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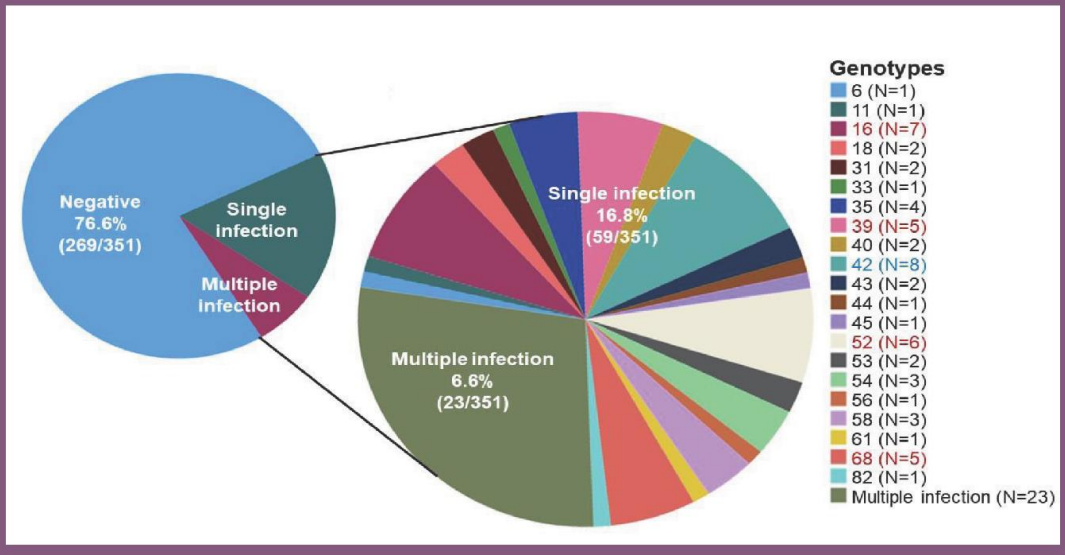

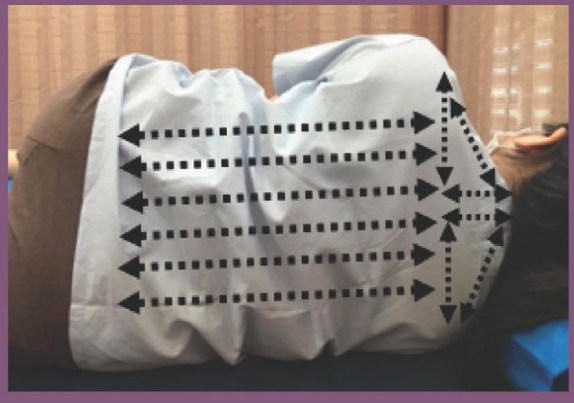

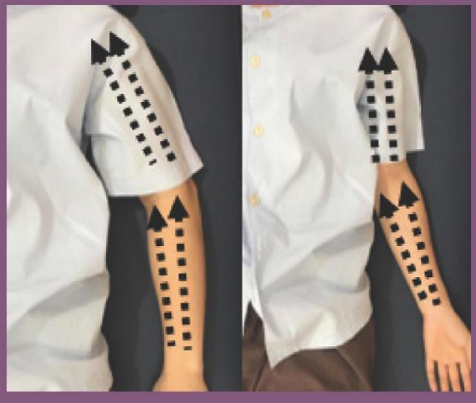

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ORIGINAL ARTICLE

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Detection of Mycobacterium tuberculosis complex in formalin-fixed, paraffin-embedded tissue by Multiplex Polymerase Chain Reaction Assay

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KEYWORDS
Mycobacterium;
C-PCR;
M-PCR;
Formalin-Fixed;
Paraffin-Embedded (FFPE) Tissue.

ABSTRACT

Tuberculosis (TB) is a common infectious disease and a serious public health problem in Thailand. The causative agents of TB are a group of closely related bacteria known as the Mycobacterium tuberculosis complex (MTBC). One accepted method for the diagnosis of tuberculous infection is the detection of their DNA by conventional polymerase chain reaction (C-PCR) assay. However, this method is complicated and time-consuming, and thus unsuitable for mass screening. A simpler and faster multiplex PCR (M-PCR) assay has been developed to overcome these problems. The objective of this study is to prove that detection of MTBC DNA from formalin-fixed, paraffin-embedded (FFPE) tissue by M-PCR assay can be acceptable in comparison to the previous C-PCR assay. Paraffin-embedded tissue samples of one hundred and fifteen suspected cases of tuberculosis referred to the Institute of Pathology, Department of Medical Services were retrieved for DNA extraction. IS6110 and B-globin gene were examined by M-PCR and C-PCR assays for the detection of M. tuberculosis complex. The results of the M-PCR assay agreed with the C-PCR assay (K=1.00, 95% CI 1.00-1.00). In conclusion, M-PCR assay is a simpler, faster, and less costly method that can be an efficient and effective alternative to C-PCR assay for the DNA detection of M. tuberculosis complex from formalin-fixed, paraffin-embedded (FFPE) tissue.

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Introduction

Tuberculosis (TB) is the crucial cause of death from infectious diseases worldwide and a serious public health problem in Thailand[1,2]. The causative agents of TB are a group of closely related bacteria known as the *M. tuberculosis* complex (MTBC)[1,3].

Diagnosis of MTBC infection has been implemented by multiple methods. Culture is a standard method but it takes more than eight weeks to complete[4,5]. Sputum smear for acid-fast bacilli (AFB) is easy, fast, and inexpensive, but its efficiency is limited by relatively low sensitivity and cannot distinguish MTBC from non-tuberculous mycobacteria (NTM)[6,7]. For the tissue section, Ziehl-Neelsen staining and careful search for acid-fast bacilli should be made to diagnose tuberculosis. Its efficiency is also limited. Acid-fast bacilli are frequently missed under light microscopy on formalin-fixed, paraffin-embedded tissue[8].

Currently, nucleic acid amplification test (NAAT)-based assays are being actively conducted for the detection of MTBC[4,6,9]. Conventional polymerase chain reaction (C-PCR) assay is generally reliable but has limitations because of two separate reactions between the IS6110 gene target and the beta-globin gene target.

Multiplex polymerase chain reaction (M-PCR) refers to the use of PCR to amplify several different DNA sequences simultaneously as if performing many separate PCR reactions all together in one reaction[10-12]. M-PCR can be performed in a single PCR reaction. This decreases the duration and risk of contamination. In addition, the M-PCR assay uses lesser amount of reagents causing lower cost than the C-PCR. We aimed to evaluate that this more beneficial method can be accepted as the C-PCR assay.

Materials and methods

Clinical Samples

One hundred and fifteen formalin-fixed paraffin-embedded (FFPE) tissue samples suspected of tuberculosis that were referred to the Institute of Pathology, Department of Medical Services were retrieved for DNA extraction. The study was approved by the Ethics Committee of the Institute of Pathology (IOP-KM-R63-001). The pathologist reexamined the histomorphology of all samples and marked the area suspected of the tuberculous lesion.

DNA extraction of FFPE tissues

Each tissue sample was manually micro-dissected from paraffin-embedded blocks. Ten µm thick ribbon sections were put in a microcentrifuge tube. Paraffin was removed from the tissue sections with xylene and rehydration with 100% ethanol. DNA was extracted from FFPE tissues and purified using a QIAamp DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions[13]. DNA quantity was determined by NanoDrop spectrophotometry (NanoDrop Technologies, Wilmington, DE). The DNA solution was adjusted to a concentration of 500 ng/µl and stored at -40 °C for further use in the following process.

Detection of M. tuberculosis complex in FFPE tissue by C-PCR Assay

The acceptable C-PCR reaction for the detection of *M. tuberculosis* complex in FFPE tissues was performed by using two tubes in each case. One was used for the IS6110 gene target and β-globin gene was performed as internal control. The first tube (25 µl in total volume) for the detection of IS6110 gene-specific MTBC gene contained 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP (New England Biolabs, USA), Amplitaq gold DNA polymerase 0.625 Unit (Applied Biosystems, USA), DNA template 500 ng/µl, adjusted volume with distilled water and 0.4 µM oligonucleotide primers: IS6110-F; 5'-CCT-GCG-AGC-GTA-GGC-GTC-GG-3' and IS6110-R; 5'-CTC-GTC-CAG-CGC-CGC-CGC-TTC-GG-3'. The second tube (25 µl in total volume) for the detection of DNA quality contained 1XPCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP (New England Biolabs, USA), Amplitaq gold DNA polymerase 0.625 Unit (Applied Biosystems, USA), DNA template 500 ng/µl, adjust the volume with distilled water and 0.4 µM Oligonucleotide primer: β-globin-F; 5-'ACA-CAA-CTG-TGT-TCA-CTA-GC-3' and β-globin-R; 5'-CAA-CTT-CAT-CCA-CGT-TCA-CC-3'. Reactions in both tubes were

amplified performed in the T100 thermocycler (Bio-Rad, USA) under the following conditions: an initial denaturation step at 95 °C for 10 min, followed by 40 cycles at 95 °C for 45 s, 63 °C for 45 s, 72 °C for 45 s, and finally 10 min at 72 °C. After amplification, the amplified products with a loading volume of 10 µl were analyzed by 6% polyacrylamide gel electrophoresis at 140 V for 45 min. Gels were stained with SYBR Green I Nucleic Acid Gel Stain (1:400, Lonza, USA) for 30 min. Two separate lanes on the electrophoresis gel were evaluated. One lane was for the MTBC. The MTBC-positive reaction showed a band at 123 bp. The other lane was for DNA quality. The good DNA quality showed a band at 110 bp. (Figure 1).

Detection of M. tuberculosis complex in FFPE tissue by M-PCR Assay

M-PCR reaction for the detection of *M. tuberculosis* complex in FFPE tissue was performed using a single tube in each case. The single-tube reaction had two main genes composed of IS6110 gene and β-globin gene. The single tube reaction (25 µl in total volume) contained 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP (New England Biolabs, USA), Amplitaq gold DNA polymerase 0.625 Unit (Applied Biosystems, USA), DNA template 500 ng/µl, 0.4 µM IS6110-F primer, 0.4 µM IS6110-R primers, 0.2 µM B-globin-F primer, 0.2 µM B-globin-R primer, and adjustable volume with distilled water. The following amplification reaction and analysis, the procedures were the same as the C-PCR assay. Each case needed only one lane on the electrophoresis gel for evaluation. The MTBC-positive reaction showed two bands at 123 bp and 110 bp. The MTBC-negative reaction showed only one band at 110 bp in case of good DNA quality.

Minimum DNA concentration for the detection of MTBC by M-PCR assay

DNA from the MTBC-positive case detected by M-PCR assay was diluted to various concentrations to evaluate the minimum DNA concentration that can be used for the detection of MTBC by M-PCR assay. The diluted DNA samples were 500, 300, 200, 100, 50, 25, 10 and 5 ng/µl. Distilled water was used as a negative control. All different DNA concentration samples of the MTBC-positive case were performed by the same M-PCR assay. Methods for amplification reaction and analysis were the same as previously mentioned in all samples. The minimum appropriate concentration for the detection of MTBC showed two bands at 123 bp and 110 bp. The inappropriate concentration showed only one band at 110 bp. or without any band.

Statistical analysis

Results of the detection of MTBC from the 115 formalin-fixed, paraffin-embedded specimens by M-PCR and C-PCR techniques were compared and were evaluated for significance using the Kappa statistics. The k-value was more than 0.81. It was considered to be significant and indicating that both methods provided almost perfect results. Statistics were carried out using SPSS software (version 19).

Results

Demographic of patients' characteristics

The demographic characteristics are presented in Table 1. Of the 115 patients, the median age was 45 years old. The male to female ratio was 1.3:1. The dominant organ was lymph node 29.6% (34/115). The most common histologic features of the sample (53.9%) were chronic inflammation and necrosis.

Table 1 Patients' characteristics of 115 samples

Characteristics	N (%)
Total patients	115
Median age (range)	45
< 20 years	13 (11.3%)
20 - 39 years	33 (28.7%)
40 - 59 years	37 (32.2%)
60 - 79 years	29 (25.2%)
80 - 99 years	3 (2.6%)
Gender	
Male	65 (56.5%)
Female	50 (43.5%)
Organs	
Lung	15 (13.0%)
Brain	2 (1.7%)
Intestine	24 (20.9%)
Gallbladder	2 (1.7%)
Skin	10 (8.7%)
Synovitis	2 (1.7%)
Lymph node	34 (29.6%)
Bone	11 (9.6%)
Soft tissue	5 (4.3%)
Others	10 (8.7%)
Histological classification no granulomatous	34 (29.6%)
Chronic, Inflammation, Necrotic	62 (53.9%)
Caseation, granuloma, Langhans giant cells	19 (16.5%)

M-PCR and C-PCR analysis of M. tuberculosis complex detection in FFPE tissue specimens

Results of M-PCR and C-PCR analysis of MTBC detection in FFPE tissue were the same. Twenty-six samples (22.61%) were positive for MTBC-DNA and 89 samples (77.39%) were negative for MTBC-DNA. All samples (115 samples, 100%) had good DNA quality (positive for B-globin-DNA). The comparison of their results by kappa analysis (Table 2) showed that the M-PCR assay had a very good agreement with the C-PCR assay (K=1.00, 95% CI 1.00-1.00). This means that the M-PCR assay is an acceptable assay as the reliable C-PCR assay. Duration for the M-PCR assay is approximately four hours shorter than the C-PCR assay. In addition, the M-PCR assay uses a lesser amount of reagents and materials than the C-PCR assay. This means that the M-PCR assay for the detection of MTBC in FFPE is faster and less expensive than the C-PCR assay.

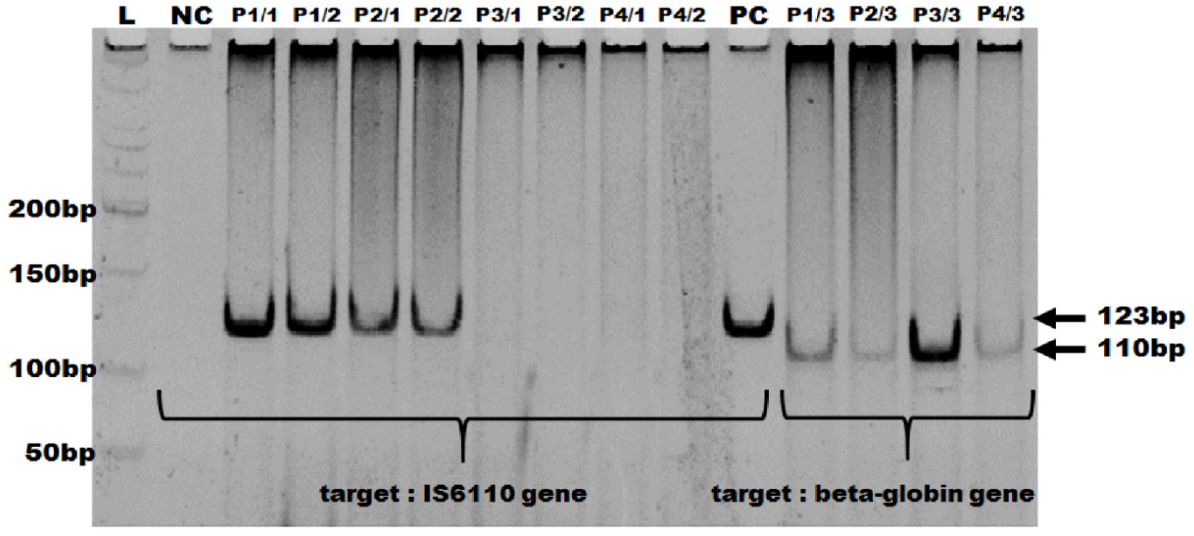

Figure 1 C-PCR assay of IS6110 and ß-globin gene for detected MTBC. Lane L: 50 bp DNA ladder. Lane NC: Negative control (IS6110 gene). Lane P1/1-P1/2 and P2/1-P2/2: By duplication reaction with clinical sample number 1 and 2 showed MTB infection; Lane P3/1-P3/2 and P4/1-P4/2: By duplication reaction with clinical sample number 3 and 4 showed no MTB infection. Lane PC: Positive control (IS6110 gene); show PCR product at 123 bp. Lane P1/3, P2/3, P3/3 and P4/3: clinical sample number 1, 2, 3 and 4 were test DNA quality show PCR product at 110 bp, respectively.

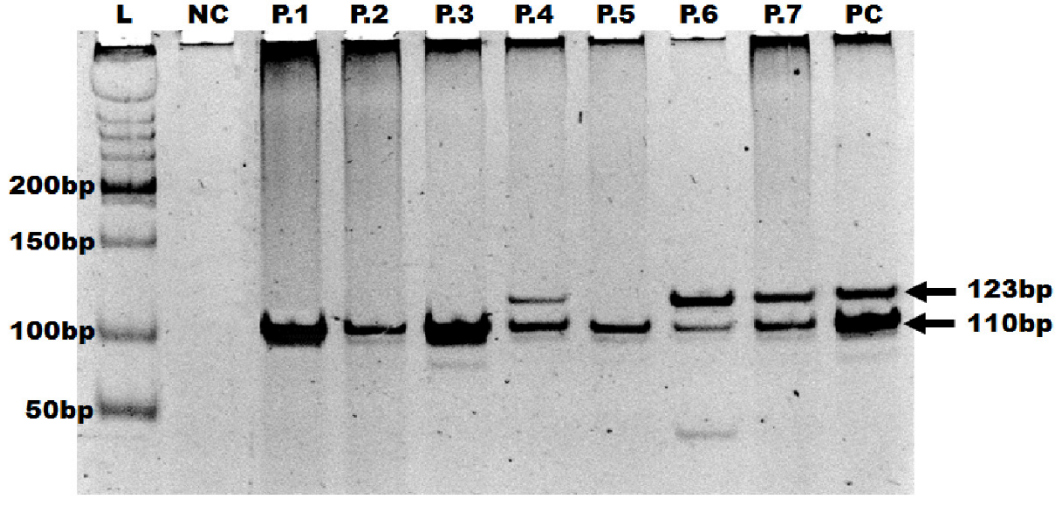

Figure 2 M-PCR assay distinguished IS6110 and beta-globin genes for detected MTBC. Lane L: 50 bp DNA ladder. Lane NC: Negative control. Lane P.1, P.2, P.3 and P.5: clinical sample number 1, 2, 3 and 5 showed no MTB infection. Lane P.4, P.6 and P.7: clinical sample number 4, 6 and 7 showed MTB infection. Lane PC: Positive control; showed PCR products at 123 bp and 110 bp.

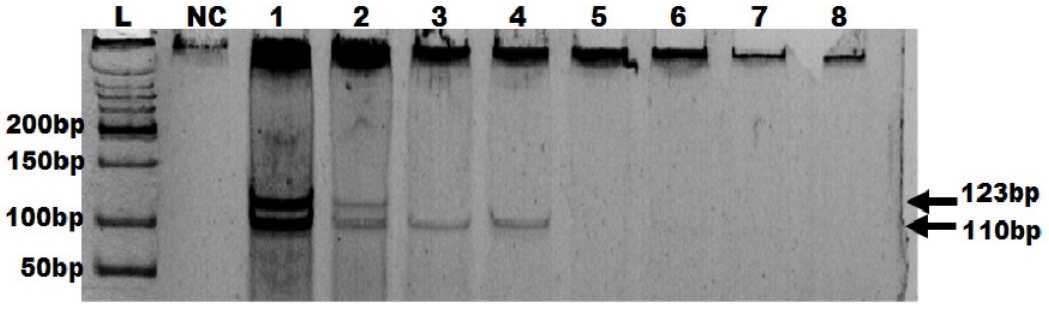

Figure 3 Minimum DNA concentration of detection by M-PCR Assay. A representative gel is shown. Dilutions of DNA template of patients with Positive-MTBC (from 500, 300, 200, 100, 50, 25, 10 and 5 ng/µl). Lane L: 50 bp DNA ladder. Lane NC: Negative control. Lane 1–8: corresponded to PCR products from 500 ng/µl to 5 ng/µl.

Table 2 Pairwise comparison and agreement analyses between C-PCR and M-PCR

Method	Sample (N)	Result				K (95%CI)	Duration (hours)
		IS6110		B-globin			
		Positive	Negative	Positive	Negative		
C-PCR	115	26	89	115	0	1.0 (1.0-1.0)	8
M-PCR	115	26	89	115	0		4

Discussion

Nucleic acid amplification test (NAAT)-based assays are recommended by the WHO as initial tools for cases suspected to have TB[14,15]. The polymerase chain reaction (PCR) for the detection of MTBC in formalin-fixed paraffin-embedded (FFPE) tissue has been widely performed[9,16]. It can give faster results than the culture and more sensitivity than the histochemical staining on the FFPE tissue.

MTBC has several DNA-regions, such as the intergenic spacer from the 16S or 23S rRNA genes, MPB64, insertion sequences 6110 (IS6110) that have been selected as NAAT PCR targets[17,18]. IS6110 is an insertion element that is found exclusively within the MTBC. It can be detected from all species of the *M. tuberculosis* complex (MTBC)[9,19,20] and has been reported to be widely used for the diagnosis of mycobacterial infection in clinical specimens[18,19,21]. To assess the specimen quality, the human beta-globin gene was included as an internal control to check for inhibition, nucleic acid extraction, and the amplification processes[9,16].

Most of the PCR based studies had reported the use of a single target like IS6110 for the diagnosis of MTB[10,22], without checking an internal control for the assessment of the DNA quality[10,22]. In addition, many PCR based studies had reported the use of two separate targets by C-PCR assay. One target was IS6110 for the diagnosis of MTB. The other was the beta-globin gene for the evaluation of internal control[9,16]. The other method, multiplex polymerase chain reaction (M-PCR) can amplify several different DNA sequences simultaneously and can be performed in a single PCR reaction. This method uses lesser amount of reagents and a shorter duration than the two separate reactions of the C-PCR assay.

To prove that M-PCR assay, the shorter duration method and lower-cost testing than C-PCR assay can be used to detect MTBC in FFPE tissue as the C-PCR assay; 115 samples of DNA extracted from the FFPE tissue were used to detect MTBC by both methods. Their results showed a superior consistency between the C-PCR assay and M-PCR (K = 1.0), which means that there was the acceptance of the similarity between the

two methods. Even though, the C-PCR method is a single target in a single reaction tube (first tube; IS6110 gene target and second tube; ß-globin gene target), which can be amplified to a PCR at the same condition. In contrast, M-PCR allows for simultaneous amplification of multiple target sequences in a single tube. However, the C-PCR method was limited and requires a larger amount of material and time.

Thus, the M-PCR proposed in this study is an effective and reliable diagnostic tool for accurate detection of *M. tuberculosis* complex infection in routine formalin-fixed, paraffin-embedded (FFPE) tissue. In addition, we also tested that the minimum concentration that can be used to detect the positivity for MTBC in the FFPE tissue is 300 ng/µl.

Conclusion

In conclusion, the M-PCR assay can be used for the detection of *M. tuberculosis* complex in FFPE tissue, by performing the IS6110 gene and B-globin gene in a single tube. Compared to the C-PCR assay, both showed the same testing results, but the M-PCR assay is easier to perform, faster, and more cost-effective. This method has a great potential to improve the clinicians' ability for early and rapid diagnosis of MTBC, thus ensuring early treatment and preventing further transmission of disease.

Clinical implication

- The single-tube reaction had two main genes composed of IS6110 gene and ß-globin gene detection of MTBC in FFPE tissue.
- Minimum concentration that can be used to detect the positivity for MTBC in the FFPE tissue is 300 ng/µl.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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ORIGINAL ARTICLE

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Discriminative ability of the Spinal Cord Independence Measure III on levels of independence among ambulatory individuals with spinal cord injury

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KEYWORDS
Clinical tool;
Rehabilitation;
Neurology;
Mobility;
Burden of care.

ABSTRACT

Spinal Cord Independence Measure (SCIM) III has been proposed as a test battery to discriminate independence of individuals with spinal cord injury (SCI). However, there is no clear evidence to support this claim. This study compared the SCIM III scores among 45 ambulatory individuals with SCI who had different levels of independence. Eligible participants were assessed for their demographics, SCI characteristics, and SCIM III scores (Thai version). The requirement of external assistance and/or devices while completing the tasks of SCIM III were used to categorize the participants into three groups (15 participants/group), including need assistance, modified independence (MoID), and independence (ID).The differences among the groups were compared using the Kruskal-Wallis test.The findings indicated significant differences in the total and subscale SCIM III scores among the three groups (p-value < 0.05), except the respiration and sphincter management between the MoID and ID participants (p-value > 0.05). The current findings clearly confirmed the use of SCIM III scores to discriminate and monitor independence among ambulatory individuals of SCI.

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Introduction

Injury to the spinal cord commonly distorts the neural conduction, i.e., sensorimotor and autonomic functions, across the lesion sites according to the severity and the levels of injury[1,2]. Consequently, individuals with spinal cord injury (SCI) suffer from myriad consequences, including self-care, respiration, bowel and bladder control, and mobility that subsequently increase health care cost and burden of care from family members[3,4]. Therefore, apart from the development of rehabilitation treatments, the use of an effective measure is important for data transferring, communication, and periodic follow-up for the change over time of these individuals.

The Spinal Cord Independence Measure (SCIM) has been claimed as a thorough, comprehensive test battery to indicate independence of individuals with both tetraplegia and paraplegia, as well as those with complete and incomplete SCI. After development, the tool has been gradually modified to the third version, as the so-called the SCIM III. It is divided into three subscales with the total of 19 items, including self-care (six items; range from 0-20 scores), respiration and sphincter management (four items; range from 0-40 scores), and mobility (nine items; range from 0-40scores). Thus, the total SCIM III scores range from 0 to 100, with a higher score inferring the requirement of little assistance or fewer aids to complete basic daily activities and life support activities[5-8]. However, to the best of the researchers' knowledge, there was no clear evidence to confirm the discriminative ability of SCIM III scores on the independence of individuals with SCI. Therefore, this study compared the SCIM III scores (discriminative ability) among ambulatory individuals with SCI who had different levels of independence as determined using external assistance and devices required while completing daily activities.

Materials and methods

Subjects

This study was cross-sectionally conducted in ambulatory individuals with SCI who were consecutively admitted to a tertiary rehabilitation center. The inclusion criteria were age at least 18 years old, having motor incomplete SCI as determined using criteria from the American Spinal Injury Association (ASIA) Impairment Scale (AIS) C and D from traumatic or non-progressive causes, and being at a sub-acute or chronic stage of injury[1,9]. Individuals with SCI were excluded if they presented with any conditions that might affect outcomes of the study or participation in this study, such as having unstable medical conditions, brain involvement, visual deficits, deformity in the joints, leg length discrepancy, pain in the musculoskeletal system with the visual analog scale more than 5 out of 10, and other medical conditions that might affect participation in the study.The sample size calculation (using pilot data of SCIM III scores, 15 participants, with a standard deviation of 4.93 and effect size of 3.06, with set a power of test at 0.8 and an alpha level of 0.05) indicated that the study required at least 14 participants/group. The research protocol was approved by the institutional this committee for human research (HE611371). A written informed consent form was obtained from all participants prior to participation in the study.

Research protocols

Participants were interviewed and assessed for their demographics (i.e., bodyweight, height, sex, age, underlying disease, if any), and SCI characteristics (i.e., post-injury time, causes, levels and severity of injury using criteria from the ASIA impairment scale (AIS C or D)[9].Subsequently, participants were assessed for their SCIM III scores (Thai version) via interview and observation by an experience rater who had excellent reliability in using the SCIM III (Thai version) (intraclass correlation coefficients > 0.9) with the confirmation from their care givers (Figure 1). Their ability and the requirement of external assistance and/or devices while completing the tasks of SCIM III were used to arrange the participants into three independent levels [9,10] including

- Assistance (ASST) referred to those who required external assistance to perform daily movements.

- Modified independence (MoID) inferred to those who were able to execute daily movements independently with external devices.
- Independence (ID) referred to those who were able to complete daily movements independently without external assistance and devices.

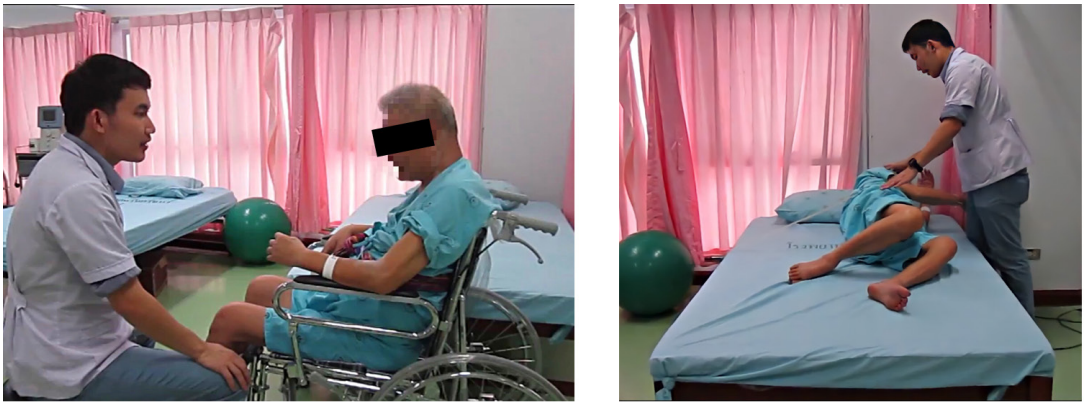

Figure 1 Data collection for the SCIM III scores using interview and observation.

Statistical analysis

Descriptive statistics were applied to explain demographics and SCI characteristics. With non-normal distribution, data among the groups were compared using the Kruskal-Wallis test. Then every pairwise comparison was further analysed using the Mann-whitney U test. A level of statistical significance was set at p-value < 0.05.

Results

Forty-five ambulatory individuals with SCI completed this study (15 participants/group). Most of them were middle-aged (53.6 ± 13.8 years) female (69%), being at a chronic stage (n = 34, average post-time injury of nearly 5 years, Table 1). All participants could breathe independently using room air. All ID participants were paraplegia with AIS D, whereas some ASST participants were tetraplegia and most of them had AIS C (p-value < 0.05, Table 1). However, other demographics and SCI characteristics showed no significant differences among the groups (p-value > 0.05, Table 1). The findings indicated that ID participants had the highest SCIM III scores, both total and all subscales, followed by those with MoID, and ASST individuals, respectively (Figures 1A-D). The ID participants had the total SCIM III of nearly 96 scores whereas ASST individuals had the total SCIM III of approximately 46 scores (Figure 2D). The findings indicated significant differences among the groups, both the total and all subscale scores (p-value < 0.05, Figures 2A-D), except the respiration and sphincter management domain between MoID and ID participants (p-value > 0.05, Figure 2B).

Table 1 Demographics and spinal cord injury (SCI) characteristics of the participants

Variable	Level of independence (n=45)			*p*-value
	Assistance (n=15)	Modified ID (n=15)	ID (n=15)	
Age (year)[a]	56.7 ± 10.8 (50.7-62.7)	51.6 ± 15.6 (42.9-60.2)	52.6 ± 14.8 (49.5-57.8)	0.572
Body mass index (kg/m^2)[a]	23.0 ± 4.4 (20.5-25.4)	23.6 ± 4.2 (21.2-26.0)	22.5 ± 3.5 (20.6-24.5)	0.770
Post time injury (month)[a]	57.9 ± 80.4 (13.3-102.4)	88.1 ± 85.4 (40.8-135.4)	49.6 ± 51.6 (21.0-78.2)	0.335
Gender (n; %)[b]				
- Female	12 (80)	10 (67)	9 (60)	0.484
- Male	3 (20)	5 (33)	6 (40)	
Cause of SCI (n; %)[b]				
- Traumatic	7 (47)	8 (53)	11 (73)	0.306
- Non-traumatic	8 (53)	7 (47)	4 (27)	
Level of injury (n; %)[b]				
- Cervical (C3-5)	7 (46)	5 (33)	0	0.012*
- Thoracic (T1-10)	4 (27)	2 (13)	1 (7)	
- Lumbar (L1-5)	4 (27)	4 (27)	7 (47)	
- Cauda equina	0	4 (27)	7 (47)	
Severity of SCI (n; %)[b]				
- AIS C	11 (90)	1 (7)	0	< 0.001*
- AIS D	4 (10)	14 (93)	15 (100)	

Note: The data are presented using [a] mean ± standard deviation (95% confidence interval) and [b] number (%). *Indicate significant difference between groups. ID, independence; AIS, American Spinal Injury Association (ASIA) Impairment Scale; AIS C: more than half of the key muscles below the neurological level of injury have muscle grade < 3; AIS D: more than half of the key muscles below the neurological level of injury have muscle grade ≥ 3.

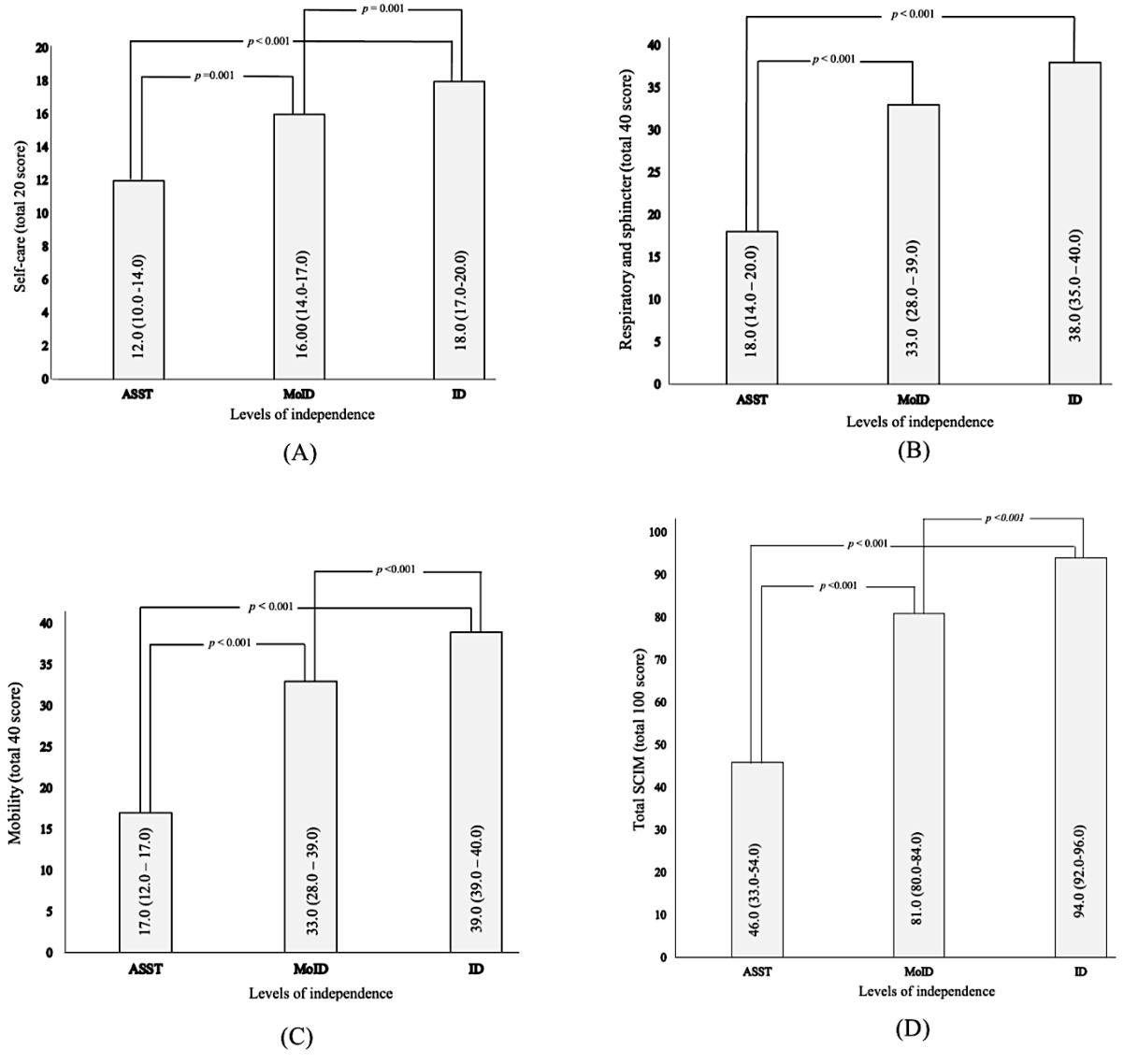

Figure 2 Spinal Cord Independence Measure (SCIM) III scores of participants with different levels of independence for
A: Self-care subscale
B: Respiration and sphincter management subscale
C: Mobility subscale
D: Total SCIM III scores

Note: Data among the groups were compared using the Kruskal-Wallis test and every pairwise comparison was analyzed using the Mann-Whitney U test. ASST, need assistance; MoID, modified independence; ID, independence.

Discussion

Without clear evidence to confirm the use of SCIM III to indicate independence in ambulatory individuals with SCI, this study compared the SCIM III scores among individuals with different levels of independence. The finding clearly indicated that the SCIM III scores, both total and in all subscales, could significantly discriminate ambulatory

individuals with SCI who had different levels of independence (p-value < 0.05), except the respiration and sphincter management subscale between MoID and ID participants (p-value > 0.05, Figure 2B).

The significant differences found for the levels and the severity of injury among the groups (Table 1) represented normal characteristics of those with different potential of independence[7,11], and appropriateness of standard criteria used to verify discriminative ability of the SCIM III scores. A large proportion of ASST participants were those with tetraplegia and AIS C (Table 1). Thus, these participants needed external assistance to execute their self-care, respiration and sphincter management, and mobility; and they had the lowest total SCIM III scores (approximately 46 scores, Figure 2D). On the contrary, all ID participants had paraplegia and mild lesion severity (AIS D). Consequently, these participants could complete their daily activities independently without assistance from persons and devices. The findings indicated that these participants had nearly total scores possible (96 out of 100 scores, Figure 2D). The findings were coherent with the previous reports[6,12] that reported the discriminative ability of the SCIM III scores in individuals with complete and incomplete SCI, and those with different degrees of lesion severity (AIS A-D). The present findings further confirm the discriminative ability of the SCIM III scores among ambulatory individuals with SCI who had different independence in daily activities especially.

The non-significant differences in the respiration and sphincter management subscale between MoID and ID participants may reflect characteristics of participants and defect in the SCIM III items. All participants in this study could breathe independently using room air; thus they had a full score that suggests the ceiling effects of this item. On the contrary, individuals with SCI commonly experience bladder and bowel control, even in ID individuals. Thus, the findings indicated the non-significant differences in the respiration and sphincter management subscale between MoID and ID participants (p-value > 0.05, Figure 2B). Ackerman et al. (2010) also reported that the respiration item of the SCIM III scores has ceiling effects, whereby individuals with SCI who were discharged from a post-acute rehabilitation program achieved the highest score possible in this item[13].

The present findings confirm the discriminative ability of the SCIM III scores among ambulatory individuals with SCI with different independence. Thus, individuals with the different SCIM III scores could reflect those with different independence in their daily living. However, the findings were derived from only ambulatory individuals with SCI with three levels of independence. In addition, this study cross-sectionally gathered the data; thus the findings may not clearly confirm the use of SCIM III score to monitor independence of individuals with SCI overtime. Further study in a large number of participants with data analysis separately for wheelchair-bounded and ambulatory individuals with various degrees of independence and the use of SCIM III scores to monitor independence of these individual overtime would further provide clinical benefit of the SCIM III scores for individuals with SCI.

Conclusion

The current findings clearly confirmed the discriminative ability of the SCIM III scores for independence among ambulatory individuals with SCI, whereby the individuals with the different SCIM III scores reflect those with different independence in their daily living.

Take home messages

The SCIM III is a comprehensive disability test battery for individuals with SCI. The different SCIM III scores reflect those with different independence in their daily living.

Conflicts of interest

The authors declare no conflict of interest.

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Effects of positive expiratory pressure and breath stacking training on pulmonary function in cardiac surgery patients: a randomized controlled trial

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KEYWORDS
Positive expiratory pressure;
Breath stacking;
Chest physical therapy;
Cardiac surgery;
Pulmonary function.

ABSTRACT
This study aimed to investigate the effects of adding positive expiratory pressure (PEP) and breath stacking (BS) training to routine chest physical therapy after cardiac surgery on pulmonary function (PF), respiratory muscle strength (RMS), and chest wall expansion (CWE) in comparison to receiving routine chest physical therapy alone. Thirty-four cardiac surgery patients were assigned randomly to either the PEP (n=10), BS (n=12), or control group (CON) (n=12). All participants received routine chest physiotherapy. The PEP training consisted of 5 breaths/set, 6 sets/session, 2 sessions/day for three days postoperatively via a BreathMAX device, while the BS training involved 5 breaths/set, 3 sets/session, 2 sessions/day for three days postoperatively. All participants were assessed for PF, RMS, and CWE. Results showed that after training, all groups showed a significant increase in force vital capacity, vital capacity, total lung capacity, and CWE (p-value < 0.01) compared to postoperative day 2. The PEP and CON groups also exhibited a significant increase in peak expiratory flow rate and forced expiratory volume in one second. Moreover, a significant increase in maximal inspiratory pressure and maximal expiratory pressure on postoperative day 5 was observed in the BS and CON groups compared to postoperative day 2. However, no significant differences between the groups were found. The three protocols were equally efficacious concerning PF recovery during the first 5 postoperative days. When compared with routine therapy, BS tended to yield greater RMS. Meanwhile, PEP tended to produce better PF and CWE than the other two techniques. Therefore, physiotherapists should consider post-operative management as a key role in these patients, especially when using the chest physical therapy technique, since this technique has different method and is beneficial for the reduction in post-operative complications.

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Introduction

Cardiac surgery is an effective treatment for patients with coronary heart disease. However, this approach may affect the respiratory function significantly. Pulmonary impairments include postoperative pain from median sternotomy, respiratory muscle dysfunction, lung volume decrease, and impaired mucociliary clearance function[1], which can lead to a prolonged length of hospital stay, higher health care cost, morbidity, and mortality[2]. The cause of pulmonary impairment is multifactorial; the most commonly reported risk factors in the early postoperative period are pain, a limited ability to take a deep breath, and sternal pain[3-6]. In the early post-cardiac surgery period, lung functions, measured in terms of forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1), usually decline by 40% - 50%[3]. Respiratory muscle strength also decreases during the first days after surgery[3].

Chest physiotherapy is an established recommendation to prevent pulmonary impairments in cardiac surgery patients[8,9]. Postoperative treatment includes airway clearance techniques, early mobilization, positioning, and deep breathing exercises[10]. Various mechanical devices have also been used to improve postoperative pulmonary function, e.g., incentive spirometry (IS) and positive expiratory pressure (PEP). IS encourages patients to perform sustained maximal deep breathing through a visual biofeedback mechanism[11]. The PEP device is used in airway clearance therapy to enhance function of the diaphragm and improve atelectasis after surgery[7]. However, the ability of a patient to perform IS and PEP can be impaired by pain, dyspnea, and weakened respiratory muscle function[12]. Therefore, an increasing interest in alternative methods to promote lung expansion without pain has been observed. A newer technique is breath stacking (BS), which can be used in postoperative patients with post-surgery pain to help increase inspiratory volume and maintain inspiration for a long period of time[13-15].

To our knowledge, there have been no studies comparing chest physiotherapy with IS, PEP, and BS in terms of their effects on pulmonary function and respiratory muscle strength in cardiac surgery patients. The aim of this study was, therefore, to evaluate the efficacy of routine physiotherapy plus PEP and routine physiotherapy plus BS in comparison with a control group that received routine physiotherapy plus IS in improving pulmonary function and respiratory muscle strength in patients undergoing heart surgery. Our hypothesis was that a significant difference in pulmonary function and respiratory muscle strength would be found after training program.

Materials and methods

Trial design

This was a single-blinded randomized controlled trial involving cardiac surgery patients, who were randomly allocated into three groups—the positive expiratory pressure (PEP), breath-stacking (BS), and control (CON) groups—before the first visit using block allocation. This study was approved by the Human Research Ethics Committee (HREC), Faculty of Medicine, Prince of Songkla University (No. 56-400-11-2).

Participants

We recruited cardiac surgery patients from Songklanagarind Hospital. The inclusion criteria were surgery via median sternotomy, good ability to communicate, no respiratory disease before surgery that affects the respiratory system, and cardiac surgery performed between September 2013 and December 2015. Meanwhile, the exclusion criteria were intubation for > 48 hours after surgery, need for reintubation for 5 postoperative days, hemodynamic instability (heart rate >120 beats per minute, systolic blood pressure < 90 or >140 mmHg, respiratory rate > 30 breaths per minute, or oxygen saturation < 90.0%), hemodynamic complications (cardiac arrhythmia, mean arterial blood pressure < 70 mmHg, or intraoperative myocardial infraction), re-median sternotomy, and postoperative complications (pneumonia, pulmonary edema, pleural effusion, or pneumothorax). A total of 67 patients were recruited, 31 of whom were excluded due to various reasons; the remaining 36 patients were randomly allocated to the PEP, BS, and CON groups (Figure 1

and Table 1) by an independent investigator. The data related to the patients who died were also excluded from the statistical analysis. All participants received the same routine postoperative chest physical therapy, which included an optimal treatment for pain control. A verbal pain score was obtained via a numeric rating scale (NRS)[16], and physiotherapy was initiated on postoperative day 1 in all cases.

Sample size calculation

Based on the results of Baumgarten MC[17] and an estimation on the basis of IS and BS training measured using FVC and inspiratory volume on postoperative day 5, and assuming a power of 80%, a significance level of 5%, and a dropout rate of 20%, a minimum sample size of 36 was required for this study in order to detect a clinically meaningful difference between groups using FVC and inspiratory volume.

Interventions

Before the operation, the patients received general information about postoperative chest physical therapy routines provided by same physical therapists for all patients. Demographic, functional, and surgical data were recorded. All patients received chest physical therapy once daily as normally performed during the first 5 postoperative days. Therapy consisted of early mobilization and secretion removal, instructions on breathing exercises including breathing control and deep breathing, deep breathing with a device using an incentive spirometer (TRIFLO II™, Sherwood Medical, St. Louis, MO, USA), supported coughing and huffing, daily active limb exercises, chest mobilization with correct posture, and assistance with turning from side to side and sitting (out-of-bed). The patients were mobilized as early as possible by the physical therapists. The patients sat out-of-bed and/or stood on the first postoperative day, walked in the room or a short distance in the ward corridor on postoperative days 1-3, and walked a longer distance in the ward corridor or up and down stairs on postoperative day 4. The patients were randomly allocated into three groups; those in the control (CON) group received only the procedures described above. Meanwhile, the PEP group participants, the physical therapist beside them and while in the supine position with the head of the bed elevated 45°, they were instructed to inspire slowly while expiring slowly and long at functional residual capacity (FRC) in order to open the airway and prevent alveolar collapse with a load of 6 cmH_2O using a BreathMAX device for 5 breaths/set, 6 sets, twice a day, for three days (postoperative days 3-5) and resting for at least two minutes between sets.[18-20]. The BS group participants, on top of the procedures of the CON group, practiced inspiratory efforts using a face mask with a unidirectional valve[17]. Patients, in the supine position with the head of the bed elevated 45°, were asked to inspire while wearing a mask that was adjusted to allow only inspiration while occluding the expiratory branch. They were asked to perform successive inspiratory efforts for a period of 20 seconds, and then the expiratory branch was opened to allow expiration; the procedure was performed twice a day for three days (postoperative days 3-5), 3 sets of 5 maneuvers/set, with a rest of at least two minutes between sets[15,21]. The intervention for all patients were conducted by the same physical therapist.

For safety purposes, their cardiovascular and respiratory parameters were monitored, and the interventions would have been stopped in case hemodynamic instability, i.e., respiratory rate > 30 breaths/min, heart rate > 120 beats/min, or oxygen saturation <90.0%, was detected. However, this eventuality did not occur during the procedures in our trial.

Outcomes

Outcome measurements were carried out both pre- and post-breathing training during the first 5 postoperative days by an independent investigator blinded to the interventions. The patients performed the lung function tests of forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), vital capacity (VC), total lung capacity (TLC), peak expiratory flow rate (PEFR), maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), and chest wall expansion (CWE).

The lung function tests were performed both before and after breathing training using a portable computerized spirometer (BTL-08 MT Plus ECG, BTL Group Ltd., UK). The measurement procedures followed the standard guideline of ATS/ERS (2005)[22].

Inspiratory and expiratory muscle strength was assessed using maximal inspiratory pressure (Micro RPM, Micro Medical, Inc., Chatham Maritime, Kent, UK) in accordance with the measurement procedures delineated in the standard guideline of ATS/ERS (2002)[23].

Chest wall expansion was measured at the xiphoid process level via a flexible measuring tape (cm) with a control traction force of 1 kg. The patient was seated on the chair and asked to perform three normal breaths, followed by a deep expiration and then a deep inspiration. This was repeated for three times, and the maximum value was recorded.

Statistical analysis

The outcomes of this study were the detected changes in pulmonary functions (FVC, FEV_1, VC, TLC, and PEFR), MIP, MEP, and CWE. The data were cleaned and then imported into the R software version 3.5.2 for analysis. Continuous variables were presented as mean with a standard deviation, and categorical variables were presented as frequency and percentage. The distribution of the variables was checked via the Kolmogorov-Smirnov test. For the comparison between groups, the linear mixed-effects model was employed, meanwhile the Wilcoxon signed-rank test was used for within-the-group comparisons. The statistical significance was set at a p-value < 0.05.

Results

Sixty-seven cardiac surgery patients were initially recruited as potential participants in this study. Of those, 31 were excluded:12 patients were intubated for > 48 hours, five had cardiac arrhythmia, four had pleural effusion, three had pulmonary edema, three had pneumonia, two underwent re-median sternotomy, and two patients experienced sudden cardiac arrest. The remaining patients were divided into three groups of 12 patients each. Two patients in the PEP group were lost to follow-up due to cardiac arrhythmia; therefore, 34 patients completed the study. A flow diagram detailing this study's participant inclusion/exclusion is shown in figure 1.

The mean patient age was 56.2 ± 16.0 years in the PEP group, 56.5 ± 13.1 years in the BS group, and 52.8 ± 10.6 years in the CON group. The average body mass index (BMI) was 22.9 ± 4.6 kg/m^2 in the PEP group, 23.7 ±3.1 kg/m^2 in the BS group, and 22.9 ± 4.1 kg/m^2 in the CON group.

The weight, height, systolic blood pressure, heart rate (HR), pulmonary functions (FVC, FEV_1, VC, TLC, and PEFR), MIP, MEP, and CWE were not significantly different among the groups, except for diastolic blood pressure (Table 1).

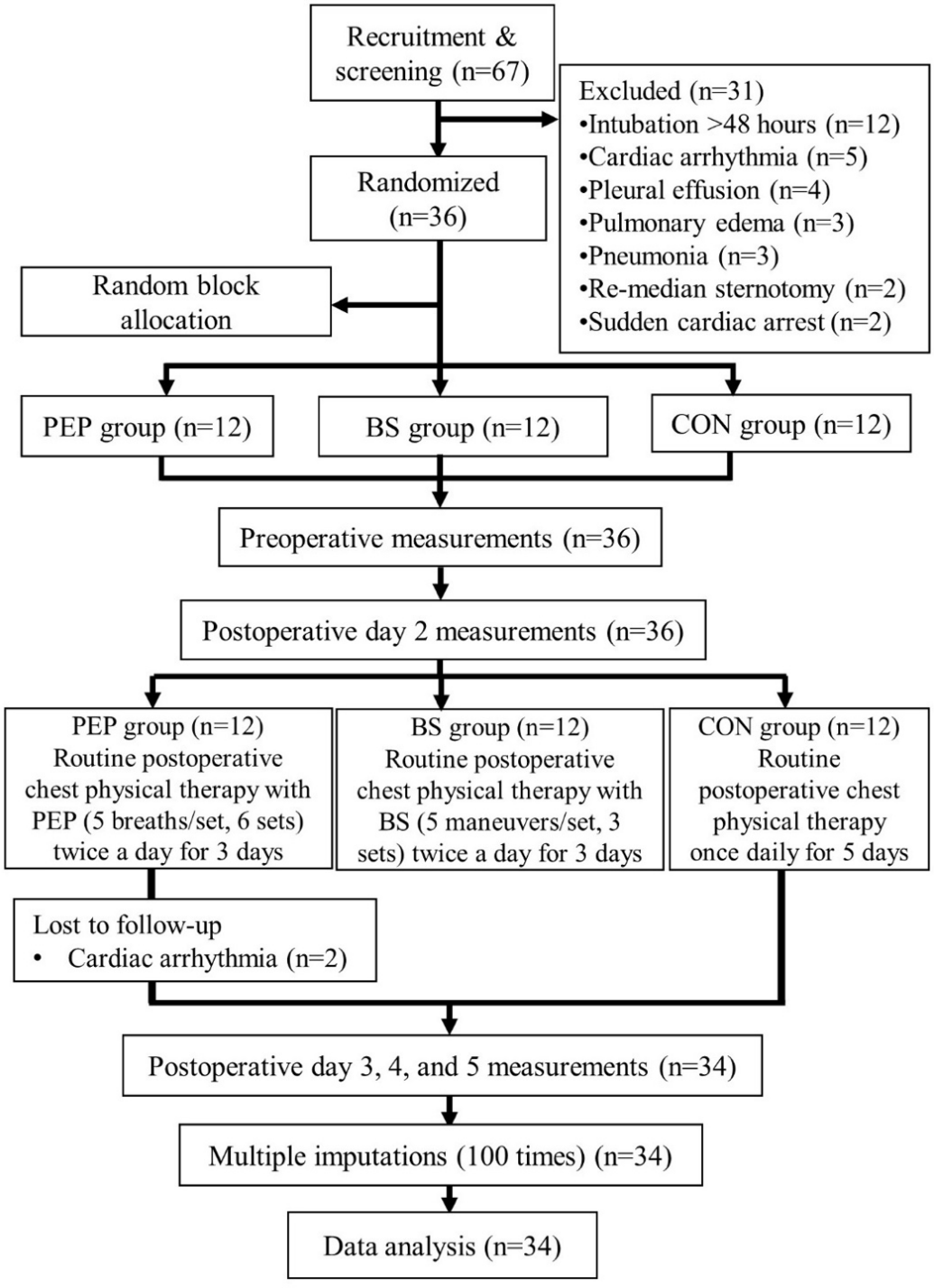

Figure 1 Flow of participants thorough the study. PEP, Positive expiratory pressure; BS, breath stacking training; CON, control.

Table 1 Demographic characteristics of participants (n = 34)

Characteristic	PEP (n = 10)	BS (n = 12)	CON (n = 12)	*p*-value
Age (years), mean (± SD)	56.216.0))	56.5 (13.1)	52.8 (10.6)	0.75
Sex, n (%)				
Male	6.060.0))	7.0 (58.3)	6.0(50.0)	
Female	4.0 (40.0)	5.0 (41.7)	6.0 (50.0)	
Functional class: n (%)				
1	1.0 (10.0)	4.0 (33.3)	3.0 (25.0)	
2	6.0 (60.0)	3.0 (25.0)	5.0 (41.7)	
3	2.0 (20.0)	4.0 (33.3)	4.0 (33.3)	
4	1.0 (10.0)	1.0 (8.4)	0.0 (0.0)	
Type of surgery: n (%)				
CABG	3 (30.0)	5 (41.7)	4 (33.3)	
Valve replacement	5 (50.0)	3 (25.0)	4 (33.3)	
ASD closure	2 (20.0)	4 (33.3)	3 (25.0)	
VSD closure	0 (0.0)	0 (0.0)	1 (8.3)	
Intubation time during surgery (hr), mean (± SD)	4.4 (1.1)	4 (0.9)	4.3 (0.9)	0.95
Weight (kg), mean (± SD)	58.2 (10.3)	61.5 (12.4)	58.99 (12.5)	0.78
Height (cm), mean (± SD)	159.8 (9.0)	160.6 (9.3)	160.3 (8.9)	0.98
BMI (kg/m^2), mean (± SD)	22.9 (4.6)	23.7 (3.1)	22.9 (4.1)	0.85
Systolic BP (mmHg), mean (± SD)	118.516.4))	116.8 (14.1)	118.3 (11.6)	0.95
Diastolic BP (mmHg), mean (± SD)	75.4 (10.1)	66.8 (6.1)	74.8 (8.1)	0.03*
HR (bpm), mean (± SD)	67.0 (12.0)	72.9 (9.7)	76.2 (10.5)	0.15
FVC (L), mean (± SD)	1.8 (0.8)	2.1 (0.8)	2.5 (1.3)	0.23
FEV_1 (L), mean (± SD)	1.3 (0.4)	1.6 (0.7)	2.1 (1.1)	0.07
VC (L), mean (± SD)	1.9 (0.7)	2.4 (0.7)	2.8 (1.0)	0.07
TLC (L), mean (± SD)	3.2 (0.6)	3.2 (1.3)	3.2 (1.3)	1.00

Table 1 Demographic characteristics of participants (n = 34) (cont.)

Characteristic	PEP (n = 10)	BS (n = 12)	CON (n = 12)	*p*-value
PEFR (L/min), mean (± SD)	2.9 (1.8)	3.5 (2.0)	4.4 (2.2)	0.24
MIP (cmH_2O), mean (± SD)	53.3 (23.6)	65.0 (27.6)	71.8 (40.5)	0.43
Relative MIP (cmH_2O)/Body weight (kg), mean (± SD)	0.9 (0.3)	1.0 (0.4)	1.2 (0.6)	0.27
MEP (cmH_2O), mean (± SD)	56.2 (35.2)	70.4 (32.2)	86.2 (55.3)	0.27
Relative MEP (cmH_2O)/Body weight (kg), mean (± SD)	0.9 (0.6)	1.1 (0.4)	1.5 (1.0)	0.23
CWE (cm), mean (± SD)	3.2 (1.4)	4.2 (1.9)	3.9 (1.94)	0.44

Note: Values are means ± SD n = 34 (15 females, 19 males). CABG, coronary artery bypass graft; ASD, Atrial Septal Defect; VSD, Ventricular septal defect; BMI, Body mass index; Systolic BP, Systolic blood pressure; Diastolic BP, Diastolic blood pressure; HR, Heart rate; FVC, Forced vital capacity; FEV_1, Forced expiratory volume in 1 second; VC, Vital capacity; TLC, Total lung capacity; PEFR, Peak expiratory flow rate; MIP, Maximal inspiratory pressure; MEP, Maximal expiratory pressure; CWE, Chest wall expansion; PEP, Positive expiratory pressure; BS, Breath stacking; CON, Control; kg, Kilogram; cm, Centimeter; kg/m^2, Kilograms per meter squared; mmHg, millimeters of mercury; bpm, Beats per minute; L, liter; L/min, Liters per minute; cmH_2O, Centimeters of water; * *p*-value < 0.05 (indicative of significant difference between groups).

All pulmonary function and CWE parameters on postoperative day 2 were significantly decreased in every group (*p*-value < 0.01) compared to preoperative values. After training, on postoperative day 5, a significant improvement in both FVC and VC was observed in all groups (*p*-value < 0.01); the same was true for TLC (*p*-value < 0.05 overall; *p*-value < 0.01 for PEP, BS, and CON groups). After training, CWE improved significantly in all groups (*p*-value < 0.05 for BS, and < 0.01 for PEP and CON groups, respectively) compared to the postoperative day 2 values (Figure 2).

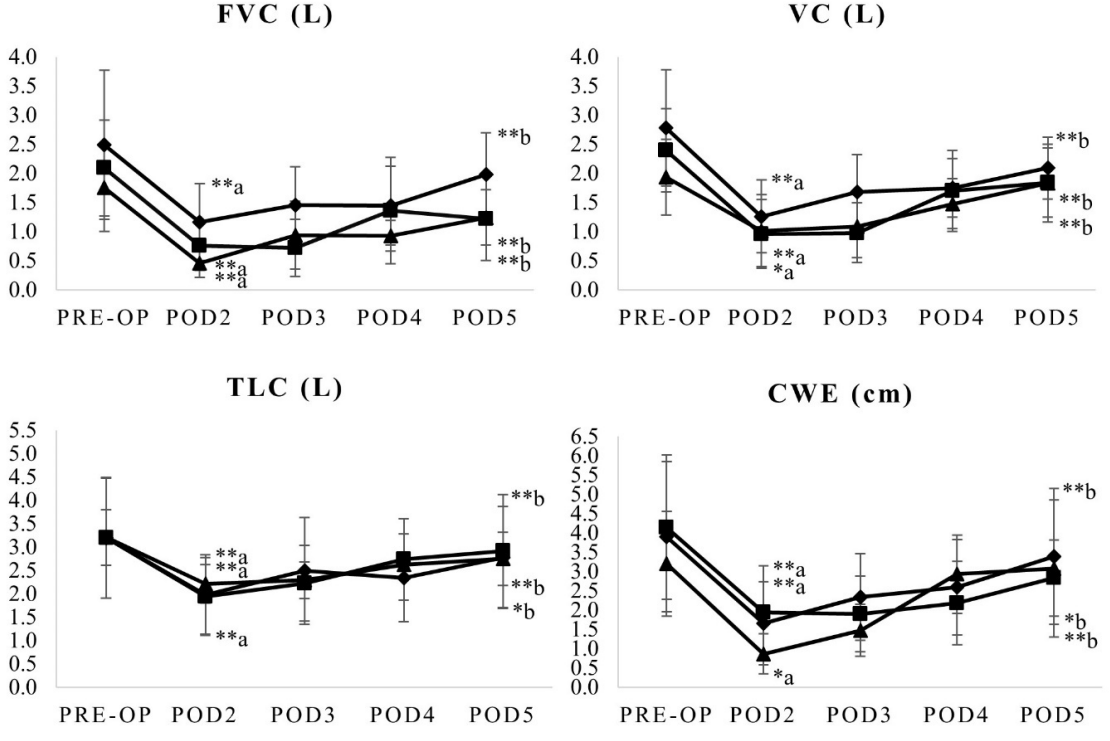

Figure 2 Mean of FVC, VC, TLC, and CWE values before surgery and on postoperative days 2, 3, 4, and 5 following cardiac surgery. Values are means ± SD n = 34 (15 females, 19 males). PRE-OP, Preoperatively; POD2, postoperative day 2; POD3, postoperative day 3; POD4, postoperative day 4; POD5, postoperative day 5; FVC, forced vital capacity; VC, vital capacity; TLC, total lung capacity; CWE, chest wall expansion; L, liter; cm, centimeter; ^{*a}*p*-value (preoperatively-postoperative day 2) < 0.05; ^{**a}*p*-value (preoperatively-postoperative day 2) <0.01; ^{*b}*p*-value (postoperative days 2-5) < 0.05; ^{**b}*p*-value (postoperative days 2-5) <0.01; control group = diamond symbols; breath stacking group = square symbols; positive expiratory pressure group = triangle symbols.

Meanwhile, a significant improvement in PEFR and FEV_1 was seen only in the PEP and CON groups (*p*-value < 0.05 and < 0.01, respectively) compared to postoperative day 2. However, there was no statistically significant difference between the three groups. Respiratory muscle strength values are given in figure 3. On postoperative day 2, a significant decrease in MIP was found in the BS group (*p*-value < 0.01), while MEP decreased significantly in the BS and CON groups (*p*-value < 0.01) compared to preoperative values. After training, on postoperative day 5, MIP and MEP improved significantly in the BS and CON groups (*p*-value < 0.05) (Figure 3).

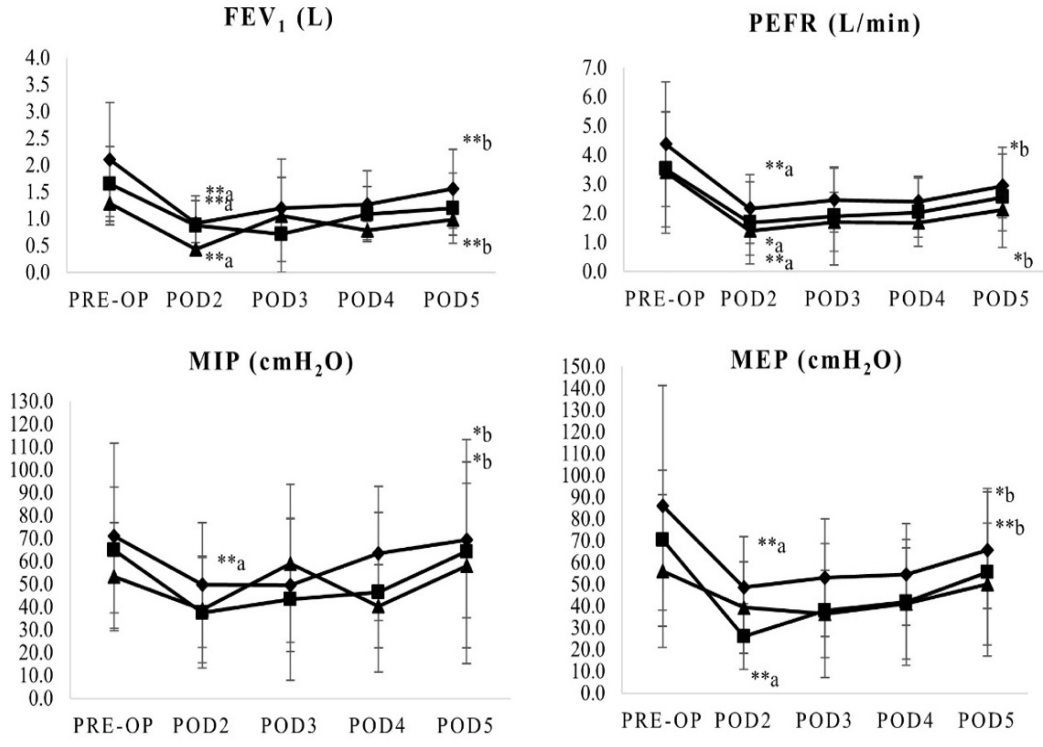

Figure 3 Mean of FEV_1, PEFR, MIP and MEP values preoperatively and on postoperative days 2, 3, 4, and 5 following cardiac surgery. Values are means ± SD n = 34 (15 females, 19 males). PRE-OP, preoperatively; POD2, postoperative day 2; POD3, postoperative day 3; POD4, postoperative day 4; POD5, postoperative day 5; FEV_1, forced expiratory volume in 1 second; PEFR, peak expiratory flow rate; L, liter, L/min, liters per minute; MIP, maximal inspiratory pressure; MEP, maximal expiratory pressure; cmH_2Ocentimeters of water; $^{*a}p$-value (preoperatively-postoperative day 2) < 0.05; $^{**a}p$-value (preoperatively-postoperative day 2) < 0.01; $^{*b}p$-value (postoperative days 2-5) < 0.05; $^{**b}p$-value (postoperative days 2-5) <0.01; control group = diamond symbols; breath stacking group = square symbols; positive expiratory pressure group = triangle symbols.

It was also found that pain on the 2nd postoperative day was significantly higher in the three groups (p-value < 0.01) compared to preoperative values. However, the reported pain scores on postoperative day 5 were significantly lower in the CON and BS groups (p-value < 0.01 and < 0.05, respectively) compared to the 2nd postoperative day values; there was no statistically meaningful difference between the groups (Table 2).

Table 2 Pain data before surgery and on postoperative days 2, 3, 4, and 5 following cardiac surgery (mean ± SD)

Variable	Group	PRE-OP	POD2	POD3	POD4	POD5	% of mean difference
NRS	PEP	0.0 (0.0)	4.7 (1.6) **a	2.4 (2.3)	3.5 (2.1)	2.0 (2.7)	-57.5
	BS	0.0 (0.0)	4.1 (2.6) **a	4.0 (2.4)	3.7 (2.7)	2.0 (1.9)*b	-51.2
	CON	0.0 (0.0)	3.9 (1.7) **a	3.5 (2.4)	2.8 (1.6)	1.1 (1.0)**b	-71.8

Note: Values are means ± SD n = 34 (15 females, 19 males). PRE-OP, Preoperatively; POD2, Postoperative day 2; POD3, Postoperative day 3; POD4, Postoperative day 4; POD5, Postoperative day 5; NRS, Numeric rating scale; PEP, Positive expiratory pressure; BS, Breath stacking; CON, Control; **a*p*-value (preoperatively-postoperative day 2) < 0.01; *b*p*-value (postoperative days 2-5) < 0.05, **b*p*-value (postoperative days 2-5) < 0.01.

Discussion

The aim of this study was to compare the effectiveness of PEP and BS added to the routine chest physical therapy with that of the routine chest physical therapy alone on pulmonary function, respiratory muscle strength, and CWE in cardiac surgery patients. It was found that, on the 5th postoperative day, pulmonary function deteriorated severely in all groups (between 60% and 75% of the preoperative values). The reduction found in our study is consistent with those reported by several previous studies[3,21,24]. The drop in expiratory flow rates, respiratory muscle strength, and CWE impairs both the cough mechanism and the secretion clearance function and the postoperative pain reduces one's ability to cough. In this study, the NRS on the 5th postoperative day was similar in all groups. There is some evidence that regular chest physiotherapy significantly decreases the incidence of pulmonary complications after cardiac surgery[25,26].

In our study, all groups demonstrated a significant improvement in FVC, VC, TLC, and CWE after three days of training. This was consistent with findings from the previous studies, which have indicated an improvement in pulmonary function after routine chest physical therapy with PEP[18] and BS[15] among cardiac surgery patients. A significant decrease in pulmonary function, persisting up to four months after cardiac surgery, has been previously reported[27]. Therefore, the results of the present study support the hypothesis that chest physical therapy facilitates the recovery of pulmonary functions within one week after cardiac surgery, which may lead to a reduction in the incidence of respiratory complications and a shorter length of hospital stay[28].

The patients who participated in the PEP group exhibited a better recovery in terms of both PEFR and FEV_1 on postoperative day 5. This indicates the clinical importance of PEP, which encourages patients to perform forced expiration through water resistance and prolong the expiratory time, resulting in decreased respiratory rate, increased lung volume, and better expiratory flow rate[29]. The results of our study are consistent with those of Borghi-Silva et al. (2005) who reported a better recovery of pulmonary functions in the group that received deep breathing training via PEP and early mobilization compared to those receiving deep breathing training without PEP and early mobilization[30]. They concluded that the use of PEP was more effective in restoring pulmonary function[30]. This is similar to the findings of the Westerdahl et al. (2005) study, which reported that coronary artery bypass graft (CABG) surgery patients, who performed exercises using PEP, experienced smaller atelectatic improvements and less reduction in FEV_1 and FVC on postoperative day 4 compared to control group participants, who performed no exercises[18].

Respiratory muscle dysfunction after cardiac surgery may lead to alveolar hypoventilation due to a reduction in pulmonary functions such as tidal volume, vital capacity, and total lung capacity. In the present study, a significant reduction in both MIP and MEP was observed in the BS group on the 2nd postoperative day. Moreover, we found an improvement in respiratory muscle strength among CON and BS group participants on postoperative day 5 (28.2% for MIP and 25.9% for MEP, and 39.5% for MIP and 48.0% for MEP, respectively). A recent study has shown that BS training is associated with a significant recovery of respiratory muscle strength, and that this recovery is directly related to improvement in pulmonary function. Thus, it can be concluded that the use of BS stimulates the maximum sustained inspiration volume[15], which is associated with improved collateral ventilation, lung re-expansion, and stretching of the intercostal muscles to their optimum length; this leads to an effective restoration of respiratory muscle function as demonstrated by the increases in MIP and MEP values. The results of our study are consistent for clinically significant changes in the MIP and MEP, which is usually more than 60 cm H_2O and associated with a improve ability to cough and secretions clearance.

The present study, however, has the limitations including a relatively small sample size and the specificity of its study population. Therefore, our results cannot be extrapolated to other surgical populations. Further studies are needed to investigate the effectiveness of PEP and BS in relation to clinically relevant outcomes such as the prevention of pulmonary complications (atelectasis and pneumonia) and their impact on the length of hospital stay. Future study should be directed toward confirming our findings and expanding this area of research.

Conclusion

This randomized controlled trial demonstrated that the addition of 5 days of PEP and BS training postoperatively to routine chest physiotherapy resulted in a faster recovery of pulmonary function, respiratory muscle strength, and CWE. However, we found no major differences between the three study groups on the 5th postoperative day. A relative increase in pulmonary function and CWE tended to be associated with PEP, while BS training tended to increase respiratory muscle strength more than the other techniques.

Take home messages

The present study demonstrated that the addition of PEP and BS training to routine chest physiotherapy. A relative increase in pulmonary function and CWE associated with PEP, while BS training tended to increase respiratory muscle strength. However, no major differences between the three study groups. Therefore, physiotherapists should consider post-operative management as a key role in these patients, especially when using the chest physical therapy technique, since this technique has different method and is beneficial for the reduction in post-operative complications.

Conflicts of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

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A combination of urinary neutrophil gelatinase-associated lipocalin and urine albumin creatinine ratio as potential biomarkers for the early diagnosis of CKD in type 2 diabetic patients

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KEYWORDS
Neutrophil gelatinase-associated lipocalin; Diabetes; Diabetic nephropathy.

ABSTRACT

One-third of patients with type 2 diabetes (T2DM) tend to develop chronic kidney disease (CKD) called diabetic nephropathy. The aim of this study was to investigate neutrophil gelatinase-associated lipocalin (NGAL) as a new potential biomarker to predict CKD in T2DM patients. A total of 93 participants were enrolled in the study and are divided into 3 groups including T2DM group (n = 16), T2DM with CKD group (n = 52) and healthy control group (n = 25). All the participants were tested for urinary NGAL (uNGAL) levels by enzyme-linked immunosorbent assay. The results revealed that the T2DM patients with CKD had a significantly higher level of uNGAL (21.0 ± 32.6 ng/mL) than the healthy controls (1.9 ± 2.0 ng/mL *p*-value = 0.005), and the uNGAL level in the T2DM patients with CKD was increased, but not significantly, compared to that in the T2DM patients without CKD (7.6 ± 6.4 ng/mL, *p*-value = 0.1067). Moreover, the analysis revealed moderate correlations between uNGAL and estimated glomerular filtration rate (eGFR) (r^2 = -0.450, *p*-value = 0.001) and between uNGAL and urine albumin creatinine ratio (ACR) (r^2 = 0.489, *p*-value = 0.000). The ROC curve analysis showed that uNGAL was more sensitive (69.23%) and specific (80.49%) than eGFR but could not reach the standard of ACR. Therefore, the study concludes that uNGAL can be a potential predictor of CKD in T2DM when combined with ACR.

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Introduction

Diabetes mellitus (DM) a major clinical and public health problem and affects approximately half a billion people worldwide; type 2 DM itself accounts for nearly 90% of the burden[1]. The condition is associated with several complications, such as cardiovascular disease, neuropathy, stroke and renal failure. A previous study showed that approximately one-third of diabetic patients develop diabetic nephropathy (DN)[2]. In Thailand, the 2015 renal replacement therapy report (TRT) revealed that 38.57% of end stage renal disease (ESRD) patients undergoing dialysis suffered from DN[3].

Glomerular damage is crucial for the initiation of DN due to continuous exposure to many metabolic and hemodynamic factors that lead to injury[4]. Renal tubule-interstitial fibrosis secondary to initial glomerular damage, however, is a potential factor of chronic kidney disease (CKD) progression in diabetes patients[5]. The early diagnosis of DN, as in other kidney diseases, is crucial for better treatment, preventing the progression to ESRD and thus increasing life expectancy and lowering health costs. Clinically, DN is diagnosed according to the guidelines of Kidney Disease Improving Global Outcomes (KDIGO), in which the estimated glomerular filtration rate (eGFR) derived from serum creatinine and daily urine albumin are used as standard kidney function markers[6]. However, an increase in serum creatinine above the physiological level is observed only when half of the kidney function is lost[7]. In other words, serum creatinine cannot accurately explain kidney health until a critical level of kidney function is reached. Urinary albumin is the gold standard for diagnosing and classifying the stages of DN[8] and accurately reflects the degree of glomerular damage; however, it is unable to fully reflect the degree of renal tubular injury[9]. Therefore, there is an immense requirement for a sensitive biomarker for the early diagnosis of DN as well as other causes of CKD, and neutrophil gelatinase-associated lipocalin (NGAL) is a potential candidate.

NGAL is a 25-kDa glycoprotein expressed in cells and tissues, such as neutrophils, monocytes, colon, stomach, trachea, lung, salivary glands, prostate, uterus, and kidney[10]. NGAL belongs to the lipocalin family, a protein family that transports small hydrophobic molecules (*e.g.*, steroids and lipids) and is associated with inflammation, transportation of pheromones, and synthesis of prostaglandins[11]. The levels of NGAL increase in both serum and urine within two hours upon acute tubulointerstitial injury, as NGAL is highly expressed by tubular renal epithelial cells, and indicates acute renal injury before the rise in serum creatinine concentrations[11,12]. Additionally, both serum NGAL and urine NGAL (uNGAL) were also related to tubule-interstitial fibrosis in various CKD models[13]. In a study carried out among patients with DN, an elevated uNGAL level was reported to be associated with the progressive course of the disease leading to ESRD[14]. Similarly, an observational follow-up study revealed that high uNGAL levels at baseline correlated with a rapid decline in eGFR levels in T2DM patients[15]. Therefore, NGAL can be a promising novel biomarker for the early diagnosis of tubular damage.

The current study aimed to investigate uNGAL as a potential marker for CKD in diabetic patients. The levels of uNGAL were analyzed and correlated with the standard biomarkers of kidney function, eGFR and urine albumin creatinine ratio (ACR), in T2DM patients with CKD, T2DM patients without CKD and healthy controls to evaluate the most appropriate biomarkers for the detection of CKD occurrence and severity in T2DM patients.

Materials and methods

Study design and population

This cross-sectional study was conducted among 93 participants grouped into healthy controls (n = 25), T2DM patients without CKD (n = 16) and T2DM patients with CKD (n = 52). The control group was composed of 25 volunteers who were undergoing an annual health checkup without a history of hypertension, diabetes, cancer, and diseases of the cardiovascular,

inflammatory, renal, lung and endocrine systems and who were not receiving any medical treatment. The diabetic participants had been cared for one year or longer at the Udon Thani Hospital, which provides tertiary care and is in the northeast of Thailand approximately 550 km from the capital city. Diabetic patients who had leukocytosis, i.e., white blood cell count > 10,000 cells/μL, and incomplete data for analyses were excluded. The study was approved by the Medical Ethics Committee of Khon Kaen University (HE621170) and conducted between January and December 2019.

A total of 68 T2DM patients were then categorized into two groups, i.e., with and without CKD based on their previous eGFR and urine ACR. CKD was defined according to the guidelines of Kidney Disease Improving Global Outcomes (KDIGO), indicating the presence of kidney damage and/or reduction in estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2 for 3 months or longer[6]. In brief, T2DM patients with ACR < 30 mg/g and eGFR > 60 mL/min/1.73 m^2 were categorized into the T2DM without CKD group (n = 16) and patients with ACR > 30 mg/g or eGFR < 60 mL/min/1.73 m^2 ≥ 3 months into the T2DM with CKD group (n = 52). The T2DM with CKD group was further categorized into five subgroups based on eGFR as CKD stage 1 (eGFR ≥ 90 mL/min/1.732 m^2), CKD stage 2 (eGFR 60-89 mL/min/1.732 m^2), CKD stage 3 (eGFR 30-59 mL/min/1.732 m^2), CKD stage 4 (eGFR 15-59 mL/min/1.732 m^2), and CKD stage 5 (eGFR < 15 mL/min/1.732 m^2). Additionally, albuminuria was classified as A1 (normal to mildly increased, ACR < 30 mg/g), A2 (moderately increased, ACR = 30-300 mg/g), and A3 (severely increased, ACR > 300 mg/g).

Collection and preparation of samples

The participants' information on sex, age, systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI) and diabetes duration were recorded with their consent. Fasting blood was collected by performing venipuncture followed by centrifugation at 3,500 rpm for 10 min at room temperature, and approximately 10 mL midstream urine was collected in a plastic container. The samples were analyzed immediately or aliquoted into sterile tubes and stored at -80 °C until analysis.

Sample analysis

The samples were analyzed for fasting blood sugar (FBS), hemoglobin A_1C (HbA_1C), serum creatinine (SCr), estimated glomerular filtration rate (eGFR), cholesterol (CHO), triglyceride (Tri), urine creatinine (UCr), urine microalbumin (UAlb), urine albumin/creatinine ratio (ACR), and uNGAL. The levels of SCr, FBS, and UAlb were measured by a fully automated chemiluminescent ARCHITECT platform (Abbott Diagnostics, Lake Forest, IL, USA), whereas HbA_1C was quantified by a G8 automated high-performance liquid chromatography (HPLC) glycohemoglobin analyzer (HLC-723G8 HPLC analyzer, Tosoh Co., Tokyo, Japan). uNGAL was measured by using an enzyme-linked immunosorbent assay (ELISA) kit (Millipore, Saint Louis, MO, USA) in 96-well plates coated with anti-human lipocalin-2. According to the protocol from the manufacturer, 100 μL of diluted NGAL-calibrator (range 4-1000 pg/mL) and diluted urine samples were added to the respective microwells followed by incubation at room temperature for 2.5 hours. Next, the wells were washed, and a biotinylated detection antibody specific for the target protein was added. After washing away unbound biotinylated antibody, horseradish peroxidase (HRP)-conjugated streptavidin was pipetted into the wells. The wells were washed again, and tetramethylbenzidine (TMB) substrate solution was added. Finally, the absorbance of the solution was measured at 450 nm [16]. All the standards and urine samples were tested in triplicate.

Statistical analysis

Statistical analysis of the data was performed using STATA version 14.2 (StataCorp, Texas, USA) and GraphPad Prism v.6 (GraphPad Software, Inc., La Jolla, CA, USA) software. Continuous variables were reported as means ± standard deviation or medians (minimum-maximum). The differences of all groups were established by one-way ANOVA or Kruskal-

Wallis test where appropriate. The differences between two groups were analyzed by unpair t-test as parametric test or Mann-Whitney U tests for nonparametric variables. The correlation was calculated using the Spearman test. A *p*-value of < 0.05 was considered statistically significant. The cut-off point, sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), and area under-ROC curves were also determined.

Results

Demographic and clinical data

The detailed demographic data and laboratory findings of the three groups are shown in Table 1. The average age of the patients in the T2DM with CKD group (59 ± 13) was higher than that in the T2DM without CKD (53 ± 12) and control (49 ± 11) groups. On the other hand, the eGFR was lower in the T2DM with CKD group (73.2 ± 32.6) than in the T2DM without CKD group (95.0 ± 19.4) and the healthy control group (99.4 ± 6.4). The ACRs in the T2DM with CKD, T2DM without CKD and control groups were 931.3 ± 2300, 13.7 ± 7.8 and 13.3 ± 8.38 mg/g Cr, respectively. The levels of uNGAL were significantly different among the three groups (*p*-value < 0.001, the *post hoc test* revealed *p*-value = 0.01 for control vs. DM without CKD groups, *p*-value < 0.001 for control vs. DM with CKD groups and *p*-value = 0.02 for DM without CKD vs. DM with CKD). The uNGAL level was approximately three times higher in the T2DM with CKD group (21.0 ± 32.6) than in the T2DM without CKD group (7.6 ± 6.4).

Table 2 shows the demographic and clinical data of the diabetic patients with various stages of CKD. The highest number of patients in the T2DM with CKD group was in stage I (42%), and the lowest average age was also in this group (50 ± 10). Levels of uNGAL gradually increased with the stages of CKD; the uNGAL levels of patients with CKD stages 1 and 2 were significantly lower than those of patients with CKD stages 4 and 5 (all *p*-value < 0.05) and significantly lower than those of patients with CKD stage 3 (*p*-value = 0.054).

Table 1 Demographic and clinical data of healthy control, T2DM without CKD and T2DM with CKD groups

Characteristic /Marker	Healthy control (n = 25)	T2DM without CKD (n = 16)	T2DM with CKD (n = 52)
Sex (Male/Female)	11/14	7/9	23/29
Age (years)*	49 ± 11	53 ± 12	59 ± 13
Systolic BP (mmHg)*	119 ± 12	132 ± 23	139 ± 17
Diastolic BP (mmHg)	76 ± 7	76 ± 15	77 ± 12
BMI (Kg/m^2)*	23.7 ± 3.3	26.3 ± 4.4	25.9 ± 4.0
Diabetes duration (years)	-	3 (range1-8)	3 (range1-8)
FBS (mg/dL)*	89 ± 9.1	188 ± 90	191 ± 76
HbA1C (%)*	5.4 ± 0.5	9.2 ± 2.4	9.4 ± 2.1
SCr (mg/dL)*	0.8 ± 0.2	0.79 ± 0.17	1.24 ± 0.96
eGFR (mL/min1.73/ m^2)*	99.4 ± 6.4	95.0 ± 19.4	73.2 ± 32.6
CHO (mg/dL)	192 ± 18	181 ± 58	203 ± 85
Tri (mg/dL)*	130 ± 46	122 ± 44	220 ± 146
UAlb (mg/dL)*	8.9 ± 4.2	16 ± 12	812 ± 1798
UCr (mg/dL)	101.0 ± 84.4	140.4 ± 90.3	108.3 ± 68.2
ACR (mg/g Cr)*	13.3 ± 8.38	13.7 ± 7.8	931.3 ± 2300
uNGAL (ng/mL), mean ± SD*	1.9 ± 2.0	7.6 ± 6.4	21.0 ± 32.6
median (min-max)*	0.9 (0-7.7)	5.1 (0.9-22.1)	8.3 (1.2-146.9)

Note: * p-value < 0.05 compared among three groups. ACR, albumin creatinine ratio; BP, blood pressure; BMI, body mass index; CHO, cholesterol; eGFR, glomerular filtration rate; FBS, fasting blood sugar; HbA1C, hemoglobin A1C; Kg, kilogram; Tri, triglyceride; SCr, serum creatinine; UAlb, urine albumin; UCr, urine creatinine; uNGAL, urinary neutrophil gelatinase-associated lipocalin; SD, standard deviation; min, minimum; max, maximum.

Table 2 Demographic and clinical data of diabetic patients with various CKD stages

Characteristic /Marker	T2DM with CKD				
	Stage1 (n =22)	Stage2 (n = 10)	Stage3 (n =13)	Stage4 (n = 5)	Stage5 (n = 2)
Sex (Male/Female)	11/11	3/7	5/8	3/2	1/1
Age (years)*	50 ± 10	63 ± 10	67 ± 13	65 ± 6	65 ± 18
Systolic BP (mmHg)	139 ± 16	142 ± 11	134 ± 17	143 ± 28	169 ± 11
Diastolic BP (mmHg)	82 ± 12	74 ± 11	74 ± 10	77 ± 16	74 ± 17
BMI (Kg/m^2)	26.7 ± 4.3	24.8 ± 4.0	26.3 ± 3.9	25.6 ± 3.5	22.0 ± 2.4
Diabetes duration (years)	4 (1-8)	3 (1-6)	3 (2-6)	2 (1-3)	3 (2-4)
FBS (mg/dL)	200 ± 78	169 ± 46	188 ± 67	165 ± 41	291 ± 231
HbA1C (%)	10.0 ± 2.3	9.0 ± 1.6	9.2 ± 1.9	7.7 ± 0.6	10 ± 5.4
SCr (mg/dL)*	0.7 ± 0.2	0.9 ± 0.1	1.3 ± 0.29	2.6 ± 1.0	4.6 ± 1.2
Egfr (mL/min1.73/ m^2)*	104.9 ± 12.6	73.0 ± 7.6	48.2 ± 5.3	24 ± 6.7	11.5 ± 4.3
CHO (mg/dL)*	206 ± 56	178 ± 60	211 ± 118	184 ± 62	401 ± 383
Tri (mg/dL)	226 ± 162	161 ± 89	244 ± 149	189 ± 79	326 ± 148
UAlb (mg/dL)*	583.3 ± 1440.7	164 ± 141	1142 ± 1598	594 ± 361	4970 ± 6949
UCr (mg/dL)	80.9 ± 42.1	135.3 ± 94.1	143.0 ± 76.4	94.3 ± 44.2	83.3 ± 12.3
ACR (mg/g)*	814.5 ± 2024.6	145.3 ± 156.3	918 ± 1393	762.6 ± 594	6651 ± 9320
uNGAL (ng/mL), mean ± SD*	9.2 ± 6.6	12.4 ± 18.6	29.5 ± 42.7	47.0 ± 37.5	73.2 ± 95.4
median (min-max)*	7.6 (1.2-26.2)	5.6 (3.0-64.5)	9.6 (1.3-146.9)	36.8 (8.4-91.7)	73.2 (5.7-140.7)

Note: * *p*-value < 0.05 compared among five groups. ACR, albumin creatinine ratio; BP, blood pressure; BMI, body mass index; CHO, cholesterol; eGFR, glomerular filtration rate; FBS, fasting blood sugar; HbA1C, hemoglobin A1C; Kg, kilogram; Tri, triglyceride; SCr, serum creatinine; UAlb, urine albumin; UCr, urine creatinine; uNGAL, urinary neutrophil gelatinase-associated lipocalin; SD, standard deviation; min, minimum; max, maximum.

The distribution of albuminuria severity in the T2DM with CKD group was 9.6%, 53.9% and 36.5% for the A1, A2 and A3 categories, respectively. The mean and median uNGAL levels in the A1 group (n = 5) were 5.9 ± 5.3 and 2.9 (1.3-14), A2 group (n = 28) were 10.9 ± 13.7 and 6.4 (1.2-70.5) and A3 group (n = 19) were 39.9 ± 46.0 and 17.1 (1.6-146.9) ng/mL. The uNGAL level in the A3 group was higher than that in the A1 and A2 groups, with statistically significant *p*-value of 0.005 and 0.015, respectively (Figure 1).

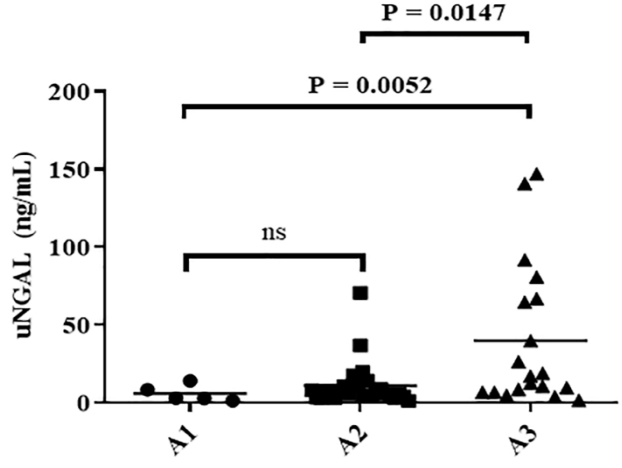

Figure 1 The comparison of uNGAL (mean ± SD) among various stages of albuminuria in T2DM with CKD subgroups.

Correlation between the uNGAL and eGFR/ACR levels in all participants

The association of the uNGAL levels with clinical parameters (eGFR or ACR) in all 93 participants is shown in Figure 2A and 2B. The analysis revealed moderate correlations between uNGAL and eGFR (r = -0.51, *p*-value < 0.001) and between uNGAL and ACR (r = 0.53, *p*-value < 0.001).

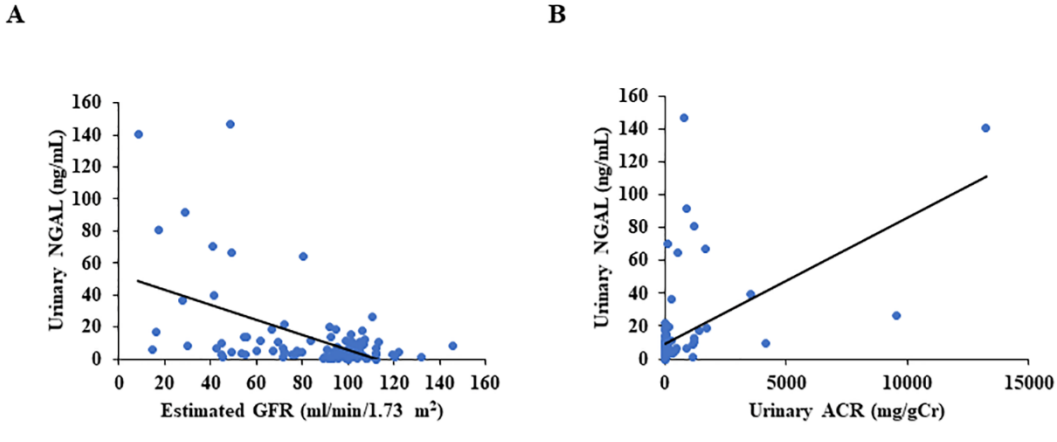

Figure 2 The correlation between (A) uNGAL and eGFR and (B) uNGAL and ACR in all participants.

Comparison of uNGAL, ACR, and eGFR biomarkers in the diagnosis of T2DM with CKD

ROC curve analysis was performed to elucidate whether the uNGAL, ACR and eGFR levels can be used to distinguish the T2DM with CKD group from the T2DM without CKD and healthy control groups (Figure 3A). The results showed that the AUC for uNGAL was 0.811; standard error (SE) = 0.045; 95% confidence interval = 0.722–0.899 with cut-off uNGAL level at ≥ 5.7 ng/mL and sensitivity = 69.23%, specificity = 80.49%, positive predictive value = 81.8% and negative predictive value = 67.3% (see Supporting Information, Table S1). The AUC for ACR was 0.952; SE = 0.022; 95% CI = 0.908–0.994. The AUC for eGFR was 0.281; SE = 0.053; 95% CI = 0.175–0.387 lower sensitivity and specificity than uNGAL.

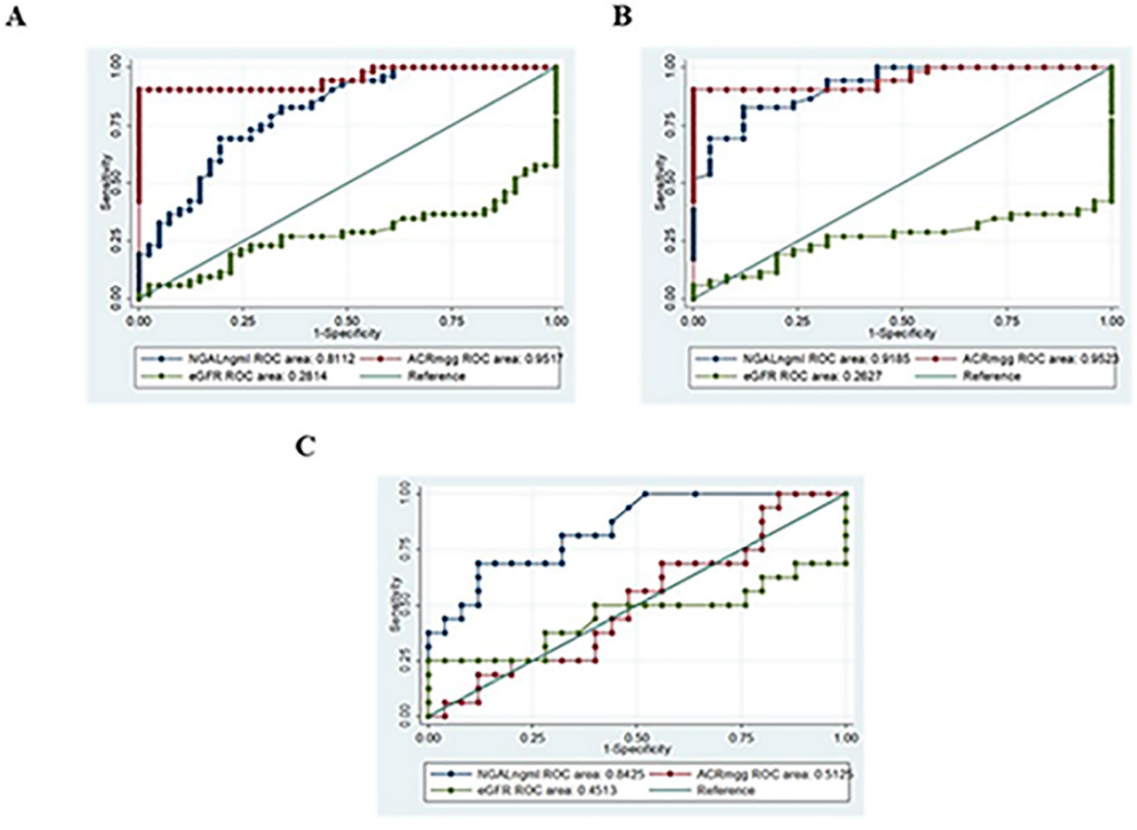

Figure 3 ROC curves of uNGAL, ACR, and eGFR (A) among the healthy control, T2DM without CKD and T2DM with CKD groups, (B) between the healthy control and T2DM with CKD groups, (C) between the healthy control and T2DM without CKD groups.

We further compared the ROC curves of uNGAL, ACR and eGFR between the healthy control and T2DM with CKD groups (Figure 3B). The AUC for uNGAL was 0.919; SE = 0.030; 95% CI = 0.857–0.979, which was comparable to the AUC for ACR (0.952; SE = 0.022; 95% CI = 0.908–0.996). The AUC for eGFR was 0.263; SE = 0.055; 95% CI = 0.153–0.372.

The comparison of the uNGAL, ACR and eGFR ROC curves between the healthy control and T2DM without CKD groups is shown in Figure 3C. The AUC for uNGAL was highest (0.842; SE = 0.060; 95% CI = 0.723–0.961). The AUC for ACR was 0.512; SE = 0.093; 95% CI = 0.328–0.696, and the AUC for eGFR was 0.451; SE = 0.109; 95% CI = 0.237–0.665.

ROC analyses of uNGAL levels in the diagnosis of various stages of T2DM with CKD

ROC analyses of uNGAL in T2DM patients with CKD (categorized according to the levels of albuminuria) demonstrated AUC (95% CI) as 0.69 (0.35-1.00) for A1 vs. A2, 0.83 (0.63-1.00) for A1 vs. A3 and 0.75 (0.48-1.00) for A1 vs A2 plus A3 (see Supporting Information, Figure S1).

Discussion

Previous studies clearly revealed that NGAL is a novel biomarker of CKD progression[13,18]. The predictive data of eGFR suggests already impaired renal function and it is an important marker for kidney damage progression. Both uNGAL and sNGAL represented a most powerful predictor even compared to adjustment of eGFR; therefore NGAL could predict CKD progression beyond the eGFR information[13]. Tubular injury may lead to glomerular damage in diabetic patients and NGAL is able to use as specific biomarker to predict DN even earlier to incipient nephropathy. Both sNGAL and uNGAL are noninvasive tools for predicting albuminuria and for diagnosis, staging, and monitoring of DN progression[17]. We proposed uNGAL as a potential biomarker of CKD progression in T2DM patients and compared it with classical kidney function markers, such as ACR and eGFR, in healthy controls and T2DM patients with or without CKD. The uNGAL level in T2DM patients with CKD was significantly higher than that in T2DM patients without CKD and normal healthy controls. Moreover, the uNGAL levels corroborated the severity of CKD based on albuminuria and eGFR in this study. A typical parallel correlation between increased uNGAL and albuminuria was observed among the patients, but the trends of uNGAL and eGFR were opposite. Although the absolute numbers may differ, these findings are similar in pattern to previous studies[17-19]. For example, a study conducted in Malaysia reported the average uNGAL levels in control subjects as 4.75 (0.1–27.5) ng/mL, the uNGAL levels in normoalbuminuria and microalbuminuria patients as 19.05 (range 1.1–60) ng/mL and 26.9 (range 3.7–603.5) ng/mL, respectively, and the uNGAL levels in DN patients as 28.55 (range 0.7–1500) ng/mL[20]. Our ROC curve analysis of uNGAL, ACR and eGFR was able to distinguish the T2DM with CKD group from the T2DM without CKD and healthy control groups. uNGAL as a marker was more sensitive (69.23%) and specific (80.49%) than eGFR but could not reach the standard of ACR.

NGAL is highly expressed in tubular renal epithelial cells following tubulointerstitial injury and is reflected in serum and urine within two hours[21] compared to serum creatinine, which is elevated after 24 hours of reperfusion[22]. Although NGAL was initially detected in acute kidney injury (AKI), it has now been used for evaluating CKD patients[23]. As such, findings suggest that uNGAL may represent a real-time indicator of renal damage and an independent predictor of renal disease progression in specific kidney diseases, such as glomerulonephritides[19]. uNGAL has also been shown to be an early biomarker for the degree of chronic tubule-interstitial injury in patients with IgA nephropathy[24]. Of importance, NGAL might also be elevated in other conditions, not necessarily pertaining only to renal injury[15]. Therefore, we excluded patients with infection, neoplasia, and inflammation from the study.

Herein, we found that some of the patients had normal albuminuria, but the uNGAL level was already significantly increased compared to that in the normal healthy controls. Our results corroborate earlier published reports[25,26]. This suggested that some kidney injury may have already occurred in the patients, which can be detected by measuring uNGAL but not albuminuria. The NGAL level is also known to increase in acute and severe pancreatitis in animal studies[27]. A high level of NGAL in pancreatic fluid was reported in a study in humans with chronic pancreatitis[28]. Therefore, there is an equal possibility that high uNGAL in diabetic patients may be due to pancreatic injury, inflammation or even silent kidney disease. NGAL is an acute phase protein, similar to C-reactive protein, that is produced during inflammation but mainly with transportation and bacteriostatic function.

A correlation between uNGAL and hyperlipidemia was revealed in the study, which may be related to metabolic syndrome. It has

been suggested that NGAL plays a significant role in both glucose and lipid metabolism[29]. Moreover, the NGAL concentration is reported to correlate linearly with obesity, hyperglycemia, and hyperglycemia in patients with metabolic and cardiovascular diseases[30]. An association between uNGAL and lipidemia in the study patients may thus be linked to the fact that NGAL antagonizes the insulin resistance-enhancing effects of tumor necrosis factor α in adipocytes and macrophages to protect against inflammation.

Previously, urinary and serum NGAL were reported to predict CKD progression independent of age and GFR[13]. A published report mentioned that the increase in NGAL in CKD is the consequence of sustained production by inflamed tubular cells, whereas the contraction of GFR is the mere passive result of a general loss of functional cells or nephrons. Importantly, our finding has implications for using uNGAL as a marker of CKD progression in patients with normal albuminuria or microalbuminuria when eGFR is still preserved above 60 mL/min/1.73 m^2. Finally, the present study has some limitations. This was a single-center study with relatively small numbers of patients. Additionally, the design of this research was cross-sectional, which limited the observations on change over time. A multicenter follow-up study may be essential for more detailed and precise findings.

Conclusion

The present study points out that uNGAL was more sensitive as a biomarker to predict CKD progression in T2DM patients compared to than eGFR but less profound than ACR. Our findings can be vital in an early diagnosis of CKD advancement especially in patients with preserved eGFR and normal or microalbuminuria. It can thus aid in timely treatment and proper management behavior to prevent CKD progression to ESRD.

Take home messages

The present study points out that uNGAL was more sensitive as a biomarker to predict CKD progression in T2DM patients compared to eGFR but less profound than ACR. Our findings can be vital in an early diagnosis of CKD advancement especially in patients with preserved eGFR and normal or micro albuminuria.

Conflicts of interest

The authors declare that there is no conflict of interest.

Acknowledgments

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Supplementary

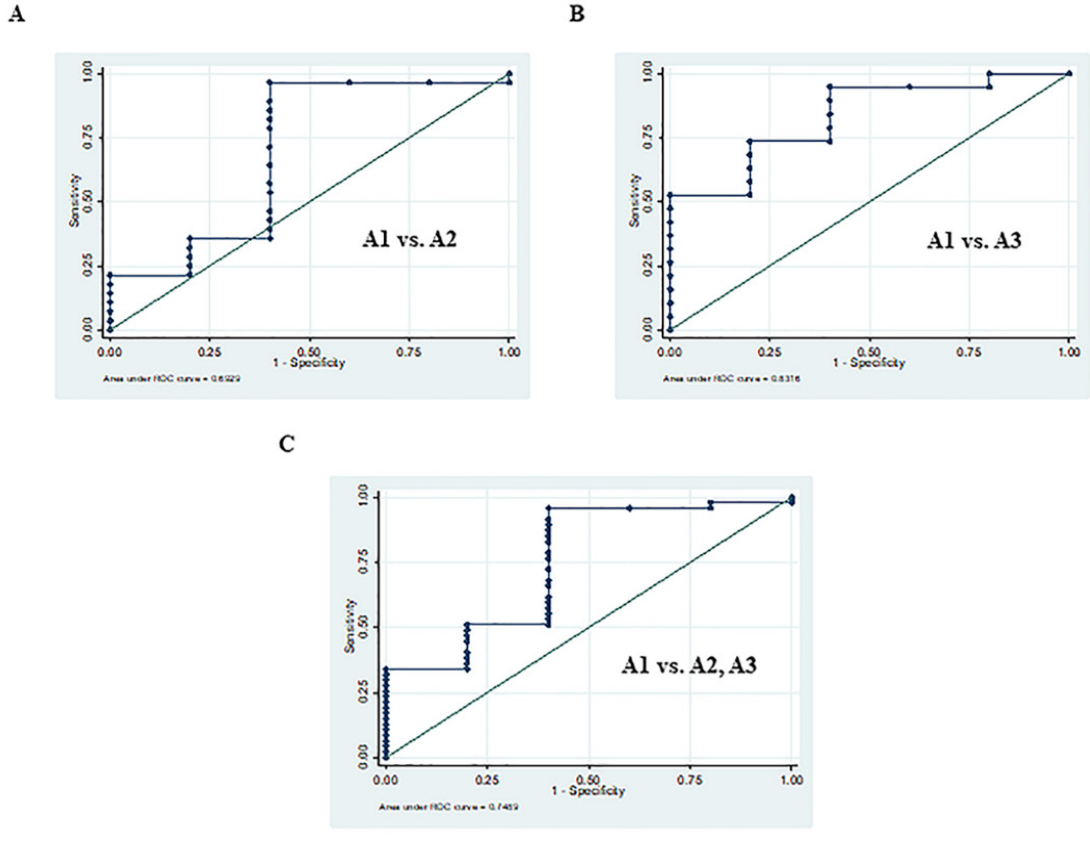

Figure S1 ROC analyses of uNGAL levels in the diagnosis of various stages of T2DM with CKD. (A) A1 vs. A2. (B) A1 vs. A3. (C) A1 vs. A2 and A3.

Table S1 Cut off uNGAL values between normal plus DM non-CKD and DM with CKD

uNGALvalue (ng/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
≤5.7	69.23	80.49	81.8	67.3	3.54	0.38

Note: PPV, positive predictive value; NPV, Negative predictive value; +LR, Positive likelihood ratio; -LR, negative likelihood ratio.

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Does the arm swing exercise benefit spatio-temporal parameters for female elderly?

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KEYWORDS
Shuai Shou Gong Training;
Elderly Females;
Stance Phase;
Swing Phase;
Stride Length.

ABSTRACT
Shuai Shou Gong (SSG) is a simple form of arm swing exercise that has been developed and applied in China for over a thousand years. It has a profound impact on maintaining physical health, especially for older people. While the spatio-temporal parameters (STP) of gait worsen in most elderly and lead to the risk of falls, the beneficial effects of SSG on these parameters of gait have not yet been verified. This study investigated the effects of SSG on the STP of gait in elderly females. Fifty-six elderly females who lived in urban communities in Khon Kaen province were recruited. They were randomly allocated into either an exercise group (EG) or a control group (CG). The EG took part in the SSG training program for eight weeks (40 minutes per day and three days per week). The CG maintained their daily life without any exercise during the same period. ANCOVA analysis revealed that SSG produced significant improvements in stance phase (left value and right value), swing phase (left value and right value), first double support phase (left value and right value), single support phase (left value), and stride length (left value and right value) in the EG compared to the CG (p-value < 0.05). Therefore, SSG could improve some temporal and spatial parameters of gait in elderly females.

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Introduction

Human gait relies on a complex interplay of crucial parts of the nervous, musculoskeletal, and cardiopulmonary systems. Spatio-temporal parameters control a person's mode of ambulation or locomotion, which involves movements of the whole body; a particular kind of gait is a specific way of walking[1]. The gait of the elderly is characterized by the strength of the back-foot decreasing as they walk, leading to a shorter step length, which relates to a lower walking speed[2]. These phenomena lead to more gait problems when the elderly are walking. The strength of the back foot in the beginning is less, which leads to a shorter step size. Shorter steps lead to a slower pace and longer feet support time. Shorter steps lead to more steps on the same road[3].

Among the elderly, the stride length decreases, the cycle time increases, and the walking base increases; the duration of the stance phase increases as a percentage of the gait cycle; stride length divided by cycle time almost always decreases[4]. The fear of falling affects the gait spatial-temporal parameters of the elderly; the gait will be slower, the stride length will be shorter, so the standing support time of the limbs is prolonged[5].

Kinematic gait analysis involves the description of gait components. It deals with motion, not dynamics, which studies the forces acting on objects. Therefore, we can use distance (space) and time parameters to analyze the STP of gait[5]. When scholars describe the kinematic characteristics of gait of the elderly, they usually include gait period, support phase, step length, stride length, gait cycle duration, and walking speed. Among scholars, the walking speed index has been widely used[6]. Older adults appear to have poor gait performance due to general muscle weakness, joint stiffness, and poor dynamic balance. The prevalence of gait abnormalities is 35% among people 70 years and older who live in the community[7]. Compared with younger adults, older adults walk slower, with a reduced stride length and an increased stance width[8]. A faster pace can be found in some elderly peoples' walk, because they might take more steps on the same path. Not only age itself, but also pathological conditions can affect gait in the elderly. Osteoarthritis and Parkinson's disease become more commonly found with age, which results in a shorter stride length. Typically, age-related gait changes occur between the ages of 60 and 70, where the stride length decreases, the cycle time increases, and the walking base increases[5].

The gait of older adults is characterized by the upper torso swinging back and forth and the body tilting slightly forward. Standing sway increases with age, after which the postural reflexes become sluggish. It could be that older people are more dependent on accurate feedback data to keep their balance. In addition, it has been observed that older adults decrease in the strength of the hindfoot during walking, resulting in a shorter stride length[9]. Therefore, any exercise mode that improves muscle strength of the leg and trunk might lead to improvement of gait parameters and to less risk of falls.

Shuai Shou Gong (SSG) is a simple form of arm swinging exercise that has been developed and applied by Chinese people. It is easy to practice individually and as a group exercise in a community. Typically, SSG is done by swinging the arms rhythmically in a standing position with straight legs alternating with knee bending. Based on the principles of training, SSG could result in increased muscle strength and balance control of lower limbs and trunk simultaneously. Several studies have found positive effects of SSG, including increased range of shoulder motion and reduced forward head posture in adults[10], improved exercise capacity and peak oxygen consumption in the overweight and normal weight sedentary young adults[11], glycaemic control of type 2 diabetes subjects[12], cognitive performance in older women with mild cognitive impairment[13], cardiac autonomic function in patients with chronic obstructive pulmonary disease[14], and reduced waist circumference with obesity participants[15]. However, the effects of SSG on the STP of elderly females in the community have not been verified. Since these components of physical capabilities could contribute to normal gait in humans, it is therefore hypothesized that SSG would have a positive effect on STP gait parameters.

Materials and methods

An experimental design was conducted in a subdistrict of Khon Kaen province, Thailand. The researcher selected two communities in Khon Kaen province based on similar age group and Barthel activities of daily living index (BADLI). Then the two communities were randomly assigned to an exercise group and a control group. This study was approved by the Research Ethics Committee of Khon Kaen University, Thailand (HE612355). The study is registered with the Clinical Trials Registry of Thailand (TCTR20200709001).

Sample

The sample size was calculated by the following formula[16].

$$\text{n/group} = \frac{2\sigma^2(z_\alpha + z_\beta)^2\,(1-\rho^2)}{(\mu 1 - \mu 2)^2}$$

Since the current study was a part of the main study that verifies the effects of SSG on standing posture, the sample size calculation was based on data from a previous study using occiput-wall distance as a major outcome measure. Thus, $\mu 1$ = 6.0 cm.; $\mu 2$ = 7.88 cm.; α = 0.05 ($Z_{0.05}$ = 1.645) and β power was set to 80%, ß=0.2 ($Z_{0.2}$ = 0.842)[17]. The dropout rate was set at 20%, because the intervention period was eight weeks, which is a relatively long time. The sample size was 28 per group, and the total sample size of this experiment was 56.

The inclusion criteria for participants were being older women, between 60 and 80 years old, who lived in Khon Kaen province during the study period and who could communicate in Thai. The participants' mental state scores, tested by the Thai version of the Mini-Mental State Examination, were between 10-24 points[18]. Moreover, the participants had to be able to walk independently and as indicated by the Barthel Activity Daily Living Index, BADL, with a score of 75 or more [19]. The exclusion criteria were people who had a history of recent severe joint pain or injury, history of related diseases affecting the movement system, history of significant injury due to a fall in the last year, were smokers or drinkers and had regular physical exercise during the past six months. The termination criteria were as follows: (1) death, (2) one or more missing interventions, (3) experienced unexpected conditions during the intervention, such as severe illness or injury, (4) unable to complete data measurement or comply with the requirements, and (5) required to withdraw.

Data collection

Participants were recruited through a public announcement. Ninety-two elderly females were recruited in this study, but 56 passed the criteria. The flow diagram of this study is shown in Figure 1. All participants gave written informed consent prior to participation. The STP were measured at baseline, day 1, week 4 and week 8.

Intervention

The fifty-six participants were randomly allocated into a Shuai Shou Gong exercise group (EG) and a control group (CG). The participants in the EG attended a supervised SSG training for eight weeks. The training frequency was three days per week, while each training session was 40 minutes per day[20]. Two trainers and six volunteers took part in every training program. The participants' heart rate of the EG was controlled at 40-50% of their age adjusted maximum heart rate. The metronome and music were used to control the rhythm of the movement of arm swing during the exercise. The participants could stop for a while if they felt uncomfortable during the exercise, after which they could resume the exercise program when they were recovered.

During SSG steps, the participants stood with their feet a shoulder width apart. The arms were actively raised to shoulder height with comfortably straightened fingers. While their trunk and neck were kept upright, their arms swung back and forth naturally following the preset tempo of the metronome, to follow the gradual principle of training. The metronome and music were set so that participants performed 15 arm swings per minute during the first two weeks of SSG training, 20 arm swings per minute during weeks 3 to 4, and 25 arm swings per minute

during weeks 5 to 8. There was a two-minute break halfway through the 30 minutes of the SSG. The participants were asked to breathe through their noses; they breathed in during the upswing and breathed out on the downswing. Each set of SSG consisted of five arm swings. The action of the 1st to 4th swing were performed with knee straight, followed by knee bending and dipping down twice at the 5th swing[10] (Figure 2).

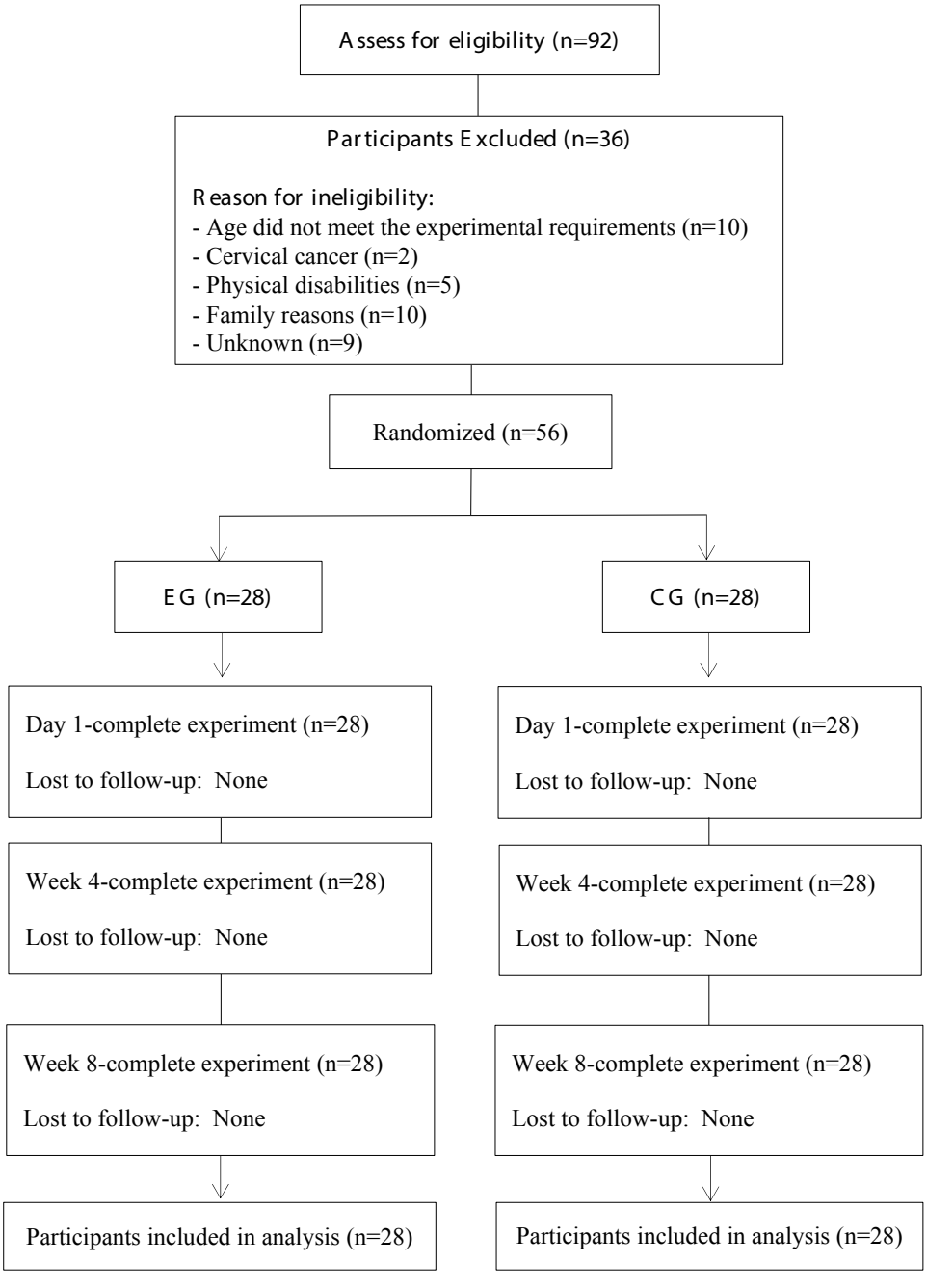

Figure 1 Flow diagram of the study
Note: EG, exercise group; CG, control group.

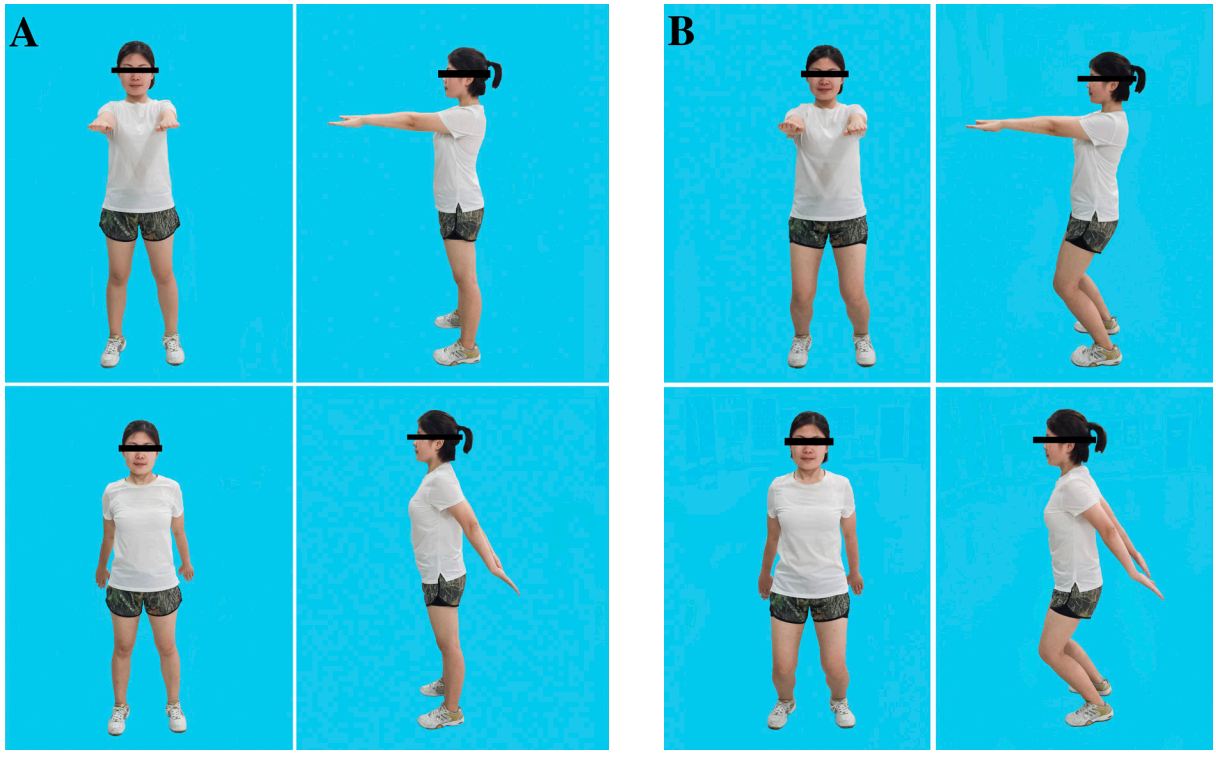

Figure 2 The movement postures of the Shuai Shou Gong exercise (SSG). A single set of SSG performance consists of the first four swings with knees extended (A) in the standing position and the fifth swing (B) with slightly bent knees.

Twenty-eight participants in the CG were asked to maintain their regular life and did not participate in the SSG training and other exercise classes. All participants in this group were offered the SSG training at the end of the experiment. The SSG program for CG was performed with a similar duration and frequency as EG carried out.

Outcome measurements

In the RCT, the immediate and longer-term benefits of SSG were studied. The following gait parameters were measured: stance phase, swing phase, first double support phase, single support phase, stride length, % stride length, and step length for both groups at baseline (seven days before the first session of SSG), immediately after the first session of SSG or no SSG (of the CG) on day 1, after 4 weeks and after 8 weeks. The CG also maintained the same measurement period, but they did not undergo SSG training during the 8-week intervention cycle. STP was measured and recorded by the G-walk (BTS Bioengineering Corporation, Quincy, Massachusetts, USA) gait analysis system. The G-walk is a wearable device embedded with an accelerometer and a gyrometer attached to a special belt around the pelvis at the sacrum level. It has been found reliable for measurement of the spatial-temporal gait parameters with excellent concurrent validity (ICC values ranging from 0.85 to 0.99)[21]. The participants kept a preferred walking speed on a 20-meter track. The movement information was collected by the sensor and sent to a computer via Bluetooth. At the end of each measurement, G-walk software displayed an automatic report which contained all the parameters recorded during the walk.

Statistical analysis

The continuous variables were reported as mean ± standard deviation (SD). To compare the differences for each variable between the two groups, the analysis of covariance (ANCOVA) was applied. One-Way Repeated-Measures ANOVA was used for within group comparison. The differences

between EG and CG in the 95% confidence interval were calculated. The researcher used the Statistical Package for the Social Sciences Software for statistical analysis (version 26.0 IBM, Armonk, NY, USA licensed for Khon Kaen University), *p*-value < 0.05 was considered statistically significant.

Results

Demographic information of participants Ninety-two participants were screened for eligibility in this study. There were 56 participants who met the criteria. The participants' characteristic data included average age, body mass index (BMI), blood pressure, Barthel activities of daily living index (BADLI), and Rosenberg self-esteem scale (RSES) as shown in Table 1. There was no significant difference in participants' characteristic data between the EG and the CG.

Table 1 Baseline demographic information for participants in the exercise group (EG) and control group (CG)

Characteristics	EG (n=28) (Mean ± SD)	CG (n=28) (Mean ± SD)	Total (n=56) (Mean ± SD)
Age (years)	68.3 ± 5.6	69.4 ± 4.4	68.8 ± 5.0
BMI (kg/m^2)	26.1 ± 3.8	25.1 ± 2.6	25.6 ± 3.2
Blood Pressure			
Systolic blood pressure (mmHg)	122.1 ± 22.6	134.6 ± 14.4	128.4 ± 18.5
Diastolic blood pressure (mmHg)	67.6 ± 12.6	66.9 ± 9.1	67.3 ± 10.9
BADLI (score)	96.00 ± 3.8	96.79 ± 2.8	96.39 ± 3.3
RSES (score)	25.43 ± 2.8	24.89 ± 2.3	25.01 ± 5.2

Note: EG, exercise group; CG, control group; BADLI, Barthel activities of daily living index; RSES, Rosenberg self-esteem scale.

The results for gait spatial-temporal parameters (STP) in the EG and the CG are presented in Tables 2 and 3, and Figure 2. Two-way repeated ANOVA results showed that first double support phase left value (DSLV), first double support phase right value (DSRV), single support phase left value (SSLV), stride length left value (SLLV), and stride length right value (SLRV) changed significantly for within-group comparison in the EG (Table 2).

For the between-group comparison (Table 3), ANCOVA revealed that STPLV and STPRV decreased significantly in the EG relative to the CG after completion of the 8-week SSG training. On the contrary, SWPLV and SWPRV were significantly increased in the EG relative to the CG after completion of the 8-week activity.

DSLV and DSRV decreased significantly in the EG relative to the CG after completion of the eight weeks of SSG. SSLV and SSRV were significantly decreased in EG relative to CG. However, there was no between-group difference for %SLLV, %SLRV, SLHLV and SLHRV parameters.

Table 2 Within-group comparison using two-way repeated ANOVA of outcome measures at all assessment time points

Outcome	Group (EG=28) (CG=28)	Baseline (Mean ± SD)	Day 1 (Mean ± SD)	Week 4 (Mean ± SD)	Week 8 (Mean ± SD)
STPLV (Cycle %)	EG	62.17 ± 2.11	61.44 ± 1.84	62.02 ± 1.85	61.78 ± 1.99
	CG	60.91 ± 2.17	60.68 ± 1.97	61.67 ± 1.60	62.73 ± 1.91*
STPRV (Cycle %)	EG	60.93 ± 1.81	61.07 ± 1.55	61.02 ± 2.22	60.99 ± 2.37
	CG	60.65 ± 1.62	61.39 ± 1.48	61.39 ± 1.34	62.65 ± 2.12*
SWPLV (Cycle %)	EG	37.83 ± 2.11	38.42 ± 1.76	37.92 ± 1.43	38.19 ± 1.97
	CG	39.19 ± 2.25	38.50 ± 1.41	37.77 ± 2.01	37.74 ± 1.69
SWPRV (Cycle %)	EG	39.07 ± 1.81	38.72 ± 2.25	38.04 ± 2.20	39.11 ± 2.07
	CG	39.06 ± 1.62	38.24 ± 1.91	38.36 ± 1.60	37.71 ± 2.19*
DSLV (Cycle %)	EG	11.49 ± 2.85	11.54 ± 1.86	12.16 ± 1.72	12.91 ± 1.37*
	CG	11.40 ± 1.91	11.22 ± 2.34	11.69 ± 2.96	10.80 ± 1.50
DSRV (Cycle %)	EG	10.65 ± 1.67	10.70 ± 1.34	12.14 ± 1.30	12.63 ± 1.50*
	CG	11.73 ± 1.58	11.57 ± 1.55	11.46 ± 2.84	11.72 ± 1.67
SSLV (Cycle %)	EG	39.06 ± 1.55	38.00 ± 2.08	38.20 ± 1.75*	37.53 ± 2.27*
	CG	39.07 ± 1.82	38.62 ± 2.21	38.82 ± 2.62	38.81 ± 1.84
SSRV (Cycle %)	EG	38.95 ± 2.22	39.09 ± 2.06	37.27 ± 2.17*	37.99 ± 1.87
	CG	37.79 ± 2.07	38.45 ± 1.93	37.91 ± 1.79	38.10 ± 1.90
SLLV (m)	EG	1.12 ± 0.13	1.12 ± 0.13	1.16 ± 0.14	1.27 ± 0.14*
	CG	1.12 ± 0.09	1.12 ± 0.10	1.11 ± 0.10	1.10 ± 0.10
SLRV (m)	EG	1.09 ± 0.11	1.10 ± 0.13	1.11 ± 0.13	1.25 ± 0.16*
	CG	1.12 ± 0.09	1.12 ± 0.10	1.11 ± 0.10	1.12 ± 0.11
%SLLV (% height)	EG	74.85 ± 9.68	73.15 ± 8.48	72.36 ± 8.33	72.87 ± 8.83
	CG	73.96 ± 5.30	73.88 ± 5.28	73.75 ± 5.39	73.79 ± 5.97
%SLRV (% height)	EG	74.25 ± 9.04	73.11 ± 8.25	72.24 ± 8.43	72.60 ± 9.16
	CG	73.96 ± 5.30	73.90 ± 5.28	73.29 ± 5.44	73.66 ± 6.13
SLHLV (% str height)	EG	49.89 ± 2.32	49.29 ± 2.21	49.51 ± 2.62	49.83 ± 2.55
	CG	49.47 ± 2.28	49.63 ± 2.62	49.54 ± 2.11	49.12 ± 2.09
SLHRV (% str height)	EG	50.48 ± 1.91	50.72 ± 2.21	50.49 ± 2.62	50.25 ± 2.51
	CG	49.47 ± 2.28	50.37 ± 2.62	50.21 ± 2.27	50.88 ± 2.09

Note: EG, exercise group; CG, control group; ANCOVA, Analysis of Covariance; STPLV, stance phase left value; STPRV, stance phase right value; SWPLV, swing phase left value; SWPRV, swing phase right value; DSLV, first double support phase left value; DSRV, first double support phase right value; SSLV, single support phase left value; SSRV, single support phase right value; SLLV, stride length left value; SLRV, stride length right value; %SLLV, % stride length left value; %SLRV, % stride length right value; SLHLV, step length left value; SLHRV, step length right value. * Indicates statistically significant difference within the groups (p-value < 0.05).

Table 3 Between-group comparison of adjusted mean and 95% CI of outcome measures (adjusted for baseline using ANCOVA) at each of the assessment time points

Outcome	Group (EG=28) (CG=28)	Adjusted Baseline (Mean)	Mean	Day 1 Difference (95%CI)	Mean	Week 4 Difference (95% CI)	Mean	Week 8 Difference (95% CI)
STPLV (Cycle %)	EG	61.54	61.20	0.28 (95%CI -0.69 to 1.26)	61.85	0.01 (95%CI -0.91 to 0.93)	61.56	-1.39 (95%CI -2.41 to -0.38)*
	CG	61.54	60.92		61.84		62.95	
STPRV (Cycle %)	EG	60.79	61.01	-0.44 (95%CI -1.18 to 0.30)	60.97	-0.46 (95%CI -1.41 to 0.49)	60.93	-1.79 (95%CI -2.93 to -0.65)*
	CG	60.79	61.45		61.43		62.72	
SWPLV (Cycle %)	EG	38.51	38.68	0.43 (95%CI -0.34 to 1.20)	38.22	0.75 (95%CI -0.08 to 1.57)	38.38	0.83 (95%CI -0.15 to 1.81)*
	CG	38.51	38.24		37.48		37.55	
SWPRV (Cycle %)	EG	39.07	38.72	0.47 (95%CI -0.53 to 1.47)	38.04	-0.32 (95%CI -1.24 to 0.60)	39.11	1.40 (95%CI 0.32 to 2.48)*
	CG	39.07	38.25		38.36		37.71	
DSLV (Cycle %)	EG	11.45	11.54	0.31 (95%CI -0.82 to 1.44)	12.15	0.45 (95%CI -0.84 to 1.74)	12.90	2.09 (95%CI 1.35 to 2.83)*
	CG	11.45	11.23		11.70		10.81	
DSRV (Cycle %)	EG	11.19	10.88	-0.52 (95%CI -1.29 to 0.26)	12.27	0.93 (95%CI -0.31 to 2.17)	12.73	1.11 (95%CI 0.22 to 2.0)*
	CG	11.19	11.39		11.34		11.62	
SSLV (Cycle %)	EG	39.06	38.00	-0.61 (95%CI -1.70 to 0.47)	38.20	-0.61 (95%CI -1.57 to 0.35)	37.53	-1.27 (95%CI -2.33 to -0.21)*
	CG	39.06	38.62		38.81		38.81	
SSRV (Cycle %)	EG	38.37	38.86	0.19 (95%CI -0.83 to 1.20)	36.95	-1.29 (95%CI -2.18 to -0.40)*	37.84	-0.42 (95%CI -1.43 to 0.59)
	CG	38.37	38.68		38.23		38.25	
SLLV (m)	EG	1.12	1.12	0.01 (95%CI -0.04 to 0.43)	1.16	0.05 (95%CI 0.00 to 0.10)	1.28	0.17 (95%CI 0.10 to 0.23)*
	CG	1.12	1.12		1.12		1.11	
SLRV (m)	EG	1.10	1.12	0.01 (95%CI -0.03 to 0.05)	1.12	0.02 (95%CI -0.03 to 0.07)	1.26	0.13 (95%CI 0.06 to 0.21)*
	CG	1.10	1.11		1.10		1.13	
%SLLV (% height)	EG	74.40	72.88	-1.28 (95%CI -4.05 to1.49)	72.11	-1.90 (95%CI -4.82 to 1.03)	72.60	-1.46 (95%CI -4.65 to 1.73)
	CG	74.40	74.15		74.00		74.06	
%SLRV (% height)	EG	74.11	73.01	-0.99 (95%CI -3.71 to 1.74)	72.15	-1.24 (95%CI -4.06 to 1.58)	72.51	-1.26 (95%CI -4.44 to 1.92)
	CG	74.11	74.00		73.39		73.77	
SLHLV (% str height)	EG	49.68	49.13	-0.65 (95%CI -1.57 to 0.27)	48.82	-0.91 (95%CI -2.20 to 0.38)	49.67	0.40 (95%CI -0.47 to 1.27)
	CG	49.68	49.78		49.73		49.28	
SLHRV (% str height)	EG	49.97	50.81	0.52 (95%CI -0.81 to 1.85)	50.44	0.19 (95%CI -1.17 to1.55)	50.23	-0.66 (95%CI -1.94 to 0.63)
	CG	49.97	50.29		50.25		50.89	

Note: EG, exercise group; CG, control group; ANCOVA, Analysis of Covariance; STPLV, stance phase left value; STPRV, stance phase right value; SWPLV, swing phase left value; SWPRV, swing phase right value; DSLV, first double support phase left value; DSRV, first double support phase right value; SSLV, single support phase left value; SSRV, single support phase right value; SLLV, stride length left value; SLRV, stride length right value; %SLLV, % stride length left value; %SLRV, % stride length right value; SLHLV, step length left value; SLHRV, step length right value. * Indicates statistically significant difference between the groups (p-value < 0.05).

Discussion

The study aimed to verify the effects of SSG on spatio-temporal parameters of gait in healthy elderly females. The main findings of the study using ANCOVA revealed the 8-week SSG training could significantly change STPLV, STPRV, SWPLV, SWPRV, DSLV, DSRV, SSLV, SLLV, and SLRV in the EG compared to the CG for between-group comparison. The improved STP demonstrated by the exercisers at the completion of the trial indicated the SSG training program could improve the age-related decline in the functional gait of older women. Therefore, the SSG training program might have an appropriate nature, adequate duration, and exercise intensity sufficient to produce considerable STP improvements.

The participants in the EG group showed a significant decrease in the STPLV and STPRV, but not those in the CG group. The increment of the stance phase accompanied a decrease of SWPLV and SWPRV, indicating that the CG participants walked slower, as is normal with reduced stability following aging. On the contrary, the decreased STPLV and STPRV in combination with increased SWPLV and SWPRL in the EG after completion of the 8-week SSG training suggest that they walked faster, which could be due to improved muscle strength and joint mobility[22]. The SSG exercise may also reflect a coordinated movement involving the hamstrings and quadriceps, which may increase the strength and stability of these muscles. These results are consistent with the report by Shigematsu et al. that a combination of aerobic dance and balance exercises can improve lower limb muscle strength, one-leg balance, and functional stretching in adults[23]. A meta-analysis also reported that therapeutic exercise generally improves habitual gait markers in older adults in a community study and found that progressive resistance training improves strength and balance. Quadriceps muscle strength plays a vital role in standing, walking, squatting, and other functional activities. The simple task of standing from a sitting position was affected by quadriceps strength such that active older adults need less time to complete activities from sit to stand[24]. The combination of aerobic dance and balance training is also very effective for improving muscle strength, as it always involves movements similar to those in SSG such as standing on one leg, squatting, and walking with heel contact[25].

The SSG increases in the first double support phase, but not in the single support phase because these changes may represent an adaptation to changes in the sensory or motor system to produce a safer and more stable gait pattern[26]. Individuals with greater stability should have longer first double support phase and single support phase. This improvement is always accompanied by a reduction in stance duration and increase of swing time. During SSG, the body was in a straight line, head and shoulders back, chest forward, feet shoulder width apart, and this position allowed the entire body to be activated; it may improve the elderly core muscle control, lower limb muscle strength, effectively promoting their core strength, core endurance, flexibility, and mobilizing the spinal joints[27].

The body's trunk is at its lowest vertical position, and its highest forward speed during the double support phase; forward speed is also at its highest in the first half of the single support phase. The trunk is lifted by the supporting legs, converting some kinetic energy into potential energy when it slows down. During the later single support phase, the trunk drops again in front of the supporting leg, and with the lower altitude simultaneously speeds up again[5]. The first double support phase is kept as a stable interval, which can promote the body's speed moving forward, and the trunk can generate more forward kinetic energy.

It has been seen that the 8-week SSG training increases participant's stride length significantly in the present study. The average range of stride length in normal healthy older adults is 1.22-1.84m (50-64 years) and 1.11-1.71m (65-80 years)[5]. Increased stride length is associated with improved hip extension strength and hip flexion strength[28]. Heels rise less during front swing and at the initial contact, the posture of the foot is closer to the horizontal direction. Both changes are associated with a decrease in stride length. The angle of the toe out in the elderly also

increases, and the posture and movements of the arms change [29].

There was no significant change in % stride length and step length. This might be due to the SSG training being performed in a standing posture rather than as a walking exercise. Therefore, it is apparently a non-specific training for the gait spatio-temporal parameters. In addition, the measurement of gait parameters in this study indicates that the participants must walk comfortably. Although their legs are strengthened, they still walk with the same speed and pattern (conservation of energy). The changes of spatio-temporal parameters might be seen if the participants are asked to walk as fast as they can. This could be due to SSG being performed in a standing position which was a non-specific training for walking[30]. No specific walking training was included in the study; therefore, it is not surprising that SSG did not show significant changes in some other STP. Should a study want to get excellent spatio-temporal parameters of gait, gait-specific training may be included. Lastly, regarding participants' characteristics, most of them were relatively healthy with a normal range of BMI and BP. The SSG could produce limited effects to them such that some STP parameters could not be significantly changed. Even for those with statistical significance, the magnitude of changes was less than 5%.

There are some limitations of the study which should be acknowledged. There was no blinded assessment in this study. This could be prone to giving bias, since the assessor could have a trend to favor positive results in either known groups. For future study, a single blind (blind the assessor) could reduce the potential bias. The results of this study could not be applied to elderly males because all the participants were females. Physiological and psychological responses could be different between the two genders. Further research could be done on the male population. Lastly, this study did not either confirm muscle strength change or muscle activity during exercise. Therefore, it is recommended to monitor muscle strength as well as perform electromyography during the SSG in a future study.

Conclusion

SSG produced significant but modest improvements in stance phase, swing phase, first double support phase, single support phase, and stride length in the EG compared to the CG. Therefore, SSG could improve some but not all spatial-temporal parameters of gait in elderly females. Further study should explore the physiological mechanism of muscle activation during SSG.

Take home messages

The Shuai Shou Gong could modestly improve some of the spatio-temporal parameters of gait in elderly females. It has provided older women with a simple and practical daily exercise for improvement of gait.

Conflicts of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

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Multiple genotype infection of human papilloma virus is associated with cervical cytological abnormalities

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KEYWORDS
Human Papilloma Virus; Cervical cancer; Liquid-based cytological test; High-risk HPV; Low-risk HPV.

ABSTRACT
Development of cervical cancer is associated with persistent infection of human papillomavirus (HPV). The present study is aimed to determine the association between HPV infection and cervical cytological abnormalities. HPV genotypes and liquid-based cytological analyses were performed in 351 cervical brush samples obtained from women visiting Gynecology Clinic, Khon Kaen Hospital, Khon Kaen, Thailand. HPV was detected in 23.36% (82/351) of the cases. Among the HPV positive case, 71.95% (59/82) was found with single infection and 28.05% (23/82) were found with multiple infections. Of 351 tested specimens, 7.6% (27/351) were presented with abnormal cytology, and 62% (17/27) of these 27 cases were found to be positive for HPV. The cytological anomalies presented in HPV positive cases were 52.94% (9/17) of ASCUS, 28.41% (5/17) of LSIL and 17.64% (3/17) of HSIL. Our data showed that cytological abnormalities were more frequently observed in patients with multiple HPV infection, compared to those with single infection (p-value < 0.001). However, among the patients with single HPV infection, cytological data were not different between patients with high-risk HPV (HR-HPV) and low-risk HPV(LR-HPV). In conclusion, our present study revealed the association between multiple HPV infection and cervical cytological abnormalities. This information emphasizes the importance of HPV genotype analysis for cervical cancer screening and surveillance. Those women with multiple HPV infection are strongly suggested to be treated and frequently followed up.

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Introduction

Utility of conventional Papanicolaou (Pap) screening test has a limitation for detecting high-grade squamous intraepithelial lesions (HSIL) of uterine cervix due to its low sensitivity and reproducibility[1]. Liquid-based cytological study has been used to improve test sensitivity and diagnostic accuracy. In addition, the collected samples were also applicable for molecular testing. Combination of human papillomavirus (HPV) genotypes with cytology has been recommended to enhance the diagnostic accuracy for screening of cervical cancer. Several genotypes of HPV have been identified and divided into low-risk HPV (LR-HPV) and high-risk HPV (HR-HPV) depending on the clinical characteristics of infection. HR-HPV covers HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82; whereas, LR-HPV includes HPV 6,11, and 81[2].

Previous reports showed that persistent HPV infection plays role in the development of cervical cancer and precancerous lesions, and 99% of cervical cancer patients are associated with HR-HPV infection[3]. HPV16 and HPV18 are the most prevalent HR-HPV genotypes of cervical cancer worldwide. However, HPV16 and/or HPV18 detection alone is insufficient for predicting the development of abnormal cytology. In contrast to the Western population, the high prevalent HR-HPV genotypes among population in East Asia are HPV52 and HPV58[4,5]. The most common genotypes among Thai women were HPV-16, HPV-58, and HPV-18 and the prevalence of HR-HPV with cervical intraepithelial neoplasia grade 2-3 (CIN2-3) ranged from 64.8% to 90.1%[6]. The identification of HR-HPV is important to early detection of the disease and for efficient prophylactic vaccines[7,8]. Due to the large number of HPV genotypes, mixed infection by different HPV genotypes is commonly found in 20%-50% of HPV infected patients. The previous studies suggested that multiple-type HPV infections are associated with the development of cervical cancer and more likely to be an initiator of carcinogenesis than single-type infections[9-11]. Co-infection of HR-HPV and LR-HPV was found to be a risk to increase disease progression and morbidity. However, this information is still controversy, some other studies showed no significant differences in the risk of cervical cancer in women with single and multiple HPV infections[12]. Many studies reported that HPV-based cervical cancer screening has greater sensitivity and negative predictive values for detection of carcinoma *in situ* and adenocarcinoma, compared with cytology-based screening[13,14]. The objective of cervical cancer screening is to detect the precursor abnormalities that lead to invasive cervical cancer or to detect the early stage of cervical cancer. The Royal Thai College of Obstetricians and Gynaecologists (RTCOG) 2021 suggests to screen women between 25-65 years with cytology every two years or co-testing (HPV testing and cytology) every 5 years[15]. In our present study, we have investigated the association between HPV genotypes either single or multiple infections and the cervical cytological changes. In addition, the prevalence of HPV infection was also calculated.

Materials and methods

Study population

A retrospective study was performed in the women who attended the Obstetrics Clinic, Out-Patient Department of Khon Kaen Hospital, Khon Kaen, Thailand, during January 2014 to December 2020. The clinical data of women who underwent detection of HPV DNA and liquid-based cervical smear cytology test (LBC) in cervical exfoliated cells were enrolled. The data were retrieved from the Hospital Information System (HIS) and Laboratory Information System (LIS). The exclusion criteria were as follows: 1) patients with a history of pelvic chemotherapy or radiotherapy, 2) patients with a history of total hysterectomy or cervical resection, 3) patients who have received cervical physical therapy, and 4) patients with a result HPV DNA or LBC only. This study was approved by Khon Kaen Hospital Institute Review Board in Human Research (KEX63007).

Liquid-based cytological test

Cervical exfoliated cell samples (n=351) were collected during January 2014 to December 2020 by cyto-brush and were analyzed using ThinPrep Pap test (Hologic Inc.,

USA.). Cytological slides were examined by two expert cytotechnologists and the results were approved by pathologist at the Department of Anatomical Pathology, Khon Kaen Hospital. The LBC results were graded, according to the 2001 Bethesda System (TBS) classification: no intraepithelial lesions or malignancy (NILM), atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), squamous carcinoma, and adenocarcinoma. The cytological grades higher than NILM were classified as positive cytological change.

HPV genotyping

HPV genotype was determined in liquid-based cytology samples by multiplex real time PCR using Anyplex™ II HPV 28 Detection (Seegene®, Seoul, Korea), according to the manufacturer's instructions. The multiplex real-time PCR for HPV allows for simultaneous detection and genotyping of 19 HR-HPV genotypes including HPV-16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82 and 9 LR-HPV genotypes including HPV-6, 11, 40, 42, 43, 44, 54, 61 and 70 in a single reaction[16].

Statistical analysis

The statistical analyses were performed in SPSS software version 27 using likelihood ratio. The *p*-value less than 0.05 were considered statistically significant.

Results

Population characteristics

All 351 cervical exfoliated cells specimens were used to examine the HPV genotyping and liquid-based cytological study by ThinPrep Pap test. The mean age of subjects recruited in our study was 46 years, minimum of 18 years and maximum of 68 years. The association between age and cytological changes was not observed (Table 1). The prevalence of HPV infection was 23.36% (82/351). The subjects with HPV infection exhibited a significantly higher proportion of abnormal cytology (*p*-value < 0.001, Table 1). The cytology characteristics of HPV positive patients were 79.2% negative for intraepithelial lesion or malignancy (NILM), 10.9% with ASCUS, 6.1% with LSIL, and 3.7% with HSIL (Table 1).

The patients with single HPV infection were presented with different cytological changes: 83.0% were with NILM, 10.2% with ASCUS, and 6.8% with LSIL. The HSIL was not observed in these patients. In single HPV-genotype infected patients, the cytological characteristics between HR-HPV and LR-HPV infection were not different. Those with HR-HPV presented 12.5% ASCUS and 7.5% LSIL and LR-HPV shown 5.3% ASCUS and 5.3% LSIL. In 23 cases with mixed-infected by multiple genotypes of HPV, the cytological abnormalities were also frequently found in this group, where 13.0%, 4.3%, and 13.0% were graded as ASCUS, LSIL and HSIL, respectively. Comparing between patients with single and multiple infections, our result showed that patients with multiple infection exhibited higher grade of cytological abnormalities (*p*-value < 0.05, Table 1).

Table 1 Association between cervical cytological grades and age and HPV infection status

	Parameter	N NILM	Cytological grading (N, %)				*p*-value*
			ASCUS	LSIL	HSIL		
Age	< 25	4	3 (75)	1 (25)	0	0	0.756
	26-35	54	46 (85.2)	5 (9.3)	1 (1.8)	2(3.7)	
	36-45	105	98 (93.3)	4 (3.8)	2 (1.9)	1 (1.0)	
	46-55	137	129 (94.2)	6 (4.4)	2 (1.4)	0	
	56-65	48	45 (93.8)	2 (4.2)	1 (2.0)	0	
	> 65	3	3 (100)	0	0	0	
HPV	Negative	269	259 (96.3)	9 (3.3)	1 (0.4)	0	< 0.001
	Positive	82	65 (79.2)	9 (10.9)	5 (6.1)	3 (3.7)	
HPV	Single infection	59	49 (83.0)	6 (10.2)	4 (6.8)	0	< 0.001
	Multiple infections	23	16 (69.5)	3 (13.0)	1 (4.3)	3 (13.0)	

Note: *Likelihood ratio, HPV=Human Papillomavirus, NILM=negative for intraepithelial lesion or malignancy, ASCUS=atypical squamous cells of undetermined significance (ASCUS), LSIL=low-grade squamous intraepithelial lesions, HSIL=high-grade squamous intraepithelial lesions.

Single and multiple infections with abnormal cytology association

Of those 82 HPV positive cases, 59 cases (16.8% of total) were presented with single infection and 23 cases (6.6% of total) had multiple infections (Figure 1). The most common single HPV genotype infection was HPV 42 and HPV 16, followed by HPV 52, 68, 39, 35, 54 and 58. The HPV16, HPV35, HPV39, HPV52 and HPV 68 accounted for 41.2% (7/17) of all cervical cytology abnormalities. Notably, HPV16 and HPV 52 were the most common type in HR-HPV with both single and multiple infections.

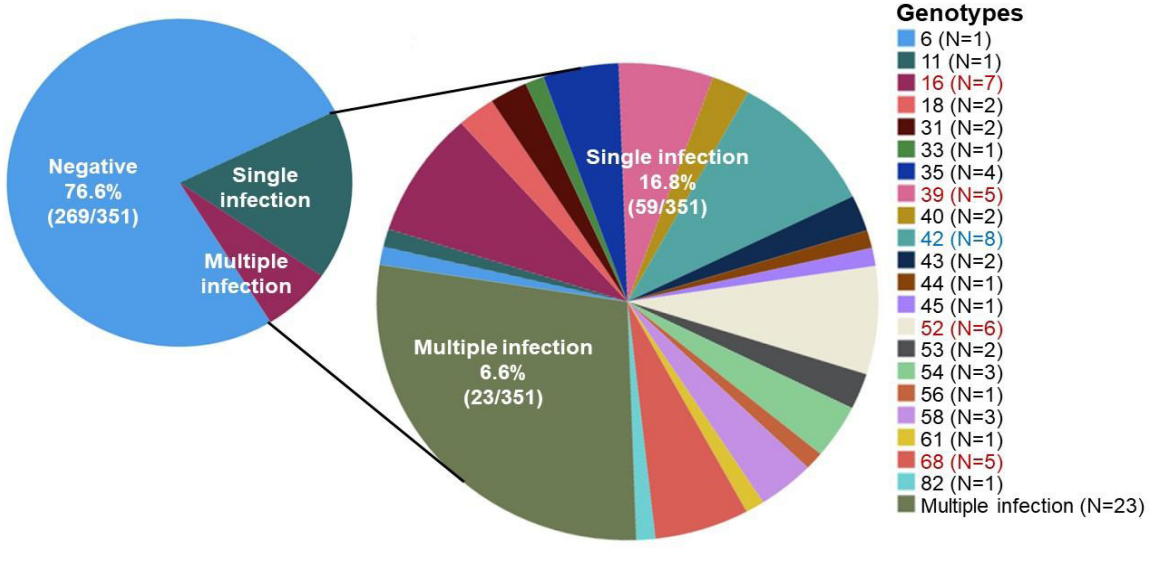

Figure 1 HPV infection status in women visited Khon Kaen Hospital, Thailand during January 2014 to December 2020.

Comparing among the patients with multiple infections, those infected with HR-HR or LR-HR were frequently presented with cytological abnormalities, comparing with LR-LR infection (Table 2, *p*-value < 0.05). However, comparing among the single-genotype infected patients, the abnormality in cytology was not different between patients with HR and LR.

Table 2 Cervical pathological grades in HPV infected patients

HPV infection	N	Cervical Pathological grading (N, %)				*p*-value*
		NILM	ASCUS	LSIL	HSIL	
Single infection						
High risk	19	17(89.0)	1(5.3)	1(5.3)	0	0.612
Low risk	40	32(80.0)	5(12.5)	3(7.5)	0	
Multiple infection						
LR and LR	2	2(100)	0	0	0	0.015
LR and HR	12	9(75)	1(8.3)	1(8.3)	1(8.3)	
HR and HR	9	5(55.6)	2(22.2)	0	2(22.2)	

Note: * Likelihood ratio, HPV=Human Papillomavirus, NILM=negative for intraepithelial lesion or malignancy, ASCUS=atypical squamous cells of undetermined significance (ASCUS), LSIL=low-grade squamous intraepithelial lesions, HSIL=high-grade squamous intraepithelial lesions, HR=high risk, LR=Low risk

Discussion

Our study presented the distribution of HPV genotypes and the association with cytological characteristics in 82 women with HPV positive in Khon Kaen, Thailand. Similar to other parts of the world, our data showed that HPV16, HPV52, HPV68, and HPV 39 were the most predominant HR-HPV genotypes among positive women. The results are in consistent with those in the previous studies performed in the same geographical area[17,18]. Among the identified HPV genotypes; HPV16, HPV52 and HPV68 were most frequently found in the samples with abnormal cytology. Also, HR-HPV genoypes with relatively high proportion in our study (i.e., HPV16, and HPV52) were consistent with the study in Asia but different from Europe, Latin America and Caribbean, Africa, and Oceania[19,20]. Multiple infections promote the development of cervical lesions and the occurrence of cervical cancer[21]. We also found that multiple HPV genotype infections increased the risk of HSIL compared with LR-HPV. The multiple infections with HR-HPV were significantly higher in women with abnormal cytology grading. It is interesting that a result of HR-HPV infection without HPV16 might be a contributor to cytological changes. Our result showed that HPV52 positivity infection seems to be associated with HSIL. This result is agreed with the previous studies in China that HPV52 infection could increase the risk of HSIL[17,22,23]. The distribution of HPV genotype and the risk of cytology abnormalities in the present study are different from those reported in the previous studies. This might be due to the variations in the study design, specimen types, and screening methods[24]. Patient's age was previously reported as one of the risks of cytological abnormalities[25]; however, our study showed the risk of HSIL was not different between the patients with different age. As a genotype analysis provided the qualitative data of HPV infection; therefore our present study could only demonstrate the association between single or multiple HPV genotype and cervical cytological changes, regardless of the infection level. Further quantitative analysis of HPV infection and its association with clinical outcomes may provide better clinical relevance. Consequently, our results suggested that the national policy for cervical cancer screening should pay attention

to the population age both of women younger and older than 46 years. Patients detected with multiple HPV infections are strongly suggested to closely followed up. Therefore, further studies either preclinical or clinical studies are required to analyze the impact of single and multiple HPV infections on development cervical diseases.

Conclusion

HPV infection is a well-established risk of cervical cancer. We have determined the occurrence of HPV in women visiting Khon Kaen Hospital for screening of cervical cancer. In the high-risk HPV, genotypes 16, 52, 68, and 39; were frequently found in the pre-cancerous lesions. Moreover, the infection with multiple genotypes of HPV was found to associated with the presentation of cytological abnormalities. This information emphasized the importance of HPV infections for cervical cancer screening method, especially in the patients infected by multiple genotypes of HPV that should be closely monitored.

Take home messages

Multiple HPV infections were more credible to a degree of cervical lesions than single infection. The multiple infection with HR-HPV was associated with cytological grading especially for HPV 16, 52, 68 and 39. The diagnosis of HR-HPV and multiple infections were important for treatment and monitoring in HPV infected women.

Conflicts of interest

The authors declare no conflict of interest.

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Immediate effect of Thai massage on timed-up-and-go test in elderly: a pilot study

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KEYWORDS
Elderly;
Thai massage;
Balance;
Pilot study.

ABSTRACT
The purpose of this study was to determine the immediate effects of Thai massage on Time-up-and-go test (TUGT) in elderly aged 60-80 years. A preliminary comparative study was used. Sixteen elderly participants (12 females and 4 males) participated. They were randomly allocated into a Thai massage group and a control group. Participants in the Thai massage group received 1-hour session of whole-body Thai massage while those in the control group were assigned to rest for one hour. TUGT were measured at the baseline and immediate after the intervention with opal sensors and APDM mobility lab software. Primary outcome from software was TUGT duration. Other outcomes from software were turn angle, turn duration, sit-to-stand duration, sit-to-stand lean angle, stand-to-sit duration, and stand-to-sit lean angle. For parametric data, dependent t-test and independent t-test were used to calculate the within-group and between-group differences, respectively. Wilcoxon signed rank test and Wilcoxon rank sum test were used to compare the outcome difference of within-group and between-group for non-parametric data, independently. A p-value < 0.05 was considered statistically significant. There were no significant differences for both within-group (0.19 s, p-value =0.15) and between-group (0.50 s, p-value =0.55) in the duration of TUGT duration and other parameters. We concluded that one session of Thai massage could not provide immediate effect to improve balance performance.

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Introduction

The elderly global population has been increased every year[1]. The health status of older people is declined by physical changes in many systems with a reduction in capabilities of physiological processes[2]. Furthermore, these physiological changes also affect the balance performances of older people[3] that are considered fall risk and affected to health problems[4].

Exercises were found to be a helpful intervention for improving balance performances of older adults by improving muscle strength, delivery and extraction of oxygen to the muscle, and joint range of motion[5]. A passive intervention like therapeutic massage was also found to improve body balance[6,7]. Theoretically, massage therapy could arouse somatosensory systems by activating the proprioceptors, Golgi tendon organ, joint receptors, and muscle spindle. It was also believed to stimulate the resetting of unproductive reflex actions[8,9]. Moreover, there are many advantages of massage, such as improved blood flow, raised skin temperature, improved tissue flexibility, diminished pain, and muscle spasm[10]. It may improve arousal or body awareness by stimulating proprioception resulting from brain activating response[8,9]. By these mechanisms, massage may have a positive effect on balance performance.

Thai massage, a type of deep therapeutic massage, has been commonly practiced for general relaxation. Practically, the massage therapist applied manual or thumb pressure on meridian lines situated along with striated muscles of limbs, back, and neck regions[11]. It has been found to provide muscle relaxation and reduce muscle pain in patients with myofascial pain syndrome[12]. A previous study also found that Thai massage improved walking performance and balanced performance in older adults using the timed-up-and-go test (TUGT)[13]. However, the immediate effect of Thai massage on subdivision component of dynamic balance has not been verified.

To determine the immediate effect of Thai massage on TUGT in older people, more details of subdivision components of TUGT would be required. The standard TUGT could provide only the sequel total time spent of sitting on a chair to standing, 3-meter walking, turning around, 3-meter walking, and sitting on the chair[14,15]. However, inertial sensor systems have played an essential role in movement analysis, especially the dynamic balance during walking[16]. The device with these sensor systems includes the tri-axial accelerometer, triaxial gyroscope, and magneto-meter signals[17]. It could provide measurement of TUGT with subdivision components analysis during the whole process of TUGT including duration of TUGT, sit-to-stand duration, sit-to-stand lean angle, turn duration, turn angle, stand-to-sit duration, and stand-to-sit lean angle. These movement components are essential for analyzing dynamic balance in patients with movement disorders and older people[18]. This preliminary study aimed to explore the immediate effects of one-hour Thai massage on TUGT using an inertial sensor system in older adults aged 60-80 years.

Materials and methods

A preliminary comparative study was conducted at Nong Waeng Community Health Center, Khon Kaen Hospital, Thailand. The study protocol was approved by the ethics committee of Center for Ethics in Human Research, Khon Kaen University, and Khon Kaen Hospital Institute Review Board in Human Research. Elderly participants were recruited from Khon Kaen province using bulletin boards and orals. Sixteen participants, aged between 60 and 80 years, who could walk independently for 10 meters or more and had good co-operation were included. They were not enrolled if they had history of brain injury, acute or unstable chronic illness, uncontrolled hypertension, communicable diseases, osteoporosis, deep vein thrombosis, acute arthritis at upper and lower limb, vestibular, neurological and cardiovascular problem, fracture and dislocation, deformities of lower extremities, pain more than 5/10 on visual analog scale, opened wound at back, upper and lower extremity,

and visual problems that could not be solved by glasses.

Sixteen older participants met the criteria and were randomly assigned to either the Thai massage group (TM group) or the control group by using block randomized allocation with block sizes of 2 and 4, which resulted in 8 participants per group. Interventions in this study were Thai massage (TM group) and supine resting (Control group). Participants in TM group received a session of one-hour Thai massage onto the whole body along the ten meridian lines by one massage therapist. Based on principles of traditional Thai massage, including thumb or palm pressure, along the meridian lines, and combined with muscle stretches at the end of the session, this protocol of Thai massage has been designed by one of the authors who has been a master of Thai massage for 30 years[20]. The details of the protocol of Thai massage in this study are shown in Figure 1-4. More specifically, Thai massage was to press by thumbs, fingers, or palms, of which the pressure was slowly applied until the participant felt mild discomfort. The pressure was temporarily sustained for 5-10 seconds and repeated five times at each point along the meridian lines[20]. Following the massage, stretching was performed. Participants in the control group rested in a supine lying position for one hour in the same environment as the TM group.

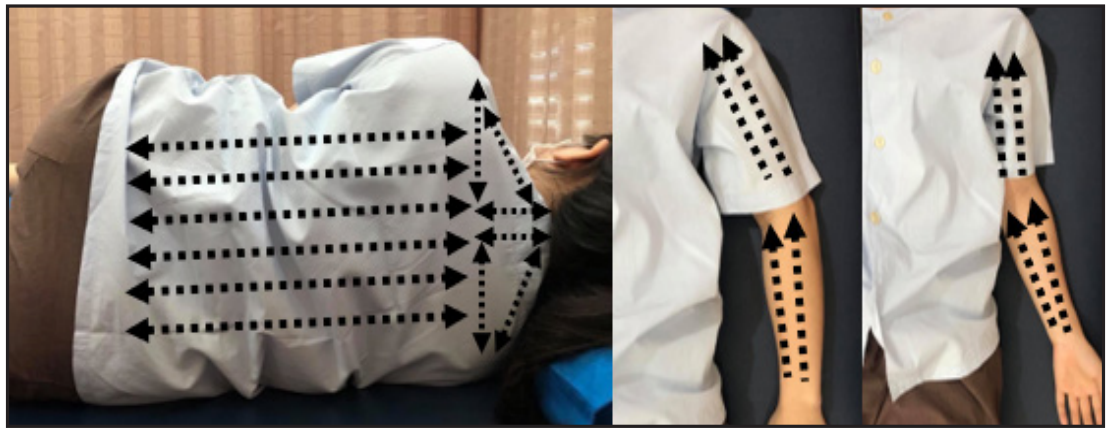

Figure 1 Back, neck, and arm massage lines.

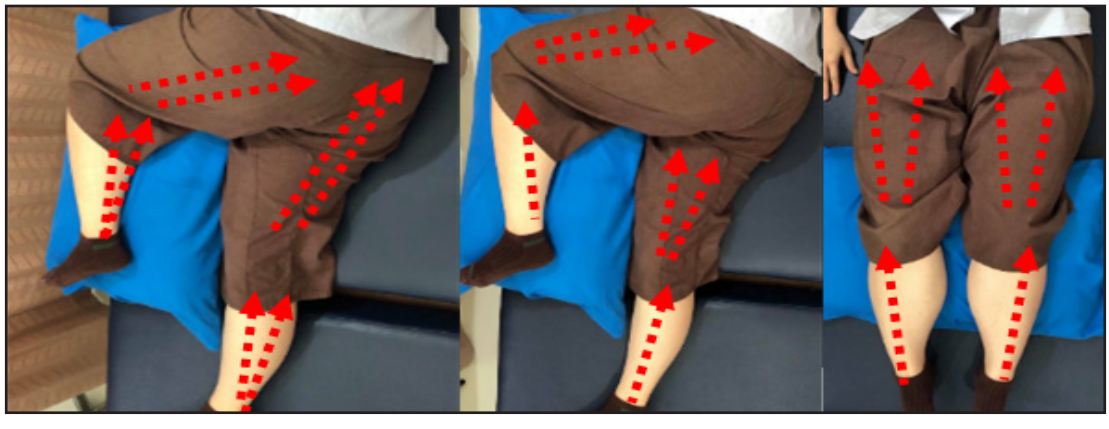

Figure 2 Leg massage lines.

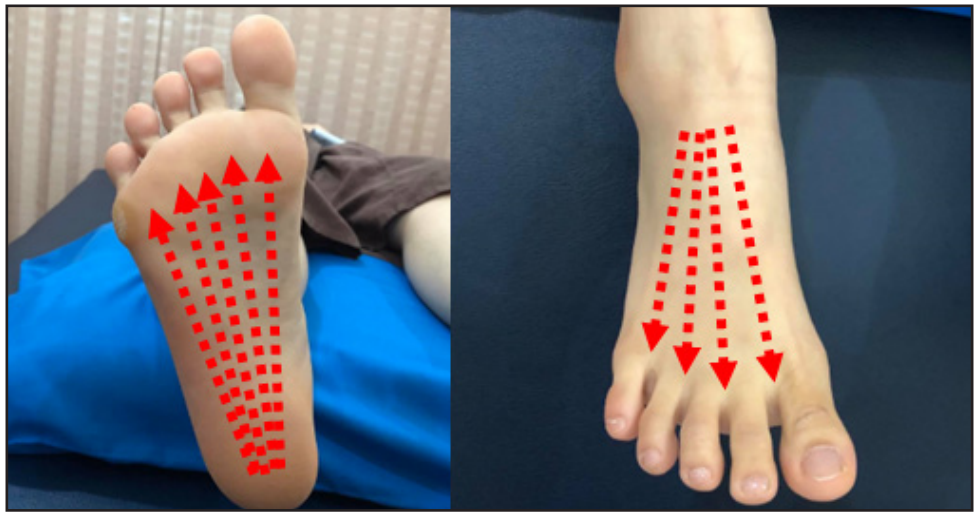

Figure 3 Foot massage lines.

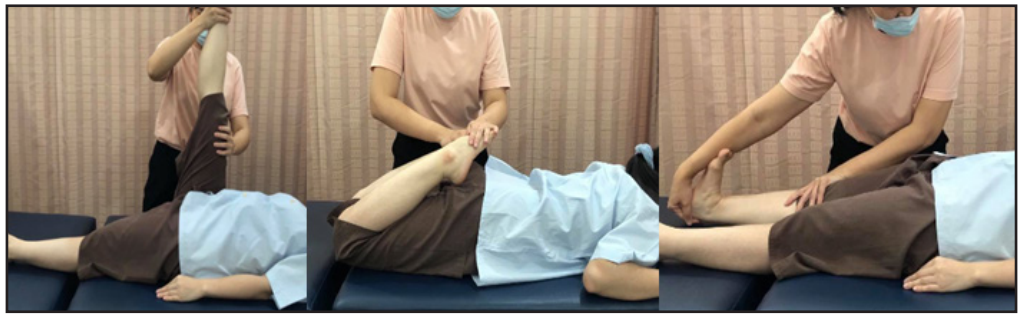

Figure 4 Stretching.

The pre-test and post-test were proceeded on both groups by one physical therapist. The TUGT was measured for dynamic balance by timing five sequences, including standing up from the armchair, walking 3 meters, turning back, walking to the chair, and sitting down with maximal performances[14,15]. Three consecutive trials were performed for TUGT using an inertial sensor system (APDM Inc, http://apdm.com). While measuring, the participants wore the APDM Opal sensors: one at the sternum, one at the lower back, and two on both feet (Figure 5). Participants were asked to sit without leaning on backseat. When participants were ready, assessor pressed start and data were recorded in 3 seconds after pressing start. Assessor said "go" in the third second to command participants. Participants raised body up to do test. Data were stopped capturing when participants sat steady[18]. The first trial was a practice trial, whereas the other two trials were recorded. The best value of the two trials was chosen. Reliability of the inertial sensor were reported to be good and excellence in the study Sankarpandi, Baldwin, and Ray in 2017 that TUG test showed good within and between sessions' reliability with mean intraclass correlation coefficients (ICC) values of 0.81 and 0.69, respectively in patients with vestibular disorder[19] TUGT duration, sit-to-stand duration, stand-to-sit duration, and turn duration from software were reported in seconds. Turn angle, sit-to-stand lean angle, and stand-to-sit lean angle were reported in degrees[18].

Figure 5 Participant wore Opal APDM Sensors.

Statistical analysis

The data from sensors were sent to the computer and analyzed by APDM Mobility Lab™ software. All results were analyzed by STATA Version 10. Data distributions were calculated by Shapiro-Wilk test. Descriptive statistics were used to describe the continuous and categorical data, including the number of participants, age, weight, sex, etc. Mean and standard deviations of the values were calculated for each variable. For parametric data, dependent t-tests were used to compare outcome variables at baseline with the immediate result after each group's intervention. An independent T-test was used to compare the outcome between each group. For non-parametric data, Wilcoxon signed-rank test was used to compare outcome variables at baseline with the immediate result after each group's intervention. Wilcoxon rank-sum test was used to compare the outcome between each group. A difference at the level of *p*-value < 0.05 was considered statistically significant.

Results

Demographic data of participants are presented in Table 2. Sixteen participants enrolled in this study; 12 were female, and 4 were male. The mean age of the TM group and control group were 64.9 and 71.5 years, respectively. Their mean height was 156.6 centimeters for the TM group and 159 centimeters for the control group. The mean body weight of participants in the TM and control groups was 64.9 and 61.3 kilograms, respectively.

Table 1 Demographic data of participants

Characteristics	TM	Control	Total	*p*-value
Number of participants (Female/Male)	8 (6/2)	8 (6/2)	16 (12/4)	
Age (years)	64.9 (4.1)	71.5 (5.8)	68.2 (6)	0.04
Weight (kg)	64.5 (11.6)	61.3 (9.7)	62.9 (10.4)	0.47
Height (cm)	156.6 (6.1)	159 (10)	157.8 (8.1)	0.54
Body mass index (kg/m^2)	26.4 (5.3)	24.3 (3.7)	25.6 (4.6)	0.39

Within-group and between-group comparisons of outcome measures in TM and control group are shown in Table 3. No statistically significant differences were found for both within- and between-group comparisons.

Table 2 Within-group and between-group comparison of outcome measures in the Thai massage and control group

Parameters	Group	Pre-intervention			Post-intervention			Pre- vs Post-	
		Mean (SD)	Different (95% CI)	*p*-value	Mean (SD)	Different (95% CI)	*p*-value	Different (Mean, SD)	*p*-value
TUGT duration (s)	TM	9.01 (1.29)	0.31 (-1.54, 2.17)	0.70	8.65 (1.24)	0.50 (-1.40, 2.40)	0.55	0.19 (0.33)	0.15
	Control	9.32 (1.09)			9.15 (1.22)				
Sit-to-stand									
Duration (s)	TM	0.88 (0.12)	0.05 (-0.06, 0.15)	0.33	0.88 (0.12)	0.04 (-0.04, 0.13)	0.28	0.00 (0.13)	0.96
	Control	0.93 (0.08)			0.93 (0.08)				
Turn									
Turn duration (s)	TM	2.00 (0.14)	0.05 (-0.18, 0.27)	0.65	1.96 (0.15)	0.12 (-0.13, 0.37)	0.31	0.07 (0.17)	0.28
	Control	2.05 (0.27)			2.08 (0.40)				
Turn angle (deg)	TM	183.50 (7.21)	1.38 (-7.03, 9.78)	0.71	184.88 (6.38)	2.25 (-5.24, 9.74)	0.50	0.88 (4.45)	0.60
	Control	182.13 (3.87)			182.63 (4.60)				
Stand-to-sit									
Duration (s)	TM	0.73 (0.17)	0.06 (-0.14, 0.26)	0.72	0.76 (0.18)	0.04 (0.13 to 0.21)	0.72	0.02 (0.31)	0.86
	Control	0.79 (0.15)			0.80 (0.12)				
Sit-to-stand									
Lean angle (deg)	TM	23.10 (21.10, 25.20)[b]	2.60 (-22.75, 4.05)[b]	0.57	23.05 (19.7, 26.6)[b]	4.50 (-19.00, 4.50)[b]	0.21[a]	0.70 (-2.90, 2.95)[b]	0.92[a]
	Control	27.05 (19.80, 42.80)[b]			28 (23.2, 38.3)[b]				
Stand-to-sit									
Lean angle (deg)	TM	20.90 (15.40, 26.90)[b]	0.65 (-14.95, 10.94)[b]	0.79[a]	22.7 (15.8, 31.3)[b]	0.70 (-8.43, 9.10)[b]	0.83[a]	2.65 (-9.82, 11.05)[b]	0.94[a]
	Control	19.20 (15.50, 37.50)[b]			19.65 (17.75, 38.4)[b]				

Note: [a] Results were calculated by the non-parametric statistic, [b] Results were reported by median and interquartile range.

Discussion

This study showed that one session of 1-hour whole body Thai massage could improve TUGT, despite not statistically significant. Thai massage was characterized by pressing and holding by manual deep pressure along ten meridian lines that cover the whole body and combining with muscle stretching at the end of the session. The massage was believed to increase blood circulation, increase temperature, buffer blood pH of muscle, and remove waste products. These help improve performance and recovery of muscle[21]. The possible mechanisms of Thai massage were included neuro-mechanical and mechanical effects.

Older people normally had decreased flexibility and limited range of motion. This affected balance performance by decreasing the activation of muscle spindle and amplitude of stretch reflex which are the important components of proprioception senses[22]. Massage may provoke proprioceptive nerve endings, change sarcomere length, stretch muscle, tendon, and ligament structures that originate reflex of muscle relaxation[23,24]. Mechanical pressure of massage helped reduce tissue adhesion by lengthening decreased or adhered connective tissue that gave rise of body flexibility[25] as measured by the range of motion[26]. Thai massage was also found to improve trunk flexibility in the normal adult with the tightness of hamstrings[27] and older adults[28]. Moreover, massage was found to arouse the proprioceptors on cutaneous, Golgi tendon organ, joint receptors, and muscle spindles. Massage also stimulated the readjustment of unproductive reflex works that was affected to improve somatosensory information and balance performance[29].

Unexpectedly, the results of this study could not support the findings of previous studies that Thai massage could improve balance performance. Thai massage on the lower leg was found to decrease the duration of TUGT in elderly with knee pain[30]. Moreover, many researchers studied the effects of other types of massage that is similar to Thai massage. One-hour whole-body therapeutic massage might improve balance performance in the elderly[6]. Negative results found in the current study could be due to either too short duration (single session) of exposure to Thai massage or small sample size. TUGT is one of the fall predictors for older adults. The TUGT of equal or more than 13.5 seconds was used to identify persons at higher risk of falling[31]. Mancini et al. in 2016 compared turning mobility between older adults who were non-faller, single fall, and recurrent falls. The results found that the turn duration of older adults who had recurrent falls was significantly higher than that of older adults who were non-faller. Moreover, mean peak speed of turn and mean turn angle of older adults who had the recurrent falls were lower than those of older adults who were non-faller[32]. In this study, the turn duration of Thai massage group was slightly decreased and turn angle of Thai massage group was slightly increased immediately after intervention.

Meanwhile, the control group was somewhat improved only for turn angle. This could be believed that Thai massage may modestly improve balance in the elderly by these parameters. Further study with larger sample size is recommended to verify its effect.

Moreover, there were no significant differences in sit-to-stand and stand-to-sit parameters. This could be because we used only one session of one-hour Thai massage. The future study should investigate for the multiple sessions and long-term result.

Furthermore, older participants in Thai massage group were significantly younger than control group as mean age of Thai massage and control group were 64.9 and 71.5 years, respectively. The TUGT duration of Thai massage group was also lower than control group at baseline. Previous study was established the TUGT normative data among community dwelling older adults, stratified base on cognitive status, gender, and age groups. It was found that TUGT duration was increased by aged[33] and it was consistent with our baseline result. Thus, this could be the limitation of this study that could not possibly detect the large effects by ceiling effects of healthy participants' performances. Further study should allocate participants to each group with

stratification of sampling by age and other factors that are related to balance performance.

As the immediate results of TUGT were not improved after one session of 1-hour whole body Thai massage. Further study on multiple sessions of Thai massage on TUGT should be investigated.

Conclusion

According to the results of this preliminary comparative study, we concluded that one session of 1-hour Thai massage session could not provide immediate improvement of TUGT parameters. In further study, long-term treatment with follow-up are suggested.

Take home messages

One session of one-hour Thai massage may not improve dynamic balance in older adults as measured by the timed-up-and-go test.

Conflicts of interest

The authors declare no conflict of interest.

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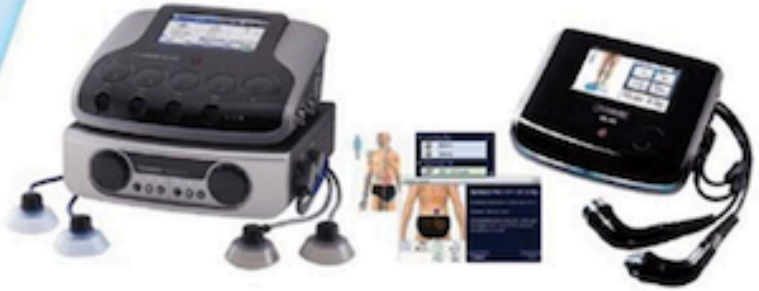

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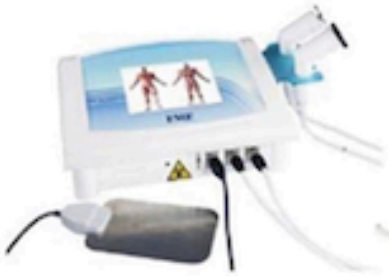

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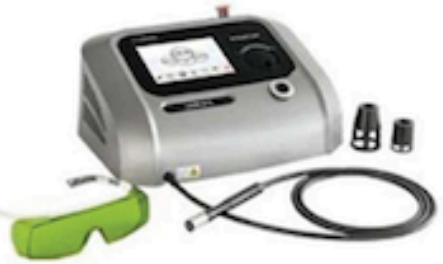

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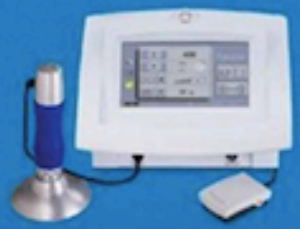

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