylococci. *S. aureus* expressed protein analyzed higher than the other 3 species. The highest protein expression in *S. aureus* implies the most virulence of this strain.

Conflicts of Interest

All of the authors declare that there are no commercial, personal, political any other potentially conflicting interests related to the submitted manuscript.

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Evaluation of a new method for metal artifact reducing in computed tomographic images

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ARTICLE INFO

ABSTRACT

Background: The common streak artifacts in computed tomographic (CT) images result from metal implants in patients. Metal artifact suppresses and obstructs diagnosis or misdiagnoses as it occurred in ten percent of the patients' tomographic images.

Objectives: To develop the method for metal artifact reduction in CT images using MATLAB software and implement it in phantoms with the metal artifact as well as in patients with the metal artifact in the head and neck region.

Materials and methods: The new method of metal artifact reduction in CT images using MATLAB software. The homogeneous polymethylmethacrylate (PMMA) phantom, the Alderson Rando phantom, and patients with a metal implant in the head and neck region were scanned by the Philips Brilliance Big Bore CT system. Commercial orthopedic metal artifact reduction (OMAR) software and a new method software were applied to the CT images of phantoms and patients. The quantitative analysis of image quality on a metal artifact of the head and neck region was evaluated in the percent noise. The qualitative analysis in clinical imaging was evaluated in scoring by two radiologists with the same experience.

Results: In the Alderson Rando phantom, the new algorithm indicated higher efficiency in metal artifact reduction than OMAR software. In contrast, for the patient at head and neck CT images with metal artifact reduction, OMAR, and the new method showed comparable results. The new method suppressed the artifact in homogeneous PMMA, Alderson Rando phantoms, and patients with a metal implant in the head and neck region with approximately 40%, 40%, and 60% percentage of noise reduction, respectively. The qualitative analysis by two radiologists showed comparable results of OMAR and the new method.

Conclusion: The efficiency of metal artifact reduction of the new method is better than no correction and OMAR in homogeneous PMMA phantom and Alderson Rando phantom. However, the efficiency of OMAR is better than the new method, and no correction regarding the percent noise.

Introduction

Computed tomographic (CT) imaging was introduced in clinical practice in the early 1970s.[1] The evolution of the X-ray CT system resulted in high image quality and improved diagnostic capabilities. Since normal tissues are not superimposed on the image as is the case in conventional X-ray imaging, CT has become one of the most important modalities for diagnostic imaging, nuclear medicine, and radiation therapy because it provides cross-sectional images of the whole body. The clinical potential of

CT became evident during its early period, and the output solidified the role of computers in medical imaging. Recent advances in acquisition geometry, detector technology, multiple detector arrays, and x-ray tube design have led to scanning time now measured in fractions of a second.[2-7]

One common artifact in CT images results from metal implants by multiple mechanisms related to the metal itself and some to the reconstruction algorithm. The metal implant can cause beam hardening, scattered effect, photon starvation, and increased Poisson noise. Metal artifact suppresses and obstructs diagnosis or misdiagnoses as it occurred in ten percent of the patients' tomographic images.[8] The source of artifacts in the oral cavity of CT images is metal fillings in the teeth. It suppresses the diagnosis, causes misdiagnoses, and hampers organ delineation in CT images.

Several methods for reducing the metal artifact in the CT images include the iterative reconstruction technique and dual-energy technique.[9] Orthopedic metal artifact reduction (OMAR) is the first commercial product available that implements a robust algorithm to mitigate artifacts caused by metal implants in CT images. The crux of the OMAR implementation is an iterative loop where the output correction image is subtracted from the original input image. The resultant image can then become the new input image and the process can be repeated.

OMAR can induce some minor artifacts when the metal implant is close to the body surface or the low-density tissue. A spine with metal screws can create a problem when using OMAR. Similarly, a pacemaker can also create a problem in an orthopedic implant. Its proximity to the lung with metal wires entering the heart and lung area can cause the streaking artifacts when using OMAR which is not present in the non-corrected image.

In this work, the new metal artifact reduction method was developed to improve the images of metallic artifacts using MATLAB software. This study aims to evaluate the new method of metal artifact reduction in head and neck CT images and compare the resulting images with no correction and those obtained using a commercially available metal artifact reduction algorithm.

Materials and methods
Study of phantom

The concept of a new method of metal artifact reduction is the B-spline and averagely weighted interpolation techniques by MATLAB software. The new method of metal artifact reduction is shown in Figure 1. The B-spline curve is a piecewise polynomial curve whose shape is controlled by control points and the degree of its polynomial basis. In practice, the metal artifacts appear in only one or two slices. The information lost due to the artifact can be inferred from the slices immediately above and below (inter-slice) and within its slice (intra-slice). The information from inter-slices and intra-slice is fused to estimate the missing information in our method. The metal artifact causes very bright and dark streaks in an image to be easily detected by thresholding for the area with very high and low intensities. B-spline interpolation is used to find the intra-slice information. The intensity lost by the artifact is interpolated from the intensity within the same slice. The weighted average between the intensities of the slices above and below is used as the inter-slice information. The missing intensity is estimated according to the following equation.

$$I_{interslice,k}(x,y) = \alpha I_{k-1}(x,y) + (1-\alpha) I_{k+1}(x,y)$$

where I (inter-slice, k) (x,y) and I_{-k} (x,y) are the weighted average and the original intensities at (x,y) in the k-th slice, respectively. α is the weight and has a value between 0 and 1. The weighted average is then applied
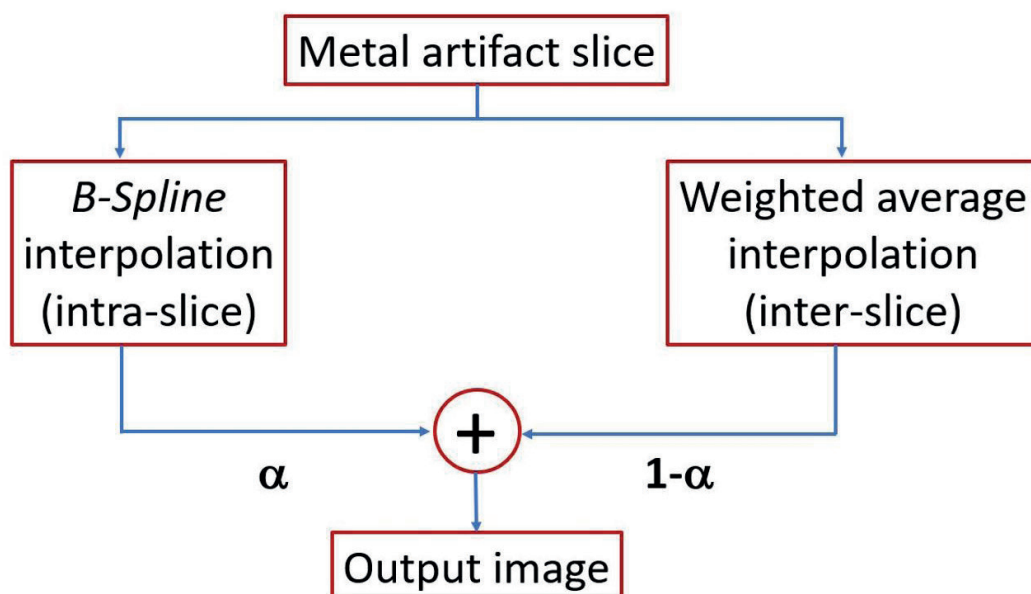
Figure 1. *Concept of the new method.*

to fuse the intra-slice and the inter-slice information. Since only a few slices are degraded by metal artifact, α for information fusion is manually set (all case set to 0.5). The concept of the new method was a simple and effective method to suppress the metal artifact in the CT image because the image space-based method in image processing was used together with the DICOM file format from CT.

The scanning parameter of Philips Brilliance CT Big Bore was 120 kVp, 3 mm slice thickness, helical mode, 512×512 matrix size, and field of view covering the body contour of the patient. In this study, a CT image from filtered back projection (no correction) was used as the original image. Upon completion of the algorithm development, the homogeneous PMMA phantom and Alderson Rando phantom images with and without amalgam inserts were used to evaluate the algorithm's utility in phantoms. The homogeneous PMMA, cylindrical phantom is 16 cm in diameter and 14 cm in length. Alderson Rando phantom incorporates the materials to simulate various body tissue muscle, bone, lung, and air cavities. It is made of tissue-equivalent material based on synthetic isocyanate rubber. The phantom material is processed chemically and physically to achieve a density of 0.985 g/cm^3 and an effective atomic number of 7.3 based on the International Commission on Radiation Units and Measurement (ICRU). The phantom is shaped into a human and sectioned transversely into slices 2.5 cm thick. Both phantoms were scanned by 16 detector rows Philips Brilliance Big Bore CT simulator with OMAR.[10] The tube voltage was fixed at 120 kVp, and the tube current time was varied at 100, 150, 200, and 250 mAs respectively for all phantom studies. The acquisition was repeated three times per mAs setting. The quantitative analysis of image quality in the phantom was obtained from the percent noise (standard deviation divided by the mean of CT number at the region of interest). The region of interest in the area of 1 cm^2 in rectangular shape was close to the metal artifact region.

Clinical study

This study was approved by the Institutional Review Board (IRB), Faculty of Medicine, Chulalongkorn University (IRB 627/60). Fifty-two head and neck CT images with metal artifact images from cancer patients were subjected to the metal artifact reduction method. All patients were acquired by a 16 detector rows Philips Brilliance Big Bore CT simulator with OMAR software. The efficacy of the metal artifact reduction method was assessed using quantitative and qualitative analysis. The quantitative analysis of image quality in patients was obtained from the percent noise. The qualitative image quality was determined by two independent radiologists to score the images. Both had the same experience in CT image interpretation (10 years of experience). This study was blind observations for two radiologists, and the weighted Kappa for inter-observer reliability was analyzed by SPSS version 22. The guidelines of image quality criteria are score 1 = very dissatisfied, score 2 = dissatisfied, score 3 = satisfied, and score 4 = very satisfied.

Result and discussion
Study of phantom

Examples of the transverse axial images from the homogeneous PMMA phantom and Alderson Rando phantom with and without artifact reconstructed by no correction, OMAR, and the new method algorithms are shown in Figure 2, 3, 4, and 5 respectively. The no correction, OMAR, and new method showed the same image quality in the homogeneous phantom and Alderson Rando phantom without metal which is shown in Figure 2 and Figure 4. The image quality of the new method showed better than no correction and OMAR in the homogeneous phantom and Alderson Rando phantom with metal as shown in Figure 3 and Figure 5.

The average percent noise of the homogeneous phantom and Alderson Rando phantom with and without artifact of no correction, OMAR, and the new method are shown in Table 1 and Table 2, respectively. As expected, the percent noise of the homogeneous phantom and Alderson Rando phantom with and without artifact decreased when mAs increased.

The new method's effectiveness in reducing the average percent of noise in homogeneous and Alderson Rando phantoms was slightly better than no correction and OMAR 17.4% and 9.7%, respectively. The results were consistent when compared with Wagenaar D *et al.* regarding the percentage of noise reduction.[9]
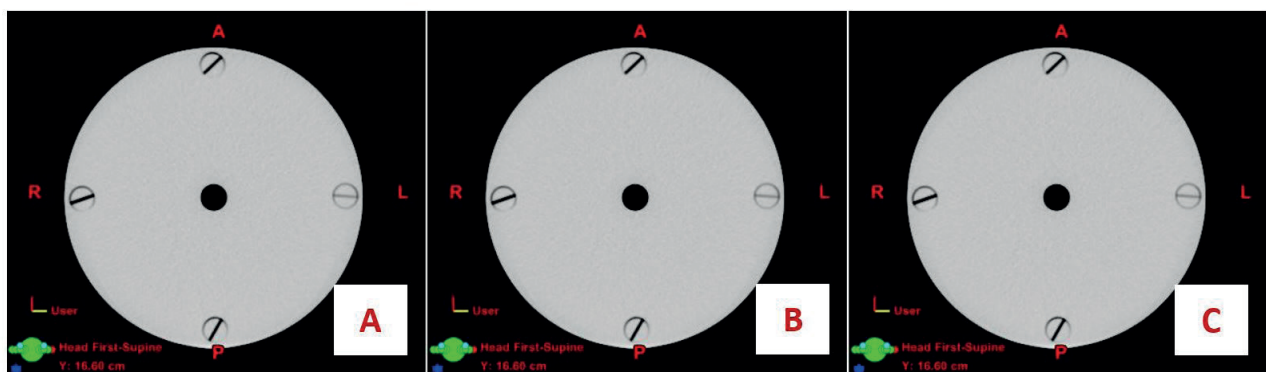
Figure 2. *Homogeneous phantom without metal artifact reconstructed by 3 different methods.*
A: FBP, B: OMAR, C: new method.
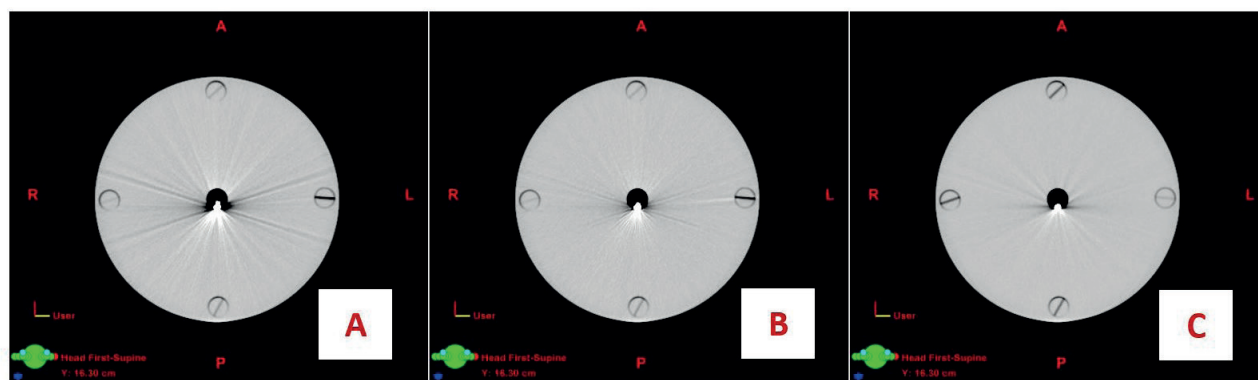
Figure 3. *Homogeneous phantom with metal artifact set close to the center of phantom reconstructed by 3 different methods. A: FBP, B: OMAR, C: new method.*
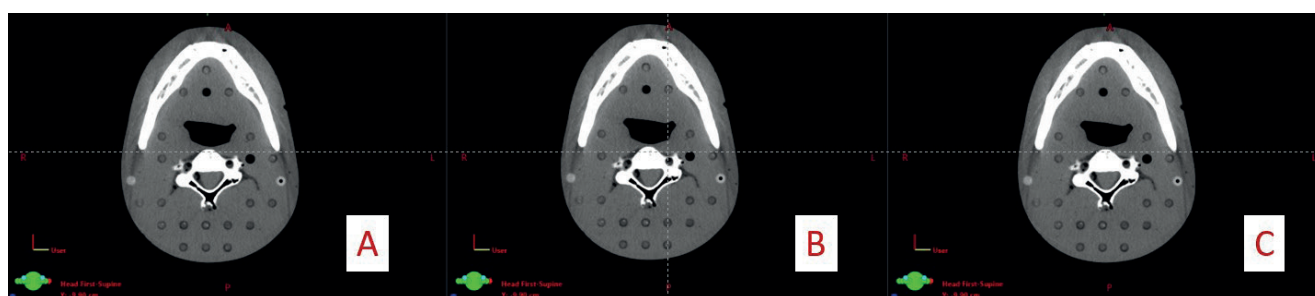
Figure 4. *Transverse axial image of Alderson Rando phantom, head, and neck part, without metal artifact reconstructed by 3 different algorithms. A: FBP, B: OMAR, C: new method.*
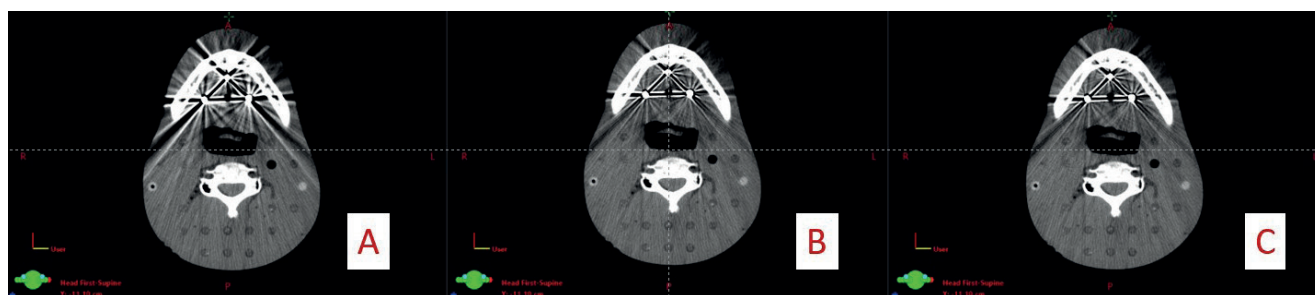
Figure 5. *Transverse axial image of Alderson Rando phantom, head, and neck part, with metal artifact reconstructed by 3 different algorithms. A: FBP, B: OMAR, C: new method.*

Table 1 Average percentage of the noise of homogeneous phantom with increasing tube-current time.

mAs	Average %noise (without metal)			Average %noise (with metal)		
	FBP	OMAR	New method	FBP	OMAR	New method
100	8.3±0.6	8.3±0.6	6.4±0.7	34.7±5.3	26.4±2.5	12.1±1.4
150	7.6±0.9	7.6±0.9	5.4±0.2	32.6±3.7	21.2±1.5	12.0±0.3
200	5.9±0.6	5.9±0.5	4.5±0.3	24.8±3.9	19.2±2.1	11.6±1.7
250	5.6±0.5	5.6±0.5	4.2±0.3	23.8±3.6	18.2±1.0	10.5±0.6

Table 2 Average percentage of the noise with increasing tube current time studied in Alderson Rando phantom without and with the metal artifact.

mAs	Average %noise (without metal)			Average %noise (with metal)		
	FBP	OMAR	New method	FBP	OMAR	New method
100	66.3±31.0	66.0±30.5	27.9±2.0	290.0±38.7	288.4±22.3	162.6±22.0
150	62.5±38.2	62.6±38.2	22.4±3.2	281.6±44.4	286.8±21.4	105.6±30.0
200	60.1±36.1	59.9±36.2	18.9±1.9	277.3±34.7	283.5±27.6	1.7±31.7
250	54.2±32.1	54.5±32.1	18.9±1.5	264.3±29.6	264.3±28.1	92.4±30.0

Clinical study

Three of fifty-two CT images of head and neck cancer patients with metal artifacts reconstructed by no correction, OMAR, and the new method are shown in Figure 6. The percent noise of CT patient images with a metal artifact of no correction, OMAR, and new method are shown in Table 3. The average percentage noise of CT patient images of the new method was less than no correction and more than OMAR. Twenty of fifty-two CT images of the new method showed better image quality than no correction and OMAR, but two of fifty-two were worse than no correction and OMAR.

The performance of both metal artifact reduction methods is shown regarding the percent of metal artifact reduction in CT patient images as in Table 4. The OMAR was slightly better, 55.5±0.15% reduction of the percentage of noise than a new method of 41.2±0.23% reduction percentage of noise. The performance of a new method showed better image quality than no correction and OMAR in Figure 6.

Two independent radiologists with similar experience evaluated the image quality by scoring the CT images among no correction, OMAR, and the new method for metal artifact reduction. The image quality scoring is shown in Table 5. Both radiologists' evaluation confirms the agreement on the new method and OMAR has better image quality than no correction image. Two of fifty-two CT images that resulted from a new method show more artifacts than no correction and OMAR because of many streak artifacts from metal in adjacent CT images.

The result in homogeneous PMMA phantom and Alderson Rando phantom with metal artifact shows that the metal artifact suppressed by a method is better than no correction and OMAR. In contrast, the clinical study indicated that the suppression of the metal artifact using OMAR is better than the new method and has no correction. The major limitation in clinical application is that OMAR could not be used in DICOM file format when processing the images. Therefore, the CT images from other vendors could not be processed by OMAR. Therefore, it is necessary to develop a new method using the DICOM file format for CT images from other vendors. A new metal artifact reduction method can be used in several CT scanner vendors because it uses the DICOM format in image processing.

Several publications reported the performance of the metal artifact reduction algorithm by sinogram technique.[3-6,11-13] and forward projection metal artifact reduction technique.[3] Most new methods show much better performance than no correction images in qualitative and quantitative evaluations. Most publications studied in the phantom or simulation phase without clinical applications. This study shows both phantom and clinical applications in which the new method could reduce metal artifacts
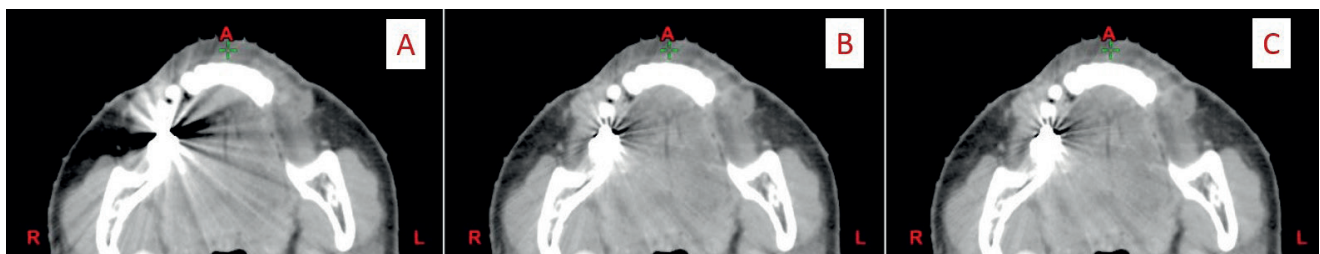
Figure 6. *Patient computed tomographic images with metal artifacts reconstructed by 3 algorithms. A: FBP, B: OMAR, C: new method.*

Table 3 Average percentage of the noise of computed tomographic patient images of three algorithms.

Average %noise (FBP)	Average %noise (OMAR)	Average %noise (new method)
111.2±65.8	46.6±30.0	63.8±54.7

Table 4 The percent of metal artifact reduction in computed tomographic patient images.

Percent of metal artifact reduction (%)	
OMAR	New algorithm
55.5±0.15	41.2±0.23

Table 5 Image quality of patients with metal artifacts using three methods, scored by two radiologists.

	Average scores on image quality, with metallic artifacts		
	FBP	OMAR	New method
1st Radiologist	1.2±0.4	2.5±0.8	2.3±1.0
2nd Radiologist	1.5±0.5	3.5±0.5	3.0±1.0

in phantoms and patients. The performance of the new method shows better image quality than the no-correction image. However, the new method does not perform better than OMAR in a heterogeneous environment. It improves the image quality regarding metal artifact reduction, but it underperforms the current commercial metal artifact reduction algorithm. There is an unexpected new artifact consequence where a new method may modify more artifacts due to metal artifacts in the previous and next CT images. The radiologist and radiation oncologist should always compare filtered back projections and a new method dataset in clinical applications.

Conclusion

The efficiency of metal artifact reduction of the new method is better than no correction and OMAR in homogeneous PMMA phantom and Alderson Rando phantom in the clinical range of mAs. However, the efficiency of OMAR is better than the new method, and no correction regarding the percent noise. The image scoring by two independent radiologists with the same experience shows comparable efficiency results of the new method and OMAR.

Acknowledgements

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Morphological patterns of the cerebral arterial circle of Willis: Implication in subjects with ischemic stroke

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ARTICLE INFO

ABSTRACT

Background: A stroke or cerebrovascular accident is associated with defects in the circle of Willis. The present research assessed whether differences in the anatomy of the circle of Willis were implicated in subjects affected by stroke.

Materials and methods: A retrospective descriptive (cohort) study of images of 340 male and female subjects aged 15 to 75 years, referred for either brain Computed Tomography Angiography (CTA) or Magnetic Resonance Imaging (MRI) scan indicative of suspected stroke, was employed. A convenient sampling technique was used to obtain images from selected hospitals and radio-diagnostic centers with Computed Tomography (CT) and MRI scanners. Approval was obtained from the Federal Health Research Ethics Committee in accordance with institutional guidelines and principles, following permission and clearance (Approval Number: FHREC/2019/01/51/13-05-19). Patterns of morphology observed in the circle of Willis were data collected and stored in a non-identifiable format. Data obtained were analyzed with the Statistical Package for Social Science (SPSS) Inc, Chicago, IL, USA version 25.0.

Results: Of the total 340 images evaluated, 256 (75.29%) subjects had ischemic stroke while 84 (24.71%) subjects had no stroke and were thus, considered to be apparently normal. Structural patterns in the circle of Willis mostly observed were the absence of the anterior communicating artery (10.94%) and the bilateral absence of the posterior communicating artery (10.16%).

Conclusion: Morphological patterns of the cerebral arterial circle of Willis observed, were implicated in subjects affected with stroke in the present study population.

Introduction

Stroke or cerebrovascular accident (CVA) is associated with defects in the circle of Willis.[1,2] The *circulus arteriosus cerebri* Willisi (cerebral arterial circle of Willis) is an important polygonal complex structure first described and officially named after a seventeenth-century English Physician; Sir Doctor Thomas Willis in his *Cerebri anatome* in the year 1664, where he first indicated the collateral potential of the circle.[3] The circle of Willis is the principal arterial anastomotic trunk, located at the root substance between the two vertebral arteries of the vertebrobasilar system and the right and left internal carotid arteries that supply the brain.[1-9] Studies have demonstrated a classical and complete pattern of a circle of Willis where all the vessels are intact and crucial for a continuous supply of blood.[6,8,10-15]

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The circle of Willis is essential for the maintenance of a stable and constant blood flow to the brain. Any changes or defects in its structure may cause the appearance and severity of syndromes of vascular inefficiency, which may predispose the individual to CVA with attendant consequences. The special activity of the circle Willis relies on a continuous circular method of its formation. However, there are distinct morphological patterns of arteries that form the circle of Willis, and knowledge of these variants is essential for timely diagnosis and subsequent management of CVA. Also, these morphological patterns may provide important information on the calibre (size and shape) of the circle of Willis for better interpretation of angiographic images and for a deeper understanding of cerebral pathology such as CVA.[1-3,5-6,9-13,16-19] The significance of the patterns of the circle of Willis using methods such as the cadaveric gross dissection (GD), injection technique (IT), magnetic resonance imaging (MRI), and recently, contrast-enhanced computed tomography (CECT) imaging have been highlighted.[12,13,20,21]

Currently, medical sciences have recorded a shift from invasive to non-invasive techniques, especially in blood vessel examinations. For example, CVA therapy is aimed at re-opening clogged arteries to increase cell survival at the site of injury followed by physiotherapy.[22] It, therefore, becomes important for the healthcare givers to have sufficient knowledge of morphological patterns of the circle of Willis including other vessels involved in stroke development.[2,23] A better understanding of patterns of the circle of Willis is imperative for tailoring prevention and early detection in the management of ischemic stroke.[2,23]

In the past decades, there have been studies on stroke.[23-32] However, knowledge of how the morphological patterns of the circle of Willis are associated with stroke is lacking. It is unclear whether the computed tomography angiography (CTA), magnetic resonance angiography (MRA), and or magnetic resonance imaging (MRI) circle of Willis in subjects with stroke could provide features associated with future risk of major secondary complications, underlain by the variations in the circle of Willis in subjects affected by stroke.

It is well known that blockage or defect of the major arteries of the circle of Willis leads to stroke with the severity of stroke directly related to the severity of obstruction. An open question as to whether the arteries affected influence the severity of obstruction from stroke or the duration and extent of obstruction needs to be answered. To address these deficiencies, it is important to investigate how morphological patterns of the circle of Willis influence stroke development using CTA and MRI as diagnostic tools. However, the role of CTA and MRI in the prognosis of stroke is poorly understood. An understanding of image appreciation or pattern recognition of the circle of Willis associated with impending CVA, affected regions of the brain, and patient outcome is crucial in designing interventions to improve the outcome of people with stroke.

The CTA and MRI produce images that allow clear visualization of the location and extent of arterial occlusion, collateral circulation, extracranial stenosis, and ischemic changes within the brain tissue.[33-37] In addition, CTA and MRI are important diagnostic tools for stroke and the identification of causal factors. These imaging modalities provide information about the severity and prognosis of the disease. The present research, therefore, focused on whether differences in anatomy (structural patterns) of the circle of Willis were implicated in subjects affected with stroke using CTA and MRI.

Materials and Method

Research design

A retrospective descriptive (cohort) research design of images of 340 male and female subjects aged 15 to 75 years referred for either brain CTA or MRI scan. The images were sourced from selected hospitals and radio-diagnostic centers with CT and MRI scanners with their specifications (Table 1 and 2) coded A, B, C, D, E, and U, V, W, X, Y, Z respectively. The subjects scanned, had a non-contrast CT before the CTA protocol while those for the MRI were scanned using the three-dimensional MRI time of flight sequence.

Sampling technique and criteria for selection

A convenient non-probability sampling technique was used to select the images with inclusion criteria; the subjects must be referred for either brain CTA or MRI whose indication was suspected stroke, an acute neurological deficit of not less than nine hours' duration, and subjects with unknown time of symptoms onset. However, subjects with fresh bleeding such as intracerebral hemorrhage, subarachnoid hemorrhage, and or tumor, subjects with known contrast allergy or previous renal failure

Table 1 Specifications of CT scanners used in the present study.

S/n	Hospital/ radio-diagnostic centre	Name	Make	Country	Machine s/n	Date of manufacture	Output	
							Max. kVp	mAs
1	A	Bright-Speed 4-Slice	GE	USA	16507017m4	2007	140	300
2	B	Optima 64-Slice	GE	USA	369366HMO	2014	140	800
3	C	Brivo 385 Series 16 slice	GE	India	96369B14	2014	140	200
4	D	Brivo 385 16-Slice	GE	China	353806HM3	2013	140	180

Table 2 Specifications of MRI scanners used in the present study.

S/n	Hospital/ radio-diagnostic centre	Name	Make	Country	Date of manufacture	Output (Tesla)
1	U	Somatom	Siemens	Germany	2007	0.2
2	V	Multiva	Philips	Holland	2016	1.5
3	W	Signa Ovation	GE	USA	2014	0.35
4	X	Magnetom Concerto	Siemens	Germany	2014	0.2
5	Y	Brivo 235	GE	USA	2014	0.5
6	Z	Brivo 235	GE	India	2014	0.5

at the time of admission, and subjects with a history of brain surgery and or radiotherapy of the head and neck were excluded from the study. All the subjects' data were treated with a high level of confidentiality and privacy following standards for conducting research. Data were collected and stored in a non-identifiable format.
Landmarks

The images were evaluated using the following structures as landmarks; from the skull base to the vertex at the level of the corpus callosum or the caudate nuclei. This was to ensure appropriate visualization of the cerebral arterial circle of Willis. The subjects' images were assessed at the radiology workstations using the RadiAnt digital imaging and communication in medicine (DICOM) and the MicroDicom workstations.

Method of data collection

A direct measurement using archived primary images of CTA and MRI was employed as the method of data collection. The vessels evaluated were terminal branches of the internal carotid artery (the anterior cerebral artery and the middle cerebral artery), the basilar artery, and its terminal branches, these include:

1. the A_1 segment/pre-communicating part of the anterior cerebral artery (from the termination of the internal carotid artery (ICA) to the junction with the anterior communicating artery (ACoA) bilaterally.
2. the M_1/sphenoidal segment (from the termination of the ICA to its bi or trifurcation) bilaterally.
3. the P_1 segment (from the basilar bifurcation to the junction) with the posterior communicating artery (PCoA) bilaterally.

Patterns of morphology observed in the circle of Willis were noted. During the evaluation, abbreviations were used to show the different structures assessed. Three observers (two consultant Radiologists and one Radiographer with work experience of >15 years) together, assessed the images obtained in the present study. The inter-observer reliability of the three observers was taken into consideration during the course of the study.

Data analysis

Data obtained were expressed using basic elements of descriptive statistics and analyzed with the Statistical Package for Social Science (SPSS) version 25.

Results

This anatomic-imaging study assessed morphological patterns of the circle of Willis in 340 male and female subjects with suspected stroke, aged 15 to 75 years (40.18 ±1.1 and 43.68±1.18). From the images evaluated, results showed that 256 (75.29%) subjects had an ischemic stroke while 84 (24.71%) subjects were without stroke with normal morphology (size, shape, and structure) of the circle of Willis and were therefore, considered to be apparently normal (Figure 1). Patterns of morphology noted in subjects with ischemic stroke of the present study were the absence of anterior communicating artery (ACoA) (10.94%) and bilateral absence of the posterior communicating artery (PCoA) (10.16%) demonstrated in Figure 2 and 3 respectively.

Discussion

A stroke occurs when blood flow to the brain is disrupted due to blood clots or ruptures in cerebral vessels, cutting off the supply of oxygen and nutrients contained in the blood and resulting in damage to the brain tissues.[1,19,38] Stroke is associated with defects in a circle of Willis whose hemodynamics is predisposed by variants in the calibre of segments, this affects its major role as an anastomotic channel.[2] In the present study, morphological patterns observed in subjects (male and female) with stroke were mostly the absence of the anterior communicating artery and the bilateral absence of the posterior communicating artery. A study has demonstrated that the anterior communicating artery does not allow any mingling of blood between the two anterior cerebral arteries where it does act as a by-pass channel, where one internal carotid artery is occluded.[39] Result of the present study confirms a similar work that observed a 5% definitive absence of the anterior communicating artery in the microsurgical anatomy of common aneurysm sites.[17] In addition, the absence of an anterior communicating artery noted in the present study is in tandem with the reports that recorded similar morphological patterns with the use of contrast-enhanced computed tomography scan in a sampled Nigerian population.[13,14]

Similarly, the posterior communicating artery is very important as it joins the right and left posterior cerebral arteries to form the posterior segment of the circle of Willis. The present study recorded 10.16% bilateral
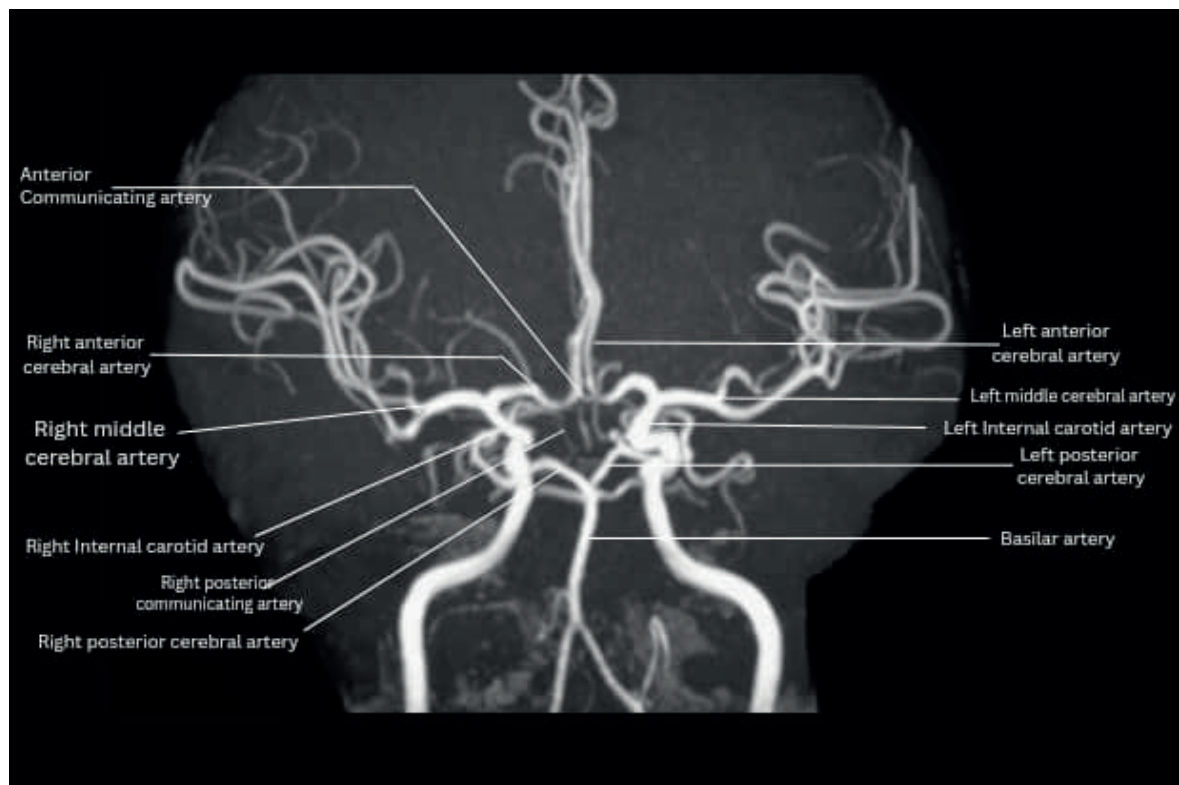
Figure 1. *Magnetic Resonance Angiogram of the circle of Willis in the present study.*
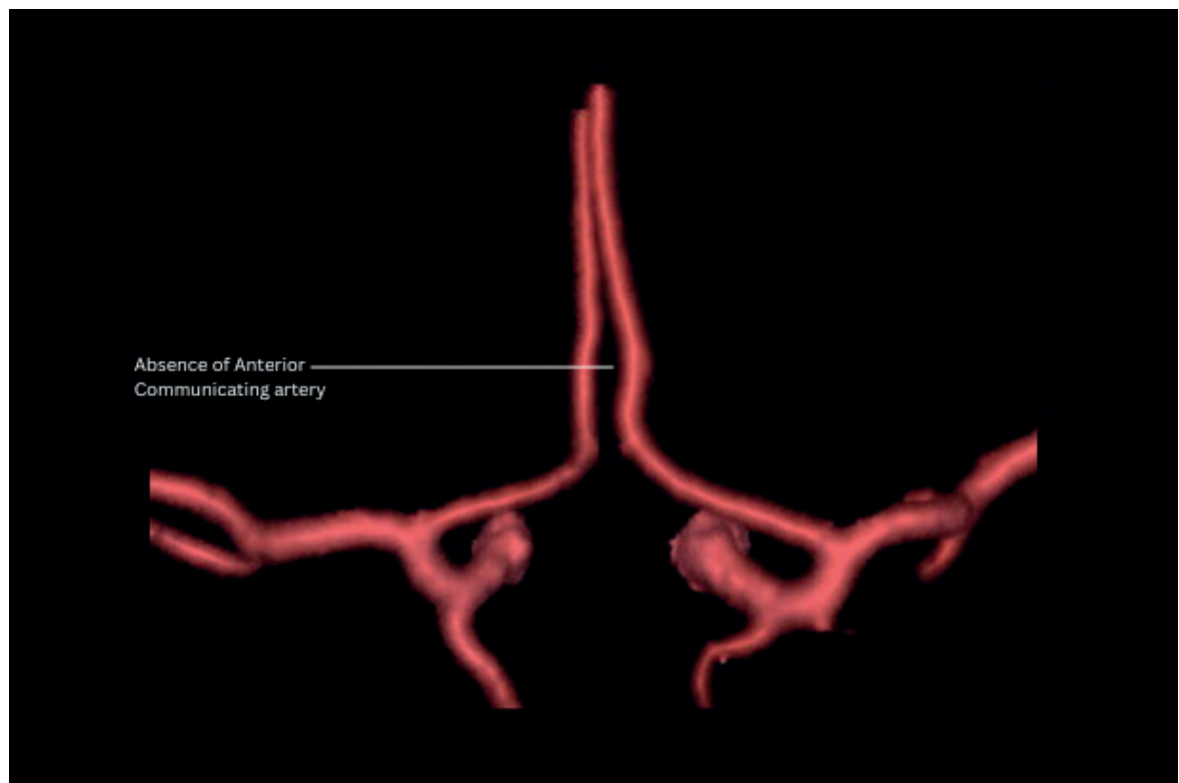
Figure 2. *Volume Rendering CTA showing absence of ACoA of the circle of Willis in subjects with stroke.*
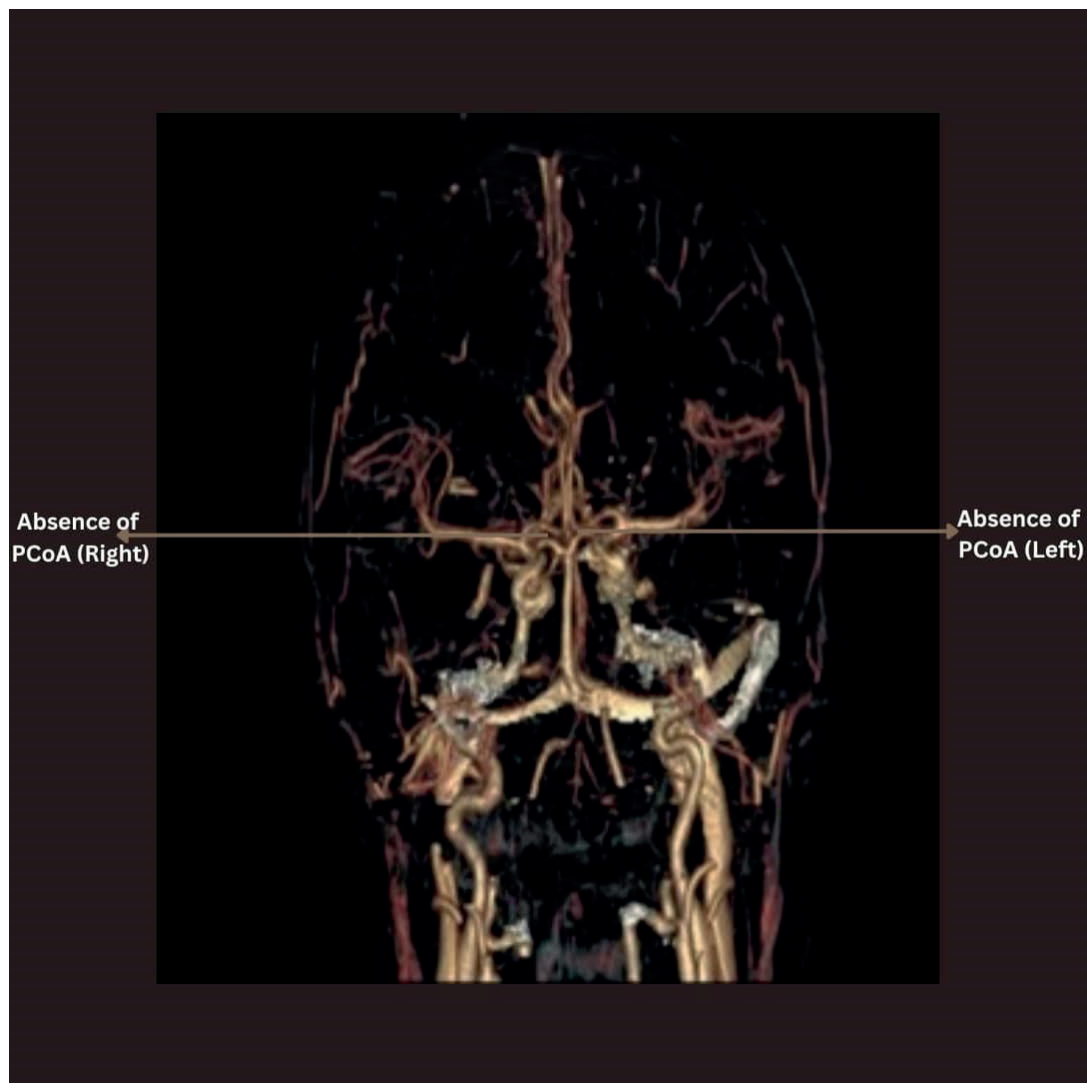
Figure 3. *Volume Rendered (VR) CTA image of the circle of Willis showing bilateral absence of PCoA.*

absence of a posterior communicating artery, which supports similar studies where the absence of a bilateral posterior communicating artery was the most common pattern noted in the circle of Willis.[14,40-41] Also, the result of the present study supports work that stipulates that the incomplete circle of Willis was mostly due to the absence of a posterior communicating artery.[8]

The absence of an anterior communicating artery and posterior communicating artery observed may be implicated in subjects affected with stroke in the present study. This is necessary as it provides important information on the calibre of the circle of Willis that may be useful for better interpretation of angiographic images. Knowledge of the patterns of presentation of the circle of Willis noted might be helpful in improving quality patient care and outcome during surgical planning and in interventional procedures thereby reducing fatalities resulting from CVA. Although the rates of global CVA death have decreased especially in developed countries, the number of people with stroke is still on the increase [42,43] as the incidence of stroke is in the ascendancy worldwide.[29-32] In Nigeria for example, stroke has assumed a frightening

dimension where about 190,000 people suffer the bug annually.[32] The effects of CVA are not only limited to the aged but also among younger people.[29-32,44] This affects the workforce population and more concerning, is the fact that even young people are becoming more vulnerable to the disease.[29,44,45] The present study has thus, provided important information on the morphological variation of the arterial circle of Willis, which can be factored into risk stratification and intervention programs in the management of stroke and its secondary complications. This will significantly improve the health status of the study population.

Conclusion

The absence of anterior communicating artery and bilateral absence of posterior communicating arteries identified as morphological patterns in the cerebral arterial circle of Willis were implicated in subjects affected with ischemic stroke in the study population.

Study limitations and clinical implications

Though, standard scanning techniques with standard

protocol and sequence were observed throughout the study. The following limitations were, however, encountered; different CT and MRI scanners from different manufacturers used for the study may have introduced differences in image quality. Also, inter-operator errors may have introduced some differences in the patterns noted in the present study. The clinical implications of the present research are that knowledge of the patterns of the morphology of the circle of Willis noted will help medical professionals in improving quality patient care and outcome during surgical planning and in interventional procedures to repair/remove the affected arteries. Also, these morphological patterns may provide important information on the calibre (size and shape) of the circle of Willis for better interpretation of angiographic images and for a deeper understanding of cerebral pathology such as CVA.

Conflict of Interest

There were no conflicts of interest declared.

Ethical approval

Approval was obtained from the Federal Health Research Ethics Committee in accordance with the institutional guidelines and principles, following permission and clearance (Approval Number: FHREC/2019/01/51/13-05-19). In addition, permission was obtained from the selected hospitals and radio-diagnostic centers.

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Prevalence of iron deficiency in non-anemic heart failure patients

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ARTICLE INFO

ABSTRACT

Background: To study the prevalence of iron deficiency in non-anemic heart failure patients.

Materials and methods: This is a single-center cross-sectional study done in the Department of General Medicine at the Institute of Medical Sciences and SUM Hospital, Odisha, between November 2020 and June 2022. The selection of participants was made based on Framingham criteria. An iron profile was used to detect iron deficiency anemia during the study period. According to the WHO, Anemia is defined as having a hemoglobin (Hb) level below 12 gm/dL in females and 13 gm/dL in males. Serum ferritin below 100 ng/mL is considered an absolute iron deficiency, and serum ferritin between 100 and 300 ng/mL with low transferrin saturation (TSAT <20%) is regarded as functional Iron deficiency.

Results: A total of 100 individuals with non-anemic heart failure (53% men and 47% women) were included in the study. Most of the individuals were of class IV New York Heart Association (NYHA) (N=39). Iron deficiency was present in 60% of individuals, with 32% having absolute iron deficiency and 28% having functional iron deficiency. Men and women had almost the same prevalence of iron deficiency (62.2% vs 57.44%). The levels of mean Hb, ferritin, and transferrin saturation in the iron deficiency group (i.e., 13.2 gm/dL, 139.7 ng/mL, and 14.4%) were less when compared to the non-iron deficiency group (i.e., 13.3 gm/dL, 391.35 ng/mL and 36.1%).

Conclusion: Our research emphasizes the impact of iron deficiency on heart failure patients in India, which is still underappreciated and ignored. This study recommends more in-depth analysis to define this manageable illness correctly and explore habitual testing in upcoming national recommendations.

Introduction

Heart failure is a prevalent issue that affects 1-2% of people and is a leading reason of death, illness, and poor quality of life (QoL).[1,2] Anemia is a common co-morbid condition in stable heart failure candidates, and it worsens morbidity by causing regular hospitalizations, reduced exercise ability, poor quality of life, and increased death.[3] Heart failure is frequently accompanied by iron deficiency with or without anemia. Even though iron deficiency is the

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most prevalent nutritional deficit globally, impacting more than one-third of the total population, its link to heart failure with or without anemia is gaining attention.[4,5-7]

Iron deficiency is an appealing therapeutic target since replacement therapy improves outcomes in individuals with heart failure-a notion verified in many recent research studies.[8,9] Diagnosis and management of heart failure guidelines from the European Society of Cardiology (ESC), for the first time, iron deficiency was identified as a co-morbidity in heart failure in 2012. It was also suggested that the identification of iron deficiency depends on the iron values in all individuals with suspected heart failure.

It has recently been identified that individuals with heart failure are more likely to acquire iron deficiency as a consequence of depleted iron reserves or processes evident in anemia or chronic disease.[10,11] Advanced age, renal disease, female sex, malnutrition, chronic inflammation, decreased iron absorption, increased iron loss, and the severity of heart failure have all been found to be independently linked with ID in HF. It should be noted that many of the risk variables listed above are hypothesized based on observational research and have not yet been shown to cause HF in patients, therefore they are still uncertain.[12,13]

Iron deficiency, whether with or without anemia, reduces working capacity, causes tiredness and activity intolerance, and exacerbates the clinical manifestations of heart failure individuals.[14] A recent randomized, double-blind research has shown that intravenous iron supplementation improved functional outcomes and living standards in Iron deficiency individuals with or without anemia and heart failure.[15]As a result, Iron deficiency has recently taken on a distinctive function in heart failure and is the topic of new research.

Most of the research on the prevalence of iron deficiency in people with heart failure came from foreign countries. The study conducted by Chobufo *et.al* in the USA concluded that anemia should not be considered a prerequisite for screening for iron deficiency in patients with heart failure.[16] Very few studies have looked into this link in Asian people.[17,18] However, there are presently no data from India that can be used to estimate the prevalence of iron deficiency in people with non-anemic heart failure. This study aims to determine the prevalence of iron deficiency in non-anemic heart failure patients. It may aid in formulating potential recommendations for regular iron deficiency evaluation in heart failure individuals in India.

Materials and methods

This is a single-center cross-sectional study done in the Department of General Medicine at IMS and SUM Hospital, Odisha, between November 2020 and June 2022. In this research, 100 non-anemic heart failure patients of IMS & SUM Hospital satisfying the inclusion criteria were included based on guidelines from the ESC and the Framingham criteria heart failure is diagnosed.

Inclusion criteria

1. Patients with symptoms of heart failure confirmed by 2d-echo showing systolic and diastolic dysfunction with raised NT- PROBNP levels.
2. Patients with heart failure having hemoglobin levels of more than 13 (gm/dL) in males and 12 (gm/dL) in females.

Exclusion criteria

1. Heart failure patients with chronic kidney disease.
2. Heart failure patients with recent blood transfusions history.
3. Pregnant females with heart failure.

All the study individuals underwent clinical examination, routine hematological investigations, and 2D-Echo using standard instruments. Participants were classified as having heart failure with reduced ejection fraction (HfrEF) (EF <40%); heart failure with mid-range ejection fraction (HfmrEF) (EF 41-50%); or heart failure with preserved ejection fraction(HfpEF) (EF>50%).

These individuals' iron status was evaluated in addition to standard hemograms by assessing their complete iron profile. Hemoglobin concentrations (gm/dL) were measured using an automatic system Coulter LH 750 Hematology Analyzer (Beckman Coulter, Krefeld), Serum iron measurement was performed with calorimetric assay, and transferrin was assessed using an immune-turbidimetric assay on a modular automated clinical chemistry analyzer. Serum ferritin was measured using an immunoassay based on electrochemiluminescence. All measurements were performed with Modular E170 (Roche/Hitachi). Plasma concentration NT-PRO BNP (ng/l) was measured using an immunoassay based on electrochemiluminescence on the Modular system.

According to the WHO, anemia is defined as having a Hb level of less than 12 gm/dL for females and less than 13 gm/dL for males. Serum ferritin of 30 ng/mL is a typical cut-off level for diagnosing absolute Iron deficiency. Still, in heart failure, as there is both increased iron buildup intracellularly and ferritin tissue overexpression (driven by inflammation), there will be a generalized rise in its level in the blood. In these circumstances, a serum ferritin level of 100 ng/mL is used as a cut-off value to diagnose absolute Iron deficiency. Functional Iron deficiency was described as serum ferritin between 100-300 ng/mL with low TSAT (<20%), whereas absolute Iron deficiency was considered serum ferritin <100 ng/mL.[19]

Statistical analysis

The chi-square test and Student's t-test were used to calculate the *p* value. Using SPSS software version 20.0, collected data were evaluated with the application of appropriate statistical methods, and $p<0.05$ were considered statistically significant.

Results

During the study timeframe, 100 individuals admitted to IMS & SUM Hospital with a clinical diagnosis of HF were included, out of which 53 (53%) were men and 47 (47%) were women, with a greater number of individuals in class IV NYHA, i.e., 39 (39%). Iron deficiency was seen in 60% of individuals (N=60), with absolute iron deficiency in 32% and functional Iron deficiency in 28%. Co-morbidities like Type 2 diabetes mellitus and hypertension were present in 46% (N=46) and 46% (N=46) patients. Out of 100 individuals, 41% (N=41) came to the hospital with features of acute pulmonary edema, and 15% (N=15) with atrial fibrillation. Mean ejection fraction (EF) and NT-PRO BNP levels were 43.64%, and 1699.5 ng/L respectively. The baseline features of all study individuals grouped into Iron deficient and non-iron deficient groups are depicted in Table 1.

As depicted in the above table, the prevalence of multiple heart failure features was higher in individuals in the Iron deficiency group compared to the Non-iron deficiency group (Rales, *p*=0.002, Raised jugular venous pressure (JVP), *p*=0.003). Also, there is a considerable difference in the use of ACE- inhibitors, with more individuals in the Iron deficiency group using them (N=31). The levels of mean Hb, Ferritin and Transferrin saturation in the Iron deficiency group were less when compared to the non-iron deficient group (Table 2).

Men and women had almost the exact prevalence of ID (62.2% vs 57.44%). Absolute iron deficiency was seen in 32 (32%) individuals. Functional Iron deficiency was seen in 28 (28%) individuals. Thus, Iron deficiency was seen in 60 (60%) individuals including both absolute and functional Iron deficiency.

Based on New York Heart Association (NYHA) functional class, patients were further categorized into Iron Deficit and Non-iron deficiency groups (Table 3). This study showed a statistically significant positive association between NYHA class and iron deficiency. The prevalence of iron deficiency increased with the class of dyspnea with a *p* value of 0.0001.

Table 1 Baseline characteristics of the study population.

Feature	Iron deficient heart failure patients	Non-iron deficient heart failure patients	*p* value
History Of Previous MI	18	15	0.5
History Of Hypertension	29	17	0.6
History Of Diabetes Mellitus	29	17	0.6
History Of Use Of ACEi's	31	19	0.8
Orthopnea	26	22	0.3
Rales	58	30	**0.002**
Gallop	13	4	0.1
Elevated JVP	49	22	**0.003**
Pedal Edema	40	23	0.3
Atrial Fibrillation	10	5	0·5
Chest X Ray Showing Heart Enlargement	16	14	0.3
Pulmonary Edema	28	13	0.1

Table 2 Comparison of mean values between Iron deficient and non-iron deficient groups.

Parameter	In total individuals (Iron deficient heart failure patients (Mean±SD)	Iron deficient heart failure patients (Mean±SD)	Non-iron deficient heart failure patients (Mean±SD)
Hb (gm/dL)	13.26±0.49	13.2±0.32	13.3±0.23
Ferritin (ng/mL)	240.4±103	139.7±66.6	391.35±191.7
Transferrin saturation (%)	23.1±10.9	14.4±5.9	36.1±15.7

Table 3 Grouping of patients with iron deficiency as per their functional class.

NYHA class	Total number of patients	Iron deficient Heart failure patient's N (%)	Non-iron deficient heart failure patient's N (%)	95% CI		*p* value
				Upper	Lower	
1	6	2(33.3)	4(66.7)	0.12	0.02	
2	25	9(36)	16(64)	0.34	0.16	**0.0001**
3	30	15(50)	15(50)	0.39	0.21	
4	39	34(87.1)	5(12.9)	0.49	0.29	

Based on left ventricular systolic & diastolic function, patients were further categorized (Table 4).

It was seen that 55 individuals had systolic dysfunction, out of which 39 had ID (i.e., 70.9% patients, *p*=0.01); 85 individuals had diastolic dysfunction, out of which 52 had Iron deficiency (i.e., 61.1%, *p*=0.56). This showed a statistically significant association between systolic dysfunction and the presence of iron deficiency, but no significant association was found between diastolic dysfunction and Iron deficiency. Further, individually within subgroups, a statistically significant association was found in left ventricular systolic function (LVEF), and no significant association was found in the type of diastolic dysfunction with Iron deficiency (Table 5).

Table 4 Grouping of patients with iron deficiency as per their left ventricle function.

Left Ventricle	Dysfunction	Total	Iron deficient heart failure patients N (%)	Non-iron deficient heart failure patients N (%)	95% CI		*p* value
					Upper	Lower	
Systolic	Present	55	39(71)	16(29)	0.64	0.44	0.01
	Absent	45	21(46.66)	24(53.34)	0.55	0.35	
Diastolic	Present	85	52(61.17)	33(38.82)	0.91	0.76	0.56
	Absent	15	8(53.33)	7(46.66)	0.23	0.08	

Table 5 Sub-grouping of patients with Iron deficiency as per their LVEF and type of diastolic dysfunction.

LV dysfunction	Type	Total number of patients	Iron deficiency N (%)	Non-iron deficiency N (%)	95% CI		*p* value
					Upper	Lower	
Systolic	HFrEF	27	19(70.3)	8(29.7)	0.36	0.18	**0.04**
	HFmrEF	28	20(71.4)	8(28.6)	0.37	0.19	
	HFpEF	45	21(46.6)	24(53.4)	0.55	0.35	
Diastolic	Type 1	34	23(67.6)	11(32.4)	0.59	0.21	0.5
	Type 2	31	31	18(58)	13(42)	0.47	
	Type 3	20	20	11(55)	9(45)	0.33	

Discussion

The results of this study show that people with heart failure in the Indian community have an astonishingly high frequency of iron deficiency. The outlook is not good because Iron deficiency is a primary contributor to anemia. Iron deficiency is common even in individuals who do not have anemia, highlighting its significance as a single factor with a lousy prognosis in individuals with heart failure. Anemia prevalence among hospitalized individuals varied from 14 to 70% in extensive clinical studies and heart failure registries.

According to future research, iron insufficiency was prevalent in 61.3% of community-dwelling people in the USA with self-reported heart failure.[20] Iron insufficiency prevalence rates in Europe range from 37 to 50%.[21,22] The prevalence of non-anemic iron deficiency identified in the research was 60% (N=60), much higher than that seen in prior investigations. This demonstrates the severity of this issue among Indian individuals with heart failure.

According to a sex-based assessment, males with heart failure had somewhat higher Iron deficiency rates than women (55% vs 45%). Most of the study's participants were post-menopausal, making menstrual blood loss - a typically prevalent basis of Iron deficiency in females - an extremely improbable reason for Iron deficiency. This result contradicts earlier research that claimed the female gender to be a sovereign indicator of Iron deficiency in heart failure.[21,23]

In this research, 60% of the participants did not have anemia but had Iron deficiency. Therefore, a large portion of the potential iceberg would have been overlooked if hemoglobin values were considered to evaluate Iron deficiency in heart failure individuals. Functional Iron deficiency contributes significantly to the burden of the disease and has a prevalence of 46.6%. If TSAT and serum ferritin is carefully considered throughout the workup, this subgroup will be noticed. In a recent article, Yeo *et al*. emphasized the importance of functional iron deficiency evaluation and linked it to clinical features independent of ejection fraction.[24] These results highlight the need to provide heart failure individuals with a complete iron profile, including TSAT.

In contrast, the Sharma *et al.* study indicated that anemia affected 63.3% (N=95) of the 150 individuals with heart failure.[25] It was discovered that 51.3% (N=77) of these individuals had iron deficiency. In comparison, the other 12% (N=18) had anemia for various other reasons. Additionally, it was shown that among non-anemic individuals, 37 (24.7%) had an iron shortage, highlighting the need to check the iron profile in all heart failure individuals, regardless of Hb status.

Based on analyses of ferritin (a metric of deposited iron) and TSAT, the ESC suggested Iron deficiency evaluation in heart failure individuals in its 2016 Recommendations.[26,27] Ferritin is an acute-phase reactant, however. It may be artificially increased if there is an

underlying infection. Absolute Iron deficiency is shown by a low ferritin level. TSAT (<20%) can be used to diagnose Iron deficiency if ferritin levels are elevated (functional). Since the estimated result depends on the serum iron, TSAT's sole drawback is the diurnal variations. The combination of these two measures' thresholds, like in the FAIR-HF research (ferritin <100 ng/mL or ferritin 100-300 ng/mL if TSAT <20%), is advised due to their inherent limitations. The ideal indicator is aspiration from bone marrow and concrete iron staining. The necessity to diagnose and manage Iron deficiency in individuals with chronic heart Failure is being incorporated into global recommendations.[28] According to the study, Iron deficiency is a frequently overlooked burden among Indian patients with heart failure, necessitating more regular testing in upcoming Indian offers.

In this research, heart failure individuals with or without Iron deficiency differed significantly regarding their NYHA functional class. Prior extensive research has also demonstrated a relationship between heart failure with Iron deficiency, NYHA functional class, and job capability.[29,30]

Four randomized, placebo-controlled trials and two open, noncontrolled studies with positive results for iron supplementation in heart failure have all been reported. Supplementing with iron has been established to enhance echocardiographic markers of cardiac muscle function in addition to NYHA class and walking distance.[31,32] Unfortunately, Indian patients lack participation in these studies. This study aims to build the groundwork for future massive multicenter observational research in Indian participants and randomized interventional investigations. Absolute iron deficiency in heart failure can be brought on by lower intake as a result of anorexia, cardiac cachexia, poor iron absorption brought on by intestinal edema, and hepcidin-induced downregulation of iron transporters such as ferroportin. The functional iron deficit in HF is caused by processes related to those that cause anemia of chronic diseases or inflammatory conditions. HF is associated with higher amounts of inflammatory cytokines that promote hepatic hepcidin production which binds, internalizes, and degrades ferroportin. This leads to impairment of iron absorption into the blood from enterocytes and entrapment of iron in the storage pool (liver and reticuloendothelial cells). Together, these effects result in relative iron depletion.[33]

This study was done at a tertiary care facility in eastern India as a single-center study. It is challenging to generalize the results in India due to the many cultures and eating customs, needing multicenter, more extensive investigations. Second, it's essential to realize that research is observational. This research article has yet to be framed to explain the underlying pathogenesis of Iron deficiency in individuals with non-anemic heart failure. There were no controls obtained to analyze Iron deficiency in participants with or without heart failure.

Limitations of the study
1. This investigation was carried out at a tertiary care facility in Odisha as a single-center study. Given the size of India and its many cultures and eating customs, it is challenging to generalize the results, needing much bigger investigations.
2. It is essential to recognize that our study was observational.
3. The research needs to frame to clarify the underlying processes of Iron deficiency in heart failure individuals.
4. There were no controls obtained to assess Iron deficiency in participants with or without heart failure.
5. Without a detailed assessment of the three patients with HFrEF, HFmrEF and HFpEF were included.

Conclusion
Our research emphasizes the impact of Iron deficiency on heart failure patients in India, which is still underappreciated and ignored. It also stresses that some data supports the effects of iron deficiency on healthy heart function (including both systolic and diastolic); however, extensive research is required to generalize the findings. This article recommends more in-depth analysis to correctly define this manageable illness and explore routine testing in upcoming national recommendations.

Funding

Conflict of interest
None declared.

Ethical approval
The study was approved by the Institutional Ethics Committee, Institute of Medical Sciences, and SUM hospital (ECR/627/Inst/OR/2014/RR-20).

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Journal of Associated Medical Sciences

The usefulness of the modified deep convolutional neural network model in improving the detection of COVID-19 on chest X-ray images

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ARTICLE INFO

ABSTRACT

Background: The COVID-19 pandemic has rapidly spread worldwide, leading to a global health crisis. Although the real-time polymerase chain reaction (RT-PCR) test is highly specific and sensitive in detecting COVID-19, chest X-rays have emerged as an optional diagnostic tool for COVID-19-induced lung lesions. Artificial intelligence (AI), particularly deep learning, is a rapidly evolving field with significant potential in medical image analysis, including the quick detection of COVID-19 to improve accuracy.

Objectives: This study aims to enhance the accuracy of COVID-19 image detection on chest X-ray images by modifying the deep convolutional neural network.

Materials and methods: We conducted lung segmentation and COVID-19 image classification experiments using a dataset of chest X-rays. The U-net algorithm was utilized for lung segmentation of COVID-19 and non-COVID-19 images. We developed a Modified Deep Convolutional Neural Network (MD-CNN) to classify the two image classes. The MD-CNN model was compared with two other models, ResNet and AlexNet, and evaluated for accuracy, sensitivity (recall), specificity, positive predictive value (precision), F1-score, and area under the curve (AUC).

Results: Our experimental results demonstrate that the MD-CNN model achieved an accuracy of 97.95%, outperforming ResNet and AlexNet, which achieved 90.25% and 78.95%, respectively. The MD-CNN model also exhibited better sensitivity, F1-score, and AUC than the other models, while its specificity and precision were comparable to those of the ResNet model.

Conclusion: The proposed MD-CNN model demonstrates significant potential for high accuracy in COVID-19 image detection compared to ResNet and AlexNet. It can serve as a useful tool for radiologists in the COVID-19 screening process, potentially reducing the workload, and improving the efficiency of COVID-19 diagnosis.

Introduction

The infectious Coronavirus (COVID-19) has spread worldwide, and as of April 3, 2023, the World Health Organization (WHO) has reported 762,201,169 confirmed cases of COVID-19, including 6,893,190 deaths.[1] The highly specific and sensitive tool for diagnosing the COVID-19 virus is real-time polymerase chain reaction (RT-PCR). However, WHO recommends reliable and accurate self-testing tools, such as SARS-CoV-2 Ag-RDTs, for self-testing for the COVID-19 virus.[2]

During the COVID-19 pandemic, chest X-rays and computed tomography are imaging instruments used to

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classify the types of lung diseases, such as tuberculosis, pneumonia, and COVID-19.[3] Ground glass appearance indicates increased whiteness due to dense density and progresses to consolidation with the complete loss of lung markings on the lung.[4] Anterior-posterior (AP) positioning of chest X-ray on a portable machine produces poor image quality compared to posterior-anterior (PA) chest imaging demonstrated in dedicated radiography facilities. The chest radiograph may appear normal in up to 63% of COVID-19 cases, while 1-3% may present nodules, pneumothorax, or pleural effusion that might be incidental, caused by COVID-19, or comorbidities such as tumors or emphysema.[4] Computer-aided diagnosis (CAD) is an artificial intelligence that helps prove the efficiency of radiologist diagnostics.[5] Common applications include the detection of cancer on mammograms[6] and lesions on chest images[7]. Machine learning and deep learning are two techniques used for classifying pathology or the class of image lesions in radiology[8].

Deep learning enables automatic lesion detection and image classification. Various studies have employed convolutional neural networks (CNNs) for image classification, with research indicating the effective and accurate diagnostic potential of CNNs for COVID-19.[9-11] However, the application of image augmentation techniques to enhance performance is limited by the constraints of a small dataset.

The integration of CNN and recurrent neural network (RNN) replaced the fully connected layers with an RNN model, presenting the combined model with high accuracy.[12] However, it is not suitable for low-resource devices with a minimum of 19 GB RAM consumption for model training. Another work introduced BasicCovn Architecture with Log Scaling, which classified COVID-19 with 95.8% accuracy, 0.989 AUC, and suggested splitting techniques, such as k-fold data splitting, that could improve effective training.[13] The development of the CNN model remains a challenge for classifying COVID-19 images by chest X-ray because of the difficulty in identifying the tissue. The objective of this study is to modify deep convolutional neural networks (MD-CNN) and compare them with two models (ResNet, AlexNet) in classifying images of COVID-19 and normal chest X-rays.

Materials and methods

This study consists of three sections: the first is lung segmentation on chest X-ray images, the second is COVID-19 and non-COVID-19 image classification, and the third is the evaluation of the performance of three models.

Lung segmentation
Dataset for lung segmentation study
We collected a total of 704 chest X-ray images containing chest X-ray images and mask images. These images were separated into two sets: a training set consisting of 563 images and a testing set consisting of 141 images. An example image is shown in Figure 1. The images were acquired from Kaggle, which obtained them from the Montgomery County X-ray Set.[14] The images were in Portable Network Graphics (PNG) format with 8 bits, and the image size was 256 x 256 pixels.

U-Net architecture for lung segmentation
The architecture of the U-Net model used in this study is shown in Figure 2.[15] The input consists of 704 images (256x256x1 pixel) processed by the U-Net model. The model consists of a contracting path and an expansive path. The contracting path follows the typical architecture of a convolutional network, with the repeated application of two 3x3 convolutions (unpadded convolutions), each followed by a rectified linear unit (ReLU) and a 2x2 max pooling operation with stride 2 for downsampling. At each downsampling step, the number of feature channels is doubled. Every step in the expansive path consists of an upsampling of the feature map followed by a 2x2 convolution (up-convolution) that halves the number of feature channels, a concatenation with the correspondingly cropped feature map from the contracting path, and two 3x3 convolutions, each followed by a ReLU. The cropping is necessary due to the loss of border pixels in every convolution. At the final layer, a 1x1 convolution is followed by a sigmoid.
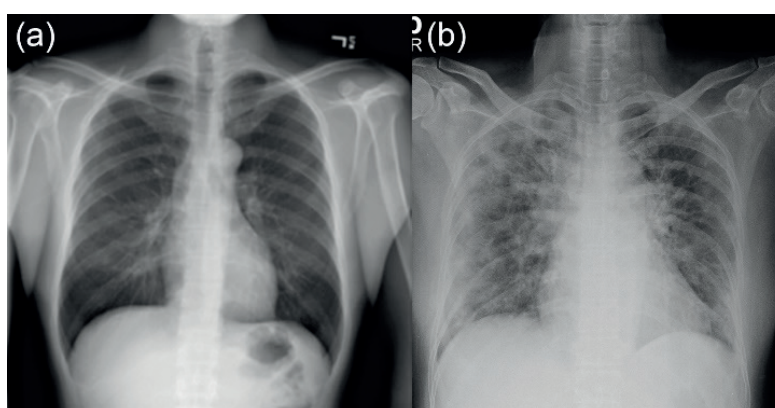
Figure 1 *Example image.*
(a: normal, b: CIVID-19 radiography)
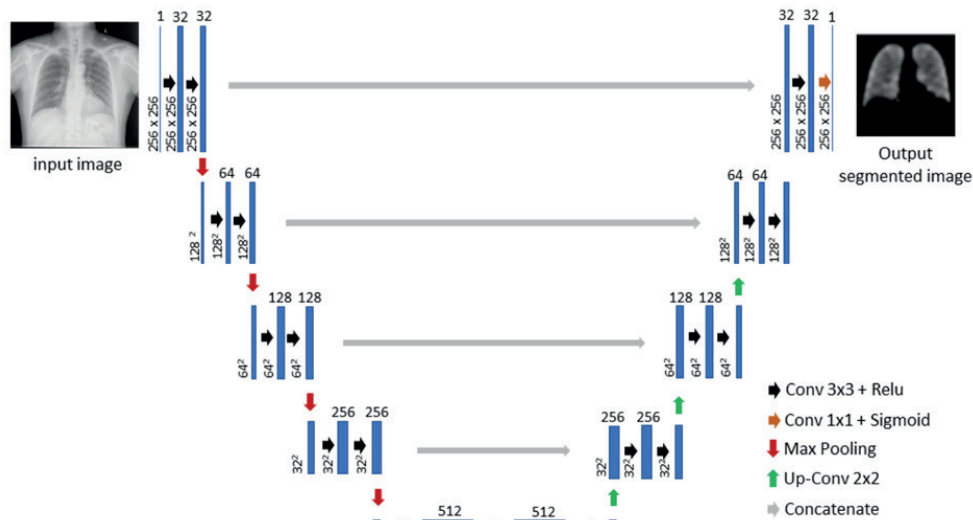
Figure 2 *U-Net architecture of this study.*

The image segmentation model was trained using a learning rate of 0.2 with a lower bound on a learning rate of 0.000001. The network training was set to a batch size of 32 for 70 epochs. The trained model was used to segment the lung region of the initial image in the image classification section.

The Dice similarity coefficient (DSC) was used to analyze the similarity between mask images and predicted segmentations, as shown in Equation 1.

$$DSC = A \cap B/(|A|+|B|) \qquad (1)$$

A is the mask area, *B* is the segmentation predicted area.

Dataset for image classification

A total of 20,000 chest X-ray images were collected, including 10,000 images labelled as COVID-19 (COVID) and 10,000 images labelled as normal (non-COVID-19). These images were randomly selected from the Kaggle dataset and segmented using the U-Net architecture for lung segmentation.[16]

Proposed image classification model: The modified deep convolutional neural network (MD-CNN)

The proposed model for the modified deep convolutional neural network (MD-CNN) architecture is shown in Figure 3. The proposed model differs from the conventional CNN in several aspects, including the input image size, the number of blocks for the convolutional layer, the dense layer, and the dropout rate. The overall parameters of the model are 4,947,162, which are all trainable. The model's details are as follows: the input layer receives 256x256x1. The feature extraction step consists of five blocks for the convolutional layer with a ReLU function, followed by a MaxPooling layer. The first and second blocks have two convolutional layers (3x3) with a ReLU function and are followed by a MaxPooling layer (2x2), while the third to fifth blocks have three convolutional layers (3x3) with a ReLU function followed by a MaxPooling layer (3x3). The output of feature extraction is sent to a flattened layer to convert the image data to a one-dimensional vector. The classification part of the proposed model consists of two dense layers (512), followed by a 0.2 dropout layer, and the last dense layer
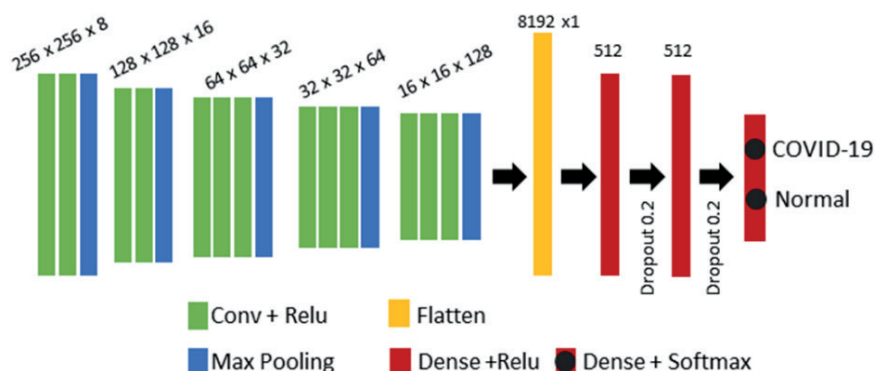
Figure 3 *The proposed model architecture.*

with SoftMax activation function produced four neurons that classify the output into two groups of image classes: COVID-19 and non-COVID-19.

To compare the performance of the proposed model, two models (ResNet and AlexNet) were selected in terms of their performance. Information on ResNet and AlexNet is shown below.

ResNet model

The input layer receives 256x256x1 images. The feature extraction step consists of five blocks. The first block has a convolutional layer with a batch normalization layer, followed by the Relu function layer, and then a MaxPooling layer. The second to fifth layers have a ResNet block layer with different filter sizes, followed by the Global Average Pooling layer. The classification layer includes a dense layer with SoftMax activation function, producing four neurons that classify the output into two groups of image classes: COVID-19 and non-COVID-19.

AlexNet model

The input layer receives 256x256x1 images. The feature extraction step consists of five blocks. The first and second blocks have a convolutional layer with a Relu function, followed by a batch normalization layer, and then a MaxPooling layer. The third and fourth blocks have a convolutional layer with a Relu function. The fifth block has a convolutional layer with a Relu function, a batch normalization layer, followed by the MaxPooling layer. The output of feature extraction is sent to a flattened layer to convert image data into a one-dimensional vector. The classification layer includes two dense layers (4096) with Relu, and the second dense layer is followed by a 0.5 dropout layer. The output layer is processed by a dense layer with SoftMax activation function, producing four neurons that classify the output into two groups of image classes: COVID-19 and non-COVID-19.

Training image classification process

The image classification dataset is separated into three sets, consisting of 14,000 images for the train set, 4,000 images for the validation set, and 2,000 images for the test set. The train set images are trained by three models (MD-CNN, ResNet, and AlexNet), set to a batch size of 32 for 70 epochs. The validation set uses the same parameters as the training process. All processes run on Google Colaboratory (Tesla P100-PCIE: GPU).[17]

Statistical analysis

The two-confusion matrix, which classifies two types of chest X-ray images (COVID-19 and non-COVID-19), describes the performance of a classifier in four terms:

True Positives (TP): the model detects correctly classified COVID-19 images.

True Negatives (TN): the model detects non-COVID-19 images on non-COVID-19 images.

False Positives (FP): the model detects COVID-19 images on non-COVID-19 images.

False Negatives (FN): the model detects non-COVID-19 images on COVID-19 images.

The performance of models is evaluated using a confusion matrix. Statistical analysis: The accuracy, sensitivity (recall), specificity, positive predictive value (precision), and F1-score are given in equations (2)-(6). The receiver operating characteristic curves (ROC) and area under curves (AUC) are also evaluated.

$$\text{Accuracy} = \frac{TP+TN}{TP+TN + FP+FN} \qquad (2)$$

$$\text{Positive predictive value(precision)} = \frac{TP}{TP+FP} \qquad (3)$$

$$\text{Sensitivity (recall)} = \frac{TP}{TP+FN} \qquad (4)$$

$$\text{Specificity} = \frac{TN}{TN+FP} \qquad (5)$$

$$\text{F1 - score} = 2 \times \frac{\text{precision x recall}}{\text{precision + recall}} \qquad (6)$$

Results

Figure 4 shows the learning accuracy of the training and validation of the proposed method (MD-CNN). The accuracy of the training set reaches 0.95 after 10 epochs, while the accuracy of the validation set fluctuates between 30 and 60 epochs. The accuracy of the ResNet model at the training phase reaches 0.9879, while it is 0.7689 for the AlexNet model.

Lung segmentation

Figure 5 shows the performance of the U-Net for lung segmentation. The Dice similarity coefficient (DSC) indicates the high efficiency of U-Net. Therefore, the lung region in the images used for image classification was completely segmented for the learning of the three models.

Performance evaluation of COVID-19 and non-COVID-19 image classification

Table 1 shows the confusion matrix of the three models. The number of true positive images in the proposed model was equal to that of ResNet and higher than that of AlexNet. Additionally, the true negative of the proposed model was higher than that of both ResNet and Alex.

Figure 6 shows the performance of three models, including accuracy, sensitivity, F1-score, and the area under curve (AUC). The proposed model's accuracy, sensitivity, and AUC were significantly higher than ResNet and AlexNet. However, the specificity and precision of the ResNet model were slightly higher than those of the proposed model.

Figure 7 shows the receiver operating characteristic curves and area under the curves of three models. The curve of the proposed model is closely located in the top-left corner, indicating that the proposed model has the best performance with an AUC equal to 0.9788. The second-best performance was achieved by the ResNet model (AUC = 0.9184), and the AlexNet model had the lowest performance (AUC = 0.7957).
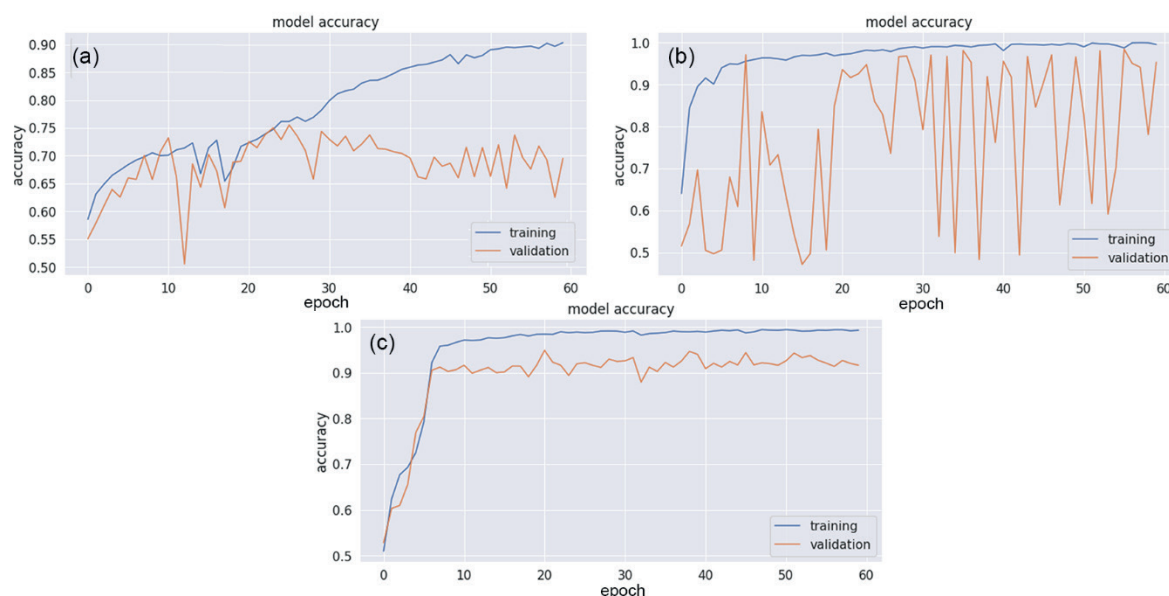
Figure 4 *Accuracy of the training and validation phase.*
(a: AlexNet, b: ResNet, c: the proposed method)
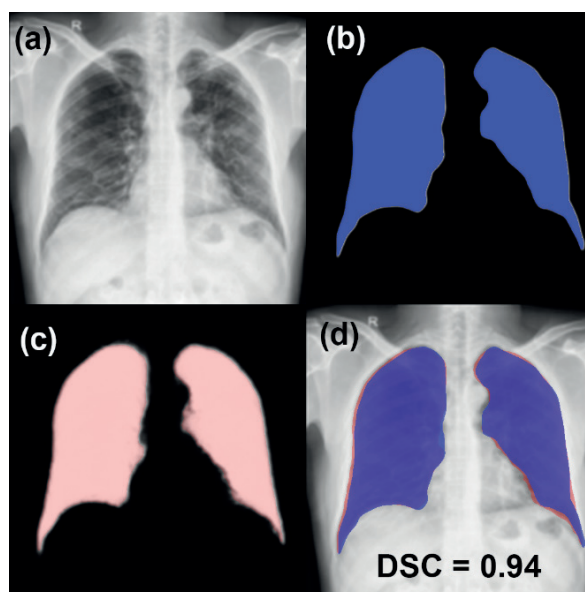
Figure 5 *The lung segmentation using U-Net.*
(a: the initial CXR image, b: ground truth mark (blue), c: the segmented predict (pink),
d: the overlap of (b) and (c) on (a) with an average of Dice similarity coefficient = 0.94)

Table 1 Confusion matrix of three models

Models	TP	FP	FN	TN
Proposed method	966	34	7	993
ResNet	1,000	0	195	805
AlexNet	862	138	283	717

*TP: true positive, FP: false positive, FN: false negative, TN: true negative
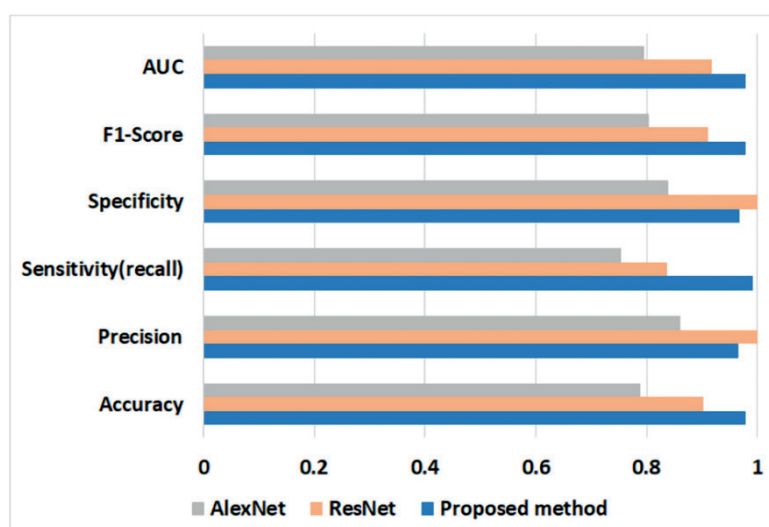
Figure 6 *The performance of three models including accuracy, precision, sensitivity, specificity, F1-score, and are under curve.*
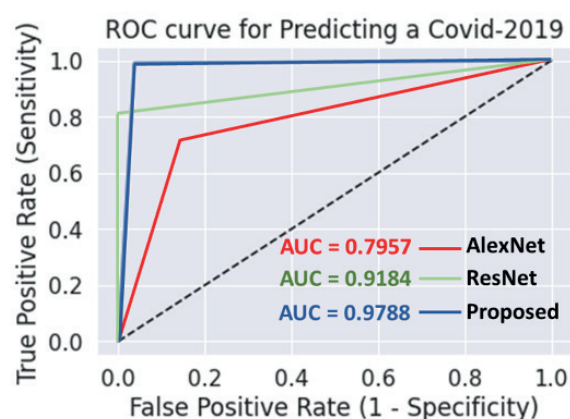
Figure 7 *Comparison of ROC and AUC of AlexNet, RetNet, and the proposed model.*

Discussion

This study consists of two sections including lung segmentation by U-net and image classification by three models (the proposed MD-CNN model, ResNet, and AlexNet). Lung segmentation is one factor that affects the efficiency of image classification on chest X-rays, which can be estimated by the Dice similarity coefficient (DSC) value. The DSC value shows the performance of image segmentation, with an ideal value of 1 indicating perfect segmentation. In this study, the average DSC was equal to 0.94, indicating that U-net can completely segment the lung region. Figure 5(d) shows the predicted lung segmentation closely overlapping the lung ground truth marks area in Figure 5(c).

To modify the convolutional neural network (CNN), we adapted the network structure including five blocks for feature extraction, a fully connected layer consisting of a flattened layer followed by two dense layers and 0.2 dropouts, and the last classification layer using Softmax activation function for classifying two-class images. The MD-CNN structure is very simple and highly performing compared to ResNet.

The accuracy, sensitivity, F1-score, and area under the curve of the proposed MD-CNN model were the highest (97.95%, 99.28%, 97.92%, and 0.98 AUC, respectively). The second-best was the ResNet model (90.25%, 83.68%, 91.12%, and 0.92 AUC, respectively). The lowest was the AlexNet model (78.95%, 75.28%, 80.37%, and 0.79 AUC, respectively). The precision and specificity of the ResNet model were 100% and 100%, respectively, because the ResNet model can correctly detect all 1,000 non-COVID-19 images. The efficiency of ResNet indicates that the complex structure affects the learning process in deep learning. The precision and specificity of the MD-CNN model (96.60% and 96.69%) were slightly lower than the ResNet model. The precision and specificity of the AlexNet model (86.20% and 83.86%) were the least efficient.

To compare the proposed MD-CNN model with the previous work that studied COVID-19 detection on X-ray images, the results of each study are shown in Table 2. Narin compared ResNet50, ResNet101, ResNet152, InceptionV3, and Inception-ResNetV2, and found that ResNet50 was the best model in terms of accuracy (96.1%) and F1-score (83.50%).[18] Medhi developed a CNN

Table 2. Comparative analysis of previous works.

Author	Image	Method	Accuracy	F1- score	AUC
Narin 2020[18]	341 COVID-19, 2,800 healthy	ResNet50	96.10	83.50	-
Medhi 2020[19]	14,000 images	CNN	93.00	-	-
Apostolopoulos 2020[20]	700 COVID-19, 1,204 healthy	VGG 19	98.75	-	-
Guefrechi 2021[21]	623 COVID-19, 3,000 healthy	VGG 16	98.30	98.00	-
Akter 2021[22]	26,000 COVID-19, 26,000 healthy	Modified MobileNetV2	98.00	97.00	-
Ismael 2021[23]	180 COVID-19, 200 healthy	ResNet50 Features + SVM	94.74	94.79	0.99
Our proposed	10,000 COVID-19, 10,000 healthy	MD-CNN	97.95	97.92	0.98

architecture with two blocks of two convolutional layers followed by max pooling layers using ReLu function, a kernel size equal to 3x3 in the feature extraction section, and a fully connected layer consisting of a flatten layer and a Softmax activation layer.[19] The accuracy of the CNN was 93.00%. Apostolopoulos compared VGG19, MobileNet v2, Inception, Xception, and Inception ResNet v2, and found that the accuracy of VGG19 was the highest (98.75%).[20] Guefrechi evaluated three models (VGG16, ResNet50, and InceptionV3), and found that VGG16 had the best efficiency in accuracy for COVID-19 prediction, with an accuracy of 98.30% and an F1-score of 98.00%.[21] Akter selected several models to experiment on COVID-19 detection, including VGG19, VGG16, InceptionV3, ResNet50, ResNet101, GoogleNet, MobileNet, AlexNet, EfficientNet B7, DenseNet121, NFNet, and compared the developed approach (Modified MobileNetV2).[22] Modified MobileNetV2 had the best accuracy (98.00%) and F1-score (97.00%). Ismael developed a hybrid deep learning (ResNet50 model) and machine learning (Support Vector Machine: SVM) and compared the performance with Fine-tuning of ResNet50, End-to-end training of CNN, and the Binarized Statistical Image Features (BSIF)+SVM. The ResNet50+SVM had the best accuracy in training (94.74%), F1-score (94.79%), and 0.99 AUC.[23]

The comparison indicates that the accuracy of several works was lower than our work, while the accuracy of several works was similar to our study. Several studies in Table 2 used a small dataset size, which is important to note as it may lead to overfitting.[18-23]

Limitations

The limitation of this work is the concern of the overfitting problem. Overfitting is a common pitfall in deep learning where small training data size, a long time of training on a single set of data, noise data with a large amount of irrelevant information, or a complex model can lead to inaccurate predictions. The way to avoid overfitting is by using a large number of datasets, adding weight regularization, and dropping out.

In this study, we used 20,000 images compared to the data of previously published work, and the number of images used in this study is larger than several works.[18,21,23] However, this may still cause overfitting. In the case of using a small dataset, overfitting can be treated by image augmentation.

Image augmentation is a method of increasing the image dataset by varying the characteristics of the

image such as reflection, rotation, scaling, shearing, and translation. In the case of chest X-rays, the structure of the lung is complex, and image augmentation may not help to improve the performance of prediction. Elgendi *et al.* reported that the use of geometrical image augmentation in X-ray images may not be effective for detecting COVID-19.[24] The accuracy of COVID-19 prediction with augmentation was 93.42%, while the accuracy of COVID-19 prediction without augmentation was 97.95%. The pattern of the chest image is an important factor for image augmentation. Therefore, it is necessary to choose a suitable technique for the lung organ.

Another very important factor is dropout, which is a regularization method that can help reduce irrelevant image data. Our work set two dropouts at 20% in the fully connected layers, after a dense layer. Tan *et al.*[25] studied the effect of different dropout percentages on training accuracy, comparing dropout rates of 20%, 40%, 60%, and 80%. The results showed higher training accuracy for 20%, 40%, and 60% dropout rates, while 80% resulted in lower training accuracy. Dropout rates of 20% and 40% resulted in lower training loss, while a 60% dropout rate resulted in high training loss, and an 80% dropout rate resulted in the highest training loss.

The image resolution of the input image is another overfitting parameter. In this work, the input image had a size of 250x250 pixels, which is a large image matrix size. The image size influences the learning of the model, with larger sizes being more prone to overfitting than smaller sizes.[26] Therefore, the large image matrix size affects the training time. However, a high resolution can improve the performance of image classification.[27,28]

Conclusion

In conclusion, the classification of chest X-ray images to distinguish between COVID-19 and non-COVID-19 cases is a challenging task. In this study, we developed a deep learning model based on the modified deep convolutional neural network (MD-CNN) architecture, which demonstrated superior performance compared to the ResNet and AlexNet models. Our proposed MD-CNN achieved a classification accuracy of 97.95%, highlighting its potential as an effective tool for COVID-19 diagnosis. However, the limited size of the dataset used in this study could be a potential limitation.

The results of this study also suggest that overfitting can be a significant issue when working with a small dataset, and techniques such as image augmentation may

be necessary to address this challenge. Future work could focus on exploring other deep learning architectures or ensemble methods to further improve the accuracy of COVID-19 diagnosis. Additionally, efforts to collect larger and more diverse datasets of chest X-ray images could enable the development of even more robust models for COVID-19 diagnosis.

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Two-stage method for hepatocellular carcinoma screening in B-mode ultrasound images

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ARTICLE INFO

ABSTRACT

Background: Hepatocellular carcinoma (HCC) is a significant global health concern that requires early detection for effective treatment.

Objectives: The objective of this study was to develop a system for screening HCC in B-mode ultrasound images.

Materials and methods: The dataset consisted of 1665 hemangioma (HEM) images, including 961 typical HEM, 704 atypical HEM, and 543 HCC images. Four YOLOv4 models were trained: one for HCC detection, one for the conventional two-class detection of HEM and HCC, one to detect typical HEM and suspicious lesions, and the last one was our two-stage model consisting of a detector and classifier. In the first stage, a YOLOv4-based detector with ResNet-50 as the backbone was used to identify focal liver lesions. The second stage utilized ResNet-50 as a classifier to classify the lesions into HCC, atypical HEM, or typical HEM. Differentiating between HCC and atypical HEM is not necessary, as both require further investigation with CT or MR imaging.

Results: The evaluation of the developed HCC screening system using ten-fold cross-validation showed that grouping HCC and atypical HEM together significantly increased precision from 0.74 to 0.88 and improved HCC recall from 0.64 to 0.68. Furthermore, employing the two-stage method further improved HCC recall from 0.68 to 0.72.

Conclusion: The results indicate that combining HCC and atypical HEM into a single class and using a two-stage approach for detection led to substantial improvements in precision and HCC recall. These findings highlight the potential of the developed system for effective HCC screening in B-mode ultrasound images. The two-stage method provided better detection than the detector-only method. More accurate detection was achieved when lesions were classified based on appearance and clinical protocols.

Introduction

Ultrasound imaging is the common liver screening protocol and often the first tool to detect the early stage of hepatocellular carcinoma (HCC), the most common liver cancer. However, it must be followed by other imaging modals (contrast-enhanced ultrasound: CEUS, computed tomography: CT, or magnetic resonance imaging: MR) for definite diagnosis due to the shared sonographic appearance of HCC and hemangioma (HEM) (Figure 1).[1,2] Deep learning models have been applied for liver lesion classification as well as detection.[3-11] It is hypothesized that the model is capable of capturing the difference invisible
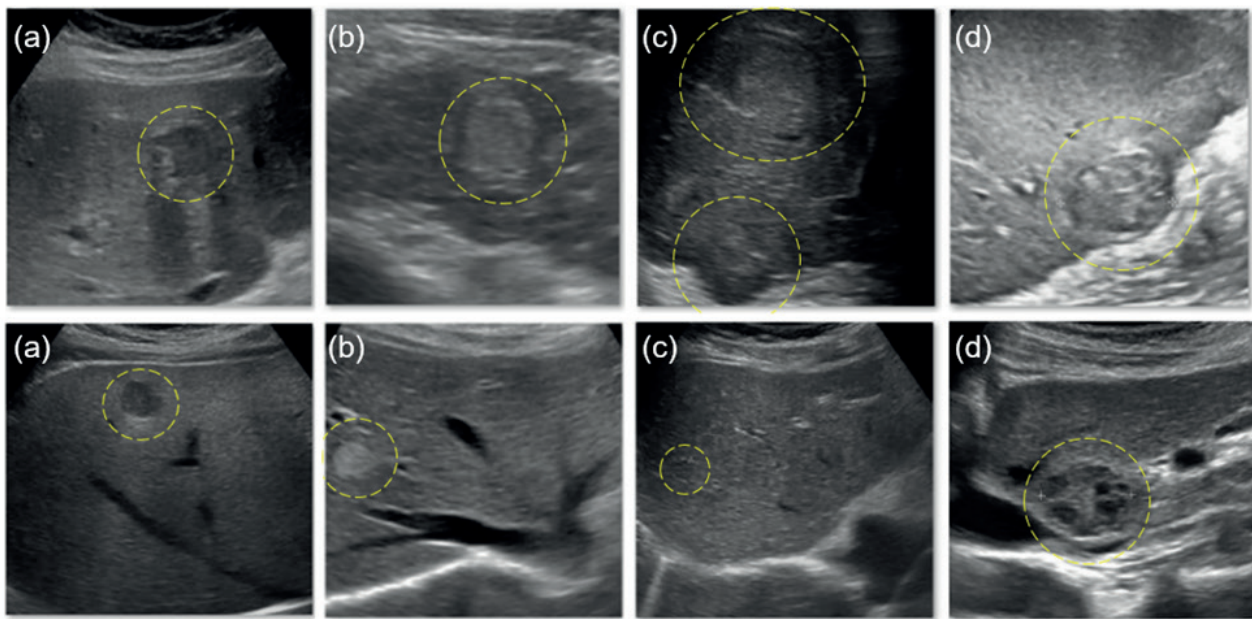
Figure 1 *Echogenic patterns of HCC and HEM lesions. Top row: HCC lesions. Bottom row: HEM lesions.*
a: hypoechoic, b: hyperechoic, c: isoechoic, d: mixed echoic appearances.

to humans, if it has been trained by a sufficiently large dataset. The limited dataset is among the major problems of deep learning models. From our survey, the RetinaNet in Tiyarattanachai *et al* was trained by the largest dataset (20432 lesions which included 2414 HCC).[12] Nevertheless, the recall of HCC was the lowest among other liver lesions and had the highest deviation. The high deviation indicated that the number of data was too small.

In this paper, we hypothesized that the available dataset was not sufficient for differentiating HCC from HEM. Thus, we followed the clinical protocol and categorized HEM into two groups, typical and atypical. The typical HEM is uniform hyper-echogenicity and a well-defined margin.[13] It will be monitored for change during the follow-up. The atypical hemangioma exhibits various imaging features and shares similarities with HCC.[13] Both are grouped into suspicious lesions and will be sent for further investigation by CT or MR imaging.[2,14,15] We tackled the problem of a limited dataset by applying the two-stage method which had the detection of HCC and HEM lesions followed by the classification to typical HEM, atypical HEM, and HCC.

Materials and methods
Dataset
The abdominal ultrasound images used in this retrospective study encompass both upper abdominal ultrasound images and whole abdominal ultrasound images. The study received approval from Chulabhorn Research Institute, Thailand (CRI No. 098/2563), as well as the Institution Review Board of the Faculty of Medicine, Chulalongkorn University, Thailand (IRB No. 485/2563). The images utilized were obtained from the period 2015 to 2019 at these two institutions. The inclusion criteria for selecting the images are outlined below.

- 2D ultrasound images from a curvilinear transducer. Due to the diverse range of ultrasound machine brands employed across the two hospitals, the dimensions and resolution of the images varied.
- HCC and HEM were confirmed by CT or MR reports.

The dataset consists of 961 typical HEM, 704 atypical HEM, and 543 HCC images. A skilled sonographer drew the lesion boundary. Compared to previous studies, Our dataset is larger than previous studies, however, it is much smaller than Tiyarattanachai *et al*.[12,16,17]

For data preparation, all images were converted to grayscale and cropped to focus on the liver by removing extraneous black areas. They were resized to 224x224 pixels to fit the input requirement of the ResNet-50 model. Patient information was removed. Some images contained markers, but their presence did not significantly impact the detector due to the mixed presentations (Figure 2). Therefore, the markers were not removed in this study.

Methods
The proposed two-stage method is depicted in Figure 3. The detector in the first stage was trained to detect focal liver lesions. Both HCC and HEM are focal liver lesions, so the training data becomes a combination of HCC and HEM images. The size of the training dataset was larger than the model where HEM and HCC were separately considered. Furthermore, the shared sonographic appearance of HEM and HCC can be exploited for better detection.

The result of the first stage was resized to 224x224 images and inputted to the classifier in the second stage. The classifier categorized lesions into three classes: typical HEM, atypical HEM, and HCC. Differentiating HCC from atypical HEM was not crucial as both required further investigations.
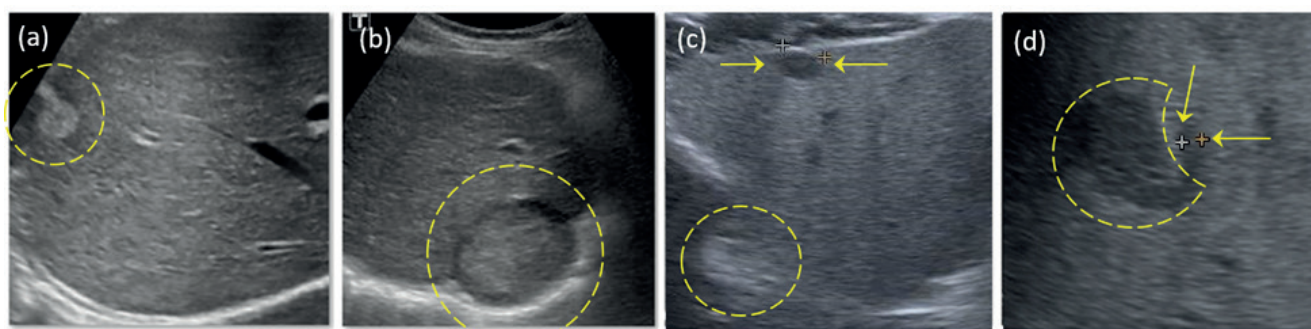
Figure 2 *Ultrasound images with and without markers. HEMs and HCCs show inside a dashed circle. a: HEM without a marker, b: HCC without a marker, c: a marker for hepatic cyst in HEM image, d: a marker for vessels near HCC.*
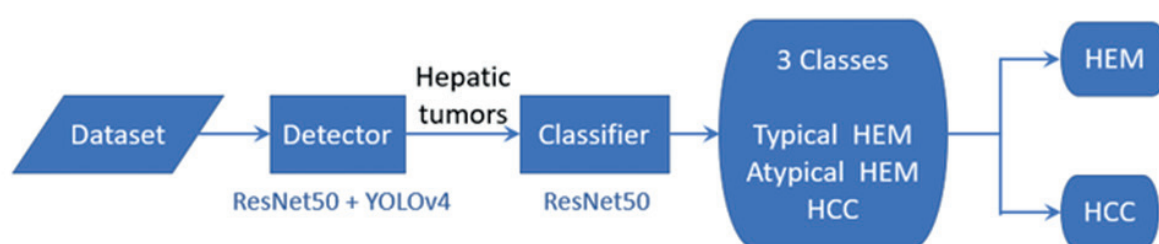
Figure 3 *Proposed two-stage method.*

The models for the detector and the classifier were selected from the available models in the Deep Learning Toolbox of MATLAB 2022a (license number 40662904). In the preliminary experiment, we compared the performance of four detectors: regions with convolutional neural networks (R-CNN), single shot detector (SSD), You Only Look Once (YOLO) v2, and YOLOv4. SSD failed to provide accurate detection. R-CNN and YOLO had comparable accuracy, but R-CNN required much longer training times. YOLOv2 and YOLOv4 had comparable accuracy but YOLOv4 offered a more precise lesion location. Thus, YOLOv4 was chosen as the detector. The architecture of YOLO consists of a backbone, neck, and head. The backbone acts as the feature extractor, while the neck is used to connect the features to the head, which provides the detection output. Pretrained convolutional networks are used as the backbone. In our preliminary experiment, ResNet-50 provided a performance better than CSPDarkNet53. Therefore, we used YOLOv4 with ResNet-50 as the backbone of this study. For the classifier, GoogLeNet, VGG-16, ResNet-18, and ResNet-50 were tested. ResNet-50 offered the highest accuracy, so it was selected as the classifier. All CNN networks were pre-trained using the ImageNet database.

Setting
All models were implemented in MATLAB 2022a on a personal computer (CPU: Intel Xeon, RAM: 128 GB, Video Card: NVIDIA 16 GB). Ten-fold cross-validation was used for performance comparison. We compared the proposed two-stage method with the following three detectors.
1. Model 1: HCC detector trained by HCC images only.
2. Model 2: HCC and HEM detector where HCC and HEM were considered as separate classes.

3. Model 3: typical HEM and suspicious lesion detector. HEM was divided into a typical HEM and an atypical HEM. Atypical HEM and HCC were grouped into suspicious lesion classes.

In most previous works detectors were trained to find HCC as a distinct lesion from HEM.[3,7,12,16,17] So, the first two models were used as the baseline models. The third model follows the clinical protocol and divided the lesions into typical HEMs for future monitoring and suspicious lesions for additional investigation. These three models were compared with Model 4, which is the initial stage of our two-stage method. Model 4 was trained to detect focal liver lesions which combine HCC and HEM in the same class.

To address the variability in the direction of the ultrasound beam, which can depend on the user (radiologist/sonographer), image data augmentation techniques were employed. Specifically, a rotation of ±5 degrees and vertical/horizontal flipping were applied. The dataset for the classifier consisted of manually drawn lesion areas extracted from the image dataset used to train the detector. These lesion areas were resized to 224x224 pixels.

The experiment was divided into three parts to assess the performance of the proposed two-stage method. The first part focused on investigating the detector's accuracy and error. The second part examined the classification accuracy of the ResNet-50 model. Finally, the overall accuracy of the two-stage method was evaluated against the detector-only method.

Performance evaluation
Intersection over Union (IoU) is the ratio of the

area of overlap and the area of union. It is often used to evaluate the result of a detector. In this experiment, the result of the detector was considered correct if the IoU was at least 50%. The classification was then evaluated by the following metrics.

$$accuracy = \frac{TP + TN}{TP + TN + FP + FN} \qquad (i)$$

$$recall = \frac{TP}{TP + FN} \qquad (ii)$$

$$precision = \frac{TP}{TP + FP} \qquad (iii)$$

$$negative\ predictive\ value = \frac{TN}{TN + FN} \qquad (iv)$$

$$F1 - score = \frac{2 \times precision \times sensitivity}{precision + sensitivity} \qquad (v)$$

where *TP, TN, FP* and *FN* are the number of true positive, true negative, false positive, and false negative, respectively.

In these evaluation metrics, a value close to 1 indicates good performance, while lower values indicate poorer performance. In addition, the average precision was also used. The average precision is the precision averaged over all the detection results. The higher the average precision indicates the better detector. Since ten-fold cross-validation was used, all metrics were averaged from the 10 experiments. Note that recall will be mostly focused since it is the most important metric for screening tools.

Result and discussion
Performance evaluation: detector

The detection result is presented in Table 1. The target lesion was considered positive. All models were tested with both HCC and HEM images. The results indicated that the detector trained to specifically detect HCC (Model 1), achieved a higher recall rate compared to the two-class model used in Model 2, but it came at the cost of the inability to differentiate HEM from HCC (low precision). The recall of Model 2 varied from 0.53 to 0.97

which indicated low repeatability. Model 3 had a high recall rate for detecting suspicious lesions, but when only HCC was considered, the recall rate dropped to 0.68.

The finding is consistent with other studies on the detection of malignant tumors, such as Cao *et al.* who used SSD to detect breast tumors in ultrasound images, and Tanaka *et al*. who developed a computer-aided diagnosis (CAD) system for classifying breast cancer but achieved a detection rate of less than 50% of breast tumors in ultrasound images.[18,19] A recent study in 2021 by Tiyarattanachai *et al*.[12] reported a high recall of 0.74 for HCC detection using RetinaNet, but this was achieved by lowering the IoU threshold to 0.2.

The best detection result was achieved by Model 4. The combination of HEM and HCC in the focal liver lesion group provided a larger dataset that could be used to train the detector to identify the distinct characteristics of both types. Notably, the detector successfully detected HCC lesions missed by the first three models, as shown in Figure 4.

To ensure that the higher recall of Model 4 led to better HCC detection. The detection result was categorized into 3 classes: HCC, HEM, and others (incorrect detection) and shown in Table 2. It is worth noting that certain images contained multiple HEM/HCC lesions, and YOLOv4 did not detect all of them. Some lesions were detected multiple times, as shown in the last row of Table 2 and Figure 5. The result indicated that Model 4 outperformed the other three models, with recall rates of 0.78 for HCC and 0.86 for HEM.

Model 4 exhibited two types of detection errors. The first type involved the failure to detect focal liver lesions, impacting the recall of the two-stage method. The second type was the misdetection of other areas/lesions as focal liver lesions, affecting precision. The second type of error constituted less than 5% of the total test data and could be easily dismissed by radiologists during follow-up.

Among 135 undetected HCC, 118 lesions (87%) did not have the sonographic appearance of HCC. Most of these lesions displayed features such as faint opacity, isoechoic tumor, or incomplete border. These lesions were

Table 1 Detection results of four YOLOv4 models. The values in the parenthesis were the range of the matrices.

	Model 1 **1 class** **543 HCC**	**Model 2** **2 classes** **1) 1665 HEM** **2) 543 HCC**	**Model 3** **2 classes** **1) 961 typical HEM** **2) 1247 suspicions**	**Model 4** **1 class** **2208 HEM and HCC** **like lesions**
Accuracy	0.52 (0.39-0.61)	0.85 (0.73-0.89)	0.72 (0.70–0.77)	0.86 (0.82-0.88)[#]
Precision	0.54 (0.43-0.61)	0.74 (0.50-0.88)	0.88 (0.84-0.93)[#]	0.88 (0.82-0.91)
Recall	HCC 0.67 (0.54-0.86)	HCC 0.64 (0.53-0.97)	Suspicion 0.70 (0.63-0.75)	HEM and HCC 0.84 (0.79-0.89)[#]
F1-score	0.71 (0.64-0.87)	0.68 (0.51-0.93)	0.78 (0.75-0.82)	0.86 (0.83-0.89)[#]
NPV*	0.60 (0.48-0.70)	0.87 (0.82-0.92)[#]	0.72 (0.65-0.76)	0.86 (0.81-0.88)
mAP**	0.50 (0.32-0.68)	0.49 (0.37-0.61)	0.60 (0.51-0.65)	0.76 (0.73-0.84)[#]

Note: [#]*the best result for the given evaluation matrix, *NPV: negative predictive value, **mAP: mean average precision*
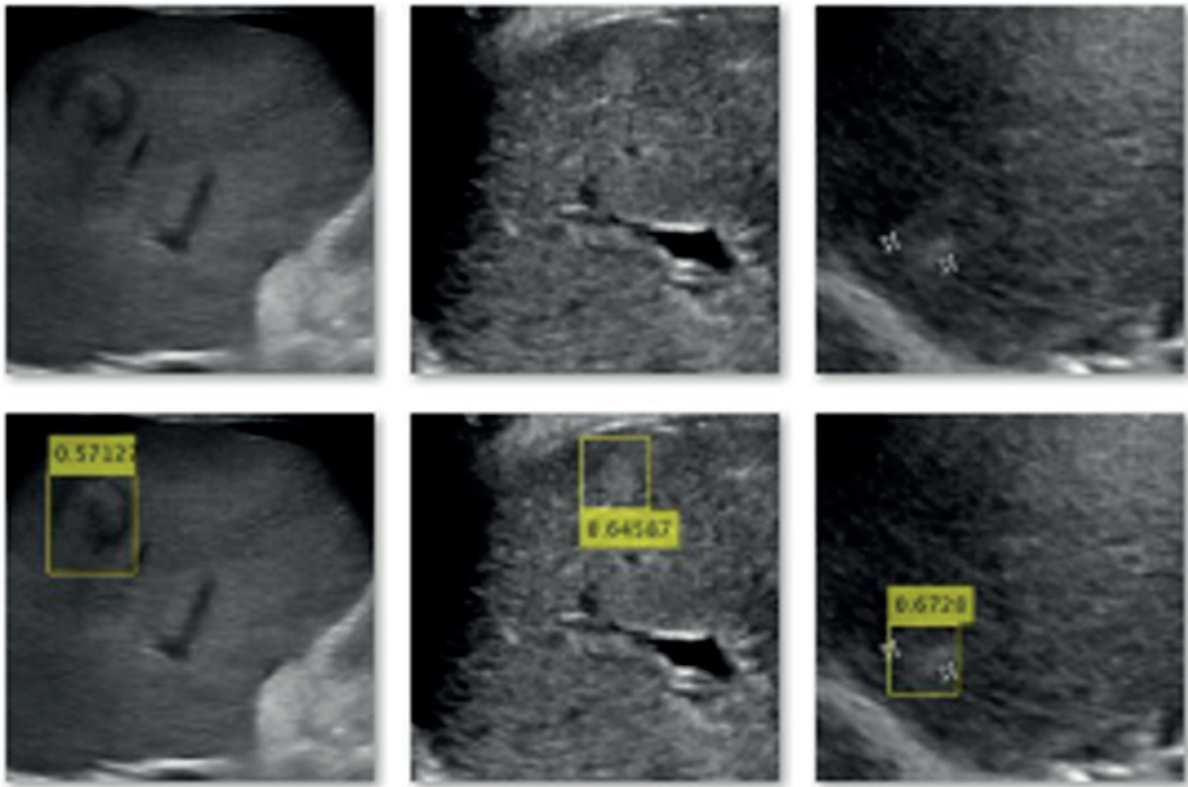
Figure 4 *Training YOLOv4 to detect HEM and HCC as one class improved HCC detection (bottom row) compared to training them as separate classes (top row) which failed to detect the lesions.*

Table 2 Detection results of Model 4 as grouped by lesion type.

Detector model YOLOv4	The number of detected lesions (actual value)			
	HCC	**HEM**	**Others**	**Total***
Images	472 (543)	1455 (1665)	68 (0)	1927 (2208)
	86.92%	87.39%		87.73%
Lesions	480 (615)	1479 (1721)	68 (0)	1954 (2336)
	78.05%	85.94%		83.64%
Lesion + Redundancy	489 (624)	1494 (1734)	68 (0)	1983 (2358)
	78.37%	86.16%		84.10%

*Total is the sum of the HCC and HEM only. Other lesions were not considered.
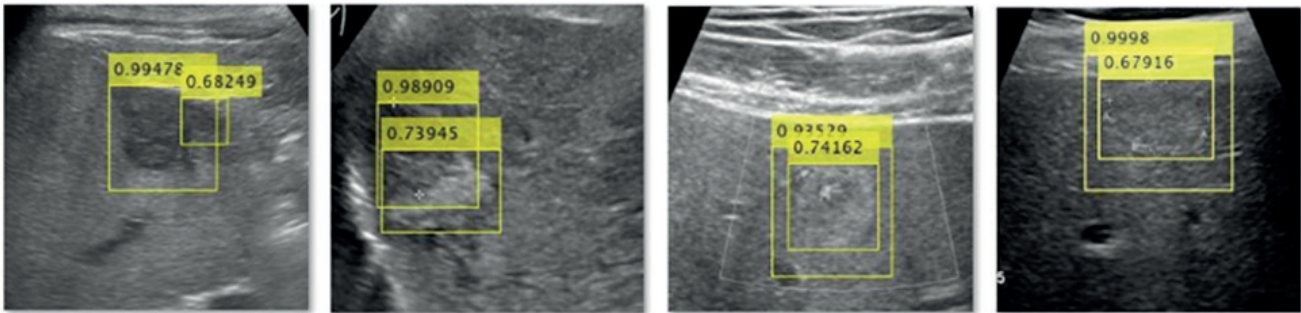
Figure 5 *Examples of multiple detection of the same lesion by YOLOv4.*

detected in further CT or MR scanning. In clinical protocol, if a new lesion appears where nothing was shown in the previous scanning, irrespective of the appearance, CT or MR scan is requested. Without the previous records, it is impossible to detect these HCC. Furthermore, if one HCC is detected, the entire liver will be scanned by CT or MR imaging. Thus, the detections of every HCC or at least one in an image have the same outcome. In this sense, it is possible to conclude that YOLOv4 could detect 86.92% of HCC patients. However, it is not guaranteed that at least one lesion would be detected, so all undetected HCC was considered false negative in this study. We concluded that the focal liver lesion detector had a 0.78 recall rate for HCC.

Performance evaluation: classifier

ResNet-50 was applied to classify focal liver lesions into three classes: typical HEM, atypical HEM, and HCC. Table 3 presents the confusion metric of the classification, where only the correct results of the first stage were considered. The 472 HCC images detected by the first-stage detector had 480 lesions (from Table 2), and the 1455 HEM images had 1479 lesions (570 atypical and 909 typical HEMs). Note that the number of detected HCC was 489 due to the multiple detection of some HCC lesions.

With the limited dataset, it is impossible to prove whether the deep learning model can differentiate the difference between atypical HEM and HCC in a B-mode ultrasound image. However, the further treatment plan for both lesions is the same, i.e., scheduled for CT or MRI examination. Therefore, the detection of HCC as atypical HEM did not pose a health risk. Table 3 was modified to Table 4 where the HCC incorrectly detected as atypical HEM is accepted as the correct classification. According to Table 4, the HCC recall rate of 0.92 (448/489) was achieved. The accuracy and the negative predictive value (HEM = negative) were 0.90 and 0.97, respectively. When HCC was considered positive, the precision (0.74) was much lower than the other values. This is because ultrasound imaging is not a tool to differentiate HCC from atypical HEM. Among 157 errors, 59 images were atypical HEMs. If atypical HEM was considered the same class as HCC (instead of HEM), the precision would jump to 0.82.

A more serious problem was an HCC incorrectly classified as a typical HEM. Out of the 41 HCC incorrectly classified as typical HEM, 22 lesions closely resembled typical HEM. These 22 lesions were well-defined and hyperechoic (Figure 6). Some of these lesions were detected in further CT or MR examinations because they were either presented 1) in a liver with multiple HCCs or 2) new lesions that appeared in the area without any lesions during the previous ultrasound screenings. Furthermore, some misclassification occurred, because the detector did not extract enough area of the HCC lesion as shown in the leftmost image of Figure 5.

The classifier was trained by the ground truth lesions. The classification result would be better if the classifier was also trained using the detection result. However, we would like to evaluate the performance independent of the detector, so the ground truth was used.

Performance comparison: two-stage method

In this experiment, we compared the proposed two-stage method (Model 4) with the detector-only model (Model 3). Since atypical HEM and HCC have the same appearance and require further CT or MR examination, distinguishing between them is unnecessary. We compared the results of Model 3 with the proposed two-stage method. HCC was considered positive, while HEM was considered negative. The incorrect detection of Model 4 was not classified but would be considered as getting a negative (HEM) classification. The accuracy and the recall rate were calculated based on the number of actual HCC (not the number of detected areas). If an HCC lesion was

Table 3 Results of YOLOv4 Detector and ResNet50 Classifier on 3x3 confusion matrix.

	Actual Class			
Class	HCC	Atypical HEM	Typical HEM	Total
HCC	337	59	98	494
Atypical HEM	111	486	135	732
Typical HEM	41	29	687	757
Total	489	574	920	1983

Table 4 Results of modified 3x3 confusion matrix as 2x2 confusion matrix.

	Actual Class		
Class	HCC	HEM	Total
HCC	448	157	605
HEM	41	1337	1378
Total	489	1494	1983
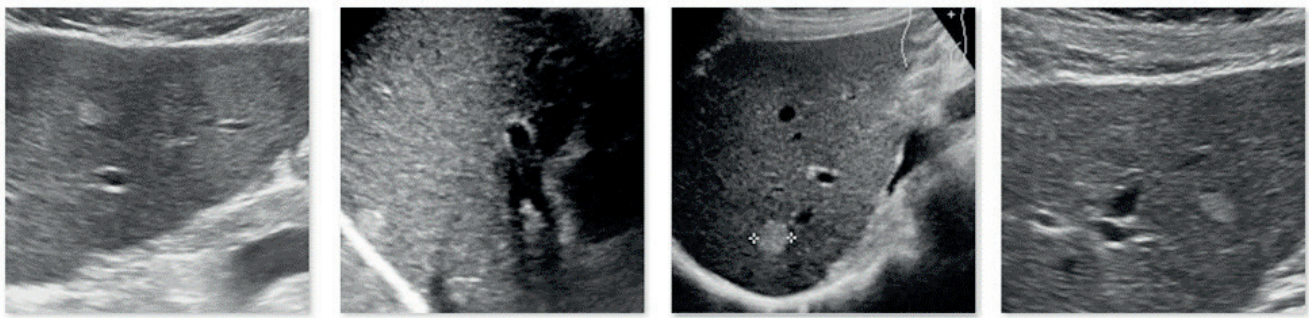
Figure 6 Small oval-shaped hyperechoic HCC lesions were misclassified as typical HEM by the Classifier.

Table 5 Results of HCC detection by the detector only and the two-stage methods.

	Model 3	Two-stage method		
		Model 4	Classifier	Overall
Accuracy	0.72	0.86	0.90	0.77*
Precision	0.88*	0.88*		
Recall	Suspicious 0.70	HCC+HEM 0.84	0.90	HCC+HEM 0.76*
	HCC 0.68	HCC 0.78	HCC 0.92	HCC 0.72*
F1-score	0.78	0.86*		
Negative predictive value	0.72	0.86		
Mean average precision	0.60	0.76*		

*Note : * the best result for the given evaluation matrix.*

detected more than once, only one instance classified as HCC was enough for further examination and would be considered as correct. The result is presented in Table 5. Except for precision, the proposed two-stage method provided better performance. Both detectors-only and two-stage methods provided the same precision.

The two-stage method outperformed the detector-only method in the experiment, improving the HCC recall from 0.68 to 0.72. This enhancement signifies a meaningful improvement in the ability to correctly identify and detect HCC cases. Despite using out-of-the-box models not specifically designed for medical imaging, the achieved recall rate of 0.72 was comparable to previous findings.[12] The dataset in our work is smaller so the number of the training image was much lower (615 HCC vs 2414 HCC). Furthermore, Tiyarattanachai *et al*.[12] reported a recall rate of 0.74 by setting the accepted IoU threshold to 0.2, which was considered incorrect detection in our work. There were other works that demonstrated high accuracy.[16,17] However, the database was too small to make a solid conclusion.

Our two-stage method allows for easy improvement as the detector and classifier can be trained separately. YOLOv4, the detector used in our study, has been surpassed by the more recent YOLOv8 (available at https://ultralytics.com/yolov8). Replacing YOLOv4 with YOLOv8 would lead to quick improvements in our method. Additionally, while ResNet-50 provided good classification, optimal results could be achieved by pre-training the network with medical images instead of the ImageNet database. We are currently developing a shallow network specifically for lesion classification in liver ultrasound images due to the limitations of training ResNet-50 with a small database.

Limitation

Two limitations of this experiment are dataset limitations and lack of external validation in real clinical settings that could limit the reliability and real-world applicability of the developed model. Additionally, the use of YOLOv4, as a deep learning model, may present challenges in understanding the decision-making process.

Conclusion

The proposed method for HCC detection from ultrasound images is a two-stage approach. In the first stage, a detector was trained to capture all focal liver lesions. In the second stage, the classifier was trained to distinguish HCC, atypical HEM, and typical HEM. The classification of HCC was not strict in the sense that HCC is allowed to be detected as atypical HEM since the future plan for HCC and atypical HEM is the same. The experiment showed that the two-stage method outperformed the detector-only method in HCC detection. The findings suggest that training separate models: detection and

classification models, led to higher efficiency and accuracy in detecting and classifying hepatic lesions.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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The measurement of radiation doses in brachytherapy using an alanine dosimeter

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ARTICLE INFO

ABSTRACT

Background: Brachytherapy involves the use of high radiation doses to treat cancer patients, making it essential to have appropriate dosimeter properties to confirm the accuracy and precision of the dose delivered to the patient.

Objectives: This study was to investigate the relationship between radiation dose and electron spin resonance (ESR) signal and to evaluate the optimal conditions for the ESR technique. Additionally, the radiation dose from brachytherapy was measured in a solid phantom using alanine in combination with the ESR technique.

Materials and methods: The alanine dosimeter (FWT-50-10, Steris, USA), ionization chamber (TW30013, PTW Freiburge, Germany), and transferring tube were positioned inside the solid phantom (Krieger, T9193, PTW Freiburge, Germany), with the BEBIG Co-60 source located at the center of the phantom. Dwell times were calculated to obtain a radiation dose range of 0.06-1.5 Gy. Following irradiation, the alanine derivative was measured using an ESR spectrometer, and a graph was generated to determine the relationship between radiation dose and ESR signal. The uncertainty and fading of the ESR signal were also evaluated.

Results: The results indicate that there is a linear relationship ($R^2 = 0.877$) between the radiation dose range of 0.49-1.5 Gy and ESR signal, with a microwave power of 1.5 milliwatt. The uncertainty of the ESR signal was found to be in the range of 0.12% - 3.79%. Signal fading was observed to be in the range of 7.2% - 27.4% over a period of two weeks.

Conclusion: Alanine and ESR technique can be used to measure absorbed dose in brachytherapy. The dose response of alanine was linear for radiation doses above 0.49 Gy. The advantages of alanine dosimetry are that alanine is tissue equivalent, nondestructive, small in size, and has low signal uncertainty.

Introduction

Brachytherapy is a cancer treatment that involves the insertion of a radioactive source enclosed in a catheter into or near a tumor. This treatment is suitable for well-circumscribed cancers, such as cervix, prostate, and breast cancer. The most common radioactive sources used in brachytherapy are Iridium-192 (energy 0.38 MeV), Cobalt-60 (energy 1.25 MeV), and Cesium-137 (energy 0.66 MeV). The beta and gamma radiations emitted from these sources have higher energy than diagnostic X-rays. The effectiveness of radiotherapy lies in delivering high-energy radiation to the tumor while minimizing damage to normal tissue. High-dose and high-energy radiation can increase the likelihood of destroying and controlling

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cancer cells, whereas an insufficient dose may reduce treatment efficacy. Therefore, it is important to ensure the accuracy and precision of the radiation doses delivered to the patient using reliable dosimeters capable of measuring and responding to the range of radiation doses delivered during therapy. The International Commission on Radiological Units and Measurements (ICRU) and the World Health Organization (WHO) have established that an acceptable difference between the calculated dose and the actual dose delivered to a patient is equal to or less than 5%.[1,2]

The alanine/electron spin resonance (ESR) technique is widely used in radiotherapy applications. The advantages of the alanine dosimeter are that it is small and easy to place on the patient's skin, as it is tissue equivalent. The interaction with ionizing radiation produces free radicals proportional to the absorbed dose, and the ESR signal is linearly responsive to the radiation dose. Furthermore, it is energy-independent at energies above 100 keV and is non-destructive after reading or recording the spectrum, making it possible to re-read with low signal fading.[3] However, its limitations include a limited radiation dose response above 1 Gy, sensitivity to temperature and humidity, and an expensive ESR spectrometer requirement.[3,4] Additionally, the ESR technique requires optimal configuration parameters such as microwave power, amplitude, time constant, number of scans, and sweep time.[5] Alanine dosimeters have been used to determine the dose in clinical radiotherapy[4,6,7] and quality assurance.[3,8] Using alanine to measure radiation in the range of 0.6 - 0.8 Gy showed an error of 3%, while thermoluminescent dosimeters (TLDs) have an error of 5%. Other reports found an uncertainty of 1.5 - 3.5% for measuring the dose range of 2-5 Gy.[9]

The aim of this study was to measure the radiation dose of a cobalt-60 brachytherapy machine in a solid phantom using an alanine dosimeter in combination with the electron spin resonance (ESR) technique. Additionally, this work aimed to investigate the relationship between radiation dose and ESR signal and to evaluate the optimal conditions for ESR technique.

Materials and methods

The dwell position accuracy was verified daily as part of quality control, with a tolerance set to within ±1 mm. The position of the dwell was selected at the location where the charge is maximum. A 0.6 cm³ farmer-type ionization chamber connected to an electrometer was positioned at 0° of a solid phantom to measure the charges. The catheter connected to the Co-60 brachytherapy machine was placed at the center of the solid phantom, and the BEBIG Co-60 source (BEBIG HDR, Germany) was transferred into the catheter. The dwell positions were set at three distances of 0.25 cm, 0.50 cm, and 0.75 cm. The dwell time was set to 70s for each distance. The charges were recorded in nanocoulomb (nC) with an interval time measurement of the 70s. The distance that gives the most charge will be designated as a dwell position for irradiation in the next experiment.

The ionization chamber and an electrometer were calibrated by the Department of Medical Sciences, Thailand Ministry of Public Health. Temperature and humidity were recorded during the measurements. The ionization probe and electrometer certificate data indicate that the reference temperature is 20 °C, the reference air pressure is 1013.25 hPa, and the calibration factor is 54.22 mGy/nC. Other correction factors used in the calculation were based on the research of Azhari H. *et al.*, including air density=1.014, polarity=1.000, saturation=1.000, phantom calibration factor=1.274, K_Q=1.000, K_A=1.027, g_w=0.0028, and $t\frac{en}{w/a}$=0.900.[10] The reference air kerma rate (K_R) was calculated as shown in equation 1, and then the radiation doses (K_R) and dwell time of 22s were calculated to obtain a dose in the range of 0.06-1.5 Gy.[11]

$$k_R\left(\frac{mGy}{h}\right) = \left(\frac{1}{1-g_w}\right) \times \frac{1}{t\frac{en}{w/a}} \times K_{wp} \times K_{zp} \times K_A \times K_p \times k_s \times k_r \times K_Q \times N_w\left(\frac{mGy}{nC}\right) \times M(nC) \quad ....(1)$$

where

g_w: Fraction of energy of the electrons from the source decay liberated by photons in water that is lost to radiative processes (mostly bremsstrahlung) = 0.0028.

$t\frac{en}{w/a}$: Ratio water/air of the mean mass-energy absorption coefficients for Co-60 = 0.900.

K_{wp}: Correction factor accounting for the differences in scatter and distortion of the radiation field between water and PMMA =1.000.

K_{zp}: Correction factor accounting for the differences in scatter and absorption in the PMMA phantom surrounding the measuring probe in comparison to free-in-air condition = 1.276

K_r: Air density correction for differing temperature and air pressure from reference conditions = 1.014

K_A: Correction factor for attenuation and scatter by the applicator = 1.027

K_p: Correction factor for the polarization effect of the ionization chamber =1.000

k_s: Correction factor for recombination losses in the ionization chamber = 1.000

K_Q: Correction factor for the different response of the ionization chamber at the measured radiation quality in comparison to the calibration quality Co-60 = 1.000

N_w: Calibration factor of ionization chamber in terms of absorbed dose to water = 54.22 mGy/nC

M: Reading in nC

Measurement using solid phantom

A solid cylindrical phantom was attached on a tripod at a distance at least 1 m away from the floor and wall in order to avoid scattered radiation. Solid cylindrical phantom has a tissue equivalent. The atomic number H:O ratio is 2:1, density is 0.998 g/cm^3, 20 cm in diameter, 12 cm in height. There is one hole in the middle and 4 around the perimeter at 0°, 90°, 180° and 270°, each hole is 8 cm from the middle hole. The end of the catheter was connected with the high dose rate brachytherapy machine (Multisource, Multi-source HDR), and the other end was inserted at the center hole of the phantom. The 0.6 cm^3 farmer-type ionization chamber with an electrometer was inserted into the 0° hole, and the electrometer was set at a voltage of 300 volts. The irradiation setup was placed in the room for at least one hour to allow the gas in the ionization chamber to reach the same temperature and humidity conditions. The initial temperature and humidity were recorded, and two alanine pellets with a diameter of 0.3 cm from Far West Technology (FWT-50-10, Steris, USA) were inserted into the 180° hole, as shown in Figure 1. The BEBIG cobalt-60 source was then moved through and stopped at the predetermined dwell time was set.

The irradiation was performed with a dwell time specified in the treatment plan to achieve a dose of 0.06 Gy. The charge from the electrometer was recorded, and two irradiated alanine pellets were then stored in a plastic bag to prevent contamination from humidity. The experiment was repeated with two un-irradiated alanine pellets placed in the phantom. The dwell time was adjusted to obtain a dose range of 0.06-1.50 Gy. The ESR signal of the irradiated alanine was then determined using an ESR spectrometer.
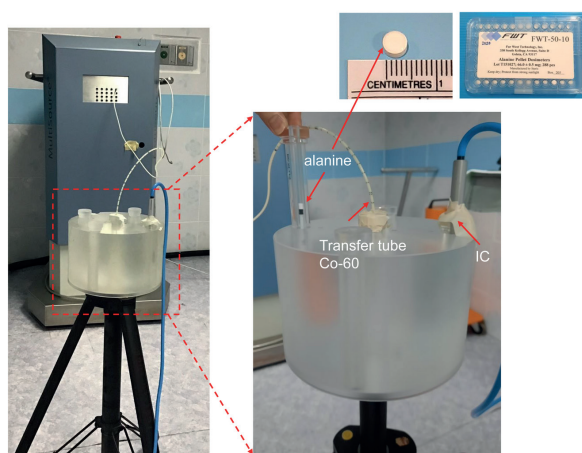
Figure 1 *The irradiation setup using a farmer-type ionization chamber and alanine in the solid cylindrical phantom.*

Electron spin resonance dosimetry with alanine

The ESR signal of alanine was determined using a Bruker A300 ESR spectrometer with a microwave frequency of 9-10 GHz. The alanine was inserted into a sample tube and placed in the center of the ESR spectrometer, also known as the 'resonator', which amplifies the ESR signal of the sample. The ESR signal intensity depends on the positioning of the sample tube within a uniform sensitivity of 1-2 mm from the center. The sample tube was immobilized using a device that does not interfere with the magnetic field and allows for measurements to be taken in the same position (see Figure 2).

The parameters of the ESR spectrometer were adjusted to achieve a high signal-to-noise ratio (SNR) and minimize uncertainties that can occur during ESR signal measurements.[5,9] This was necessary because microwave power can affect the shape of the ESR spectrum, signal intensity, and amplitude. The microwave power modulation was started as low as 0.5 milliwatts (mW) and gradually increased with fine-tuning to achieve a stable ESR signal intensity and avoid saturation effects, which was beneficial for obtaining accurate ESR signals. The parameters of the ESR signals are shown in Table 1. The ESR signal was then recorded under room temperature conditions. The ESR spectrum represents the number of free radicals occurring after alanine was irradiated. The ESR signal curve is expressed as the first derivative of the absorption spectra as a function of the magnetic field. The ESR signals were measured for each dose range, and the relationship between the radiation doses and ESR signals was analyzed.

Results

The results demonstrated that the highest charge reading of 451 pC was obtained at 0.75 cm from the catheter tip. Thus, this distance was selected as the dwell position for alanine irradiation. The corresponding dwell time, charge (nC), and radiation doses are presented in Table 2.

The ESR spectrum and magnetic field intensity were analyzed to determine the relationship between ESR signal intensity and the irradiated dose of alanine. Data analysis showed that the ESR signal had varying signal intensities in each dose range, and alanine had a linear response to radiation doses with the equation y = 0.0479x+0.0515. The correlation coefficient (r^2) was 0.8607 (figure 3). The uncertainty of ESR signals from 0.06-1.5 Gy was 0.1231%-3.7870% (Table 2). The signal fading experiment demonstrated that after 2 weeks, the ESR signal reduced by 7.2%-27.4%.
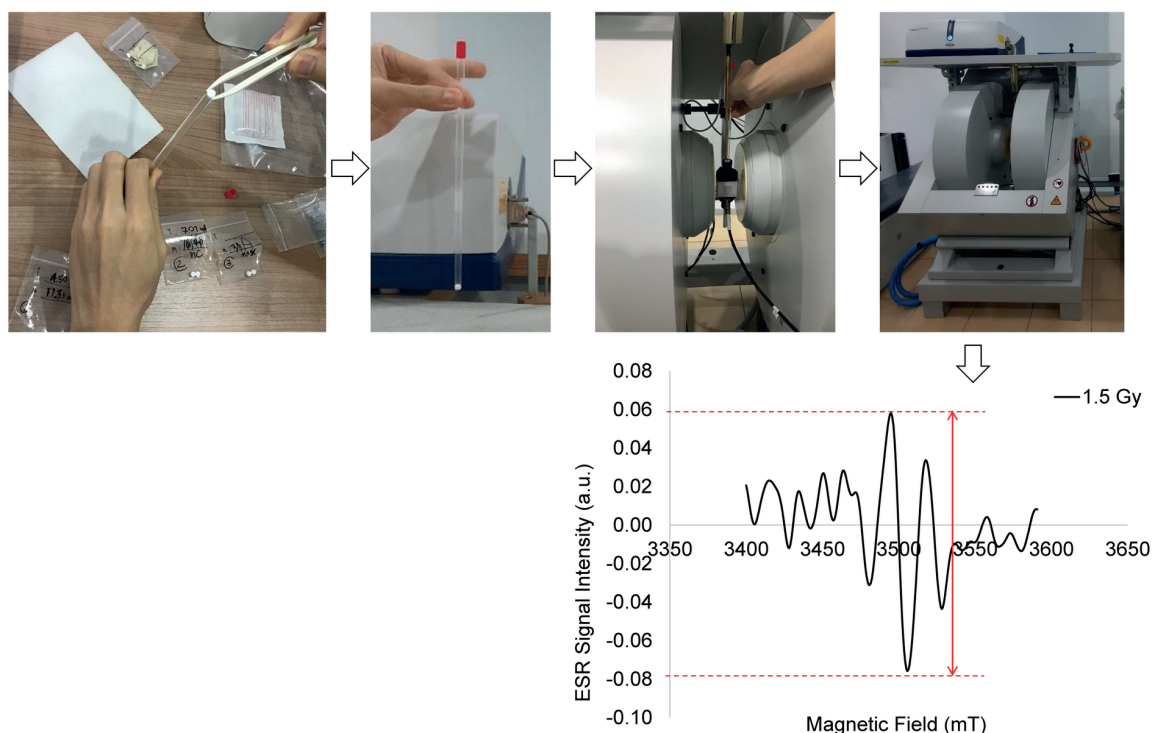
Figure 2 Illustration of alanine was placed in a sample tube and inserted into the ESR spectrometer. The ESR spectrometer generated a magnetic field and applied microwave power, resulting in a characteristic ESR spectrum of alanine. The signal intensity is quantified by measuring the peak-to-peak height of the spectrum. In the example, the ESR spectrum of irradiated alanine with a 1.5 Gy dose was obtained. The peak-to-peak height was measured, providing ESR signal intensity.

Table 1 Parameter of ESR spectrometer.

Parameter	Value	Unit
Microwave power	0.5 - 2.5	mW
Modulation amplitude	0.70	mT
Center field	350	mT
Sweep width	2.00	mT
Microwave frequency	9.87	GHz
Time constant	NA	ms
Sweep time	20.02	s
Number of scans	3	time

Table 2 Calculated radiation dose, mean, and uncertainty of ESR signal intensity.

Calculated dwell time (s)	Electrometer reading (nC)	Calculated radiation dose (Gy)	ESR signal Intensity (a.u.)	Standard deviation (SD)	%Uncertainty (%)
113	0.8700	0.0600	0.0692	0.0033	0.3313
226	1.7270	0.1200	0.0571	0.0016	0.1595
339	2.5800	0.1800	0.0625	0.0033	0.3342
452	3.4660	0.2400	0.0580	0.0036	0.3575
564	4.3130	0.3000	0.0604	0.0012	0.1231
677	5.0790	0.3600	0.0625	0.0028	0.2830
790	6.0230	0.4200	0.0749	0.0025	0.2514
903	6.9250	0.4900	0.0707	0.0024	0.2369
1016	7.7600	0.5400	0.0815	0.0019	0.1907
1887	14.6300	1.0000	0.0855	0.0036	0.3583
2831	21.5000	1.5000	0.1342	0.0379	3.7870

y = 0.0479x + 0.0515
R² = 0.8607
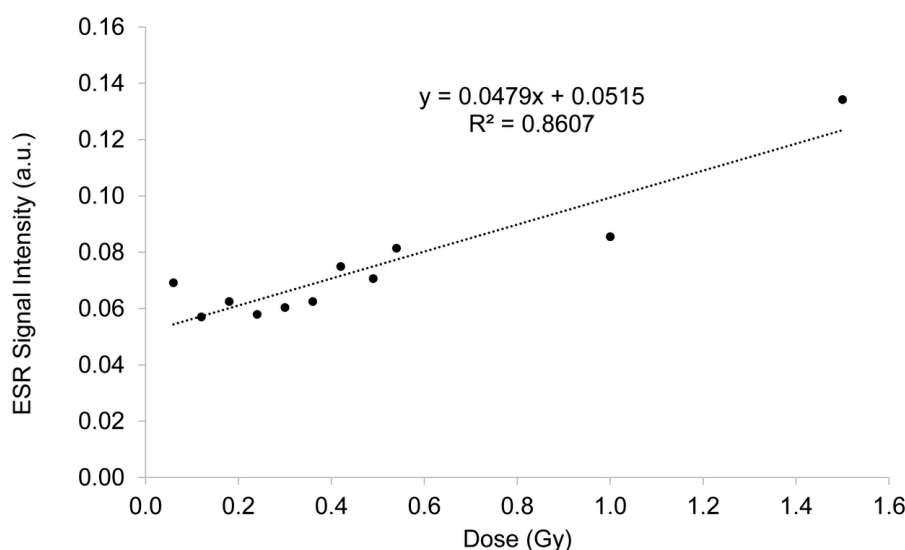
Figure 3 *Relationship between radiation dose and ESR signal intensity.*

Discussion

The accurate measurement of radiation dose using an ionization chamber requires the application of correction factors, including temperature, pressure, ion recombination, beam quality, and a phantom calibration factor of 1.276 as manufactured. The calibration factor $N_{D,w,Q0}$, which was determined by the Department of Medical Sciences, Thailand Ministry of Public Health, is 54.22 mGy/nC. The radiation doses were calculated according to the recommendations of DIN6809-2 and DGMP report 13.[10] Additionally, it is necessary to check the dwell position to determine the distance that gives the maximum dose response.[12]

A phantom study has the advantage of reproducibility in the same position with accuracy and decreases the effect of tissue inhomogeneity. However, the size of the phantom is an important factor that influences the dose distribution around the source.[13] In this study, the alanine dosimeter was placed 8 cm away from the source. In Brachytherapy, the dose decreases rapidly from the source, also known as "rapid fall-off." If the distance is increased, the dose decreases according to the inverse square law. Measuring doses in a large-diameter phantom is difficult and requires a long irradiation time. The result is consistent with Schaeken B. *et al.*, who conducted a study to determine the response of alanine in the HDR source. They found that the source-to-detector distance of less than 5 cm significantly affects the dose distribution in brachytherapy.[14]

Alanine, in combination with electron spin resonance, was used to determine the radiation dose by analyzing the peak-to-peak signal amplitude. The results showed that the first alanine derivative in the middle, where the highest signal intensity occurs, is directly proportional to the number of free radicals generated after irradiation. Alanine is suitable for measuring radiation doses in radiotherapy due to its ability to determine doses in the high dose range accurately. The relationship between radiation dose and ESR signal is linearly responsive from 0.1 to 20 Gy, with an R^2 value of 0.9999.[3,12] However, at low doses, the relationship between radiation dose and ESR signal is non-linear due to the limitations of alanine in doses below 1 Gy.[15] This study measured a dose range from 0.49-1.5 Gy (R^2=0.8744) because the ionization chamber can measure charges not exceeding 23 nC, resulting in the measurement of radiation doses up to only 1.5 Gy.

Correcting signal fading is crucial for accurate dose measurements using alanine dosimeters. The signal fading may occur due to various factors, including storage conditions after irradiation, humidity, and dose range. The signal fading can be corrected by applying correction factors to the ESR signal based on the irradiation temperature and time elapsed after irradiation.[5,16] To improve the accuracy and reproducibility of ESR signal measurements, it is recommended to create a calibration curve, increase the number of signal readings, and optimize the measurement parameters, including selecting an appropriate microwave power to reduce saturation effects. Additionally, the ESR signal of irradiated alanine should be read out within 2 hours to avoid significant signal fading.[17] The rectangular cavity has better sensitivity than the circular one and reduces the effects of conditions inside the room that may cause sensitivity changes.[18]

Alanine is a small, tissue-equivalent dosimeter that offers convenient use without wire setup, and the signal endures after reading. However, the electron spin resonance technique used with alanine dosimeters for measuring low radiation doses is relatively time-consuming, and parameters must be determined to improve signal quality and increase the signal-to-noise ratio while taking four minutes per reading. Therefore, it is crucial to consider the time used to increase accuracy.[9] An alternative dosimeter, such as lithium formate, can also be used with the ESR technique.[19] In the future, we suggest using lithium formate instead of alanine, as it provides a better response and has similar properties to human tissue.

Conclusion

In conclusion, the alanine dosimeter with ESR technique proves to be a reliable and valuable method for measuring radiation doses in brachytherapy. The dose-response of alanine demonstrates linearity above 0.49 Gy, rendering it suitable for high-dose range measurements, particularly under specific experimental conditions where the sample tube is accurately positioned in the central region of the ESR spectrometer. It is crucial to optimize the parameters of the ESR spectrometer to ensure precise and accurate readings. This conclusion assumes the ESR signal was recorded at room temperature and that readings should be taken at the appropriate time after irradiation. By adhering to these specified conditions, the accuracy of alanine dosimeters can be significantly enhanced, leading to reliable and precise measurements of radiation doses. The dosimeter offers convenience, exhibits tissue-equivalent properties, and provides a non-destructive measurement process with minimal signal fading. Considering these advantages, alanine can be used in various radiotherapy applications.

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Lived experiences of Filipino occupational therapists amid the COVID-19 pandemic in the East Coast, USA: compassion fatigue and compassion satisfaction

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ARTICLE INFO

ABSTRACT

Background: Occupational therapists and other healthcare workers are characterized as having a higher probability of experiencing work-related stress and mental health issues than other professionals. Moreover, occupational therapists utilize mindful, compassionate care and full empathetic engagement in every clientele that draws from a source of wellness, strength, resilience, and presence. Occupational therapists provide client-centered care and highlight the therapeutic use of self by showing empathy and genuine engagement. This approach often exposes occupational therapists to clients' experienced trauma during service delivery, leading to other problems for the healthcare practitioner. However, there is limited literature about burnout, compassion fatigue, and other mental health issues experienced by occupational therapists. More so, as accentuated by the past years of the COVID-19 pandemic.

Objectives: This study addresses the following research objective, to explore the lived experiences of Filipino occupational therapists working in the East Coast, USA, amid the COVID-19 pandemic. Through their lived experiences, we can gain insight into how their experiences shape compassion fatigue and compassion satisfaction, which can then be used to attenuate its adverse effects on the well-being of occupational therapists.

Materials and methods: Ten Filipino occupational therapists who managed COVID-19 cases in the East Coast, USA, served as the participants in individual in-depth interviews centered around their experience of COVID-19 patient care. Transcripts served as qualitative data sets and underwent thematic analysis.

Results: Three themes emerged from the analysis and include: (1) Professional Experience: Waging War against the Pandemic, (2) Compassion Fatigue, and (3) Compassion Satisfaction.

Conclusion: Despite facing conflict, uncertainty, and worry during the height of the COVID-19 pandemic, Filipino occupational therapists find fulfillment in helping their patients recover and achieve optimal performance in affected occupations. Compassion fatigue themes are adverse effects of COVID-19, heavy workload combined with less manpower, and difficulty separating work and personal life. Compassion satisfaction includes participants' view of their work as heroic and support from co-workers. This requires occupational therapists to highlight setting boundaries and doing everyday life routines to take a break from work and motivation to provide for themselves and their families. Moreover, this posits a need for healthcare organizations to re-examine strategies to attenuate compassion fatigue and highlight compassion satisfaction.

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Introduction

Occupational therapists and other healthcare workers are characterized as having a higher probability of experiencing work-related stress and issues with mental health in contrast to other professionals. [1] Occupational therapists utilize mindful, compassionate care and full empathetic engagement in every clientele that draws from a source of wellness, strength, resilience, and presence.[2] Occupational therapists provide client-centered care and highlight the therapeutic use of self by showing empathy and genuine engagement. This approach often exposes occupational therapists to clients' experienced trauma during service delivery, leading to other problems for the healthcare practitioner.[3] However, there is limited literature about burnout, compassion fatigue, and other mental health issues experienced by occupational therapists. More so, given the rising incidence of these problems prompted by the COVID-19 pandemic. The pandemic brought numerous challenges and stressors in healthcare practice, some of which, but are not limited to, continuous stress, fatigue, and exposure to trauma.[4,5] These stressors can lead to compassion fatigue and may result in burnout if the former is not addressed well.[6,7]

Based on a systematic review of healthcare workers globally, they experienced high levels of burnout and stress compared to the general population in the first year of the pandemic.[4] Anxiety and depression were among the factors that were positively related to the level of burnout experienced. The high-stress level was attributed to the lack of support and personal protective equipment. In the same systematic review, burnout positively correlated with compassion fatigue. The experience of compassion fatigue affects the empathy of the healthcare worker and can influence the quality of patient care.[1]

In this study, the construct of compassion fatigue can be viewed through the Compassion Stress and Fatigue model, which focuses on the assumption that an individual's capacity for empathy and ability to engage or enter a therapeutic relationship is central to compassion fatigue.[8] This model describes individuals who display prominent levels of empathy and empathic response to a patient's pain, suffering, or traumatic experience are more vulnerable to experiencing compassion fatigue. This model linked empathy with the caregiver's capability to connect with and help a client. Compassion fatigue is a process that starts with compassion stress, which, if not managed by a sense of satisfaction or disengagement and with continued exposure to stress, develops into compassion fatigue.[9]

Compassion is an awareness of or sensitivity to the pain and suffering of others that results in taking a verbal, nonverbal, or physical action to try to alleviate, remove or reduce the impact of such affiliation.[10] In contrast, empathy is recognizing and understanding a person's experience. Still, it does not necessarily involve responding to that experience, though it does not necessarily result in better health care provision. Therefore, compassion influences the provision of occupational therapy and would serve as an ember that keeps the passion of occupational therapists

to provide interventions that optimally fit the client's needs and current functions.[10,11] However, excessive exposure to a patient's experience may be detrimental to one's own health and mental stability as a professional healthcare provider. It may influence a person's emotions outside of work and may impact a worker's professional quality of life.[12]

Occupational therapists provide "mindful, compassionate care and full empathetic engagement" to patients as a source of wellness, strength, resilience, and presence. Aligned with that, stress, compassion fatigue, and burnout are factors that decrease job satisfaction and influence the attention of patients.[2] Consequently, there is a need for self-care protocols or programs that carry the goals of increasing positive outcomes to improve the professional quality of life, client-care, wellbeing, burnout, presence, mindfulness, and self-management. To prevent and manage compassion fatigue, healthcare professionals should continuously self-monitor their symptoms and regularly educate on the importance of self-care.

In the USA, Filipino healthcare workers are one of the top on the frontline during the pandemic providing healthcare services, such as home health and many others. They are often labeled as invisible or unseen in the industry which places them more at risk during the pandemic.[13] The additional risk that Filipino health care workers are subjected to exacerbates existing issues pervading the mental health of Filipinos living in the USA.[14] Specifically for Filipino occupational therapists, the concern for mental health exacerbated by the pandemic further adds to the prevalent struggle concerning burnout.[15]

This study aims to address the following research objective, to explore the lived experiences of Filipino occupational therapists working on the East Coast, USA, amid the COVID-19 pandemic. Through their lived experiences, we can gain an insight into how their experiences shape compassion fatigue and compassion satisfaction, which can then be used as a basis to attenuate its adverse effects on the mental health and wellbeing of occupational therapists.

Materials and methods:
Design

This study utilized a qualitative approach, specifically phenomenology,[16,] which was appropriate in examining the lived experiences of Filipino OTs and compassion fatigue while working in the East Coast in the United States of America. The interview transcripts served as the data sets for this qualitative study. Moreover, the researchers adopted a constructivist worldview to situate shared themes on the experiences of Filipino OTs, which may reflect the impact of compassion fatigue. Both researchers are Filipino OTs, with one having a master's background in occupational therapy and experience working as an immigrant in the USA. The other researcher has a doctorate and teaching background in occupational therapy.

Participants and Sampling

Participants were 10 Filipino OTs situated in the

East Coast in the United States of America. Purposive sampling was used in this study with the following criteria in selecting the participants: (1) has more than two years of clinical experience, (2) is employed as an occupational therapist working amidst the COVID-19 pandemic, and (3) involved in direct patient care. Participants were recruited through the first author's clinical affiliation. Then the recruited participants were asked to refer other possible participants from other affiliations that they know of around the East Coast. Specifically, they came from Florida, Maryland, New Jersey, and New York. The East Coast was chosen as the locale as this was where the first author was employed and was deemed convenient to recruit participants based on the set inclusion criteria. A summary of the participants' socio-demographic data is presented in Table 1.

Table 1. Summary of Participants' profiles (N=10)

Variables		Frequency (%)
Gender	Male	2 (20)
	Female	8 (80)
Age (years)	35-39	6 (60)
	40-45	3 (30)
	46-49	1 (10)
Years of experience	1-5	2 (20)
	6-10	6 (60)
	11-15	2 (20)
Working hours (per day)	10	10 (100)

Recruitment of participants was stopped after data saturation was achieved. The interview with the 8th participant yielded no new information. To confirm data saturation, an interview was conducted with the 9th and then the 10th participant, after which recruitment stopped.

Data Collection

The study utilized individual in-depth interviews to gather data. Using a semi-structured interview with open-ended questions aided in the collection of qualitative data about the experiences of Filipino OTs working in the East Coast and compassion fatigue. Participants were contacted primarily through the first author's workplace and were sent emails informing them about the study and then seeking their consent to participate.

The interview time and place were based on the participants' preference, either in-person or via Zoom Communications. Permission to audio record the interviews was also sought from the participants. The interviews lasted for at least an hour and were then transcribed after completion.

Data Analysis

Data analysis started as soon as the first interview was conducted. For this study, thematic analysis was employed in generating the themes from the narratives of Filipino OTs about their experiences.[17] The following steps were followed during the process of analyzing data: (1) the recorded interviews were transcribed; (2) researcher familiarized the transcribed data through reading and review; (3) researcher isolated phrases, sentences, and paragraphs which presented significant meaning; (4) coded the data; (5) found commonalities and classified them into cluster; (6) generated themes from the clusters; (7) named and defined themes, and lastly (8) presented and summarized the themes generated based from interviews.

Rigor and Trustworthiness

To ensure rigor and trustworthiness, the researchers followed the principles outlined by Lincoln and Guba.[18] To ensure confirmability, reflexivity was employed by the authors using journaling to minimize projecting any bias toward the participants' responses during the interviews. Credibility was ensured by having the participants review the generated themes and ensure that it was reflective of their experiences. Dependability was ensured by having researchers with a background in qualitative research review the process and findings of the study.

Ethical Considerations

This study complied with the requirements set by the Cebu Doctors' University Institutional Ethics Review Committee and was assigned the code "2022-185-Dag-um-WorkingAmidstCovid-19".

Results

Three themes emerged from the analysis and include (1) Professional Experience: Waging War against the Pandemic, (2) Compassion Fatigue, and (3) Compassion Satisfaction are summarized in Table 2. To aid in illustrating the themes, quotations, translated from the Cebuano language to English, are included.

Professional Experience: Waging War Against the Pandemic

Participants expressed mixed emotions working during the COVID-19 pandemic. However, all of them are uncertain and nervous because of the threats posed by the pandemic. Some of them treated the situation as an opportunity to serve and to achieve fulfillment for the cause of their duty. The following statements exemplify the mixed emotions highlighted by the participants:

I was busy then, so it had the opposite effect on me. It was a blessing … because I can work

Table 2. Summary of themes and subthemes.

Themes	Subthemes
Professional experience: waging war against the pandemic	Mixed emotions Patient-focused care
Compassion fatigue	Fear and anxiety Challenge of understaffing Finding balance
Compassion satisfaction	True to their oath We are all in this together

here despite COVID. It was also terrifying, but I haven't got sick. (Participant 4)

I was fearful about getting infected. I gave my insurance cards and passwords because I had to work and had no idea what it was back then. (Participant 5)

Participants were all working in nursing homes and described their line of work as focused and intensive. The pandemic accentuated the demand for work. Participants expressed that there was a need for rehabilitation professionals, especially OTs, because of complications due to COVID-19 infection as stated by the participant:

I started in a school, then I followed by covering shifts at a nursing home. I kept covering shifts at a nursing home until January when a facility hired me because of COVID. (Participant 4)

Participants also described the patient load that they experienced. Participants usually handle orthopedic cases, while some handle neurologic cases. They have heavy workloads per week and must reach a certain quota. The spread of COVID-19 has significantly disrupted established patterns of behavior and procedures in the workplace. The participants highlight this:

We accept post-COVID patients. So, we were dealing with low oxygen saturation, and some exercises were not done because they got tired quickly. (Participant 1)

[OTs] usually treat 20-30 patients for our caseload for short-term care patients. Mostly orthopedic cases. (Participant 6)

Compassion Fatigue

Participants were negatively affected by COVID-19 by working with the feeling of fear and anxiety of contracting the disease. Fear of the unknown was common among occupational therapists, and social distancing techniques contributed to feelings of isolation, anxiety, and changes in social behavior. Occupational therapists bring a distinct perspective to the table when it comes to detecting problems with behavioral health and offering support to patients and their peers.

Our caseloads increased, and you get traumatized and hypervigilant with the people you are encountering as you do not know if your patients have encountered someone with COVID. (Participant 1)

The dificulties the participants face are heavy workloads combined with less manpower in their respective workplaces. The role of occupational therapy in establishing safe discharge recommendations became increasingly difficult as the number of post-acute care options available to patients who tested positive for COVID-19 decreased. Transferring patients to post-acute care settings were complex because many post-acute care units refused to accept patients due to the medical complexity of the patients and the possibility of disease transmission.

OTs pulled out from therapy due to short staffing of nurses. OTs need to help for 20-45 minutes and still have to do work. (Participant 6)

Demotivation, pressure, exhaustion, and frustration are the things that give negative feelings to their work. When the COVID-19 pandemic first started, OTs, on average, went through a wide range of emotions. There has been a significant increase in the likelihood of psychological distress among the participants due to the pandemic caused by COVID-19.

There are a lot of stressful patients who complain and act out a lot since they can't adjust to their situation, and they act out on you. (Participant 3)

Compassion Satisfaction

Participants view their work as a heroic act to serve people. The risks posed to medical professionals during the COVID-19 pandemic are noticeably more significant than those present during regular practice. The added expenses include physical and mental exhaustion, the pain of making difficult triage decisions, the grief of losing patience and colleagues, and the chance of catching the infection. It has also been observed that spending extended periods away from vulnerable family members to maintain employment might have an emotional impact.

Of course, when [patients] get to go home, it feels very good knowing that you were part of

the reason why they can walk again, or they are independent again … All in all, it is adorable to be able to contribute to enriching another person's life and independence. (Participant 3)

When you see progress from your patients, no matter how small it is but you see that they are also progressing and when they appreciate what you are doing for them. (Participant 4)

Co-workers of the participants served as their support system in their duty as OTs. Participants expressed it made them feel better having their colleagues around as a source of support.

… And then, at work, you have your colleagues with you no matter how hard it is. You can vent out to them, and in that way, you can leave your frustrations at work; have people you can vent to. (Participant 6)

Discussion:

Individuals subjected to situations wherein there is uncertainty and transitions may cause negative emotions. The advent of the pandemic placed undue stress on the global healthcare system, and one of those affected by this overwhelming demand were occupational therapists. Healthcare workers already experience high levels of mental and physical stress.[19,20] However, the increasing mortality of healthcare workers managing COVID-19 cases adds to these existing elevated levels of stress.[21] Based on the results of this study, it can be inferred that occupational therapists underwent transitions in their work roles given the ever-changing demand of the healthcare landscape. This transition of roles to fit the demands set by COVID-19 exemplifies constructs under occupational adaptation. [22] Successful adaptation warrants successful acclimation to the new roles' individuals have in the workplace. However, unsuccessful acclimation can lead to feelings of isolation, anxiety, and stress that may have had an impact on their psychological health and their ability to cope due to the disruption in roles that promoted a sense of belonging and connectedness.[23,24]

The phenomenon of compassion fatigue and compassion satisfaction is widely experienced among healthcare professionals.[1] In literature, healthcare workers experienced increasing levels of compassion fatigue since 2010.[25] Healthcare workers in Asia predominantly experience compassion fatigue. However, the findings of this study support the notion that healthcare workers in the Americas also experience compassion satisfaction. It is essential to note that the participants are Filipino occupational therapists working in the American context. In the bigger picture, it warrants further investigation as to why the cultural context of healthcare practice influences the incidence of compassion fatigue or compassion satisfaction over the other.

Occupational therapists experience work-related stress, which is considered an occupational hazard.

More so under the overwhelming demands of health emergencies such as the pandemic. Under the construct of compassion fatigue, identified risk factors are fewer years of experience, qualifications, and job-related factors.[26] This notion is further supported by findings in this study, indicating that higher workload, understaffing, and switching to a new role added to the occupational stress experienced by healthcare workers, which can attenuate their compassion satisfaction. It is essential to highlight compassion satisfaction for occupational therapists since they are helping patients achieve their optimal performance in occupations after being infected with COVID-19 despite exposure to stressful work-related situations. As was highlighted in the themes, occupational therapists find satisfaction in seeing progress in their patients as they recover from the detrimental effects of the COVID-19 infection and slowly recover their optimal function. However, with other job-related factors such as understaffing, occupational therapists are subjected to more stressful situations and fewer opportunities to appreciate their patients' recovery process fully.

The findings of this study further support that compassion fatigue arises from the exposure of healthcare workers to trauma experienced by their patients, their practice of empathy, and intense emotions.[1] Filipino occupational therapist highlighted patient-focused care in their experiences providing services during the pandemic. The patient-focused care led Filipino occupational therapists to practice empathy more, resulting in compassion fatigue that led them to struggle to find balance. Moreover, it was established in the literature that burnout during the pandemic was caused due to overwhelming workloads, which the participants also experienced. The limitations of being understaffed further led to the stress experienced by the participants, which is also evident among other healthcare workers.[4] In terms of compassion satisfaction, the results of this study may be a basis for further investigation. Literature posits that healthcare workers in Asia exhibit lower levels of compassion satisfaction than those in the Americas. [25] However, Filipino occupational therapists in this study, who are Asian, reported experiencing both compassion fatigue and compassion satisfaction, citing that despite the challenge of understaffing, they still hold to their oath of giving patient-focused care. Further investigation may be significant to administrators in addressing compassion fatigue.

As highlighted in this study, compassion fatigue can be detrimental to the healthcare industry. This rising concern over compassion fatigue is not limited only to the period when the COVID-19 pandemic was at its peak but also to other contexts wherein there is unnecessary occupational stress. Healthcare administrators should investigate factors that can affect the incidence of compassion fatigue, such as increased patient load and availability of support which ultimately affects the quality of life of healthcare workers.[4] In addition, a multi-faceted approach is necessary to manage compassion fatigue, which includes raising awareness of the issue, identifying

current self-care practices, addressing problematic thinking and behavioral patterns, developing skills to cope with stress, identifying barriers, and problem-solving.[27] Further implications of this study can be investigating self-care, strategies preventing fatigue, and promoting resilience among healthcare workers in high work-related stress environments. The literature cites that burnout, compassion fatigue, and compassion satisfaction may be affected by various factors, such as the individual's context (workplace and culture). Further investigation may be needed to determine the working conditions of healthcare workers from varying cultural backgrounds vary while working in the USA.[4]

Conclusion:

Despite facing conflict, uncertainty, and worry during the height of the COVID-19 pandemic, Filipino occupational therapists find fulfillment in helping their patients recover and achieve optimal performance in affected occupations. Those affected by the pandemic worked in nursing homes and managed orthopedic or neurological conditions with heavy workloads and weekly quotas. COVID-19 induced fear, anxiety, demotivation, pressure, exhaustion, frustration, and distractions. However, coworkers provided needed social support in the workplace. To cope and build resilience, occupational therapists set boundaries and engage in everyday life routines to provide for themselves and their families. The experiences of Filipino occupational therapists reveal three themes: professional experience, compassion fatigue, and compassion satisfaction. Professional experience includes mixed emotions, working privately in nursing homes, and handling heavy workloads. Compassion fatigue themes are adverse effects of COVID-19, heavy workload combined with less manpower, and difficulty separating work and personal life. Compassion satisfaction includes participants' view of their work as heroic and support from co-workers. This requires occupational therapists to highlight setting boundaries and doing everyday life routines to take a break from work and motivation to provide for themselves and their families. Moreover, this posits a need for healthcare organizations to re-examine strategies to attenuate compassion fatigue and highlight compassion satisfaction.

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Reasons, duties, commitments, and love bonds: The lived experiences of grandparents caring for children with autism spectrum disorder

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ARTICLE INFO

ABSTRACT

Background: One of the significant changes in childrearing over the past 20 years was the increasing number of grandparents raising their grandchildren. Being a primary caregiver to help nurture grandchildren is considered a principal occupation for many older adults. Grandparents frequently play a significant role in helping their families care for grandchildren diagnosed with autism spectrum disorder. However, little was known about the grandparents of these children in Thailand.

Objectives: This phenomenological qualitative research aimed to examine the forms and meanings of grandparenting for grandchildren with autism spectrum disorder aged 2-6 years.

Materials and methods: Semi-structured interviews were conducted. Seven participants who were grandmothers of children with autism spectrum disorder participated in this study. Data were transcribed verbatim, and the transcripts were coded and analyzed using a content analysis method. Data were interpreted using occupational therapy and occupational science notions to address the research questions and discussion.

Results: Three main themes were developed. The first theme was reasons for deciding to become a primary caregiver. Because of the participants' involvement in caring for grandchildren, the families could face this difficult task together, and grandchildren would be effectively raised by grandmothers, receiving the appropriate care. The second theme revealed three forms of grandparenting occupations, which involved time allocation to fit daily demands, adjusting childrearing methods to promote the development of a grandchild, and dealing with grandchildren's behaviors caused by autism spectrum disorder symptoms. The last theme was the meanings of grandparenthood. Participants addressed what it meant to be grandparents raising grandchildren diagnosed with ASD through their duties and commitments, and love bonds.

Conclusion: With a better understanding of how grandparenting occupations were managed and orchestrated to meet the needs of families and grandchildren with ASD, we could see the adaptation process in childrearing methods. Moreover, the findings suggested that engagement in grandparenting occupations was meaningful for the participants in this study.

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 doi: 10.12982JAMS.2023.063
 E-ISSN: 2539-6056

Introduction

At present, the family structure of Thai society is changing drastically. Kinship care, which is one type of family-based care within the child's extended family or with close friends of the family, has expanded. Because

parents must work to support the family in other cities or other areas, children must sometimes live with the elderly, and often they are grandparents.[1]

Raising grandchildren is one of the roles of grand-parenthood, which ranges from helping, supporting, disciplining, and nurturing grandchildren.[2] As a result of recent trends for longer lifespans, some adults today can spend several more years in the role of grandparenting.[3] The allocation of roles and duties in caring for children with autism spectrum disorder (ASD) may occur for different reasons, and/or there is a family agreement involved. For example, when the parents become unavailable to raise their children due to substance abuse, illness, or incarceration, some grandparents assume responsibility for raising a grandchild.[4,5] Some grandparents are involved in sharing responsibilities for caregiving to a grandchild due to the divorce or work commitments of their adult children.[6] In some instances, grandparents found a source of meaning and practical support from participating in raising their grandchildren, and grandparenting could also provide a significant positive social role for the elderly.[7,8] In addition to these direct effects, autism can negatively affect family members' well-being, including the grandparents, by increasing stress.[9] For example, a survey study by Marken and Howard revealed that raising grandchildren had a negative impact on grandmothers' physical and emotional well-being.[10]

When a child is diagnosed with ASD, which refers to a range of conditions characterized by challenges with social skills, repetitive behaviors, speech, and non-verbal communication,[11] families can be affected in many ways. When providing full care for a child with ASD, the roles and responsibilities of family members are different, depending on the lifestyle and limitations of each family.[12] Even though the responsibilities of raising children with ASD are the main duties of the parents, it has been found that grandparents play a major role in raising their grandchildren with ASD in certain families.[12] Moreover, the prevalence of childhood ASD is now increasing due to early detection and diagnostic criteria. Consequently, increasing numbers of adults will likely find themselves grandparenting a child with ASD.[13,14]

This research conceptual framework was embedded in occupational science and the occupational therapy profession's view of individuals as occupational beings. The individual is viewed as the active author of occupations.[15] Occupational choices happen within the context of individuals' physical, cultural, and social environments.[16,17] Therefore, we applied a qualitative method to address three questions: (a) What were the forms and meanings of these grandparents? (b) How did they perceive themselves as primary caregivers? and (c) Why had they engaged themselves in these roles? This research was a study done at the Yuwaprasart Waithayopathum Child and Adolescent Psychiatric Hospital, Samut Prakan, since this hospital serves as a tertiary care and excellence center for autism in Thailand, under the Department of Mental Health Services, Ministry of Public Health.[18]

Materials and methods
Study design

This phenomenological qualitative study utilized semi-structured interviews to explore the forms and meanings of grandparenting for grandchildren with ASD aged 2-6 years.

Participants and settings

The inclusion criteria for grandparents were being the biological grandparents, either grandfathers or grandmothers, of children diagnosed with ASD aged 2-6 years; having primary responsibility in raising grandchildren diagnosed with ASD, consistently and continuously for more than 1 year; being primary caregivers with the most hours raising grandchildren with ASD; having no psychiatric diagnosis from a physician or psychiatrist; being able to understand and speak Thai fluently; and willing to participate in the study.

Before data collection, ethical approval for the study was obtained from the Ethical Review Committee for Research in Humans, Faculty of Associated Medical Sciences, Chiang Mai University (Ref. no AMSEC-62EX-053). The Ethical Review Research in Humans at Yuwaprasart Waithayopathum Child and Adolescent Psychiatric Hospital, Samut Prakan Province, also granted approval for this research.

After receiving ethical approvals, purposive sampling was used to identify and select the participants based on the richness of the information they could provide. The research study flyers were publicized on the hospital's outpatient board. The first author contacted grandparents who registered to participate to obtain informed consent and arrange for a semi-structured interview to be carried out at a time and place of their convenience. All participants chose to be interviewed at the outpatient unit's consultation room/examination room during their available times. At the time of data collection, eleven grandmothers and two grandfathers met the inclusion criteria. However, only grandmothers were available and willing to participate after the informed consent process. As a result, seven participants who were grandmothers of children with ASD participated in this study.

Due to the COVID-19 pandemic, data collection was done entirely in a 10-month. Data obtained from the participants had reached data saturation. The first author conducted all interviews, and one open-ended audio-taped interview was done for each grandmother. Overall, the interviews ranged from about 50 to 60 minutes.

Instruments

The researchers employed semi-structured interview questions. The interview questions are shown in Table 1. Reviews of the literature, including occupational science,[19] Model of Human Occupation[20], and Occupational Adaptation,[21-22] were used to guide the formulation of the questionnaire items.

Each interview was audio-taped for later transcription. The tape-recorded interviews and field notes were transcribed, coded, categorized, and analyzed to identify themes.

Table 1. Interview questions.

Why did you take the role of primary caregiver for raising your grandchild? *[probe questions, such as when or why are you raising your grandchild?]*
What is your role in grandparenting? *[probe questions, such as what do you do during the activities with your grandchild on weekdays or holidays?]*
How do you raise your grandchild? *[probe questions, such as how do you handle when they are sick, or do you take them to see a doctor or teach them activities that are important in daily life?]*
Can you provide the meanings for raising your grandchild? *[probe questions, such as what do you think about the importance of your role in rearing your grandchild?]*

Detailed field notes became necessary for recording observations to describe the interviews' mood, atmosphere, and context. Transcription was conducted after the interview was done. While listening to the audio tapes, the participants' pauses, changes in intonation, laughter, and other nuances were tracked and recorded through a transcription system to preserve the contextual authenticity of their lived experiences. Most importantly, the anonymity of the participants was preserved using a confidentiality protocol.

Data analysis

Data analysis occurred with data gathering. It helped further focus on data gathering and explored the findings or issues raised during data collection.[23] In this study, data were analyzed using content analysis.[24] This ongoing and iterative process began with the first author becoming familiar with all the transcripts through active reading and re-reading. Then, the first author labeled the meaning unit within the transcription that leads to the processing of creating codes, categories, and themes, respectively.[25] Once core categories were thoroughly identified, themes were sought within the data coding. Table 2 gives an example of how the manifest content analysis was conducted.

Both authors interpreted and verified the emerging themes and relationships between categories. Finally, the notions of occupational therapy and occupational science focusing on the forms, functions, and meanings of occupations were used to interpret and address research questions and for discussion.

Strategies to ensure trustworthiness were carried out, including audio recording interviews, transcribing them verbatim, and checking the transcripts for accuracy. Moreover, semi-interview questions that a second author had validated were employed as methods for triangulation at the level of data collection. Peer examination was done as a method of data triangulation to increase credibility. During the data analysis process, the first author had found agreement mainly from the thesis advisor, who happens to be the second author. The process included a discussion of emerging themes and categories and the researchers' interpretations.

Results

All seven of the participants lived in central Thailand. Three of them were maternal grandmothers, and four of them were paternal grandmothers. Their ages ranged from 46 to 62 years, with the average age being 54 years. The duration of being a primary caregiver ranged from 2 years to 3 years to 10 months. All grandchildren were male. Table 3 provides a detailed overview of the participants' demographic information. Pseudonyms have been used to protect the participants' privacy.

Three central themes were discovered, including reasons for deciding to become a primary caregiver, the forms of grandparent occupations, and the meanings of grandparenthood. The themes and sub-themes illustrating grandmothers' experiences and responses were taken directly from the research questions.

Table 2. Example of content analysis in this study.

Theme	The forms of grandparenting occupations
Categories	Time allocation
Codes	Stop everything and stay with him
Condensed meaning unit	If he is awake, I cannot do anything. I have to stop everything and stay with him or play with him
Unit of Analysis of an entire interview	I will find time to do crafts. When I am stressed, I thread beads, knit, and crochet. Sometimes, I make ribbon-flowers for almsgiving. However, I must wait until my grandson goes to bed. If he is awake, I cannot do anything. I have to stop everything and stay with him or play with him.

Table 3. Demographic information of participants.

Pseudonym	Gender of a primary caregiver	Age (years)	Relationships to a grandchild with ASD	Duration of being a primary caregiver	Gender of a grandchild with ASD	Age of a grandchild with ASD	Health condition (s) or chronic disease (s) of a caregiver	Number of grandchildren
Crystal	Female	47	Maternal Grandmother	2 years 2 months	Male	2 years 8 months	NO	1
Ruby	Female	46	Maternal Grandmother	2 years 9 months	Male	2 years 10 months	Herniated Nucleus pulpous & Diabetes with Hypertension	1
Pearl	Female	57	Maternal Grandmother	2 years 8 months	Male	3 years	NO	1
Diamond	Female	55	Paternal Grandmother	3 years 10 months	Male	4 years	NO	1
Garnet	Female	55	Paternal Grandmother	3 years 4 months	Male	3 years 5 months	Hypertension	2 (1 grandchild with ASD and 1 typical development)
Sapphire	Female	57	Paternal Grandmother	2 years	Male	2 years 4 months	Hypertension with Hyperlipidemia	1
Opal	Female	62	Paternal Grandmother	3 years 6 months	Male	4 years 11 months	Hypertension	1

Reasons for deciding to become a primary caregiver

Grandmothers in this study explained their reasons for being primary caregivers of their grandchildren with ASD in two sub-themes: "doing it for my adult children" and "doing it for my grandchild". By doing so, the families could walk this road together, and the grandchildren would be adequately raised by grandmothers and receive the appropriate care they need.

Doing it for my adult children

This sub-theme illustrated that these grandmothers had decided to take the role of primary caregiver for grandchildren diagnosed with ASD because of the work commitments of their adult children. There were two explanations: their daughters or daughters-in-law needed to return to work since maternity and childcare leave had ended, or they had to return to take care of their business. The participants' choice to help carry out their caregiving roles and duties in raising grandchildren was immediately made, coupled with the need to earn income to cover expenses for the whole family. Pearl said, "Well, my daughter-in-law wanted to return to work and make money. So I decided to help her take care of her baby boy". Crystal explained her situation,

It's about the right time. At first, my daughter wanted to go back to school to finish her degree after giving birth. But we had many expenses to bear, so she decided not to pursue her degree but to return to work instead. Even though I was sick and resigned from my job, I assumed the leading role in raising my grandson.

Garnet was another informant who spoke about the reason for her decision to raise grandchildren, as she stated,

"No one takes care of my grandchild. His parents must work to earn a living. So, I have to raise him because my adult child has to go to work to earn money to support the family".

Doing it for my grandchild

It was also found that there was another reason why participants had to take over the role of raising grandchildren with ASD. The health and well-being of a grandchild made a grandmother's decision to reach out for help. Opal told her stories in a sad tone of voice.

When my grandchild was one and a half years old, his parents divorced and separated. My adult child informed me about the separation. He also asked me whether I could take care of his son. I said, 'Yes, I will take care of him'. I knew that they might not be able to make it. So, if they were separated, I would take complete care of my grandson. At first, she said she would take him. However, she left him with us when she departed. I pitied my grandson as I watched television news about a child her stepfather abused. I was so afraid that it could happen to my grandson. I decided to take him into my total care.

Crystal explained her concerns by stating, "I was so afraid that he might be beaten or bullied. I wouldn't be able to accept that. It'd be hard for me not to take him. Uh… so, I decided to raise him".

In sum, by raising grandchildren, their adult children could return to work and/or carry out their employment. The findings also revealed that taking on the role of raising grandchildren was necessary for the grandchildren's health and well-being.

The forms of grandparenting occupations

This second theme revealed three forms of grandparenting occupations, which involved time allocation to fit daily demands, adjusting childrearing methods to promote the development of a grandchild, and dealing with grandchildren's behaviors caused by ASD.

Time allocation

After deciding to take the leading role in raising grandchildren, the participants had to adjust their time and routines to fit their daily demands and their grandchildren's needs. Crystal talked about daily activities with her grandchild, where they spent all day together. Crystal was always involved in all other activities that the grandchild had engaged in, either watching from a distance or playing together. If she found that he could not do any tasks by himself, she would teach him to provide attentive care and instruction on increasing his abilities, such as social communication skills.

In the mornings, our family members spend time eating breakfast and talking. We often watch the news on TV in the afternoons. While I was eating, my grandson played with his toys. If he didn't play with the toys properly or had no idea, I'd teach him how it's supposed to be done. Reading the alphabet and knowing colors were also our goals to teach him. Sometimes, I taught him to speak or call out the animals' names. In the evenings, we watch the news on TV and soap operas and then go to bed together.

All participants also discussed how they allocated time to fit well with their grandchildren's activities. They ensured that their grandson's daily routines were done thoroughly, and then they would take care of their daily activities or chores. Garnet said she had to manage her time to finish chores as quickly as possible. As she stated,

When using the restroom, sometimes I must bring him in. There is also no time for me to enjoy my meals. However, when he sleeps, I must hurry to do the laundry. Taking care of a child is time-consuming, busy, and takes dedication. I find time for myself. Anyhow, he comes first. I will adjust my time to suit what I must do for him.

As shown in Table 3, some participants have health conditions or chronic diseases, such as hypertension or diabetes. This subtheme showed that grandmothers were concerned about their medication schedules, managing their time to care for personal health conditions or diseases. Crystal expressed her concerns by stating that,

I will not let my health conditions interfere with other things that I have to do in my life. As I have to raise my grandson, I must ensure everything will be all right. I have tried my best to take care of my health. When I feel something is wrong with my health, I immediately take pills to prevent or alleviate the symptoms.

They were worried about their grandchildren's future if they were no longer available due to illness or disability. One participant expressed, "If I could not take care of him due to my illness, my grandson would be sent away or sent to someone else's custody. His parents would not take him back to their care".

Apart from their physical health, their mental health also needed attention and management. Through the use of time allocation, they managed and arranged their spare time to enjoy some relaxing activities to deal with stressors. Diamond stated, "I often went to the temple to join the merit-making ceremony. I arranged my time to participate in this activity for my spiritual well-being. It helps comfort my soul and mind. When I went there, I brought my grandson with me".

Ruby has spent her leisure time doing her favorite arts and crafts to relieve her stress. She stated, "I will find time to do crafts. When I am stressed, I thread beads, knit, and crochet. Sometimes, I make ribbon flowers for almsgiving. However, I must wait until my grandson goes to bed. If he is awake, I cannot do anything. I must stop everything and stay with him or play with him".

Adjusting childrearing methods to promote development

This finding reflected childrearing methods that were adjusted to meet the needs of grandchildren with ASD. Grandmothers were the first to spot autism in their grandchildren and realize the significance of searching for help and adjusting childrearing ways to promote development. Sapphire recalled,

I knew something was wrong. I wondered what was happening with him and asked what I should do. When I told my daughter, we both agreed to make a doctor's visit. However, her husband didn't agree to do so. He said, 'No need to see a doctor. If a child did not want to speak, just let it be.' But I insisted and begged them to bring the child to a doctor.

After several arguments and consultations among family members, it was found that they decided to take the child to be examined by a child and adolescent psychiatrist. Subsequently, the child has been diagnosed with ASD. Since then, the physician's recommendations have adjusted the ways to raise the child. Crystal shared her story by stating,

Before his diagnosis, I often opened YouTube for him to watch ABC and listen to other kids' songs. Those programs were fun and easy to understand for young kids. Therefore, I thought what I did was right and good for him to help him develop his language skills. But it turned out that he neither responds to verbal commands nor speaks. Now, I avoid anything that seems risky to keep him

from talking. I don't let him watch TV as much as he used to. I will follow the doctor's suggestions because I want my grandson to improve.

Adjusting how to raise a grandchild with ASD also involves teaching the grandchild to do various activities of daily living on their own as much as possible. One grandmother said, "I told him to take a bath. He did it by himself, even though it's still not as good as I did for him. He often comes to me and asks me for help since sometimes he still has soap all over his body". They expect that their grandchildren's development will improve. One participant told her story to illustrate her teaching about improving her grandson's toilet training skills. She said, "I taught him to take off his diapers. If he needs to pee, he uses gestures or takes me to the restroom. Right now, he will have a diaper on only at nighttime".

Dealing with grandchildren's behaviors caused by ASD

The following short quotes, such as "I raised my kids. None of them did what my grandson does," or "It is different from my child," or "I don't know why he keeps doing such behaviors," were repeatedly expressed by these grandmothers when they talked about their grandchildren's behaviors.

Data from interviews with grandmothers showed that they struggled to manage their grandchild's tantrums and inappropriate behaviors, especially in public places. One participant said, "It was unlike my child. My grandson behaves differently. I don't know how to manage". Another grandmother said, "I told him to stop. He didn't. He kept spinning and spinning".

Grandmothers have learned to use effective methods or strategies, such as taking a child out of the situation and removing the stimulus that triggers the behavior.

I took him outside the house to walk when I found that he sometimes could not control himself. However, sometimes it didn't work at all. He ran so fast that I could not catch him, so other people came to help. Raising him is pretty tricky. I often have to leave him inside the room and lock the door. However, most of the time, he needs close attention and cannot be out of sight. Occasionally, he bangs his head on the floor. Even though I tell him to stop, he does not listen. I had to pull him out or remove things that triggered these behaviors.

Sometimes such methods cannot stop the behavior of the grandchildren. Other methods, such as hitting, are needed to manage the behavior. Grandmothers knew hitting might not be the best solution. During the data collection process, all participants had not yet been trained or had practiced methods suggested by the hospital to manage tantrums and inappropriate behaviors.

The meanings of grandparenthood

When the participants were asked about the meanings of being primary caregivers for children with ASD, they spoke about "duty and commitment" and "love bonds".

Duty and commitment

Opal shares what it means to be a grandmother raising a granddaughter diagnosed with ASD. "It was a job... so we adopted the grandchildren". Sapphire sentimentally said, "Actually, I think it's my duty. I have to raise him. It's what I have to do. Now, I think I must do it well". The participants said that they help raise their grandchildren so that they can grow up under their care. Ruby revealed her feelings about that in the following remark.

I was so delighted when I had a grandson. I felt like my family was getting more members. It's excellent, even though raising him took much energy. I was tired, but still, I was more than happy to childcare for him. He is my offspring. One day when I am gone, he will still be here. He is a part of me.

Grandmothers provided information on raising grandchildren, starting with the duties of taking care of their offspring. The more they were together, the more a connection grew.

Love bonds

When asked what the idea of grandparenthood meant to them, the participants answered the questions and conveyed their responses in simple but meaningful words that expressed love and care.

The following excerpt is drawn from semi-structured interviews conducted with Opal. The interview questions included, "What does grandparenting mean to you?" She immediately said, "I love him. He is my grandson. I love him so much. I cannot find the right words to express how much I love him". Crystal commented on the bonding of love by saying,

Well, my grandson is my love. I didn't know when our bonding began. I only know that I love him very much. When I had my first child, it was pretty automatic in terms of how I felt and what I had to do as a parent. However, when my grandson came to live with me, I didn't know how love could bring us this far. My adult child sometimes said I love my grandson more than him. He teases me about it, saying, 'Oh… you love your grandchild more than you love me' (Crystal smiled shyly at the end of this sentence).

When taking grandchildren into their care, some participants accepted what they did because of duties and blood ties. However, as time passed, the more they stayed together, the more bonding occurred. Grandchildren with ASD often find themselves attached to their grandchildren. Their bonds of love keep growing and strengthening.

Discussion

This section will discuss the findings of this research concerning grandparenting occupations, occupational therapy, and occupational science. The strength of this study lies in its understanding of the grandparenting occupations of seven grandmothers.

The findings that were presented on the first theme - reasons for coming into the role of raising grandchildren with ASD - can be supported by the Model of Human Occupation or MOHO.[20] When humans interact with their environment, they modify their occupations to meet environmental demands. What humans choose, analyze, and execute in their occupations as they interact with the environment presents a dynamic, open-cycle system of human actions.[20] Within this model, three essential terms are key to understanding human actions and occupations: volition (motivation), habituation (habits, roles, and patterns), and performance. Deciding to become a primary caregiver for a grandchild with a diagnosis of ASD was an expression of a grandmother's intent to help their family to walk this difficult road together. Through these efforts, an adult child could return to work as the grandchild is now in good hands. It also allowed family members to continue their lifestyle activities within the context of each family. Likewise, a study by Ochiltree examined the role of grandparents as a key player in raising grandchildren due to changing family and social structures.[3] It has been reported that in the case of families with children diagnosed with ASD, grandparents have prioritized and been concerned about the well-being of the father and mother of the grandchildren.[13] The study results also revealed that when grandmothers decided to take roles in childrearing, they became the center of the family's mental and emotional care and became the cornerstone of the family.

The findings of this study revealed that grandparenting was an essential occupation in the participants' family contexts.[26] These grandmothers' lives had been changed according to occupational contexts and roles.[3] They had made every effort to endure by focusing on ensuring the best development possible for their grandchildren, helping them to do activities of daily living, teaching them to communicate, managing their tantrums and inappropriate behaviors, and, more importantly, giving love and care to them.[2] By doing so, specific patterns of grandparenting occupations (forms of occupation) emerged, such as allocating time in response to grandchildren's needs and engaging in daily routines, adjusting childrearing methods, and finding strategies to deal with and manage grandchildren's behaviors. Grandmothers in this study engaged in promoting the development of grandchildren with ASD, providing relevant additional data to combine with other studies that reported the main focus of grandparents in caring for their grandchildren's development and well-being.[27-29]

From an occupational science perspective, childrearing methods were considered adaptations because grandmothers had chosen those occupations by adapting them in a way that enabled them to meet their grandchildren's needs, and they could fulfill their grandparenthood roles if they had the abilities and available resources. This is consistent with what has been stated in the Occupational Adaptation Model that individuals will adjust in order to develop their abilities to perform activities or occupations that they desire or are expected to do.[21-22] Adaptation focuses on changing the demands of occupations, changing the physical or social environment, modifying the occupation itself, and/or applying or using assistive devices.[30] Grandmothers in this study illustrated that adaptation in childrearing methods involves adjusting the daily routines and occupations through time allocation and changing how to deal with their grandchildren's needs and behavioral problems so that they can continue their involvement in valued occupations.

According to Polatajko, the significant value of an occupation is that "it gives meaning to life and organizes behavior".[31] Grandmothers in this study engaged their lives, organized their occupational behaviors, and found meanings through "duty and commitment" and "love bonds". Although there were obstacles from the grandmothers' physical and mental health problems that limited their abilities to care for their grandchildren, they perceived the value and meaning of their love and devotion to their grandchildren. These grandmothers then had a clear goal of helping their grandchildren grow and develop as much as possible. According to Zero to Three, love and duty were the power to pull grandparents into caring for grandchildren.[32] Although Zero to Three did not study Thai grandparents, its ideas fit with the meanings expressed and perceived by the grandmothers in this study. This can also be supported by Bruner, who stated that an individual attempts to make sense of his or her action.[33] The taking of caregivers' roles could nourish a sense of belonging and contribution in families, that later provided meanings to the grandmothers in this study.

Limitations and future research

Based on a small number of participants, the findings of this study could not be generalized to the heterogeneous population of all Thai grandmothers raising grandchildren with ASD. Also, the findings of this study were not interpreted as the final or definitive information on this topic. This study offered a perspective on how these grandmothers engaged in grandparenting occupations and the subjective meanings of these valued occupations. It presented a view of how and why they became primary caregivers, how they adapted and adjusted to their childrearing occupations, and how they dealt with their grandchildren's behaviors caused by ASD. It also provided rich and detailed descriptions, making it applicable for further studies of grandmothers of children with ASD in the future.

Longitudinal studies on grandparenting occupations of children with ASD are necessary to understand changes across time. Further research is needed to identify and reflect on experiences in engaging in grandparenting occupations for grandfathers who have raised grandchildren with ASD.

Conclusion

The grandmothers in this study responded to the research question asking how grandparenting occupations for grandchildren with autism spectrum disorder were managed and orchestrated. Data from this study showed the challenges they experienced and the adaptations made by grandmothers in their daily lives as primary caregivers of children with ASD. Findings from this study also suggested that engagement in grandparenting occupations was meaningful for grandmothers in this study. This study could add a new layer to occupational therapy's understanding of grandparenting occupations' multiple and holistic meanings.

Conflicts of interest

The authors declare no competing interests in this research.

Acknowledgements

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Journal of Associated Medical Sciences

Health-related quality of life among Thai children and adolescents with cleft lip and/or palate: a qualitative study

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ARTICLE INFO

ABSTRACT

Background: Having cleft lip and/or palate (CLP) can be challenging for children and adolescents and appears to impact their health-related quality of life (HRQoL). To date, there is limited empirical work in this field, demonstrating a need for further exploration of qualitative research, especially with children and adolescents with CLP in Thailand.

Objectives: To explore the HRQoL perceptions of Thai children and adolescents with CLP.

Materials and methods: The present study was a qualitative approach. Eight children and adolescents with CLP, ages 9 to 14, were selected at the speech therapy clinic at Chiang Mai University, Thailand. Data were collected through a semi-structured interview guideline. The data were analyzed using thematic analysis.

Results: Four main themes were identified: (1) physical well-being, (2) psycho-emotional well-being, (3) social well-being, and (4) school functioning. Five subthemes further described this experience: communicative competence; teasing or bullying; self-perception and self-esteem; family and friend support; and difficulties in academic performance. Each theme was described with illustrative quotes.

Conclusion: The findings of this study provide a more thorough picture of HRQoL in children and adolescents with CLP. They have communicative competence, psycho-emotional well-being, social functioning, and school functioning issues rather than significant physical symptoms. Professionals should continue intervening as part of an interdisciplinary strategy to avoid or at least lessen negative effects on these children and adolescents with CLP.

Introduction

Orofacial clefts (OFCs) comprise cleft palate (CP), cleft lip (CL), and cleft lip and/or palate (CLP). OFCs involve structures around the oral cavity and can spread to facial structures, causing defects in the oral, facial, and craniofacial structures.[1-2] One in every 500-700 births globally is affected by OFCs[3], as well as one in every 500 Asian or Indian American births and one in every 2,500 newborns of African heritage.[4] In Thailand, CLP affected 2.2% of newborns in Chiang Mai Province between 2015 and 2019.[5]

The growing body of literature concerning having CLP can be challenging for children and adolescents and appears to impact their health-related quality of life (HRQoL). To date, a growing body of quantitative research

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supports this.[6-10] However, there was little qualitative research conducted in Thailand.[11]

HRQoL is a broad and multifaceted concept that includes a person's physical, social, and mental health as well as the symptoms of diseases/disorders or health conditions and the side effects of treatment.[12] Pediatric HRQOL instruments should be based on a conceptual framework considering the child's physical, emotional/psychological, social, and developmental concerns.[13] In a recent systematic review, the primary conceptual framework of CLP QoL concerns, however, was categorized, including physical (e.g., communication, pain, physical function, and general health), psychological (e.g., self-concept, behavior, satisfaction with appearance, psychological distress, and cognition), and social health (e.g., peer relations, school, family, social support, and social function).[14]

Regarding physical aspects, previous studies have found that individuals with CLP have speech problems, including compensatory articulation disorders and dysphonia.[15-17] Bickham and colleagues reported that 26.9% to 34.3% of children had issues with families understanding their speech, others understanding their speech, having a conversation with a group of familiar listeners, or having a telephone conversation.[6] Moreover, individuals with CLP may have a negative impact on various aspects of oral-health-related quality of life (OHRQoL).[7]

Regarding psycho-emotional aspects, Glener and colleagues found that throughout middle school, children with CLP were more affected by aesthetic concerns, bullying, and challenges with friendship and interaction with others and less so during elementary and high school.[8] Furthermore, Lovegrove and Rumsey found that adolescents were affected by teasing or bullying regarding their appearance, which caused considerable distress.[9] A qualitative study by Chimruang and colleagues reported that some Thai adolescents with repaired unilateral and bilateral cleft lip and palate expressed negative views such as classmate harassment and overprotective parents.[11] Also, Kanthawong and colleagues reported that self-esteem may be caused by an awareness of their values in general, such as physical characteristics and self-confidence. In addition, the study found that Thai adolescents with cleft lip and palate who received the lowest scores on self-esteem had the most severe clefts, whereas those who received the highest scores had less severe clefts.[18] However, Locker and colleagues reported that adolescents with CLP were well-adjusted and capable of coping with the challenges they confronted as a result of their conditions.[10]

In addition, regarding social aspects, Chimruang and colleagues reported that sociability problems had a more significant impact than self-image problems. Also, the study reported that friends and family were generally cited as sources of support and fun.[11] Furthermore, some studies found other relevant aspects in terms of academic ability. Richman and colleagues found that children with CP were more likely to have general language difficulties than children with cleft lip and palate, which could lead to long-term reading disabilities. Children with cleft lip and palate appeared to have reading difficulties that resolved with age.[19] When compared to their peers, children with oral clefts underperformed in all academic areas and grade levels.[20]

It can be seen that children and adolescents are affected by CLP, which can lead to a decrease in HRQoL in all domains. Also, the fact that there is limited empirical work in this field demonstrates that there is a need for further exploration of qualitative research, especially with children and adolescents with CLP in Thailand. This study aimed to explore the perceptions of HRQoL of Thai children and adolescents with CLP. This may lead to a better understanding of their quality of life and provide them with a voice so that relevant health professionals may learn from them and manage plans since the approaches must be multidisciplinary and timely.

Materials and methods

The present study was a qualitative approach based on the philosophy of phenomenology because its purpose was to explore the perceptions of HRQoL of Thai children and adolescents with CLP. This approach was used to gain more insight and understanding of subjective experiences and capture the complexities of in-depth experiences, such as those of adolescents with CLP.[11] In this study, we adopted the conceptual framework of CLP QoL described by Klassen and colleagues.[14]

The Ethics Committee, Faculty of Associated Medical Sciences, Chiang Mai University (CMU), Thailand (AMSEC-65FB-007) approved the study. The interviews were conducted while respecting the participants' privacy in a private and quiet space in the speech therapy clinic. The purposes and details of this study were fully explained to the participants and their caregivers. Written informed consent was obtained from participants and their caregivers before entering the study participants who requested to be removed from the research study without providing a reason. The participants' confidentiality and privacy were protected throughout the data collection process.

Participants

Eight children and adolescents with CLP, ages 9 to 14, were selected at the speech therapy clinic at Chiang Mai, University, Thailand. All participants were treated at the craniofacial center and the speech therapy clinic, using a standardized procedure. The research team chose a purposive sample of eight people out of a total of eighteen people. Participants were included in the study if they: (1) were diagnosed as having CLP by a pediatrician or plastic surgeon; (2) were willing to participate in this study and give written consent, as well as their caregiver; and (3) had adequate Thai language skills to contribute to the interview and express their views productively. We continued to recruit participants for the study until we reached data saturation, at which point no more information for the resulting notion could be obtained.[21]

Data collection

Data were collected through a semi-structured interview guideline,[22] designed to obtain reliable and comparable data.[23] All face-to-face interviews were held between August and October 2022. The semi-structured interview guideline, questions, and prompts were related to the perceptions of HRQoL of children and adolescents with CLP (Table 1). These interviews were audio-recorded using a recorder application on Vivo, and notes were also taken during the interviews. The duration of the interviews varied from 30 to 45 minutes. These interviews were transcribed verbatim.

Table 1. Semi-structured interview questions.

Domains	Questions
Physical	1. Do or how do you have a problem with your physical health? 2. Do or how do you have a problem with hearing? 3. Do or how do you have a problem with your oral health, such as teeth, or a problem chewing or choking on food when you eat it? 4. Do or how do you have trouble speaking when talking to other people? If any, can you tell me how difficulties with speaking affect your life?
Psychological	5. How do you feel about your appearance, and how do you think other people see you? 6. Can you describe what things you think you are good at and bad at? 7. How do you feel about yourself now, and how do you see yourself in the future?
Social	8. Who are you closest to in the family, and how do family members take care of you? 9. Do you have a best friend, and why are you so close to this friend? 10. Can you tell me about your experiences with bullying, such as pranks and mocking, and how you dealt with it? 11. Do you have to stop studying often to see a doctor or therapist, and how does the absence of school affect your life?
School	12. Do you want to go to school, play, or talk to your friends? 13. What school subjects do you like most and least; how are your grades; and are you satisfied with your grade?

Data analysis

The data were analyzed using thematic analysis.[24] The first step started with the project leader manually transcribing the interviews and reading them several times to become familiar with the content. Then, initial codes were generated and applied to the data set. Codes featuring similar content were then grouped, yielding themes that describe HRQoL as related to physical, psychological, social, and academic factors. Later, the researchers reviewed and refined the main themes and provided definitions for the themes. Finally, the researchers report data analyses are based on the whole sample, which is analyzed in Thai, and the excerpts used to show the results in the paper are translated into English.

Study rigor

Credibility, transferability, dependability, and confirmability were the criteria used to increase trustworthiness, including the prolonged engagement of the researchers in the study, multiple interviews and notes, member checking, and triangulation.[25,26]

Results

Table 2 illustrates the demographics of the participants' characteristics of eight children and adolescents with CLP aged 9 to 14. The average age of the participants was 10 years old. There were 5 females (62.5%) and 3 males (37.5%). The participants' cleft conditions included craniofacial clefts (N=1), cleft lip and palate (N=3), CP (N=3), and CL (N=1). Participants comprised 6 elementary school students (75.0%) and 2 middle school students (25.0%). All of them were in mainstream schools.

According to the data analysis, four main themes were identified: (1) physical well-being, (2) psycho-emotional well-being, (3) social well-being, and (4) school functioning. Five subthemes further described this perception: communicative competence; teasing or bullying; self-perception and self-esteem; family and friend support; and difficulties in academic performance, as shown in Table 3. Each theme was described with illustrative quotes.

Physical well-being
Communicative competence

The perceptions of all participants vividly noticed and expressed concerns about the impact of CLP on communicative competence. They mentioned how their cleft had affected their confidence in having conversations and how they tried to avoid having conversations, whether in person or front of a class:

"I have problems pronouncing aware consonants, such as [j] and [d]. When I speak, I am overly concerned with whether I will express something

Table 2. Demographics of the participants.

Interviewee	Age (Year)	Gender	Type of Cleft	School	Education
1	9.8	Female	Craniofacial clefts	Elementary school	Mainstream
2	11.7	Female	Cleft lip and palate	Elementary school	Mainstream
3	9.1	Female	Cleft palate	Elementary school	Mainstream
4	9.8	Female	Cleft lip and palate	Elementary school	Mainstream
5	9.7	Male	Cleft palate	Elementary school	Mainstream
6	14.2	Male	Cleft palate	Middle school	Mainstream
7	10.6	Female	Cleft Lip	Elementary school	Mainstream
8	11.11	Male	Cleft lip and palate	Middle school	Mainstream

Table 3. Interview themes.

Themes	Subthemes
Physical well-being	Communicative competence
Psycho-emotional well-being	Teasing or bullying
	Self- perception and self- esteem
Social well-being	Family and friend support
School functioning	Difficulties in academic performance

correctly. It induces stuttering... My communication causes me concern. Therefore, I lack self-assurance; I do not dare to speak and am frightened that others will not comprehend me. I lack confidence when addressing my peers in front of the class." (Interviewee 7)

"It is difficult for others to understand me as I have difficulty speaking clearly, and my voice is hoarse. I will stop talking and be silent. Even though no one makes fun of me at school, I am not very confident." (Interviewee 8)

Psycho-emotional well-being
Teasing or bullying

More than half of the participants reported having experienced negative emotions such as worry about other people's opinions, sadness, or moodiness in the past because they were teased or bullied:

"I was bullied at school. I got teased a lot when I was in kindergarten. I was angry at that time. There was one friend who kicked me in the back. I did not know why he bullied me. But he did not get teased about looks. Also, some friends stared at me. Now I am used to it anyway. So, if someone looks at me, let them look." (Interviewee 1)

"I was being bullied about having a cleft at school. I told my teacher that I was teasing. My teacher summoned that friends and warned him that he would be expelled if he did it again." (Interviewee 4)

"My friends joked that my nose was crooked, and my face was not pretty. At first, it hurt quite a bit." (Interviewee 2)

Self-perception and self-esteem

However, as they grew older, they nurtured positive emotions both in self-perception and self-esteem. They were able to accept this fact and see the positive aspects of themselves. All of the participants were able to adapt and adjust to their condition:

"I am not worried anymore because I cannot change anything. I feel good about myself now. At school, my friend asked me to help them study. (Interviewee 1)

"I am not worried about my condition. I feel confident. I know my strength. I am a fun person, and I am good at art." (Interviewee 4)

"Now I feel nothing. Some other friends told me that I am beautiful, and I rate my beauty at 7 out of 10. I feel confident, and I am happy for who I am." (Interviewee 2)

Social well-being
Family and friend support

Overall, all participants reported having good relationships with family and friends and enjoying attending school. They believed their friendships were valuable, that they were welcomed by their friends, that they could communicate with and rely on their friends, and that they felt cheerful when in the presence of friends:

"My parents look after me perfectly. They support me, so I feel confident in my life. I like to go to school. I have three best friends. I like to do manly things. I like playing football with them." (Interviewee 3)

"My parents will be the ones to give me advice. My parents will warn me when I do bad things."

He also said, "I like to go to school. I have many friends, but I do not know which one is my best friend. They are all close to me. After school, we played in the neighborhood." (Interviewee 6)

School functioning
Difficulties in academic performance

Most participants mentioned their academic achievements. They did not like academic subjects such as Thai language, English, and mathematics. They preferred physical education and the arts, as they felt that they got good grades without studying hard:

"I like physical education and art. I do not like math. I do not understand and do not know how to do it. Overall, I am not satisfied with my GPA." (Interviewee 4)

"I do not like English. I couldn't remember what grade I got last semester, but I did not get an A. My father told me if the regular school system does not work for me when I finish high school, he wants me to study vocational education instead." (Interviewee 5)

"I love reading comics, drawing, and painting. At school, I am waiting for the art class. It makes me feel free and relaxed. I don't like mathematics because I do not know how to solve math problems, and there is too much homework." (Interviewee 7)

Discussion

This study specifically focused on exploring the perceptions of HRQoL of Thai children and adolescents with CLP. The findings of our study found that, in terms of physical aspects, all of the participants mentioned difficulty in communication competence with others understanding their speech and having a conversation with familiar listeners such as friends, similar to Bickham *et al*.[6] Participants also reported speech problems, including articulation and resonance disorders, even though they had received cheiloplasty, palatoplasty, velopharyngeal insufficiency (VPI) correction and evaluation, and speech therapy according to the protocol for cleft lip and cleft palate at the craniofacial center and at the Chiang Mai University, Thailand. Compensatory articulation disorders can be found in individuals with CLP, resulting in reduced speech intelligibility.[15,16] In addition, dysphonia is characterized by breathiness, hoarseness, and low intensity of voice and can also be found during speech tasks.[17] Therefore, speech pathologists should be involved in early intervention to prevent or help CLP-related communication problems. Individuals living with cleft lip and palate were determined to maintain their sense of dignity after having their speech treatment.[27]

In contrast to Defabianis *et al*.,[7] none of the participants with CLP in this study reported experiencing problems with their physical functioning in terms of oral health. Similar to Konan *et al*.,[28] Thai children and adolescent patients with CLP had generally positive OHRQoL. Our participants could have received dental care since there was a treatment protocol for cleft lip and palate at the craniofacial center in northern Thailand. That cleft lip and/or palate patients should receive immediate care from a dentist.

Additionally, most participants reported that they experienced social stigma due to their appearance. They were teased or bullied in grades 1 or 2 in elementary school. They were in psychological distress. Like Lovegrove and Rumsey, adolescents were affected by teasing or bullying regarding their appearance, which caused considerable distress.[9] However, all participants reported that, at present, they were satisfied with their appearances. They also should be notified that they may face challenges when they become concerned about their appearance as they age.[8] In contrast, Prathanee found that Thai children with CLP experienced social stigma due to speech and language problems after surgical treatment[29] but not due to their appearance. Our findings provide direct input into teachers' practice. It is critical to help children who have been bullied because of their appearance feel safe at school. This could be facilitated by having a clear school policy on what teachers should do and how they should act.

Importantly, it should be noted that our findings did not explore social stigma for children and adolescents with CLP in the perceptions of their family or friends. Chung *et al*. found that the perception of a child with CLP in low-resource areas is negatively influenced by parental blame or the belief that the cleft results from a punishing supernatural force. A child with CLP is bullied, rejected, and socially isolated, sometimes even from their family. This misery continues in school, resulting in a dropout and adding a further barrier to finding their preferred job. An inability to fully participate in the workforce results in significant financial consequences and decreased productivity.[30] Therefore, further study can provide the opportunity to explore in greater depth.

Furthermore, in our findings, female participants reported positive self-esteem as they grew older, while male participants did not mention it. Kanthawong *et al.* found no statistically significant relationship between self-esteem and personal characteristics such as gender or age.[18] Similar to Locker *et al*. it might also be that children born with CLP are well-adjusted and can cope with the adversities they experience due to their conditions.[10] Also, Eiserman found that children born with CLP showed positive self-perception and psychosocial adjustment.[31] Therefore, it can be seen that self-perception is critical to the adjustment process and is one of the key indicators of positive adjustment.[32]

This study also found that participants reported having support from their families and a good relationship with friends at school. Kramer *et al*. mentioned that family functioning is a key component in preventing children with CLP from being exposed to influences that decrease their quality of life.[33] Those who received constant support from

parents, friends, and professionals were also more likely to have a favorable health status and higher treatment satisfaction.[11]

Our study found that most participants preferred physical education and the arts to academic subjects such as Thai, English, and mathematics. Richman *et al.* found that children and adolescents with CLP had a high rate of reading disabilities, particularly in children.[19] Children with clefts were approximately one-half grade level behind their peers, had greater academic underachievement rates, and used higher rates of special education services.[20] On the other hand, Collett *et al.* found that children with clefts did not perform worse than control participants on neurocognitive and academic achievement assessments.[34] Further investigation into additional diagnoses, such as cognitive impairments linked to reading difficulties, is needed. As a result, it is essential to consider that children must be evaluated and receive early intervention for academic difficulties to be identified and supported.

Limitation

Several limitations were noted in this study, such as the small sample size because our study was qualitative. The participants included in the study were not representative. Future mixed-method research, such as exploratory research that includes quantitative research with a larger sample size, might be used. Also, more research comparing the views of typically developing children and children with CLP is needed to better understand the importance of these various HRQoL elements in children with CLP.

Conclusion

The findings of this study provide a more thorough picture of HRQoL in children and adolescents with CLP. They have communicative competence, psycho-emotional well-being, social functioning, and school functioning issues rather than significant physical symptoms. Professionals such as a dentist, speech therapists, a teacher, and parents should continue intervening as part of an interdisciplinary strategy to avoid or at least lessen adverse effects in these children and adolescents with CLP.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this paper.

Acknowledgements

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Validity and reliability of the Thai Adaptation of Naming Test-Revised (TANT-Revised)

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ARTICLE INFO

ABSTRACT

Background: The Thai Adaptation of Naming Test (TANT) is useful for assessing naming ability among Thais with naming deficits. However, the TANT was developed in 2004, meaning some words have become less common. Consequently, the TANT was revised for modernity and suitability for the current Thai cultural context and to measure the psychological properties.

Objectives: To assess the validity and reliability of the Thai Adaptation of Naming Test-Revised (TANT-Revised).

Materials and methods: The TANT was revised by adjusting certain items for suitability to the current Thai cultural context, consisting of 2 parts: Pictures of words and semantic cues. Subsequently, the TANT-Revised was assessed for content validity index (CVI), content validity for the item (I-CVI), and content validity Index for scale (S-CVI) test by five expert speech-language pathologists (SLPs). Afterward, the TANT-Revised was examined for test-retest reliability by intraclass correlation coefficient (ICC) in an aphasia group and a normal group, with 13 people per group.

Results: The results of testing for the validity of TANT-Revised by expert SLPs revealed that the validity for pictures (CVI = 0.96, S-CVI = 0.99, and I-CVI = 1) and semantic cues (CVI = 0.95, S-CVI = 0.99, and I-CVI = 1) have high reliability in the normal group at 0.79 and the aphasia group at 0.96.

Conclusion: This study assessed the psychological properties of the TANT-Revised instrument used to test naming ability. The validity of the revision test was high, and the TANT-Revised was deemed acceptable by experts. In addition, the test-retest reliability of TANT-Revised was high in both the normal and aphasia groups, implying the TANT-Revised is an efficient instrument for confrontation naming ability. However, this study was a preliminary test revision, and insufficient norm data and factors are affecting the test score.

Introduction

People who have difficulty retrieving words correctly or lose the ability to produce a specific word in different contexts at the time desired suffer from "word-finding difficulty."[1, 2] In patients with aphasia, it is a prominent attribute that indicates lexical retrieval impairment.[3]

The Boston Naming Test (BNT) is the most popular standard visual picture-naming test for lexical processing abilities, consisting of 60 black & white pictures with common easy–difficult words.[4] The BNT is suitable for testing the naming ability of children, adults with brain

injury or dysfunction, aphasic patients, and of normal adults.[5] The BNT has been developed in various forms, starting from the original version published by Kaplan *et al*. in 1978 as a trial with 85 items.[5] Subsequently, Kaplan, Goodglass, and Weintraub[4] revised it in 1983 with 60 items. Later, a study on normative data was carried out by Van *Gorp et al*. on the elderly using the initial BNT 60-item.[6]

Research related to the validation of BNT has been carried out previously, though most studies on the validity of BNT intended to examine the validity of participants at that time to measure the sensitivity of naming ability.[7-10] Axelrod, Ricker, and Cherry[7] studied the concurrent validity of the BNT in patients diagnosed with psychiatric disorders, while Locascio, Growden, and Corkin[8] examined the validity of the BNT to detect Alzheimer's disease (AD). Zgaljardic *et al*.[9] studied the validity of the BNT in patients with acquired brain injuries to find the convergent validity of the NAB Naming Test correlated with BNT. Furthermore, the validity of the BNT for normal people was studied by Yochim, Kane, and Mueller[10] to assess the convergent validity and discriminant validity of the NAB Naming Test among the elderly and analyze the relationship with BNT. In addition, Madore *et al*.[11] studied the validity of the Verbal Naming Test and the BNT in older veterans to study the convergent and discriminant validity.

For the reliability test, the instrument has been demonstrated to be stable and compatible,[12] especially the revised version of the test for reliability testing. In addition, most of the methods selected to analyze reliability are test-retest. The study of the range of reliability in BNT has retest periods within two weeks, 1 month, 2 months, 8 months, and once a year.[13-17] Most previous studies tested healthy people with no neurological disorders.[13-15,17] The study by Flanagan and Jackson showed a test-retest reliability for BNT at 0.91, while Murray revealed a test-retest reliability for BNT at 0.96, and Sawrie *et al*.[13,15,16] showed a test-retest reliability for BNT at 0.94. Mitrushina and Satz carried out a test-retest for the reliability of BNT the first and second time at 0.62, the second and third time at 0.65, and the first and third time at 0.89.[17] Except for the study by Sawrie *et al*. tested the abilities of patients with epilepsy, the test-retest reliability of BNT was 0.94.[16]

The original BNT was in English. However, when translated for use in other languages, BNT's effectiveness in assessing naming ability is affected due to English's possible unsuitability with the translated languages' native cultures.[18-21] Therefore, the BNT-60 item was constructed for development in different languages, including Brazilian Portuguese, Korean, Malaysian, Greek, Spanish, Indonesian, and Thai.[14,20,22-26]

In Thailand, Koonchit developed the Thai Adaptation of Naming Test (TANT) to study naming ability among Thai people in Bangkok aged between 20-86 years.[14] The results revealed that younger subjects scored significantly higher than elderly subjects ($p<0.01$). TANT's testing and scoring criteria are constructed in accordance with the standard instruction in the original BNT.[14] The TANT is also an instrument used by SLPs for naming ability tests in patients with aphasia. Prasurdeengam used the TANT for a comparative study on naming ability in Thai patients with aphasia.[27] She found that a group of patients with pathological conditions, including posterior aphasia, had significant mean scores for spontaneous correct responses and percentage for naming ability after semantic cues than those with anterior aphasia ($p<0.05$); semantic cues may have improved word comprehension difficulties in this group of patients.[27]

As time passes, the frequency of some words used in the TANT does not communicate the current age or the scarcity of use in today's daily communication. Therefore, the researcher aimed to revise the TANT for improved suitability to the current context. The TANT-Revised was prepared by word replacement using the same semantic categories. Words in the same categories were matched with their semantic cues, which would describe the words for the particular subjects in the case of naming failures. The selected naming words were sought from the Royal Thai Institute Dictionary 2011 and the corpus of Bandhumedha.[28,29] Then, all 60 items were re-ordered by word frequency from the Thai National Corpus (TNC).[30]

According to the test process, the standard instructions from the original BNT and TANT were used for each picture, allowing 20 seconds to answer. Test completion was stopped in the case of six consecutive failures. In addition, stimulus cues were used in any case of misperception or lack of recognition. However, phonemic cues were used in the case of incorrect answers after cueing, which included the initial consonant and vowel for that word.[4,14,27,31] The TANT-Revised uses different scoring criteria compared to the TANT in that if a subject responds with a spontaneous answer, it scores 1 point, while a correct answer after using the semantic cue scores 0.5 points, and a correct answer after using the phonemic cue scores 0.25 points. The total score is the sum of all correct answers.

The TANT revised version has not been assessed for psychometric properties. Thus, this research aims to study the validity and reliability of the TANT-Revised.

Materials and methods

This study was designed as an experimental study. The study was divided into two stages. The first stage was validity testing by five expert SLPs. The second stage was reliability testing by using the TANT-Revised in the aphasia group and the normal group.

Data collection started from April 2021 to February 2022 after the project was approved by the Human Research Ethics Unit for the Faculty of Medicine, Ramathibodi Hospital (COA. No. MURA2021/318 Ref. 1335), and informed consent was received from all participants.

Subjects

Participants were recruited for this study. They would attend to the stage of reliability testing. The subjects include patients, caretakers, and visitors at the Speech and Language Clinic and Department of Rehabilitation

Medicine from the Faculty of Medicine Ramathibodi Hospital and Golden Jubilee Medical Center, Faculty of Medicine Siriraj Hospital. They were adults aged ≥ 20 years, divided into 2 groups comprising an aphasia group and a normal group consisting of 13 subjects in each group.

The inclusion criteria for the normal subjects in this study included: (1) healthy adults; and (2) a score for the Mini-Mental State Examination Test (MMSE-Thai) >23 points.[32] The exclusion criteria for normal subjects included having a medical history of neurological disorders, mental disorders, or problems with communication disorders. In the aphasic subjects, inclusion criteria included: (1) being diagnosed by neurologists as having aphasia for at least 6 months post-onset; (2) having pathological conditions for a left-hemisphere stroke; and (3) having scored ≥40 points for the Aphasia Quotient (AQ) from the Thai Adaptation of Western Aphasia Battery Test (WAB) or having a mild to moderate severity level due to being severe if the score is lower than this.[27] All participants are native Thai speakers living in Bangkok and the Metropolitan Region, graduated from elementary school or higher, and have no hearing and eyesight/vision problems.

Data collection and statistical analysis
Validity testing
The TANT-Revised with adapted pictures that suited the cultural context was forwarded for content validity testing. The measurement of content validity consists of Content Validity Index (CVI), Content Validity for Item

(I-CVI), and Content Validity Index for Scale (S-CVI). Five licensed SLPs with more than ten years of experience considered the test as containing contents and pictures that met the objectives of the test and could be used for the naming ability test. The test was revised along with suggestions from the experts in the case of incongruence. The CVI of TANT-Revised was based on criteria of ≥0.80, I-CVI should not be less than 0.78, and S-CVI should not be less than 0.9.[33,34]

Reliability testing
TANT-Revised was used to test the reliability of the participants. Test-retest reliability was used to check the stability of the test for naming ability scores. TANT-Revised also tested both groups. After two weeks, the subjects were re-tested using the same methods and scoring criteria. The total test-retest scores for the subjects were calculated for reliability using ICC and interpreted according to Koo and Li.[35]

Results
Validity of the TANT-Revised
The content validity was tested by five expert SLPs. The validity test of the TANT-Revised was divided into two parts. Part 1 comprised new black & white pictures drawn with lining similar to the original ones, while Part 2 included semantic cues for the test pictures. The results of these two parts found that the CVI, S-CVI, and I-CVI passed the criteria and were accepted by the experts, as in Table 1.

Table 1. Validity of TANT-Revised.

TANT-Revised	CVI	S-CVI	I-CVI Median (range)
Part 1: Picture of words	0.96	0.99	1 (0.8-1.0)
Part 2: Semantic cueing	0.95	0.99	1 (0.8-1.0)

Participants' characters
The normal group included four males and nine females aged between 32 and 71 (Mean=55.84; SD=10.65). In the aphasia group, eight males and five females aged between 34-73 years (Mean=56.38; SD=9.42). Their educational level, the MMSE scores for the normal group, an AQ score for the aphasic group, and the aphasia type are presented in Table 2.

According to the test-retest for the naming ability of the TANT-Revised in both groups of participants, the scores for some correct answers used a spontaneous response (SR), semantic cue (SC), and phonemic cue (PC), including incorrect answer or no answer. The normal group obtained the scores in Table 3, while the aphasia group obtained the scores in Table 4.

When data were analyzed by STATA showing the measurement and ICC of the participants in the normal group, which were higher than in the aphasia group, the ICC in the normal group was 0.79 (95% CI=0.16-0.94), and the subjects with aphasia was 0.96 (95% CI=0.45-0.99) (Table 5). These test-retest reliability values implied that

the normal group had good reliability, whereas the aphasia group had excellent reliability for test-retest reliability.

Discussion
Validity of the TANT-Revised
The TANT-Revised included some updated words and all new drawings. The psychometric properties of the picture words and the developing semantic cues were studied. The researchers tested its validity. Scoring for itemized pictures and semantic cues was implemented by five experienced assessors. Test completion processes and scoring criteria were arranged, reviewed, and given feedback by the experts. The experts suggested adjusting some of the contents of semantic cues in items 1 and 23 (Appendix 2) due to the descriptions of the words not being appropriate or clear. The researchers revised the test for more suitability, as suggested by the experts. The results shown in Table 1 conclude that the validity of the TANT-Revised, in terms of CVI, I-CVI, and S-CVI was at a very high level for content validity, similar to the study of Koonchit,[14] and accepted

Table 2. General data of participants.

No.	Normal				Aphasia					
	Gender	Age (year)	Education	MMSE score	Gender	Age (year)	Education	AQ score	Aphasia type	Duration of illness (year)
1	Male	71	Bachelor degree	24	Male	61	Doctoral degree	94.4	Anomic	3.1
2	Female	69	Bachelor degree	29	Female	66	Elementary school	53.6	Broca's	2.5
3	Male	32	Bachelor degree	28	Male	34	Senior high school	51.8	Broca's	5.5
4	Female	59	Bachelor Degree	26	Female	53	Senior High School	67.1	Transcortical motor	12
5	Female	63	Bachelor degree	29	Male	57	Bachelor degree	57.6	Transcortical motor	2.5
6	Female	56	Senior high school	27	Female	60	Middle school	72.7	Anomic	18
7	Female	47	Vocational certificate	29	Male	60	Diploma degree	44.4	Broca's	9.1
8	Female	53	Bachelor degree	29	Male	46	Elementary school	93.8	Anomic	5.5
9	Male	43	Bachelor degree	27	Male	52	Middle school	67.7	Transcortical sensory	0.8
10	Male	60	Elementary school	28	Male	73	Bachelor degree	69.7	Transcortical sensory	1.2
11	Female	63	Doctoral degree	26	Female	57	Bachelor degree	93.4	Anomic	11.8
12	Female	53	Bachelor degree	26	Female	55	Bachelor degree	91.7	Anomic	12
13	Female	57	Bachelor degree	26	Male	59	Middle School	61.6	Conduction	1.5

Table 3. Scores from TANT-Revised in the normal group from test-retest measurement.

No	The number of answered items								Interpreted score	
	Spontaneous response		Semantic cue		Phonemic cue		No answer/incorrect		SR+SC+PC	
	Test	Retest	Test	Retest	Test	Retest	Test	Retest	Test	Retest
1	57	57	1	2	1	0	1	1	57.75	58.00
2	53	56	5	2	0	2	2	0	55.50	57.50
3	51	57	2	1	2	0	5	2	52.50	57.50
4	58	58	0	0	0	0	2	2	58.00	58.00
5	54	59	3	1	1	0	2	0	55.75	59.50
6	53	56	4	2	2	2	1	0	55.50	57.50
7	52	57	1	2	4	0	3	1	53.50	58.00
8	57	56	2	3	1	1	0	0	58.25	57.75
9	54	54	1	2	1	2	4	2	54.75	55.50
10	46	43	5	9	3	4	6	4	49.25	48.50
11	55	56	3	3	1	1	1	1	56.75	57.75
12	55	56	2	3	2	0	1	1	56.50	57.50
13	56	59	4	1	0	0	0	0	58.00	59.50
Mean									55.53	57.11
SD									2.58	2.76

Table 4. Scores from TANT-Revised in the aphasia group from test-retest measurement.

No	The number of answered items								Interpreted score	
	Spontaneous response		Semantic cue		Phonemic cue		No answer/incorrect		SR+SC+PC	
	Test	Retest	Test	Retest	Test	Retest	Test	Retest	Test	Retest
1	49	53	5	2	1	2	5	3	51.75	54.50
2	32	29	3	5	5	5	20	21	34.75	32.75
3	17	15	2	5	14	14	27	26	21.50	21.00
4	13	18	5	6	10	18	28	18	18.00	25.50
5	27	33	0	2	12	12	17	13	30.00	37.00
6	34	41	5	2	9	7	12	10	38.75	43.75
7	6	8	2	3	12	14	32	29	10.00	13.00
8	41	54	8	2	3	1	8	3	45.75	55.25
9	45	50	8	2	5	5	2	3	50.25	52.25
10	24	25	1	2	8	14	22	19	26.50	29.50
11	44	49	3	1	4	6	9	4	46.50	51.00
12	38	45	8	6	9	4	5	5	44.25	49.00
13	35	48	9	3	8	4	8	5	41.50	50.50
Mean									35.34	39.61
SD									13.21	14.11

Notes: In the first test, Subjects 4, 5, 7, and 10 in the aphasia group had six consecutive failures. Thus, the subjects had to stop completing the test, which had already gone through until the final part. Moreover, In the second test, Subject 7 also had six consecutive failures in the retest. Thus, this subject had to stop completing the test, which had already gone through until the final part.

Table 5. Display of test-retest reliability utilizing ICC analysis for the normal group and aphasia group.

Group	ICC (Average)	95 % Confident interval
Normal	0.79	0.16-0.94
Aphasia	0.96	0.45-0.99

by the experts as being an appropriate test for naming ability. This test conformed to a previous study about the measurement of the BNT, which concluded that the BNT had concurrent validity, sensitivity, verbal intellectual abilities, and naming performance with significantly excellent interest agreement, including high convergent validity and discriminant validity of the NAB naming test.[7,9,10] In addition, the BNT was suitable for the detection of sensitivity and differences between patients with mild and severe Alzheimer's Disease.[8]

Picture naming in TANT-Revised

A few subjects in the normal group (3.58%) had incorrect answers at different orders along the test. It was found that the number of incorrect answers increased for the last ten items of the test, which conformed to the study of Nicholas *et al*.[36] These wrong answers for the normal group could occur for those who might name words that were semantically related to the target words, e.g., the name "Lion" instead of "Leo," (item 4). When semantic cues were given, they could answer correctly. Some words that only a few subjects could name correctly might need consideration for future test revision. Incorrect

answers were different in the aphasia group, possibly due to brain injuries or different neurological dysfunctions.[27, 37] The item with the least correct answers was "Asparagus" (item 53), ranked as one of the last ten items in the test. This incorrect answer may be associated with verbal paraphasia and semantically related to the target word. Thus, a replacement was required, e.g., named "Water bamboo shoot"; and perceptual misnaming, e.g., named "Candle".

Reliability of TANT-Revised

The TANT-Revised reliability was evaluated using test-retest among the participants (normal and aphasic groups). It was analyzed by ICC. In Table 3, a strong intraclass correlation coefficient (ICC) was presented and interpreted based on the results from the study of Koo and Li as a tool that had high stability for the test-retest examination within 2 weeks.[13] The results were the same as a previous study by Koonchi in which the test-retest reliability was 0.94 (r=0.8-1.0).[14]

The TANT-Revised uses a 60-item format that also had results for test-retest reliability that were similar to the study of Thompson and Heaton, which could

better differentiate patients with naming impairment.[38] Moreover, it had a high correlation with the 85-item version (r=0.96) used to test patients with mental and neurological disorders. The reliability in this study had a strong result similar to the study of a normal group by Mitrushina and Satz to find reliability, which found that test-retest reliability was 0.62-0.89.[17] Murray found the reliability was 0.96 (*p*<0.001).[15] The results also conformed to the study of Flanagan and Jackson, with test-retest reliability of 0.91.[13] Likewise, the study of Sawrie *et al*.[16] demonstrated that test-retest reliability was 0.94 in patients with epilepsy.

The TANT-Revised in this study had high reliability, implying that it could be a good tool for assessment in both normal and aphasia groups. Because of several issues, such as selecting words for naming from the Thai dictionary, which is a source of data for accepted terms, the frequency of words is more suited for communications, including the test-retest reliability process within two weeks according to the theory in the study by Streiner and Norman.[12] Moreover, the lining of word pictures from the TANT-Revised implied that the words were good for the imagination of participants. Whenever they see the pictures, they can recognize and name them.[39]

Limitations of the study

All participants were recruited from clinical settings. It might not represent all aphasia patients in the community. The TANT-Revised has no sufficiently normative data or cut-off points for naming ability assessment in a study for large groups. Thus, those using the TANT-Revised should be aware of the word frequency because the frequency data from the Thai National Corpus may sometimes need to be updated. As a result, the word frequency remains subject to change. Therefore, they might be reordered for a new set in future studies.

Future study

Data should be collected to find the norms and cut-off points in larger groups, which could be used as the norms of Thais for naming ability. This study does not control for gender, age, or education factors. Thus, it could not be concluded whether such factors have any effect on the score of the test. There should be studies on the factors of education, age, gender, or aphasia type that might affect word retrieval ability by TANT-Revised.

Conclusion

This study aimed to assess the validity and reliability of the TANT (revised version). The test was revised to reflect updated words and suitable semantic cues for better understanding and accuracy in terms of score calculation.

The revised test was examined for validity by five expert SLPs, with their opinions toward target words correlated with pictures and semantic cues. The CVI, S-CVI, and I-CVI were high, implying that the acceptance from the experts confirmed its suitability as a naming ability test. The reliability measurement with the test-retest method

in the normal and aphasia groups used ICC. It was found that the tool had high reliability for both groups.

Conflict of interest

The authors declare no conflict of interest.

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Appendix 1. Word items and semantic cue of TANT

No	Item	Semantic cue	No	Item	Semantic cue
1	ต้นไม้	-	31	วงเวียน	สำหรับวาดกลม
2	กรรไกร	ใช้ตัด	32	ตั๊กแตน	แมลง
3	หวี	ใช้แต่งผม	33	ฮิปโปโปเตมัส	-
4	ดอกไม้	-	34	เปลญวน	ใช้นอน
5	เลื่อย	เครื่องมือช่าง	35	หนุมาน	ตัวละครในรามเกียรติ์
6	แปรงสีฟัน	ใช้ในปาก	36	สมอ	อุปกรณ์เรือ
7	ดอกเห็ด	กินได้	37	บ่วง	ใช้แขวนคอ
8	ไม้แขวนเสื้อ	พบในตู้	38	บ้านเรือนไทย	-
9	ธนู	ใช้ยิง	39	รถเข็นล้อเดียว	-
10	พวงหรีด	ใช้ในงานศพ	40	บุ้งกี๋	-
11	ตะเกียบ	ใช้คีบอาหาร	41	กระบองเพชร	พืชในทะเลทราย
12	กลอน	อยู่ที่ประตู	42	หอยทาก	-
13	เตียง	ใช้นอน	43	เกมส์โดมิโน	-
14	ดินสอ	ใช้เขียน	44	นกกระจอกเทศ	-
15	ขลุ่ย	เครื่องดนตรี	45	กล้องจุลทรรศน์	-
16	ไม้กวาด	ใช้ทำความสะอาด	46	หน่อไม้ฝรั่ง	-
17	ชฎา	ที่สวมศีรษะ	47	หน้าจั่ว	ส่วนประกอบของบ้าน
18	ประทัด	ใช้ในเทศกาลจีน	48	เรือสำเภาจีน	-
19	บันไดเลื่อน	-	49	พิณ	เครื่องดนตรี
20	ลูกคิด	ใช้คิดเลข	50	สิงห์	สัตว์ในวรรณคดี
21	กรวย	ใช้ในการเท	51	โตก	ใช้ใส่อาหาร
22	คีม	เครื่องมือ	52	หีบเพลงปาก	เครื่องดนตรี
23	อูฐ	สัตว์ที่อยู่ในทะเลทราย	53	ตะกร้อปาก	ใช้กับสุนัข
24	อนุสาวรีย์ประชาธิปไตย	-	54	บายศรี	ใช้ในงานพิธี
25	เฮลิคอปเตอร์	-	55	ตั่ง	ใช้นั่ง
26	โปรแทรกเตอร์	ใช้วัดมุม	56	ไม้ระแนง	ใช้ในสวน
27	ตาลปัตร	เครื่องใช้ของพระ	57	แอก	ใช้ในงานเกษตร
28	มะม่วงหิมพานต์	-	58	หีบเพลงมือ	เครื่องดนตรี
29	ภูเขาไฟ	ภูเขาชนิดหนึ่ง	59	ฝาละมี	ฝาหม้อโบราณ
30	ม้าน้ำ	สัตว์ทะเล	60	ทับทรวง	เครื่องประดับบริเวณหน้าอก

Note: (-) Semantic cues of the original words from TANT that had not been available before.

Appendix 2. Word items and semantic cue of TANT-Revised

No	Item	Semantic cue	No	Item	Semantic cue
1	ดอกไม้	ส่วนที่ผลิออกจากต้นหรือกิ่งของต้นไม้	31	คีม	เครื่องมือ
2	ต้นไม้	เป็นพืชทั่วไป	32	ลูกคิด	ใช้คิดเลข
3	เตียง	ใช้นอน	33	ชฎา	ที่สวมศีรษะ
4	สิงห์	สัตว์ในวรรณคดี	34	ชักโครก*	ที่นั่งถ่ายอุจาระ
5	กลอน	อยู่ที่ประตู	35	หนุมาน	ตัวละครในรามเกียรติ์
6	ธนู	ใช้ยิง	36	เต็นท์*	ที่พักอาศัยชั่วคราว
7	ดินสอ	ใช้เขียน	37	ฉิ่ง*	เครื่องดนตรีประเภทตี
8	ไม้แขวนเสื้อ	พบในตู้	38	บ้านเรือนไทย	ที่อยู่อาศัย
9	พิณ	เครื่องดนตรี	39	ม้าลาย*	เป็นสัตว์ที่มี 4 ขา
10	หวี	ใช้แต่งผม	40	ประทัด	ใช้ในเทศกาลของจีน
11	ตะเกียบ	ใช้คีบอาหาร	41	บายศรี	ใช้ในงานพิธี
12	อนุสาวรีย์ประชาธิปไตย	สถานที่ตั้งอยู่กึ่งกลางวงเวียนบนถนน	42	หอยทาก	สัตว์ที่มีเปลือกแข็งหุ้ม
13	พวงมาลัย*	อุปกรณ์บังคับรถหรือเรือ	43	แปรงสีฟัน	อุปกรณ์ที่ใช้ทำความสะอาดในช่องปาก
14	กรวย	ใช้ในการเท	44	นกกระจอกเทศ	สัตว์ปีกขนาดใหญ่
15	ภูเขาไฟ	ภูเขาชนิดหนึ่ง	45	โจงกระเบน*	ผ้านุ่งชนิดหนึ่ง
16	ไม้กวาด	ใช้ทำความสะอาด	46	พวงหรีด	ใช้ในงานศพ
17	อูฐ	สัตว์ที่อยู่ในทะเลทราย	47	ลูกเต๋า*	วัตถุทรงเหลี่ยมมีหกหน้า
18	กรรไกร	ใช้ตัด	48	ม้าน้ำ	สัตว์ทะเล
19	ขลุ่ย	เครื่องดนตรี	49	แว่นขยาย*	ใช้สำหรับส่องดูให้เห็นเป็นภาพขยาย
20	เรือใบ*	พาหนะทางน้ำชนิดหนึ่ง	50	บันไดเลื่อน	ใช้เดินขึ้นลง
21	เลื่อย	เครื่องมือช่าง	51	โต๊ะหมู่บูชา*	ที่วางเครื่องสักการะต่าง ๆ
22	เฮลิคอปเตอร์	พาหนะที่เดินทางบนฟ้า	52	มะม่วงหิมพานต์	ไม้ผลชนิดหนึ่ง
23	บ่วง	เชือกที่ทำเป็นวงสำหรับคล้อง	53	หน่อไม้ฝรั่ง	เป็นผักชนิดหนึ่ง
24	ระนาด*	เครื่องดนตรีประเภทตี	54	โตก	ใช้วางอาหาร
25	สมอ	อุปกรณ์เรือ	55	ฝาชี*	สำหรับครอบอาหาร
26	ตั๊กแตน	แมลง	56	กระบองเพชร/ตะบองเพชร	พืชในทะเลทราย
27	มอเตอร์ไซค์*	รถที่มีล้อสองล้อ	57	ตาลปัตร	เครื่องใช้ของพระ
28	กล้องจุลทรรศน์	อุปกรณ์สำหรับมองวัตถุขนาดเล็ก	58	บุ้งกี๋/ปุ้งกี๋	ใช้โกยดิน
29	วงเวียน	สำหรับวาดวงกลม	59	เปลญวน	ใช้นอน
30	มังคุด*	ผลไม้ชนิดหนึ่ง	60	เกือกม้า*	เหล็กรูปโค้ง

Note: Asterisk (*) = Revised word items.

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Development of a Thai naming application for clients with aphasia

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ABSTRACT

Background: Aphasia, as brain damage resulting from a stroke, results in language impairment, including speaking, auditory comprehension, reading, and writing. Modern technology enhances self-administered recovery training, allowing clients to restore and improve communication.

Objectives: This study developed and assessed the effectiveness of an application on Android mobile devices for language rehabilitation with an emphasis on picture naming.

Materials and methods: A small sample research design was conducted with five primary caregivers and five clients with aphasia. The study was divided into two steps: application development and implementation using a single-subject experimental research design. This research design was divided into three phases: (1) Phase A1, a baseline of 2 weeks before applying the training; (2) Phase B, a training period of 6 weeks; and (3) Phase A2, a withdrawal period of 4 weeks. The total study period was 12 weeks.

Results: The naming rehabilitation application was assessed by visual graphical data analysis, with differences analyzed at each phase interval. After using the application for six weeks, the naming scores of the participants as clients were measured by the Thai Adaptation of the Western Aphasia Battery test (WAB). Results showed increased naming scores while the clients developed their naming skills using the application.

Conclusion: This application enhanced the clients' naming skills and reduced the time taken to name the words when conducting repetitive stimulation exercises in the form of intensive training combined with various cueing techniques. This application promoted the ability of clients with word-finding difficulties to retrieve words.

Introduction

Aphasia is a language disorder that affects speech, auditory comprehension, reading, and writing. Approximately 33% of stroke clients with a lesion in the language-dominant brain hemisphere suffer from aphasia.[1] This condition can significantly impact social communication and quality of life for sufferers and their families.[2,3] Common symptoms of aphasia are difficulty with word retrieval and word recall, which negatively impacts speech expression.[4]

The role of the speech-language pathologist (SLPs) is crucial in providing a rehabilitation program in the early stages of recovery and must be consistently maintained.[1,5] However, receiving a conventional rehabilitation approach

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in hospitals is challenging due to the limited availability of SLPs and the clients' economic status, with caregivers having to provide transport. Hence, in several countries, there is growing interest in developing a rehabilitation approach that caregivers can carry out at home. This option provides clients access to language and speech services without incurring high costs. Modern technology has now been integrated into the rehabilitation process as an additional tool to enhance the effectiveness of home treatment.[5]

The recent upsurge in research studies conducted abroad regarding the implementation of computer programs or smartphone-based applications for the rehabilitation of clients with aphasia has revealed that the integration of technology with conventional training can facilitate the recovery and enhancement of speech quality.[5-7] Results demonstrated that this protocol facilitated improved naming skills and enhanced the quality of non-verbal communication.[8] However, currently, in Thailand, only programs are used for communication with service recipients with aphasia that are developed based on the principles of augmentative and alternative communication (AAC). This rehabilitation approach involves devices to assist individuals with aphasia in expressing their needs and feelings or understanding various things around them according to their specific requirements. It utilizes simple devices such as communication boards with pictorial representations to more advanced technology like tablet-based applications.[9,10] No specific computer programs developed for language rehabilitation encompass speech, auditory comprehension, repetition, reading, writing, and naming skills.

In Thailand, the availability of smartphone or tablet-based language rehabilitation applications is limited because most of the existing programs are in foreign languages, expensive, and are exclusively for the iOS operating system.

An application focusing on word retrieval tailored to the Thai language's structure was developed to address this issue to rehabilitate communication impairment. After development, the caregivers used this application for 12 weeks to study its effectiveness in improving their clients' language abilities. Caregivers can utilize the results of this research to enhance word recall among clients with aphasia and better achieve the expected communication capacity.

Materials and methods
Sample group
Five pairs of caregivers and clients with aphasia were selected by purposive sampling using the following inclusion criteria.

1) Caregiver's eligibility criteria for inclusion were as follows:
- 1.1) no abnormal vision or use eyeglasses to correct for normal vision
- 1.2) have no history of hearing impairment
- 1.3) use Thai as the primary language of communication

- 1.4) demonstrate proficiency in reading and writing the Thai language
- 1.5) can use a smartphone or tablet device with a screen size of at least 5.5 inches with an Android operating system version 4.1 or higher
- 1.6) serve as the primary caregiver for service recipients who possess the characteristics outlined in item 2

2) The criteria for the selection of clients were as follows:
- 2.1) were diagnosed with aphasia by qualified SLPs and underwent assessment using the WAB test while also attaining a comprehension score that exceeded 4.[11]
- 2.2) Both genders with aphasia were aged 40 years and over.
- 2.3) normal vision or use of eyeglasses to correct their vision.
- 2.4) no history of hearing impairment or communication difficulties before the stroke.
- 2.5) used the Thai language as the primary language of communication and effectively communicated before experiencing aphasia.
- 2.6) could read and write Thai before experiencing aphasia from the stroke.

Research Methodology
This research was divided into two steps as follows:

Development of the "Thai Naming" application
The development of the *"Thai Naming"* application was divided into four steps as follows:

- 1.1) The application utilized a list of fundamental vocabularies from early childhood up to grade six provided by the Bureau of Academic Affairs and Educational Standards, Office of the Basic Education Commission (OBEC).[12] Twelve verbs and 58 nouns were categorized into two groups. Each word was assigned a picture, phonation, and six types of prompts, including semantic cueing (Q1), first letter (Q2), written word (Q3), sentence completion (Q4), phonological cueing (Q5), and spoken word (Q6).
- 1.2) The content validity of each item, such as words, pictures, and cues used in the application, was evaluated by three qualified SLPs utilizing the index of item objective congruence (IOC). The goal was to ascertain whether the items and the proposed measurement construct were congruent. Items with an average IOC score of 0.5 or higher were considered to cover the content adequately. In contrast, items with an IOC score lower than 0.5 were revised according to the experts' recommendations. The content was then integrated into an Android Studio application by programmers and reviewed again by experts to ensure its validity. After confirming that the content aligned with the research objectives, the application was tested and
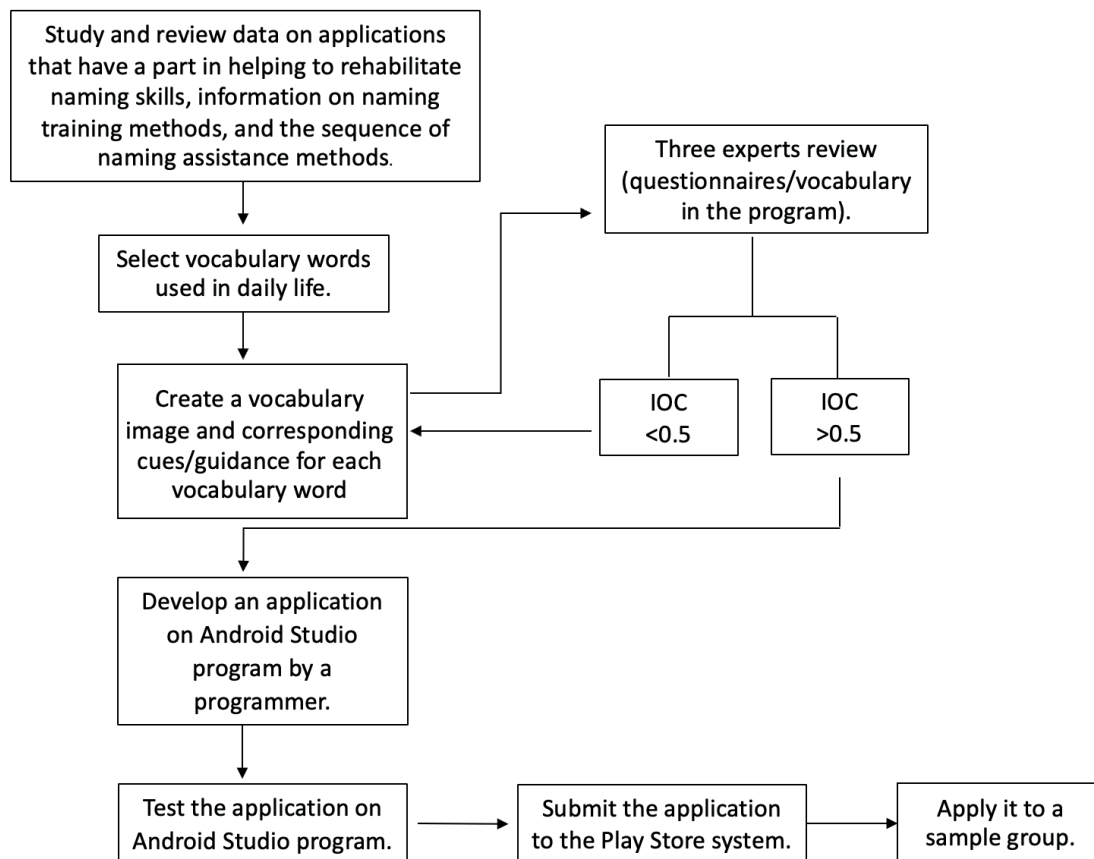
Figure 1 *Development process of "***Thai naming***" application.*

published on the Play Store, as shown in Figure 1.

1.3) After correction, the new application version was sent back to the three experts to evaluate the content. The final version of the application was uploaded to the Play Store. Figure 1 shows the process of application development.

Application of "*Thai Naming*" in the sample group

Purposive sampling was conducted to select five stroke clients with aphasia and their caregivers. The clients were undergoing rehabilitation at the Speech Therapy Unit of the Faculty of Associated Medical Sciences, Chiang Mai University, and the Industrial Rehabilitation Centre Region 3 in Mae Rim District, Chiang Mai Province. The inclusion criteria were as follows:

2.1 Stroke clients were assessed using the WAB test by the researchers.[11]

2.2 The *"Thai Naming"* application was installed on the caregiver's smartphone or tablet, and the utilization steps were clearly explained.

2.3 The application was implemented over 12 weeks, divided into three phases, as shown in Figure 2.

1) Phase 1 was the baseline or pre-application period, which lasted for two weeks. The researchers collected data at week 0 and week 2 before implementing the application.

2) Phase 2 was the experimental period during which clients and their caregivers practiced with the application for 30 minutes every day for six weeks. The researchers collected data on vocabulary practice three times during this phase at weeks 4, 6, and 8.

3) Phase 3 was the withdrawal period when the caregivers no longer used the application. The researchers collected data twice during this phase at weeks 10 and 12. At the end of the 12th week. The parameters of this study were as follows: Naming scores from the WAB test[11] and the score obtained using the application will be recorded as

 - A number of words are named using the application.
 - Average time of word naming.
 - (Sum of Response Times) / (Number of Trials).
 - The average frequency of using cues during naming.
 - (Total Number of Cues Used) / (Number of Trials with Cues).

Results
The content design of the application.

After undergoing content validation by three experts, the Index of Item-Objective Congruence (IOC) for the interview form yielded a total score of 0.90. Additionally, revisions were made in terms of visual representation of
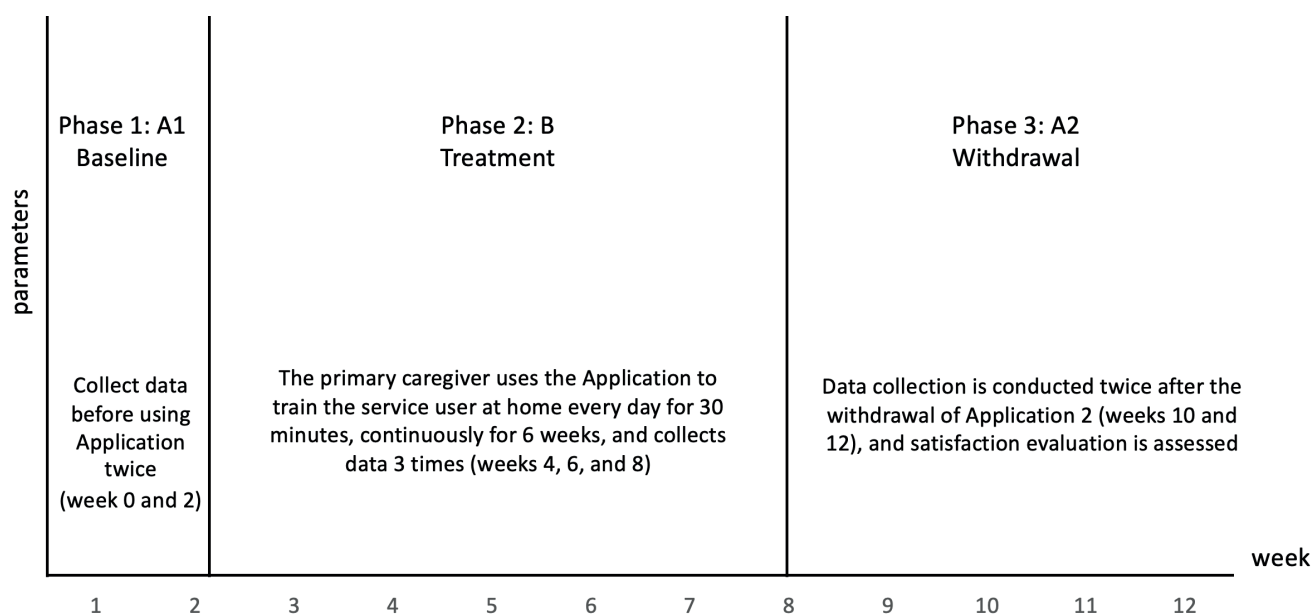
Figure 2 *Data collection for the A-B-A research design during the 12-week study period.*

vocabulary words and cueing methods, such as semantic cueing, first letter, and written word cues. Consequently, the vocabulary used in the application consists of 70 commonly known and frequently used words in daily life. Examples include "apple," "table," "shirt," and "pants," as well as verbs like "walk," "sit," and "sleep." These words will be displayed in the application, as depicted in Figure 3.

Demographic information of the caregivers of the clients

The five caregivers who participated in the study comprised three males and two females. Four of the caregivers were between 40 and 49, while the other was over 60 years old. All five caregivers were native Thai speakers. Three caregivers had a high school education, a diploma, and a postgraduate degree. They provided care for three clients for periods ranging from one to twelve hours and two clients for periods ranging from thirteen to twenty-four hours. The Thai Naming application was utilized for the five groups of clients using four smartphones with a screen size of 5.5 inches or more and a tablet with a screen size of 7 inches.

Demographic information of the clients

The five stroke clients with aphasia were four males and one female, with an average age of 53. Three clients completed primary school, one had a bachelor's degree, and one had a master's degree. Four clients had a history of stroke two years ago, one had a history of stroke one year ago, and one had a history of recurrent strokes. The WAB test [11] was used to assess the results both before and after the *Thai Naming application*, as presented in Table 1.

Visual data analysis results

The results from implementing the application for naming training with the primary caregivers over 12 weeks were presented as line graphs, divided into three phases. The analysis included the four variables recorded from the program, as shown in Table 2.

WAB naming score

The WAB naming Scores of the five clients were evaluated during Phase 1 (baseline), Phase 2, and Phase 3. Results showed an increase in naming scores during Phase 2.
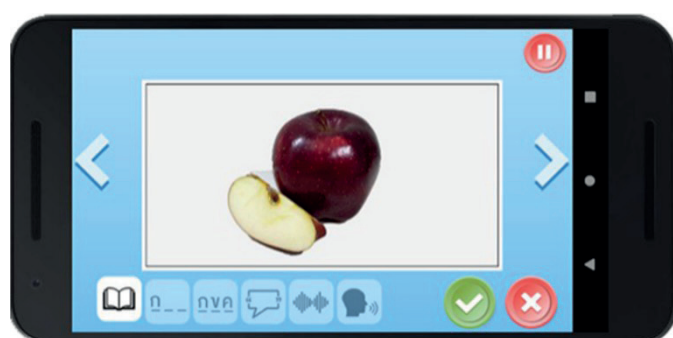

Figure 3 *Example of vocabulary display on the 'Thai Naming' application.*

Table 1. Demographic information of the stroke clients (N=5).

Order	Age (years)	Gender	Education level	Post-stroke duration (years)	Recurrent (times)	Baseline evaluation		Difference in AQ scores	Type of aphasia
						T-WAB (AQ) Pre-week 0	T-WAB (AQ) Post-week 12		
1	75	Female	Elementary education	1	-	23.7	43.8	20.1	BA
2	45	Male	Master's degree	2.4	-	33.6	36.9	3.3	WA
3	53	Male	Elementary education	2.1	-	37.3	56.2	18.9	BA (pre) TA (post)
4	51	Male	Elementary education	3.2	1	63.2	70.2	7	Anomic
5	43	Male	Bachelor's degree	2.8	-	41.3	53.7	12.4	TA

Note: WAB: Thai Adaptation of the Western Aphasia Battery, AQ: aphasia quotient, BA: Broca's aphasia, TA: transcortical motor aphasia, WA: Wernicke's aphasia, BA (pre): Broca's aphasia (pre-test week 0), TA (post): transcortical motor aphasia (post-test week 12)

Table 2. Clients' scores from four variables (N=5).

Oder	Week \ Case	Baseline (A1)		Treatment (B)			Withdrawal (A2)	
		0	2	4	6	8	10	12
WAB naming score	1	2.5	2.1	4.2	5.9	7.1	7.6	7
	2	2.3	2.6	2.6	3.6	4.7	4.8	3.9
	3	4	5.7	5.6	6.8	7.1	7.1	7.5
	4	7.6	7.9	7.8	8.3	9.2	8.8	8.8
	5	2.9	4.5	5.2	6.5	6.9	7.9	7.6
Number of words named using the application	1	0	6	34	47	62	55	55
	2	9	7	24	30	27	18	31
	3	3	8	21	29	23	22	29
	4	60	61	64	67	67	68	68
	5	3	4	18	39	38	42	33
Average time of word naming (second)	1	106	53	32	26	26	26	26
	2	56	72	30	30	30	44	28
	3	56	46	32	31	31	31	26
	4	26	26	26	26	26	26	26
	5	82	58	38	27	27	26	26
Average frequency of using cues during naming (time)	1	15	27	24	22	6	14	14
	2	20	18	23	30	32	22	32
	3	28	29	33	30	31	37	39
	4	9	8	5	2	2	1	1
	5	20	26	28	27	27	28	34

Note: Average time of word naming = (sum of response times) / (number of trials), Average frequency of using cues during naming = (total number of cues used) / (number of trials with cues)

After withdrawing the application at the end of the 10th week, the scores of all clients still improved. Figure 4 shows the WAB naming scores from week 0 to week 12.

Number of words named using the application.

Results in Figure 5 showed a significant improvement in naming ability when using the application (Phase 1 vs.

Phase 2). During the withdrawal (Phase 3), the number of words all five clients could spontaneously name remained stable until the completion of the 12th week. Findings suggested that using the application for six consecutive weeks effectively enhanced the clients' naming ability. However, the severity and type of client aphasia may impact the results.
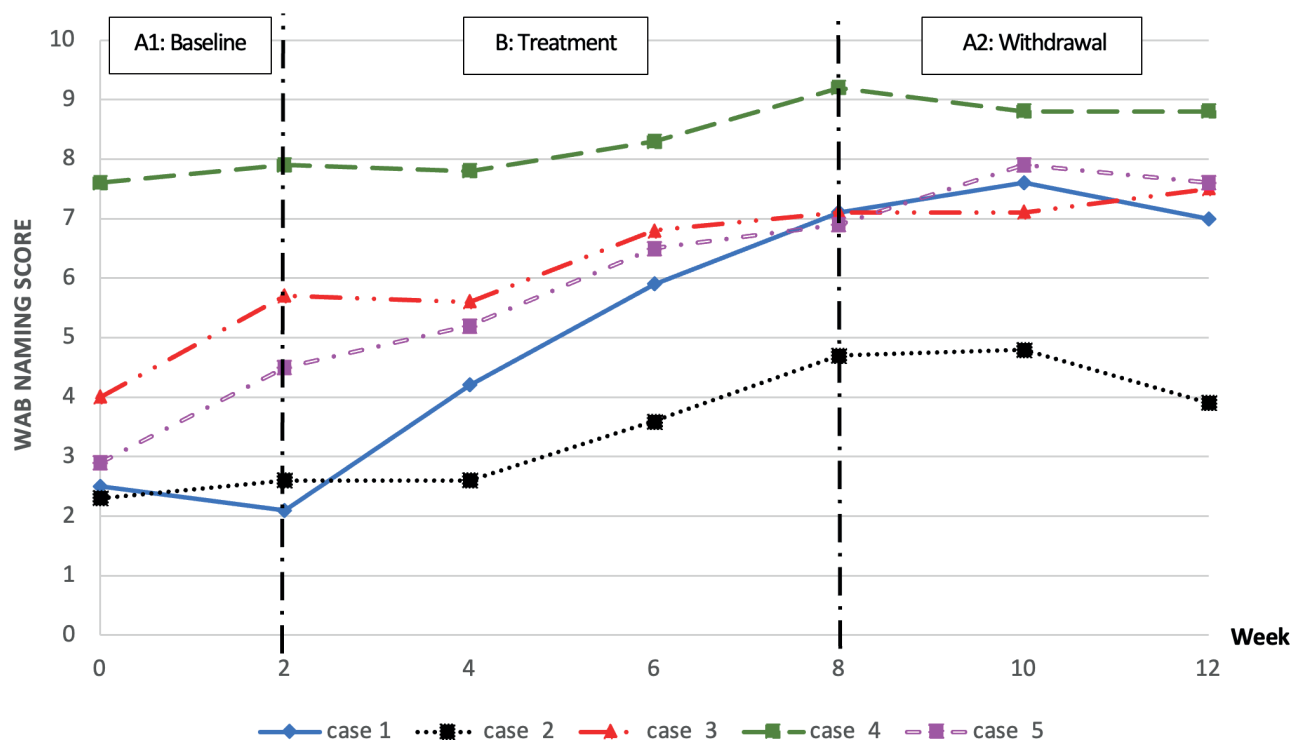
Figure 4 *Naming scores from the WAB test[11] during the 12-week study period.*
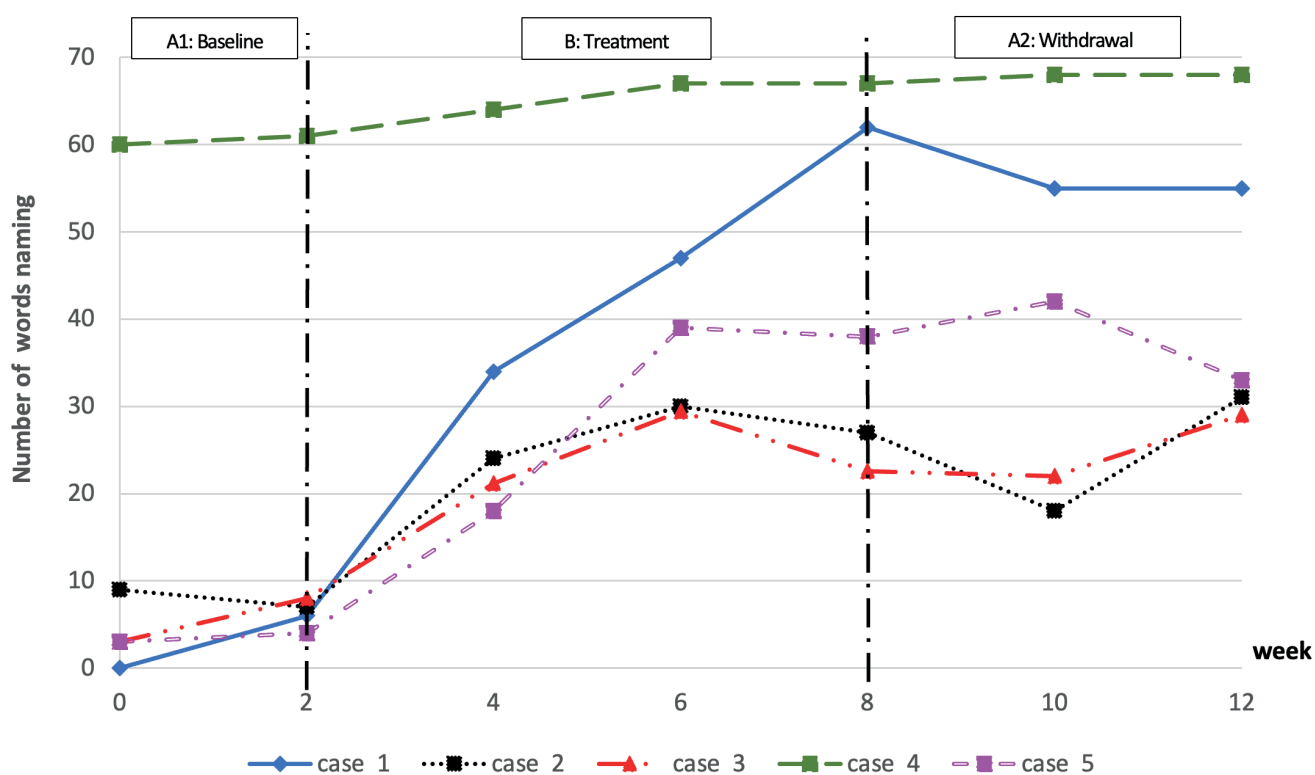

Figure 5 *Number of words named by the five clients during the 12-week study period.*

Average time of word naming by application

Figure 6 demonstrates a significant decrease in time required to spontaneous naming one word in Cases 1, 2, 3, and 5 following instructions using the Thai Naming program. This improvement in naming speed persisted during the 10th and 12th weeks of the withdrawal period (Phase 3), with no significant changes observed in case 4. Results suggested that the application was effective in improving naming speed.

Average frequency of using cues by application

Results revealed a decreasing use of cues among cases 1 and 4 across all three study phases. By contrast, the number of cues used increased in cases 2, 3, and 5 (Figure 7 and 8).

Results in Table 1 and Figure 7 show that five participants had chronic strokes, while participants with shorter stroke durations tended to develop spontaneous naming, as observed in case 1. Those with less severe stroke conditions tended to name spontaneously using fewer cueing, as seen in case 4. Figure 8 shows the frequency of using each cue during naming among the five service recipients. Participants with good auditory comprehension skills most utilized semantic cueing (Q1). This motivated and stimulated individuals with Motor Aphasia, including Broca's Aphasia and Transcortical Motor Aphasia (cases 1, 3, and 5), show enhanced vocabulary. For individuals with Wernicke's Aphasia (case 2), more cueing levels were necessary, including the use of suggesting the first letter (Q2), written words (Q3), sentence completion (Q4), and spoken word (Q6). Cueing effectively enhanced naming skills without relying solely on auditory comprehension,

which presents a significant challenge for those with Wernicke's Aphasia.

Discussion

Results showed that the "Thai Naming" application improved the ability to name and increased retrieve words. The training method followed the principles of neuroplasticity, involving short-term, high-intensity sessions. This approach demonstrated improved outcomes in terms of learning and recovery within the nervous system compared to less intensive training methods.[13] This study aligned with previous research, highlighting the effectiveness of utilizing tablets for word retrieval in clients who experienced difficulties with naming due to stroke.[5, 14-16]

The use of cueing, designed with varying levels of guidance, duration, and presentation techniques, is particularly beneficial in improving the word recall capacity of clients with Motor Aphasia, including Broca's Aphasia and Transcortical Motor Aphasia (cases 1, 3, and 5). The effectiveness of this application was demonstrated by a significant increase in the total score of the Aphasia Quotient after training. Results indicated that this application increased spontaneous naming and decreased the response time required for naming, even after a break in training activities. However, in case 4 with mild Anomic Aphasia (according to WAB AQ score (Table 2), the application did not show remarkable improvement in spontaneous naming. It could be due to the lower severity of aphasia at the beginning of the study WAB AQ score at week 0 (Table 1), resulting in a slight difference in WAB AQ scores compared to week 0 (scored at 60) and week 12
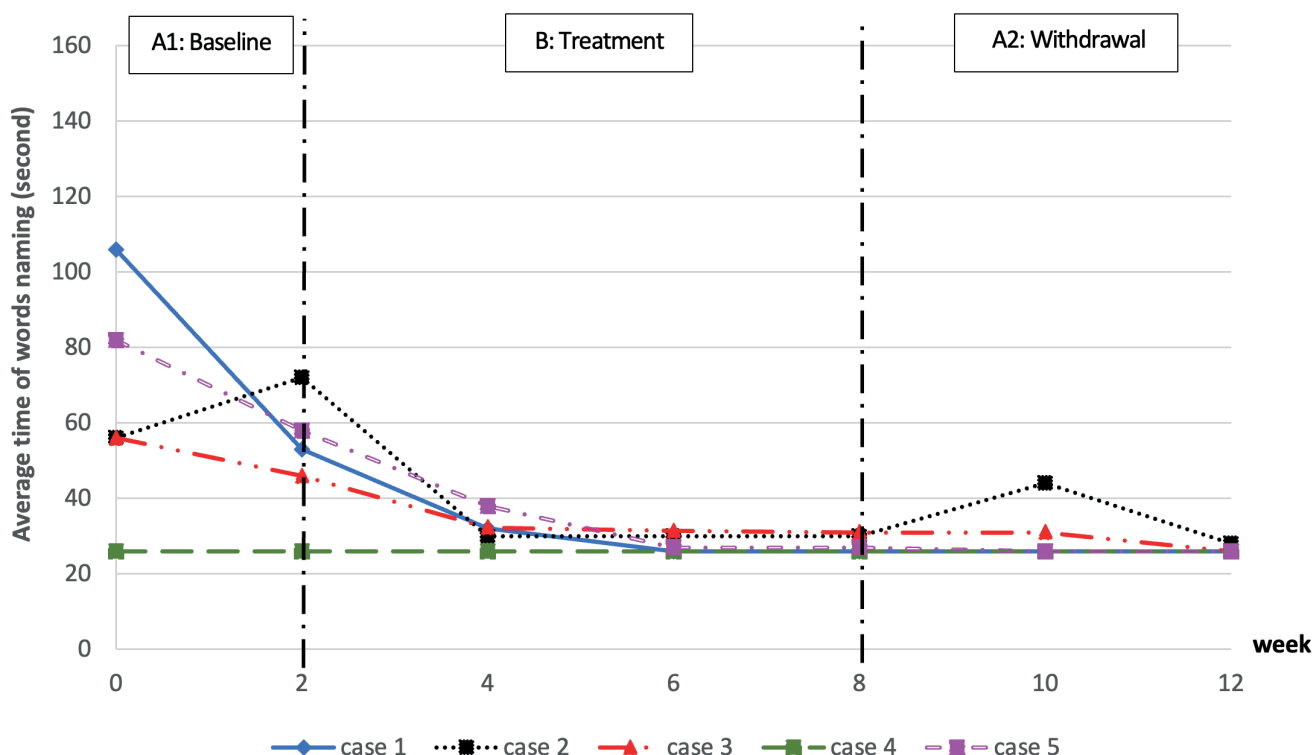

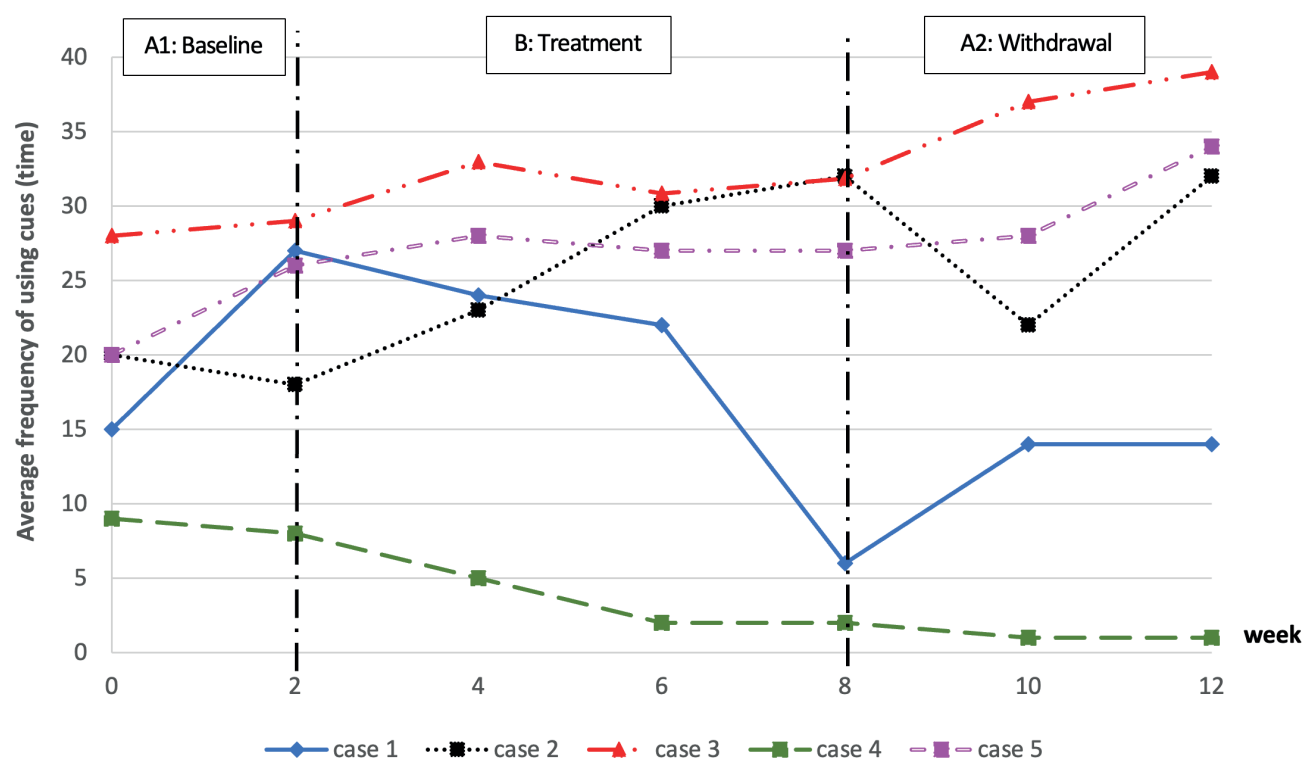**Figure 6** *Average times of words named by the five clients during the 12-week study period.*

Figure 7 *Average frequency of using cues during naming among the five service recipients.*
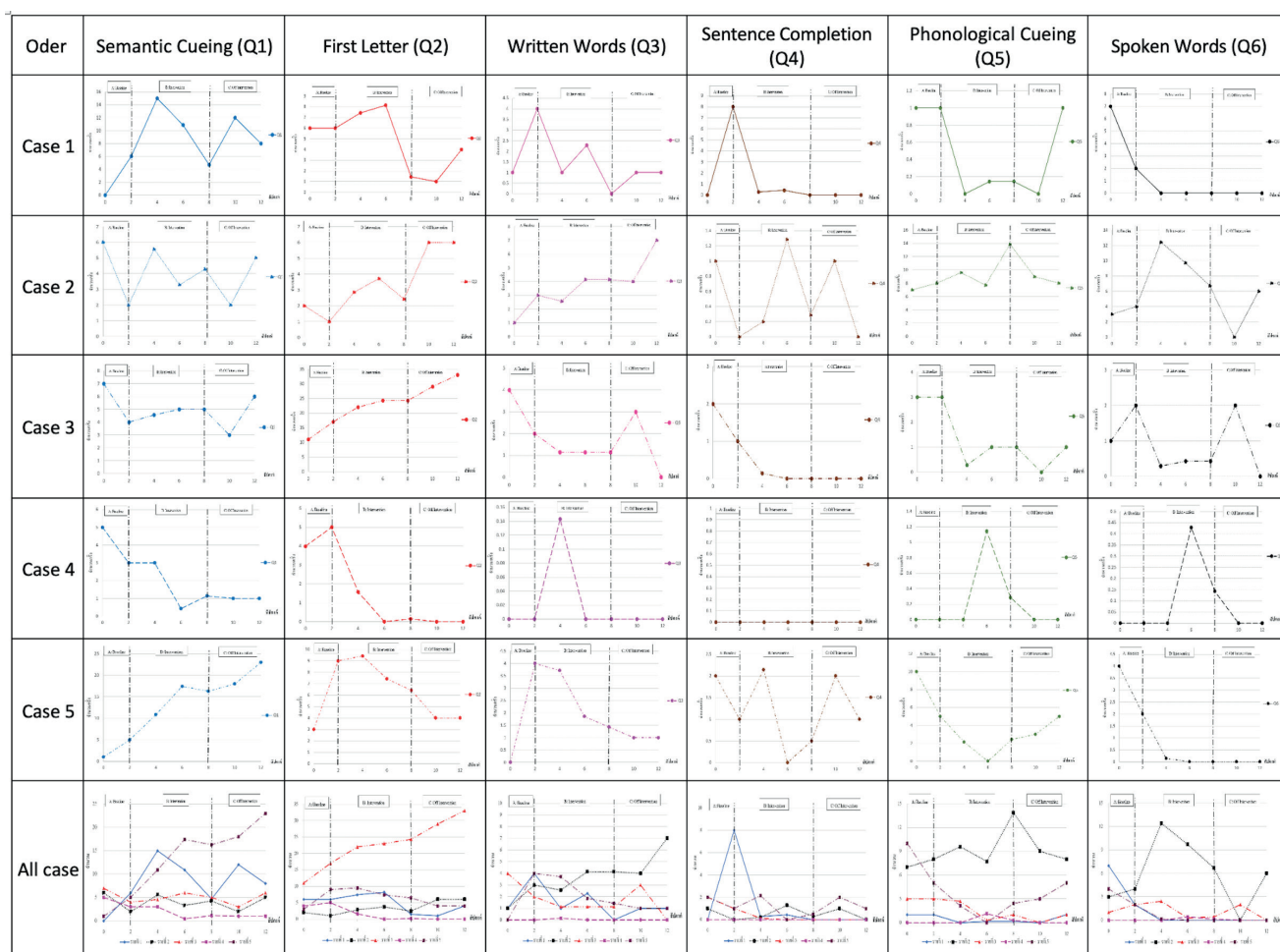

Figure 8 *Frequency of using each cue during naming among the five service recipients.*

(scored at 68). Although recurrent episodes were noted, the client's symptoms were only headache and motor weakness, but the participant showed rapid recovery. Therefore, no remarkable change in the time required for naming was found during data collection (Figure 6).

Results showed that individuals with non-fluent aphasia and/or auditory comprehension skill deficits such as Motor Aphasia (e.g., Broca's Aphasia and Transcortical Motor Aphasia; cases 1, 3, and 5) used semantic cueing (Q1) at a low level to aid in naming. Those who received education at the primary level or higher used letter cueing as a visual aid to help them name objects. By contrast, those with auditory comprehension deficits such as Wernicke's Aphasia (case 2), used phonological cueing (Q5) at a higher level, first letter cueing (Q2), written word cueing (Q3), sentence completion cueing (Q4) and spoken word cueing (Q6) respectively. The cueing method that provided phonological cues was often used. The principle of brain functioning can explain this. When we hear a sound, the brain separates the sound signals into linguistic information such as pitch, rate of sound, and tone of sound sent to the Wernicke's area for further analysis. This area collects information from visual, auditory, and tactile experiences, uses the cognitive and linguistic processes involved in recalling and matching vocabulary with sounds, and sends this data to the Broca's area to organize the response process through communication.[17-19] The Dual Stream Model of Speech and Language explains the roles of the brain's left and right hemispheres in speech perception and production, as well as language comprehension.[20] People with Broca's aphasia typically have a relatively intact receptive language, with the areas of the brain responsible for interpreting sound meaning, specifically the temporal lobe and parietal operculum, still functioning well.[17-20] Therefore, semantic cueing may be sufficient for naming. Individuals with Wernicke's Aphasia, characterized by damage to the parietal and temporal regions that are crucial for interpreting incoming auditory signals and perception, used more effective cueing strategies such as phonological cueing (Q5) or spoken word (Q6) to assist with naming. These cues helped those with WA retrieve words similar to their previous experiences and closer to the target words, as shown by the study results of Case 2. The application did not show remarkable improvement in spontaneous naming.

The result demonstrated that using the application for six weeks improves the ability of clients to do spontaneous naming. After completing the training, clients with motor aphasia could maintain their progress. However, more than simply training on specific words through concentration may be required to result in practical usage without further practice. Short-term training can enhance learning, but further research is needed to determine the long-term capacity for daily communication.[13] Holland *et al.*[21] concluded that sustained and improved language development needs 5-6 months of regular recovery because clients with aphasia have difficulty recalling words.[4] Prolonged training sessions and duration activate neurons in the brain, enhancing the retention and recall of word information stored in long-term memory, consistent with Hebb's theory.[22] Previous research showed that tablets and computer-based interventions improved clients' word recall, but these techniques have not yet been proven effective in improving communication skills with untrained vocabulary.[14-16]

The results of this study indicated that remote rehabilitation, such as the "*Thai Naming*" application, has the potential to increase the intensity of treatment for clients who cannot receive regular therapy or reside in regions with limited access to SLPs. Findings reveal that this application potentially improves clients' naming skills. Results concurred with previous studies conducted by Sukcharoen and Saksiri,[10] Choi *et al*.[23] and Wall *et al*.[24]

Conclusions

The *"Thai Naming"* application designed for training naming skills in clients with chronic aphasia could improve the ability of clients with word-finding difficulties to retrieve words and improve their spontaneous naming. This benefits their rehabilitation by involving the clients and their primary caregivers in the treatment process and helping to intensify the therapy sessions.

Research limitations

Vocabulary should be grouped according to difficulty levels to conform to each client's capabilities. It is also crucial to incorporate a tracking system to record the number of training words during the therapy program to monitor progress and effectiveness. To encourage client engagement and motivation, the application system should be interactive and include online activities such as video chats which can build trust and provide effective treatment. Incorporating medical terminology and concepts in the application can also enhance its relevance and applicability in speech and language therapy. In addition, providing guidance and training to caregivers before using the application at home is crucial to ensure proper understanding and enhance efficiency in its usage.

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Journal of Associated Medical Sciences

Effects of short foot exercise on Q-angle in individuals with flatfoot

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ARTICLE INFO

ABSTRACT

Background: Flatfoot is a predominant chronic condition associated with lower extremity musculoskeletal injuries. Current studies have illustrated that a flatfoot deformity may result in an increased Q-angle which can cause knee disorders. Short foot exercise (SFE) is a broadly acknowledged strategy for strengthening the intrinsic foot muscles. The exercise is recommended to be performed to improve the medial longitudinal arch (MLA). However, there is a lack of studies examining the effects of SFE on the Q-angle.

Objectives: The primary purpose of this study was to investigate the effects of SFE on Q-angle in individuals with flatfoot. The second purpose was to examine the effects of SFE on the navicular drop test (NDT).

Materials and methods: This randomized controlled trial study included 16 participants aged 18-25 years with flexible flatfoot on both feet based on the results of NDT. Q-angle and NDT were measured at the beginning of the study. The participants were randomly assigned to either the experimental or control groups. The experimental group performed SFE three days a week for five consecutive weeks, while the control group did not perform the exercise. Q-angle and NDT were reassessed after five weeks of the exercise.

Results: Q-angle significantly decreased in the exercise group after the program for both legs (Right leg: from 21.62±1.87 to 19.83±1.63 degrees; Left leg: from 21.42±1.92 to 20.04±1.89 degrees). In addition, NDT significantly improved in the exercise group after SFE for both feet (Right foot: from 11.08±1.18 to 6.83±2.09 mm; Left foot: from 10.58±1.50 to 6.58±1.60 mm).

Conclusion: This present study demonstrated that SFE effectively improved Q-angle and NDT in individuals with flexible flatfoot.

Introduction

Flatfoot or excessive foot pronation, appearing as low or absent height of the medial longitudinal arch (MLA) while bearing weight, is a predominant chronic condition with an incidence of 2-23% in adults.[1] It is associated with lower extremity musculoskeletal injuries. In individuals with flexible flatfoot, changes in foot shape cause overuse injuries of the lower extremity regions, such as medial tibial stress syndrome, plantar fasciitis, and patellofemoral pain syndrome (PFPS).[2,3]

Current studies have demonstrated that a flatfoot deformity may result in an increased quadriceps (Q)-angle.[4,5] Excessive foot pronation leads to internal rotation of the lower extremity and may result in knee valgus with an increased Q-angle.[6,7] Moreover, there are studies presenting the impact of the flatfoot on the Q-angle

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and its association with tibiofemoral mechanics. Q-angle of more than 20° imposes a risk of the patella being pulled by the quadriceps. This increases the chance of knee disorders, especially PFPS.[8,9] Rearfoot alignments and Q-angle measurements are usually evaluated in individuals with PFPS.[10] Abnormalities of the rearfoot result in compensatory movement at the subtalar joint to maintain normal function of the lower leg and foot during the gait cycle.[11]

The MLA is stabilized by the intrinsic foot muscles, which are the abductor hallucis, flexor digitorum brevis, and quadratus plantae. These muscles moreover control arch deformation.[3,12,13] Contraction of the abductor hallucis can increase flexion and supination of the first metatarsal bone, inversion of the calcaneus, and medial rotation of the tibia in conjunction with elevation of the MLA.[3,14] Impairment of the abductor hallucis can lead to increased navicular drop.[15]

Short foot exercise (SFE), also known as sensory-motor training, is the most broadly acknowledged method for strengthening the intrinsic foot muscles.[3,16,17] The exercise is performed by pulling the heads of the metatarsal bones towards the calcaneus without flexing the toes and lifting the forefoot and heel from the ground.[3] As a result, this increases the height of the MLA.[16,18]

Several previous studies investigated the effects of SFE on the MLA.[18] Some studies demonstrated the presence of a flatfoot deformity resulting in an increased Q-angle.[4,5] However, there is a lack of studies examining the effects of SFE on the Q-angle. Multiple studies have claimed that NDT is a clinical assessment and is one of the simplest methods to provide clinical information regarding the pronation of the foot affecting the MLA.[2,15] A finding that SFE can improve navicular drop may provide initial evidence for the prevention and treatment of flexible flatfoot.

Thus, the main purpose of this study was to investigate the effects of SFE on the Q-angle in individuals with flatfoot. The second purpose was to examine the effects of SFE on NDT.

Materials and methods
Participants
This randomized controlled trial study (RCT) included 16 participants sampled from students enrolled at University of Phayao, Thailand, with the following inclusion criteria: 1) 18-25 years of age, 2) flexible flatfoot on both feet based on results of navicular drop test (NDT), and 3) body mass index (BMI) between 18.50-22.90 kg/m^2. The study excluded volunteers who presented with 1) a history of foot and ankle pain within the past six months and 2) a leg length discrepancy of more than 1.5 cm. Participants were randomly allocated into an intervention group and a control group using the drawing lots method. The sample size was calculated using G*Power 3.1 (University of Dusseldorf, Dusseldorf, Germany) based on a previous study.[17] A statistical significance level of 0.05 and power of 95% were used in the sample size calculation, which yielded 8 participants in each group.

Intraclass correlation coefficient (ICC)
ICC was used to estimate the intra-rater reliability of three researchers for performing Q-angle measurement and NDT in the study. NDT and the Q-angle were assessed in 10 volunteers on the same day. The exact process was repeated with a separation of 24 hours. The results were then analyzed for ICC. An investigator who presented with an ICC value mostly closer to 1 was selected to perform the measurements. An ICC of 0.70 was selected as the minimally acceptable value.[19] ICC values of 0.85 and 0.92 were found for assessments of the Q-angle and NDT, respectively.

Procedure
The study was approved by University of Phayao Human Ethics Committee (UP-HEC 1.2/050/65), and the protocol was approved by the Thai Clinical Trials Registry (TCTR20230706002). All participants provided written informed consent prior to testing. The participants were randomly assigned to either the experimental or control groups by researcher 3. All baseline data, Q-angle, and NDT were measured at the beginning of the study by researchers 1 and 2. The assessment researchers were unaware of the group allocation. The volunteers were asked to refrain from discussing their group activities with the assessment staff. The experimental group performed SFE three times a week for five weeks, while the control group did not receive any intervention during the study. The Q-angle and NDT were reassessed after five weeks of the exercise by researchers 1 and 2, who remained blinded to the group allocation. The flow chart of the RCT testing procedure is shown in Figure 1.

Outcome measurements
Quadriceps angle: Q-angle was characterized as the point made by the crossing point of 2 lines: one interfacing the anterior superior iliac spine (ASIS) and the middle of the patella, and the other interfacing the middle of the patella and the tibial tuberosity. This measurement can be done using a universal goniometer as the method appears to have high reliability (ICC=0.88).[19] The angle was examined by researcher 1 while a participant was standing in a neutral position with full weight bearing. Researcher 1 palpated for three landmarks, which were ASIS, the middle of the patella, and the tibial tuberosity. Subsequently, each marker was placed on each landmark. A universal goniometer was placed with the axis over the marker in the middle of the patella; the goniometer arms were adjusted to position the imaginary line joining the marker on ASIS and the line joining the marker on the tibial tuberosity. The angle on the goniometer was perused as the Q-angle. Both left and right legs were measured for each subject. Each leg was assessed three times, and an average degree was used for the analysis.[20]

Navicular drop test: NDT was assessed for both feet by measuring the navicular height difference between non-weight bearing and weight bearing in millimeter (mm). The test has been used to measure the MLA and has been shown to have high reliability for examining navicular
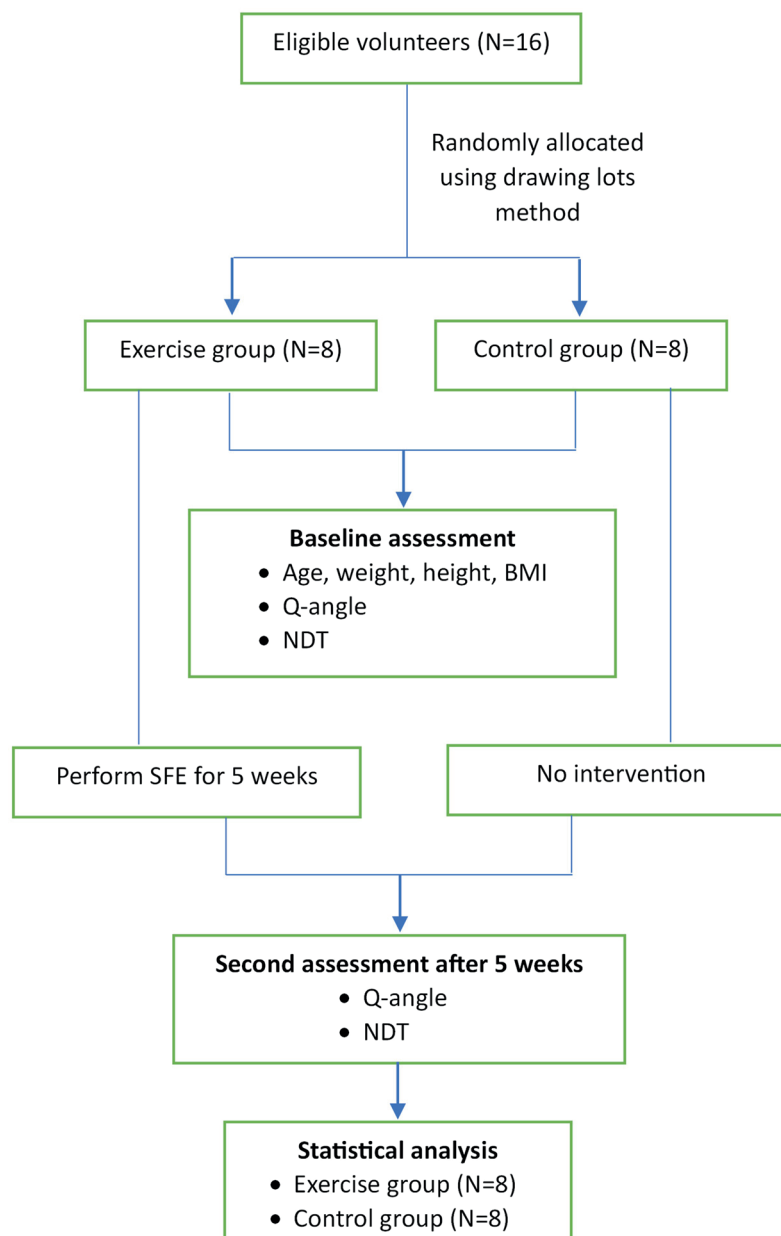
Figure 1 Flow chart of RCT testing procedure.

height (ICC > 0.94).[16] A participant sat on a chair and flexed his/her hip and knee at 90° while maintaining a neutral foot position. Researcher 2 palpated the navicular tuberosity and marked it with a pen. A cardboard was vertically placed on the ground next to the medial side of the foot and was marked at the same level as the navicular tuberosity. The participant then stood up, and the same cardboard was marked at the same level of the navicular tuberosity while still in a standing posture. Two marks on the cardboard would create the navicular height difference. Flatfoot was defined as a navicular drop exceeding 10 mm.[1,16] This test was used as a screening tool for participant recruitment and as one of the outcomes in this present study.

Exercise protocol

Before the experiment, researcher 3 demonstrated SFE for the individuals in the experimental group, and the individuals performed the exercise to familiarise themselves with the intervention. The exercise was performed while a volunteer sat on a chair with 90° of hip, knee, and ankle flexion. A towel was placed under both feet as the participant had to exercise 2 feet simultaneously. After that, the participant was instructed to move the head of the 1st metatarsal bone towards the heel without bending the toes. The position was maintained for 20 seconds to form the MLA. This process was performed for 30 minutes each session, three sessions a week for five weeks in total.[17] The participants performed SFE under researcher 3's supervision for every session.

Statistical analysis

SPSS statistical package version 26 (SPSS Inc, Chicago, IL) was used to analyze the data. All baseline data were expressed as mean±SD. ICC estimates of intra-rater reliability for performing Q-angle measurement and NDT were calculated based on ICC (3,1). The Shapiro-Wilk

test was used to explore the normality of all variables. Significant differences in the baseline data between groups were examined using an Unpaired t-test except gender proportion using a Chi-squared test. The dependent variables were analyzed using a two-way mixed ANOVA on two factors, which were one between-subjects factor (group) with two levels (groups: exercise, control) and one within-subjects factor (time) with two levels (time: pre, post). A significant level was set at $p<0.05$. In addition, the Bonferroni correction was used to compare the difference between the two times for each group and the *p*-value was adjusted to <0.01. According to Cohen, effect sizes were classified as small (d=0.2), medium (d=0.5), and large (d≥0.8).[21]

Results

A total of 16 participants with flexible flatfoot were recruited. No participants left the trial. Baseline characteristics of the participants are presented in Table 1.

Table 2 reveals a significant effect of SFE on Rt. Q-angle ($F_{(1,14)} = 22.211$, $p<0.001$) and Lt. Q-angle ($F_{(1,14)} = 32.628$, $p<0.001$) with the effect sizes of 0.61 and 0.70, respectively, indicating a medium effect size. The results also presented a significant effect of SFE on Rt. NDT ($F_{(1,14)} = 34.294$, $p<0.001$) and Lt. NDT ($F_{(1,14)} = 35.700$, $p<0.001$) with

the effect sizes of 0.71 and 0.72, respectively, indicating a medium effect size.

Figure 2-5 represents significant interaction effects for the time x group, indicating that SFE significantly improved Q-angle and NDT in individuals with flexible flatfoot.

Discussion

The main findings of this study were that Q-angle and NDT decreased after the exercise program for both legs. Importantly, the exercise program for individuals with flatfoot resulted in a medium effect size. Therefore, the results suggested that the exercise training showed a moderate positive effect on Q-angle and NDT outcomes. The results of NDT support studies of Unver *et al.,*[1] Park and Park,[16] and Kim and Kim[17] indicating that SFE was effective at reducing distance of the navicular bone. In the present study, navicular height significantly decreased from 11.08±1.18 mm to 6.83±2.09 mm for the right foot and from 10.58±1.50 mm to 6.58±1.60 mm for the left foot in the exercise group. This could be explained by improved intrinsic foot muscle strength and decreased rearfoot angle. As a result, the height of the MLA increased.[17] The results of the present study were also supported by Hara *et al.* who found that ≥ five weeks of SFE was

Table 1 Baseline characteristics of the participants with flexible flatfoot (N=16).

	Exercise group (N=8)	Control group (N=8)	*p* value
Gender (male/female)	2/6	2/6	1.00
Age (Year)	21.13±0.35	20.88±1.13	0.52
Weight (Kg)	51.13±6.15	54.00±7.48	0.24
Height (cm)	161.75±7.30	161.50±6.85	0.88
BMI (Kg/m^2)	19.52±1.25	20.62±1.58	0.12
Rt. Q-angle (°)	21.62±1.87	23.58±4.35	0.27
Lt. Q-angle (°)	21.42±1.92	22.96±4.24	0.37
Rt. NDT (mm)	11.08±1.18	10.29±1.49	0.26
Lt. NDT (mm)	10.58±1.50	10.83±1.07	0.71

Table 2 Mean±SD of Q-angle and NDT in the exercise and control groups.

Variables	Group	N	Pre-test	Post-test	*F*	*p* value	Partial Eta Squared
Rt. Q-angle (°)	Exercise	8	21.62±1.87	19.83±1.63#	22.211	0.000	0.613
	Control	8	23.58±4.35	24.67±3.78			
Lt. Q-angle (°)	Exercise	8	21.42±1.92	20.04±1.89#	32.628	0.000	0.700
	Control	8	22.96±4.24	24.83±4.49			
Rt. NDT (mm)	Exercise	8	11.08±1.18	6.83±2.09#	34.294	0.000	0.710
	Control	8	10.29±1.49	10.87±0.89			
Lt. NDT (mm)	Exercise	8	10.58±1.50	6.58±1.60#	35.700	0.000	0.718
	Control	8	10.83±1.07	11.17±1.17			

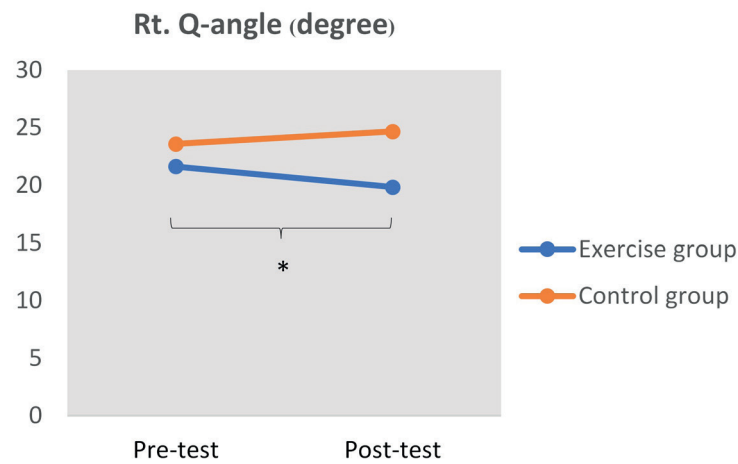# The Bonferroni correction p-value less than 0.01

Rt. Q-angle (degree)

Figure 2 *Interaction effect for time x group. F=22.211, df=1, p<0.001. There was a significant improvement in Rt. Q-angle only in the exercise group following the 5-week exercise (p<0.01).*

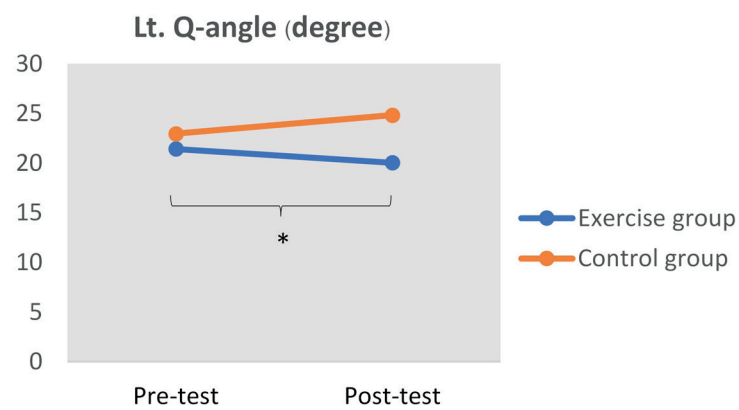### Lt. Q-angle (degree)

Figure 3 *Interaction effect for time x group F=32.628, df=1, p<0.001. There was a significant improvement in Lt. Q-angle only in the exercise group following the 5-week exercise (p<0.01).*
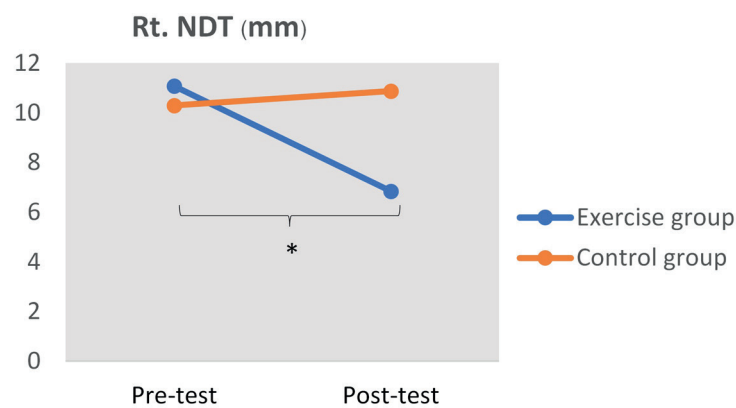
Rt. NDT (mm)

Figure 4 *Interaction effect for time x group. F=34.294, df=1, p<0.001. There was a significant improvement in Rt. NDT only in the exercise group following the 5-week exercise (p<0.01).*

Lt. NDT (mm)

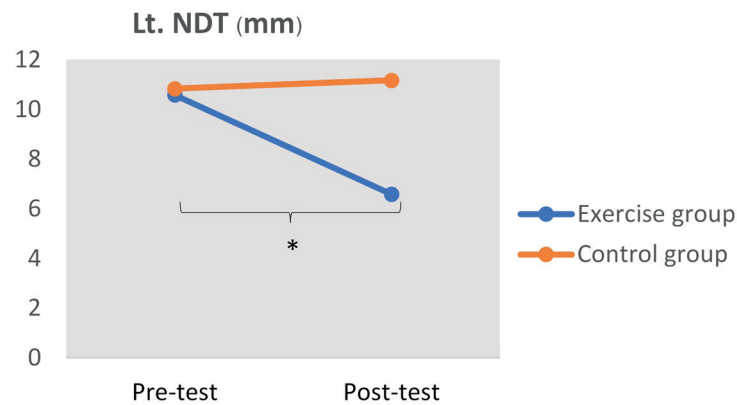**Figure 5** *Interaction effect for time x group. F=35.700, df=1, p<0.001. There was a significant improvement in Lt. NDT only in the exercise group following the 5-week exercise (p<0.01).*

effective in improving the MLA.[18] The plantar fascia is a major supporter of MLA retention.[22] It overlaps the intrinsic foot muscles and connects to the flexor digitorum brevis. In this manner, intrinsic foot muscle strengthening plays an important role in maintaining the MLA.[23] Some studies reported improvements in muscle thickness and muscle activity after SFE.[18] Additionally, there was a study presenting an increase in muscle cross-sectional area of the abductor hallucis muscle after SFE.[3] However, the effects of SFE on the MLA require further investigation on morphological changes of the intrinsic foot muscles as this present study did not examine a relationship between the intrinsic foot muscles and the MLA.

The Q-angle is considered as an essential clinical parameter that has substantial biomechanical impacts on the knee.[4] This study found that the Q-angle significantly decreased for both legs in the intervention group after the exercise program. The result could be explained by the association between the Q-angle and flatfoot. Ullah (2020) demonstrated that flexible flatfoot was strongly correlated with an increased Q-angle in individuals.[4] Moreover, AlKhouli *et al*. examined that the Q-angle was greater in individuals with flatfoot compared to healthy individuals.[5] Kalbouneh *et al*. also found that pes planus deformity was accompanied by a higher risk of developing a higher Q-angle.[24] The possible reason for the result in the present study is that SFE strengthened the intrinsic foot muscles and reduced excessive foot pronation, resulting in increased MLA and decreased Q-angle.[1,16,17] Excessive Q-angle can cause biomechanical stress during repetitive activities related to the knee as it interferes with smooth movements of the patella within the trochlear groove.[25] Additionally, excessive Q-angle can also lead to excessive pronation of the foot, which causes excessive internal rotation of the tibia. This will alter the quadriceps mechanism and lateral tracking of the patella.[26] Therefore, controlling foot pronation can often diminish the detrimental effects of an abnormal Q-angle.[27]

There are some limitations found in the present study. Firstly, the study's results cannot be generalized to all age groups as the current study was conducted in volunteers aged 18-25 years. Secondly, the study only examined the short-term effects of SFE. Thus, future studies with a

longer follow-up period are suggested. Lastly, the present study did not consider any symptoms of flexible flatfoot. Further studies should include the effectiveness of SFE on symptomatic individuals.

Conclusion

This present study demonstrated that SFE effectively improved Q-angle and NDT in individuals with flexible flatfoot. The results of the current study may provide implications for physiotherapists to treat flexible flatfoot with increased Q-angle and NDT.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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Journal History

Established in 1968
- 1968-2016 As the Bulletin of Chiang Mai Associated Medical Sciences
  - Vol1, No1 - Vol.49, No3
- 2017, the Journal of Associated Medical Sciences

  - Vol.50, No1 and forward.

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