JOURNAL OF ASSOCIATED MEDICAL SCIENCES

JAMS

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# Journal of Associated Medical Sciences

## Aims and scope

The Journal of Associated Medical Sciences belongs to Faculty of Associated Medical Sciences (AMS), Chiang Mai University, Thailand. The journal specifically aims to provide the platform for medical technologists, physical therapists, occupational therapists, radiologic technologists, speech-language pathologists and other related professionals to distribute, share, discuss their research findings, inventions, and innovations in the areas of:

1. Medical Technology
2. Physical Therapy
3. Occupational Therapy
4. Radiologic Technology
5. Communication Disorders
6. Other related fields

Submitted manuscripts within the scope of the journal will be processed strictly following the double-blinded peer review process of the journal. Therefore, the final decision can be completed in 1-3 months average, depending on the number of rounds of revision.

## Objectives

The Journal of Associated Medical Sciences aims to publish integrating research papers in areas of Medical Technology, Physical Therapy, Occupational Therapy, Radiologic Technology, and related under peer-reviewed via double-blinded process by at least two internal and external reviewers.

## Types of manuscript

Manuscripts may be submitted in the form of review articles, original articles, short communications, as an approximate guide to length:

- **Review articles** must not exceed 20 journal pages (not more than 5,000 words), including 6 tables/figures, and references (maximum 75, recent and relevant).
- **Original articles** must not exceed 15 journal pages (not more than 3,500 words), including 6 tables/figures, and 40 reference (maximum 40, recent and relevant).
- **Short communications** including technical reports, notes, and letters to the editor must not exceed 5 journal pages (not more than 1,500 words), including 2 tables/figures, and references (maximum 10, recent and relevant).

## Peer review process

By submitting a manuscripts to Journal of Associated Medical Sciences, the authors agree to subject it to the confidential double-blinded peer-review process. Editors and reviewers are informed that the manuscripts must be considered confidential. After a manuscripts is received, it is assigned by a specific Associate Editor. The Associate Editor prepares a list of expert reviewers, which may include some suggested by the Editor-in-Chief. Authors can indicate specific individuals whom they would like to have excluded as reviewers. Generally, requests to exclude certain potential reviewers will be honored except in fields with a limited number of experts. All potential reviewers are contacted individually to determine availability. Manuscripts files are sent to at least two expert reviewers. Reviewers are asked to complete the review of the manuscripts within 2 weeks and to return a short review form. Based on the reviewers' comments, the Associate Editor recommends a course of action and communicates the reviews and recommendations to the Editor-in-Chief for a final decision.

The Associate Editor considers the comments made by the reviewers and the recommendation of the Editor-in-Chief, selects those comments to be shared with the authors, makes a final decision concerning the manuscripts, and prepares the decision letter for signature by the Editor-in-Chief. If revisions of the manuscripts are suggested, the Associate Editor also recommends who should review the revised paper when resubmitted. Authors are informed of the decision by e-mail; appropriate comments from reviewers and editors are appended.

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# Validity and reproducibility of chest expansion measurement by a device using an ultrasonic sensor

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**ABSTRACT**

**Background**: Chest expansion is a type of physical examination used by physicians and physical therapists to diagnose various pulmonary diseases. Despite the fact that it can be performed using various equipment. However, it has some limitations while operating in the clinic. Therefore, a prototype device was developed.

**Objectives:** The study was carried out to assess the validity and reproducibility of a chest expansion measurement device using an ultrasonic sensor. Furthermore, the opinion to the device was gathered by the questionnaire.

**Materials and methods:** The study included 110 healthy subjects ranging in age from 20-60 years old. Two examiners measured upper, middle, and lower chest expansion independently and at random, and the measurements were repeated one day later. The Pearson correlation coefficients and Bland-Altman plotting were then used to assess validity and reproducibility. In addition, a questionnaire yielded suggestions from 10 experienced physical therapists.

**Results:** The results showed that the validity, as measured using Pearson's correlation coefficient, had a moderate association in the lower part ($r$=0.69, $p$<0.001), whereas the other levels had the lowest and lowest association. There was also a strong correlation between intra-observer reproducibility (upper and middle: $r$=0.81, lower: $r$=0.84, all $p$<0.001). According to the questionnaire responses, some aspects of the device's appearance should be improved.

**Conclusion:** The device's validity appears to be very low to moderate depending on the expansion levels measured. Additionally, the reproducibility is considerably high, while some details of the device need to be improved to maximize its efficiency.

## Introduction

Chest expansion measurement was first described by Moll *et al*. in 1972.[1] It is a useful method for assessing disease conditions, such as asthma and chronic obstructive pulmonary disease (COPD),[2] as well as the effects of treatment techniques.[3,4] Commonly, it is measured as the difference between the thoracic girth measurement after maximal inspiration and at the end of maximal expiration.[1] Clinical measurements can be taken using simple methods that do not necessitate the use of complex devices, such as a cloth tape[5, 6] or a caliper.[7] Even if the method is simple, the validity of the measurable distance is dependent on various factors, including the equipment, the examiner's

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expertise, and the patient's cooperation. In the literature on chest expansion, it is mainly measured in a standing position.[1,2] However, according to the preceding facts, measurement will be difficult in the case of unconsciousness or an uncooperative patient, such as a patient with brain injury or spinal cord injury. It is also difficult in patients who have thoracic wall wounds or who have known or suspected infections that represent an increased risk of contact transmission. Currently, numerous inventions of devices used to measure chest wall dimensions[8-12] may compensate for the limitations discussed previously.

These devices, however, must be used in conjunction with other instruments, such as body markers, computers, camcorders, etc., which may not be suitable for clinical use. Based on the fact that the expansion was the length, a previously reported device for measuring length was adapted from an ultrasound sensor.[13,14] It consists of a transmitter and a receiver that can transmit and receive ultrasonic sound. The sensor's principle is to measure the time to flight of an ultrasonic soundwave from the sensor to a detected object. It provides noncontact range detection with high validity. It is unaffected by sunlight or dark materials.[15] Furthermore, it can be used without any other instruments. As a result, the researchers attempted to develop a chest expansion measurement prototype device using an ultrasonic sensor, and the current study was conducted to evaluate its validity and reproducibility in comparison to standard equipment. In addition, evaluating opinions on the device from experienced physical therapists was also conducted. The data provided will aid researchers to improve the device so that it can be used more effectively and alleviate the limitations of chest expansion measurement in some clinical conditions.

## Materials and methods

The study was approved by the Khon Kaen University Ethical Committee (HE621260), and written informed consent was obtained from all subjects.

### *Developing the device*

The device consists of two parts: the body, in which the set of sensors is located, and the standing part that fixes into the bed rail, as shown in Figure 1(A). It was produced by adapting twenty-five HC-SR04 ultrasonic sensors (version 1.0) and an aluminium structure. The sensor was a product of Cytron Technologies® Sdn. Bhd., Johor, Malaysia. The measurement range of the sensor is 2-400 cm and the precision is 0.3 cm.[15] The set of sensors consists of two ultrasonic transducers: the transmitter and the receiver. To measure chest expansion, device's body is set at a height of 60 cm above the mattress by fix the standing part to the bed's upper handrail, and the start button that is located on the body is pressed. The sensor will then transmit ultrasonic bursts one by one at a frequency of 40 kHz. The sound then travels through the air and finds the mattress or the subject's chest wall. After that, it bounces back to the module and the reflected wave is detected by the receiver. The time between the transmission and reception of the signal allows the processor board settled inside the device's body to calculate the distance. After that, the received distance is processed again to obtain the results from the calculations between several sensors installed to indicate the chest wall distance from side to side. Finally, the distance that represents the chest expansion is expressed on the LCD screen.

### *Subjects for chest expansion measurement*

Sample size of the first study was calculated for correlation analysis, based on the level of correlation coefficient =0.8, and estimated drop out of 10%.[16] One hundred and ten subjects between the ages of 20 and 60 were recruited. Personal information was gathered using a self-administered questionnaire. All subjects were healthy and had no medical conditions. Their BMIs were between 18.5-22.9 kg/m$^2$. In addition, they were asked to keep their normal physical activity while participating

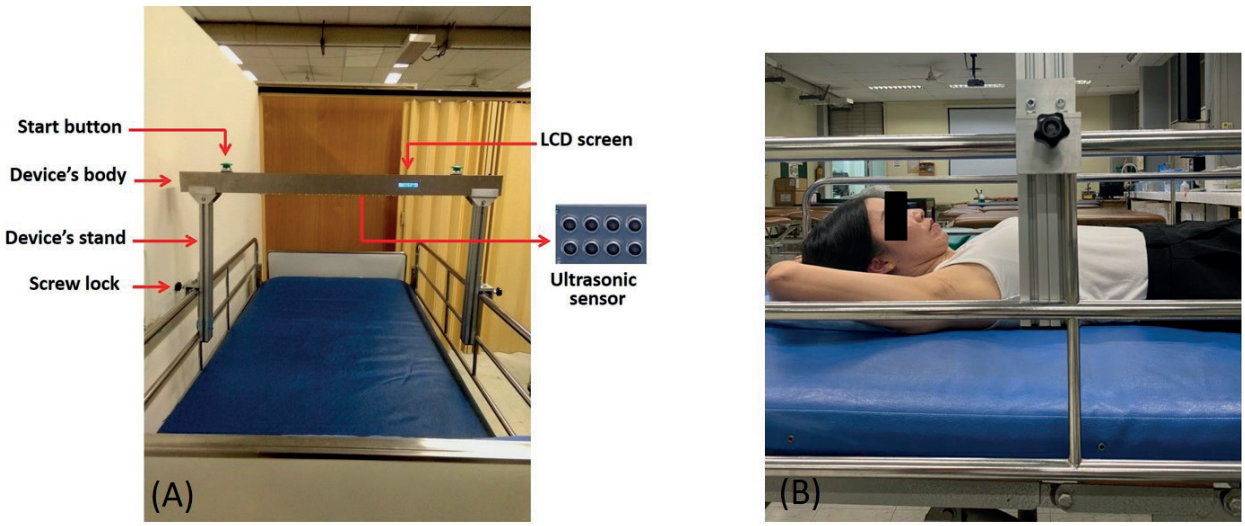


***Figure 1.*** *Device's component (A) and the measurement position (B).*

in the study. Subjects were excluded if they had physical situations altering respiratory mechanics, i.e., scoliosis or kyphosis, or had a history of fainting while taking a deep breath or holding a breath, syncope of unknown origin, or a history of clavicle, rib, or sternum fracture less than six months prior.

***Chest expansion measurement***

To evaluate the device's validity, chest expansion in the transverse dimension was measured by two different instruments: a 20-inch-scale Martin Breadth Caliper as the standard and the device. To evaluate the device's reproducibility, chest expansion was evaluated under two conditions. On the first visit ($V_1$), it was measured by two examiners: a professional ($Ex_1$) and a general ($Ex_2$) physical therapist whose order was random. On the second visit ($V_2$), it was only re-assessed by the $Ex_1$. The protocol is shown in Figure 2. Each subject was instructed to perform breathing exercises at three levels—the upper, middle, and lower chest-to maximise each level of expansion. Upper chest expansion was measured at the level of the third intercostal space.[5] Middle and lower chest expansion were measured at the level of the fourth intercostal space[17] and the level of the xiphoid process, respectively.[1,5,18] The test procedure was standardized, and the two examiners involved were trained in testing before the study started to avoid measurement errors. In this study, block randomization was employed using computer-generated random numbers for the examiners and the subjects. The subjects were divided at random into the caliper then device group (CD group) and the device then caliper group (DC group). All subjects were measured in a supine position with their hands placed on their head, as shown in Figure 1(B). For the caliper measurement, the chest expansion was measured by placing the C-shaped arm end over the left to right chest wall at the mid-axillary line.[1] For the device measurement, the chest expansion was measured by operating method that previously mention in the "developing the device" topic. Three trials at each level were measured, with a 5-minute rest between the levels. The best value was then selected. After resting for 15 minutes, the CD group and the DC group exchanged instruments. Finally, measurements using the device were repeated on $V_2$ one day apart by the $Ex_1$. All measurements in the study were conducted in a room with the temperature set at 26±2 °C to avoid environmental factors affecting the ultrasonic sensor's capability.

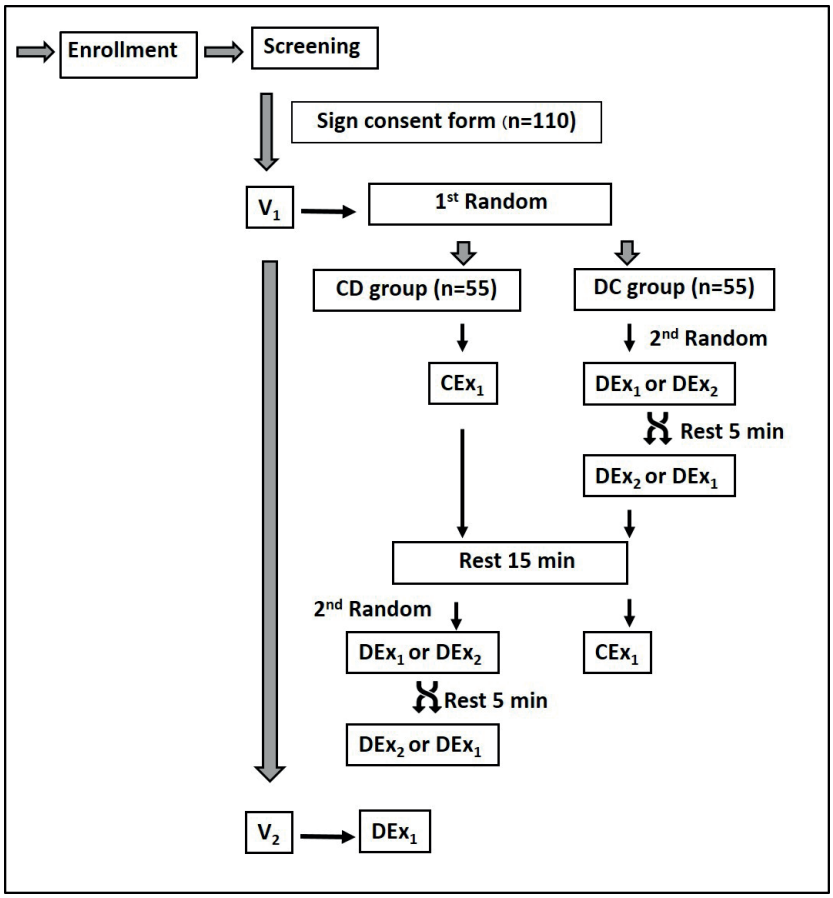


***Figure 2*** *Consort diagram of the study.*
*$V_1$: First visit, $V_2$: second visit, CD group: caliper then device group, DC group: device then caliper group, $CEx_1$: caliper measured by professional physical therapist, $DEx_1$: device measured by professional physical therapist, $DEx_2$: device measured by general physical therapist.*

***Experienced physical therapists and the questionnaire***

The second sample consisted of 10 experienced physical therapists who were invited to participate in the study through purposive sampling. All had been working at a hospital for more than two years. The instrument was a two-part self-administered questionnaire. The first part was divided into three questions. The first question dealt with the structure of the device and gathered opinions about its material, strength, weight and size, and overall appearance. The second question elicited feedback on device stability, safety, portability, and ease of use. The last question assessed opinions on device cleaning and storage, as well as overall satisfaction. In this part, each physical therapist was asked to rate their satisfaction on a five-point Likert scale ranging from 1 ("disagree") to 5 ("strongly agree"). Furthermore, the second part required all of them to include any additional suggestions or comments about the device. Following the demonstration of device usage, the questionnaire was distributed and collected within seven days by asking respondents to return it via postal mail. Following that, all suggestions were compiled.

***Statistical analysis***

Descriptive information, i.e. the general characteristics of the subjects and the opinions expressed in the questionnaires, are presented in the form of percentages. Data are expressed as the mean±SD. The statistical analysis was performed using the STATA programme version 10.5 software. Pearson's correlation analysis and Bland–Altman plotting were performed for the assessment of validity and reproducibility. Values of $p<0.05$ were considered to indicate statistical significance.

## Results

Personal information about the subjects is depicted in Table 1. To evaluate the device's validity, the correlation between the caliper-measured expansion and the device is shown in Table 2. Between the upper and middle chest expansions, the lowest and lowest correlations were found. The lower chest expansion, on the other hand, had a medium correlation, and these patterns were seen in both examiners (Table 2). For reproducibility, a good correlation between device measurements taken on different trial visits by the $Ex_1$ was 0.81 ($p<0.001$) for the upper and the middle, and 0.84 ($p<0.001$) for the lower chest expansion. The sample of Bland-Altman plots between the two instruments and two trial visits by the $Ex_1$ are shown in Figures 3(A) and 3(B). As this shows, almost all of the values are within a mean±2SD deviation.

**Table 1.** General characteristics of the subjects for chest expansion measurement (n=110).

| Characteristics | Mean (min-max) |
|---|---|
| Male: Female [n (%)] | 25: 85 (23%: 77%) |
| Age (years) | 32.0±12.8 (20-58) |
| Body weight (kg) | 56.1±6.72 (44.4-78.2) |
| Height (cm) | 161.6±7.77 (146.0-185.0) |
| BMI (kg/m2) | 21.5±1.58 (18.0-22.9) |
| Waist circumference (cm) | 73.9±7.88 (58.0-90.0) |
| Hip circumference (cm) | 93.2±5.36 (78.0-109.0) |
| WHR | 0.79±0.07 (0.66-0.94) |

***Note:*** *Values are mean±SD unless otherwise indicated. BMI: body mass index, WHR: waist-to-hip ratio.*

**Table 2.** Chest expansion and device correlation.

| | | Upper | Middle | Lower |
|---|---|---|---|---|
| Expansion (cm) | Caliper | 1.15±0.29 | 1.34±0.43 | 2.97±0.88 |
| | (min-max) | (0.6-2.2) | (1.0-3.0) | (1.2-5.0) |
| | Device $Ex_1V_1$ | 1.49±0.31 | 2.15±0.53 | 3.47±1.35 |
| | (min-max) | (0.9-2.5) | (1.1-3.5) | (0.6-8.9) |
| | Device $Ex_1V_2$ | 1.64±0.33 | 2.38±0.54 | 4.17±1.51 |
| | (min-max) | (0.9-2.6) | (1.2-4.0) | (1.1-10.0) |
| Correlation | $Ex_1V_1$ | 0.10 | 0.20 | 0.69 |
| (*p* value) | | (0.302) | (0.033) | (<0.001) |
| | $Ex_2V_1$ | -0.19 | -0.02 | 0.42 |
| | | (0.503) | (0.847) | (<0.001) |
| | $Ex_1V_2$ | 0.01 | 0.11 | 0.65 |
| | | (0.922) | (0.263) | (<0.001) |

***Note:*** *Data expressed in mean±SD. $Ex_1$: professional physical therapist, $Ex_2$: general physical therapist, $V_1$: the first visit and $V_2$: the second visit.*

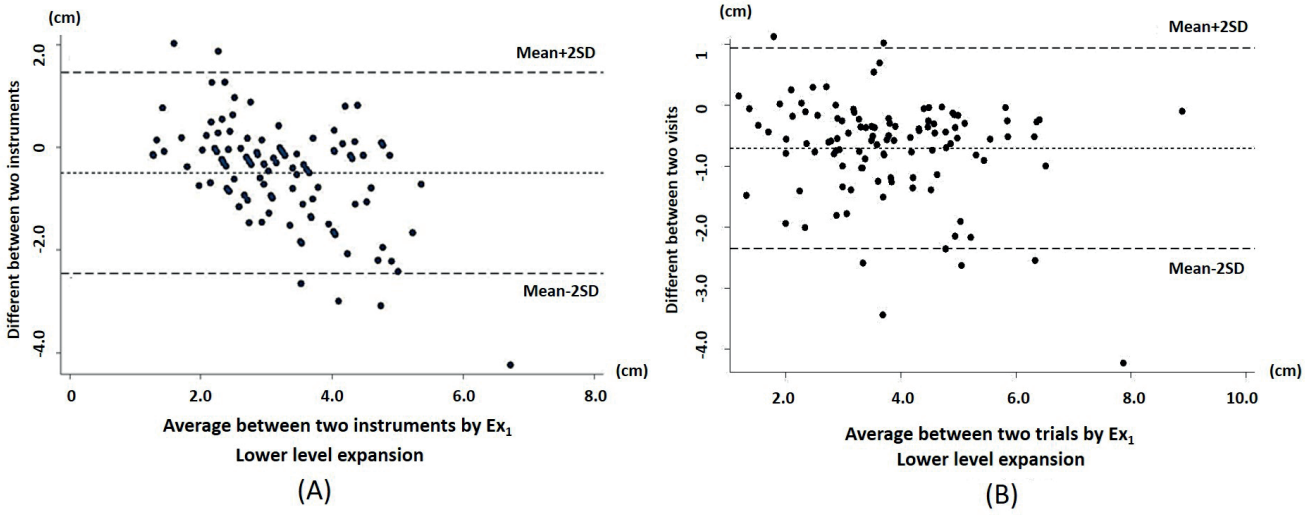


***Figure 3.*** *Bland-Altman plots of the lower-level expansion between the two instruments (A) and two trial visits (B) by the* $Ex_1$.

Ten experienced physical therapists provided their opinions on the device. Almost all (80%) strongly agreed with the device's material, which is made from aluminum. Furthermore, 70% and 60% of them strongly agreed on the device's strength and stability, respectively. Only 60% of them agree with its weight and size and ease of use. Furthermore, they strongly agreed and agreed with the device's safety and ease of cleaning and storing on a half-to-half basis. However, almost all of them indicated that the devices were difficult to transport (neutral 50%, slightly disagree 40%). Furthermore, it should improve its overall appearance (40% agree and 30% neutral) and overall satisfaction (20% strongly agree and 80% agree). The interesting additional suggestion and comment from the last section included the charging component should be adapted outside the device. It would be preferable if it could be easily adjusted up to the bed's headboard or down to the footboard. One opinion suggested that it may not appear sufficiently like a medical device if the material is made with a carbon or fiber frame.

## Discussion

Validity and reproducibility are two of the key features of evaluation instruments. The present study found that Pearson's correlation coefficients of intra-examiner reproducibility were considerably high. Almost all values in the Bland-Altman plots were within a 2SD deviation of the mean. However, the validity was unsatisfactory. These findings might be explained by the following reasons.

According to the capability of the invented device, one by one, the twenty-five HC-SR04 transmitters emit a high-frequency sound at 40 kHz. The sound then travels through the air and finds the mattress or the subject's chest wall. After that, it bounces back to the module. Based on the product user's manual, even though the sensor is capable of excellent performance, it has some limitations.[15] First, its limitations are related to the object's size and the distance between the sensors. The sensor and the object must be no more than 4 m or less than 2 cm apart, and the object must be no smaller than 0.3 cm in order to reflect enough sound waves back to the sensor. The object evaluated in the study was the thorax; therefore, the factor attributed to the device's validity may not be accounted for by the object's size. Additionally, the device's stand was designed to be able to adjust the distance above the bed's lower handrail to a maximum of 50 cm. In the study, the device's stand was installed by attaching it to the bed's upper handrail. Therefore, device's body was set 60 cm above the mattress to provide a comfortable space for the subject's body size. As a result, the sensor's distance from the chest wall was within the measurement range.

Another limitation is that the ultrasound wave generated by the sensor used in the study was classified as low-frequency ultrasound (20-200 kHz), which is usually applied in industrial and therapeutic applications.[19] Although the sensor's functionality is not affected by light or color. However, it was limited by the shallow reflective surface angle (<15 degrees), which meant that the sound wave could not be reflected back towards the receiver.[15] Moreover, there is the surface of the object to consider. Based on the theory of sound, there are many different phenomena leading to a loss in strength and distortion of the signal between the transmitter and receiver. These phenomena include reflection and transmission at the boundaries between media, attenuation in the media and loss in received sound energy due to dispersion.[20] To avoid false detection of the boundary of the chest wall by the shoulder, all subjects were measured in the supine position with their hands on their heads. They also provided a white cotton tank top that fit their bodies and allowed the female subjects to wear their bras. Given these circumstances, it was obvious that their bras almost covered the measurement areas. As a result, the device's poor validity, particularly at the upper and middle levels, could be explained by the bra fabric transmitting the sound wave and then attenuating the reflection to the sensor. In accordance with the clinical situation, the patients' chest expansion was measured while wearing only a hospital

patient gown. To validate this possibility, the next study will be conducted only on male subjects or on both genders, but only in the context of wearing hospital patient attire.

Previous studies have reported the greatest expansion was found at the lower level.[5,6,21] Notably, these patterns were discovered in the current study. Under normal physiological conditions, the upper ribs more move upward than outward; however, the lower ribs have more freedom of motion, this contributed to the lower level's broadening expansion.[22,23] It was observed that the mean expanded value at the upper chest level was 1.15 cm and the minimum value was 0.6 cm, while the sensor resolution was 0.3 cm. Based on the limitations of the sensor, less movement might have the potential to affect device validity. In addition, the device measured the transverse dimension of the chest wall, whereas the upper ribs move more upward than outward; this additional fact may have contributed to the study's lowest and lowest of the Pearson's correlation found at upper and middle chest level. Furthermore, during measurement, the lower level of the male subjects was only covered by the tank top, whereas some of the female subjects were also covered by their bras. This condition could be related to the medium level of the correlation finding at the lower level. As a result, not only the further study that was mentioned earlier but also a sensor replacement with a higher validity ultrasonic sensor generation or additional with another sensor type will be performed to achieve greater validity.

The researchers agree with the experienced physical therapist's that suggested to improve the device. For the usage, the device's stand should be redesigned so that it can be easily moved up to the headboard or down to the footboard by adjusting along the bed's handrail. In addition, because the battery was installed inside the device's body, it could not be moved to charge outside. According to this suggestion, the researcher will widen the charger slot to make it easier to plug in and disconnect the battery cable. For the structure, the size and weight should be smaller and lighter to enhance its portability to carry around when used in the clinic. Although, the aluminum structure might be suitable to be strong, lightweight, durable, and resistant to corrosion when cleaning. However, it should be noted that the higher the number of sensors, the greater the number of processor boards. As a result, the number of sensors and apparatus located in the body accounts for nearly all the device's weight. Furthermore, in conjunction with the previously discussed HC-SR04 sensor, the researchers intend to minimize its limitations to improve its validity, reduce its overall weight and size, and improve its appearance.

### *Study limitations*

There were four limitations in the study. Firstly, based on the fact that measuring thoracic expansion during a clinical workout in the unconscious or even in those with mobility problems or muscle weakness, such as quadriplegia patients or patients with myasthenia gravis or Amyotrophic Lateral Sclerosis, etc., is difficult because they cannot be upright. According to the preceding condition, the supine position was chosen in the study to mimic the problems encountered while performing in the clinic, despite the fact that it limited the motion of the chest to expand. Secondly, there was no statistical test involve in the reliable tests of the professional examiner. Thirdly, the study was unable to validate that the cloth absorbed ultrasound waves through the bare skin. This is because clinical measurements of chest expansion are taken while wearing clothing. Lastly, to conduct the study, subjects with a normal BMI were used. As a result, for other body sizes, the validity may be different, and the results may differ from those found in this study.

## Conclusion

Based on the results, it is possible to conclude that, while the device's reproducibility with an ultrasonic sensor is quite high, its validity appears to be very low to moderate, depending on the expansion levels measured. Furthermore, the sensor should be modified to improve its validity, and the device's overall appearance should be improved to maximize its aesthetics.

## Conflicts of interest

The authors declare no conflicts of interest.

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# Comparative effects of wearing N95, surgical, cloth, and $PM_{2.5}$ masks during six-minute walk test on dyspnea, breathing effort, oxygen saturation, and functional capacity in pre-aging individuals

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**ABSTRACT**

**Background**: Wearing a face mask became a part of daily life due to the COVID-19 pandemic and fine particulate matter ($PM_{2.5}$) air pollution in Thailand. However, performing activities while wearing different types of masks can cause different physiological effects, especially in the pre-aging population that still performs outdoor activities regularly.

**Objectives**: To compare the impacts of different types of the mask (no mask, N95, surgical, cloth, $PM_{2.5}$ mask) on dyspnea, breathing effort, blood oxygen saturation ($SpO_2$), and functional capacity (six-minute walk distance and estimated $VO_2$max) during the six-minute walk test (6MWT) in pre-aging individuals.

**Materials and methods**: Twenty-four healthy pre-aging individuals (54±3 years) performed five trials of 6MWT wearing different types of masks. Pre-test and post-test of dyspnea, breathing effort, and blood oxygen saturation values were recorded. The estimated $VO_2$max was calculated from 6MWT. The satisfaction for each type of mask was collected using questionnaires.

**Results**: $SpO_2$, walking distance, and estimated $VO_2$max were not significantly different ($p$>0.05) among all trials. Changes in dyspnea, breathing effort, and systolic blood pressure values were statistically different ($p$=0.026, $p$=0.031, and $p$=0.028, respectively). Despite post hoc analysis revealing a nonsignificant difference in delta changes in dyspnea between walking trials, there was a clinical difference in dyspnea while wearing N95 compared to no mask as well as surgical masks compared to other masks (MCID for Borg scale ≥1). Wearing an N95 or surgical mask resulted in higher dyspnea. Moreover, wearing an N95 mask also resulted in higher breathing effort and systolic blood pressure compared to wearing no mask.

**Conclusion**: Wearing various types of masks in the pre-aging population during moderate-intensity activity did not result in differences in oxygen saturation and functional capacity. Wearing an N95 resulted in higher levels of dyspnea, breathing effort and systolic blood pressure than wearing no mask. Wearing a surgical mask may have resulted in higher levels of dyspnea than wearing no mask, a cloth mask, or a $PM_{2.5}$ mask.

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## Introduction

COVID-19 pandemic has had a global impact on human health. Since the beginning of the COVID-19 outbreak, at the time of writing, the number of confirmed cases globally has surpassed 584 million, with over 6.4 million deaths.[1] To prevent further cases and deaths, people should follow individual preventive behaviors, i.e., social distancing, frequent handwashing, and mask-wearing.[2] Since March of 2012, Thailand has faced an air pollution crisis with fine particulate matter ($PM_{2.5}$) that exceeds the standard safe level, especially in the north of Thailand.[3] $PM_{2.5}$ can cause irritation of the eyes and respiratory tract. In addition, the long-term effects of $PM_{2.5}$ may cause chronic obstructive pulmonary disease (COPD), cardiovascular disease, and cancer, which are leading causes of death.[4] Therefore, the Ministry of Public Health has advised people to avoid outdoor exercise or, if necessary, to wear a mask.[5]

Wearing a mask has become a part of daily life. Various types of masks with different particle filtration efficiency as well as distinct shapes and materials are widely used in Thailand.[6] The N95 respirator is one of the most commonly used because of the high filtration efficiency that prevents droplet and airborne transmission of COVID-19 and protects from $PM_{2.5}$. However, wearing an N95 respirator for a long time may have adverse health effects, e.g., decreased ventilation[7-8] can cause hypoxemia and hypercapnia,[9] leading to dyspnea and decreased functional capacity. In addition, performing activities while wearing an N95 respirator may induce a reduction in exercise capacity and functional capacity.[10]

The pre-elderly (age 50-59) population is a high-risk group for adverse effects when wearing a mask, as mentioned above.[11] Because this population regularly performs activities while wearing a face mask, despite the fact that their bodies are undergoing age-related deterioration.[12,13] Furthermore, the daily routine of the pre-aging population consists of moderate-intensity activities, such as brisk walking, gardening, and housework.[14] As a result, it is critical to assess the impact of performing activities while wearing various face masks on this population. However, there are no studies on the physiological effects of performing activities while wearing different masks in the pre-aging population. Previous research mainly studied the impact of wearing only one type of mask.[15,16] The few studies that compared the effects of wearing different types of masks were all in younger populations.[17,18]

To evaluate a pre-aging population's capacity for moderate-intensity activity, the 6MWT is a suitable test.[19] Therefore, this study aims to determine the physiological effects of wearing N95 respirators, surgical masks, cloth masks, and $PM_{2.5}$ masks during the 6MWT for pre-aging participants to further recommend the most appropriate type of mask to protect from COVID-19 or $PM_{2.5}$.

## Materials and methods

### Participants

First, the number of participants was determined using data from a previous study[18] that compared the effects of different masks on the 6MWT in healthy subjects. G-Power version 3.1.9.4 calculations revealed that the study required data from 7 participants. However, the researchers considered this to be an insufficient number for study in the pre-aging population. As a result, the effect size for partial $\eta^2$ was changed to 0.06 at the medium effect size level, the power was set to 0.80, and the alpha error was 0.05. Data were collected from 24 participants.

Inclusion criteria were healthy pre-elderly (age 50-59) and capable of self-walking safety. Exclusion criteria included respiratory disease (acute or chronic), smoking history ≥20 packs a year or having quit less than a year prior, unstable cardiovascular diseases, taking medication affecting outcome measurement, and other diseases that prevent the participant from safely performing the walking test. All participants signed a written informed consent before any procedures started.

### Outcome measurements

Physiological variables were measured at rest, the end of test, and the recovery period. The primary outcome was dyspnea (Borg CR10 scale), and the secondary outcomes were breathing effort (Borg CR10 scale), blood oxygen saturation ($SpO_2$), and functional capacity (six-minute walking distance and estimated $VO_2$max).[20] $VO_2$max ($ml/kg^{-1}/min^{-1}$) was calculated from 70.161 + (0.023 × 6MWT [m]) - (0.276 × weight [kg]) - (6.79 × sex, where male =0, female =1) - (0.193 × resting HR [beats per minute]) – (0.191 × age [y]).[21] Furthermore, heart rate recovery at 1 and 2 minutes was recorded, which was calculated from the heart rate at the end of the 6MWT and the heart rate after one or two minutes, respectively.

### Study Design

This study is a randomized, prospective, cross-over trial. The study protocol was approved by the ethics committee of the institute (AMSEC-64EX-029). After familiarization with the test, each participant performed five trials (two on the first day and three on the second day) of the 6MWT with no mask and wearing four different types of masks; N95 respirator (3MTM 1870+ N95, Minnesota, USA), surgical mask (Medima SKTM, Bangkok, Thailand), cloth mask (handmade mask with 2 layers fabric), $PM_{2.5}$ mask (UnicharmTM 3D mask, Japan). The sequence of face masks used in each trial was generated using a balanced Latin square method. The 6MWT was performed in accordance with the American Thoracic Society guidlines.[22]

The participants rested for at least 30 minutes before starting each walking trial (dyspnea Borg CR10 scale =0). Then the Self-perceived dyspnea (Borg CR10 scale), breathing effort (Borg CR10 scale), blood pressure (OmronTM HEM-7130, Kyoto, Japan), heart rate, and $SpO_2$ (NoninTM Onyx II 9550, Minnesota, USA) were measured while wearing a mask for 5 minutes, at the beginning and end of the test, and then at minutes 1, 2, and 5 after the test. The six-minute walk distance was also recorded to calculate the estimated $VO_2$max. Using two questionnaires, participants were asked to rate their level of satisfaction with a mask at the end of each walking trial (Supplementary Table S1). Furthermore, after completing all of the walking trials, the participants ranked their satisfaction with the four types of masks (Supplementary Table S2).

### Statistical analysis

All data were tested for normality using the Shapiro -Wilk test. Depending on whether the data is normally distributed, one-way repeated ANOVA or Friedman test was used for the comparative analysis. The statistically significant level was set at 0.05. If the data were statistically significant, pairwise comparisons between group means (post hoc analysis) were performed using Bonferroni or Wilcoxon signed-rank test with Bonferroni-adjusted significant level value, which the Bonferroni-adjusted significant level value is calculated as the significant level value divided by the number of pairwise comparisons, 0.05 divided by 10, so the value is 0.005 ($p \leq 0.005$). Data with a normal distribution were presented as mean±SD, whereas data with a non-normal distribution were presented as median (upper-lower quartile).

## Results

Twenty-four participants (male: female 8: 16; age 54±3 years; weight 63±13 kg; height 158±8 cm; BMI 25.1±4.4 kg/m$^2$) participated in this study.

### Physiologic variables at rest

Physiologic variables at rest are presented in Table 1. Most of the variables were not significantly different between the walking trials ($p>0.05$), with systolic blood pressure (SBP) ($p=0.006$) being the exception.

**Table 1** Comparison of the physiologic variables at rest.

| Outcome | No mask | N95 | Surgical | Cloth | $PM_{2.5}$ | *p* value | Effect size |
|---|---|---|---|---|---|---|---|
| | median (upper quartile - lower quartile) | | | | | | |
| Dyspnea (Borg (CR10) scale) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | - | - |
| Breathing effort (Borg (CR10) scale) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | - | - |
| Oxygen saturation (%) | 98.0 (97.0-98.0) | 98.0 (98.0-98.0) | 98.0 (97.0-98.8) | 98.0 (98.0-98.0) | 98.0 (97.0-98.0) | 0.483 | 0.036[b] |
| Heart rate (bpm) | 79 (69-88) | 81 (72-88) | 80 (72-88) | 78 (71-90) | 78 (68-90) | 0.143 | 0.072[b] |
| | mean±SD | | | | | | |
| Systolic blood pressure (mmHg) | 120±18 | 115±17 | 116±20 | 113±17 | 115±19 | **0.006*** | 0.153[b] |
| Diastolic blood pressure (mmHg) | 74±11 | 74±10 | 73±9 | 73±9 | 74±10 | 0.814 | 0.012[a] |

*Note: One-way repeated ANOVA reported with mean±SD, Friedman test reported with median (upper-lower quartile). *Statistically significant indicating that at least one pair of masks is significantly different ($p \leq 0.05$), [a] Effect size estimates of one-way repeated ANOVA are Partial Eta Squared ($\eta^2$) = SSeffect / SSeffect + SSerror; $\eta^2$ =0.01 indicate small effect, $\eta^2$ =0.06 indicate medium effect and $\eta^2$ = 0.14 indicate large effect, [b] Effect size estimates of the Friedman test is Kendall's W value (W) =$\chi 2$ / N(K-1); w =0.10 indicates a small effect, w =0.30 indicates a medium effect, and w =0.50 indicates a large effect.*

### Physiologic variables after wearing a mask for five minutes

Between-group comparisons of physiologic variables after wearing a mask for five minutes showed significant differences in dyspnea and breathing effort ($p=0.042$ and 0.001, respectively) (Table 2).

### Physiologic variables at the end of the 6MWT

At the end of the 6MWT, generally there were no statistically significant differences on physiological variables except for dyspnea, breathing effort, and SBP ($p=0.026$, $p=0.031$, and $p=0.028$, respectively) (Table 3).

**Table 2** Physiological variables after wearing a mask for five minutes.

| Outcome | No mask | N95 | Surgical | Cloth | $PM_{2.5}$ | *p* value | Effect size |
|---|---|---|---|---|---|---|---|
| | median (upper quartile - lower quartile) | | | | | | |
| Dyspnea (Borg (CR10) scale) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | **0.042*** | 0.103[b] |
| Breathing effort (Borg (CR10) scale) | 0 (0-0) | 0 (0-0.5) | 0 (0-0) | 0 (0-0) | 0 (0-0) | **0.001*** | 0.190[b] |
| Oxygen saturation (%) | 98.0 (97.3-98.0) | 98.0 (97.0-98.0) | 98.0 (97.0-98.0) | 98.0 (97.0-98.0) | 98.0 (97.0-98.0) | 0.687 | 0.024[b] |
| Systolic blood pressure (mmHg) | 118 (108-128) | 112 (97-126) | 111 (105-122) | 108 (96-119) | 108 (99 -126) | | |
| Change in systolic blood pressure (mmHg) while resting and wearing a mask five minutes | 0 (0-0) | -1.50 (-6.00-2.75) | -3.50 (-8.25-1.75) | -2.50 (-8.50-3.50) | -5.00 (-8.00-0.25) | 0.176 | 0.066[b] |
| Diastolic blood pressure (mmHg) | 74 (66-80) | 72 (67-77) | 72 (66-79) | 70 (66-77) | 72 (64-78) | 0.268 | 0.054[b] |
| | mean±SD | | | | | | |
| Heart rate (bpm) | 78±11 | 78±10 | 80±10 | 78±10 | 77±10 | 0.345 | 0.047[a] |

*Note: One-way repeated ANOVA reported with mean±SD, Friedman test reported with median (upper-lower quartile). *Statistically significant indicating that at least one pair of masks is significantly different (p≤0.05), [a] Effect size estimates of one-way repeated ANOVA are Partial Eta Squared ($\eta^2$) = SSeffect / SSeffect + SSerror; $\eta^2$ =0.01 indicate small effect, $\eta^2$ =0.06 indicate medium effect and $\eta^2$ =0.14 indicate large effect, [b] Effect size estimates of the Friedman test is Kendall's W value (W) = $\chi 2$ / N(K-1); w =0.10 indicates a small effect, w =0.30 indicates a medium effect, and w =0.50 indicates a large effect.*

**Table 3** Physiological variables at the end of 6MWT.

| Outcome | No mask | N95 | Surgical | Cloth | $PM_{2.5}$ | *p* value | Effect size |
|---|---|---|---|---|---|---|---|
| | median (upper quartile - lower quartile) | | | | | | |
| Dyspnea(Borg (CR10) scale) | 0.50 (0.50-1.75) | 2.00 (0.50-3.00) | 1.50 (0.63-3.00) | 1.00 (0.50-2.00) | 1.00 (0.50-2.75) | | |
| Change in dyspnea (Borg (CR10) scale) during wearing a mask for five minutes and the at the end of test | 0.50 (0.50-1.75) | 1.00 (0.50-2.75) | 2.00 (0.50-3.00) | 1.00 (0.50-2.00) | 1.00 (0.50-2.00) | **0.026*** | 0.115[b] |
| Breathing effort (Borg (CR10) scale) | 0.50 (0-1.00) | 0.50 (0-2.00) | 1.50 (0.50-2.75) | 0.50 (0-2.00) | 0.50 (0-1.00) | | |
| Change in breathing effort (Borg (CR10) scale) during wearing a mask for five minutes and at the end of test | 0.50 (0.00-1.00) | 1.00 (0.50-2.00) | 0.50 (0.00-2.00) | 0.50 (0.00-1.88) | 0.50 (0.00-1.00) | **0.031*** | 0.011[b] |
| Oxygen saturation (%) | 98.0 (97.0-98.0) | 98.0 (97.0-98.0) | 98.0 (97.0-98.0) | 98.0 (97.0-98.0) | 97.0 (96.3-98.0) | 0.202 | 0.062[b] |
| Systolic blood pressure (mmHg) | 140.13±22.85 | 140.88±21.18 | 143.38±22.66 | 135.75±17.93 | 136.83±20.54 | | |
| Change in SBP (mmHg) during wearing a mask for 5 minutes and the end of test | 18.50 (10.25-30.75) | 22.50 (18.25-42.00) | 26.00 (15.25-41.25) | 20.50 (13.50-37.75) | 24.00 (14.50-37.75) | **0.028*** | 0.113[b] |
| | mean±SD | | | | | | |
| Heart rate (bpm) | 112±20 | 115±19 | 116±17 | 114±17 | 112±18 | 0.479 | 0.033[a] |
| Diastolic blood pressure (mmHg) | 76±10 | 78±9 | 77±8 | 76±10 | 77±10 | 0.533 | 0.031[a] |
| HRR1 (bpm) | 18±9 | 21±7 | 20±7 | 20±7 | 19±11 | 0.427 | 0.039[a] |
| HRR2 (bpm) | 25±10 | 26±9 | 27±10 | 26±9 | 27±14 | 0.099 | 0.084[a] |
| 6MWT distance (meter) | 509.86±63.15 | 502.08±66.75 | 507.75±62.79 | 506.55±61.58 | 508.39±61.60 | 0.715 | 0.018[a] |
| Estimated $VO_2$ max (ml/kg/min) | 34.84±5.60 | 34.66±5.40 | 34.79±5.55 | 34.77±5.29 | 34.81±5.43 | 0.715 | 0.018[a] |

*Note: HRR: heart rate recovery, HRR1: heart rate at the end of 6MWT - heart rate after 1 minute, HRR2: heart rate at the end of 6MWT - heart rate after 2 minutes. One-way repeated ANOVA reported with mean±SD, Friedman test reported with median (upper-lower quartile). *Statistically significant indicating that at least one pair of masks is significantly different (p≤0.05), [a] Effect size estimates of one-way repeated ANOVA are Partial Eta Squared ($\eta^2$) = SSeffect / SSeffect + SSerror; $\eta^2$ =0.01 indicate small effect, $\eta^2$ =0.06 indicate medium effect and $\eta^2$ =0.14 indicate large effect, [b] Effect size estimates of the Friedman test is Kendall's W value (W) =$\chi 2$ / N(K-1); w =0.10 indicates a small effect, w =0.30 indicates a medium effect, and w =0.50 indicates a large effect.*

There was no difference between the delta changes of dyspnea between walking trials, according to the post hoc analysis value obtained from the Friedman test ($p$>0.005) (Figure 1).

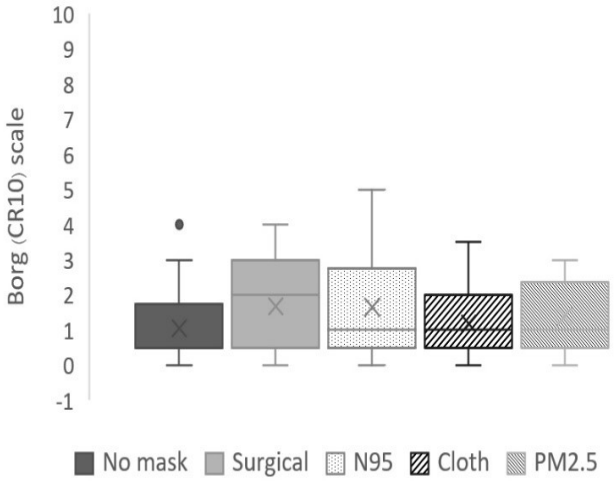
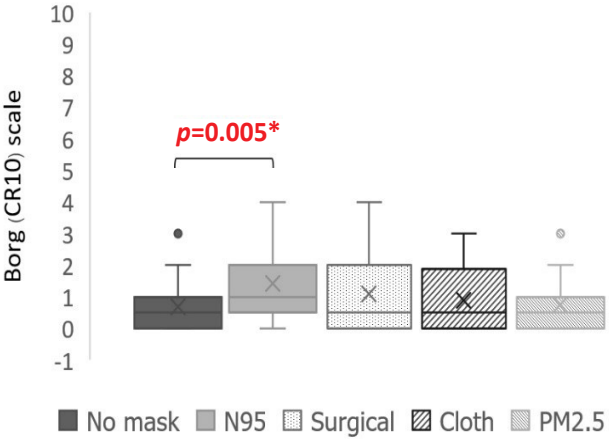



***Figure 1*** *Post hoc analysis of delta changes of dyspnea.*

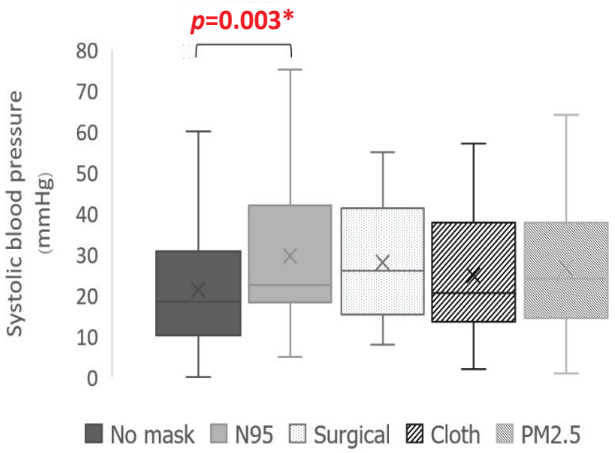
There was a statistically significant difference in the delta changes of breathing effort between the trials with no mask and an N95 mask ($p$=0.005) (Figure 2).




* Bonferroni-adjusted significance level $p$≤0.005 indicate a statistically significant difference between the two-walking trial, which was obtained from the Friedman test.

***Figure 2*** *Post hoc analysis of delta changes of breathing effort.*

There was a statistically significant difference in the delta changes of SBP score between the trials with no mask and an N95 mask ($p$=0.003) (Figure 3).




* Bonferroni-adjusted significance level $p$≤ 0.005 mean there is a statistically significant difference between the two-walking trial, which was obtained from the Friedman test.

***Figure 3*** *Post hoc analysis of delta changes of systolic blood pressure.*

**The satisfaction level of wearing a mask**

In summary of supplementary Table S1, $PM_{2.5}$ mask received the highest comfort satisfaction rating (4.8±0.4), while the N95 respirator and the cloth mask were rated the lowest (both are 4.1±1.2). The N95 respirator and surgical mask received the highest score for face and mouth fit (4.7±0.7 and 4.7±0.6, respectively), while the cloth mask received the lowest rating (3.6±1.3). The highest scores in breathability were for the $PM_{2.5}$ mask (4.5±0.8), while the lowest score was for the N95 respirator (3.8±0.9). $PM_{2.5}$ mask received the highest score for heat-cooling category (4.3±0.8), while the N95 respirator was rated the lowest (3.4±1.1). In the category of confidence, the surgical mask and $PM_{2.5}$ mask received the highest scores (4.7±0.6 and 4.7±0.7, respectively), while cloth mask received the lowest (4.1±1.2). Finally, N95 respirator received the highest ratings in the appearance category (4.7±0.7), while cloth mask received the lowest (3.8±1.3).

## Discussion

The main findings indicated that, there were no statistically significant differences in most physiological parameters between walking trials while wearing different masks, but statistically significant differences were found for dyspnea, breathing effort, and SBP ($p$≤0.05) (Table 3). Wearing an N95 mask resulted in higher breathing effort and SBP compared to no mask ($p$=0.005 and 0.003, respectively) (Figure 2 and Figure 3). While post hoc analysis could not detect a significant difference in delta changes in dyspnea between walking trials ($p$>0.005) (Figure 1), wearing a surgical mask tended to increase dyspnea compared to wearing other types of masks (Table 3, median 2.0 (0.5-3.0)).

The N95 respirator and surgical mask are effective at preventing the spread of infection. However, the characteristics of these masks (such as being made of nano-micro polypropylene, having shapes designed to fit the mouth, and having multiple layers) result in limited ventilation.[8, 23] Wearing these masks while doing moderate-intensity exercise may have an effect on pre-aging individuals. As a result, type of mask used should be determined by the activity and situation.

**Effects of physiologic variables at rest**

At rest, most of the variables were not significantly different between the walking trials ($p$>0.05) (Table 1), with SBP being the exception ($p$=0.006) (Table 1). SBP can vary from 2.5 to 10 mmHg,[24] depending on various factors, e.g., deterioration of the cardiovascular system at pre-aging that could impair SBP adaptation.[13] In this study, the greatest difference in median score of SBP between trials was 7 mmHg. Consequently, these differences were considered normal body adaptations and had no clinical significance.

**Effects of physiologic variables after wearing a mask for five minutes**

After wearing mask for five minutes, significant differences in dyspnea and breathing effort were found ($p$=0.042 and 0.001, respectively) (Table 2). However, post hoc analysis could not identify the pairs of masks that differed ($p$>0.005) (Table 2) and the median scores were not different among the five trials (dyspnea and breathing effort score=0) (Table 2). Hence, these statistical differences were not clinically significant. Therefore, wearing the masks for five minutes at rest does not seem to change any physiological parameters.

**Effects of different mask types during 6MWT on dyspnea**

At the end of 6MWT, the dyspnea scores were significantly different ($p$=0.026) (Table 3). However, due to a small effect size, post hoc analysis could not identify the pairs of masks that resulted in different scores ($p$>0.005) (Figure 1). Even so, since the change in dyspnea exists with minimal clinically important difference (MCID) (≥1 on the Borg CR 10 scale).[25] It could be implied that wearing a surgical mask while performing moderate-intensity activities resulted in higher dyspnea compared to other masks. Moreover, wearing an N95 mask while performing moderate-intensity activities resulted in higher levels of dyspnea than no mask. This finding was consistent with the meta-analysis of Shaw *et al*.[26] and other studies in healthy adults.[18] The characteristics of an N95 respirator and surgical mask (such as material, shapes, and number of layers) are high-filtration effective. However, while performing tasks of moderate intensity, the characteristics of the mask can result in decreased ventilation and impaired gas exchange.[8, 23, 27] Furthermore, it may cause more dyspnea than a cloth and $PM_{2.5}$ mask.

Surprisingly, the N95, which has higher carbon dioxide rebreathing, was found to result in less dyspnea compared to the surgical mask.[27] It is possible that this is the case because some N95 respirators use an electrostatically charged filter to increase filtration effectiveness by trapping microorganisms on the fiber wall. As a result, the manufacturer can design a more open structure without having to reduce the fiber size, maintaining the N95 respirator's good breathability.[8, 28] Additionally, while the surgical mask has a lower fiber resolution and thickness than the N95 respirator, it cannot form 3D shapes like the N95 respirator, therefore, the fiber might be sucked into the nose and mouth while deeply inhaling, resulting in the possibility of increased dyspnea.[23, 29] In contrast, the higher open space of N95 expands the respiratory area while decreasing the velocityof airflow entering the respiratory tract resulting in increased ventilation and gas exchange.[3]

**Effects of different mask types during 6MWT on breathing effort**

The result indicated that wearing an N95 respirator while performing moderate-intensity activities resulted in higher levels of breathing effort than wearing no mask (Figure 2). This was consistent with a study in healthy young adults by Dacha *et al*.[18] However, despite being statistically significant, there were still no clinically significant effects (<1 on the Borg scale).

Dyspnea has no direct effect on breathing effort.[31] Breathing effort is caused by the perceived mechanism of respiratory loading and capacity imbalance, which is the normal respiratory response to an increase in physical activity or a limit on ventilation.[32,33] On the other hand, in low-intensity activities carried out by healthy adult, dyspnea is uncommon[34-35] because a normal respiratory function is capable of balancing receptor afferent feedback and neural respiratory drive.[32-33] However, the increasing the neural respiratory drive for this study may come from mechanical factors (such as increasing the intensity or duration of exercise) and chemical factors (such as a ventilation-perfusion mismatch and carbon dioxide rebreathing due to wearing a mask), both of which result in dyspnea.[35]

The shapes, structures, and materials of the mask could result in increased dyspnea and breathing effort. For example, the N95 respirator is made from nanometer to micrometer fibers of polypropylene and has a shape that fits closely to the face,[8, 23] which may lead to limited ventilation[8] and carbon dioxide rebreathing.[27] This was consistent with the lowest satisfaction scores for breathability, comfort, and heat-cooling (Supplementary Table S1).

**Effects of different mask types during 6MWT on SBP**

Wearing an N95 respirator during 6MWT resulted in higher levels of SBP than wearing no mask ($p$=0.003) (Figure 3). Similarly, Cano Carrizal's study[36] found that wearing a surgical mask while performing the Bruce protocol caused a higher level of SBP than wearing no mask. It is possible that the shape and material of the mask cause retention and rebreathing of carbon dioxide.[27] The high level of carbon dioxide stimulates the sympathetic nervous system in the brainstem, causing vasoconstriction and a rise in SBP.[37] The high level of carbon dioxide in the blood can induce a feeling of discomfort and breathlessness which was consistent with the above discussion about

dyspnea and breathing effort. Also, the mask-wearing satisfaction questionnaire results indicated that the N95 respirator scored lowest for comfort and breathability (Supplementary Table S1).

### Effects of different mask types during 6MWT on oxygen saturation

Wearing different types of masks did not affect oxygen saturation ($p$>0.05) (Table 3), which was consistent with the previous studies except for a study by Salles *et al*.[38] in post COVID-19 patients.[18,26] In general, oxygen saturation may decrease after activity or exercise,[39] especially when the exercise is prolonged until $VO_2$max is attained.[40] However, since the level of exercise in this study was not extreme, most participants were still able to maintain a good oxygen transport balance. As a result, oxygen saturation was only slightly reduced and not clinically significant (Table 3), causing only a short-term effect.

### Effects of different mask types during 6MWT on functional capacity (six-minute walk distance and estimated $VO_2$max)

Indirect maximum oxygen consumption (estimated $VO_2$max) was calculated. There were no statistically significant differences ($p$>0.05) (Table 3) in walking distance and $VO_2$max, which was consistent with the previous studies on the effects of mask wearing on 6MWT.[15,18,26] The 6MWT is a submaximal exercise test that measures functional capacity. Therefore, this test could not directly represent cardiorespiratory endurance. However, evidence suggests that wearing an N95 respirator or surgical or cloth mask during cardiopulmonary exercise tests (CPET) can lower the maximum oxygen consumption ($VO_2$max), heart rate, and exercise time.[10, 41-42]

### Strengths, limitations, and recommendations

A key strength of this study was that the research questions were relevant to current problems. The prospective crossover trials on the same participant eliminate the interference effect of between-group variance. However, the post hoc analysis could not determine the pair of masks that differ, indicating a small sample size. Therefore, the result should be interpreted with caution. Future studies should investigate a larger sample size and consider the effect of activity duration if the activity is performed for more than 6 minutes.

### Clinical implications

Pre-aging individuals should choose an appropriate mask for each activity and situation. For example, wearing an N95 mask is tolerable when performing low-intensity activities. However, when performing moderate to high-intensity activities, wearing other masks might be a better option to reduce the impact of dyspnea and breathing effort. Furthermore, masks should not be worn while performing vigorous activities.

## Conflict of interest

All authors declare that they have no conflicts of interest that are relevant to the publication of this research.

## Conclusion

For pre-aging individuals, wearing various types of face masks during moderate-intensity activity did not result in differences in blood oxygen saturation and functional capacity. However, wearing an N95 mask during moderate-intensity activity resulted in higher levels of dyspnea, breathing effort, and systolic blood pressure than wearing no mask. In addition, wearing a surgical mask during moderate-intensity activity may result in higher dyspnea than wearing no mask, a cloth mask, or a $PM_{2.5}$ mask.

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**Supplementary Tables**

The scoring criteria ranged from 1 to 5, with a score of 1 being the lowest level of satisfaction and a score of 5 being the highest level of satisfaction. Table S1 shows the mean and standard deviation of the satisfaction scores of masks.

**Table S1** Satisfaction when wearing each type of mask.

| Categories | Satisfaction level (mean±SD) | | | |
|---|---|---|---|---|
| | Surgical | N95 | Cloth | $PM_{2.5}$ |
| (1) Comfort | 4.6±0.7 | 4.1±1.2 | 4.1±1.2 | 4.8±0.4 |
| (2) Fit for the face and mouth | 4.7±0.6 | 4.7±0.7 | 3.6±1.3 | 4.6±0.9 |
| (3) Breathability | 4.4±0.8 | 3.8±0.9 | 4.3±1.1 | 4.5±0.8 |
| (4) Heat-Cooling | 4.1±0.9 | 3.4±1.1 | 4.0±1.2 | 4.3±0.8 |
| (5) Confidence | 4.7±0.6 | 4.6±0.7 | 4.1±1.2 | 4.7±0.7 |
| (6) Appearance | 4.5±0.7 | 4.7±0.7 | 3.8±1.3 | 4.5±0.7 |

The participants ranked their satisfaction with the four types of masks by filling in numbers 1 to 4. Number one indicated the most satisfied of all groups of masks, and four indicated the least satisfied mask. The sequence of satisfaction was presented by number and the percentage of participants rating on each mask in Table S2.

**Table S2** Masks ranked in order of satisfaction.

| Title | order | Satisfaction (number (%)) | | | |
|---|---|---|---|---|---|
| | | Surgical | N95 | cloth | $PM_{2.5}$ |
| (1) Comfort | 1 | 6 (25.0%) | 4 (16.7%) | 5 (20.8%) | 9 (37.5%) |
| | 2 | 11 (45.8%) | 4 (16.7%) | 4 (16.7%) | 5 (20.8%) |
| | 3 | 7 (29.2%) | 7 (29.2%) | 4 (16.7%) | 6 (25.0%) |
| | 4 | 0 (0%) | 9 (37.5%) | 11 (45.8%) | 4 (16.7%) |
| (2) Fit for the face and mouth | 1 | 7 (29.2%) | 9 (37.5%) | 2 (8.3%) | 6 (25.0%) |
| | 2 | 7 (29.2%) | 5 (20.8%) | 6 (25.0%) | 6 (25.0%) |
| | 3 | 10 (41.7%) | 4 (16.7%) | 3 (12.5%) | 7 (29.2%) |
| | 4 | 0 (0%) | 6 (25.0%) | 13 (54.2%) | 5 (20.8%) |
| (3) Breathability | 1 | 3 (12.5%) | 4 (16.7%) | 9 (37.5%) | 8 (33.3%) |
| | 2 | 11 (45.8%) | 1 (4.2%) | 6 (25.0%) | 6 (25.0%) |
| | 3 | 10 (41.7%) | 3 (12.5%) | 3 (12.5%) | 8 (33.3%) |
| | 4 | 0 (0%) | 16 (66.7%) | 6 (25.0%) | 2 (8.3%) |
| (4) Heat-Cooling | 1 | 2 (8.3%) | 3 (12.5%) | 9 (37.5%) | 10 (41.7%) |
| | 2 | 11 (45.8%) | 0 (0%) | 6 (25.0%) | 7 (29.2%) |
| | 3 | 10 (41.7%) | 4 (16.7%) | 6 (25.0%) | 4 (16.7%) |
| | 4 | 1 (4.2%) | 17 (70.8%) | 3 (12.5%) | 3 (12.5%) |
| (5) Confidence | 1 | 11 (45.8%) | 6 (25.0%) | 1 (4.2%) | 6 (25.0%) |
| | 2 | 9 (37.5%) | 3 (12.5%) | 3 (12.5%) | 9 (37.5%) |
| | 3 | 3 (12.5%) | 5 (20.8%) | 8 (33.3%) | 8 (33.3%) |
| | 4 | 1 (4.2%) | 10 (41.7%) | 12 (50.0%) | 1 (4.2%) |
| (6) Appearance | 1 | 3 (12.5%) | 10 (41.7%) | 0 (0%) | 11 (45.8%) |
| | 2 | 14 (58.3%) | 3 (12.5%) | 3 (12.5%) | 4 (16.7%) |
| | 3 | 6 (25.0%) | 5 (20.8%) | 6 (25.0%) | 7 (29.2%) |
| | 4 | 1 (4.2%) | 6 (25.0%) | 15 (62.5%) | 2 (8.3%) |

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# Nasalance scores of Thai cleft palate children using the Thai Simplified Nasometric Assessment Procedures Test (Thai SNAP Test)

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**ABSTRACT**

**Background:** Nasometry in children with cleft lip and/ or palate (CLP) aged 4-6 years is necessary for diagnosis and planning treatments to promote decreasing velopharyngeal insufficiency (VPI) or resonance disorders that affect good speech intelligibility, prevent compensatory errors and other speech and language problems. Most Thai nasometric speech stimuli are passages suitable for literate patients. Using nasometric passages in young children who cannot read takes a long time to complete and gives unreliable nasalance scores. Due to the limitations, The Thai Simplified Nasometric Assessment Procedures Test (the Thai SNAP Test) was developed and assessed for validity and reliability, revealing that the Thai SNAP Test is proper for evaluating the speech resonance of illiterate patients. However, there is no study on nasometry in children with repaired cleft lip and/ or palate (RCLP) using the Thai SNAP Test.

**Objectives:** To study nasalance scores between the control (non-cleft) group and the RCLP group assessed by the Thai SNAP Test and to describe the influential factors that affected nasalance scores.

**Materials and methods:** The subjects were Thai children aged 4-7. The two groups of children were the RCLP and the control groups, and 36 children in each group. Nasalance scores were measured by a Nasometer II (model 6450). The child was asked to repeat 25 speech stimuli from the Thai SNAP Test, and then the scores were computed using a t-test or Mann-Whitney U test, depend on data distribution. The mean difference in nasalance scores between the two groups and the 95% Confident Interval (95% CI) were analyzed by the two-sample t-test with equal variances and the bootstrap confidence interval method, respectively.

**Results:** The nasalance scores of the RCLP group were significantly higher than the control group ($p<0.05$) when using high-pressure oral speech stimuli. However, using nasal speech stimuli, the RCLP group's nasalance scores were significantly lower than the control group ($p<0.05$), except for nasal syllable repetition (/na/ and /ni/), which did not find a significant difference ($p\geq0.05$). This study emphasized that influential factors for the difference in the nasalance scores between the two groups were abnormal structures and functions articulators, especially the velopharyngeal port that was affected by the CLP, which caused resonance disorders, misarticulations, voice disorders, obstruction in the vocal tract, and hearing impairment. However, the phonological features used in the speech stimulus caused the difference in the mean nasalance scores of the same group.

**Conclusion:** The trends in nasalance scores suggested that the Thai SNAP Test could identify speech resonance disorders in Thai children aged 4-7. The speech and language pathologist (SLP) or evaluator should consider factors influencing the nasalance scores. For accurately diagnosing or evaluating the progression of treatments, nasalance scores from Nasometer should be applied together with other information from various methods or instruments.

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## Introduction

Nasometry is one of the most popular methods of speech resonance assessment. Nasometry is a non-invasive instrument that measures the acoustic energy from oral and nasal cavities; then, a microcomputer in a nasometer computes the data and gives numerical data called nasalance scores. Nasalance scores are the results of oral acoustic energy divided by oral and nasal acoustic energy and then multiplied by 100, shown in the percentage. The mean nasalance scores were used to represent the scores of each speech stimulus.[1]

Pediatric nasometry is essential and needs reliable results for accurate diagnosis and treatment. However, speech and language pathologists (SLPs) frequently confront problems while assessing nasalance scores. One of the critical problems is nasometric passages, which are long and composed of complex words that are hard to understand and produce correctly, causing an extended period of nasometry and resulting in unreliable nasalance scores.[1,2] Passages and duration of nasometry are inappropriate for illiterates, patients who cannot tolerate wearing a headset, or with limited attention spans.[1]

From these limitations, Kummer modernized the SNAP Test, named the MacKay-Kummer Simplified Nasometric Assessment Procedures Test Revised 2005 (the SNAP Test-R), and re-normed using a Nasometer II in 246 American children without speech and language problems aged 3-9 years.2 Because the SNAP test-R is easy to use, gives reliable results, does not require reading skills, and can select some items, so a patient can produce and relate to suspected resonance problems.[1,2] The Test was adapted to other languages, such as Turkish, Persian, and Thai.[3-5]

In Thai, Liadprathom developed the Thai Simplified Nasometric Assessment Procedures Test (Thai SNAP Test) based on the SNAP Test-R, including speech sound selection, assessment processes, and instructions.[5,2] Consonants selection considered the sound acquisition of Thai children.[6] The content validity and reliability of Thai SNAP test were analyzed in 51 children aged 4-6 years who speak central Thai and have age-appropriate speech and language development. The results showed excellent accuracy and a high correlation coefficient of test-retest reliability, indicating that Thai SNAP Test is valid for evaluating speech resonance in children aged 4-6 years. With a test of 2 subtests (25 speech stimuli), Thai SNAP Test includes the syllable repetition/ prolonged sound subtest I and the picture-cued subtest II.[5]

The literature review found that many studies compared the nasalance scores of the RCLP and the non-cleft groups using the SNAP Test or simple nasometric speech stimuli (syllables, words, or simple sentences). Most studies reported that the scores of the RCLP group were significantly higher than those of the control group when using oral speech stimuli but could not find a significant difference when using nasal speech stimuli [7-10] and concluded that factors that influenced the nasalance scores are children with RCLP have abnormal structures and functions of articulators, misarticulations,[11] voice disorders,[12,13] obstructions in the vocal tract,[14]snoring,[15,16] hearing impairment,[17,18] and phonological features.[8,9] Many studies on nasalance scores of children with RCLP compared with the non-cleft group, but no research on nasalance scores in children with RCLP using Thai SNAP Test. This research aims to study the nasalance scores and factors affecting nasalance scores in Thai children aged 4-7 assessed with Thai SNAP Test.

## Materials and methods

### *Subjects*

The subjects include group of children with RCLP and typically participants. The RCLP group is patients in the Speech and Language Clinic or who visit plastic surgeons at the Cleft Clinic. The control group is children without CLP who attended the Well-baby Clinic or came to Ramathibodi Hospital. Research participants are boys and girls, aged 4-7 years, with no age- and gender-matched between groups, and all 72 use the central Thai language. Children were excluded if they met one of the exclusion criteria: a child's parent refusing to participate in the research, children have any abnormalities (visual impairments, neurological conditions, syndromes), or on the day of data collection, they have upper respiratory tract infection (URI), e.g., cold and asthma, have uncooperative behavior.

### *Data collection and analysis*

Data collection was provided at Ramathibodi Hospital from October 2018 to September 2019 after receiving the Certificate of Approval (Protocol Number 07-61-45) from a full board of the Human Research Ethics Unit, the Faculty of Medicine Ramathibodi Hospital.

Before starting nasometry each day, the nasometer (Nasometer II model 6450; Kay Elemetrics Corporation, Lincoln Park, NJ, USA) was calibrated according to the manufacturer's standard calibration procedure[19] in a quiet room. After receiving consent, the child's medical, health information, hearing ability, and language-speech development were taken. The screening test began with the oral-peripheral examination, language skills were evaluated by the language developmental screening test for diagnostic and treatment plans[20], and speech skills were assessed by asking a child to repeat four sentences from the Thai articulation screening test[21]. The child's visual and hearing abilities were observed while evaluating language and speech skills to confirm that all children have sufficient visual and hearing capabilities for nasometry.

The child was trained to produce all 25 items from Thai SNAP Test with actual loudness and speech rate. Nasalance scores were assessed by asking the child to repeat random speech stimuli after the instructor. If the child made a mistake or a long pause had to skip to another item and re-evaluate this item later. In case of the child was repeatedly misarticulated or unsure about speech sounds, visual and auditory cues were given to inform or correct each sound's production and to ensure that the child knew the right target sound.

Data were analyzed by a statistical software package Stata (version 16.0): the mean, SD, median, and minimum and maximum nasalance scores of each speech stimulus

were calculated, then independently compared the mean nasalance scores between the control and the RCLP groups following this condition: normal distributions were examined by the t-test, and the non-normal distributions were calculated by Mann-Whitney U test. The mean differences in nasalance scores between the two groups were analyzed using the two-sample t-test with equal variances. The bootstrap confidence interval method was used to calculate the 95% Confident Interval (95% CI).

## Results

Table 1 presents the age range of subjects in this study from 4-7 years, with a mean age of 5 years and 5 months. Both groups have more girls than boys. All non-cleft children in the control group had typical structures and functions of articulators and did not have speech-language and hearing problems that influenced nasalance scores. In contrast, children in the RCLP group have defective structures and functions of articulators due to the CLP, which were influential factors that affected the nasalance scores, including cleft lip and/ or palate, oronasal fistula (ONF), abnormal structure and function of velum, missing teeth/ malocclusion, obstructions in the vocal tract, and snoring. Moreover, children with CLP also led to speech-language and hearing problems, such as misarticulations, resonance disorders, nasal air emission (NAE), voice disorders, language-delayed development, and middle ear diseases.

Table 2 shows that most children with RCLP have hypernasality at least in one speech sound, and only 3 children with hyponasality that were detected while screening the speech skills. In addition, children with RCLP have nasal air emissions that can occur with hypernasality or compensatory errors and may cause higher nasalance scores. Nasal grimaces were found in 5 children with RCLP, which present excessive acoustic energy in the nasal cavity. The results of the articulation screening test showed that each child with RCLP had more than an error, finding that compensatory errors were as many as phonological disorders. The most common compensatory errors are nasalization, pharyngeal stops, and glottal fricatives, respectively. Children with RCLP frequently use substitutions for the target sound, such as backing, stopping, and gliding. In addition to substitution, also found distortion and omission. Children with RCLP who always use compensatory laryngeal adjustments to complete the velopharyngeal port led to voice disorders, another common speech problem in the RCLP group; this study found these problems characterized by hoarseness, breathiness, low pitch, or inconsistent loudness.

**Table 1** General data and oral-peripheral examination results.

| Variables | Control group (N=36) | RCLP group (N=36) |
|---|---|---|
| Age (years) | 5.5 (4.1, 6.8) | 5.5 (4.0, 7.0) |
| Gender | | |
| - Boy | 11 (30.56%) | 13 (36.11%) |
| - Girl | 25 (69.44%) | 23 (63.89%) |
| Cleft type | | |
| - Cleft palate | - | 13 (36.11%) |
| - Cleft lip and palate | - | 23 (63.89%) |
| Oronasal fistula (ONF) | - | 15 (41.67%) |
| Size of ONF | | |
| - Small (<2 mm) | - | 10 (27.78%) |
| - Medium (3-5 mm) | - | 4 (11.11%) |
| - Large (> 5 mm) | - | 1 (2.78%) |
| Site of ONF | | |
| - Junction soft/ hard palate | - | 9 (25%) |
| - Hard palate | - | 3 (8.33%) |
| - Lingual alveolar | - | 3 (8.33%) |
| Structure and function of velum | | |
| - Short velum | - | 34 (94.44%) |
| - Poor velar movement | - | 28 (77.78%) |
| Class III malocclusion | - | 27 (75%) |
| Missing upper and/or lower central incisors | 3 (8.33%) | 6 (16.67%) |
| Abnormal nose structure | - | 21 (58.33%) |
| - Cleft nose | - | 16 (44.44%) |
| - Cleft nose with nostril stenosis | - | 5 (13.89%) |
| Snoring problem | - | 12 (33.33%) |

Moreover, the language screening test showed that children with RCLP have language-delayed development with a limited vocabulary range, use simple sentence structures, or have less detail in the description. More than half of the children in the RCLP group have middle ear diseases, which the chronic middle ear diseases can lead to various types and degrees of hearing loss. This study found that some children with RCLP had unilateral moderately severe sensorineural hearing loss (the worse-hearing ear has hearing loss between 56 and 60 dB in speech frequencies), which may cause misarticulations in high-pressure oral consonants, especially fricatives and affricates by substitution, distortion, and omission, respectively. In this case, visual and auditory cues were

**Table 2** Numbers of speech-language and hearing problems assessed by the screening test.

| Variables | Control group (N=36) | RCLP group (N=36) |
|---|---|---|
| Resonance disorders | - | 33 (91.67%) |
| - Mild hypernasality | - | 13 (36.11%) |
| - Moderate hypernasality | - | 12 (33.33%) |
| - Severe hypernasality | - | 5 (13.89%) |
| - Hyponasality | - | 3 (8.33%) |
| *Nasal air emission (NAE)* | - | 16 (44.44%) |
| - Nasal rustle with hypernasality | - | 10 (27.78%) |
| - Visible NAE with hypernasality | - | 6 (16.67%) |
| *Nasal grimace* | - | 5 (13.89%) |
| Misarticulations | - | 33 (91.67%) |
| *Compensatory errors* | | |
| - Nasalization | - | 24 (66.67%) |
| - Pharyngeal stops | - | 17 (47.22%) |
| - Glottal fricatives | - | 14 (38.89%) |
| - Posterior nasal fricatives | - | 13 (36.11%) |
| - Pharyngeal fricatives | - | 13 (36.11%) |
| - Double glottal stops | - | 12 (33.33%) |
| - Mid-dorsum palatal stops | - | 9 (25%) |
| - Glottal stops | - | 8 (22.22%) |
| - Weak consonants | - | 5 (13.89%) |
| *Phonological errors* | | |
| - Distortion | - | 17 (47.22%) |
| - Omission | - | 11 (30.56%) |
| - Backing | - | 8 (22.22%) |
| - Stopping | - | 6 (16.67%) |
| - Gliding [j, w /l, r] | - | 6 (16.67%) |
| - Fronting | - | 5 (13.89%) |
| - Affrication | - | 5 (13.89%) |
| - Deaffricates | - | 5 (13.89%) |
| - Labialization | - | 4 (11.11%) |
| - Gliding of affricates [j, w/tç$^{h}$, tç] | - | 2 (5.56%) |
| - Gliding of fricatives [j, w/s, f] | - | 2 (5.56%) |
| - Vowel substitution | - | 2 (5.56%) |
| *Voice disorders* | - | 11 (30.56%) |
| *Delayed language development* | - | 4 (11.11%) |
| *Middle ear diseases and hearing impairment* | - | 21 (58.33%) |
| - Serous otitis media (SOM) | - | 13 (36.11%) |
| - SOM with unilateral mild to moderate conductive hearing loss (hearing loss between 30 and 55 dB in the speech frequencies in the worse-hearing ear) | - | 6 (16.67%) |
| - SOM with unilateral mild to moderately severe sensorineural hearing loss (hearing loss between 35 and 60 dB in the speech frequencies in the worse-hearing ear) | - | 2 (5.56%) |

used to prevent misunderstanding the target sounds, which may cause lower speech sound production accuracy.

***Nasalance scores of the control and the RCLP groups***

Nasalance scores from most speech stimuli were non-normal distributions. The normal distributions were found in data from nasal syllable repetition (/ma/ and /na/), prolonged /i/, and nasal sentences.

Table 3 showed that the nasalance scores of the RCLP group were significantly higher than the control group in all speech stimuli ($p$<0.05) with exception nasal syllables (/ma/, /ŋa/, /mi/, and /ŋi/), prolonged /m/, and nasal sentences, the scores of the RCLP were significantly lower than the control group ($p$ < 0.05). However, when data was assessed with nasal syllable repetition (/na/ and /ni/), the scores of the RCLP group were lower than those of the control group, with no significant difference ($p$≥0.05).

This study found that phonological features are another factor influencing nasalance scores, such as the mean nasalance scores of syllables consisting of a high-pressure oral consonant and vowel /a/ were lower than those with vowel /i/ by approximately 10%. For nasal syllables found, trends of the mean nasalance scores of syllables composing a nasal consonant and vowel /a/ were lower than those with /i/ by approximately 20%. The highest mean nasalance scores of both groups came from sustained /m/, whereas the lowest mean nasalance scores of each group were different; the lowest of the control group came from prolonged /s/ (0%), but the lowest of the RCLP group came from high-pressure oral syllable repetition, including /$k^h$a/ and /t$ç^h$a/ (20.06%).

Nasalance scores of the RCLP and the control groups are close when assessed with nasal syllable repetition (/na/ and /ni/), which may result from 3 RCLP children with hyponasality. Therefore, subgroup analysis was provided by excluding 3 children with hyponasality. Hence, the RCLP group had 33 children left and 36 children in the control group. The results of the subgroup analysis are shown in Table 4.

**Table 3** Nasalance scores (%) of the control and the RCLP groups.

| Subtest | Speech stimuli | Mean±SD | | Median (min, max) | | Mean difference (95% CI) |
|---|---|---|---|---|---|---|
| | | Control group (N=36) | RCLP group (N=36) | Control group (N=36) | RCLP group (N=36) | |
| **Subtest I:** Syllable repetition/ Prolonged sound | 1. /$p^h$a/ | 7.56±2.12 | 21.36 ± 16.44 | 7 (4, 13) | 16 (5, 55) | 13.81 (8.3-19.32) |
| | 2. /$t^h$a/ | 7.53±1.75 | 21.39 ± 15.44 | 7 (5, 12) | 13 (5, 61) | 13.86 (8.7-19.03) |
| | 3. /$k^h$a/ | 7.44±1.5 | 20.06 ± 14.37 | 8 (4, 10) | 15 (4, 55) | 12.61 (7.81-17.42) |
| | 4. /sa/ | 7.81±2.32 | 24.06 ± 17.11 | 7 (5, 14) | 22 (5, 60) | 16.25 (10.51 - 22) |
| | 5. /t$ç^h$a/ | 7.94±2.62 | 20.06±14.8 | 7.5 (5, 17) | 14.5 (4, 54) | 12.11 (7.11-17.11) |
| | 6. /$p^h$i/ | 19.94±7.19 | 35.25±22.44 | 21 (8, 38) | 25 (6, 76) | 15.31 (7.47-23.14) |
| | 7. /$t^h$i/ | 18.22±5.72 | 36.69±22.17 | 18 (8, 29) | 31 (9, 75) | 18.47 (10.86-26.08) |
| | 8. /$k^h$i/ | 22.11±6.19 | 36.08±21.58 | 22.5 (11, 35) | 31 (7, 81) | 13.97 (6.51-21.44) |
| | 9. /si/ | 16.58±5.71 | 36.53±23.21 | 16 (8, 29) | 27.5 (6, 79) | 19.94 (12-27.89) |
| | 10. /t$ç^h$i/ | 18.78±5.18 | 35.97±21.33 | 19 (12, 30) | 30.5 (9, 77) | 17.19 (9.9-24.49) |
| | 11. /ma/† | 58.92±8.27 | 54.14±10.44 | 60.5 (41, 75) | 53.5 (33, 79) | -4.78 (-9.21- -0.35) |
| | 12. /na/*† | 56.47±9.07 | 53.58±10.95 | 58 (32, 77) | 55 (31, 79) | -2.89 (-7.61-1.84) |
| | 13. /ŋa/ | 63.44±8.03 | 54.58±11.96 | 65 (42, 76) | 55 (27, 82) | -8.86 (-13.65 - -4.07) |
| | 14. /mi/ | 80.47±6.53 | 74.06±10.65 | 82 (67, 90) | 77 (48, 90) | -6.42 (-10.57- -2.26) |
| | 15. /ni/* | 76.22±7.69 | 71.58±11.95 | 76.5 (51, 86) | 72.5 (49, 89) | -4.64 (-9.36-0.08) |
| | 16. /ŋi/ | 78.92±6.9 | 72.97±11.04 | 82 (64, 88) | 75.5 (49, 92) | -5.94 (-10.27 - -1.62) |
| | 17. prolonged /a/ | 14.53±14.48 | 24.86±14.37 | 7 (4, 52) | 23.5 (5, 59) | 10.33 (3.55-17.12) |
| | 18. prolonged /i/ † | 20.97±7.79 | 47.39±22.44 | 21 (8, 35) | 45 (9, 89) | 26.42 (18.52-34.31) |
| | 19. prolonged /s/ | 0±0 | 26.44±35.50 | 0 (0, 0) | 0 (0, 96) | 26.44 (14.64-38.25) |
| | 20. prolonged /m/ | 93.58±2.55 | 91.25±6.28 | 94 (86, 97) | 93 (60, 97) | -2.33 (-4.59- -0.08) |
| **Subtest II:** Picture-cued sentences | 21. Bilabial plosives | 10.08±3.73 | 27.39±20.4 | 10.5 (4, 19) | 19 (5, 73) | 17.31 (10.41-24.2) |
| | 22. Lingual-alveolar plosives | 9.31±2.95 | 23.97±17.34 | 9 (5, 18) | 17.5 (6, 62) | 14.67 (8.82-20.51) |
| | 23. Velar plosives | 9.5 ± 3.01 | 22.47±15.48 | 10 (5, 19) | 16.5 (5, 61) | 12.97 (7.73-18.21) |
| | 24. Sibilant fricatives | 9.67 ± 3.14 | 26.08±17.17 | 10 (5, 18) | 25.5 (5, 64) | 16.42 (10.61-22.22) |
| | 25. Nasals † | 56.69 ± 6.31 | 51.89±10.04 | 56 (47, 76) | 50 (32, 72) | -4.81 (−8.75- -0.86) |

***Note:*** **not significant, † t-test*

**Table 4** Subgroup analysis of nasalance scores (%) of the control and the RCLP groups.

| Subtest | Speech stimuli | Mean±SD | | Median (min, max) | | Mean difference (95% CI) |
|---|---|---|---|---|---|---|
| | | Control group (N=36) | RCLP group (N=33) | Control group (N=36) | RCLP group (N=33) | |
| **Subtest I:** Syllable repetition/ Prolonged sound | 1. /p$^{h}$a/ | 7.56±2.12 | 21.42±16.91 | 7 (4, 13) | 15 (5, 55) | 13.87 (8.2, 19.54) |
| | 2. /t$^{h}$a/ | 7.53±1.75 | 21.36±15.79 | 7 (5, 12) | 12 (5, 61) | 13.84 (8.55, 19.12) |
| | 3. /k$^{h}$a/ | 7.44±1.5 | 20.24±14.79 | 8 (4, 10) | 14 (4, 55) | 12.8 (7.85, 17.74) |
| | 4. /sa/ | 7.8±2.32 | 23.18±16.93 | 7 (5, 14) | 20 (5, 60) | 15.38 (9.69, 21.06) |
| | 5. /tç$^{h}$a/ | 7.94±2.62 | 20.55±15.32 | 7.5 (5, 17) | 15 (4, 54) | 12.6 (7.43, 17.78) |
| | 6. /p$^{h}$i/ | 19.94±7.19 | 36.42±22.85 | 21 (8, 38) | 26 (6, 76) | 16.48 (8.48, 24.48) |
| | 7. /t$^{h}$i/ | 18.22±5.72 | 38.03±22.45 | 18 (8, 29) | 38 (9, 75) | 19.81 (12.08, 27.53) |
| | 8. /k$^{h}$i/ | 22.11±6.19 | 37.27±21.91 | 22.5 (11, 35) | 36 (8, 81) | 15.16 (7.57, 22.76) |
| | 9. /si/ | 16.58±5.71 | 36.85±23.87 | 16 (8, 29) | 27 (6, 79) | 20.27 (12.09, 28.44) |
| | 10. /tç$^{h}$i/ | 18.78±5.18 | 36.7±21.93 | 19 (12, 30) | 26 (9, 77) | 17.92 (10.41, 25.43) |
| | 11. /ma/$^{\dagger}$ | 58.92±8.27 | 55.21±10.18 | 60.5 (41, 75) | 55 (33, 79) | -3.7 (-8.15-0.74) |
| | 12. /na/$^{*\dagger}$ | 56.47±9.07 | 54.64±10.65 | 58 (32, 77) | 56 (31, 79) | -1.84 (-6.58-2.91) |
| | 13. /ŋa/ | 63.44±8.03 | 55.64±11.79 | 65 (42, 76) | 56 (27, 82) | -7.81 (-12.62- -3) |
| | 14. /mi/ | 80.47±6.53 | 74.88±10.26 | 82 (67, 90) | 78 (48, 90) | -5.59 (-9.69- -1.5) |
| | 15. /ni/$^{*}$ | 76.22±7.69 | 72.82±11.47 | 76.5 (51, 86) | 74 (49, 89) | -3.4 (-8.06-1.25) |
| | 16. /ŋi/ | 78.92±6.9 | 73.67±10.94 | 82 (64, 88) | 76 (49, 92) | -5.25 (-9.61- -0.89) |
| | 17. prolonged /a/ | 14.53±14.48 | 25.55±14.68 | 7 (4, 52) | 26 (5, 59) | 11.02 (4-18.03) |
| | 18. prolonged /i/ $^{\dagger}$ | 20.97±7.79 | 48.61±22.67 | 21 (8, 35) | 46 (9, 89) | 27.63 (19.62-35.64) |
| | 19. prolonged /s/ | 0±0 | 28.3±36.46 | 0 (0, 0) | 0 (0, 96) | 28.3 (16.18-40.42) |
| | 20. prolonged /m/ | 93.58±2.55 | 91.24±6.52 | 94 (86, 97) | 93 (60, 97) | -2.34 (-4.68-0) |
| **Subtest II:** Picture-cued sentences | 21. Bilabial plosives | 10.08±3.73 | 28.45±20.87 | 10.5 (4, 19) | 20 (6, 73) | 18.37 (11.31-25.43) |
| | 22. Lingual-alveolar plosives | 9.31±2.95 | 24.85±17.72 | 9 (5, 18) | 18 (6, 62) | 15.54 (9.56, 21.52) |
| | 23. Velar plosives | 9.5±3.01 | 22.76±15.8 | 10 (5, 19) | 15 (6, 61) | 13.26 (7.9, 18.61) |
| | 24. Sibilant fricatives | 9.67±3.14 | 26.45±17.37 | 10 (5, 18) | 26 (5, 64) | 16.79 (10.91, 22.66) |
| | 25. Nasals $^{\dagger}$ | 56.69±6.31 | 52.85±9.91 | 56 (47, 76) | 51 (32, 72) | -3.85 (-7.8-0.11) |

***Note:*** **not significant, † t-test*

## Discussion

This study aims to apply the Thai SNAP Test to assess nasalance scores in Thai children with RCLP aged 4-7 years. Due to the limited sample size (36 children per group), therefore could not analyze nasalance scores separately to gender or age range. However, trends of nasalance scores agreed with previous studies that reported gender and age are not influential factors for nasalance scores in children aged 4 to 7. Because at this age, all children had similar shapes and sizes of the vocal tract, resulting in similar mean nasalance scores and could represent the scores of the entire group.[2,4,5]

Nasalance scores of the RCLP group were significantly higher than the control group when evaluated with high-pressure oral speech stimuli ($p$<0.05). In contrast, the nasalance scores of the RCLP group were lower than the control group when using nasal speech stimuli, which agreed with several studies.[7-10] Moreover, this study agreed with the previous research that informed factors that influenced nasalance scores, including abnormal structures and functions of articulators, misarticulations,[11] voice disorders,[12,13] obstructions in the vocal tract,[14] snoring,[15,16] hearing impairments,[17,18] and phonological features.[8,9] Each factor affects the nasalance scores in different aspects, as follows.

Higher nasalance scores than the control group can describe by the RCLP group having defective structures and functions of articulators resulting from CLP including ONF, and VPI, which can cause various types of misarticulations and hearing impairments.[17,18] This study found that 10 children with RCLP (27.78%) have a small penetrating ONF and 12 of 15 children have ONF at the anterior palate region, which this ONF's size and the site usually impact high-pressure oral consonants because the ONF locates at the same area as the placement of articulation of high-pressure oral consonants.[22-24] When a lifted tongue opposes an ONF, the tongue pushes acoustic energy into the nasal cavity, causing nasal resonance. Moreover, this study found 44.44% of the RCLP group have some energy rub against the edge of the ONF, creating nasal emission that disturbs speech and can cause increased nasalance scores.[1,12,22]

The VPI caused by short velum or poor velar movement mainly affects high vowels or voiced oral

consonants because while producing these sounds, acoustic energy leaks through a velopharyngeal gap and then generates nasal resonance, resulting in increased nasalance scores. The acoustic energy leakage caused 5 children with RCLP (13.89%) to tense facial or nasal muscles to close the velopharyngeal gap or diminish excessive nasal resonance, resulting in nasal or facial grimaces.[12]

This study found that 33 of 36 children (91.67%) in the RCLP group have misarticulations, compensatory and phonological errors equally. Children with RCLP have compensatory errors with nasal rustle and hypernasality, such as mid-dorsum palatal stops, posterior nasal fricatives, and pharyngeal fricatives may increase nasalance scores.[12] The frequent pattern of phonological errors is substitutions because children with RCLP have atypical articulators. Children cannot produce the target sound correctly and need to substitute the sound they can produce for the target sounds. Substitutions affect the validity of nasalance scores because the scores may come from a substituted sound, not a target sound, such as a child substitutes a nasal consonant for an oral consonant.[1,12] These findings conform to Nandurkar who reported that all children with RCLP misarticulated at least one position in a pressure-sensitive word, the most common was substitution (19.44%) also found that nasalance scores of the RCLP group were higher than the non-cleft group.[8]

CLP affects the Eustachian tube's functions and causes middle ear disease. In this study, most children with RCLP have a history of serous otitis media (SOM), or SOM remains, and 8 of 36 children with RCLP (22.22%) have SOM with unilateral hearing impairments, which levels of hearing loss between 30 and 60 dB in speech frequencies.[25] Hearing-impaired children may have higher nasalance scores because children with hearing issues have a slower speech rate than those with normal hearing, causing incomplete velopharyngeal closure, resulting in the acoustic energy leak through the velopharyngeal gap to the nasal cavity, creating nasal resonance and causing hypernasality.[18] Moreover, hearing impairment causes misarticulations, especially in voiceless consonants. Because these consonants are low-intensity and hard for hearing-impaired children to produce correctly. Misarticulations, such as substitutions, or distortions due to hearing loss may be another factor that affects the validity of the nasalance scores.[17]

The significantly lower nasalance scores of the RCLP group, when assessed with nasal speech stimuli, are consistent with a study by Abou-Elsaad *et al*.[7] reported that nasalance scores of the RCLP group were significantly lower than those of the control group when assessed with nasal syllable repetition (/ma/, /na/, /mi/, and /ni/) from the Arabic SNAP Test. This study found that hyponasal speech in 3 RCLP children might result from an obstruction in the vocal tract and voice disorders.

This study found that the obstruction in the vocal tract, including the cleft nose with or without nostril stenosis and hypertrophic structures with snoring that occludes the energy from exiting through the nasal cavity,[14-16] resulting in hyponasality.[1] Furthermore, this study agreed with Sadjadi *et al*. reported that having voice disorders cause decreased acoustic energy and lower nasalance scores than children without voice disturbance.[26] Voice disorders occur in children with mild VPI who used to strain vocal cords to close the symptomatic ONF or a small velopharyngeal gap for having sufficient vocal loudness.[12,13,27] The present study found that 30.56% of children with RCLP had voice disorders with low pitch or soft voice, resulting in the RCLP having lower nasalance scores than the control group.

Only two nasal syllable repetitions (/na/ and /ni/), which nasalance scores of the RCLP group were lower than those of the control group, but not a significant difference ($p \geq 0.05$). These results can be described by the range of nasalance scores of the two groups are almost no difference. A large range of nasalance scores means low validity.[1] Vary nasalance scores may result from most children with RCLP having misarticulations in /n/. Because the children could not raise the tip of the tongue to precisely reach the alveolar ridge, an /n/ sound was distorted or substituted [ŋ/n], decreasing the validity of nasalance scores because the scores did not come from a target sound. Moreover, thirteen children with RCLP had mild hypernasality, which challenged a nasometer to identify speech resonance problems and cause nasalance scores close to the control group that comply with Dalston *et al*. which informed that a nasometer could accurately determine resonance disorders in patients with more than mild hypernasality.[28,29] The results of this study correlate with the previous studies, suggesting nasal speech stimuli are proper for measuring hyponasality, not hypernasality. Because nasal consonants mainly resonate in the nasal cavity. It is difficult for a nasometer to differentiate between normal nasal resonance and hypernasality.[1,3,9]

Comparing nasalance scores within the same group revealed that the impact factors were the phonological features of consonants and vowels used in each stimulus. This study found that nasalance scores from nasal speech stimuli were significantly higher than high-pressure oral speech stimuli; for example, the scores from prolonged /m/ were the highest because the nasal resonance mainly occurred. [2,5,7]

In contrast, the lowest nasalance scores (0%) were found in the control group when sustained /s/. Because /s/ is an oral voiceless consonant, only oral resonance occurs; children in the control group could close the velopharyngeal port tightly, with no energy entering the nasal cavity.[1] However, in 44.44% of the RCLP group, the mean nasalance scores were higher than 0% because children may have nasal resonance due to nasal emission or have compensatory errors with a nasal rustle while prolonging /s/.[12] These results correspond to Watterson *et al*. which indicated that different numbers of nasal phonemes impact the nasalance scores; a speech stimulus with more nasal phonemes results in higher nasalance scores than with fewer or without nasals.[9] Because of the assimilation phenomenon of nasal consonants, a nasal consonant assimilates to an adjacent vowel, causing a nasalized vowel, which was accepted as normal

nasalization.[24,30,31] Furthermore, phonological features of vowels also influenced nasalance scores. In high vowels, an elevated tongue causes a narrower oral cavity and rising intraoral pressure that pushes acoustic energy to the nasal cavity, resulting in oronasal resonance generating higher nasalance scores. In contrast, low vowels, a lower tongue with a wide mouth, decreasing intraoral pressure, and the energy cannot flow into the nasal cavity. Thus, only oral resonance occurs, causing lower nasalance scores.[1,2]

## Conclusion

For further studies, this research recommends assessing screening tests with the instruments, including screening hearing levels using audiometry test and assessing the velopharyngeal port by direct measurements such as nasopharyngoscopy or multi-view videofluoroscopy, which present the degree of hearing and illustrate the size of the velopharyngeal gap, and the types of VPI may reveal the correlation between these factors and the nasalance scores. The present study found that ONF is another influential factor with the limited number of subjects that could not specify that ONF or VPI impacted the nasalance scores. Further study should increase the sample size and group children in the RCLP group into two groups, including RCLP children with ONF and RCLP children without ONF, then compare the nasalance scores of the two RCLP groups with the control group may identify the main factor affecting the nasalance scores. Accents and dialects also impact nasalance scores therefore, researchers should evaluate nasalance scores in Thai children who use different regional dialects. Moreover, researchers should study normative nasalance scores of Thai SNAP Test with a substantial sample size to represent each gender and age range and show the impact of gender and age on nasalance scores can be used as the reference data for measuring speech resonance disorders.

In conclusion, this study revealed the trends in nasalance scores, which suggested that Thai SNAP Test could identify speech resonance disorders in Thai children aged 4-7 years. However, for diagnosing or evaluating the progression of treatments, the SLP or evaluator should consider factors that affect the nasalance scores, as mentioned above. For precise diagnosing and assessing the progression of the therapy should apply nasalance scores together with other results, including perceptual nasality ratings evaluated by experienced SLPs in cleft palate speech, which is the gold standard, integrated with the results from various methods or instruments.

## Conflict of interest:

The authors declare no conflict of interest.

## Ethics approval:

This research was approved on September 17, 2018, and expired on September 16, 2019, with COA. No. MURA2018/ 532.

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**Appendix 1:** Nasalance scores of the control group

| Code | Syllable repetition | | | | | | | | | | | | | | | | Prolonged sound | | | | Picture-cued sentences | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pʰa | tʰa | kʰa | sa | tɕʰa | pʰi | tʰi | kʰi | si | tɕʰi | ma | na | ŋa | mi | ni | ŋi | a | i | s | m | Bilabials | Alveolar plosives | Velars | Alveolar fricatives | Nasals |
| C-01 | 7 | 9 | 8 | 8 | 8 | 12 | 12 | 16 | 11 | 15 | 56 | 57 | 64 | 75 | 68 | 73 | 37 | 10 | 0 | 96 | 6 | 9 | 8 | 10 | 55 |
| C-02 | 4 | 5 | 4 | 5 | 6 | 19 | 19 | 14 | 17 | 19 | 64 | 60 | 76 | 86 | 84 | 82 | 4 | 27 | 0 | 97 | 7 | 7 | 6 | 6 | 54 |
| C-03 | 12 | 11 | 10 | 10 | 12 | 26 | 23 | 28 | 18 | 17 | 61 | 65 | 73 | 86 | 81 | 83 | 9 | 15 | 0 | 94 | 11 | 7 | 10 | 10 | 67 |
| C-04 | 6 | 6 | 6 | 5 | 5 | 8 | 9 | 14 | 10 | 13 | 51 | 48 | 52 | 70 | 66 | 74 | 7 | 13 | 0 | 95 | 5 | 6 | 6 | 5 | 48 |
| C-05 | 12 | 8 | 9 | 8 | 9 | 17 | 18 | 22 | 14 | 14 | 62 | 59 | 68 | 81 | 74 | 79 | 7 | 8 | 0 | 92 | 9 | 11 | 11 | 10 | 53 |
| C-06 | 7 | 7 | 8 | 6 | 5 | 8 | 8 | 14 | 8 | 13 | 49 | 47 | 42 | 87 | 83 | 78 | 8 | 8 | 0 | 94 | 4 | 5 | 7 | 5 | 53 |
| C-07 | 13 | 8 | 8 | 13 | 9 | 25 | 16 | 26 | 27 | 19 | 67 | 63 | 65 | 69 | 65 | 74 | 6 | 29 | 0 | 96 | 11 | 13 | 11 | 10 | 64 |
| C-08 | 6 | 6 | 6 | 5 | 6 | 17 | 14 | 15 | 8 | 13 | 45 | 32 | 52 | 81 | 69 | 66 | 12 | 13 | 0 | 91 | 5 | 6 | 5 | 7 | 48 |
| C-09 | 6 | 7 | 10 | 7 | 5 | 13 | 11 | 11 | 8 | 12 | 63 | 66 | 65 | 80 | 75 | 81 | 9 | 11 | 0 | 95 | 7 | 7 | 10 | 9 | 53 |
| C-10 | 5 | 6 | 7 | 6 | 7 | 8 | 13 | 18 | 11 | 12 | 52 | 53 | 52 | 73 | 65 | 67 | 5 | 17 | 0 | 92 | 6 | 9 | 7 | 6 | 50 |
| C-11 | 8 | 8 | 8 | 7 | 7 | 24 | 18 | 32 | 15 | 22 | 42 | 43 | 56 | 76 | 66 | 72 | 7 | 22 | 0 | 94 | 8 | 10 | 8 | 10 | 53 |
| C-12 | 5 | 7 | 6 | 5 | 5 | 10 | 11 | 15 | 15 | 14 | 56 | 52 | 65 | 90 | 83 | 83 | 39 | 23 | 0 | 96 | 6 | 7 | 7 | 7 | 56 |
| C-13 | 9 | 8 | 8 | 10 | 9 | 21 | 19 | 22 | 20 | 21 | 54 | 55 | 62 | 71 | 75 | 69 | 20 | 19 | 0 | 90 | 11 | 8 | 11 | 10 | 52 |
| C-14 | 11 | 11 | 10 | 12 | 11 | 33 | 18 | 17 | 16 | 23 | 60 | 60 | 67 | 82 | 77 | 84 | 9 | 13 | 0 | 93 | 13 | 13 | 11 | 11 | 67 |
| C-15 | 8 | 9 | 8 | 9 | 10 | 12 | 15 | 15 | 16 | 16 | 54 | 49 | 68 | 83 | 76 | 69 | 7 | 17 | 0 | 95 | 13 | 11 | 11 | 12 | 58 |
| C-16 | 7 | 7 | 7 | 6 | 7 | 21 | 17 | 16 | 8 | 13 | 54 | 46 | 50 | 67 | 70 | 64 | 7 | 16 | 0 | 90 | 8 | 7 | 6 | 6 | 48 |
| C-17 | 6 | 7 | 8 | 7 | 6 | 17 | 19 | 26 | 17 | 18 | 43 | 59 | 59 | 86 | 81 | 83 | 5 | 18 | 0 | 91 | 8 | 6 | 8 | 8 | 51 |
| C-18 | 10 | 8 | 8 | 8 | 8 | 18 | 27 | 28 | 12 | 21 | 69 | 67 | 64 | 75 | 79 | 82 | 42 | 33 | 0 | 93 | 16 | 10 | 10 | 11 | 61 |
| C-19 | 7 | 7 | 6 | 9 | 7 | 22 | 21 | 29 | 21 | 24 | 66 | 59 | 68 | 89 | 83 | 84 | 43 | 26 | 0 | 96 | 15 | 15 | 16 | 18 | 76 |
| C-20 | 8 | 6 | 6 | 7 | 7 | 14 | 12 | 25 | 15 | 21 | 59 | 48 | 64 | 76 | 71 | 82 | 6 | 22 | 0 | 96 | 6 | 9 | 6 | 6 | 56 |
| C-21 | 7 | 7 | 8 | 6 | 6 | 23 | 15 | 28 | 13 | 23 | 65 | 62 | 67 | 81 | 80 | 84 | 5 | 15 | 0 | 92 | 11 | 10 | 10 | 8 | 58 |
| C-22 | 7 | 8 | 7 | 9 | 8 | 19 | 15 | 23 | 17 | 14 | 75 | 77 | 75 | 88 | 85 | 87 | 8 | 25 | 0 | 97 | 10 | 8 | 10 | 10 | 62 |
| C-23 | 8 | 7 | 6 | 6 | 7 | 12 | 14 | 17 | 11 | 12 | 50 | 53 | 62 | 85 | 75 | 80 | 7 | 12 | 0 | 93 | 7 | 8 | 10 | 8 | 55 |
| C-24 | 8 | 9 | 8 | 9 | 10 | 21 | 25 | 28 | 20 | 21 | 67 | 62 | 65 | 85 | 84 | 87 | 7 | 15 | 0 | 89 | 15 | 11 | 11 | 10 | 56 |
| C-25 | 7 | 7 | 7 | 6 | 5 | 28 | 26 | 25 | 20 | 23 | 67 | 56 | 65 | 85 | 79 | 84 | 7 | 20 | 0 | 94 | 17 | 9 | 8 | 8 | 55 |
| C-26 | 11 | 12 | 9 | 14 | 17 | 30 | 22 | 29 | 21 | 30 | 65 | 56 | 66 | 83 | 76 | 85 | 35 | 30 | 0 | 95 | 15 | 18 | 14 | 13 | 60 |
| C-27 | 9 | 11 | 10 | 11 | 11 | 23 | 25 | 26 | 26 | 25 | 68 | 67 | 71 | 84 | 81 | 80 | 7 | 24 | 0 | 96 | 19 | 15 | 19 | 17 | 62 |
| C-28 | 7 | 8 | 8 | 9 | 12 | 25 | 22 | 26 | 19 | 19 | 64 | 53 | 64 | 70 | 72 | 82 | 8 | 32 | 0 | 86 | 11 | 7 | 7 | 10 | 47 |
| C-29 | 7 | 7 | 9 | 8 | 9 | 28 | 13 | 21 | 14 | 21 | 66 | 61 | 76 | 86 | 83 | 88 | 52 | 26 | 0 | 92 | 10 | 10 | 10 | 10 | 54 |
| C-30 | 6 | 7 | 6 | 7 | 8 | 19 | 20 | 23 | 16 | 16 | 59 | 59 | 66 | 85 | 84 | 82 | 6 | 24 | 0 | 93 | 11 | 8 | 10 | 10 | 60 |
| C-31 | 6 | 5 | 6 | 7 | 6 | 22 | 22 | 24 | 22 | 26 | 41 | 37 | 47 | 68 | 51 | 64 | 18 | 30 | 0 | 90 | 11 | 7 | 8 | 13 | 49 |
| C-32 | 8 | 9 | 9 | 11 | 10 | 27 | 29 | 35 | 29 | 27 | 64 | 72 | 70 | 82 | 86 | 85 | 7 | 35 | 0 | 94 | 14 | 14 | 14 | 15 | 64 |
| C-33 | 6 | 7 | 7 | 7 | 8 | 38 | 27 | 32 | 28 | 25 | 57 | 57 | 69 | 87 | 81 | 87 | 7 | 31 | 0 | 96 | 11 | 10 | 13 | 15 | 58 |
| C-34 | 6 | 5 | 5 | 5 | 5 | 21 | 13 | 18 | 14 | 12 | 63 | 60 | 69 | 84 | 86 | 83 | 49 | 17 | 0 | 94 | 7 | 8 | 8 | 7 | 56 |
| C-35 | 6 | 5 | 6 | 7 | 10 | 24 | 29 | 21 | 24 | 27 | 63 | 60 | 64 | 82 | 75 | 77 | 4 | 34 | 0 | 96 | 11 | 9 | 7 | 8 | 62 |
| C-36 | 6 | 6 | 6 | 6 | 5 | 13 | 19 | 17 | 16 | 15 | 60 | 53 | 56 | 79 | 75 | 77 | 7 | 25 | 0 | 96 | 8 | 7 | 8 | 9 | 60 |

**Appendix 2:** Nasalance scores of the RCLP group

| Code | Syllable repetition | | | | | | | | | | | | | | | | Prolonged sound | | | | Picture-cued sentences | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pʰa | tʰa | kʰa | sa | tɕʰa | pʰi | tʰi | kʰi | si | tɕʰi | ma | na | ŋa | mi | ni | ŋi | a | i | s | m | Bilabials | Alveolar plosives | Velars | Alveolar fricatives | Nasals |
| R-01 | 11 | 28 | 16 | 20 | 11 | 16 | 26 | 25 | 39 | 19 | 53 | 66 | 68 | 75 | 74 | 75 | 44 | 44 | 64 | 93 | 10 | 14 | 18 | 26 | 48 |
| R-02 | 11 | 12 | 9 | 11 | 8 | 13 | 15 | 11 | 15 | 11 | 45 | 44 | 55 | 61 | 59 | 66 | 26 | 33 | 0 | 89 | 13 | 17 | 9 | 15 | 45 |
| R-03 | 41 | 18 | 19 | 32 | 37 | 51 | 39 | 61 | 37 | 44 | 44 | 58 | 53 | 53 | 61 | 58 | 17 | 37 | 0 | 91 | 44 | 39 | 29 | 31 | 47 |
| R-04 | 7 | 8 | 5 | 5 | 6 | 13 | 16 | 13 | 14 | 13 | 33 | 42 | 37 | 67 | 61 | 62 | 5 | 25 | 0 | 94 | 8 | 12 | 7 | 7 | 47 |
| R-05 | 9 | 8 | 11 | 8 | 10 | 20 | 26 | 26 | 23 | 26 | 72 | 52 | 66 | 67 | 70 | 69 | 5 | 35 | 0 | 93 | 14 | 13 | 11 | 12 | 57 |
| R-06 | 6 | 11 | 6 | 7 | 6 | 13 | 15 | 15 | 16 | 14 | 53 | 46 | 47 | 72 | 70 | 64 | 30 | 14 | 0 | 93 | 10 | 20 | 11 | 9 | 44 |
| R-07 | 6 | 6 | 6 | 7 | 6 | 24 | 16 | 16 | 21 | 17 | 62 | 59 | 65 | 81 | 82 | 83 | 5 | 38 | 0 | 96 | 7 | 9 | 9 | 11 | 56 |
| R-08 | 40 | 43 | 38 | 43 | 34 | 62 | 57 | 52 | 60 | 47 | 56 | 57 | 55 | 67 | 69 | 66 | 41 | 70 | 92 | 91 | 55 | 46 | 42 | 44 | 52 |
| R-09 | 24 | 36 | 11 | 55 | 49 | 61 | 75 | 53 | 73 | 77 | 58 | 56 | 51 | 87 | 88 | 86 | 27 | 65 | 71 | 96 | 41 | 46 | 19 | 45 | 55 |
| R-10 | 33 | 35 | 29 | 46 | 11 | 22 | 21 | 30 | 52 | 35 | 46 | 39 | 36 | 57 | 51 | 58 | 19 | 48 | 18 | 89 | 18 | 13 | 21 | 40 | 41 |
| R-11 | 42 | 33 | 26 | 27 | 29 | 41 | 41 | 39 | 32 | 39 | 37 | 31 | 27 | 66 | 55 | 53 | 17 | 47 | 0 | 88 | 36 | 29 | 30 | 25 | 40 |
| R-12 | 21 | 22 | 17 | 44 | 21 | 36 | 36 | 32 | 28 | 36 | 44 | 51 | 51 | 58 | 54 | 60 | 25 | 39 | 0 | 90 | 24 | 24 | 32 | 21 | 38 |
| R-13 | 6 | 14 | 7 | 6 | 8 | 20 | 14 | 16 | 12 | 18 | 41 | 34 | 36 | 61 | 51 | 55 | 21 | 38 | 0 | 89 | 13 | 13 | 11 | 9 | 49 |
| R-14 | 8 | 8 | 11 | 7 | 6 | 29 | 26 | 23 | 11 | 26 | 52 | 44 | 45 | 78 | 61 | 72 | 11 | 41 | 18 | 91 | 7 | 6 | 7 | 5 | 37 |
| R-15 | 7 | 6 | 5 | 6 | 7 | 6 | 14 | 12 | 21 | 19 | 59 | 57 | 70 | 84 | 78 | 73 | 12 | 18 | 19 | 93 | 6 | 9 | 6 | 7 | 63 |
| R-16 | 53 | 61 | 46 | 60 | 50 | 76 | 70 | 81 | 72 | 74 | 79 | 79 | 82 | 81 | 83 | 84 | 59 | 80 | 96 | 95 | 73 | 62 | 61 | 64 | 72 |
| R-17 | 5 | 10 | 18 | 8 | 7 | 11 | 48 | 45 | 6 | 18 | 51 | 62 | 61 | 69 | 74 | 76 | 9 | 63 | 0 | 91 | 6 | 8 | 15 | 9 | 48 |
| R-18 | 17 | 23 | 25 | 27 | 29 | 33 | 44 | 46 | 45 | 42 | 58 | 60 | 50 | 80 | 71 | 78 | 28 | 50 | 0 | 91 | 29 | 13 | 15 | 30 | 57 |
| R-19 | 55 | 53 | 55 | 52 | 54 | 74 | 74 | 74 | 76 | 70 | 69 | 66 | 57 | 82 | 79 | 86 | 49 | 83 | 68 | 95 | 61 | 59 | 55 | 54 | 67 |
| R-20 | 6 | 11 | 12 | 9 | 8 | 26 | 13 | 25 | 21 | 23 | 49 | 47 | 53 | 81 | 81 | 78 | 21 | 30 | 0 | 87 | 12 | 6 | 9 | 7 | 40 |
| R-21 | 5 | 5 | 7 | 5 | 5 | 16 | 16 | 21 | 19 | 22 | 55 | 51 | 59 | 83 | 86 | 83 | 5 | 46 | 0 | 94 | 9 | 6 | 8 | 8 | 51 |
| R-22 | 7 | 8 | 7 | 8 | 10 | 16 | 13 | 18 | 14 | 15 | 60 | 63 | 64 | 76 | 76 | 73 | 8 | 19 | 0 | 94 | 13 | 7 | 9 | 12 | 51 |
| R-23 | 34 | 41 | 28 | 30 | 34 | 62 | 58 | 60 | 57 | 59 | 54 | 45 | 46 | 70 | 49 | 66 | 13 | 61 | 16 | 60 | 48 | 45 | 29 | 35 | 45 |
| R-24 | 29 | 31 | 23 | 34 | 28 | 53 | 55 | 59 | 48 | 57 | 61 | 57 | 61 | 88 | 83 | 92 | 35 | 69 | 0 | 94 | 44 | 36 | 40 | 34 | 66 |
| R-25 | 41 | 27 | 32 | 24 | 23 | 67 | 67 | 58 | 55 | 57 | 46 | 54 | 56 | 80 | 79 | 83 | 41 | 67 | 55 | 94 | 46 | 31 | 31 | 39 | 46 |
| R-26 | 53 | 40 | 29 | 42 | 40 | 64 | 60 | 62 | 65 | 68 | 58 | 62 | 46 | 90 | 89 | 84 | 42 | 89 | 69 | 97 | 46 | 48 | 39 | 44 | 66 |
| R-27 | 25 | 22 | 38 | 32 | 21 | 49 | 54 | 39 | 58 | 40 | 70 | 67 | 62 | 81 | 86 | 81 | 49 | 71 | 84 | 95 | 30 | 18 | 28 | 32 | 66 |
| R-28 | 42 | 50 | 51 | 43 | 45 | 72 | 72 | 76 | 79 | 74 | 68 | 68 | 76 | 86 | 82 | 86 | 28 | 81 | 82 | 95 | 58 | 46 | 56 | 55 | 65 |
| R-29 | 17 | 12 | 13 | 41 | 15 | 22 | 38 | 40 | 71 | 43 | 57 | 51 | 56 | 80 | 83 | 76 | 26 | 51 | 92 | 81 | 45 | 32 | 24 | 53 | 63 |
| R-30 | 20 | 12 | 42 | 10 | 17 | 61 | 61 | 36 | 27 | 56 | 66 | 67 | 64 | 84 | 85 | 86 | 37 | 77 | 0 | 94 | 51 | 32 | 30 | 27 | 62 |
| R-31 | 8 | 8 | 8 | 11 | 12 | 9 | 9 | 7 | 19 | 13 | 37 | 36 | 42 | 80 | 69 | 78 | 8 | 15 | 0 | 95 | 5 | 6 | 5 | 5 | 45 |
| R-32 | 15 | 12 | 14 | 18 | 17 | 17 | 23 | 19 | 22 | 13 | 49 | 56 | 51 | 48 | 53 | 49 | 22 | 42 | 73 | 86 | 20 | 20 | 15 | 19 | 32 |
| R-33 | 6 | 8 | 7 | 40 | 8 | 20 | 9 | 8 | 10 | 9 | 47 | 50 | 48 | 72 | 64 | 64 | 22 | 9 | 0 | 90 | 6 | 6 | 10 | 35 | 43 |
| R-34 | 43 | 32 | 36 | 33 | 32 | 61 | 65 | 66 | 68 | 60 | 47 | 38 | 40 | 65 | 70 | 65 | 32 | 69 | 0 | 94 | 59 | 54 | 43 | 47 | 60 |
| R-35 | 11 | 9 | 11 | 10 | 14 | 17 | 16 | 16 | 17 | 20 | 61 | 64 | 70 | 70 | 70 | 77 | 39 | 14 | 0 | 94 | 9 | 10 | 12 | 14 | 55 |
| R-36 | 5 | 7 | 4 | 5 | 4 | 16 | 19 | 19 | 12 | 21 | 52 | 50 | 59 | 86 | 81 | 82 | 17 | 28 | 35 | 93 | 10 | 8 | 13 | 9 | 49 |

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# Impact of health education and cognitive behavioural therapy intervention during tobacco cessation session for smokers: A comparative study in Eastern India.

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**ABSTRACT**

**Background**: Tobacco use remains the leading cause of death worldwide. Smoking cessation is the only way to fight the most feared disease in the world. Current evidence-based treatment approaches to smoking cessation include several behavioural treatments and health education-such as cognitive behavioural therapy, and individual and group counseling which tobacco cessation can help control global causes of preventable death. Health professionals play an important role in combating the tobacco epidemic.

**Materials and methods**: All the current smokers who had participated in this study submitted their self-reported case record forms. Baseline data was collected, tobacco history was obtained, the Fagerstrom nicotine dependency questionnaire was administrated and for the quitter, a standardized cotinine test was used. The total number of participants enrolled in this study is 240, but only 190 participants completed the entire program study. Tobacco users who met the inclusion criteria were randomly assigned to three intervention groups: the health education (HE) group, the cognitive behavioural therapy group (CBT), and the combination of health education with the cognitive behavioural therapy (HE+CBT) group. All the participants were visited five times within the six-month duration. Pearson's Chi-square test, paired and unpaired t-tests, Fagerstrom score scale, and Fisher's exact test were used to test the effectiveness of all interventions among the groups. SPSS 22.0, IBM, USA, was used for the analysis of all statistical events.

**Results**: Both groups showed no difference in attempts to quit and had no statistical significance. The reduction in Fagerstrom's score was greater in the HE+CBT group than in the individual CBT and HE groups. Among all the groups, the HE+CBT group shows more effectiveness in cessation therapy than those in the individual groups, which improves the possibility of smoking cessation.

**Conclusion**: In term of tobacco habit reduction, the combination of HE and CBT is more effective than individual therapy for smokers. Further, it can be studied that which kind of barriers are faced by the health professionals during the practice of HE and CBT intervention in their tobacco cessation practices in order to making the sessions clinically effective and educationally more impactful, as well as the heavy tobacco users are can be given immediate attention and prior opportunity for the treatment.

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## Introduction

Tobacco is a deadly substance that brings darkness to life. For smokers, it is a pleasurable thing. Smokers are blind to seeing the toxic effect of smoking rather than enjoying it for a short moment with long-lasting health hazards. Smokers use a variety of forms of tobacco, but tobacco leaves are primarily used for smoking (cigarettes, hookahs, cigars, hookahs, pipes), and rechargeable and disposable e-cigarettes, e-cigarettes, e-pipes, and vaporizers are other forms of smoking materials.[1] More than 40,000 chemicals and 60 types of carcinogens are present in tobacco.[2] These carcinogens present in tobacco are specific aromatic amines, polycyclic aromatic hydrocarbons (PAHs), N-nitrosamines, volatile hydrocarbons, and nitro compounds phenols and aldehydes.[3]

Over 80% of the world's 1.3 billion tobacco users live in low- and middle-income countries. In 2020, 22.3% of the global population used tobacco, 36.7% of all men and 7.8% of the world's women. Tobacco kills more than 8 million people each year. More than 7 million of those deaths are the result of direct tobacco use while around 1.2 million are the result of non-smokers being exposed to second-hand smoke.[4] The WHO announced in 2008 that tobacco use is the world's largest preventable cause of death.[5]In the future, both the addiction rate and death rate can enormously about one billion people can die in this century if the consumption rate of tobacco globally remained at this level as per WHO.[3] According to data from the National Vital Statistics System recorded in 2016 it was found that 7.2% of women smoked cigarettes during pregnancy.[6]

Smoking causes sensitization of cells, causing hyper-responsiveness in the body, and inflammatory changes due to the constant entry of foreign toxins. Smoking leads to the destruction of respiratory epithelium, alters and weakens immunity, and increases the frequency and severity of getting infections, diabetes, increase risk of clot formation, slows down blood supply to ear lower limbs, fertility problems, and osteoporosis. Recently studies have shown that nicotine inhibits the expression of angiotensin-2 conversion enzyme in various organs (lungs, heart, brain) and cells.[7-9] Smoking induces Inflammation occurs as a combination of changes that include the activity of neutrophils, dendritic cells macrophages, basophils, and eosinophils. As the end of the consequence of constant inflammation, carcinogenicity becomes the outcome at the end.[10] Carcinogenic change due to smoking mostly leads to cancer of the lungs, pharynx, larynx, oral cavity, stomach, pancreas, esophagus, urinary bladder (kidney), and cervix as well as acute myeloid leukemia. In addition, smoking causes major respiratory symptoms, chronic obstructive pulmonary disease, stroke, coronary heart disease, abdominal aortic aneurysm, and subclinical atherosclerosis.[11]

No one is born with an addiction, but during the course of life stress, anxiety, depression, pressure, social environment, family problems, and dealing with problems lead an individual to choose an addictive path to forget the problem for a few moments without actually facing it. Addiction starts with enjoyment, to get relief, and to feel good, but as time passes, it gets included as a habit and day-to-day maintenance. The mortality rate in smokers is three times higher and their life expectancy gets reduced and they die an average of 10 years earlier than those non-smokers.[12] The risk of developing stroke and head and neck cancer are two to four times higher and 10 times respectively, in smokers as compared to non-smokers.[13,14]

The solution to addiction is de-addiction. Cessation of habits and maintaining them without relapse are the main steps to increasing the quality of life and expanding life span. Tobacco cessation methods include Cognitive behavioural Therapy (CBT) includes methods such as self-help which can be provided by health professionals, health education, Intensive therapy provided at cessation centres, and pharmacological means including Nicotine Replacement Therapy (NRT) and the use of antidepressants like bupropion.[15]

According to Dr. Saul McLeod, 2019, CBT is based on the idea that how we think (cognition), how we feel (emotion) and how we act (behaviour) all interact together. And this theory if we apply to change someone's habit and change of behaviour of different triggering factors that influence to do habit in terms of tobacco addiction. Similarly, several theories support the practice of health education. When identifying a theory to guide health education or disease prevention programs, it is important to consider a range of factors, such as the specific health problem being addressed, like tobacco cessation comprehensive and active awareness of the population through health promotion strategies are the primary tools for smoking prevention and cessation.

The aim of this research was not to let the end of life of smokers on ashtray. This study aimed to find the effectiveness of Cognitive behavioural Therapy (CBT) and Health Education (HE) intervention and the combination of both therapies (HE+CBT) in 3 different groups of smokers in tobacco cessation. The researcher hypothesized that CBT's effects on tobacco cessation are stronger than HE's an intervention to reduce tobacco habits in smokers. Therefore, this study was conducted to evaluate the effectiveness of the intervention (Group-1, HE only; Group-2, CBT only; Group-3, HE with CBT) in three groups of smokers in the city of Bhubaneswar. In this study, participants were between 18 and 67 years old and all participants in the three groups were male.

Quitting smoking is one of the most difficult challenges in the life of smokers. One may reach the final goal of quitting after several attempts. If the patient again starts to get attached to cigarettes after completion of tobacco cessation, then the net outcome is zero. Relapse is quite common in smoking patients after cessation of the habit.[16,17] Three out of 10 ex-smokers were observed to relapse.[18] On average, 23.8% of patients experienced relapse following the completion of CBT.[19] Previous study shows that CBT is more effective than HE when 2 groups are compared to each other. In this study, the combination of HE and CBT has a stronger effect on tobacco cessation than either the individual HE or CBT uses a combination of therapy to increase the effectiveness of quitting.

High-intensity smoking cessation interventions in hospital settings can be effective for smoking cessation in smokers with high nicotine dependence.[20] So in our study, we put extra and more intense cessation sessions by combining both Cognitive behavioural therapy and Health Education. CBT is a type of psychotherapeutic treatment that makes individuals identify and change the destructive or disturbing patterns of thought which cause a negative influence on their emotions and behaviours.[21] We also know that CBT is a promising psychological intervention for people who want to quit smoking because changing and restructuring thought processes, combined with new learning behaviours, is essential for people who want to effectively quit smoking. The thought processes of each smoker are different from one another, so it is necessary to counsel the present mental state and then put positive energy to improve quitting capabilities. So, for the effective and efficient result of tobacco cessation, multiple management of therapy should be included to help the smoking individual to defeat the smoking habit and to live long and strong. At the same time, health education will help to make a positive mind-set by spreading active awareness to the individual through health promotion strategies as it is one of the basic tools for smoking prevention and cessation.[22] So, a combination of cessation therapy may lead to a positive impact on the reduction in the prevalence of smoking. This study compared three types of counselling to continue to be positive about problem elimination by breaking it down into smaller parts and meeting points on current issues rather than focusing on the past.

## Materials and methods

A randomized controlled study was conducted to understand the effectiveness of CBT, HE, and a combination of both from December 2019 and May 2020 for 6 months among smokers attending a tertiary care hospital in Bhubaneswar, India. Ethical clearance was obtained by the Institutional Ethics Committee (DMR / IMSSH / SOA / 180338).

A Randomised control study was done with a sample size of 240. The sample size of two hundred and forty participants was analyzed using the G Power software with a statistical power of 90% with a 95% confidence interval and an effect size (d) of 0.89 calculated. All the participants are current tobacco users attending the tobacco cessation centre of the selected tertiary referral centre. All information regarding the cessation process was explained and written informed consent was obtained from all participants. A pretested customized questionnaire was administered to assess their knowledge of addiction in participants. The participants were blindly assigned into three groups based on the intervention used which is group 1 health education (HE), group 2 and Cognitive behavioural therapy only, and a combination of both HE and CBT. The examiner was competent enough to constructively provide HE and CBT to all participants in the Tobacco Cessation Centre.

The quit attempt was assessed after a week and a cotinine test and Nicotine dependency were performed to validate quitting. The sample is organized by considering the current tobacco users who have defined themselves as continuous users of smoking tobacco from the first day of recruitment in the study. 240 participants were able to fulfil the inclusion criteria which include males between ages 18-68 years, and patients with smoking habits. Various exclusion criteria were added to the study which contains patients undergoing treatment for severe psychiatric problems, alcohol users, and patients on nicotine replacement therapy (NRT), with hearing difficulty, patients > 68 years old were excluded from the study. Almost all participants belonged to a low socio-economic status with marginal educational and income levels. All the participants in enrolled group 80 and participant are randomly selected individuals (computer-generated randomization) and were assigned to Group-1 (HE), Group-2 (CBT), and Group-3 (HE+CBT) interventions. Some participants dropped out of the study after the 1st visit and they lost to follow-up after 1st visit. After dropping out of each group, the total number of participants was 62 for the HE, 67 for CBT, and 61 for the HE+CBT group. All the individuals in group 1 receiving HE counselling, who was assigned to CBT received Cognitive behavioural therapy, and group 3 participant receive both therapy of HE and CBT. All the participants were visited five times (1st Visit, follow up by 2nd, 6th , 12th and 24th weeks ) within the six-month duration.

At the 1st visit, baseline data were collected. The self-structure case record form was preparedto know the patient's detailed history; the participant must have all the information available for which a self-assessment could be completed. The case record form includes various contains like age, gender, occupation, marital status, living arrangement, and details about tobacco history (types of smoking, age of onset, socio-economic status, details of smoking history, no of year of regular use, past quit attempt tobacco addiction, alcohol history, family history of tobacco and motivational level of tobacco cessation), Fagerstrom's questionnaire form was used to know nicotine dependency. All the data are collected and translated into MS excel sheets of Microsoft office.

At the 2nd visit, the patient who was previously enrolled blindly in group 1 received health education intervention. Health education is provided through custom-made posters and videos developed in the local language (Odia, Hindi, English) which will clarify tobacco-related deaths, the chemical constituent of nicotine, various forms of tobacco use, its deadly effects on health, the benefits of quitting the habit, and the instructions of how to quit. A pamphlet was provided consisting of various for using cigarettes and written instructions on quitting process were handed over to all group 1 participants receiving. The intervention in CBT were provided through (a) provision of cognition (by self-identification of the reason for the initiation of habit, identification of the daily routines conditions, and learning to reduce and cope with the negative situation or urge associated with the withdrawal of nicotine, (b) provision of counselling to change the behaviour towards addiction(by breaking the daily urge or habit of same routine, managing the temptation and resisting it , and the various day-to-day tension or stress that provoke the usage, (c) interviewing

to motivate patient to make them understand and accept the real environment which all the normal individual are facing and accepting (through various series of questions, insight was provided to make them understand the need to quit the smoking tobacco), and (d) the patient again starts to get attached toward cigarettes after completion of tobacco cessation, then the net outcome is zero so at last the relapse prevention strategies were introduced and asked them to follow it.

During each follow-up after the intervention, a follow-up timeline was set on a calendar which was used to assess the self-reported outcome variables. At every follow-up visit, participants are screened for all parameters such as reduced consumption, point prevalence abstinence, attempted abstinence, continuous abstinence, relapse, and participant turnover rate. Fagerstrom's nicotine dependency scale and nicotine kit test have been performed in the 6$^{th}$, 12$^{th}$ and 24$^{th}$ week follow-up. A standardized cotinine test was used to validate the self-reported quit rates, the cotinine test was done by Nicotine test kit with the help of a urine sample the rapid kit include 2 types of result (cotinine "positive" / "Negative"), if the patient use any form of nicotine before 5 to 7 days. The instruction for the cotinine test has been explained back side of the test kit. If the sample is wrongly placed may be the result invalid so the researcher helps the patient how to test it.

Participants in this study had five follow-up visits, including the first, (1$^{st}$ Visit, follow up by 2$^{nd}$, 6$^{th}$ , 12$^{th}$ and 24$^{th}$ weeks) which was completed within six months of the study period. Fagerstrom's Test for Nicotine Dependence is a standard instrument for assessing the intensity of physical addiction to nicotine. It contains six items that evaluate the quantity of cigarette consumption, the compulsion to use, and dependence. In scoring the Fagerstrom's Test for Nicotine Dependence, yes/no items are scored from 0 to 1 and multiple choice items are scored from 0 to 3. The items are summed to yield a total score of 0-10. The higher the total Fagerstrom's score, the more intense the patient's physical dependence on nicotine. This questionnaire was used at the start of the study and follow-up examinations. The distribution of the mean Fagerstrom's addiction scale for smokers was assessed [Table-5]. Participants were followed up for 6 months (a total of five sessions at 1$^{st}$, 2$^{nd}$, 6$^{th}$, 12$^{th}$, and 24$^{th}$ weeks from the 1$^{st}$ visit), and follow-up reminder was done online by sending them reminder message. Pearson's Chi-square test [Table-1], Paired and unpaired t-tests, and Fisher's exact test were used to test the effectiveness of all interventions within the groups. SPSS 22.0, IBM, USA was used for the analysis of all statistical events.

## Results

One hundred and ninety patients were included in this study and divided into three groups. The groups consisted of participants between the ages of 18 to 68 years. The mean age of the three groups in this study was: Group 1 of HE 37.6±2.8 years, Group 2 of CBT 39.2±2.48, while in the HE with CBT group 40.8±3.48 years. 80% of the population were in a lower-upper class of socioeconomic status. 87% of participants are using the smoking form (Bidi, cigarette, Huka) than the smokeless form(pan, gutka and khaini) of tobacco or rest are both forms of tobacco usage. Forty-four percent of the tobacco users were alcoholics with 39% of them reporting a family history of tobacco usage, and 85% of smokers are more than 10yr of regular smoking history. 95% of the sample was in the contemplation stage of motivation at recruitment. Knowledge of the participants at the baseline was poor with a mean score of 8.63 that means Out of all groups the knowledge score average between the group is 8.63 (maximum score = 21). Age, sex, types of smoking, age of onset, socio-economic status, details of smoking history, no of the year of regular use, past quit attempt tobacco addiction, alcohol history, family history of tobacco, and the stage of motivation were compared between the groups at baseline using Pearson's Chi-square and no significant difference was found. Intention to treat analysis was done, and the dropouts in the follow-up sessions were considered to continue tobacco usage (Figure 1).

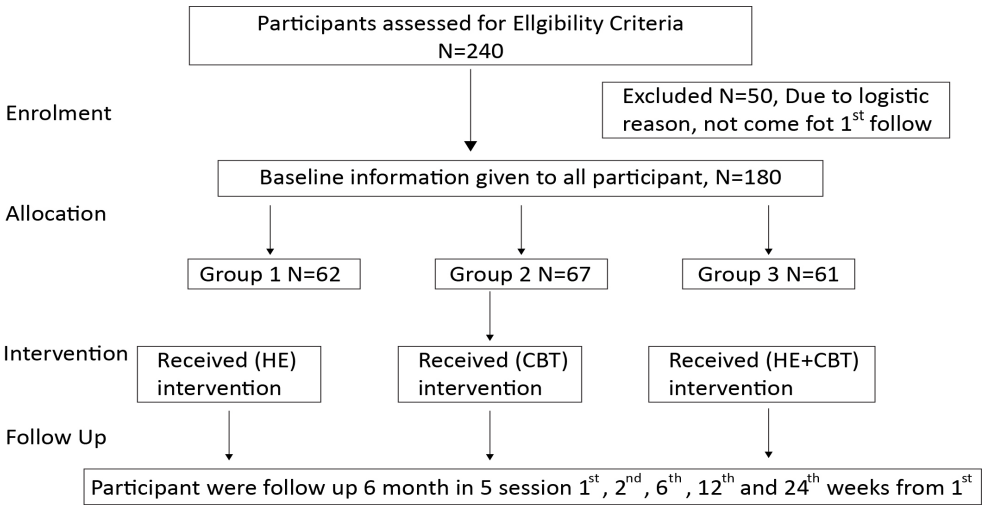


***Figure 1*** *Study design.*

The baseline addiction and knowledge scores were assessed using Mann–Whitney U test and were found to be similar between the intervention groups (Table 1).

**Table 1** Mann-Whitney U Test: Baseline assessment of addiction and knowledge score.

| | Health education | | Cognitive behaviour therapy | | Z | *p* |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | | |
| Addiction scores | - | - | - | - | - | - |
| Smoking form | 4.26 | 2.021 | 4.58 | 2.128 | 0.441 | 0.651 |
| Smokeless form | 6.02 | 2.123 | 6.78 | 2.325 | 0.958 | 0.327 |
| Knowledge score | 7.21 | 3.132 | 7.36 | 3.234 | 0.002 | 0.889 |

A reduction in the use of all groups was calculated and found that group HE+CBT (93%) was significantly higher as compared to others Group HE (71.3%) and Group CBT (82%) statistical differences in significance. ($p$=0.004). The rate of quit attempt are similar in the initial phase of the study, but later on, the quit attempt rate is slightly high in the HE+CBT group with no statistical differences in significance. Point prevalence abstinence was significantly higher in group HE (87.5%), followed by group CBT (79%) and group HE+CBT (80.6%). Continuous abstinence was significantly high in group HE+CBT (92.4%) compared to group CBT (78.4%) and group HE (42.6%).The percentage of lapse in group HE (62%) was significantly higher than in group CBT (51.5%) and group HE+CBT (34.5%). The relapse rate (3.4%) was comparatively lower than in all three groups and there is no significant difference was found. The rate of attrition was high (92%) with reasons to change of address, and change of phone number (Table 2).

**Table 2** Evaluation of the effectiveness of total 3 groups using Pearson's Chi-square test.

| Outcome Variables | HE, n 62 (%) | CBT, n 67 (%) | HE+CBT, n 61 (%) | χ2 | p |
|---|---|---|---|---|---|
| Reduced use | 44(71.3%) | 54(82%) | 57(93%) | 9.112 | 0.004$ |
| Quit attempt | 47(76.4%) | 59(88.6%) | 58(95.3%) | 8.328 | 0.063 |
| Point prevalence | 54(87.5%) | 53(79%) | 49(80.6%) | 0.067 | 0.768 |
| Continuous abstinence | 26(42.6%) | 52(78.4%) | 56(92.4%) | 33.139 | 0.000* |
| Lapse | 38(62%) | 35(51.5%) | 21(34.5%) | 4.462 | 0.029 |
| Relapse | 6(10.2%) | 5(8.5%) | 2(3.4%) | 0.051 | 0.642 |
| Attrition | 57(92%) | 55(81.4%) | 44(72%) | 2.987 | 0.061 |

*Note: *Highly significant, $: Significant, χ2: Pearson's Chi-square test, HE: health education, CBT: cognitive behavioural therapy.*

The evaluation in the 2nd session showed a significantly low reduction in consumption in group HE and significantly high abstinence attempts and point prevalence abstinence in group CBT and HE. The proportion of smokers with a frequency of more than ten cigarettes/day decreased ($p$=0.16) by the second follow-up ($p$=0.07) in group HE+CBT compared to groups CBT and HE. At the end of the 2nd follow-up, the majority of participants in both groups HE and CBT were in the contemplation phase passed while other participants in group CBT+HE had reached the preparation phase of cessation as compared to group HE. In the third session (6 weeks after the intervention), the attempt to quit, the point prevalence abstinence, and lapse were significantly higher in group HE and CBT but Group (HE+CBT) had significantly higher continuous abstinence. Relapse was more common in group-HE and Group CBT but in Group HE+CBT there is no relapse. During the 4th session (12 weeks after the intervention) abstinence attempts and withdrawals were more in group HE, while point prevalence abstinence and continuous abstinence were more in group CBT+HE. During the 5th session (24 weeks after the intervention), the continuous abstinence in group HE+CBT is highly significant than in the other two groups (Table 3). The cotinine test validated for a real quitter. The true quitters were significantly higher in HE+CBT ($p$=0.006) (Tables 4). The mean reduction in the Fagerstrom's score in the three groups was significantly higher in the combination group (CBT+HE) (2.1 3.2), followed by the CBT group (3.1 6.5) and then the HE-Group (3.4 4.4), and the difference was considered that nicotine dependency is less from baseline to 24th follow up (Table 5).

**Table 3** Percentage of outcome during follow up (follow up week 2nd, 6th, 12th and 24th).

| Outcome Variables | HE, n 62 (%) | CBT, n 67 (%) | HE+CBT, 61 (%) | χ2 | *p* |
|---|---|---|---|---|---|
| | Follow up: 2nd week after intervention | | | | |
| Reduced use | 35 (56.6%) | 40 (60.6%) | 40 (66.7%) | 11.108 | 0.000 |
| Quit attempt | 38 (61.5%) | 48 (72%) | 49 (81%) | 21.189 | 0.002 |
| Point prevalence | 20 (32%) | 30 (45.4%) | 40 (60.5%) | 8.879 | 0.000 |
| Continuous abstinence | 0 | 0 | 0 | - | - |
| Lapse | 15 (24%) | 13 (19%) | 8 (12.7%) | 0.987 | 0.032 |
| Relapse | 0 | 0 | 0 | - | - |

**Table 3** Percentage of outcome during follow up (follow up week 2nd, 6th, 12th and 24th). (continued)

| Outcome Variables | HE, n 62 (%) | CBT, n 67 (%) | HE+CBT, 61 (%) | χ2 | *p* |
|---|---|---|---|---|---|
| | Follow up: 6th week after intervention | | | | |
| Reduced use | 0 | 0 | 1 (2.1%) | 2.150 | 0.143 |
| Quit attempt | 20 (32%) | 12 (18.6%) | 5 (9.5%) | 8.567 | 0.003 |
| Point prevalence | 40 (65%) | 18 (26.6) | 7 (11.7%) | 24.569 | 0.000* |
| Continuous abstinence | 22 (36%) | 33 (48.6%) | 41 (68.7%) | 54.879 | 0.000* |
| Lapse | 28 (45%) | 15 (22.6%) | 9 (15.3%) | 3.678 | 0.015 |
| Relapse | 4 (7%) | 2 (3%) | 0 | 2.870 | 0.03 |
| Attrition | 23 (38%) | 14 (21%) | 7 (12%) | 3.982 | 0.053 |
| | Follow up: 12th week after intervention | | | | |
| Reduced use | 0 | 0 | 0 | - | - |
| Quit attempt | 7 (12%) | 6 (8.4%) | 3 (5%) | 0.213 | 0.761 |
| Point prevalence | 5 (7.5%) | 6 (9%) | 7 (11.7%) | 0.426 | 0.567 |
| Continuous abstinence | 14 (23%) | 16 (24%) | 16 (27%) | 0.433 | 0.651 |
| Lapse | 5 (8%) | 4 (6.3%) | 5 (8.4%) | 0.180 | 0.672 |
| Relapse | 0 | 0 | 0 | - | - |
| Attrition | 39 (63%) | 37 (56.5%) | 29 (48.6%) | 1.573 | 0.554 |
| | Follow up: 24th week after intervention | | | | |
| Reduced use | 0 | 0 | 0 | - | - |
| Quit attempt | 4 (6%) | 1 (2%) | 1 (1.6%) | 0.431 | 0.543 |
| Point prevalence | 0 | 5 (8%) | 3 (6.4%) | 0.321 | 0.078 |
| Continuous abstinence | 14 (23%) | 27 (40.7%) | 40 (66.5%) | 8.021 | 0.046$ |
| Lapse | 10 (16%) | 7 (11%) | 5 (9.7%) | 1.678 | 0.382 |
| Relapse | 2 (4%) | 2 (3%) | 0 | 0.056 | 0.387 |
| Attrition | 55 (88%) | 51 (76.3%) | 40 (66%) | 3.987 | 0.058 |

*Note: *Highly significant, $: significant. χ2: Pearson's Chi-square test, HE: health education, CBT: cognitive behavioural therapy.*

**Table 4** Cotinine test validation by Nicotine test kit after 6 months (based on continuous abstinence).

| Self-report | Cotinine test | HE, n (%) | CBT, n (%) | HE+CBT | χ2 | *p* |
|---|---|---|---|---|---|---|
| Quit | Positive | 10 (38.4) | 6 (11.5%) | 3 (5.3%) | 11.345 | 0.006 |
| | Negative | 3 (11.53) | 22 (42.3%) | 56 (96.4%) | | |

*Note: *Significant. χ2: Pearson's Chi-square test, HE: health education, CBT: cognitive behavioural therapy.*

**Table 5** Fagerstrom's Nicotine dependency scale for smokers at baseline and follow ups (6th, 12th and 24th).

| Fagerstrom's addiction scale | HE Group, n (%) (N=62) | | | | CBT group, n (%) (N=67) | | | | HE+CBT group, n (%) (N=61) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | 6th follow up | 12th follow up | 24th follow up | Baseline | 6th follow up | 12th follow up | 24th follow up | Baseline | 6th follow up | 12th follow up | 24th follow up |
| <=8 score | 56 (90.32) | 57 (91.93) | 58 (93.54) | 59 (95.1) | 63 (94.02) | 64 (95.52) | 65 (97.01) | 66 (98.5) | 54 (88.52) | 57 (93.44) | 60 (98.36) | 60 (98.36) |
| >8 score | 6 (9.67) | 5 (8.06) | 4 (6.45) | 3 (4.8) | 4 (5.97) | 3 (4.47) | 2 (2.98) | 1 (1.4) | 7 (11.47) | 4 (6.55) | 1 (1.63) | 1 (1.63) |
| Total | 62 (100) | 62 (100) | 62 (100) | 62 (100) | 67 (100) | 67 (100) | 67 (100) | 67 (100) | 61 (100) | 61 (100) | 61 (100) | 61 (100) |
| Mean | 4.2±5.6 | 3.8±7.2 | 3.4±4.4 | 3.5±4.7 | 5.9±4.4 | 3.7±7.3 | 3.1±6.5 | 3.3± 6.9 | 4.3±5.4 | 3.1±4.5 | 2.1±3.2 | 2.1±3.2 |

*Note: Mean scores between baseline, Fagerstrom's Nicotine dependency 1st follow up on 6th week and 2nd follow up on 12th week + 3rd follow up on 24th week; (p<0.001), CBT: cognitive behavioural therapy; HE: health education.*

## Discussion

The best way to stop the global smoking epidemic is to have good tobacco control programs via counselling. It is important to keep in mind that addiction to the use of tobacco should be treated as a chronic persistent disorder. Treatment for removing smoke in the lives of tobacco users requires persistent efforts to assist tobacco users by changing their behavioural and making them to attempts quitting it.[23]

Unhealthy behaviour is one of the main causes of human disease and behaviour management is the possible way in life smokers to make them quit. Changing unhealthy behaviour often seems very easy but in short term, the most difficult is to sustain it.[23,24] It is proven that the likelihood of achieving success in tobacco cessation can be through effective counselling. Through counselling programs, various information and resources are provided to tobacco users for developing a quit plan and addressing specific barriers to quitting and managing different withdrawal symptoms.[25] the methods and intensity of counselling vary as it varies from individual to individual based on the amount and type of support they needed.[26]

This study compares two types of counselling methods used such as counselling and CBT to continue to be positive about problem elimination and meeting points on current issues rather than focusing on the past. In the previous studies and literature searches, the age group that was included in the study was 14 to 65 years and the mean age for the CBT group was found to be 19-48.32 years.[27-32] In this present study, participants were in the age group of 18-68 years and the mean age group was found to be 37.6 years, 39.2 years and 40.8 years respectively in HE, CBT and HE+CBT groups. Gilpin *et al*. reported that smoking is believed to reduce anger and sadness in men.[33] In one previous research, only patients had been considered for the study.[34] In this study patients receiving treatment for tobacco cessation are male patients. The prevalence of tobacco consumption is largely related to the level of education and the socio-economic condition of the population. The lack of social and emotional security affects tobacco use from the lower working class and large family groups.[35] The level of Education and degree of awareness of the individuals mostly matter in controlling tobacco habits.[36] In this study age, sex, socioeconomic status, types of tobacco usage, alcohol usage, and the stage of motivation are not playing any significant role in tobacco cessation. In the current study, overall 44% of the tobacco users consumed alcohol but in the study conducted by Kumar *et al*, overall 69.5% of the study subjects gave a history of alcohol consumption and in another clinical trial done by Reddy *et al*., 43.6% shows the history of alcohol consumption.[37,38] In this study, half of the participants in the three groups started smoking in early adolescence 15-19 years, while other studies showed a little early age onset of 14.7-15.7 years.[30,31] Primarily the use of Tobacco begins during adolescence. It was found that nine out of ten individuals start smoking at the age of 18 years.[39] There is an important role of duration in quitting as it is very difficult for most smokers. Smokers often attempt to quit before successfully quitting, with some relapsing even after prolonged abstinence.[39] In the current three-group study, the majority of participants smoked for more than ten years. The average smoking duration was found to be (7.3±3.1 years) for the HE group, 7.7±3.9 years for CBT, and (7.9±2.8) for the HE+CBT group. The present study contrasts with previous studies, according to which three-quarters of the participants smoked <10 cigarettes per day as their group of participants also included smokeless tobacco.[40] The overweight of the study participants accounted for 5 years or more cigarette years in which fewer participants (12%) smoked <10 cigarettes per day. In the present study, the majority of participants smoked up to 250-285 cigarettes in one month. In the follow-up, the counselling was effective in three groups and the smokers reduced their cigarette smoking. In the three groups, the most effective advice on smoking cessation is the HE+CBT group. The mean number decreased in all three groups from baseline to subsequent follow-up. However, the difference was statistically significant in the combination of both therapies of HE & CBT.

In our study, the Fagerström's Test for Nicotine Dependence score at baseline was (4.2±5.6) in the HE, (4.7±5.9) in the CBT group, and (4.3±5.4) in the HE+CBT group. Fagerstrom's addiction score was found to be significant in two groups (CBT and HE + CBT) taking into account their initial value with the 3$^{rd}$ and 6$^{th}$ follow-up examinations ($p$<0.001), but in a previous study significant reduction in groups of CBT and groups of HE was found. 26 Fagerstrom's score reduction was greater in the HE+CBT group than in the CBT and HE groups. It was interesting to note that the reduction took place despite the interventions with equal contacts. This gives authenticity to the instruments adopted and used by the groups studied and indicates the differences in the interventions adopted. The main aim of the study is to motivate patients to give up smoking. Various studies show that CBT is more effective than HE [27,28,41-44,34] only one study shows that CET (Cognitive Enhancement Therapy) is more effective than CBT therapy.[29] Any intervention which was given to tobacco users from either HE or CBT groups was helpful to the patients in quitting the habit of tobacco. But if we add both therapies interventions HE+CBT it helps Tobacco users become aware of inaccurate or negative thinking so they can view challenging situations more clearly and respond to them more effectively. Basically, it is a matter of motivation and change of habit and behaviour towards tobacco use. The present study agrees that HE+CBT combined therapy is more effective at nicotine addiction and smoking cessation compared to the other two groups.

## Conclusion

We concluded from the study that the effectiveness of combined therapy (HE+CBT) is more effective in reducing tobacco habits than function of individual therapy among smokers. Health professionals should use a combination of HE and CBT interventions for tobacco users, especially smokers, for their quitting and heavy tobacco users are getting immediate attention for the tobacco cessation treatment. In the future, we can carry on the study with

the barriers that impact health professionals during the use of HE and CBT intervention in their tobacco cessation practices for making the sessions clinically effective and educationally more impactful.

## Limitations

There were three main factors to consider for limitations of this study; only males were included, only smokers were included, followed-up, and shorter study duration. This research also tried to establish some relationship between tobacco use cessation with appropriate education and the socioeconomic status of the participants. These findings also could not justify the general population. Finally, they used questionnaires that might be considered prepossession because the participants came to the hospital for their treatment. Multiple studies are required to test the effectiveness of CBT in this community and should run for long-term follow-up assessments with different gender distributions.

## Ethical approval

The study is approved by the Institutional Ethics Committee (DMR / IMSSH / SOA / 180338).

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## Conflict of interest

There are no conflicts of interest.

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# Impact of post-meal spot marching exercise in individuals with type 2 diabetes mellitus: A randomized clinical trial

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**ABSTRACT**

**Background**: For meaningful HbA1c reduction, improvement of cardiovascular risk profile, and weight loss, the current recommendation for people with type 2 diabetes mellitus (T2DM) is a minimum of 150 min/week of physical exercise. Post-meal exercise was both safe and effective in improving glycemic profiles in people with T2DM. However, the effects of post-meal spot marching exercise (PMSME) on glycemic control, exercise tolerance, leg muscle strength, and quality of life (QoL) in individuals with T2DM have not yet been investigated.

**Objectives**: Aim of this study was to investigate the effect of PMSME on glycemic control, exercise tolerance, leg muscle strength, and QoL in individuals with T2DM.

**Materials and methods**: Participants aged 50-70 were randomly allocated to the exercise or control groups (N=12 each). Participants in both groups received standard treatment following the Standards of Medical Care in Diabetes 2019. Only the exercise group performed moderate-intensity PMSME for 15 minutes per set, 3 sets per day, 4 days per week for 8 weeks. All participants underwent fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), Three-Minute Step Test (TMST), Five Times Sit to Stand Test (FTSST), and the 36-item Short Form Survey (SF-36) at baseline and at the end of this study.

**Results**: At baseline, there were no differences in outcomes between the two groups. At post-intervention period, the exercise group showed significant improvements in HbA1c (7.99 vs. 9.16%), heart rate recovery in the first minute after completion of the TMST (99.83 vs 113.58 bpm), FTSST (8.44 vs 10.02 sec), and SF-36 (physical function [82.50 vs. 67.50 scores], physical role [75.00 vs. 50.00 scores], vitality [75.00 vs. 57.50 scores], and total score [76.19 vs 62.81 scores] domains) compared to the control group ($p$<0.05).

**Conclusion**: PMSME could be used as an alternative home-based exercise to improve glycemic control, exercise tolerance, functional leg muscle strength and QoL in T2DM patients.

## Introduction

In emerging nations, inactivity and bad eating habits accelerate the incidence of type 2 diabetes (T2DM).[1] According to the International Diabetes Federation (IDF), there were 463 million cases of diabetes worldwide in 2019, and the number is expected to rise to 700 million by 2045.[2] Dietary modifications, weight management and increased physical activity are essential for T2DM patients.[3]

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Either aerobic activity or strength training can be used to decrease hemoglobin A1c (HbA1c) levels.[4,5] Using glucose from each meal for exercise is the ideal method of glycemic control for diabetes because the blood glucose-lowering benefits can be seen immediately.[6] Previous studies revealed that post-meal exercise in T2DM patients was both safe and able to improve glycemic profiles, hence reducing the risk of cardiovascular disease.[7-10] As post-meal blood glucose levels peak within 90 min, post-meal exercise could attenuate this peak in diabetes patients.[11,12] Spot marching was determined to be a safe and effective home-based exercise for people with chronic obstructive pulmonary disease to improve exercise endurance and quality of life.[13] Compared to arm or leg aerobic exercise alone, spot marching is a whole-body aerobic exercise that may be more beneficial in improving blood sugar levels and physical fitness.[9,14] However, there is insufficient evidence regarding the feasibility of PMSME in enhancing glycemic control, physical fitness, and quality of life in T2DM patients. Therefore, this study aimed to investigate the effect of 8-week home-based post-meal spot marching exercise (PMSME) on glycemic control, exercise tolerance, leg muscle strength, and quality of life (QoL) in individuals with T2DM.

## Materials and methods

### *Study design and participants*

The participants were recruited at Waritchaphum Hospital, Sakon Nakhon Province, Thailand, between October 2020 and January 2021. Before recruitment, each participant was screened for eligibility. A flow chart of the study enrolment is demonstrated in Figure 1. The sample size was calculated based on fasting plasma glucose (FPG) from a previous study.[15] The significant level $\alpha$=0.05 and the test efficiency 1-$\beta$=0.8 were set for bilateral test.[16] As a result, each group required 9 participants. Given a 20% rate of lost to follow-up, each group would require 12 participants.[17]

The inclusion criteria were as follows: (1) those diagnosed with T2DM, (2) age between 50 and 70 years, (3) body mass index (BMI) of 18.5-29.9 kg/m2, (4) sedentary behavior (metabolic equivalent; METs ≤1.5), and (5) capable of reading and communicating in Thai and achieving the Mini-Mental State Examination (MMSE) in Thai, depending on the participant's level of education: ≥22 scores for secondary school graduation or above ≥17 scores for elementary school graduation, and ≥14 scores for non-study.[18] The exclusion criteria were as follows: (1) FPG >300 mg/dL, (2) blood pressure <90/60 mmHg or >140/90 mmHg, (3) neurological and/or orthopedic problems, (4) risk of falls (Time Up and Go test >12 s),[19] and (5) participation in other exercise programs during this study period.

This study was a double blinded randomized controlled trial. The protocol was registered in Thai Clinical Trial Registry (ID 20210906003). Each participant was randomly allocated to the exercise group (EG) or the control group (CG) using stratified block randomized allocation with block sizes of 4 and 6 (www.sealedenvelope.com). Ages (50 to 60 years and 61 to 70 years) and gender were set as the stratified variables.

### *Intervention*

Both groups were required to maintain doses and

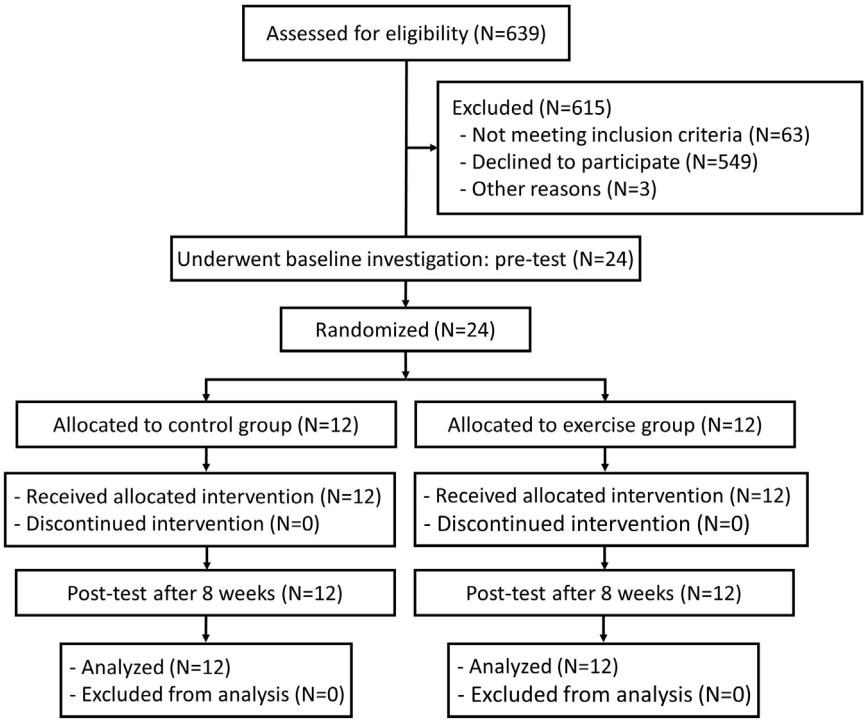


***Figure 1*** *Flow of participants in the study.*

medications during the intervention. The EG received home-based PMSME in addition to standard treatment following the Standards of Medical Care in Diabetes 2019.[20] The CG received only standard treatment. The intensity, time, and duration of PMSME in this study were derived from Pahra *et al*.[9] Participants in the EG underwent moderate-intensity PMSME with shoulder flexion ≥90° and hip flexion ≥70° (Figure 2) covering 1,400-1,600 steps for 15 min starting 30 min after each meal (breakfast, lunch, and dinner) 4 day/week for 8 weeks. Exercise intensity was achieved by using music with a rhythm of 80 to 90 beats/min, which was adjusted every 2 weeks. After each session, subjects were asked to record their step counts and any adverse events.

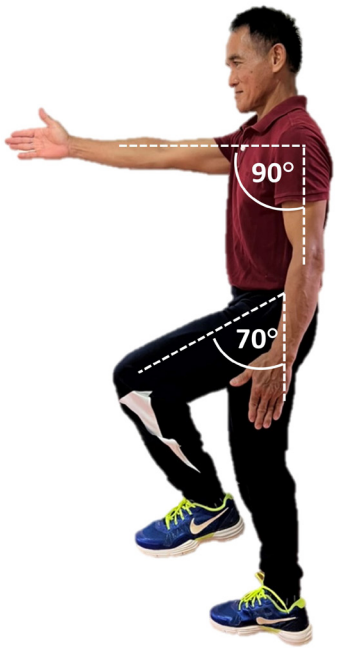


***Figure 2*** *Post-Meal Spot Marching Exercise (PMSME).*

*Outcome measures*

Five outcomes were evaluated at baseline and week 8 by a research assistant who was blinded to the study procedure.

*Glycemic control*

FPG and HbA1c tests were used to monitor glycemic control. After 8-12 hours of fasting, 5 ml of blood sample was collected in the next morning. FPG was determined using a glucose oxidase-peroxidase method, whilst HbA1c was assessed by high performance liquid chromatography (HPLC).[9,21] The FPG test determines the amount of free glucose in the blood, whereas the HbA1c test determines the amount of glycosylated hemoglobin. In addition, HbA1c is a key indicator of long-term glycemic management in T2DM patients.[22]

*Exercise tolerance test*

The Three-Minute Step Test (TMST) was used to determine cardiovascular fitness. First, all participants' resting heart rates (RHR) were determined. The participants then stepped on and off a 12-inch step at 96 beats/min for 3 min. The participants sat down, and their pulse rate was measured with a pulse oximeter immediately. Auscultation using a stethoscope was then performed to determine their cumulative heart beats (heart rate recovery [HRR]) in the first minute after completion of the test.[23]

*Lower extremity muscle strength*

Five Times Sit to Stand Test (FTSST) was used to measure functional lower extremity strength.[24] Participants sat on conventional armless chairs (43 cm seat height) with their arms crossed over their chests, backs straight, and feet flat on the floor 10 cm behind their knees. The participants had to stand with their hips and knees fully extended and sit down five times without moving their arms. From "Go" to the fifth repetition, Time was recorded in seconds. Data analysis used two attempts' average time.[25]

*Quality of life*

Self-Report Short Form-Series (SF-36) was used to monitor physical function, physical role, bodily pains, general health, vitality, social functioning, emotional role, and mental health. Each domain was scored 0-100, with higher scores indicating greater health.[26]

**Statistical analysis**

All analyses were carried out using SPSS version 28.0 (SPSS Inc., Chicago, IL, USA). The demographic data of the participants were analyzed using descriptive statistics. A Shapiro-Wilks test was used to determine the normality of the data set. The data of FPG, FTSST, HbA1c, and HRR had a normal distribution, whereas the data of SF-36 did not. Pre- and post-intervention data were compared using the paired t-test for normal distributed data and the Wilcoxon singed-rank test for non-normal distributed data. A comparison of pre- and post-intervention data between the groups was conducted using the independent t-test for normal distributed data and the Mann-Whitney U test for non-normal distributed data. The significance level for all inferential test was set at 0.05.

## Results

Table 1 shows the participants' demographics and health status. Baseline characteristics were comparable in the EG and CG. Each group had four men and eight women. The CG and the EG had 7±3.8 years and 6±3.7 years diabetes diagnoses, respectively. The majority of the participants were elderly, overweight, and had diabetic dyslipidemia with high plasma triglyceride, low HDL cholesterol, and increased LDL cholesterol levels. These lipid alterations are linked to diabetes mellitus because of the increased free fatty acid flux caused by insulin resistance.[27] The intervention completion was 100%. During the 8-week intervention, both groups had no hypo- or hyperglycemia, muscle soreness or other complications.

**Table 1** Baseline characteristics of participants in control and exercise groups.

| Variable | Control group (N=12) | Exercise group (N=12) | *p* value |
|---|---|---|---|
| Age (years) | 61 (5.74) | 62.33 (5.33) | 0.561 |
| Weight (kg) | 60.83 (8.45) | 60.25 (10.29) | 0.881 |
| Height (cm) | 159.08 (8.82) | 158.92 (5.79) | 0.957 |
| BMI (kg/m$^2$) | 24.17 (3.40) | 24.17 (3.07) | 0.999 |
| Lipid profile | | | |
| - Cholesterol (mg/dL) | 196.50 (54.10) | 178.75 (36.06) | 0.355 |
| - Triglyceride (mg/dL) | 154.25 (52.22) | 172.83 (55.22) | 0.406 |
| - LDL (mg/dL) | 128.42 (45.98) | 110.33 (26.70) | 0.251 |
| - HDL (mg/dL) | 37.47 (11.49) | 37.58 (8.60) | 0.978 |

*Note: Values are presented in mean (standard deviation), BMI: body mass index, LDL: low density lipoprotein, HDL: high density lipoprotein.*

***The effect of post-meal spot marching exercise on glycemic control***

*Fasting plasma glucose*

There was a significant reduction in FPG levels from baseline to the end of the intervention period in the CG (%change = -11.58%, $p<0.05$) and the EG (%change = -7.42%, $p<0.001$). At the end of the study, there was no significant difference in FPG levels between the CG and the EG ($p>0.05$) as shown in Table 2.

*Hemoglobin A1c*

There was a significant decrease in HbA1c after the intervention period in both the CG (%change = -2.97%, $p<0.001$) and the EG (%change = -15.98%, $p<0.001$) compared to baseline (Table 2). In addition, the decrease in HbA1c at the end of the study in the EG was significantly greater than in the CG ($p<0.05$).

*The effect of post-meal spot marching exercise on functional lower extremities muscle strength*

At the end of week 8, the EG had a significant decrease in the FTSST time scores compared to baseline (%change = -14.31%, $p<0.05$), whilst there was no difference in the CG ($p>0.05$). In addition, the EG demonstrated greater improvement in the FTSST scores than the CG at the end of the study ($p<0.05$).

*The effect of post-meal spot marching exercise on exercise tolerance*

At the end of the trial, HRR in the first minute after completion of the TMST in the EG had decreased significantly (%change = -11.20%, $p=0.001$) from the baseline, whereas there was no difference in the CG ($p>0.05$). In addition, the decrease in HRR in the EG was significantly greater than in the CG ($p<0.05$) after 8 weeks of intervention (Table 2).

**Table 2** Comparison of FPG, HbA1c, FTSST, and HRR between control and exercise groups at week 0 and week 8$^{th}$.

| Variable | Group | Week 0 | Week 8$^{th}$ | *p* value [a] | *p* value [b] |
|---|---|---|---|---|---|
| FPG (mg/dL) | Control | 175.17 (20.93) | 162.17 (12.92) | 0.001* | 0.224 |
| | Exercise | 175.50 (14.27) | 155.17 (14.43) | <0.001* | |
| HbA1c (%) | Control | 9.44 (1.71) | 9.16 (1.58) | <0.001* | 0.045* |
| | Exercise | 9.51 (1.11) | 7.99 (1.06) | <0.001* | |
| FTSST (s) | Control | 9.95 (1.35) | 10.02 (1.89) | 0.741 | 0.029* |
| | Exercise | 9.85 (1.78) | 8.44 (1.37) | 0.009* | |
| HRR (bpm) | Control | 112.75 (9.98) | 113.58 (11.94) | 0.592 | 0.004* |
| | Exercise | 112.42 (8.91) | 99.83 (8.58) | 0.001* | |

*Note: Values are presented in mean (standard deviation). [a]comparison between week 0 and week 8$^{th}$ using the Paired t-test, [b]comparison between groups at week 8$^{th}$ using Independent t-test, *statistically significant at p<0.05, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c, FTSST: five time sit to stand test, HRR: heart rate recovery in the first minute after completion of the TMST.*

*The effect of post-meal spot marching exercise on quality of life*

After completing the intervention, only the EG had significantly improved QoL ($p < 0.05$), including the physical function (%change =26.92%), physical role (%change =50.00%), bodily pain (%change =61.32%), vitality (%change =15.38%), mental health (%change =50.00%), and total score domains (%change =29.05%). At the end of the study, the increase in SF-36 scores in the physical function, physical role, vitality, and total score domains were significantly greater than in the CG ($p<0.05$), as shown in Table 3.

**Table 3** Comparison of quality of life in control and exercise groups at week 0 and week 8th.

| Domain | Group | Week 0 | Week 8th | *p* value [a] | *p* value [b] |
|---|---|---|---|---|---|
| Physical function | Control | 65.00 (28.75) | 67.50 (30.00) | 0.812 | 0.017* |
| | Exercise | 65.00 (28.75) | 82.50 (21.25) | 0.026* | |
| Physical role | Control | 50.00 (37.50) | 50.00 (43.75) | 0.655 | 0.004* |
| | Exercise | 50.00 (25.00) | 75.00 (25.00) | 0.002* | |
| Body pain | Control | 58.50 (12.00) | 57.75 (41.88) | 0.533 | 0.111 |
| | Exercise | 53.00 (11.00) | 85.50 (30.50) | 0.010* | |
| General health | Control | 55.00 (10.00) | 47.50 (34.75) | 0.504 | 0.118 |
| | Exercise | 57.50 (12.50) | 60.00 (27.00) | 0.540 | |
| Vitality | Control | 57.50 (15.00) | 57.50 (21.25) | 0.944 | 0.036* |
| | Exercise | 65.00 (12.50) | 75.00 (18.75) | 0.029* | |
| Social function | Control | 75.00 (34.38) | 75.00 (25.00) | 0.257 | 0.067 |
| | Exercise | 75.00 (18.38) | 87.50 (21.88) | 0.055 | |
| Emotional role | Control | 50.00 (33.34) | 66.67 (33.34) | 0.655 | 0.187 |
| | Exercise | 66.67 (33.34) | 66.67 (0.00) | 0.083 | |
| Mental health | Control | 56.00 (19.00) | 78.00 (23.00) | 0.105 | 0.727 |
| | Exercise | 52.00 (10.00) | 78.00 (14.00) | 0.003* | |
| Total score | Control | 59.47 (4.11) | 62.81 (14.04) | 0.136 | 0.003* |
| | Exercise | 59.04 (9.49) | 76.19 (11.51) | 0.002* | |

*Note: Values are presented in median (interquartile range). [a]comparison between week 0 and week 8th using the Wilcoxon Signed-Rank test, [b]comparison between groups at week 8th using the Mann-Whitney U test, *statistically significant at p<0.05.*

## Discussion

The present study demonstrated that the PMSME in combination with standard treatment significantly improved glycemic control, exercise tolerance, functional leg muscle strength and QoL in T2DM patients. FPG in the CG and EG decreased significantly from baseline at the end of this study. Our result is similar with previous studies that reported a decrease in FPG in T2DM patients who received dietary education.[28,29]

T2DM management is cost-effective when HbA1c decreases by 0.51%–0.89%.[30] Reduced HbA1c prevents micro- and macrovascular complications.[31] Additionally, the diabetic minimal clinically important difference (MCID) is a 0.5% HbA1c reduction.[32] Therefore, the decrease in HbA1c by 1.52% after 8 weeks of PMSME indicating it was cost-effective and clinically meaningful advantages for T2DM patients. Increased translocation of GLUT-4 (glucose transporters) to the plasma membrane during muscular contraction may explain the decrease in HbA1c.[33] Compared to Pahra *et al*,[9] our study showed a greater HbA1c reduction (0.9% vs 1.52%). A possible explanation is that the PMSME was a total-body workout, whereas brisk walking mostly targeted the lower limbs. In our study, however, neither FPG nor Hba1c returned to normal range after 8 weeks of the PMSME program.

This study also demonstrated that the PMSME can improve HRR in the first minute after completion of the TMST. Our finding was similar to a previous study on overweight patients with chronic kidney disease who engaged in home-based aerobic exercise three times per week for 12 weeks.[34] This could be explained by the fact that aerobic exercise enhances myocardial contractility and endothelial function by reducing oxidative stress and preserving nitric oxide bioavailability.[35]

After 8 weeks of PMSME, functional leg muscle strength improved significantly. In diabetics, increased functional leg muscular strength could delay age-related sarcopenia.[36,37] In addition, increased muscle mass and strength in T2DM patients can improve insulin sensitivity and glycemic control.[38,39] Since the PMSME is an accumulating aerobic exercise, its efficacy in achieving the MCID of FTSST (2.5 s) may be limited.[40]

The improvements in HbA1c, exercise tolerance and functional muscle strength observed in this study may contribute to an improvement in QoL (physical function, physical role, vitality, and total score domains of SF-36). Additionally, an increase in SF-36 scores in these domains was found to meet the MCID of SF-36.[41] The mechanism by which PMSME improves QoL in people with T2DM is unclear. The improvement in QoL is probably due to the result of a decrease in FPG and HbA1c as well as an increase in functional leg muscle strength an exercise tolerance. Our findings are consistent with previous studies suggesting that aerobic exercise training can increase QoL in sedentary, healthy men and T2DM patients.[42,43]

As far as the limitations are concerned, our study's findings may not be generalizable to other racial or regional groups due to the limited sample size and participants from only the Northeast Thailand. Therefore, the results should

be interpreted with caution. Further, we only instructed the participants to regulate their mealtime before exercise. Dietary intake before exercise has been observed to affect the exercise-induced blood glucose response.[44] Additional research should include dietary pattern measurements, e.g., eating frequency, nutritional composition, mealtime and employ dietary records to collect dietary information of the participants. Despite these limitations, the current study has several strengths including that it is a double-blind RCT with individual supervised exercise instruction and excellent 8-week intervention compliance (100% complete).

## Conclusion

According to our study, PMSME is simple to implement and highly compliant. T2DM patients can improve glycemic control, exercise tolerance, functional leg muscle strength and QoL by using the PMSME as an alternative home-based exercise program.

## Conflicts of interest

None

## Ethics approval

The study was approved by Ethics Committee of Center for Ethics in Human Research, Khon Kaen University (HE632170). All subjects provided their informed consent prior to participation.

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# Efficacy of straw phonation exercises with breathing exercises and indirect approaches for dysphonic adults with benign vocal fold lesions

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**ABSTRACT**

**Background:** The voice is essential to a people's daily life, especially in occupational voice users who are at risk for benign vocal fold lesions. It is important to use the proper vocal therapy techniques on a dysphonic patient who has benign vocal fold lesions in order to reduce behaviors that will aggravate the voice disorder and enhance behaviors that help improve voice quality.

**Objectives:** The purpose of this study was to evaluate the efficacy of straw phonation exercises with breathing exercises and indirect approaches in dysphonic adults with benign vocal fold lesions, including vocal nodules; vocal polyps, and vocal cysts.

**Materials and methods:** This study was a pilot study. Twelve participants who had benign vocal fold lesions received vocal hygiene guidance, abdominal breathing exercises, muscle relaxation, and straw phonation voice therapy. All participants received 8 weekly therapy sessions: 30 minutes per session. The vocal assessment data was collected before the first voice therapy session, and immediately after the eighth session, by subjective vocal assessment, and objective vocal assessment with Dr. Speech software version 5.

**Results:** The results after therapy showed significant improvements against the GIRBAS scales in terms of the subjective vocal assessment, in the values of SD F0, jitter, NNE, and MPT of the objective vocal assessment.

**Conclusion:** This result indicates straw phonation with breathing exercises and indirect approaches as being a worthwhile alternative method for voice therapy in dysphonic patients with benign vocal fold lesions.

## Introduction

The important role of the voice is as the medium for communication in our society. Voices matter for sharing ideas, showing an individual's emotions, and representing a personality. Besides, voice use is important for some jobs, such as singers, teachers, salesmen, and so on.[1,2] If voice quality is abnormal, an effect on the people's interpersonal relationships, communication abilities, or working ability is revealed. Additionally, occupational voice users have a high risk of voice disorder because their vocal behaviors can lead to vocal hyperfunction and develop into benign vocal fold lesions.[1] Vocal hyperfunction has been explained as a hypertonic state of intrinsic and extrinsic laryngeal musculature. Examples of vocal hyperfunction are very common, acting as excessive talking, singing with poor

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technique, excessive throat clearing, or coughing. These behaviors may lead to swelling, and to edema of the vocal fold mucosa, and subsequent vocal fatigue. If a patient does indeed have vocal hyperfunctional behaviors, benign lesions will be found on the vocal fold mucosa.[1] To prevent the effect of voice disorders, people with benign vocal fold lesions should receive treatment. The most common treatment for voice disorders is voice therapy.[1,3]

The semi-occluded vocal tract (SOVT) exercise technique is one of the voice therapy techniques which has been developed from a vocal warm-up for singers and has more recently gained popularity in the rehabilitation of the voices of dysphonic patients.[4-7] The SOVT technique includes a group of sub-techniques. Straw phonation exercise is one of those sub-techniques.[4,5,8-12] The basis of the SOVT exercises is to bring about the occlusion of some or all parts of the anterior vocal tract during phonation. Some research about SOVT exercises has shown that some physical changes take place during or after doing exercises.[4,13,14] SOVT exercises are a process that focuses on source-filter interaction to give the acoustic power of voice.[11] The result of this process is the vocal economy which is the minimum degree of vocal folds impact stress to phonate the maximum acoustic output.[5,6,12,15,16] So, the SOVT exercise technique increases the vocal economy by increasing the inertive reactance in the vocal tract.[6,8,10,12,13,15-20] Then, acoustic energy is thrown back to the vocal fold, which makes the phonation threshold pressure lower, and eases phonation.[5, 10, 18, 21, 22] The greater supraglottal pressure was attained by the semi-occlusion, resulting in reduced transglottal pressure, in turn leading to small proportionately vibrational amplitude, and barely any abduction or adduction of the vocal folds.[7,10,12,22-24] Moreover, the subglottal pressure also increases. Phonation with high subglottal pressure reduces the risk of injury to the vocal fold mucosa, making the SOVT Exercises the ideal vocal warm-up.[7,12,18,22-24] SOVT Exercises are based on resonant voice theory, that users will feel the vibration at the mid-face area when there is phonation.[4,5,10,25,26] Even if there are many other sub-techniques, such as voiced fricatives, lip-rounded vowels, nasal consonants, lip trill, tongue trill, or humming, some studies have found straw phonation exercise results in widening the throat, and enhanced voice energy than other techniques.[27] Furthermore, straw phonation exercise is one of the most commonly used SOVT exercises among occupational voice users.[28]

Therefore, to reduce the risk of lesions' progression and to prevent any socially significant effects from voice disorders, patients with benign vocal fold lesions should have efficient and suitable voice therapy. Straw phonation exercise is a sub-technique for voice therapy to produce a voice which results in minimum vocal fold impact stress, so this exercise may be suitable for treatment of hyperfunctional voice users who exhibit benign vocal fold lesions. However, previous studies did not focus specifically on the efficacy of straw phonation in dysphonic adults with benign vocal fold lesions, in order to prove the efficacy of this technique in this type of voice disorder. As such, the objective of this study is to evaluate the efficacy of straw phonation in dysphonic adults with benign vocal fold lesions.

## Materials and methods

This study was designed as a pilot study. The details were as follows.

### *Participants*

From the number of new patients with benign vocal fold lesions who came to the Speech Clinic at Ramathibodi Hospital in 2017-2019, it was found that there was an average population of 32 people per year. The sample was determined to be 28 people by using table created by Krejcie and Morgan in 1970 for calculating sample size for a finite population. However, there were twelve participants in the current study when data was collected from November 2018 to October 2020, in addition to the COVID-19 outbreak, and some participants withdrew.

Twelve participants in this study were adults with benign vocal fold lesions, and hyperfunctional vocal behavior. These lesions included vocal nodules, vocal polyps, and vocal cysts. The participants comprised 11 females (91.66%) and 1 male (8.33%). The mean age of participants was 47 years old, SD=14.04 years; with age range =24-62 years old, none of whom having any hearing problem for conversation purposes. The mean onset of voice problem were 16.41 months, SD=34.04 months, and the mean daily voice uses were 3.41 hours, SD=1.37 hours. The diagnosis of all participants included 8 participants with vocal nodules, 3 participants with vocal polyp, and 1 participant with vocal cyst. None of the participants had ever received any voice therapy.

### *Data collection procedures*

The data was collected after receiving approval from the Ethical Committee of the Ramathibodi Hospital, COA. No. MURA2018/819. The researcher would inform participants about the purpose of the study and explain the procedures to them. Subsequently, each accepted participant signed a consent form.

### *Assessments*

In this study, two types of vocal assessments were used: subjective vocal assessments and objective vocal assessments.

### *Subjective Vocal Assessment*

Voice samples of each participant were recorded before and after voice therapy by prolonging the vowel /a:/, counting 1-30, and reading the "Fon fah" passage, which includes all Thai consonant sounds. The participants' voice samples were blinded, and subsequently assessed in terms of GIRBAS scales by 3 speech language pathologists who had at least 20 years' experience in voice therapy. The subjective vocal assessment data had to have an agreement from 2 of 3 raters. GIRBAS scales included: values ranging from 0=no abnormality to 3=severe abnormality. The scale comprises 5 components: Grade

(G) is the overall vocal quality impression; Instability (I) is the fluctuation of voice quality over time; Roughness (R) is the auditory impression of irregular fluctuation of fundamental frequency; Breathiness (B) is the turbulent noise from some degree of air leakage whilst speaking; Asthenia (A) is the overall weakness or lack of power of the voice; and Strain (S) is the auditory impression of the voice produced with muscle tension or excess effort.[29-31]

***Objective Vocal Assessment***

Each participant was assessed by using the vocal assessment program of Dr. Speech Software version 5 (Designed by Daniel Z. Huang, Tiger DRS Inc, Shanghai, China). A participant sat in front of a microphone, 10 cm away from the microphone to the mouth. A participant was suggested to use their usual sound level while doing the assessment by prolonging the vowel /a:/ for as long as possible from one breath to assess maximum phonation time (MPT), and then again in 3 seconds, in order to assess each of the following acoustic parameters of voice: mean fundamental frequency (F0); standard deviations of F0 (SD F0); jitter; shimmer, and; the normalized noise energy (NNE).

***Voice therapy sessions***

After the assessment session, participants rested for 10 minutes, and then received the first session of voice therapy. The treatment period consisted of 8 sessions, a 30-minute session per week, and 20 minutes for a daily home program. Each therapy session included abdominal breathing exercises, muscle relaxation exercises, and straw phonation. For straw phonation, the method was adapted from the study of Kapsner-Smith *et al*. and Meerschman *et al*.,[5,26] the activities were added progressively in each therapy session, and all activities were straw phonations with the distal end in the air. In the first session, participants phonated /u:/ through a plastic straw of 22.5 cm in length, and 0.5 cm in diameter by placing the straw in the mouth, sealing the straw by the lips without air leaking from the nose or mouth, and focusing on the feeling of vibration in the mid-face. Then, for sessions 2-6, the activities would be added in each session as follows: (i) prolonging /u:/ with a combination of emphasis, and pitch glides, and making a pitch glide up – rest - and down, (ii) phonating /u:/ at separate pitch levels, without glides, (iii) phonating /u:/ when singing and reading sentences, without any articulation, following an intonation pattern, (iv) prolonging /u:/ with and without straw, and (v) reading short passages with and without straw. Session 7-8 would be a repeat of session 6.

After voice therapy in each session, participants were given homework to practice by themselves and to note any problems to discuss in the next session. At the last session, participants rested for 10 minutes after the therapy session, and then they were assessed again by using the same method as before the therapy assessment.

***Data analysis***

Vocal assessment data from before and after therapy were computed by utilizing the statistical package IBM SPSS Statistics 14. The results of the comparison of GIRBAS scales, before and after voice therapy, were calculated by using the Wilcoxon signed rank test.

Data from objective vocal assessment that included mean fundamental frequency (mean F0), standard deviation of fundamental frequency (SD F0), jitter as perturbation of fundamental frequency, shimmer as perturbation of amplitude, maximum phonation time (MPT), and normalized noise energy (NNE) were compared before and after therapy by using the Wilcoxon signed rank test. Level of significance was set at 0.05.

## Results

Results of the subjective vocal assessment and objective vocal assessment were shown as follows.

***Subjective vocal assessment***

From the comparison of GIRBAS scales before and after therapy by using the Wilcoxon signed rank test, the results show that there is a statistically significant difference, in terms of the p-values being <0.05; G ($p$=0.046), I ($p$=0.008), R ($p$=0.008), B ($p$=0.035), A ($p$=0.005), S ($p$=0.020). So, all severity levels of GIRBAS scales were significantly reduced after therapy. (Table 1)

**Table 1** Comparison of results of the subjective vocal assessment (GIRBAS scales) before and after therapy by Wilcoxon signed rank test.

| **Straw phonation voice therapy** | | | | |
|---|---|---|---|---|
| | **Before** | **After** | **Z** | ***p* value** |
| | median (QD) | median (QD) | | |
| **G** (grade of overall severity) | 1.00 (0.50) | 1.00 (0.00) | -2.000 | 0.046* |
| **I** (instability) | 1.00 (0.50) | 0.00 (0.37) | -2.646 | 0.008* |
| **R** (roughness) | 2.00 (0.50) | 1.00 (0.37) | -2.640 | 0.008* |
| **B** (Breathiness) | 2.00 (0.87) | 1.00 (0.37) | -2.828 | 0.035* |
| **A** (Asthenia) | 1.00 (1.00) | 0.00 (0.50) | -2.111 | 0.005* |
| **S** (Strain) | 1.00 (0.50) | 0.00 (0.37) | -2.333 | 0.020* |

**Significant at p<0.05*

***Objective vocal assessment***

Table 2 shows the comparison of voice parameters from the objective vocal assessment. The results of the Wilcoxon signed rank test show that there were statistically significant differences between before and after therapy of SD F0 ($p$=0.003), jitter ($p$=0.014), NNE ($p$=0.004), and MPT ($p$=0.010).

## Discussion

Results of straw phonation exercises with breathing exercises and indirect approaches in the current study

**Table 2** Comparison of the results of objective vocal assessment (voice parameters) before and after therapy by Wilcoxon signed rank test.

| Straw phonation voice therapy | | | | |
|---|---|---|---|---|
| Parameter | Before | After | Z | *p* value |
| | median (QD) | median (QD) | | |
| **Mean F0** | 200.47 (21.57) | 199.43 (18.59) | -0.314 | 0.754 |
| **SD F0** | 3.56 (1.28) | 1.56 (0.35) | -2.981 | 0.003* |
| **Jitter** | 0.29 (0.39) | 0.22 (0.05) | -2.447 | 0.014* |
| **Shimmer** | 1.88 (1.87) | 1.49 (0.24) | -1.334 | 0.182 |
| **NNE** | -7.43 (2.84) | -13.22 (2.63) | -2.903 | 0.004* |
| **MPT** | 5.60 (1.52) | 6.43 (1.12) | -2.589 | 0.010* |

**Significant at p<0.05*

were evident to be an effective method for patients with benign vocal fold lesions after 8 sessions of voice therapy with at least 4 days of home program during each week. These results also agree with the principle of SOVT exercise technique. For the subjective vocal assessment, all results of GIRBAS scales showed there to be statistically significant differences in scales between before and after therapy. The similarity of the results to those from the study by Peas *et al*.[32] may be attributable to the participants in the study, who were dysphonic adults. Although the study of Peas *et al.* assessed the immediate effects of voice therapy, this consistency indicated that the straw phonation was effective for better voice quality immediately after exercises and after 8 weeks of continuous therapy. Moreover, in the study of Peas *et al*., the voice sample was a natural speech sample, such as the counting number.[32] As in the current study, the counting 1-30 and reading the passage, which were used in a subjective vocal assessment, were natural speech samples, so the effectiveness of the therapy would be shown. Furthermore, the straw phonation with the distal end in the air in the current study was similar to the study of Guzman *et al*.,[25] they found that the results of the straw phonation with the distal end in the air from an auditory-perceptual assessment showed a significant improvement. Thus, it could be speculated that the straw phonation with the distal end in the air, enables auditory self-monitoring, as the participants are able to hear themselves clearly while doing tasks. Then, the auditory monitoring would lead to the improvement of voice quality.[25] However, the results of Fadel *et al*.[33] who studied the immediate effect of SOVT exercise with LaxVox tube in singers, showed that there was no difference in the results of the perceptual assessment. The different results may be due to the participants who were singers without any voice disorders, and the duration of treatment was too short to show any effectiveness.

Meanwhile, the objective vocal assessment revealed the changes in voice parameters - after therapy in SD F0, jitter, NNE, and MPT - to be statistically significant. For SD F0, which should be less than 3.0 in the vocal assessment program of Dr. Speech software, a significant reduction represented a positive effect on the voice parameters, which might affect the voice quality after therapy. Likewise, the reduction in jitter meant the frequency variations of participants' vocal fold vibration were reduced, and this affected the better voice quality.[1] Furthermore, Fadel *et al*.[33] mentioned that the straw phonation was a therapeutic option for voice disorder cases, and it brought about smoother and more efficient mobility of the vocal folds by increasing activity of the thyroarytenoid muscle instead of that of the cricoarytenoid lateralis muscle.[12] As regards NNE, or noise in voice production, normal sound should be accompanied by minimal noise.[1] The similarity of results with Peas *et al.,*[32] Fadel *et al*.[33] and Guzman was evident in the reduction of noise in voice production after straw phonation.[34] The goal of straw phonation exercises was to improve voice production efficiency, with the process focusing on source-filter interaction to provide acoustic power of voice,[35] and possibly be effective in reducing noise in voice production. In this current study, NNE was reduced significantly. That is, the noise in production, which affects the voice quality of participants, was reduced after straw phonation voice therapy. It also corresponds to the study of Guzman *et al*.[34] The result of which was that the breathy voice quality, which represented the noise in voice production, decreased significantly. These results may be due to better control of airflow, which is required to perform straw phonation.[32] Nonetheless, Guzman *et al*. noted that this change could be found after a sudden set of exercises and after several weeks of therapy in the participants who were patients with hyperfunctional voice disorders.[34] For MPT, the finding of this study was that it increased significantly after having undertaken the therapy, such that participants could produce longer phonations after straw phonation voice therapy. However, the result of MPT in this study was contrary to the MPT finding of Meerschman *et al.,*[26] there was no significant difference between the before and after straw phonation in healthy participants. Meerschman *et al*. informed that the MPT of their participants might be in a normal range before therapy.

Nevertheless, there were two voice parameters in the current study that had no significant difference before and after therapy. They were mean F0, and shimmer. Each did exhibit a tendency to decrease when comparing the median before and after therapy. For shimmer, eight participants were found to have shimmer that was within the norm of Dr. Speech software's vocal assessment program before and after therapy, but four were found to be abnormal before therapy. However, three of them were found to have normal shimmer after therapy. It was noticed that another individual who had abnormal shimmer before and after therapy tended to have reduced shimmer afterwards. Meanwhile, the F0 before therapy was found to be higher than normal in one male participant in this study. But after therapy, it was found that the F0 decreased but still did not reach the normal criteria. Before therapy, 5 of the 11 female participants had F0 that were within the acceptable range for their age and sex. The other 4 had F0 that were below average, while 2 had F0 that were above average. Three female participants

with F0 below the acceptable range had their F0raised to the acceptable range, while one female participant with F0 above the acceptable range had her F0 decrease to a level that was nearly within the acceptable range. The F0 of female participants who had previously been within the normal range had slightly decreased but remained within the range. As a result, overall F0 trend decreases. The decreasing F0 in the current study was similar to that from the study of Laukkanen *et al*.[36] which mentioned that the decreasing F0 was accompanied by comfortable and easy phonation. In contrast to the study of Fadel *et al.*[33] they found that the F0 significantly increased in female participants. They noted that the increase in F0 occurred post-SOVT exercises with high pitched sound technique by gliding and ascending scales. So, the cricothyroid muscle, which regulated the fundamental frequency, was activated during the exercise.[33] Even though the current study comprised with some sessions with pitch sound techniques, the pitch sound techniques comprised with ascending and descending scales. Also, participants of this study were dysphonic adults, and the exercise was eight weekly sessions. But the participants in the study of Fadel *et al.* were healthy singers, and their results were assessed immediately after SOVT exercise.

## Conclusion

The results of the current study have shown the efficacy of straw phonation with breathing exercises and indirect approach after therapy in the GIRBAS scales and in the values of SD F0, jitter, NNE, and MPT. Thus, this may indicate straw phonation as being another worthwhile alternative method for voice therapy in dysphonic patients with benign vocal fold lesions and the therapeutic results can be seen within a period of 8 weeks. Patients have been practicing continually during the whole course of therapy and should also be practicing by themselves at home. However, the participants also received some behavioral voice therapy methods. There is also the possibility that the participants would have better voice quality as a result of side voice therapy.

The COVID-19 pandemic had interfered with this data collection; thus, the number of research participants was limited. This study was nonetheless designed as a pilot study. For further studies, the data should be collected from a greater number of participants and compared the results from both genders of participants and should collect the physical characteristics of the vocal cords, in order to be able to study the significant changes in the lesion on the vocal folds. The comparison among other voice therapy techniques should be studied in order to achieve a comprehensive comparison of results for voice quality.

## Ethic approval

The data was collected after receiving approval from the Ethical Committee of the Ramathibodi Hospital, COA. No. MURA2018/819.

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# Reliability of transversus abdominis and internal oblique muscle thickness measurement using B-mode ultrasound imaging in standing and single leg standing positions

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**ABSTRACT**

**Background**: The thickness of the transversus abdominis (TrA) and internal oblique (IO) muscles are measured with high reliability by musculoskeletal ultrasound imaging (MSUSI) in the supine hook-lying and walking position. However, the reliability of the procedure to measure the TrA and IO muscle thickness measurement in the functional positions such as standing and single leg standing positions have not been well established.

**Objectives**: To examine the reliability of MSUSI for the TrA and IO muscles thickness measurement during standing and single leg standing positions in healthy participants.

**Materials and methods**: Ten healthy participants (aged 24.10±2.47 years) were recruited to perform MSUSI measurement of TrA and IO muscle thickness during the resting and abdominal drawing in maneuver (ADIM) in the standing and single leg standing positions. The intra-tester reliability of MSUSI measurement was performed by the same investigator in 2 different times (24 to 72 hours apart), while the inter-tester reliability was performed by 2 investigators within the same day. The intraclass correlation coefficient (ICC) whereby $ICC_{3,3}$ was used to determine the intra-tester and $ICC_{2,3}$ was used to determine the inter-tester reliability of TrA and IO muscles thickness measurement using MSUSI. The standard error of measurement (SEM) and coefficient of variation (CV) were calculated.

**Results**: The MSUSI measurements for TrA and IO muscles thickness showed high reliability in both standing and single leg standing positions ($ICC_{3,3}$ and $ICC_{2,3}$ >0.9). Moreover, the values of SEM ranged between 0.055 to 0.662 mm, MDC ranged between 0.152 to 1.834 mm and CV ranged between 0.543 to 14.001%.

**Conclusion**: The MSUSI measurement of TrA and IO muscles thickness could be performed with high intra and inter-tester reliability in standing and single leg standing positions among healthy individuals. The study findings on the MSUSI procedure to measure muscle thickness of TrA and IO would be useful for practice guidance in various research and clinical settings.

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## Introduction

Musculoskeletal ultrasound imaging (MSUSI) is an alternative method widely used for measuring trunk muscle activation as it is noninvasive and allows visual presentation of deep trunk muscle contraction and thickness.[1-5] Transversus abdominis (TrA) and internal oblique (IO) muscles are deep trunk muscles suggested to provide spinal stability.[6,7] As such, past studies had reported the clinical functioning of TrA and IO muscles thickness during lumbopelvic test among people with low back pain.[1,7] The measurement of muscle thickness represents the morphologic characteristics of the muscles and demonstrate the activation of the muscles by calculating the relative change of muscle thickness during muscle contraction to resting thickness. Past studies that investigated the reliability of the procedures using MSUSI for measuring TrA and IO muscles thickness and activation were performed in supine hook-lying and walking positions.[4,8-12] The results on the reliability of the past studies showed that there were moderate reliability in the standing (ICC=0.55) and walking positions (ICC=0.74). The factors that may affect reliability of MSUSI on abdominal muscles include transducer location, pressure, and stage of breath cycle at which the image is taken.[4,9,10,12] The position of the patient is another important aspect that may influence the reliability of the MSUSI measurements of TrA and IO as different positions such as walking and supine lying demands different levels of muscle activation to control trunk stability.[12] Therefore, it is very appropriate to investigate the reliability of the MSUSI procedure to determine muscle thickness of TrA and IO especially when the technique is applied in a functional position. This study had chosen standing and single leg standing positions as these positions required more postural control than supine hook-lying position. Moreover, the muscle thickness during contraction and resting can be used to indicate the muscle activation. Also, abdominal drawing-in maneuver (ADIM) is recommended to stimulate the activation of the TrA muscle.[13] Furthermore, the TrA and IO activation has not been well established in the functional tasks such as standing and single leg positions. Thus, the main aim of the study was to investigate the reliability of the MSUSI of the TrA and IO in the standing and single-leg standing positions during resting and ADIM. Establishing a reliable procedure for the MSUSI measurements of TrA and IO in a functional position such as single leg standing is pertinent as this may inform the reliable assessment practice of TrA and IO in any of the clinical studies and practice settings.

## Materials and methods

### Subjects

Sample size calculation in this study was based on Walter *et al*.[14] which recommended using sample size calculator (https://wnarifin.github.io/ssc/ssicc.html). An estimated sample size of 10 participants was needed for this study with the minimum acceptable reliability (ICC) was set at 0.75 and a power of 0.8 with an alpha level of 0.05. A total of 10 healthy participants (5 males and 5 females) with mean age 24.1±2.47 years and BMI 20.94±1.51 kg/m$^2$ participated in the study. Participants with lumbopelvic, hip, lower extremity injury or surgery within the past 12 months, participants with abnormalities that affect the balance, participants who had impaired standing balance and who were not able to perform single leg standing for at least 30 seconds were excluded. Participants were withdrawn if they were unable to stand still during MSUSI measurement, or who were unable to complete all MSUSI measurement conditions. Informed consent was obtained from all the participants prior to their participation in the study. This research was approved by an institutional research and ethics committee (Ethical Approval No. 299/2021, code AMSEC-64EX-034).

### Procedure

Ultrasound images of the TrA and IO muscles were obtained using an ultrasound scanner (Canon Xario-100S, TUS-X100S model) in B-mode with a 12-MHz linear array transducer. The aqua sonic gel was applied on participant's skin. The transducer of the ultrasound was placed firmly on the anterolateral aspect of the abdominal wall, superior to the iliac crest and perpendicular to the mid-axillary line while ensuring that the thoracolumbar fascia was aligned with the edge of the screen with TrA and IO muscles. The ultrasound images of TrA and IO were shown in Figure 1. The investigator had to constantly control the pressure of the transducer in all the test position while monitoring the muscles image on the screen. The images of the TrA and IO were captured at the end of participants' expiration during resting and abdominal drawing-in maneuver (ADIM). Before MSUSI, participants were instructed to practice the ADIM in supine hook-lying position (standard position). The participants were given a standard instruction which was "inhale with expanding the abdomen then exhale with flattening the abdomen. At the end of exhalation, pull the navel inward and upward towards the spine and hold it." The procedure was randomized to both the limb dominancy (dominant and non-dominant limbs) and the functional testing positions (standing and single leg standing positions). The images were captured three times for each condition and images were captured in resting position before ADIM to prevent fatigue of the muscles with a one minute rest given between each condition. The thickness of TrA and IO muscles were averaged from 3 images. The MSUSI images were collected in 2 positions including standing and single leg standing (Figure 2A and 2B). Participants were instructed to stand with dominant leg and lift non-dominant leg without dropping the pelvis. The dominant leg was determined by kicking a ball 10 meter 3 times, the leg which was used to kick the ball more frequently was taken as the dominant leg.[15] The images were captured from both sides of of TrA and IO muscle in standing and single leg standing positions. Intra-tester reliability of TrA and IO MSUSI measurements was performed by same investigator at 2 different times within 24 to 72 hours apart. Inter-tester reliability of TrA and IO MSUSI measurement was performed by 2 investigators within the same day and the investigators performed the procedures randomly without any particular order. The thickness of TrA and IO muscles were measured by using ImageJ program (NIH; version 1.8).

**Statistical analysis**

The analysis of intra-tester and inter-tester reliability of MSUSI measurements were calculated using the intraclass correlation coefficient (ICC). $ICC_{3,3}$ and $ICC_{2,3}$ were used to determine the Intra-tester and inter-tester reliability respectively, and *p*-value was set at $p<0.05$. Response stability was calculated using standard error of the measurement (SEM) and the coefficient of variation (CV).

$SEM = \sqrt{MSE}$ MSE is mean square error.

$CV = (SD/\bar{x}*100)$ $\bar{x}$ is the mean of the data, SD is the standard deviation of the muscle thickness.

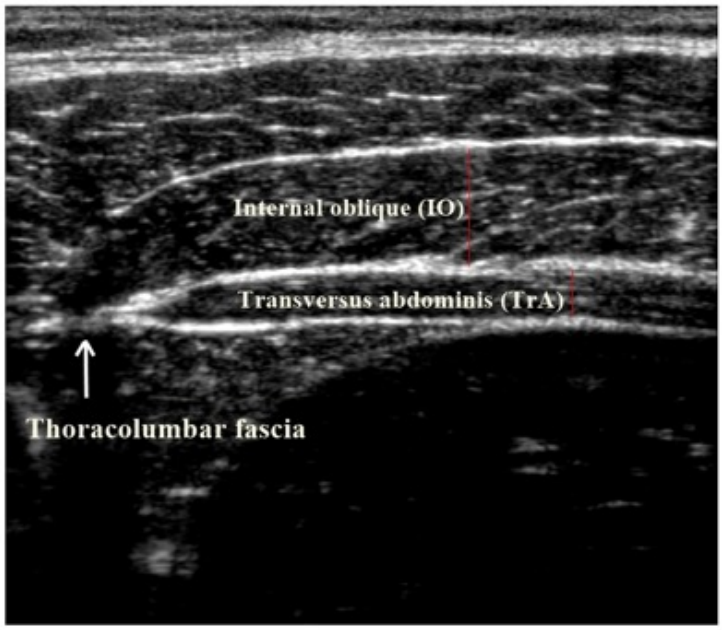


***Figure 1***. *The TrA and IO muscle image which captured by the MSUSI.*

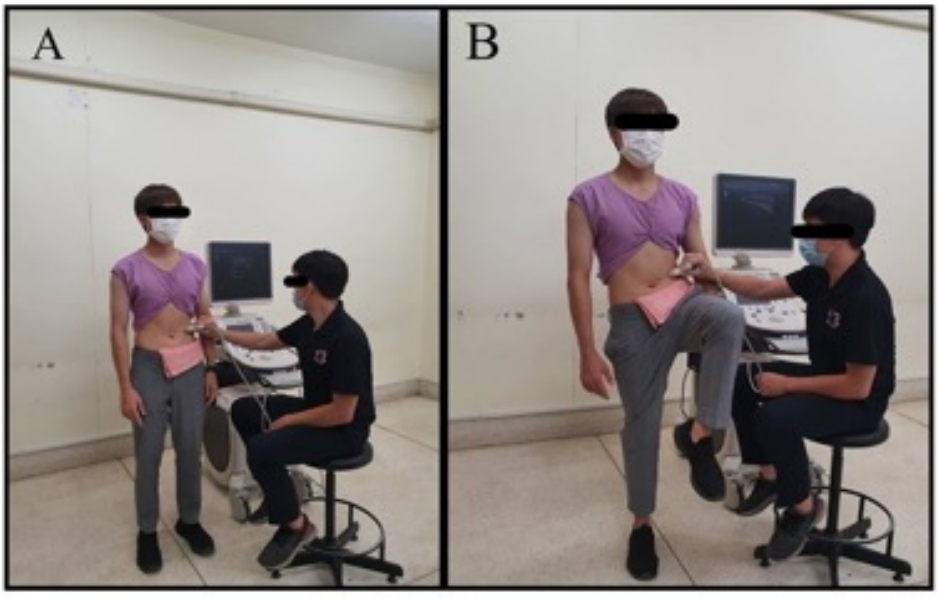


***Figure 2***. *Position set up for MSUSI measurement of TrA and IO muscles. MSUSI measurement in standing position (A) and in single leg standing position (B).*

## Results

There were 10 healthy participants (5 males and 5 females) who participated in this study with mean age 24.1±2.47 years and BMI 20.94±1.51 kg/m$^2$. Mean and SD of TrA and IO muscle thickness during standing and single leg standing are shown in Table 1. The ICC, SEM, CV, and MDC are presented in Table 2 for intra-tester reliability and Table 3 for inter-tester reliability. During standing and single leg standing position, the ICC of TrA and IO muscle thickness measurements were more than 0.9 both intra-tester and inter-tester reliability. The values of SEM ranged between 0.055 to 0.662 mm, MDC ranged between 0.152 to 1.834 mm and CV ranged between 0.543% to 14.001%.

**Table 1** Mean and SD of TrA and IO muscle thickness during standing and single leg standing from investigator 1 trial 1, investigator 1 trial 2 and investigator 2.

| Position | | Investigator 1 trial 1 | | | |
|---|---|---|---|---|---|
| | | TrA muscle thickness (mm) | | IO muscle thickness (mm) | |
| | | Dominant side | Non-dominant side | Dominant side | Non-dominant side |
| Stand | Resting | 3.55±1.05 | 3.85±0.81 | 7.74±2.84 | 8.43±2.72 |
| | ADIM | 5.24±1.61 | 5.91±1.49 | 10.67±3.19 | 10.63±2.53 |
| Single leg stand | Resting | 3.69±1.11 | 4.12±0.90 | 9.06±3.49 | 8.40±2.55 |
| | ADIM | 5.57±1.20 | 6.08±1.14 | 10.96±3.02 | 10.79±2.45 |
| **Position** | | **Investigator 1 trial 2** | | | |
| | | TrA muscle thickness (mm) | | IO muscle thickness (mm) | |
| | | Dominant side | Non-dominant side | Dominant side | Non-dominant side |
| Stand | Resting | 3.58±1.07 | 3.87±0.91 | 9.05±3.28 | 8.49±2.64 |
| | ADIM | 5.24±1.56 | 5.89±1.48 | 10.98±3.31 | 10.80±2.52 |
| Single leg stand | Resting | 3.83±1.23 | 4.08±1.00 | 9.16±3.49 | 8.41±2.62 |
| | ADIM | 5.53±1.17 | 6.00±1.12 | 10.82±2.95 | 10.65±2.37 |
| **Position** | | **Investigator 2** | | | |
| | | TrA muscle thickness (mm) | | IO muscle thickness (mm) | |
| | | Dominant side | Non-dominant side | Dominant side | Non-dominant side |
| Stand | Resting | 3.55±1.07 | 3.84±0.80 | 8.97±3.20 | 8.64±2.86 |
| | ADIM | 5.26±1.57 | 5.95±1.49 | 10.85±3.24 | 10.74±2.64 |
| Single leg stand | Resting | 3.71±1.09 | 4.08±0.89 | 9.09±3.41 | 8.47±2.55 |
| | ADIM | 5.43±1.15 | 6.01±1.10 | 10.97±3.00 | 10.81±2.45 |

***Note**: TrA: Transversus abdominis, IO: Internal oblique, mm: millimeter, ADIM: abdominal drawing in maneuver.*

**Table 2** $ICC_{3,3}$, SEM and CV of TrA and IO muscle thickness for intra-tester reliability.

| Muscle/position/condition | | Dominant side | | | | Non-dominant side | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $ICC_{3,3}$ | SEM (mm) | CV (%) | MDC (mm) | $ICC_{3,3}$ | SEM (mm) | CV (%) | MDC (mm) |
| TrA muscle thickness during standing | Resting | 0.998 | 0.055 | 1.797 | 0.152 | 0.960 | 0.239 | 6.199 | 0.662 |
| | ADIM | 0.994 | 0.170 | 3.269 | 0.472 | 0.997 | 0.114 | 1.917 | 0.316 |
| TrA muscle thickness during single leg standing | Resting | 0.987 | 0.187 | 5.714 | 0.519 | 0.989 | 0.145 | 3.563 | 0.402 |
| | ADIM | 0.994 | 0.134 | 2.498 | 0.372 | 0.998 | 0.245 | 1.599 | 0.679 |
| IO muscle thickness during standing | Resting | 0.976 | 0.662 | 14.001 | 1.834 | 0.984 | 0.281 | 3.371 | 0.779 |
| | ADIM | 0.991 | 0.430 | 4.539 | 1.192 | 0.998 | 0.161 | 1.887 | 0.447 |
| IO muscle thickness during single leg standing | Resting | 0.999 | 0.095 | 1.324 | 0.263 | 0.998 | 0.164 | 1.975 | 0.455 |
| | ADIM | 0.997 | 0.228 | 2.323 | 0.632 | 0.997 | 0.173 | 1.904 | 0.480 |

***Note**: ICC: intraclass correlation coefficient, SEM: standard error of measurement, CV: coefficient of variation, MDC: minimal detectable change, mm: millimeter, ADIM: abdominal drawing in maneuver.*

**Table 3** $ICC_{2,3}$, SEM and CV of TrA and IO muscle thickness for inter-tester reliability.

| Muscle/position/condition | | Dominant side | | | | Non-dominant side | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $ICC_{2,3}$ | SEM (mm) | CV (%) | MDC (mm) | $ICC_{2,3}$ | SEM (mm) | CV (%) | MDC (mm) |
| TrA muscle thickness during standing | Resting | 0.992 | 0.134 | 3.758 | 0.372 | 0.991 | 0.105 | 2.741 | 0.291 |
| | ADIM | 0.999 | 0.055 | 1.100 | 0.152 | 0.998 | 0.089 | 1.580 | 0.248 |
| TrA muscle thickness during single leg standing | Resting | 0.999 | 0.055 | 1.396 | 0.152 | 0.996 | 0.077 | 2.041 | 0.215 |
| | ADIM | 0.989 | 0.173 | 3.710 | 0.480 | 0.992 | 0.138 | 2.459 | 0.382 |
| IO muscle thickness during standing | Resting | 0.983 | 0.551 | 12.811 | 1.528 | 0.987 | 0.446 | 5.527 | 1.237 |
| | ADIM | 0.997 | 0.232 | 2.502 | 0.644 | 0.997 | 0.214 | 2.145 | 0.594 |
| IO muscle thickness during single leg standing | Resting | 0.999 | 0.187 | 2.068 | 0.519 | 0.999 | 0.071 | 1.075 | 0.196 |
| | ADIM | 0.999 | 0.084 | 0.787 | 0.232 | 0.999 | 0.055 | 0.543 | 0.152 |

***Note**: ICC: intraclass correlation coefficient, SEM: standard error of measurement, CV: coefficient of variation, MDC: minimal detectable change, mm: millimeter, ADIM: abdominal drawing in maneuver.*

## Discussion

The current study demonstrated high intra-tester and inter-tester reliability for MSUSI procedure to measure muscle thickness of TrA and IO muscle during standing and single leg standing positions ($ICC_{3,3}$ and $ICC_{2,3}$ >0.9). Moreover, the reproducibility of the measurement procedures were considered to be reliable as the overall SEM were 0.055 to 0.662 mm and CV ranged between 0.543% to 14.001%. which is within the acceptable measurement error of SEM less than 5% and CV were less than 15%.[14] Therefore, the results of ICC, CV and SEM showed that the use of MSUSI in this study was reliable to measure thickness of TrA and IO muscle in standing and single leg standing positions. Previous study of Mangum *et al*.[12] assessed the reliability of MSUSI for TrA muscle on tabletop, seated, standing and walking positions among the university students and they found that the MSUSI were reliable in all position. The intraclass correlation coefficient value (ICC>0.90) in the current study was comparable with the previous study which reported reliability values in tabletop position (ICC=0.903), seated position (ICC=0.613), standing position ICC=0.553), and walking position (ICC=0.737).[12] The investigators followed the exact procedure and measurement timeline from the previous study which could explain the high reliability results demonstrated by the MSUSI procedure. Previous study participants were university students, and the procedure was not tested in the single leg stand position. However, the current study participants were community adults and the current study measured in both standing and single leg standing positions thereby providing further knowledge and new data on TrA and IO during the single leg standing position which might help for subsequent research.

Using an average of three measurements of the muscle thickness values from the MSUSI procedure is reported to decrease the SEM by over 50%.[15] The fact that the current study also followed this recommendation of using an average of three measures might had contributed to high reliability of the study findings. The SEM and CV reported as intra-tester reliability measures for TrA muscle in the current study (SEM range, 0.055 to 0.245 mm and CV range, 1.797 to 6.199%) was lower than Teyhen *et al*,[16] who reported SEM of TrA (range, 0.31-0.87 mm). Similarly in the current study, the SEM and CV related to inter-tester reliability measures of TrA (SEM range, 0.055 to 0.173 mm and CV range, 1.100 to 3.758%) was lower to Springer *et al*,[17] who demonstrated SEM of TrA muscle to range between 0.13 to 0.35 mm. The study findings might therefore imply that the MSUSI procedure was highly reliable to measure muscle thickness during standing and single leg standing positions.

The present study showed high intra-tester reliability across day for TrA and IO (ICCs range, 0.960 to 0.999) and SEM range 0.055 to 0.551 mm. The high reliability might be explained due to using better resolution of the TrA and IO ultrasound images with a B-mode 12-MHz linear array transducer. Previous studies suggested using a linear transducer contributed for excellent reliability.[4,5,9,10,12,18] Linear transducers commonly have higher frequency, reduced penetration and better resolution which helped to capture a clear TrA and IO muscle structures on the MSUSI images. Furthermore, a standardized protocol was used to place the transducer whereby positioning of the transducer was established to be halfway between the lower rib and iliac crest and placed at the area of participants' abdomen capturing the thoracolumbar fascia of TrA and IO muscle on the ultrasound screen. Additionally, the participants were instructed to breath normally and the measurement using the imaging was performed at the end of the expiration, hence such standardization procedures of measurement could have helped to perform the MSUSI procedure with high reliability. All of the above procedures might have contributed to reduce the margin of measurement errors and resulted in high reliability. MSUSI measurement can be used clinically to make decision on muscle thickness change regarding to intervention such as spinal stabilization exercise.

Some limitation of current study should be considered. One of the limitations was the age, as all the participants in this study were 20-30 years old so this might cause generalization to lower age groups. In addition, participants in this study were healthy participants so the results of this study might be relevant to only healthy participants and

not applicable to a clinical population such as low back pain. Further studies could examine the reliability of performing the procedures in different populations such as chronic low back pain. Nevertheless, the current study had established a clear MSUSI procedure and measurement protocol to measure muscle thickness of TrA and IO during functional tasks such as standing and single leg standing. The current study provided a first-hand knowledge in the single leg standing positions with regard to the TrA and IO muscle activations which was not studied before. The results of this study showed that MSUSI was a reliable procedure for measuring TrA and IO muscles thickness. Thus, the MSUSI procedure could be used in future studies to determine the muscle activation and thickness of TrA and IO muscles in functional position such as single leg standing. The procedural information and findings might provide guidance to future clinical research on the functional assessment of for TrA and IO muscles in symptomatic population such as people with low back pain.

## Conclusion

MSUSI could be performed as a non-invasive procedure with high reliability to measure muscle thickness of the TrA and IO muscles during functional tasks such as standing and single leg standing among the healthy individuals.

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# Fall incidence and associated factors in spinal cord injury patients who walk with or without an ambulatory assistive device

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**ABSTRACT**

**Background:** The majority of ambulatory individuals with spinal cord injury (SCI) require an ambulatory assistive device (AAD). However, the association between falls and gait aid use is still inconsistent. Subgroup analysis may provide the opportunity to differentiate these factors more clearly.

**Objectives:** This study aimed to explore fall incidence and associated factors in SCI patients who walk with or without an AAD.

**Materials and methods:** Ninety-seven participants were interviewed for their baseline demographic data, physical activity, history of falling and fear of falling. They were divided into 2 groups, AAD and non AAD group. They were assessed with three functional tests, including the Timed Up and Go Test, Five Time Sit to Stand Test and 10 Meter Walk Test. Then fall data was followed for six months.

**Results:** More than one-third of all participants experienced at least one fall (40%). The results also showed that the proportion of falls in the AAD group was slightly greater than that of the non-AAD group (AAD=46% and non-AAD=32%). In the AAD group, a multiple variable analysis indicated a significantly higher fall rate among those who were younger than 50 years, used a cane or crutches and had moderate to high physical activity. For the non-AAD group, it was found that poor balance and a history of falls were strong factors associated with more falls.

**Conclusion:** Falls were a serious issue for the participants who walk with or without an ADD. However, the factors affecting falls in these two groups were different. Thus, therapists should consider the factors associated with falls for each group of SCI patients separately to provide proper rehabilitation that prevents falls and subsequent injury.

## Introduction

Nowadays, approximately 80% of individuals with incomplete spinal cord injury (iSCI) can regain their walking ability.[1] However, the majority of ambulatory iSCI require an ambulatory assistive device (AAD) (64%), while 36% of these individuals walk without an ADD.[2] To alleviate reduced quality of ambulation, gait aids are commonly prescribed for those with muscle weakness, and poor weight bearing and mobility[3]. Although gait aids can be helpful for maintaining balance, improper use of AAD contributes to increased energy expenditure, an unsteady gait, overload, joint damage and a higher risk of fall.[3-4] A previous study reported that AAD users were more likely to fall than non-AAD users.[5] Nevertheless,

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Phonthee *et al*. found that iSCI walking independently without gait aids reported an increased risk of fall.[6] The injuries resulting from falls in patients with SCI have been reported as including muscle pain, wounds, ligament tears and fractures.[7] Moreover, functional restriction, fear of falling, social isolation, depression, and hospitalization may occur as consequences of falls.[8] Musselman *et al*.[9] investigated intrinsic fall risk factors based on clinical and laboratory measurement. They found that lower extremity weakness, loss of balance-evaluated by centre of pressure (COP) velocity in mediolateral (ML direction)-and low gait speed were factors contributing to future falls. In addition, several other studies have indicated that functional impairments, less exercise, a history of falls, fear of falling (FOF) and walking device use were all strongly associated with fall risk in these individuals.[7,10–13]

However, the association between falls and gait aid use is still inconsistent. Subgroup analysis may provide the opportunity to differentiate these factors more clearly. Therefore, the aim of prospective study was to explore fall incidence and associated factors in ambulatory iSCI who walk with or without an ADD.

## Materials and methods

### *Participants*

One hundred and three SCI patients agree to participate in this study. However, one person in AAD group and five individuals in non-AAD group were drop out during follow up period due to the personal reasons. Therefore, 97 participants completed the study. The participants were enrolled from a tertiary rehabilitation centre and community in the northeast area of Thailand. The sample size calculation using from pilot study.

Participants were identified by clinicians from a specialty hospital and recruited at a tertiary rehabilitation centre and in communities in northeast Thailand. The participants were at least 18 years of age, included both traumatic and non-traumatic causes (American Spinal Injury Association Impairment Scale [ASIA] C and D) and were able to ambulate at least 17 meters either with or without an AAD (FIM-L scores (Functional independence measure locomotor) = 5-7). Exclusion criteria included patients who were unable to understand and follow commands, had a leg length discrepancy, were pregnant, or had any underlying disease that caused pain (visual analog scale more than 5). This study was approved by the local ethics committee (HE611187), and the eligible participants gave their written informed consent before participating in the study.

### *Protocol*

The eligible participants were interviewed for their baseline demographics, SCI characteristics, physical activity, fear of falling, fall history and associated factors using incidence of fall and associated factors Thai version questionnaire.[6] Physical activity (PA) measured by interview their activities and using MET (The metabolic equivalent of task) to indicate their intensity.[6,14] Then each participant was assessed for balance using the Timed Up and Go Test (TUGT), walking speed using the 10 Meter Walk Test (10MWT) and lower limb strength test using the Five Times Sit to Stand Test (FTSST). In addition, participants were asked to track falls for the subsequent 6 months using monthly fall calendars, and researchers visited participants during the third and sixth months to ensure that falls data-including how many falls, the circumstances of each fall and consequences of falling -was being recorded.

TUGT was used to measure balance performance. The method required stopwatch, a standard chair (height: 43 cm) and tape (mark 3 m). The subject's started in a sitting position with their back closed to the backrest 90 degree and their feet placed on the floor slightly behind the knee. Then, stood up upon assessor's command "Go" then walked for 3 meters, turned around and went back to the chair and sat down. The time would be stopped when the subjects sat on the chair against the backrest. The subjects were allowed to use an assistive device. Subjects performed 3 trials/test and calculated the average times of 3 trials.[15]

The 10-meter walk test was a walking speed assessment. The testing device was included stopwatch, and measuring tape (mark 10 meters walkway, add mark at 3 and 7 meter). The participants walked with or without assistance for 10 m, allow for 3 m acceleration and 3 m deceleration. Assistive device could be used, the test began when the iliac crest passed the 3 meters mark and stopped timing when the iliac crest passed the 7 meters mark. The test measured the time in middle 4 meters of walkway. Subjects walked with their preferred speed. Perform three attempted and average value of 3 trials were calculated.[16]

The FTSST was used to measure lower limb muscle strength during changing position form sit to stand. The equipment of the test was stopwatch and standard armless chair (with a seat height of 43 m). Participants sat with both arms cross the chest, their back closed to the chair and feet placed on the floor with slightly behind the knee. Participants were required to stand up with the hip and knee fully extension and sit down 5 times as fast and safe as possible. If the participants unable to stand up five times independently, they were allowed to press their hands down against a standard walker while performed FTSST. Timing began at "Go" and end when their back against the backrest on the fifth repetition. Participant performed 3 trial/test and calculated the average of 3 trials.[17]

### *Statistical analysis*

Data were gathered using the SPSS IBM version. A descriptive statistic was applied to explain baseline demographic and SCI characteristic data. A chi-square test was utilized to compare categorical data, and an independent t-test was used to compare normally distributed continuous data. A Mann-Whitney U-test was used for non-normally distributed continuous data. A multiple logistic regression analysis was used to determine factors associated with falls among ADD and non-ADD users. The results were reported in terms of

unadjusted and adjusted odd ratios (aOR) with 95% CI, and significance was set at $p<0.05$.

## Results

A total of 97 participants were eligible based on the criteria and agreed to participate in the study. The mean age of the participants was 54.31±13.16 years old, and the post-injury time was 6.20±5.69 years. Most participants were male (80%), paraplegic (67%) and ASIA D (89%). There were 56 participants who walked with a walking device and 41 who did not.

### *Incidence of fall and consequences*

A total of 39 (40%) participants reported at least one fall during the 6-month follow up ("fallers"), while 12 (30%) fell once ("single fall") and 27 (70%) fell twice or more ("multiple fall"). AAD group and Non AAD group experienced multiple fall 18 and 9 persons. The fall incidences among the ADD and non-ADD using participants were 46% (Figure 1) and 32% (Figure 2), respectively. Furthermore, participants in the AAD group were injured 65% of the time upon falling, and those in the non-AAD group were injured 46% of the time. The consequences of falling were wounds, abrasions, bruises, muscle pain and ligament tears. Participants in the AAD group fell mostly while walking within and near their houses, and falls occurred most frequently in the afternoon. Non-AAD group fell mostly while walking around their houses and at the workplace. These falls occur in the morning or afternoon.

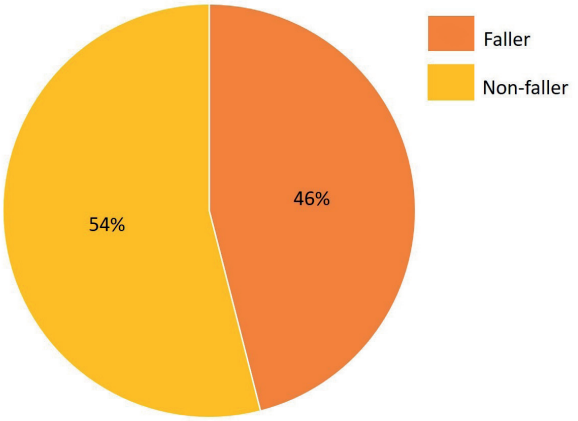


***Figure 1*** *Incidence of fall in AAD group.*

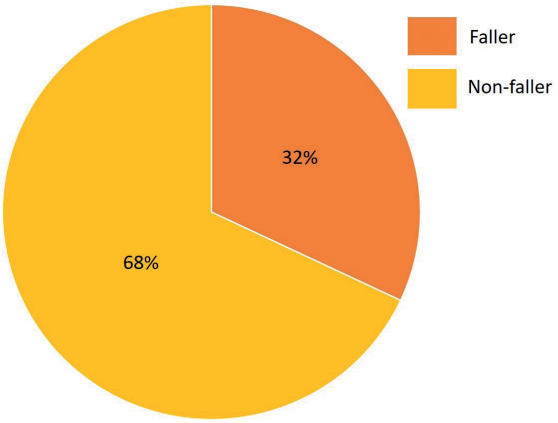


***Figure 2*** *Incidence of fall in non-AAD group.*

### *Demographics and factors associated with falls in patients with SCI who walk with an assistive walking device (the AAD group)*

Table 1 shows baseline demographics of the AAD group demonstrated that fallers were significantly younger and took a shorter time to complete the TUGT and 10MWT (which they were presented as median) than non-fallers ($p<0.05$). However, there was no significantly difference in FTSST between fallers and non-fallers. Table 2 shows the factors associated with falls in the AAD group. A multivariate logistic regression model used to investigate fallers versus non-fallers among those in the ADD group and revealed that age less than 50 years (aOR=8.21, 95%CI=1.35-50.06, $p=0.023$), moderate to high physical activity (aOR=6.81, 95%CI=1.06-43.83, $p=0.044$) and using crutches (aOR=16.06, 95%CI=1.13-229.02, $p=0.041$) or a cane (aOR=6.72, 95%CI=1.03-43.89, $p=0.047$) significantly increased the risk of falling.

**Table 1** Baseline demographics of individuals with spinal cord injury in AAD group (N=56).

| Variable | Total (N=56) | Fallers (N=26) | Non-fallers (N=30) |
|---|---|---|---|
| Age: (years) | 59 (45.25, 65.5) | 46.5 (39.75, 63) | 62 (54.75, 69)* |
| PIT: (years) | 4 (3,8) | 4 (2.75, 8) | 4.5 (2.75, 8.75) |
| Gender: Male (N) % | 46 | 21 (46) | 25 (54) |
| Level of injury: Paraplegia (N) % | 35 | 20 (57) | 15 (43) |
| Cause: Traumatic (N) % | 27 | 15 (56) | 12 (44) |
| AIS impairment: D (N) % | 45 | 21(47) | 24 (53) |
| TUGT (sec) | 29.67 (20.61, 45.13) | 23.70 (18.54, 32.16) | 40.43 (24.25, 51.94)* |
| FTSST (sec) | 15.32 (12.50, 18.68) | 14.31 (11.15, 17.60) | 15.48 (13.57, 21.09) |
| 10MWT (m/sec) | 0.34 (0.21, 0.51) | 0.45 (0.30, 0.61) | 0.24 (0.18, 0.44)* |

*Note: Continuous data was presented as median (IQR) and compared groups using the Mann-Whitney U test and categorical data using Chi-square test, PIT: post time injury, AIS: American Spinal Injury Association (ASIA) Impairment Scale TUGT: Timed Up and Go Test, 10MWT: 10 Meter Walk Test, FTSST: Five Times Sit to Stand Test, *significant differences of p<0.05.*

**Table 2** Factors associated with fall in spinal cord injury who walked with walking device (N=56).

| Variable | Total (N=56) | Fallers (N=26) | Non-fallers (N=30) | uOR (95%CI) | *p* value | aOR (95%CI) | *p* value |
|---|---|---|---|---|---|---|---|
| Gender: N (%) | | | | | | | |
| Female | 10 | 5 (50) | 5 (50) | Ref. | | - | - |
| Male | 46 | 21 (46) | 25 (54) | 0.84 (0.21-3.30) | 0.803 | | |
| Age (years): N (%) | | | | | | | |
| ≥50 | 34 | 9 (26) | 25 (74) | Ref. | | Ref. | |
| <50 | 22 | 17 (77) | 5 (23) | 9.44 (2.69-33.13) | 0.001* | 8.21 (1.35-50.06) | 0.023* |
| Physical activity: N (%) | | | | | | | |
| Low | 23 | 2 (9) | 21 (91) | Ref. | | Ref. | |
| Moderate-High | 33 | 24 (73) | 9 (27) | 28.00 (5.43-144.39) | 0.001** | 6.81 (1.06-43.83) | 0.044* |
| Type of gait aid: N (%) | | | | | | | |
| Walker | 29 | 6 (21) | 23 (79) | Ref. | | Ref. | |
| Crutches | 10 | 9 (90) | 1 (10) | 34.50 (3.63-328.28) | 0.003** | 16.06 (1.13-229.02) | 0.041* |
| Cane | 17 | 11 (65) | 6 (35) | 7.03 (1.84-26.85) | 0.004** | 6.72 (1.03-43.89) | 0.047* |
| Level of injury: N (%) | | | | | | | |
| Cervical | 21 | 6 (29) | 15 (71) | Ref. | | - | - |
| Thoracic | 11 | 8 (73) | 3 (27) | 6.67 (1.31-34.03) | 0.023* | | |
| Lumbar | 24 | 12 (50) | 12 (50) | 2.50 (0.72-8.64) | 0.147 | | |
| Cause: N (%) | | | | | | | |
| Non-traumatic | 29 | 11 (38) | 18 (62) | Ref. | | - | - |
| Traumatic | 27 | 15 (56) | 12 (44) | 2.05 (0.70-5.95) | 0.189 | | |
| AIS impairment: N (%) | | | | | | | |
| C | 11 | 5 (45) | 6 (55) | Ref. | | - | - |
| D | 45 | 21 (47) | 24 (53) | 1.05 (0.28-3.94) | 0.942 | | |

***Demographics and factors associated with falls in patients with SCI who do not use an assistive waking device (the non-AAD group)***

Table 3 shows demographic data of the non-AAD group showed that fallers took significantly longer time to complete the TUGT, FTSST and 10MWT than non-fallers ($p$<0.05). Table 4 shows the factors associated with falls in the non-AAD group. A multiple variable analysis indicated a significantly longer time to complete the TUGT (aOR=1.38, 95%Cl= 1.04-1.84, $p$=0.025) and a history of falling (aOR=7.19, 95%Cl=1.31-39.51, $p$=0.023) significantly increased the risk of fall.

## Discussion

The current study found that 39 participants (40%) at least one fall during the 6-month follow-up. These results are consistent with previous studies on ambulatory SCI patients that used prospective fall interviews (34-54%).[7,18] Our study found that fall incidence among those AAD group (46%) was slightly higher than non-AAD group (32%). Similarly, Roman de Mettelinge found that fall incidence in older adults using a walking device (68%) was dramatically higher than in older adults who did not use one (35%).[19] Some evidence has been found that persons with SCI who use an AAD have poorer balance, muscle

**Table 3** Baseline demographics of individuals with spinal cord injury in non-AAD group (N=41).

| Variable | Total (N=41) | Fallers (N=13) | Non-fallers (N=28) |
|---|---|---|---|
| Age: (years) | 56 (52, 60.5) | 55 (48.5, 61) | 56 (51.25, 60.75) |
| PIT: (years) | 5 (2, 8) | 5 (1.5, 14) | 4.5 (2, 8) |
| Gender: Male (N) % | 32 | 12 (38) | 20 (62) |
| Level of injury: Paraplegia (N) % | 30 | 8 (27) | 22 (73) |
| Cause: Traumatic (N) % | 23 | 7 (30) | 16 (70) |
| AIS impairment: D (N) % | 41 | 13 (32) | 28(68) |
| TUGT (sec) | 11.56 (9.85, 14.85) | 14.40 (11.69, 16.24) | 10.85 (9.57, 13.60)* |
| FTSST (sec) | 0.34 (0.21, 0.51) | 0.45 (0.30, 0.61) | 0.24 (0.18, 0.44)* |
| 10MWT (m/sec) | 0.87±0.20 | 0.77±0.20 | 0.91±0.19* |

*Note: Continuous data was presented as median (IQR) and mean±SD, To compared between groups using the Mann-Whitney U test and independent t-test and Categorical data using Chi-square test, PIT: post time injury, AIS: American Spinal Injury Association (ASIA) Impairment Scale , TUGT: Timed Up and Go Test, 10MWT: 10 Meter Walk Test, FTSST: Five Times Sit to Stand Test, *significant differences of p<0.05*

**Table 4** Factors associated with fall in spinal cord injury who walked with walking device (N=41).

| Variable | Total (N=41) | Fallers (N=13) | Non-fallers (N=28) | uOR (95%CI) | *p* value | aOR (95%CI) | *p* value |
|---|---|---|---|---|---|---|---|
| Gender: N (%) | | | | | | | |
| Female | 9 | 1 (11) | 8 (89) | Ref. | | - | - |
| Male | 32 | 12 (38) | 20 (62) | 4.81 (0.53-42.26) | 0.162 | | |
| Age (years): N (%) | | | | | | | |
| ≥50 | 30 | 7 (23) | 23 (77) | Ref. | | - | - |
| <50 | 11 | 6 (55) | 5 (45) | 3.94 (0.92-16.94) | 0.065 | | |
| Physical activity: N (%) | | | | | | | |
| Low | 31 | 7 (23) | 24 (77) | Ref. | | - | - |
| Mod-High | 10 | 6 (60) | 4 (40) | 5.14 (1.13-23.51) | 0.035* | | |
| Level of injury: N (%) | | | | | | | |
| Cervical | 11 | 5 (45) | 6 (55) | Ref. | | - | - |
| Thoracic | 9 | 2 (22) | 7 (78) | 0.34 (0.05-2.46) | 0.287 | | |
| Lumbar | 21 | 6 (29) | 15 (71) | 0.48 (0.11-2.20) | 0.343 | | |
| Etiology: N (%) | | | | | | | |
| Non-traumatic | 18 | 6 (33) | 12 (67) | Ref. | | - | - |
| Traumatic | 23 | 7 (30) | 16 (70) | 2.05 (0.70-5.95) | 0.189 | | |
| AIS impairment: N (%) | | | | | | | |
| C | 0 | - | - | N/A | - | - | - |
| D | 41 | 13 | 28 | | | | |
| Fear of falling: N (%) | | | | | | | |
| No | 17 | 2 (12) | 15 (88) | Ref. | | - | - |
| little-Mod | 15 | 7 (47) | 8 (53) | 6.56 (1.10-39.32) | 0.039* | | |
| High | 9 | 4 (44) | 5 (56) | 6.00 (0.83-43.29) | 0.076 | | |
| History of fall: N (%) | | | | | | | |
| No | 29 | 6 (21) | 23 (79) | Ref. | | Ref. | |
| Yes | 12 | 7 (58) | 5 (42) | 5.37 (1.25-23.05)* | 0.024* | 7.19 (1.31-39.51) | 0.023* |
| TUGT (sec) | 11.56 (9.85, 14.85) | 14.41 (11.69, 16.24) | 10.85 (9.57, 13.60) | 1.29 (1.03-1.62) | 0.028* | 1.38 (1.04-1.84) | 0.025* |
| FTSST (sec) | 11.51±3.07 | 12.47±2.23 | 11.06±3.6 | 1.17 (0.93-1.46) | 0.183 | - | - |
| 10MWT (m/sec) | 0.87±0.20 | 0.77±0.20 | 0.91±0.19 | 0.23 (0.01-1.00)* | 0.005* | - | - |

*Continuous data was presented as median and mean±SD, AIS: American Spinal Injury Association Impairment Scale, TUGT: Timed Up and Go Test, 10MWT: 10 Meter Walk Test, FTSST: Five Times Sit to Stand Test, *Odd ratio is significantly different from reference group for which the value was set at 1.00 (p<0.05)*

strength and walking ability than those who do not.[2] Thus, lower functional ability might increase the risk of fall in ambulatory individuals with SCI.

The current study found that age lower than 50 of SCI patients with AAD group had greater odds of falling than those over 50 years old for 8.21 times. This finding is consistent with a previous study that found SCI patients who fell were generally under 50 years old.[7] Similarly, Matsuda *et al*.[20] found that the occurrence of falls peaked in a middle age group of older adults with disabilities. iSCI who are younger and more active have also been reported to have a greater risk for recurrent falls.[21] Furthermore, the members of the AAD group in the current study who were more physically active were found to be at greater odds of having a fall. In other words, behavioural factors account for younger participants' higher probability of falling: younger participants tend to perform more physical activity, walk long distances, and engage in social activities while paying little attention to their movement.

On the other hand, previous studies have reported that regular physical activity is associated with a reduced risk of poor physical health, which is linked to decreased fall risk in older adults.[22,23] The elderly have poor physical health and functional declined due to aging process while, SCI patient in this study still have neurological deficits and high physical activity could walk with long distance to engage social community. Thus, ambulatory patients with SCI who were high physical activity exposed to risk of fall.

In addition, our study found that SCI patients who use a cane or crutches were at increased odds of falling compared to those who used a walker. Previous research has reported that canes and crutches support less body weight and thus provide less stability than walkers (75-100% of body weight).[24] Furthermore, reliance on a single cane or crutches is associated with walking longer distances than a walker.[25] This may contribute to an increase in fatigue and pain that induces a compensation pattern for insufficient muscle strength and abnormal gait pattern, leading to a higher fall risk.[3,26] Moreover, the use of crutches can cause a compensatory walking pattern and result in more energy consumption, both of which interfere with hip, ankle and walking control strategies.[27-29] These fall findings among SCI patients who use an AAD may imply that they challenge themselves to

attempt a variety of daily activities, but their functional performance may be insufficient (Table 1). Individuals with SCI in this study might use these devices inappropriately without guidance; however, AAD can decrease fall risk if a professional instructs and trains to use them safely and correctly.

In non-AAD group, a history of falling and taking longer to complete the TUGT were associated with higher odds of falling falling (aOR=1.38 for TUGT and 7.19 for history of fall). These findings are similar to those of Jorgensen *et al.*[12] who found that a history of falling was an important predictor for further falls in ambulatory SCI patients (OR=4.2). A history of falling can reflect both intrinsic (muscle weakness, poor balance, poor walking performance) and extrinsic (environmental hazards) factors. [30,31] These consistency with the finding of current study found participants who fell in non-AAD group had poor muscle strength, poor balance control and poor walking speed (Table 2). Extrinsic factors mostly occur in those who fall once, while those who fall multiple times are usually affected by neuromuscular performance, muscle strength and balance.[32,33] The average number of falls in the non-AAD group was also reported as three per person in the current study. These findings reflect that those who had a history of falling in the non-AAD group in this study had musculoskeletal and neurological system changes that caused a high risk for falls.

Loss of balance has also been reported in other studies as a strong factor associated with fall including in ambulatory SCI patients and the elderly.[13,32] Previous studies have reported that individuals who fall have poorer balance than non-fallers.[32,33] In addition, fallers have a problem with mediolateral sway direction interfering with balance.[9,30] The TUGT is a test that can predict fall and has a cut-off point for risk of fall of >13.5 sec.[15] The non-AAD fallers in this study scored an average of 14.40 sec. This implies that non-ADD users still have insufficient balance and abandon walking aids too soon when trying to regain the ability to participate in social activities and improve self-confidence.

The ambulatory SCI patients in both groups had high fall incidence. Especially, individuals who used an ADD appeared to be at a higher risk of falling. Participants in the AAD group who were younger, moderately to highly physically active and relied on a cane or crutches had a higher fall risk. In the non-AAD group, the factors for falls were a history of falling and loss of balance. Fallers who used an AAD had high functional performance (Table 1), which is different from those with other conditions, such as stroke or old age, while fallers in the non-AAD group had low functional performance (Table 2), similar to those other conditions. Therefore, therapists should consider falls and the associated factors in ADD and non-ADD using ambulatory SCI patients separately to provide proper rehabilitation that prevents falls and subsequent injury

There are some limitations of this study such as most of participants in this study were male (80%) paraplegic (67%) and ASIA D (89%). Therefore, the results could not be generalised to the entire population of SCI patients. Therefore, future study should be conducted in other population such as female, tetraplegic and ASIA A to C. In addition, there is a lack of information on drug intake and health conditions that might have led to a fall. These factors should be considered in further studies.

## Conclusion

The ambulatory SCI patients in both groups had high fall incidence. Especially, individuals who used an ADD appeared to be at a higher risk of falling. This current study suggest that therapists should consider falls and the associated factors in ADD and non-ADD using ambulatory SCI individuals separately to provide proper rehabilitation that prevents falls and subsequent injury. Thus, therapists should focus on young age groups, select appropriate assistive devices before discharging them from the hospital, and follow up continuously with the AAD group. In non-AAD users, rehabilitation professionals should concern fall history and emphasize balance control even if they walk independently for fall prevention.

## Conflicts of interest

The authors declare no conflict of interest.

## Ethical approval

The participants gave their informed consent before enrolling in the study. The study was approved by the Khon Kaen University Ethics Committee for Human Research (No. HE611187).

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# *Cordyceps militaris* extracts inhibit FLT3 protein expression and induce cell cycle arrest in FLT3 overexpressing leukemic cell models

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**ABSTRACT**

**Background**: Leukemia is a type of blood cancer, which is characterized by the clonal proliferation of malignant leukocytes resulting in production of abnormal blood cells. Mutations in the FMS-like tyrosine kinase 3 (FLT3) gene are one of the most frequently identified genetic alterations that affect downstream intracellular signaling pathways thereby enhancing leukemogenesis. Nowadays, drugs from herbal sources were selected as alternative drugs for studying their effectiveness on leukemia treatment.

**Objectives**: This study aimed to evaluate anti-leukemia activities of various *Cordyceps militaris* (*C. militaris*) extracts including crude extract (Crude) and fractional extracts from hexane (Hex), ethyl acetate (EtOAc), and ethanol (EtOH) on EoL-1 and MV4-11 cells.

**Materials and methods**: Cytotoxicity and effects on FLT3 protein expression and cell cycle progression were investigated and compared with standard cordycepin using MTT assay, Western blotting, and PI staining, respectively.

**Results**: The results demonstrated that EtOAc and Hex fractional extracts showed the strongest cytotoxic effects on EoL-1 cells with inhibitory concentration at 50% growth ($IC_{50}$) values of 11.0±2.9 and 11.7±0.6 µg/mL and 45.9±0.4 and 36.8±15.3 µg/mL for MV4-11 cells, respectively. Crude and EtOAc fractional extracts exhibited excellent inhibitory effects on FLT3 protein expression. Moreover, the cell cycle progression of both leukemic cells was arrested at S phase after treatment. The obtained results are in the same pattern with standard cordycepin.

**Conclusion**: Overall, this present study showed that anti-leukemia activities of *C. militaris* on EoL-1 and MV4-11 may involve the inhibition of cell proliferation by inhibiting FLT3 protein expression and inducing cell cycle arrest at S phase and cordycepin may plays a major role of these activities.

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## Introduction

Acute myeloid leukemia (AML) is a heterogenous malignant disorders characterized by abnormal proliferation and differentiation of myeloid cells. It is the most prevalent type of leukemia in adults with an increased risk of relapse and poor prognosis. Current treatments for AML patients include induction therapy, which is a combination of cytotoxic agents such as cytarabine and anthracycline as a "7+3" regimen and consolidation therapy, which consists of high doses of cytarabine for eradication of residual leukemia.[1,2] However, the use of cytotoxic agents can cause toxicities to the patients, especially in older patients who are over 65 years of age.[3] Thus, treatment of AML remains challenging, particularly in patients over the age of 65 and those with refractory or relapsed AML.[4] Therefore, the development of new drugs with lower toxicities is necessary for the treatment of AML.[5]

FMS-like tyrosine kinase 3 (FLT3) is a member of the type III receptor tyrosine kinase (RTK) which normally expressed on the surface of many hematopoietic progenitor cells, and plays a critical role in controlling cell survival, proliferation, and differentiation of hematopoietic cells.[6-9] It is one of the main mutated genes which can be found in nearly 30% of newly diagnosed AML cases; about 25% of patients with internal tandem duplication (ITD) mutations within the juxtamembrane domain (JMD) and 7-10% with point mutations in the tyrosine kinase domain (TKD), leading to the constitutive activation of kinase activity.[10-13] FLT3-ITD gene is the most common mutation that exhibits a high leukemia burden and used as a prognostic marker. It was associated with poor prognosis, increased risk of relapse and decreased overall survival.[11] Consistently, FLT3-TKD point mutations result in ligand-independent activation, phosphorylation of the receptor and constitutively activate kinase activity, thereby enhancing uncontrolled proliferation.[14] According to the previous studies, drugs from herbal sources were selected to evaluate their anti-leukemia properties.[15-17] *Cordyceps militaris* (*C. militaris*) is a species of fungus in the family Clavicipitaceae, and the genus Cordyceps which was originally described by Carl Linnaeus in 1753 as *Clavaria militaris*. It has been widely used as an herbal remedy or crude drug in oriental medicine and folk tonic foods in East Asia.

Several studies have shown that Cordyceps species contain numerous bioactive compounds which play a critical role in anti-inflammation, antioxidant, anti-microbial activity, anti-viral, enhancing immunity, anti-aging, and anti-tumor.[18-20] Aqueous extract of *C. militaris* has anti-proliferative effects on human leukemia U937 cells by enhancing apoptotic cell death through regulation of several major growth regulatory gene products and caspase protease activity.[21] Cordycepin in cultivated *C. militaris* possesses a synergistic cytotoxic effect on human leukemia cells.[22] In addition, *C. militaris* extract exhibited anti-leukemia efficacy on a xenografted leukemia model.[23] Moreover, hot water extract of *C. militaris* strongly inhibited the growth of HL-60 cells by promoting cell apoptosis through sequential activations of caspase-3 and PARP.[24] According to previous studies of Cordyceps species, they were rich in bioactive compounds and solvent polarity affected the contents of bioactive compounds in each fractional extracts which may differ in their biological properties.[19,25] Cordycepin represents one of the major bioactive compounds in *C. militaris* which is associated with several biological activities and widely used for various pharmacological properties.[26,27] Previous study of our extracts showed that the cordycepin content was demonstrated in all fractions of *C. militaris* extracts with different percentage in each fraction.[25] Therefore, it was suggested that cordycepin might involve in anti-leukemia properties presented in all fractions of *C. militaris* extracts. The mechanism by which cordycepin inhibits leukemic cell proliferation is only partially understood. As overexpression of FLT3 protein play a critical role in leukemogenesis, if cordycepin can inhibit leukemic cell proliferation in FLT3 overexpressing leukemic cell model, it may involve in the inhibition of FLT3 protein expression.

According to various biological activities and anti-leukemia properties of *C. militaris* as mentioned above, the objective of this study was, therefore, to investigate the anti-leukemia proterties of *C. militaris* extracts on FLT3 overexpressing leukemic cell lines, including the wild-type FLT3 overexpressing leukemic cell (EoL-1) and FLT3-ITD mutant leukemic cell (MV4-11).

## Materials and methods

### Plant materials

Dried *C. militaris* powder was kindly contributed by Mushroom Research and Development Center (MRDC, Chiang Mai Province, Thailand).

### Chemicals and reagents

Commercial grade cordycepin (purity≥98.0%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Roswell Park Memorial Institute (RPMI)-1640 medium, Iscove's Modified Dulbecco's Medium (IMDM), fetal bovine serum (FBS), penicillin-streptomycin, and L-glutamine were purchased from GIBCO (Waltham, MA, USA). Anti-FLT3 antibody and rabbit polyclonal anti-GAPDH antibody were purchased from Abcam (Cambridge, UK). Luminata™ Forte Western HRP Substrate was purchased from Merck Millipore (Darmstadt, Germany).

#### *C. militaris extraction*

*C. militaris* extracts used in this study composed of crude extract (Crude), fractional extracts including hexane fractional extract (Hex), ethyl acetate fractional extract (EtOAc), and ethanolic fractional extract (EtOH). The extraction process was previously mentioned by Marsup P *et al*.[25] Briefly, crude extract was obtained from maceration of dried *C. militaris* powder in 95% ethanol, followed by filtration through Whatman No.1 paper and removed the solvent with a rotary evaporator (Buchi Labortechnik GmbH, Essen, Germany). On the other hand, sequential maceration was performed to obtain the Hex, EtOAc, and EtOH fractional extracts. First, hexane fractional extract (Hex) was obtained by maceration of dried *C. militaris* powder in hexane, followed by filtration through Whatman No.1 paper to obtain the solution of the extract. After that, the hexane was removed by using a rotary evaporator to get the hexane fractional extract (Hex). Subsequently, the

residue powder was macerated in ethyl acetate, filtrated through Whatman No.1 paper and the solution of the extract was evaporated out of the solvent using a rotary evaporator to get the ethyl acetate fractional extract (EtOAc). Afterwards, the residue power was macerated in 95% ethanol, using the same method as above and ethanolic fractional extract (EtOH) was obtained from this final step.

**Leukemic cell lines and cell culture conditions**

FLT3 overexpressing leukemic cell lines, including wild-type (EoL-1) and FLT3-ITD mutant (MV4-11) were used as leukemic cell models in this experiment. EoL-1 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 1 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin. MV4-11 cells were cultured in IMDM medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. Both leukemic cell lines were cultured at 37 °C in a humidified incubator with 5% $CO_2$.

**MTT cytotoxic assay**

To check the cytotoxicity of *C. militaris* extracts on leukemic cell lines, MTT assay was performed by using EoL-1 and MV4-11 cells at 70-80% confluence in exponential growth phase. Briefly, EoL-1 cells at $3\times10^4$ cells/well and MV4-11 cells at $1\times10^4$ cells/well were plated in 96 well plates containing 100 µL medium prior to the treatment with the extracts at 37 °C in a humidified condition with 5% $CO_2$ for 24 hrs. Then, an equal volume of *C. militaris* extracts (including crude and three fractional extracts) and standard cordycepin at various concentrations (3.125, 6.25, 12.5, 25, 50, and 100 µg/mL) were added to each well, and incubated for another 48 h. After removal of 100 µL medium, 15 µL of MTT dye solution was added and the plate was incubated at 37 °C for 4 hrs. Then, 200 µL of DMSO was added to each well and mixed thoroughly to dissolve the formazan crystals. The absorbance of treated wells (test wells), vehicle control well (VC; 0.8% DMSO in culture medium), and untreated control or cell control well (CC) were measured using SpectraMax® M5 Multi-Mode Microplate Reader (Molecular Devices Crop, CA, USA) at 578 nm with a reference wavelength of 630 nm. Three independent assays were done. The average cell viability of the vehicle control was compared to untreated control. If there are no significant differences between CC and VC, the average cell viability of the treated well was calculated in respect to the vehicle control.

**Western blotting**

To evaluate the effect of *C. militaris* extracts on FLT3 protein expression in FLT3 overexpressing leukemic cell lines, Western blot analysis was done. At first, both EoL-1 cells ($3\times10^5$ cells/mL) and MV4-11 cells at ($1\times10^5$ cells/mL) were treated with the non-toxic doses ($IC_{20}$) of the extracts (including crude and three fractional extracts) and standard cordycepin, which was gained from MTT assay for 48 hrs. After that, the cells were harvested and washed 3 times with ice-cold PBS. The total cell number was counted for cell viability evaluation by using trypan blue dye solution. After that, the cell pellet was lysed in RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 0.5 mM EDTA, and 0.1% SDS) containing protease inhibitors. Then, the whole protein lysate was separated, and protein concentration was measured by using Folin-Lowry method. Later, SDS-PAGE was performed using whole protein lysate (80 µg, 25 µL/well) of tested samples and the separated proteins on were transferred to the polyvinylidene difluoride (PVDF) membrane by using 32 volts of electricity overnight in blotting buffer. Then, the membrane was blocked with 5% skimmed milk in PBS and incubated with primary antibodies, the rabbit polyclonal anti-FLT3 (1:1,000) and the rabbit polyclonal anti-GAPDH (1:16,000). The membrane was then incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:20,000) and proteins were visualized by means of chemiluminescent method using the Luminata™ Forte Western HRP Substrate. This experiment was performed in three independent experiments and the most effective C. militaris extract was selected and its effect on FLT3 protein expression was evaluated in term of dose dependent manner.

**Cell cycle analysis**

Cell cycle analysis was performed using DNA binding dye, propidium iodide (PI). In this study, various concentrations of crude extract (Crude) and ethyl acetate fractional extract (EtOAc) of *C. militaris* were used to treat EoL-1 and MV4-11 cells, respectively. The activities of extracts were compared to that of standard cordycepin. Briefly, EoL-1 cells at $3\times10^5$ cells/mL and MV4-11 cells at $1\times10^5$ cells/mL were starved for 24 hrs in 0.5% FBS RPMI-1640 and 0.5% FBS IMDM medium, respectively. Then, leukemic cells were treated with non-toxic dose ($IC_{20}$) value and 2 concentrations lower than $IC_{20}$ value ($IC_{10}$, $IC_5$) of extract for 48 hrs. After that, the cells were harvested and washed with ice-cold PBS. Next, cells were resuspended in 300 µL PBS and then, 700 µL of ethanol was added and incubated for 30 min on ice. Thereafter, cells were centrifuged at 5,000 rpm for 10 sec and washed with ice-cold PBS. Finally, leukemic cells were stained with propidium iodide (PI) solution (1 mg/mL PI, 80 mM EDTA, 1% Triton X-100, 10 mg/100 µL RNAse A) in the dark at 4 °C and the DNA content was detected by flow cytometer. This experiment was performed in three independent experiments and the data were analyzed using the FlowJo Vx software.

**Statistical analysis**

The data were expressed as the mean±SD of three independent experiments. The statistical differences were determined using unpaired Student's t-test and statistical comparison was processed by one-way ANOVA using SPSS program (SPSS Statistics Base 17.0 for Windows (SPSS License No.7 (50)). The differences were considered significant when the probability value was less than 0.05 ($p<0.05$).

## Results

### Cytotoxic effects of *C. militaris* extracts

Cytotoxicity of crude and fractional extracts of Hex, EtOAc, and EtOH were determined by an inhibitory concentration at 50% growth ($IC_{50}$). All the test compounds demonstrated the cytotoxic effects on EoL-1 and MV4-11 cells with various $IC_{50}$. It may be due to the different constituents of bioactive compounds in each extract. During fractionation, solvents are added according to the order of increasing polarity for extraction of both polar and nonpolar compounds. The polarity of solvents in the extraction process influences the yield of bioactive compounds.[28] The higher polar solvents yielded higher bioactive compounds which may differ in their biological activities. In this study, it was found that different *C. militaris* extracts exhibited different cytotoxic effects on leukemic cell lines. By comparing the results from these two cell lines, all the extracts showed the strongest inhibitory effects on both EoL-1 and MV4-11 cells (Table 1). The $IC_{50}$ values of standard cordycepin, crude, and fractional extracts of Hex, EtOAc, and EtOH on EoL-1 cells were 28.7±7.3, 43.5±9.1, 11.7±0.6, 11.0±2.9, and >100 µg/mL, respectively (Figure 1a). On the other hand, the $IC_{50}$ values on MV4-11 cells were 50.0±13.0, >100, 36.8±15.3, 45.9±0.4, and >100 µg/mL, respectively (Figure 1b).

According to the obtained results, the Hex and EtOAc fractional extracts exhibited the greatest cytotoxic effects on leukemic cells. However, the cytotoxicity in these leukemic cells were different due to the difference in leukemic cell-type. In our study, EoL-1 cells were found to be more sensitive to the extracts than MV4-11 cells. This result was well in accordance with pervious study reported that EoL-1 cell lines were sensitive to sorafenib and cytarabine than MV4-11 cells due to the presence of activated FIPL1-PDGFRα fusion gene in EoL-1 cells.[29]

**Table 1** Inhibitory concentration values of *C. militaris* extracts on EoL-1 and MV4-11 cell lines.

| *C. militaris* extracts | EoL-1 | | MV4-11 | |
|---|---|---|---|---|
| | $IC_{20}$ (µg/mL)* | $IC_{50}$ (µg/mL)* | $IC_{20}$ (µg/mL)* | $IC_{50}$ (µg/mL)* |
| **Cordycepin** | 8.3±1.1 | 28.7±7.3 | 14.9±8.0 | 50.0±13.0 |
| **Crude** | 5.8±3.8 | 43.5±9.1 | 4.3±1.8 | >100 |
| **Hex** | 1.5±0.2 | 11.7±0.6 | 2.2±1.0 | 36.8±15.3 |
| **EtOAc** | 1.5±0.2 | 11.0±2.9 | 2.9±1.8 | 45.9±0.4 |
| **EtOH** | 4.7±0.5 | >100 | 5.4±15.5 | >100 |

** Data are the mean±SD of three independent experiments.*

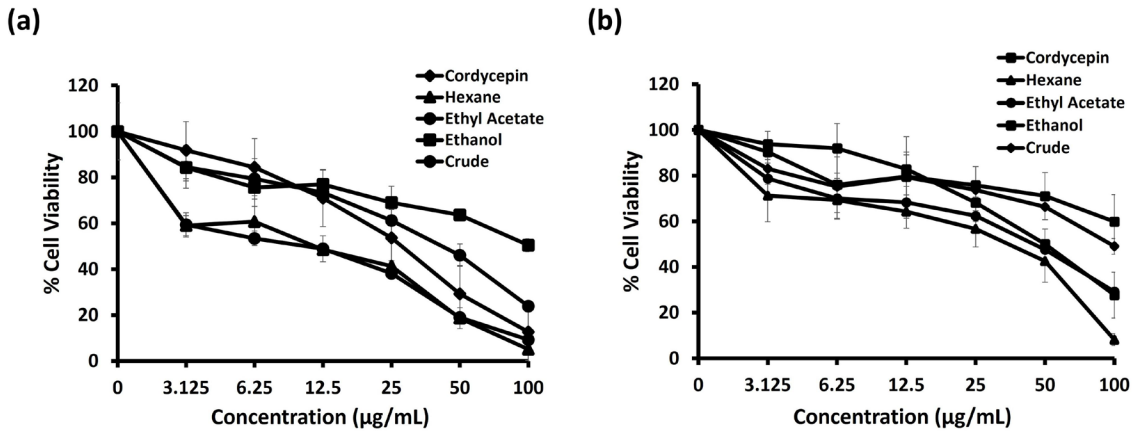


***Figure 1*** *Cytotoxicity of C. militaris extracts on (a) EoL-1 and (b) MV4-11 cell lines.*

### Effects of *C. militaris* extracts on FLT3 protein expression

The effects of *C. militaris* extracts on FLT3 protein expression in EoL-1 and MV4-11 cells were evaluated using Western blot analysis. After treatment of leukemic cells with the non-toxic doses ($IC_{20}$) of the extracts for 48 hrs, it was found that crude extract exhibited the strongest inhibitory effects on FLT3 protein expression in EoL-1 cells followed by Hex fractional extract. The FLT3 protein level was decreased by 29.1 and 20.6%, respectively. Besides, standard cordycepin demonstrated a greatest inhibitory effect on FLT3 protein expression by decreasing the FLT3 protein level for 32.3%. In addition, the total cell number after treatment was decreased in the same pattern of the decreasing of FLT3 protein level (Figure 2). By contrast, EtOAc

fractional extract has the strongest inhibitory effects on MV4−11 cells (44.7%), followed by EtOH fractional extract and crude extract which were 38.0% and 23.6%, respectively (Figure 3).

Thereafter, crude extract and EtOAc fractional extract were selected as the most effective extracts for EoL-1 and MV4-11 cells, respectively. Their effects on FLT3 protein expression were evaluated in term of dose-dependent manner by comparing to standard cordycepin. After the treatment of leukemic cells for 48 h, crude extract exhibited the strongest inhibitory effects on FLT3 protein expression in EoL-1 cells by dose-dependent manner (Figure 4). Similarly, EtOAc fractional extract showed an excellent inhibitory effect on FLT3 protein expression in MV4-11 cells by means of dose-dependent manner (Figure 5).

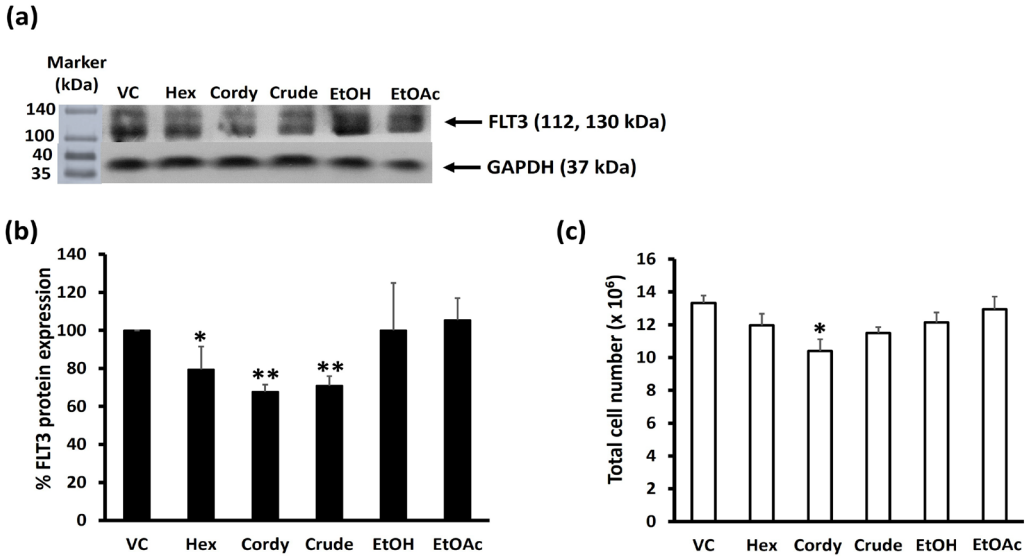


***Figure 2*** *Effects of C. militaris on FLT3 protein expression in EoL-1 cell line after 48 hrs of treatment. (a): The levels of FLT3 protein expression after treatments with standard cordycepin (Cordy), crude and fractional extracts of Hex, EtOAc, and EtOH were assessed by Western blotting. GAPDH was used as the loading control, (b): The protein levels were analyzed by a scan densitometer, (c): The total cell number after treatment was determined using trypan blue exclusion method. Data are the mean±SD of three independent experiments. The data were analyzed by unpaired, two tailed, Student's t test. Asterisks denote values that were significant difference from the vehicle control, *p<0.05; ** p<0.01.*

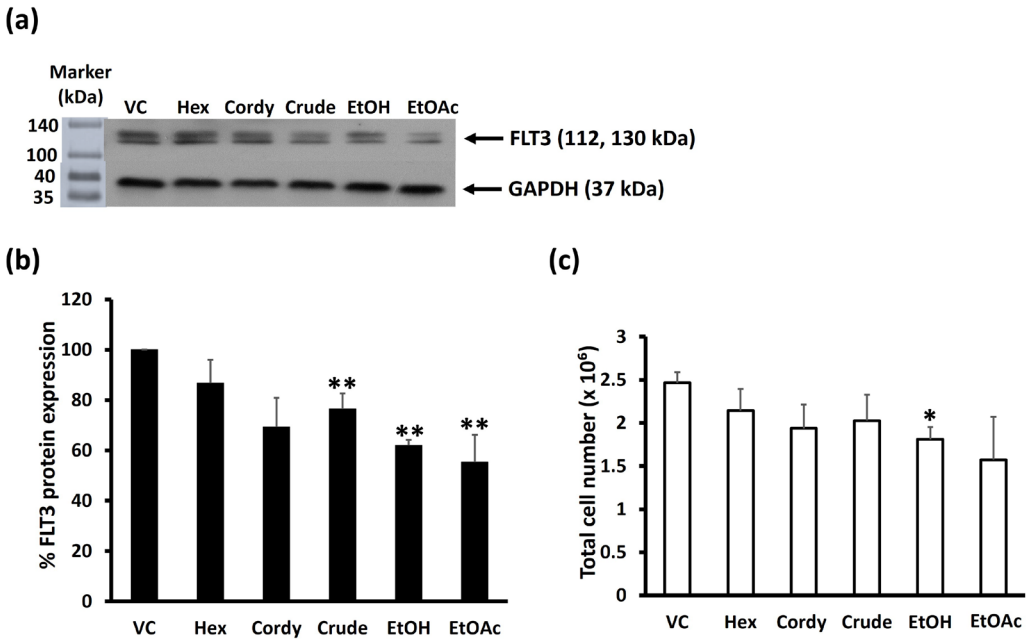


***Figure 3*** *Effects of C. militaris on FLT3 protein expression in MV4-11 cell line after 48 hrs of treatment. (a): The levels of FLT3 protein expression after treatments with standard cordycepin (Cordy), crude and fractional extracts of Hex, EtOAc, and EtOH were assessed by Western blotting. GAPDH was used as the loading control, (b): The protein levels were analyzed by a scan densitometer, (c): The total cell number after treatment was determined using trypan blue exclusion method. Data are the mean±SD of three independent experiments. The data were analyzed by unpaired, two tailed, Student's t test. Asterisks denote values that were significantly different from the vehicle control, *p<0.05; **p<0.01.*

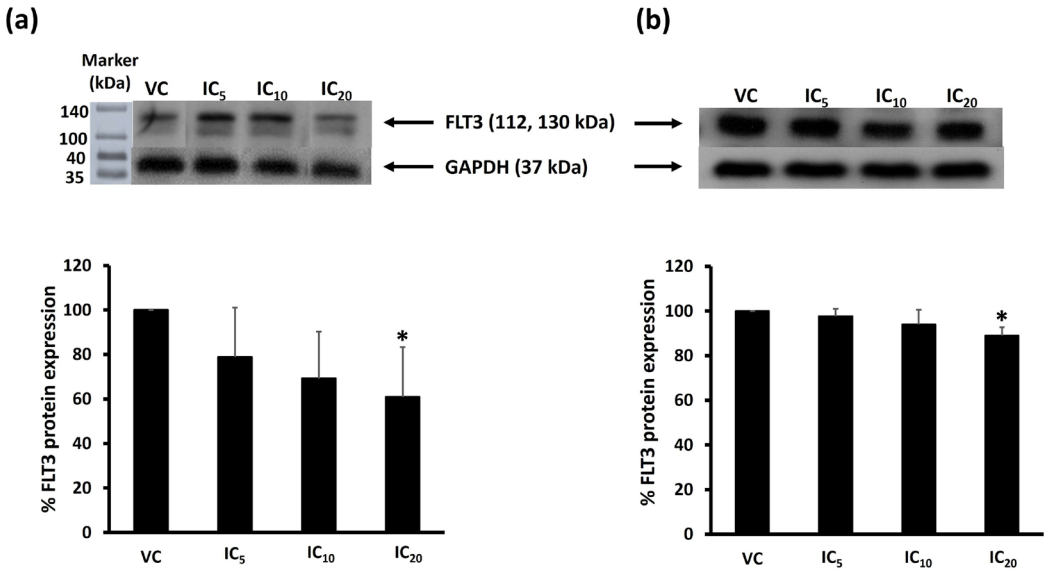


***Figure 4*** *Effects of concentration of (a) crude extract on FLT3 protein expression in EoL-1 cell lines compared with (b) standard cordycepin after 48 hrs of treatment. The levels of FLT3 protein expression after treatments with various concentrations ($IC_5$, $IC_{10}$, and $IC_{20}$) of the extracts were assessed by Western blotting. GAPDH was used as the loading control. The protein levels were analyzed by a scan densitometer. Data are the mean±SD of three independent experiments. The data were analyzed by unpaired, two tailed, Student's t test. Asterisk (*) denotes a significant difference from the vehicle control (VC) group ($p<0.05$).*

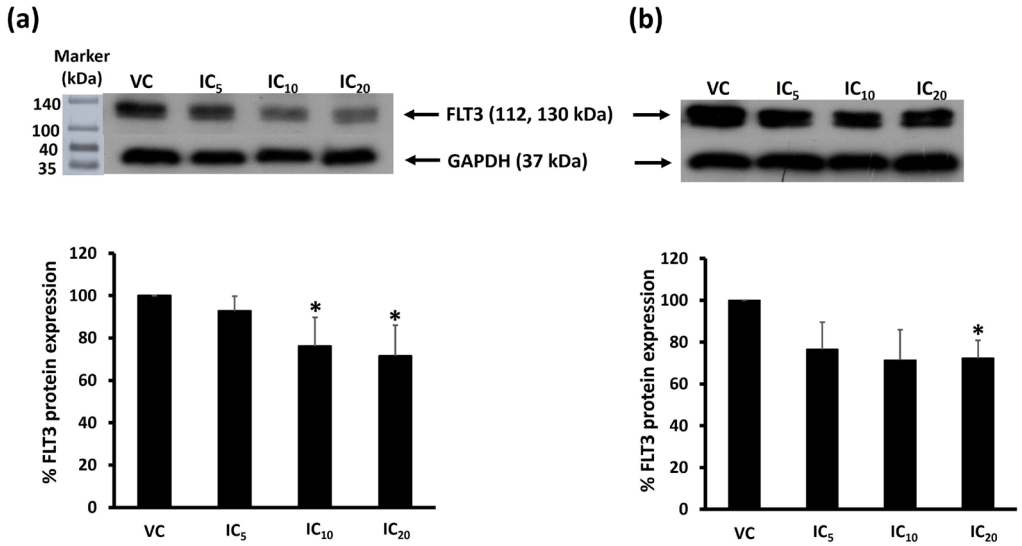


***Figure 5*** *Effects of concentration of (a) EtOAc fractional extract on FLT3 protein expression in MV4-11 cell line compared with (b) standard cordycepin after 48 hrs of treatment. The levels of FLT3 protein expression after treatments with various concentrations ($IC_5$, $IC_{10}$, and $IC_{20}$) of the extracts were assessed by Western blotting. GAPDH was used as the loading control. The protein levels were analyzed by a scan densitometer. Data are the mean ±SD of three independent experiments. The data were analyzed by unpaired, two tailed, Student's t test. Asterisk (*) denotes a significant difference from the vehicle control (VC) group at $p<0.05$.*

**Effects of *C. militaris* extracts on cell cycle progression**

In addition to the expression of FLT3 protein, the effect on cell cycle progression is also a critical parameter of the action of anti-leukemia drugs. Thus, crude extract and EtOAc fractional extract are selective as effective extracts for each leukemic cell line and cell cycle analysis was done in terms of dose dependent manner to check whether cell cycle progression is involved in decreased cell viability. Treatments of EoL-1 cells with crude extract (Figure 6a) and treatments of MV4-11 cells with EtOAc fractional extract (Figure 6b) significantly increased the cell populationat S phase by a concentration dependent manner, when compared to the vehicle control. In addition, standard cordycepin also arrested the cell cycle progression of both EoL-1 and MV4-11 at S phase (Figure 7). Moreover, total cell number of both EoL-1 and MV4-11 cells after treatment was decreased in a dose dependent manner (Table 2).

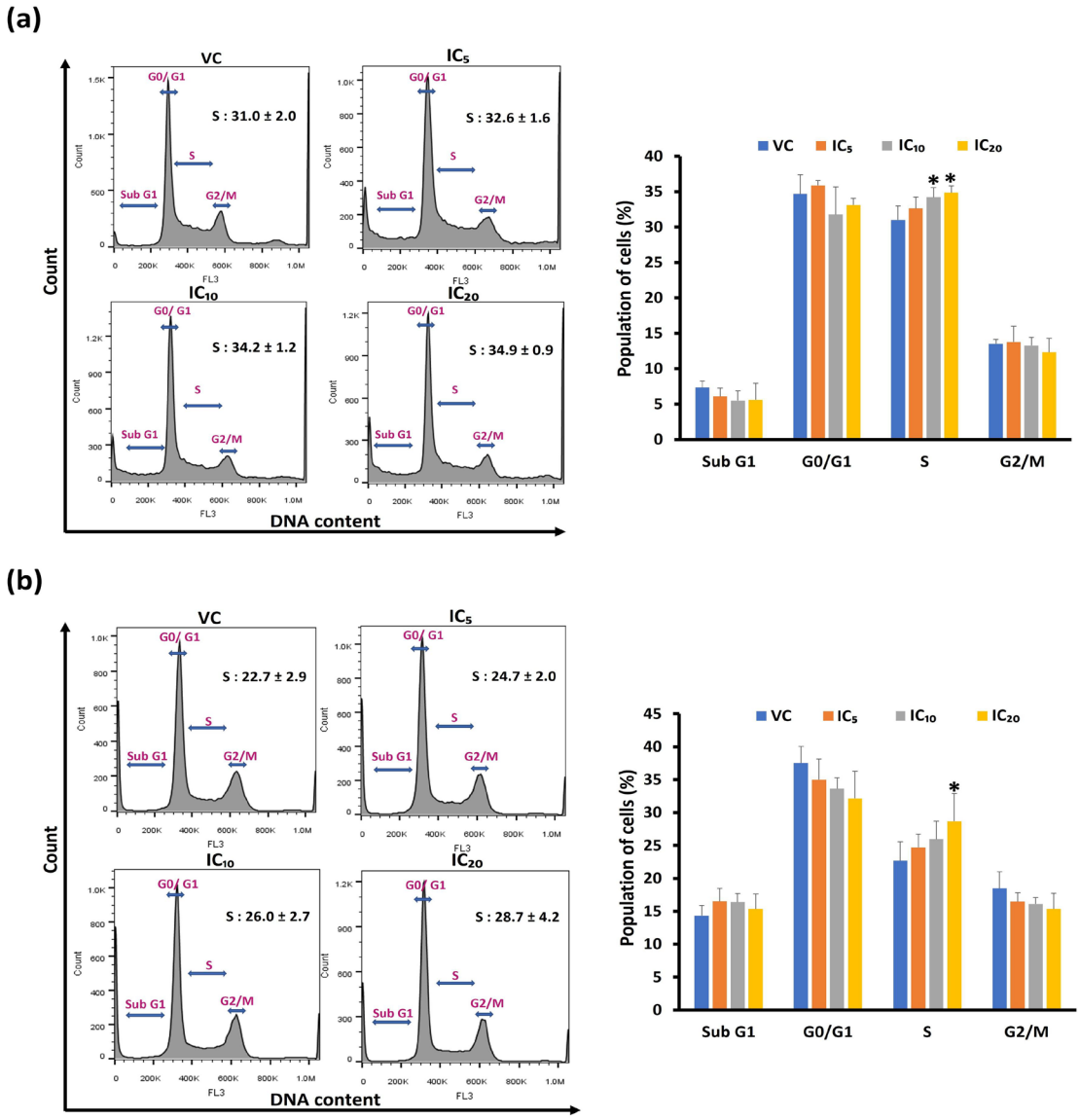


***Figure 6*** *Effects of concentration of (a) crude extract and (b) EtOAc fractional extract on cell cycle progression of EoL-1 and MV4-11 cells, respectively, was evaluated after 48 hrs of treatment. Cell population after treatments with various concentrations was assessed by flow cytometric quantitation of DNA contents using propidium iodide solution. The percent of cell population was analyzed using FlowJo Vx software. Data are the mean±SD of three independent experiments. The data were analyzed by One-Way-ANOVA test. Asterisk (*) denotes a significant difference from the vehicle control (VC) group at p<0.05.*

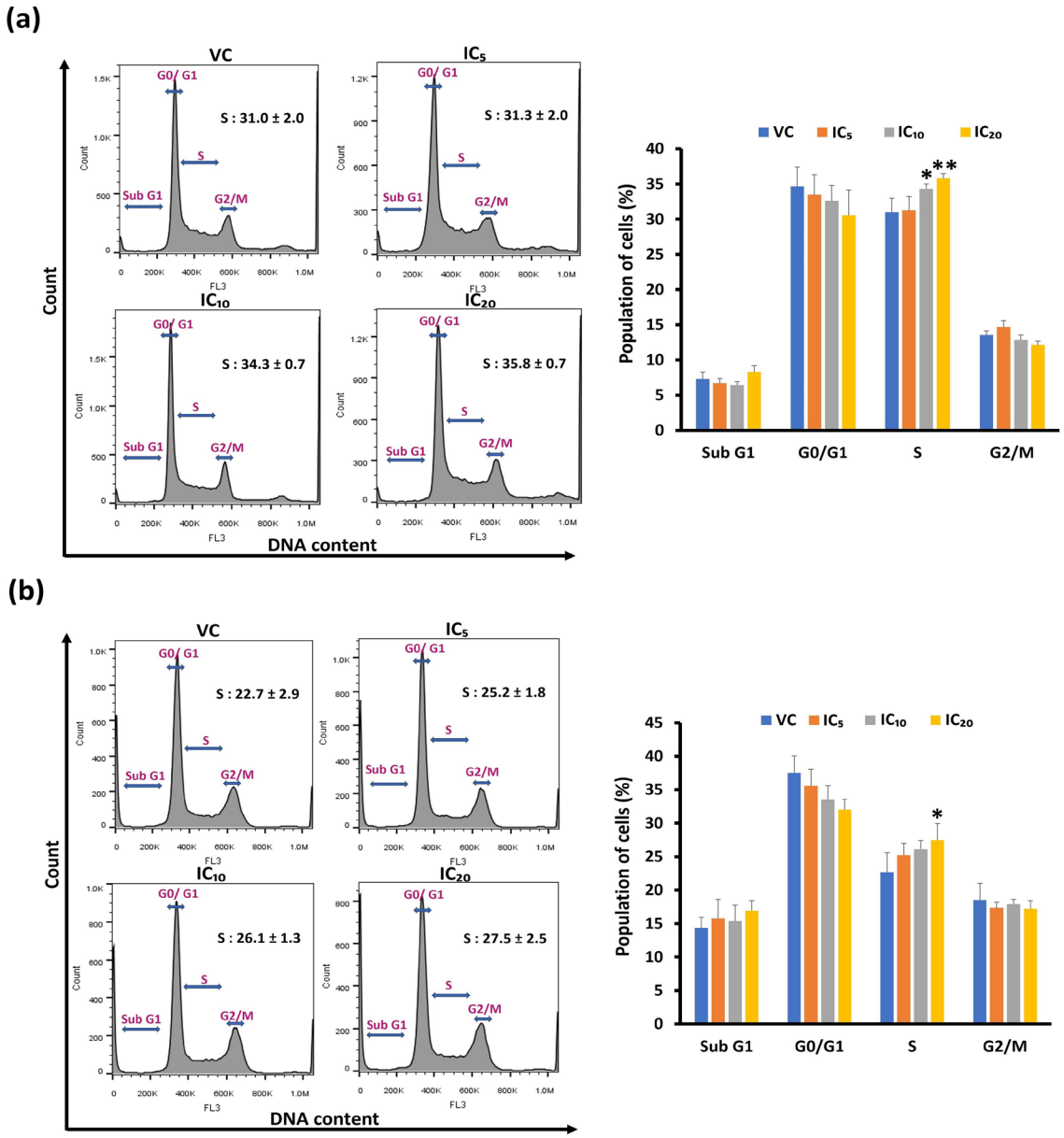


***Figure 7*** *Effects of concentration of standard cordycepin on cell cycle progression of (a) EoL-1 and (b) MV4-11 cells were evaluated after 48 hrs of treatment. Cell population after treatments with various concentrations was assessed by flow cytometric quantitation of DNA contents using propidium iodide solution. The percent of cell population was analyzed using FlowJo Vx software. Data are the mean±SD of three independent experiments. The data were analyzed by One-Way-ANOVA test. Asterisks denote values that were significantly different from the vehicle control, *p<0.05; **p<0.01.*

**Table 2** Total cell number of EoL-1 and MV4-11 cells after 48 hrs of treatment with various concentrations of *C. militaris* extracts and standard cordycepin.

| Treatment | EoL-1 (×10$^6$ cells) | | MV4-11 (×10$^6$ cells) | |
|---|---|---|---|---|
| | Crude | Cordycepin | EtOAc | Cordycepin |
| VC | 13.9±2.0 | 13.9±2.0 | 2.6±0.5 | 2.6±0.5 |
| $IC_5$ | 13.4±1.9 | 12.9±1.6 | 2.5±0.6 | 2.3±0.8 |
| $IC_{10}$ | 12.0±1.5 | 11.2±1.2 | 2.4±0.8 | 2.2±0.8 |
| $IC_{20}$ | 9.3±0.6* | 8.8±0.6* | 1.9±0.3 | 2.1±0.7 |

*Data are the mean±SD of three independent experiments. Asterisk (*) denotes a significant difference from the vehicle control (VC) group (p<0.05).*

## Discussion

AML accounts for 80% of acute leukemia in adults. The overexpression of FLT3-ITD was associated with poor prognosis and leukemia progression. Its expression promotes proliferation and inhibits apoptosis of leukemia cells. The role of FLT3-ITD in AML has been recently reviewed elsewhere.[12,14,30] FLT3 has been shown to be the most important protein marker in AML patients and its overexpression leads to the ligand-independent activation, phosphorylation of the receptor and constitutively activate kinase activity, thereby enhancing proliferation and survival of AML.[14] Although several FLT3 inhibitors have been identified and are in clinical trials, treatment of FLT3-mutated AML remains challenging. Thus, it is important to find new inhibitors to overcome the development of resistance and increase toxicities in AML patients with FLT3 mutations. Medicinal plants have been accepted that they contain a wide variety of chemical compounds which are used to perform important biological functions with low toxicity. Several researchers have been studied in their biological activities and accepted that they may be used as effective alternative drugs for treatment of a variety of malignancies. In this study, *C. militaris* is the plant of interest, which has been used for a long time as traditional medicine for many therapeutic purposes. Investigating the previous studies of plants in the genus Cordyceps, they were rich in bioactive compounds, cordycepin and other adenosine derivatives, polysaccharide, mannitol, peptides, etc.[19,31] Recently, ethanolic extract of *C. militaris* possess cytotoxic effects and inhibitory effects on WT1 protein expression and cell cycle progression in EoL-1 cells.[32] However, nothing was known concerning the effects of *C. militaris* extract on FLT3 protein expression, which is related to leukemic cell proliferation. Our previous studies demonstrated the activities of curcumin on the proliferation rate of wild-type FLT3 overexpressing leukemic cells, EoL-1.[16] The recent study demonstrated that both inhouse-*C. militaris* extracts and commercially standard cordycepin exhibited strong cytotoxic effects on both wildtype-FLT3 overexpressing EoL-1 cells and FLT3-ITD overexpressing MV4-11 cells with various $IC_{50}$ values. The Hex and EtOAc extracts exhibited the greatest cytotoxic effects on leukemic cells. However, the cytotoxicity in these leukemic cells were different due to difference in leukemic cell-type. EoL-1 cells were found to be more sensitive to the extracts than MV4-11 cells. This result was well in accordance with previous study reported that EoL-1 cell line was more sensitive to sorafenib and cytarabine than MV4-11 cell line due to the presence of activated FIPL1-PDGFRα fusion gene in EoL-1 cells which is highly sensitive to the kinase inhibitors.[29] According to the study of FLT3 protein expression in leukemic cell lines, non-cytotoxic doses ($IC_{20}$ values) of *C. militaris* extracts including crude extract, Hex fraction, EtOAc fraction, and EtOH fraction were used. After the treatment of leukemic cells for indicated time periods, crude extract exhibited the strongest inhibitory effects on FLT3 protein expression in EoL-1 cells by a dose dependent manner. Similarly, EtOAc fractional extract showed an excellent inhibitory effect on FLT3 protein expression in MV4-11 cells by means of a concentration dependent manner. Moreover, both extracts showed their ability to suppress leukemic cell proliferation when compared to the vehicle control. Thus, the results revealed that the active compounds involved in crude extract and EtOAc fractional extract have ability to inhibit cell proliferation, destroy leukemic cells at high doses and downregulate the target FLT3 protein level at non-cytotoxic doses. Previous study showed that crude extract of *C. militaris* contains the highest amount of cordycepin content and EtOAc fraction contains minimum amount of cordycepin.[25] The likely explanations were due to high polarity of cordycepin and polarity of solvents that used in extraction method which leads to differences in the yield of bioactive compounds. Higher polar solvents contained higher content of bioactive compounds due to their amine and hydroxyl groups.[25] Thus, the inhibitory effect of crude extract on FLT3 protein expression in EoL-1 cells might involve in the function of cordycepin. For EtOAc fraction, it can be explained that other compounds might exert a synergistic effect on inhibition of FLT3 protein expression in MV4-11 cells. Previous studies reported that cordycepin, adenosine, phenolic contents, flavonoid contents, polysaccharides, Ergosterol, cordycepic acid, isoflavonoid were identified in *C. militaris* extracts after extraction with polar solvent (ethanol or water) and semi-polar solvent (ethyl acetate). Not only cordycepin but also other active compounds, such as polysaccharides, cordycepic acid, flavonoids, that demonstrated anti-proliferation, antioxidant, and anti-cancer activities.[33-36] On the other hand, more than 80% of the compounds that extracted from non-polar solvent (hexane) are fatty acid, carboxylic acid and their esters. Only small amount of cordycepin was extracted by using hexane. In addition, the hexane extract demonstrated antioxidant and cytotoxic activity on HCT116 colon carcinoma cells.[37] According to the information mentioned above, various active constituents in each extract may exhibited different inhibitory effect on FLT3 protein expression. Another possible reason is the different between wild-type FLT3 in EoL-1 cells and mutant-FLT3 in MV4-11 may demonstrated the different pattern in response to the treatment and need to be investigated and described more in the future.

Crude extract and EtOAc fractional extract were selective as effective extracts on EoL-1 and MV4-11 leukemic cell lines, respectively. Cell cycle analysis were investigated whether cell cycle progression is involved in reduction of cell viability. In the previous study, ethanolic extract of *C. militaris* arrested cell cycle of K562 cells at S phase.[38] Moreover, cordycepin induced cell cycle arrest at S phase in U937 and NB-4 cells thereby inhibiting the growth of leukemic cells.[39] Consistently, our present study showed that both extracts arrested the cell cycle at S phase in leukemic cells in term of a concentration dependent manner. Previous study reported hat cordycepin-induced S phase cell cycle arrest involves inhibition of cyclin A2, cyclin E, and CDK2 expressions thereby accumulating cells in S phase.[39] In this study, the findings revealed that crude extract and EtOAc fractional extract of *C. militaris* have the ability to downregulate the target FLT3 protein level and inhibit cell cycle progression by the S phase cell cycle arrest at non-cytotoxic doses thereby inhibiting the proliferation of leukemic cells. According to our results, cordycepin may involve in S phase cell cycle

arrest of both EoL-1 and MV4-11 cells thereby decreasing the proliferations of those cell lines.

## Conclusion

In this study, the growth of leukemic cells was inhibited by *C. militaris* extracts. This effect might be caused by the inhibition of FLT3 protein expression and the inhibition of cell cycle progression at S phase. Cordycepin might plays a crucial role of this inhibition. However, the inhibitory mechanisms of active compounds in crude and EtOAc extracts of *C. militaris* are still not clear and need further investigations. These findings may be used for an important guideline for further development of alternative drug for leukemia in the future.

## Conflicts of interest

The authors declare that there are no conflicts of interest.

## Acknowledgments

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# Translation and codons

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**Letter to Editor**

In molecular biology, Central Dogma is defined as the process by which the instructions of the DNA are converted into a functional product, primarily proteins. Francis Crick first proposed it in 1958. In this sequential process, the translation is the final stage, where genetic code contained within a messenger RNA (mRNA) molecule is decoded to produce a specific sequence of amino acids in a polypeptide chain.[1] It is a complex three-stage process requiring a set of different factors in each stage: initiation, elongation, and termination. Mainly the execution of this occurs in the cytoplasm. Albeit some variations of this process in eukaryotic and prokaryotic, the essential features and results remain the same.

When DNA is transcribed to RNA, its complementary nucleic acid is paired with it. DNA codes A, G, T, and C are transferred to RNA codes U, C, A, and G, respectively. The encoding of proteins is done in groups of three, known as codons. These codons represent the sense DNA strand and are arranged in a 5′-to-3′ direction in a linear sequence of genes, which is then transcribed to RNA from the antisense strand of DNA. The standard codon table of RNA is shown below, along with the 20 respective amino acids. As seen in Table 1, the amino acids can be specified by different codons.

**Table 1** Table of codon translation.

| 1st letter | Second Letter: U | Second Letter: C | Second Letter: A | Second Letter: G | 3rd letter |
|---|---|---|---|---|---|
| U | UUU Phe | UCU Ser | UAU Tyr | UGU Cys | U |
| | UUC | UCC | UAC | UGC | C |
| | UUA Leu | UCA | UAA Stop | UGA Stop | A |
| | UUG | UCG | UAG Stop | UGG Trp | G |
| C | CUU Leu | CCU Pro | CAU His | CGU Arg | U |
| | CUC | CCC | CAC | CGC | C |
| | CUA | CCA | CAA Gln | CGA | A |
| | CUG | CCG | CAG | CGG | G |
| A | AUU Ile | ACU Thr | AAU Asn | AGU Ser | U |
| | AUC | ACC | AAC | AGC | C |
| | AUA | ACA | AAA Lys | AGA Arg | A |
| | AUG Met | ACG | AAG | AGG | G |
| G | GUU Val | GCU Ala | GAU Asp | GGU Gly | U |
| | GUC | GCC | GAC | GGC | C |
| | GUA | GCA | GAA Glu | GGA | A |
| | GUG | GCG | GAG | GGG | G |

In the translation, each codon specifies specific amino acid; therefore, the word “codon” is described as “a specific sequence of three consecutive nucleotides which is part of the genetic code on RNA molecule, and that specifies a particular amino acid sequence in protein synthesis.” This term is accurate for the start codon (AUG), which always codes methionine or f-methionine in eukaryotic and prokaryotic, respectively.

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Similarly, all the other codon that follows on the mRNA strand gives a specific amino acid sequence. However, toward the termination stage of this process, one of the three different "codons" UAA, UAG, and UGA, will terminate the translation process without adding any amino acid. In DNA, these stop "codons" are TAA, TAG, and TGA, respectively. The historical basis for ordaining the stop codons as amber, ochre and opal was initially described by Brenner S and Bob E.[2,3] Therefore, when it comes to a stop "codon", this term nullifies its definition "codon specifies amino acid".

Consequently, I strongly suggest that a stop codon should be referred to as a stop "signal" as it does not specify any amino acid. It is just a signal for the translation to stop. Henceforth appropriate terms are "start codon" and "stop signal."

## Conflict of interest

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

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# Evaluation of novel PCR-CTPP for simultaneous detection of *Mycobacterium tuberculosis* complex and identification of RpoB H526D point mutation

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**ABSTRACT**

**Background**: Tuberculosis (TB) is a chronic and highly contagious disease caused of the acid-fast bacilli, *Mycobacterium tuberculosis* complex (MTC). Currently, it has been reported that rifampicin-mono resistant *M. tuberculosis* containing histidine to aspartate replacement at residue 526 (H526D) of the beta-subunit RNA polymerase enzyme (RpoB) increased the cell wall permeability and approximately eight times more susceptible to vancomycin.

**Objectives:** To develop and evaluate the Polymerase chain reaction with confronting two-pair primers (PCR-CTPP) for simultaneous detection of MTC and identification of RpoB H526D mutation.

**Materials and methods:** PCR-CTPP was implemented for TB diagnosis. Reaction and profile were optimized and applied for detection in a total of 308 clinical samples. Sensitivity and specificity of PCR-CTPP was calculated in comparison to the acid-fast bacilli (AFB) staining and standard culture method. In addition, microscopic observation drug susceptibility (MODS) assay was modified for vancomycin susceptibility determination in 15 clinical isolates.

**Results:** Limit of detection of PCR-CTPP was approximately $2\times10^3$ bacilli and no cross-detection to other mycobacteria. PCR-CTPP was evaluated in 308 clinical samples. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of PCR-CTPP versus standard culture method were 90.95%, 79.31%, 91.78% and 77.53%, respectively. In addition, comparison of developed PCR-CTPP versus AFB staining were also represented. MODS was performed in fifteen samples, two multidrug-resistant (MDR) strains containing RpoB H526D were susceptible against vancomycin.

**Conclusion:** The established PCR-CTPP is highly sensitive, specific for investigation of MTC and identification of RpoB H526D mutation. This method could be useful for TB diagnosis together with precision medicine application in vancomycin susceptibility determination in TB patients in the future.

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## Introduction

Tuberculosis (TB) is a chronic and highly contagious disease caused of the acid-fast bacilli, *Mycobacterium tuberculosis* complex (MTC). The disease is mentioned in all ethnic groups whereas infected people are mostly found in low- and middle-income countries. A quarter of

the world's population is estimated to be latent infection and has a risk of developing active TB.[1] Recently, MTC infected people was estimated to be approximately 10 million cases and 1.2 million of which died worldwide.[2] Thailand is one of forty-eight high TB burden countries with the estimation of 105,000 cases ill with TB and about 2,500 infected with multidrugs-resistant (MDR) *M. tuberculosis.*[2] TB is a curable disease, therefore, MDR *M. tuberculosis* that resists against two effective anti-TB drugs, isoniazid and rifampicin, is a new face of a problem and nowadays becomes a major barrier to successful TB control. [3,4] Rifampicin resistant *M. tuberculosis* is always associated with the *rpoB* mutations in the 81-bp rifampicin resistance determining region (RRDR) and leading to the codon substitution at amino acid position 507 to 533 of the beta-subunit RNA polymerase enzyme (RpoB).[5,6] Currently, the cell wall metabolism and physiology of rifampicin-monoresistant *M. tuberculosis* containing a single amino acid replacement from histidine (H) to aspartate (D) at residue 526 (H526D) of the RpoB were studied. The alteration of colony morphology including the length of bacterial cells and cell wall thickness compared to rifampicin-susceptible strain were speculated.[7] Interestingly, the increasing of cell wall permeability and approximately eight times more susceptible to vancomycin of RpoB H526D mutant strain have also been emphasized.[7] Due to these previously observations, it is hypothesized that vancomycin would be a new drug of choice in either alone or in combination with other anti-TB drugs for treatment of TB containing H526D mutation of the RpoB.

Polymerase chain reaction with confronting two pair primers (PCR-CTPP) has been implemented for single nucleotide polymorphisms (SNPs)[8]. In our previous studies, this technique has been adopted for detection of MTC and discrimination of *M. bovis* in sputum specimens.[9,10]

The purposes of this study are to implement the PCR-CTPP for simultaneous detection and identification of RpoB H526D mutation and to compare the sensitivity and specificity of developed PCR-CTPP with either AFB staining or the standard solid culture method.

## Materials and methods

### *Bacterial strains*

MTC consisting of *M. tuberculosis* H37Ra, *M. tuberculosis* H37Rv, *M. bovis* BCG ATCC 35740, and *M. microti* and other mycobacteria such as *M. austroafricanum*, *M. intracellulare*, *M. kansasii, M. nonchromogenicum, M. scrofulaceum, M. terrae,* and *M. vaccae* were provided by Division of Clinical Microbiology, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University. The bacteria were cultured in either Middlebrook 7H10 or 7H9 (Becton Dickinson, USA) supplemented with 10% oleic acid, albumin, dextrose, and catalase (OADC) (Becton Dickinson, USA ) and incubated at 37 °C for 2-4 weeks.

### *Clinical samples and DNA extraction*

The approval for the research was obtained from the Institutional Biosafety Committee of Chiang Mai University (Approval Number: CMUIBC A-0563002). A total of 308 clinical specimens consisting of 274 sputum, 7 cerebrospinal fluid, 6 lymph nodes, 5 bone marrow, 5 brochoalveolar larvage and other 11 specimens were collected from the Office of Disease Prevention and Control Region 1 (ODPCR-1), Chiang Mai, Thailand. Of these, AFB staining results were available in 130 samples consisting of AFB-negative in 53 samples and AFB-positive (scanty to 3+) in 77 samples. The DNA were extracted from clinical specimens using the commercial TB detection kit (Anyplex™ MTB/NTM Real-time detection kit; Seegene Inc., Republic of Korea). The procedures were performed according to the manufacturer's instruction. The quantity and quality of DNA was measured using Eon microplate spectrophotometer (Biotek Instruments, Inc., USA). Mycobacteria in all culture-positive samples were identified and confirmed by the para-nitrobenzoic acid inhibition test and the SD Bioline TB Ag MPT64 test (Standard Diagnostics, Republic of Korea). Genotypes of first-line drug resistant were identified using GenoType MTBDRplus Ver 2.0 (Hain Lifescience GmbH, Germany).

### *PCR-CTPP*

The PCR-CTPP was established for simultaneous detection of MTC and identification of rifampicin-resistant *M. tuberculosis* containing RpoB H526D mutation. Primers used in this study were designed based on a single nucleotide alteration from cytosine (C) to guanine (G) at nucleotide position 1333 of *rpoB* (GenBank accession No. L27989) causing of RpoB H526D amino acid substitution. The primer sequences were shown in Table 1. The 25 µl of PCR reaction contains 2× Quick Taq HS dye master mix (Toyobo, Japan), 1 pmol of TB-F, 20 pmol of D526-R, 1 pmol of H526-F and 1 pmol of TB-R primer (in the ratio of 1:20:1:1) and approximately of 100 ng of DNA template. The PCR was performed in a thermal cycler (Labcycler gradient Senso-Quest, Germany) and the amplification profiles were as follows: pre-denaturation at 94 °C for 2 min, 45 cycles of 94 °C for 30 sec, and 68 °C for 30 sec, with a final extension at 68 °C for 5 min. The expected PCR product was shown in Table 1. The PCR products were electrophoresed through a 2% agarose gel staining with RedSafe nucleic acid staining solution (iNtRON Biotechnology, Inc., Korea) and visualized by a UV transilluminator (G:BOXchemi XRQ gel doc system, UK). Integrity of nucleotide changed from C to G at position 1333 identified by PCR-CTPP were confirmed by Sanger DNA sequencing (Bio Basic Inc., Singapore).

**Table 1** The nucleotide sequence used for PCR-CTPP in this study.

| Primers | Nucleotide sequences (5'-3') | Position | Amplified product (bp) |
|---|---|---|---|
| TB-F | AGACCACGATGACCGTTCCG | 2119-2138 | 315 |
| D526R | CCGACAGTCGGCGCTTGTC | 2415-2433 | |
| H526F | CCGCTGTCGGGGTTGACCC | 2397-2415 | 218 |
| TB-R | CGGTACGGCGTTTCGATGAAC | 2593-2614 | |
| TB-F | AGACCACGATGACCGTTCCG | 2119-2138 | 496 |
| TB-R | CGGTACGGCGTTTCGATGAAC | 2593-2614 | |

***Limit of detection (LOD) of established PCR-CTPP***

The LOD of developed PCR-CTPP was determined. The standard strain *M. tuberculosis* H37Rv and *M. tuberculosis* R43 clinical isolate (The RpoB H526D mutation was previously confirmed by whole genome sequencing) were used as a candidate for wild type and RpoB H526D mutation, respectively. The DNA was extracted from 2-weeks culture of bacteria in Middlebrook 7H9 supplemented with 10% OADC (M7H9-OADC) using the commercial kit (NucleoSpin Triprep, Germany). The DNA quality and quantity were measured before serially 10-fold diluted ranging from undiluted (approximately 100 ng of DNA) to $10^{-7}$ and amplified by PCR-CTPP. The amplified products were analyzed by 2% agarose gel electrophoresis. The experiments were undertaken in 3 independent experiments. The lowest copy number detected by PCR-CTPP was calculated using public software (http://cels.uri.edu/gsc/cndna.html).

***Specificity determination of established PCR-CTPP***

The specificity of established PCR-CTPP was determined in several mycobacterial strains. Bacteria were cultured separately in the M7H9-OADC at 37 °C for 2-4 weeks. The bacterial cells were pelleted, and DNA was extracted using the commercial kit (NucleSpin Triprep, Germany). One hundred nanogram of each DNA sample was mixed into the PCR reaction and the amplified product was analyzed by agarose gel electrophoresis.

***Vancomycin susceptibility testing***

MODS assay was modified for vancomycin susceptibility determination of mycobacteria in this study.[11,12] Fifteen clinical isolates consisting of 5 samples of DS *M. tuberculosis* (one of these having *rpoB* silent mutation at codon 535 (C→T)), one sample of INH-MoR, 3 samples of RIF-MoR, and 6 samples of MDR were included in this experiment. Briefly, vancomycin was two-fold serially diluted in M7H9-OADC with the concentration ranging from 10-320 µg/mL and added into 24-well tissue culture plates. Mycobacterial cells were adjusted with McFarland No. 1 ($10^7$ CFU/mL) and approximately $10^5$ CFU/mL were added into 24-well tissue culture plates. The inoculated plates were labelled, sealed with parafilm, and placed in a transparent ziplock polyethylene bag. Plates were incubated at 37 °C for 2 weeks with examined daily under an inverted microscope. Cell control (without antibiotic drug), media control (without bacterial cells and drug) and drug control (without mycobacteria) were performed and incubated in parallel. Each sample was done in duplicate and three independent experiments. Resistance ratio was calculated from the minimal inhibitory concentration (MIC) of tubercle bacilli (unknown strain) to *M. tuberculosis* H37Rv (standard laboratory strain) for interpretation of either resistant (R) or susceptible (S).[13] The resistance ratio $\leq 1$ was defined as drug sensitive whereas the ratio > 1 was interpreted to be resistant.

## Results

PCR-CTPP was established for rapid MTC detection and identification of a single C to G substitution at nucleotide position 1333. After optimization, a single band of either 218 bp specific for wild type or 315 bp specific for H526D mutation was seen. The LOD of established PCR-CTPP was determined in serial 10-fold diluted DNA extracted from both genotypes, the result indicated that the minimum quantitation observed in wild type and H526D mutant were at dilution of $10^{-4}$ and $10^{-5}$ equivalent to approximately $2\times10^3$ and $2\times10^2$ bacilli, respectively (Figure 1). Specificity determination was performed in MTC and some of mycobacteria other than tuberculosis, the result indicated that developed technique was highly specific for MTC and showed no cross-detection with other mycobacteria (Figure 2). Some of the positive samples consisting of wild type and the RpoB H526D mutation detected by PCR-CTPP were verified by direct sequencing, the sequencing results of some samples were shown in Figure 3.

The PCR-CTPP was evaluated with 308 clinical samples. Of these, 130 samples with AFB staining result available were compared with the developed PCR-CTPP. The percent positive and negative for diagnosis of MTC using the PCR-CTPP compared to AFB staining was calculated and shown in Table 2. In addition, the comparison of the developed PCR-CTPP versus the standard culture method for diagnosis of MTC in clinical samples was performed. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated and represented in Table 3. In Figure 4, total of 19 positive clinical samples were also detected with PCR-CTPP and found wild type MTC and RpoB H526D mutant in total 18 samples, while the rest was undetectable by PCR-CTPP. It supposed that this clinical sample contained other mutation besides RpoB H526B. In Figure 4, one sample (SP8) gave a positive band of approximately 500 bp in length. Sanger DNA

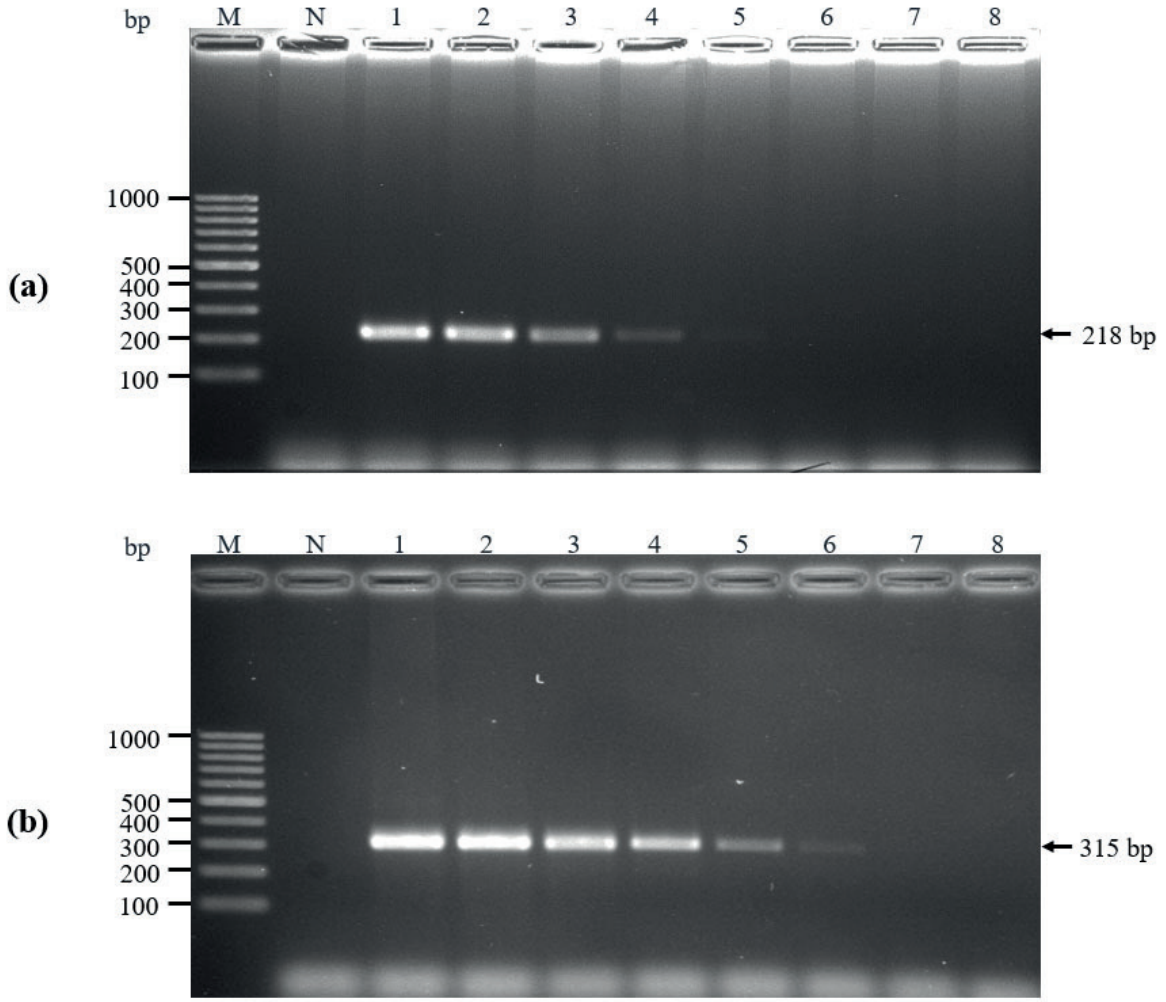


***Figure 1*** *Sensitivity determination of developed PCR-CTPP for detection of MTC (a) and RpoB H526D mutation (b). DNA separately extracted from either M. tuberculosis H37Rv or M. tuberculosis R43 (RpoB H526D mutation), 10-fold serially diluted and used as template for detection using PCR-CTPP. Lane M: standard 100 bp DNA marker, Lane N: negative control, Lane 1: DNA at 100 ng, Lane 2-8: 10-fold diluted DNA at $10^{-1}$-$10^{-7}$ dilution.*

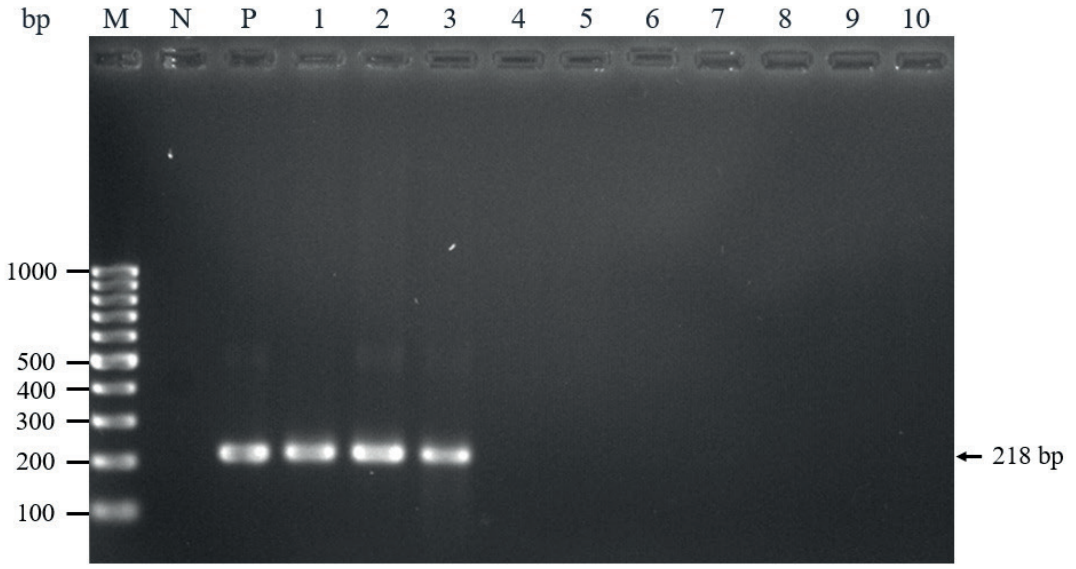


***Figure 2*** *Specificity determination of developed PCR-CTPP in several mycobacteria strains. Lane M: standard 100 bp DNA marker, Lane N: negative control, Lane P: M. tuberculosis H37Rv, Lane 1-10: M. tuberculosis H37Ra, M. bovis BCG ATCC 35740, M. microti, M. scrofulaceum, M. intracellulare, M. kansasii, M. nonchromogenicum, M. terrae, M. austroafricanum, and M. vaccae, respectively.*

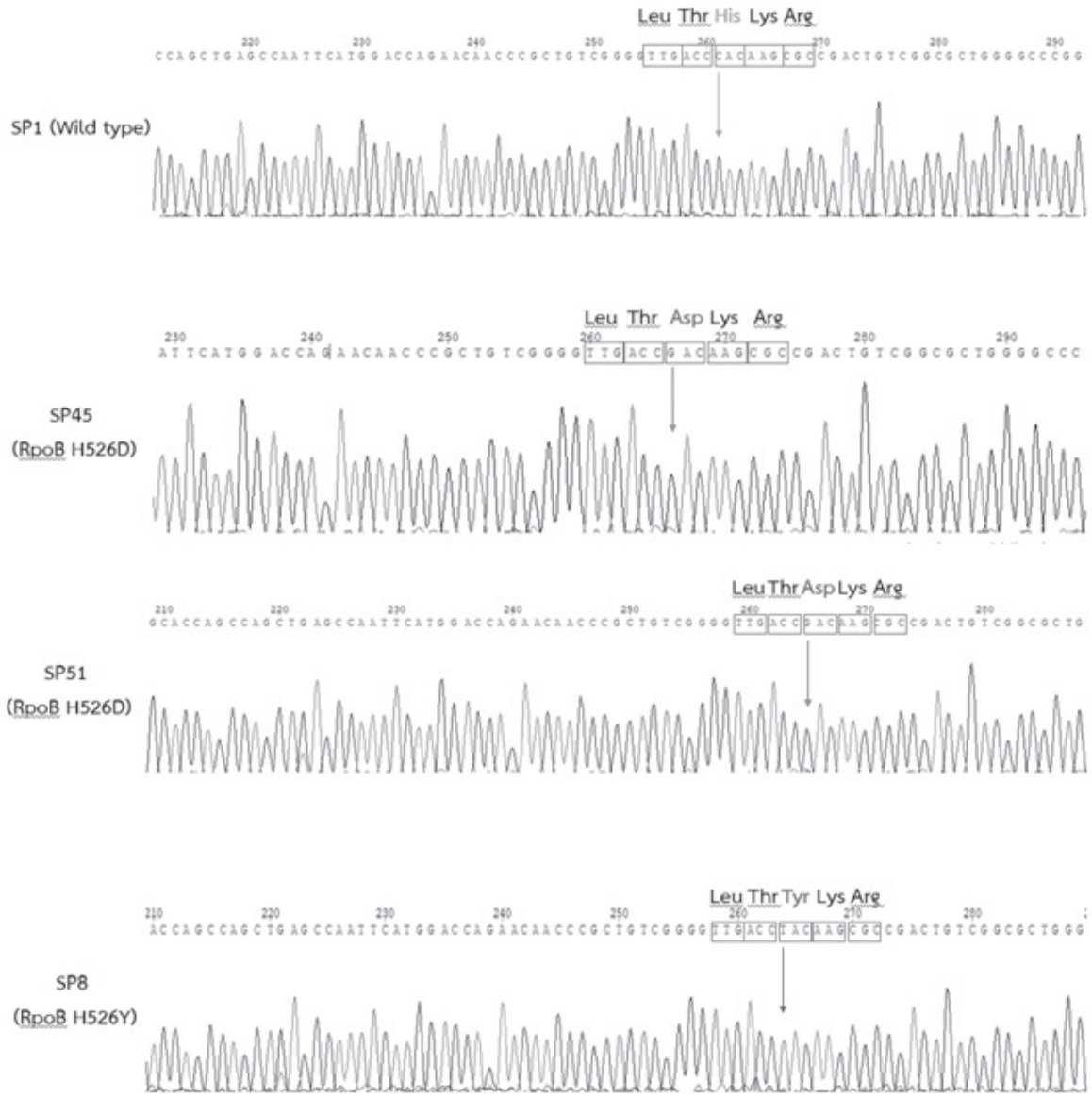


***Figure 3*** *Sequencing results of wildtype, RpoB H526D and RpoB H526Y mutants. The integrity of developed PCR-CTPP was verified using Sanger DNA sequencing. The wildtype (SP1) and a single nucleotide alteration at nucleotide position 1333 (C→G) in RpoB H526D mutant (SP45 & SP51) and 1333 (C→T) in RpoB H526Y (SP8) were indicated.*

**Table 2** The comparison of the AFB staining versus developed PCR-CTPP for diagnosis of MTC in 130 clinical specimens.

| Technique | PCR-CTPP | | Total | Kappa coefficient |
|---|---|---|---|---|
| | Positive (%) | Negative (%) | | |
| AFB staining[#] | | | | |
| 3+ | 10 (90.9) | 1 (9.1) | 11 | 0.555 |
| 2+ | 9 (75) | 3 (25) | 12 | |
| 1+ | 32 (72.7) | 12 (27.3) | 44 | |
| Scanty | 5 (50) | 5 (50) | 10 | |
| Negative | 8 (15.1) | 45 (84.9) | 53 | |
| Total | 64 (49.2) | 66 (50.8) | 130 | |

[#] *AFB staining is not available in 178 samples*

**Table 3** The comparison of the standard solid culture method versus developed PCR-CTPP for diagnosis of MTC in 308 clinical specimens.

| Techniques | Standard culture | | Sensitivity (%) | Specificity (%) | % PPV (95% CI) | % NPV (95% CI) |
|---|---|---|---|---|---|---|
| | Positive (%) | Negative (%) | | | | |
| PCR-CTPP | | | | | | |
| Positive (N) | 201 | 18 | 90.95 | 79.31 | 91.78 (88.07-94.41) | 77.53 (69.14-84.16) |
| Negative (N) | 20 | 69 | | | | |
| Total | 221 | 87 | | | | |

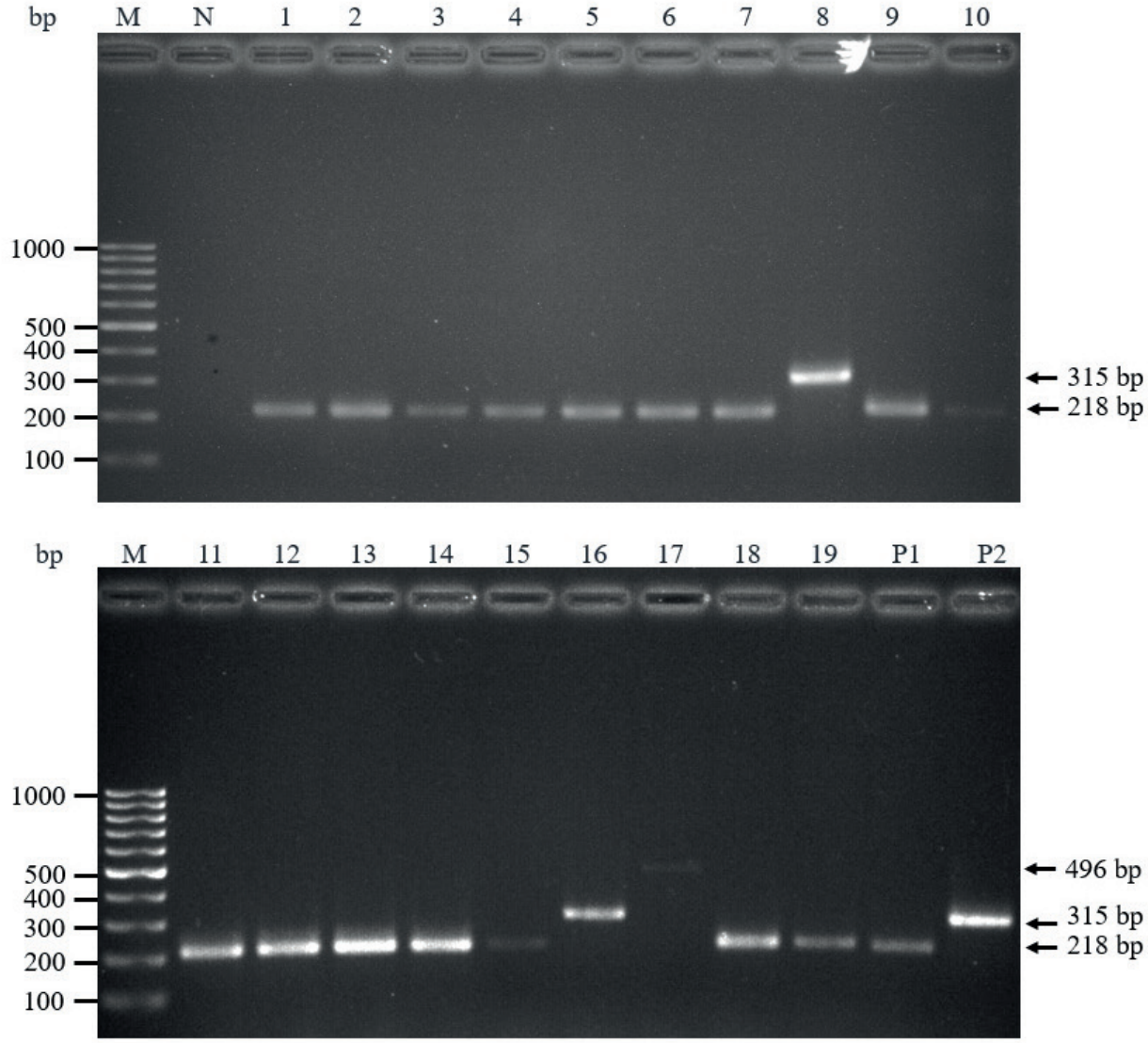


***Figure 4*** *PCR-CTPP for detection of MTC and identification of RpoB H526D mutation in clinical specimens and example of PCR-CTPP results in 19 samples. Lane M: standard 100 bp DNA marker, Lane N: negative control, Lane P1 and P2: positive amplification control for wild type (218 bp) and RpoB H526D mutant (315 bp), respectively, Lane 1-7, 9-15 and 18-19: positive amplification of wild type in clinical specimens, Lane 8 and 16: positive amplification of RpoB H526D mutant in clinical specimens, Lane 17: positive amplification of clinical samples containing other mutations at codon 526 of RpoB.*

sequencing was performed for identification of mutation in this sample, the result indicated a single nucleotide changed from C to T at position 1333 and leading to the H526Y amino acid substitution (Figure 3).

MODS assay was modified for vancomycin susceptibility determination in fifteen clinical isolates. The MIC of the standard strain, *M. tuberculosis* H37Rv, was determined to be 40 µg/mL. Resistant ratio was applied for interpretation of either resistant or susceptible in these mycobacterial strains. The results indicated that two MDR strains containing H526D amino acid substitution detected by both developed PCR-CTPP and direct sequencing were susceptible to vancomycin with the MIC ≤40 µg/mL. Another MDR with RpoB S522L also susceptible to this antibiotic drug. Other twelve clinical isolates including DS, INH-MoR, RIF-MoR, and other MDR were resist to vancomycin. The genotypes and phenotypes of these mycobacteria and vancomycin susceptibility testing were illustrated in Table 4.

**Table 4** Genotype and phenotype of samples examined for vancomycin susceptibility using MODS assay.

| Clinical isolate | Code name | Phenotype | Mutation identification | | | Vancomycin susceptibility |
|---|---|---|---|---|---|---|
| | | | rpoB | katG | inhA | |
| 1 | SP02 | DS | - | - | - | R |
| 2 | SP09 | DS | - | - | - | R |
| 3 | SP25 | DS | - | - | - | R |
| 4 | B16 | DS | - | - | - | R |
| 5 | SP31 | DS | 535(C→T) | - | - | R |
| 6 | P06 | INH-MoR | - | 327 (C→T) | - | R |
| 7 | SP01 | RIF-MoR | H526D | - | - | R |
| 8 | SP24 | RIF-MoR | L511P | - | - | R |
| 9 | SP45 | RIF-MoR | H526D | - | - | R |
| 10 | SP5 | MDR | S522L | S315T | -17 (G→C) | S |
| 11 | SP26 | MDR | S531L | S315T | - | R |
| 12 | SP4 | MDR | D516V | - | -8 (T→C) | R |
| 13 | SP8 | MDR | H526Y | - | -8 (T→C) | R |
| 14 | SP51 | MDR+SM | H526D | S315T | - | S |
| 15 | SP111 | MDR+SM | H526D | - | -15 (C→T) | S |

*DS: drug sensitive, INH-MoR: isoniazid monoresistant, RIF-MoR: rifampicin monoresistant, MDR: multidrug-resistant, MDR+SM: multidrug-resistant with streptomycin resistant, R: resistant, S: susceptible*

## Discussion and conclusion

TB remains a major public health concern worldwide. Due to the airborne transmission, TB easily spreads from infected person to another nearby through coughing and sneezing. Although new strategy to fight the global TB epidemic has launched, patients infected with MDR and extensively drug resistant (XDR) *M. tuberculosis* are increasing and becoming a new life-threatening problem.

Recently, the fitness costs *in vitro* and *in vivo* of rifampicin-resistant *M. tuberculosis* containing RpoB H526D were studied.[14] Relative to the isogenic wild type strain, it was revealed that this mutant significantly conferred long-term survival during growth-limiting conditions *in vitro* and in mouse lungs. In addition, an increasing of both cell wall permeability and susceptible to vancomycin, the classical antibiotic drug targeting to cell wall biosynthesis, was observed.[7] These previous studies inspired us to conduct the experiments for proving the hypothesis that RIF-MoR and MDR-TB containing RpoB H526D mutation might susceptible to vancomycin and this drug might be a new drug for further specific treatment of TB patients with RpoB H526D mutation of *M. tuberculosis*.

In this study, MODS assay was performed for vancomycin susceptibility determination in fifteen clinical isolates consisting of DS, INH-MoR, RIF-MoR and MDR-*M. tuberculosis*. A reference strain *M. tuberculosis* H37Rv was examined in parallel, and the result indicated the MIC of the standard strain was 40 µg/mL. The same MIC value was also observed when vancomycin was examined with another standard strain, *M. tuberculosis* CDC1551.[7] In addition, the previous data indicated that both standard strains, *M. tuberculosis* H37Rv and *M. tuberculosis* CDC1551, were not different in term of growth rate *in vitro* and *in vivo*.[15]

Of these fifteen clinical isolates, three MDR phenotypes were susceptible to vancomycin and two of which were identified as RpoB H526D mutation (Table 4). Interestingly, these two MDR strains showed different INH-resistant genotypes (SP51 was *katG* (S315T) & SP111 was *inhA* (-15 (C→T))) and associated with the resistance to streptomycin. Another MDR with RpoB S522L and double mutations of INH (S315T of katG & inhA promoter at -17 (G→C)) was also susceptible to vancomycin. This MDR strain with susceptible to vancomycin has not yet been

reported. However, in-depth characterization of this strain including whole genome sequencing is underway. In contrast to the previous report [7], two RIF-MoR strains with RpoB H526D mutation were resist to vancomycin. Considering to the RpoB H526D phenotype in both RIF-MoR and MDR strain, the resistant to INH and/or streptomycin might be the key finding of vancomycin susceptible in these MDR samples. However, a higher number of clinical samples with RpoB H526D mutation in *M. tuberculosis* would be recruited for vancomycin susceptibility testing before further application.

From the MODS assay results and the previous reports,[7] identification of *M. tuberculosis* with RpoB H526D mutation would pave the way for vancomycin susceptibility determination before TB treatment. PCR-CTPP was implemented in this study for early detection of MTC and simultaneous identification of RpoB H526D mutation. After optimization, PCR-CTPP was examined in MTC and some other nontuberculous mycobacteria and the results indicated that established method is highly sensitive with the limit of detection (LOD) of approximately 2,000 bacilli and specific to MTC. As few as 10-1000 bacilli has been reported when the nucleic acid amplification tests were applied for TB diagnosis.[16,17]

A total of 308 clinical samples consisting of sputum and other specimens, were examined by PCR-CTPP and the result was compared to AFB staining and gold standard culture method. As compared to AFB staining, it was indicated that the percent positivity of PCR-CTPP was increased from 50% to 90% when the number of tubercle bacilli was increased from scanty to 3+, respectively. The agreement between AFB staining and PCR-CTPP was represented using Cohen's kappa coefficient (K) and moderately agreement between these two methods was indicated (Table 2).[18] Similar result in which TB diagnosis by AFB staining compared to Xpert MTB/RIF was previously reported.[19] The developed PCR-CTPP was compared to the standard culture method for diagnosis of MTC in clinical samples (Table 3). Relative to the culture-based methods, the sensitivity of the established method was comparable whereas it represented less specific with the false positive result. The major drawback for nucleic acid detection-based methods is an indistinguishable between live and dead mycobacteria and these techniques are more suitable for first MTC diagnosis prior treatment.[20,21] In this study, PCR-CTPP prefers an advantage in identification of H526D mutation through a single C to G detection at position 1333 of *rpoB*. Of nucleic acid amplification-based methods, the sensitivity and the specificity of nested PCR targeting the IS6110 for *M. tuberculosis* detection have been demonstrated to be equivalent to our developed method.[22]

In this study, 12 of 308 samples were identified as MDR-TB (3.89%), which similar as information that recently reported by another team of Thai researchers in 2022 (4.1%).[23] Moreover 2 of 12 samples of MDR-TB (16.6%) were also identified to be RpoB H526D mutation. The percent of MDR with RpoB H526D ranging from 3.5-10.0% have previously been reported in Thai population.[7,24,25] However, more clinical samples must be studied to determine the accurate prevalence of MDR-TB with RpoB H526D in Thailand.

In conclusion, a highly sensitive and specific PCR-CTPP was established for investigation of MTC and simultaneously identification of RpoB H526D mutation. This method could be useful for TB diagnosis together with precision medicine application in vancomycin susceptibility determination in TB patients. However, a higher number of clinical samples with RpoB H526D mutation in *M. tuberculosis* would be recruited for vancomycin susceptibility testing before further application.

## Conflicts of interest:

The authors declare no conflict of interest.

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# Ancestry evaluation from subtrochanteric shape of femur using Tallman and Winburn's Adjusted Platymeric Index: A validation study in the Northern Thai population

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**ABSTRACT**

**Background**: Ancestry estimation of unidentified human skeletons using metric parameters of bones other than the skull, such as the subtrochanteric shape of the femur, is a challenging method in cases with limited information from cranial examination. The results of multiple studies show that the subtrochanteric shape can differentiate Asian and non-Asian individuals. However, the cut-point of the platymeric index for subtrochanteric shape might need adjustment for each region or country for higher accuracy, due to interpopulation and intrapopulation variation.

**Objectives**: This study aims to validate the cut-point adjusted for the Thai population suggested by Tallman and Winburn and observes the differences between each sex within the same population.

**Materials and methods**: The researchers conduct the study by collecting and calculating multiple parameters (such as femoral subtrochanteric diameters and shape, and platymeric index) from 130 northern Thai individuals collected from the Chiang Mai University bone collection, then making comparisons with the traditional cut-point, and comparing these parameters between different populations and sex.

**Results**: The results show that the accuracy of ancestry estimation is improved from 46.38% to 57.49%, using an adjusted cut-point. This study also notes that the platymeric index of the northern Thai samples is significantly higher ($p$<0.05) than the north-eastern samples recorded by Tallman and Winburn. Moreover, the index of the male Thai samples is significantly higher ($p$<0.05) than the female samples from the same bone collection.

**Conclusion**: This study approves the use of the adjusted cut-point in the northern Thai region and indicates an intrapopulation variation and sexual dimorphism of subtrochanteric shape which might influence ancestry assessment.

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## Introduction

In regular forensic work, understanding forensic anthropology is a crucial step in the personal identification of human remains. Forensic pathologists examine remains thoroughly to gain data on individual characteristics, called a biological profile. The police can compare this profile to missing person data.[1]

One crucial process in the biological profiling of human remains is ancestry assessment. Nowadays, forensic anthropologists use the morphological traits of the human skull (both metric and non-metric) as a significant aspect of the evaluation.[1-3] Meanwhile, the ancestry assessment of remains with incomplete or missing skulls might prove difficult. Thus, other bones such as femurs,[4-8] talus, humerus[1] and cervical spines[9] can provide the data to distinguish between ancestral origins.

Many studies indicate that subtrochanteric shape, intercondylar notch height and intercondylar shelf angle can differentiate between individuals of each descent.[4-6,8] These parameters are used by archaeologists and forensic anthropologists to distinguish between Native American remains and White and Black Americans.[8,10-12] The subtrochanteric aspects play a role in differentiating Asian remains from non-Asian remains.[6] Many researchers use the platymeric index as a representative of subtrochanteric shape. The index is calculated by dividing the antero-posterior subtrochanteric diameter by the medio-lateral subtrochanteric diameter and multiplying by 100.[13] Individuals with indices equal to or less than 84.9 are classified as platymeric (mediolaterally broad), those with indices between 85.0 and 99.9 are classified as eurymeric (round), and those with indices equal to or greater than 100.0 are classified as stenomeric (antero-posteriorly broad).[8] Many studies show that platymeric is the most common shape in the Asian population, while the non-Asian population usually exhibits a eurymeric shape.[4]

The study conducted by Tallman and Winburn shows that the platymeric index range of north-eastern Thai samples is significantly lower than the White American range. Moreover, applying the frequency distribution of the platymeric index to north-eastern Thai individuals is not accurate, using the traditional cut-point. An adjusted cut-point value for the Thai population is calculated and suggested, four units higher than the traditional value (platymeric with indices equal to or lesser than 88.9, eurymeric with indices between 89.0 and 103.9, and stenomeric with indices equal to or greater than 104.0).[4]

Within any population there is some difference in phenotype, called intrapopulation variation.[14,15] Therefore, the frequency distribution of the subtrochanteric shape in other Thai regions might differ from the north-eastern individuals and the adjusted cut-point from Tallman and Winburn's study might not be suitable for the Thai population in other areas.

Therefore, this study aims to validate the adjusted cut-point value using data from northern Thai individuals and observing the differences between each sex of the northern Thai population.

## Materials and methods

### *Sample selection*

This study uses the femurs of Thai skeletons donated for educational purposes to the Faculty of Medicine, Chiang Mai University (CMU). Before the femur examination, the researchers examined the general information on each case (ancestry, sex, age, underlying disease, evidence of trauma and orthopedic treatment). Any cases which matched the following exclusion criteria were rejected:

- Any femora that came from a deceased with a confirmed diagnosis of osteoporosis or diseases that affect bone quality (such as osteomalacia, Cushing's disease, Paget's disease or Marfan syndrome).[16]
- Any femora that had a moderate to severe degree of damage, such as fractures, deformities or post-mortem breaking.
- Any femora that had retained artificial implants or fixating material.

Of the 471 cases in the bone collection, 130 which came from the northern Thai population were randomly selected and included in the research as the northern Thai samples. The sample size was calculated using the formula:

$$n = ( Z^2 \times P \times (1 - P) )/ e^2$$

where $Z$ = value from standard normal distribution corresponding to the desired confidence interval (CI) ($Z$=1.96 for 95% CI), $P$ = expected true proportion ($P$=0.9), and $e$ = desired precision ($e$=0.05).[17]

The sample contained 79 male and 51 female cases, with ages ranging from 26 to 94 years (average 60.42 years). For the north-eastern Thai sample and foreigner sample, the researchers used the data from Tallman and Winburn's study which is available in their study's full article.[4]

### *Femur examination and data collection*

Metric dimensions of the subtrochanteric region (A-P and M-L diameter) of the left femur was measured using standard anthropometric sliding callipers (Figure 1A-1C), using the right femur if the left was missing or damaged (according to the measurement recommendation from standards for data collection from human skeletal remains).[18] Medio-lateral subtrochanteric diameter refers to the distance between the medial and lateral surfaces at the point of greatest expansion below the base of the lesser trochanter. Antero-posterior subtrochanteric diameter was defined as the distance between anterior and posterior surfaces perpendicular to the medio-lateral diameter (Figure 2).[18] Each parameter was measured three times and the platymeric index (PI) was calculated using an average of the measured values. Meanwhile, the anthropology expert measured and revised 30 samples for inter-observer bias calculation. Next, the subtrochanteric shape of each femur was classified by its calculated platymeric index compared to the traditional cut-point and adjusted cut-point from Tallman and Windburn's study (shifting the cut-point values by four units higher from the traditional cut-point).[4] The ancestry was estimated from the subtrochanteric shape and compared to the recorded ancestry of each case for validation. Difference in frequency

distribution between the traditional and adjusted methods, between each sex, and between other population results were compared to other studies.

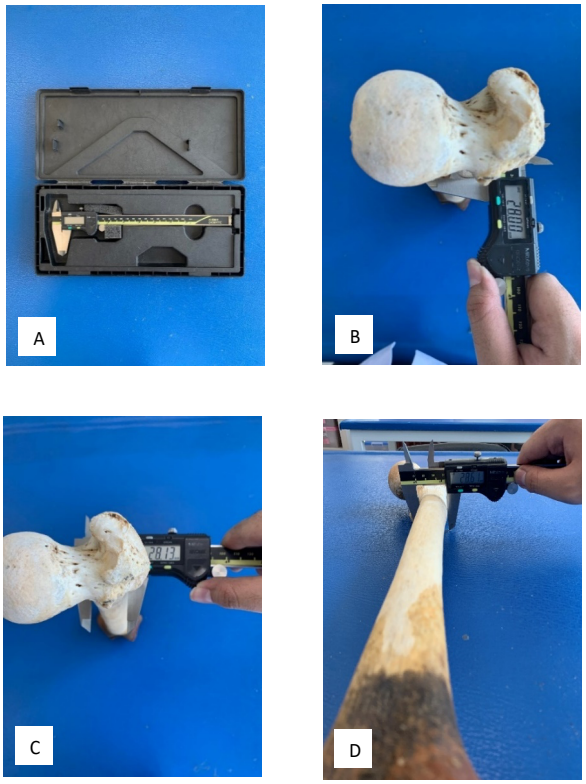


***Figure 1***. *Measurement of metric dimensions of subtrochanteric region (A-P and M-L diameter). (A): standard anthropometric sliding calipers being used in this study, (B): process of antero-posterior diameter, (C, D): medio-lateral diameter measurement from the selected femur.*

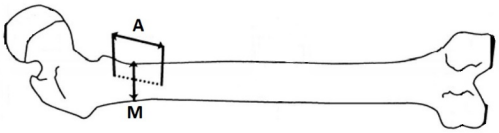


***Figure 2***. *Landmarks for antero-posterior (labelled as "A") and medio-lateral diameter (labelled as "M") measurement in human femur (modified from Figure 54. Measurements of the left femur, posterior view from Buikstra and Ubelaker's Standards for data collection from human skeletal remains, 1997).*[18]

### *Statistical assessment*

The intraclass correlation coefficient (ICC) was used to calculate intra-rater and inter-rater bias during the data collection process. The researchers used Cohen's kappa coefficient to measure reliability between the adjusted cut-point evaluation and recorded ancestry data, for adjusted cut-point validation. For comparison between the two groups, the independent t-test and Pearson's chi-squared test were used to compare the platymeric index and frequency distribution of subtrochanteric shape, respectively.

## Results

### *Platymeric index*

Platymeric index of 130 samples from the northern Thai population samples from CMU had an average of 87.03 with SD of 6.53 (range 69.99-106.67). The platymeric index had a normal distribution, shown by the Kolmogorov-Smirnov test ($p$=0.20) (Figure 3). The demographic data, average A-P and M-L diameter, and platymeric index of each sex are shown in Table 1. There was excellent reliability of the rater according to the intra-rater and inter-rater ICC values (Table 2).

**Table 1** Summation of demographic data and femoral metric data of northern Thai population samples from CMU.

| | Cases | Age | | A-P diameter | M-L diameter | Platymeric index | |
|---|---|---|---|---|---|---|---|
| | N (%) | Mean (SD) | Range | Mean (SD) | Mean (SD) | Mean (SD) | Range |
| Male | 79 (60.8) | 59.92 (12.87) | 29-91 | 27.09 (2.11) | 30.81 (2.14) | 88.08 (5.93) | 69.99-100.60 |
| Female | 51 (39.2) | 61.18 (15.32) | 26-94 | 24.17 (2.28) | 28.34 (1.98) | 85.42 (7.13) | 70.77-106.67 |
| All samples | 130 | 60.42 (13.84) | 26-94 | 25.95 (2.60) | 29.84 (2.40) | 87.03 (6.53) | 69.99-106.67 |

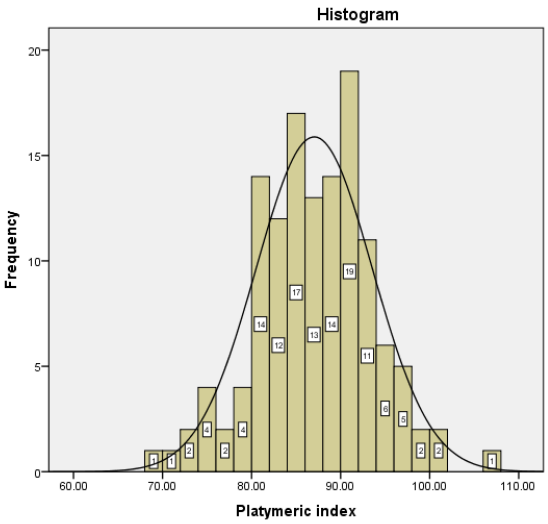


***Figure 3***. *Platymeric index distribution curve calculated from northern Thai population samples from CMU.*

**Table 2** Intraclass correlation coefficient value for intra-rater and inter-rater bias of each parameter.

| | A-P diameter | M-L diameter |
|---|---|---|
| Intra-rater bias | 0.992 | 0.990 |
| Inter-rater bias | 0.957 | 0.910 |

***Subtrochanteric shape and frequency distribution***

Using the traditional cut-point, the most common type of subtrochanteric shape of northern Thai population samples from CMU is eurymeric (80 cases; 61.5%), while only 47 cases (36.2%) have platymeric shape. After re-classification of the subtrochanteric shape using the adjusted cut-point from Tallman and Windburn's study, the number of cases with platymeric shape increased by 32 (24.6%), while cases with eurymeric and stenomeric shape decreased by 30 and 2, respectively (Table 3).

The researchers used raw data on White American males from Tallman and Windburn's study as non-Asian representatives for the validation process. Assuming that a platymeric shape is interpreted as Asian and other shapes are interpreted as non-Asian (Table 4), there is slight agreement between the recorded ancestry and estimated ancestry using the adjusted cut-point of Tallman and Winburn (κ=0.123, $p$=0.075). Comparing the traditional and adjusted cut-point accuracy, the adjusted cut-point accuracy (57.49%) is higher than the traditional (46.38%).

**Table 3** Subtrochanteric shape of northern Thai population samples from CMU, evaluated by traditional and adjusted cut-point from Tallman and Windburn's study.[4]

| | | Platymeric | Eurymeric | Stenomeric |
|---|---|---|---|---|
| | | N (%) | N (%) | N (%) |
| Traditional cut-point[a] | Male | 21 | 56 | 2 |
| | Female | 26 | 24 | 1 |
| | Total | 47 (36.2) | 80 (61.5) | 3 (2.3) |
| Adjusted cut-point[b] | Male | 42 | 37 | 0 |
| | Female | 37 | 13 | 1 |
| | Total | 79 (60.8) | 50 (38.5) | 1 (0.8) |

[a]*Platymeric (PI≤84.9), Eurymeric (85.0≤PI≤99.9), Stenomeric (PI≥100.0)*
[b]*Platymeric (PI≤88.9), Eurymeric (89.0≤PI≤103.9), Stenomeric (PI≥104.0)*

**Table 4** Number of cases for each estimated ancestry by subtrochanteric shape, compared to recorded ancestry.

| | | | Recorded ancestry | |
|---|---|---|---|---|
| | | | Asian | Non-Asian |
| Estimated ancestry | Traditional | Asian | 47 | 28 |
| | | Non-Asian | 83 | 49 |
| | Adjusted | Asian | 79 | 37 |
| | | Non-Asian | 51 | 40 |

***Subgroup comparison***

Comparing male and female Thai samples from the CMU collection, as shown in Table 1, the platymeric indexes of the female skeletons are statistically significantly lower than the male skeletons ($p$=0.023). Also, the female skeletons' AP diameters and ML diameters are statistically significantly lower than the males ($p$<0.05). There is a statistically significant difference in the frequency distribution of subtrochanteric shape between the sexes, using the traditional cut-point ($p$=0.018) and adjusted cut-point ($p$=0.028).

Platymeric index and subtrochanteric shape of male Thai samples from the CMU collection was compared to male samples from the Khon Kaen University (KKU) collection and the American White males from Tallman and Winburn's study.[4] The results show that the platymeric indexes of the male skeletons from CMU (88.08±SE of 0.67) are statistically significantly higher than the male skeletons from KKU (83.85±SE of 0.65) ($p$<0.05) but statistically significantly lower than the American male skeletons (91.36±SE of 1.14) ($p$=0.014). Moreover, there is a statistically significant difference in the subtrochanteric shape distribution between the Thai males from the CMU collection and the KKU collection and American White males from the Tallman and Windburn study, using the traditional cut-point ($p$<0.05). The frequency distribution percentage comparison of the male Asian subpopulation (data from multiple studies) and White males from Tallman and Winburn's study is presented in Table 5.

**Table 5** Number of cases for each estimated ancestry by subtrochanteric shape, compared to recorded ancestry.

| | Northern Thai | Northeastern Thai[4] | Singapore Chinese[19] | Malaysia Chinese[20] | American white[4] |
|---|---|---|---|---|---|
| Platymeric | 36.2 | 57.8 | 51.2 | 55.8 | 36.4 |
| Eurymeric | 61.5 | 39.1 | 48.8 | 30.0 | 37.7 |
| Stenomeric | 2.3 | 3.1 | 0.0 | 14.2 | 26.0 |

## Discussion

Postcranial methods of ancestry estimation (the ancestry estimation using the other bones rather than the skull) have a major role in the skeleton with a damaged or missing skull.[1] But currently, the studies about postcranial methods are still not many. One of the most common bones that are being used for ancestry studies is the subtrochanteric region of the femur. Many researchers state that humans of different ancestry have different subtrochanteric shapes due to the type of terrain and subsistence method.[8] The subtrochanteric shape is different between Asian and non-Asian populations and between each region within populations.[4] Therefore, this study aims to validate Tallman and Winburn's adjusted cut-point of platymeric index using a sub-population different from the original research.

According to the results for the northern Thai population, the accuracy of the ancestry estimation using the platymeric index along with Tallman and Winburn's adjusted cut-point is higher than the traditional cut-point (57.49% and 46.38%, respectively), and the number of Asian cases correctly classified increases by 24.6% (32 cases). The results are similar to Tallman and Windburn's original study based on the KKU collection.[4] It can be assumed that the population-specific platymeric index cut-point should be used to estimate ancestry more accurately. However, we suggest that the platymeric index should not be used solely for estimation, based on the value of Cohen's kappa coefficient (κ=0.123). Evaluation using combined data from various femoral parameters (such as intercondylar notch height and intercondylar shelf) would be more proper and effective, and give more appropriate results.

Based on the frequency distribution of subtrochanteric shape in the northern Thai sample, the most frequent type is eurymeric (61.5%), while the platymeric shape is found in only 36.2%. These numbers contrast with the north-eastern Thai sample and other subpopulations from the Asian continent, where the most common type is platymeric, and eurymeric is less common. The average value of the platymeric index calculated from the CMU collection is higher than the KKU collection (88.08 and 83.85, respectively). These differences might be caused by intrapopulation variation of the femur due to multiple factors that affect the mechanical stress on the lower limbs and nutrition status, including the terrain of the northern province and the livelihoods of the population compared to other regions.[8,14,15,21]

Because of the difference between the northern and north-eastern Thai populations, the researchers suggest that the data from this study might not be sufficient to calculate a new platymeric index cut-point, and additional data collection from other Thai provinces is recommended.

This study finds that the average A-P diameter, M-L diameter and platymeric index of the male sample is significantly higher than the female. The frequency distribution of subtrochanteric shapes for each sex is significantly different. One reason for these findings is sexual dimorphism, which is the difference in phenotype between males and females of the same ancestry. The morphological variance of the femur between males and females is explained by the principle that the axial skeleton weight of males is relatively heavier than females, and one of the major bones that are impacted by the weight transmission is the femur. Another theory suggests that the female pelvis morphology is modified for reproduction and pregnancy, leading to different stresses and strains affecting the femur.[21,22] The researchers suggest that sexual dimorphism should be considered when estimating ancestry. In routine biological profile assessment, ancestry is the first aspect to be determined, before sex, so sexual dimorphism might interfere with the ancestry interpretation.

## Conclusion

Postcranial studies for ancestry estimation are essential in the forensic field, especially in cases with limited skull data. However, few studies or databases exist in Thailand or other Asian countries. The researchers aim to contribute Asian data to further research on subtrochanteric shape and ancestry assessment. Secondly, this study validates the platymeric index cut-point adjusted by Tallman and Winburn to determine the intrapopulation variation between provinces and approve its accuracy and adaptability for use in Thailand. Lastly, this study raises one significant factor that might affect ancestry interpretation, the sexual dimorphism of the human femur. The researchers hope for the discovery of a new proper cut-point or other method for more accurate ancestry estimation using subtrochanteric shape, whatever the sex of the individual.

## Conflict of interest

The authors declare no conflict of interest.

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# Ethical conflicts in the context of the COVID-19 pandemic

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**ABSTRACT**

**Background**: Healthcare setups in India are encountering a lot of medical, ethical, legal, and social challenges endorsed by the ongoing COVID-19 crisis, modifying the healthcare protocols, which are considered a standard of care. The ethical conflicts are pressurizing the decision-makers of society, to revamp the basic principles and traditional assumptions of our present healthcare modalities.

**Objectives:** This report addresses the common but crucial ethical issues, encountered by healthcare workers e.g., doctors and paramedics in day-to-day healthcare practice during the COVID-19 pandemic.

**Materials and methods:** This report tries to cover several ethical aspects of COVID-19 such as: setting priority of screening; professional responsibility of healthcare workers; dilemma encountered by a doctor; compromise of patient confidentiality; allocation of scanty resources; end-of-life situation, and certain critical clinical scenarios.

**Results and conclusion:** COVID warriors from the health sector possess dual responsibilities of taking utmost care of COVID patients ethically, along with self-protection from this havoc. COVID-19 has taught us the lesson that, extra-ordinary time calls for extra-ordinary measures. Also, intervention performed upon the patient affects not only the patient, but also the whole of society.

## Introduction

COVID-19 is an infectious disease, that spreads more robustly than our health-service systems can tackle. As global communities try to combat the COVID crisis, lots of critical ethical, legal, and social crossroads have evolved. These ethical conflicts are pressurizing the decision-makers of society, to revamp the basic principles of our present healthcare modalities.[1] Enumerating the relevant aspects, as follows:

***1. Portion of the population, ideally to be screened for COVID-19, in limited availability of tests:***

Screening tests create a conflict ethically, due to the scanty number of tests, and are with suboptimal specificity and sensitivity. On a priority basis, symptomatic patients should be tested first because early diagnosis, and prompt interventions are critical, as most of the spread is from this said fraction of the population. As the improvement of tests in quantity and quality, gets available, the screening of asymptomatic healthcare workers can also be performed.[1,2]

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***2. Professional responsibility of healthcare workers, in the management of highly contagious COVID-19:***

Appropriate personal protective equipment (PPE) does a commendable job in the prevention of exposure, thereby restricting the spread of infection. But many healthcare organizations fail to accommodate a sufficient quantity of PPE for adequate protection of their staff, altering dynamics ethically. A certain fraction of populations such as the elderly, and healthcare workers with underlying comorbidities are more susceptible to the deleterious effects of COVID-19. They are vulnerable while dealing with COVID-19 patients, with insufficient PPE. With the surplus availability of PPE, it becomes an ethical duty of clinicians to give care to COVID-19 victims. New guidelines and Standard Operating Procedures (SOPs) can be formulated and implied. The health workers must be trained in the standard guidelines of universal precautions e.g., Personal Protective Equipment (PPE) use.[3,4]

***3. Addressing the compromise of patient confidentiality in reporting the positive cases to the public and the higher authority concerned:***

Securing COVID-19-positive patients' privacy is an ethical conflict as it possesses a potential threat to society. Currently, prejudicial stigma is not linked with testing positive for COVID. So, breakage of the seal of confidentiality is not so challenging and framing decisions on public interest is ethically less conflicting. Healthcare centers should be advised to warn their healthcare workers of the positive status of COVID-19 patients. The COVID-19 patients who agree to disclose their current health status to those contacts, which they might have put at risk, should have the opportunity of informing for tracing purposes. With the high contagiousness of COVID-19, confidentiality must be restricted for sake of mass public health. So, the conventional rule of 'Professional Secrecy' can be superseded by 'Privileged communication' in this critical scenario. It is quite pivotal that, hospitals and physicians should convey to the concerned public agencies regarding COVID positive cases, thereby fetching more data to be tabulated, analyzed, and finally processed to frame management guidelines.[4,5]

***4. Rational allocation of limited resources such as ventilators, ICU beds, as well as life-saving remedies*:**

Much more attention is being paid to the allocation of scanty resources wisely in this current pandemic. Various guidelines are now available to different healthcare facilities. Discrete opinions should be framed regarding the allocation of limited lifesaving modalities on a priority basis or the triage. Collaborative support for conserving scarce resources is a mandate in this crisis.[10]

***5. Ideal protocol to be followed in the tragic 'End-of-Life' situation:***

In such of tragic 'end-of-life' (EOL) situation, the ICU core members should not debar the family members from the golden chance of conveying the last goodbye to the patient. Visitations, may it be physical or virtual, should be made possible. The concerned family members should be prepared mentally beforehand for the impending tragic bereavement. Also, EOL-family conferences may be arranged remotely if possible and if required. Honest counseling and conversations are vital for the family members to get prepared for the saddest news of the death of their nearest and dearest ones. It is required to overcome anxiety, and withstand depression, PTSD, and complicated grief afterward.[7,8]

***6. Ethical crossroads in certain critical scenarios:***

***6.1 HIV-positive patients:***

HIV-positive patients may be more susceptible to getting extremely ill with COVID. They should implement 'COVID-appropriate behavior' (vaccination, regular mask-wearing,


***Figure 1*** *Spectrum of dilemmas encountered by treating doctor during management of COVID-19.*[1,9]


***Figure 2*** *Various ethical loopholes in critical care of COVID-19 victims in intensive care unit of hospital.*[7,8]

and practicing social distancing, exercise, and yoga) at the earliest. Despite being fully vaccinated, persons with comorbidity or immune suppressants might not be protected. They should not discontinue taking all precautions, ideally suggested for unvaccinated persons, unless advised medically.[5,9]

***6.2 Pregnancy:***

Pregnancy involves certain physiological changes, which increase vulnerability to catching respiratory viruses like COVID-19. These alterations in the body might last long, even after childbirth. Breast milk, according to current evidence, is unlikely to pass the virus to babies. Nevertheless, COVID-appropriate behavior is to have stringently adhered to. Preventive COVID vaccine is universally advised for all individuals aged 12 and above, as well as, those who are pregnant, breastfeeding, or attempting to conceive. Precautionary measures like washing hands for 20 seconds with soap and water, before breastfeeding or expressing breast milk, along with using a hand sanitizer containing at least 60% alcohol in the unavailability of soap and water. It is recommended by the precautionary guidelines for COVID-19 set by World Health Organization (WHO) and Center for Disease Control (CDC).[3,4]

## Conclusion

The COVID-19 crisis is robustly remodeling our benchmarks of priorities, may it be medical, ethical, legal, or social. But, some of our ethical obligations remain unaltered. Undoubtedly, our commitment to advocacy, transparency, and empathy for human life remains unchanged. COVID-19 has taught us the lesson that, any intervention that is performed upon the patient affects not only the patient but also the whole of society.[9,10]

## Conflicts of Interest

The authors declare no conflict of interest

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# Screening algorithms for detecting dysphagia: A validation study of Thai EAT-10 combined with the 3-Ounce Water Swallow Test

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**ABSTRACT**

**Background**: Dysphagia assessment using standard tools is not commonly performed in Thailand because it relies on equipment and experts to evaluate. This study was conducted by combining the Thai EAT-10 and the 3-ounce water swallow test, an easy-to-use tool for dysphagia screening. Therefore, it is crucial to study the accuracy and appropriateness before being widely used in the Thai population.

**Objectives:** To evaluate the validity of the combined Thai Eating Assessment Tool 10 (Thai EAT-10) and the 3-ounce Water Swallow Test (WST) screening algorithm for detecting dysphagia compared to diagnosis by an otolaryngologist.

**Materials and methods:** Analytical Cross-sectional study: sample of 260 adults (aged 20 years old or older) demographic data and past medical history were assessed using a questionnaire. The accuracy of dysphagia screening tests that combine Thai EAT-10 and the 3-ounce water swallow test with pulse oximetry was compared to the clinical swallow test assessed by an otolaryngologist. The sensitivity and specificity of the swallowing screening test for detecting dysphagia were evaluated.

**Results:** Thai EAT-10 combined with the 3-ounce Water Swallow Test has higher validity and accuracy for dysphagia evaluation than those of the Thai EAT-10 or the 3-ounce Water Swallow Test alone. There was 100% sensitivity, 93.4% specificity, 68.1% positive predictive value (PPV), and 100% negative predictive value (NPV).

**Conclusion:** The Thai EAT-10 combined with the 3-ounce water swallow test and pulse oximetry has higher reliability and validity than those of the Thai EAT-10 and the 3-ounce water swallow test alone. The reliability and validity of the combined test is closed to the gold standard. Furthermore, the algorithm is appropriate for screening dysphagia in remote areas with a large population.

## Introduction

Dysphagia, defined as having difficulty swallowing, is a prevalent symptom that is caused not only by medical diseases but also by aging.[1] Its consequences are complications include dehydration, malnutrition, bronchitis, upper airway infections, and life-threatening conditions such as aspiration pneumonia.[2, 3] There are several clinical methods for screening oropharyngeal dysphagia. The gold standard for swallowing examination is Videofluorographic Swallowing Study; VFSS and

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Fiberoptic Endoscopic Examination of Swallowing (FEES). However, FEES is difficult to conduct in all patients.[4, 5] Alternatively, swallowing screening, a minimally invasive procedure performed by speech-language therapists etc., is used to determine the likelihood of dysphagia. The systematic review by Bour *et al.* in 2009 that was conducted to determine the effectiveness and feasibility of bedside screening methods for detecting dysphagia in patients with neurological disorders. The result revealed that a water swallowing test combined with pulse oximetry was the best method for screening dysphagia in patients with high accuracy (73-98% sensitivity and 63-76% specificity).[6] The 3-ounce water swallowing (WST) is a widely known test with high sensitivity (96.5% sensitivity and 48.7% specificity) for predicting aspiration status in a large and heterogeneous patient population who are at risk for oropharyngeal dysphagia.[7] In addition, a self-assessment tool is essential for patients to clarify the severity of their symptoms. Belafsky *et al*. studied the accuracy and reliability of the Eating Assessment Tool (EAT-10), which consists of 10 questions for self-assessment of dysphagia. If the score is greater than or equal to 3, it means abnormal.[8] This test can be carried out and finished in less than 2 minutes, and is easy to use.[8] EAT-10 had high sensitivity and specificity for screening dysphagia in healthy populations (89% sensitivity and 82% specificity).[9] Moreover, former studies suggested the usage of EAT-10 as the first tool for dysphagia screening in older adults and patients with neurological disorders.[9] EAT-10 has been introduced in many countries and has been translated into various languages, including Thai.[10-15] Korwanich *et al*. examined the accuracy of the EAT-10 in Thai version on patients at the Ear, Nose, and Throat clinic, Faculty of Medicine, Chiang Mai University and found that it had 96.9% sensitivity and 93.4% specificity, which showed that the use of the Thai EAT-10 was highly valid and accurate for detecting dysphagia.[15]

However, previous studies have found that dysphagia assessments were conducted using only an objective or subjective assessment.[6-9] Combining a test to other kind of test may have the potential of bringing higher reliability and accuracy while possibly making the effectiveness of the combined tests the gold-standard. In addition, a new combination may possibly be useful to replace the gold-standard tools in remote areas.

This study aimed to evaluate the validity of the self-dysphagia assessment form, the Thai EAT-10, in combination with the 3 oz. water swallow test (WST), compared to an otolaryngologist's diagnosis and to establish appropriate and accurate assessment algorithms for daily life practice.

## Materials and methods

This study was conducted in the Ear, Nose, and Throat department, Faculty of Medicine, Chiang Mai University. The sample size calculation using 85% sensitivity[16] and 95% confidence indicated 196 subjects were required. To reduce data collection discrepancies, the number of samples was raised to 270. Ten patients were excluded from the study due to incomplete demographic information. Thus, in purposive sampling, 260 patients who visited Ear Nose Throat Clinic voluntarily were recruited in this study. All participants were without any cognitive state problems.

The inclusion criteria were as follows;

1. Male or female, aged 20 years or more.
2. Never been diagnosed with dysphagia.
3. The participants had passed the Thai Mental State Examination (TMSE). The passing level scores were depended on participants' education level. For participants with elementary education background or below, the passing score must be greater than or equal to 19. For those with higher than elementary education level background, the passing score must be greater than or equal to 24.

The exclusion criteria were as follows;

1. Patients with underlying severe medical conditions such as head and neck cancer patients, end stage of life patients.
2. Patients with a nasogastric tube or tracheostomy tube.
3. Those who did not agree to give an interview or could not communicate.
4. Patients who could not perform a water swallowing test, judge by a physician.

Data of age, gender, body mass index, past medical history, education level, career, and salary per month was collected using a general questionnaire. Thai EAT-10 and the 3-ounce WST with pulse oximetry to monitor aspiration was performed separately. If one of the following assessment criteria were met, the patient would be classified as a dysphagia group:

- Score 3 or more from the Thai EAT 10 test.
- Cannot continuously swallow 90 milliliters of water within 1 minute.
- Having wet, hoarse, or voice change after swallowing all the water within 1 minute.
- Aspiration: oxygen saturation dropped 2% or more during water swallowing or choking after swallowing all the water within 1 minute.
- Coughing after swallowing all the water within 1 minute.

All samples were clinically examined by a 7 year experience otolaryngologist and a speech-language pathologist to diagnose whether or not he or she showed dysphagia conditions using the Clinical Swallow Test (CSE), which consists of general history, history of underlying medical conditions, treatment and medications received, reporting of the patient's symptoms, and oromotor function assessment. In case samples diagnosed with dysphagia by CSE, the physician would perform a fiberoptic endoscopic evaluation of swallowing (FEES) examination.

**Screening algorithms process**

The sequential approach was used for both tests because it is practical, logistical, and preferred in rural areas.[17] The 3-ounce WST was used to confirm the result of the Thai EAT-10 (positive results and negative results confirmation). The Thai EAT-10 and 3 oz. WST were used singularly as a comparator. For this study, there are 4 algorithms of dysphagia screening processes as below (Figure 1).

**Algorithm A**: The 3-ounce WST was used if the Thai EAT-10 gave a negative result. This approach is applied to minimize a false negative.[17] A case was considered negative if both test results are negative, meaning that if the case fails to pass either test, he/she is categorized as positive.

**Algorithm B**: The 3-ounce WST was used if the Thai EAT-10 gave a positive result. This approach was applied to minimize a false positive.[17] A case is considered positive if both test results are positive, meaning that if the case passes either test, he/she is categorized as negative.

**Algorithm C**: Only the Thai EAT-10 is performed to categorize.

**Algorithm D**: Only the 3-ounce WST is performed to categorize.

Statistical analysis involved descriptive and inferential analyses with a 5% significance level where in the variables were determined by percentage, mean, and standard deviation. Other statistics performed include sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Thai EAT-10 combined with the 3-ounce WST. Receiver operating curves (ROCs) were calculated for each scale to determine the area under the curve.

## Results

Total number of samples recruited in this study was 260. The mean age was 46.5±16.4 years (20-92 years). Percentage of participants described by demographic characteristics is shown in Table 1. There were 32 participants (12.3%) who were diagnosed with dysphagia by CSE. All of them were confirmed with FEES as dysphagia positive.

The number of participants with positive and negative results in each test are shown in Figure 2. In addition, algorithm A provided the highest sensitivity and specificity with PPV of 68.1% and NPV of 100%., algorithm B provided 78.1% sensitivity and 100% specificity with 100% PPV and 97.0% NPV. Both Thai EAT-10 (algorithm C) and 3-ounce WST (algorithm D) alone showed lower values of sensitivity and specificity (Table 2). The ROC curve of algorithm A shows the highest area under curve (Figures 3 and Table 3).

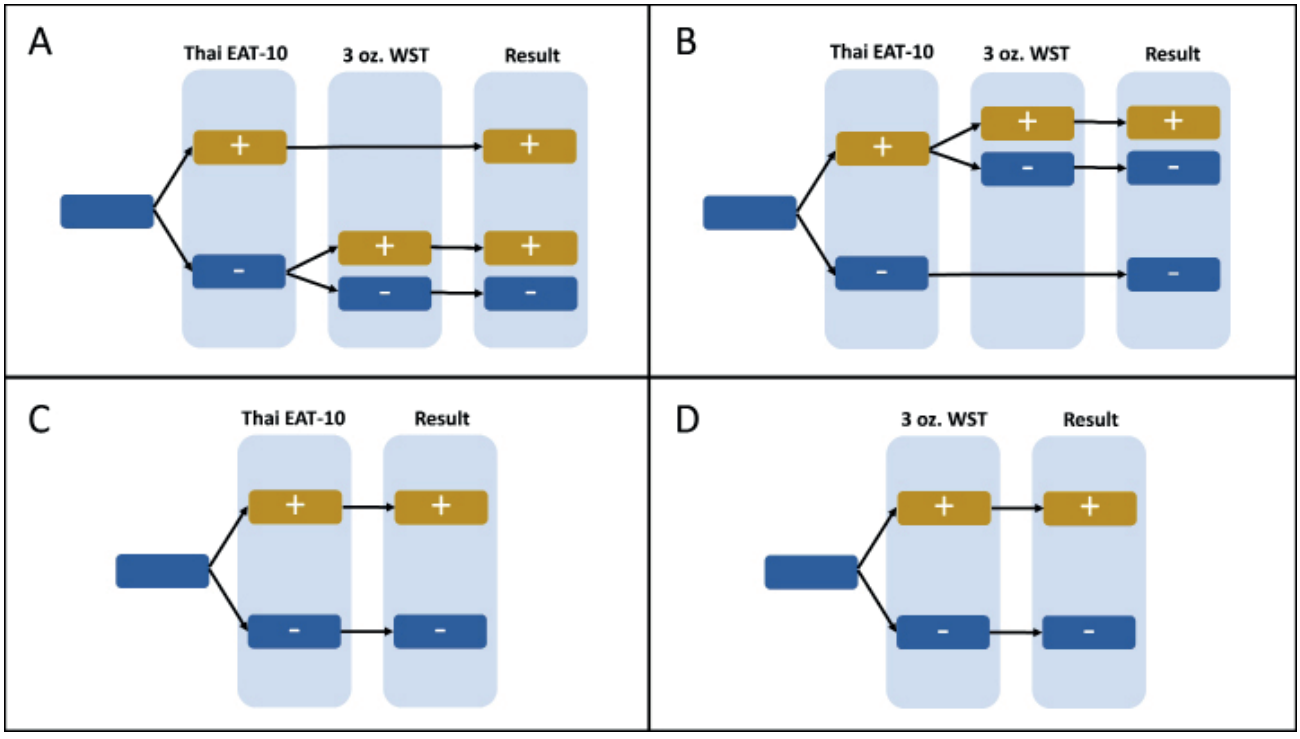


***Figure 1*** *Screening algorithms process.*

**Table 1** Characteristic of participants and dysphagia patients.

| Characteristic | | Participants (260) N (%) | Dysphagia patients (32) N (%) |
|---|---|---|---|
| Gender | Male | 108 (41.5) | 26 (81.3) |
| | Female | 152 (58.5) | 6 (18.8) |
| Age | Mean±SD (years) | 46.5±16.4 | 68.3±10.3 |
| | Min-Max (years old | 20-92 | 42-92 |
| | <30 years old | 60 (23.1) | 0 |
| | 31-59 years old | 135 (51.9) | 3 (9.4) |
| | ≥60 years old | 65 (25) | 29 (90.2) |
| BMI | Mean±SD | 23.8±4.4 kg/m2 | 23.8±4.3 kg/m2 |
| | Min-Max | 14.7-43.3 kg/m2 | 14.7-43.3 kg/m2 |
| Medical condition | No | 149 (57.3) | 6 (18.8) |
| | Yes, hypertension, cancers, hyperlipidemia | 111 (42.7) | 26 (81.3) |
| Status | Single | 102 (39.2) | 1 (3.1) |
| | Married | 152 (58.5) | 29 (90.6) |
| | Widow | 4 (1.5) | 2 (6.3) |
| | N/A | 2 (0.8) | 0 |
| Educational level | Under Bachelor's degree | 156 (60) | 26 (81.3) |
| | Upper Bachelor's degree | 102 (39.2) | 6 (18.7) |
| | N/A | 2 (0.8) | 0 |
| Salary per month | <30,000 Baht | 213 (82) | 28 (88.5) |
| | >30,000 Baht | 46 (17.7) | 4 (12.5) |
| | N/A | 1 (0.3) | 0 |

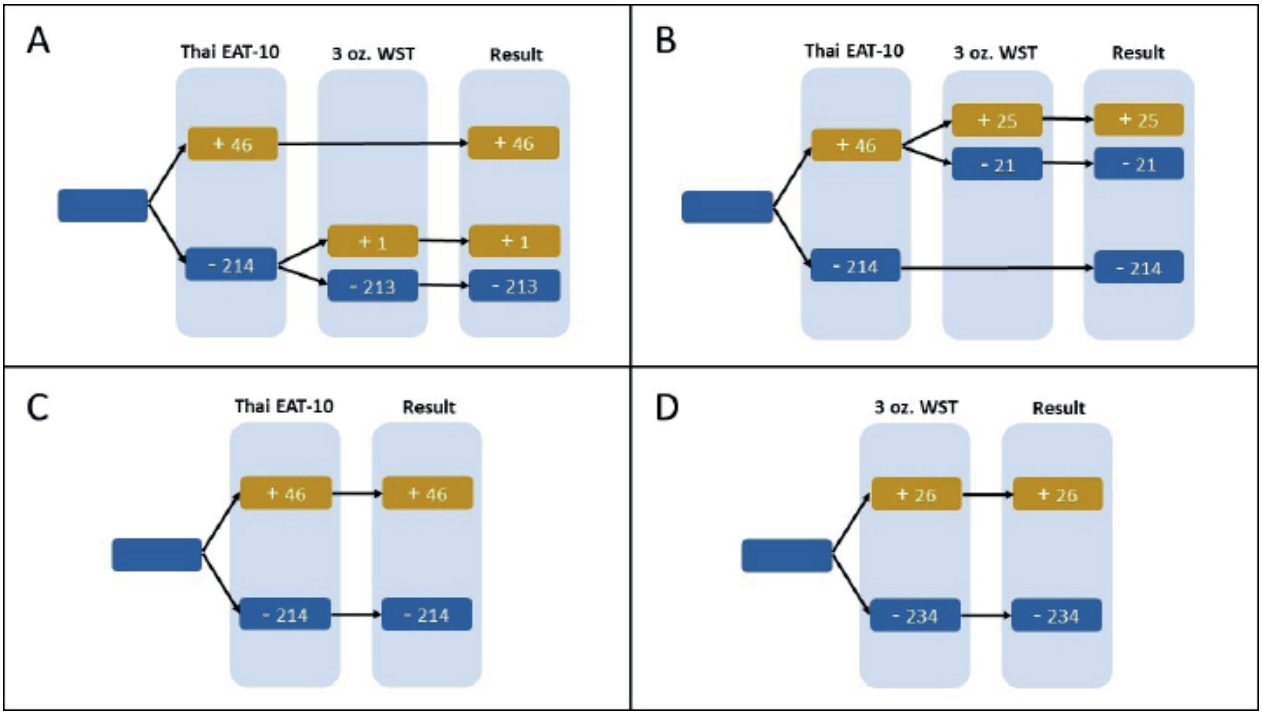


***Figure 2*** *Tests from different algorithms.*

**Table 2** Measures of validation and efficacy for dysphagia screening test.

| Algorithm | Sensitivity (95% CI) (%) | Specificity (95% CI) (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|
| A | 100 (86.7-100) | 93.4 (89.2-96.1) | 68.1 | 100 |
| B | 78.1 (59.6-90.0) | 100 (97.6-100) | 100 | 97.0 |
| C | 96.8 (82.0-99.8) | 93.4 (89.1-96.1) | 67.4 | 99.5 |
| D | 81.3 (62.9-92.1) | 100 (97.9-100) | 100 | 97.4 |

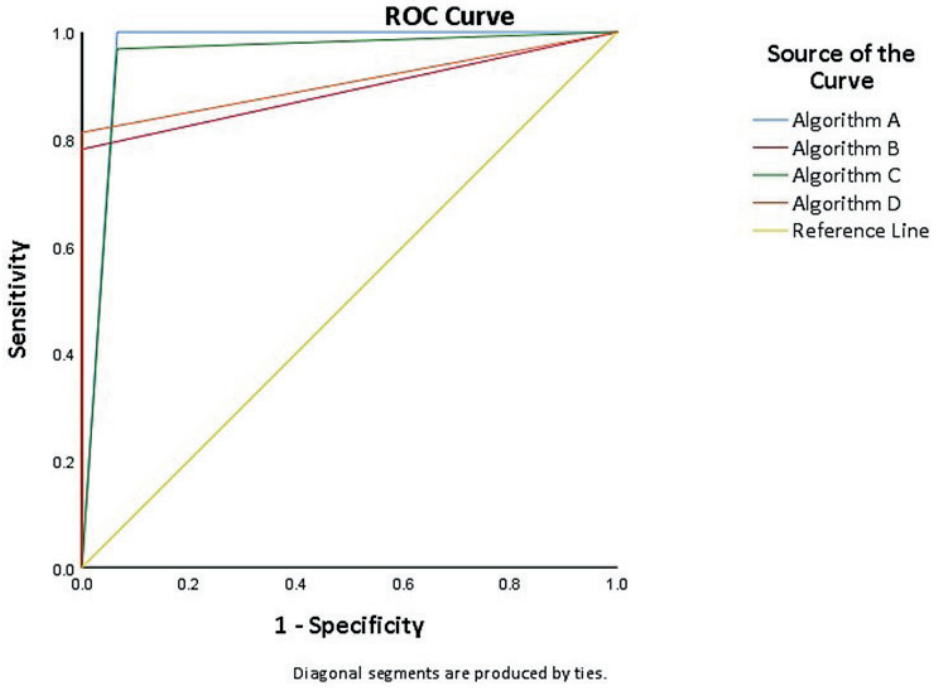


***Figure 3*** *ROC curve showed sensitivity and specificity of dysphagia screening techniques.*

**Table 3** Area under ROC curve of dysphagia screening techniques.

| Dysphagia screening technique | Estimate | SE | *p* value | 95% Confidence interval | |
|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound |
| Algorithm A | 0.967 | 0.010 | <0.001 | 0.947 | 0.987 |
| Algorithm B | 0.891 | 0.044 | <0.001 | 0.805 | 0.977 |
| Algorithm C | 0.951 | 0.020 | <0.001 | 0.912 | 0.991 |
| Algorithm D | 0.906 | 0.041 | <0.001 | 0.826 | 0.987 |

## Discussion

In Thailand, history taking, physical examination, and clinical assessment have been performed to diagnosed patients who possibly suffering from dysphagia caused by cerebrovascular disease and head and neck cancer. Widely known dysphagia diagnostic tests in Thailand are swallowing water at a volume of 3-5 milliliters[9, 10] and 90-milliliter water swallow test, such as PMR Siriraj Swallow Screening and dysphagia assessment in patients with cerebrovascular disease at Srinagarind Hospital.[18] Water swallowing test is accepted widely because of its high sensitivity. While the small volume (1-5 mL) of water swallowing test has been used in patients who were suspected to have dysphagia, neurologic disease, or respiratory disease, with a 71% sensitivity and 90% specificity,[6] it could have false-negative at a high rate if used with patients who has a mild symptom of dysphagia.[6] On the other hand, using water volume of 90-100 milliliters is considered as a high sensitivity test and suitable for screening dysphagia in a population without severe congenital disease or dysphagia-associated disease risk group. Such a protocol is needed to provide a diagnosis

and prognosis before planning treatment and rehabilitation.[6] However, the high volume of the WST has a risk of causing aspiration in severe dysphagia patients. Therefore, pulse oximetry in combination with water swallowing test and viscous powders used in combination with the WST such as the V-VST swallowing test, especially for patients at risk of aspiration, such as stroke patients [9], has been recommended to monitor silent aspiration.[6] However, many countries use self-assessment tools such as the EAT-10, including the Thai version, to screen dysphagia because it is easy to use, takes minimal chair time, and has high sensitivity and specificity. [8, 9, 14]

According to the results of this study, dysphagia assessment in participants using bothThai EAT-10 and the 3-ounce WST found that algorithm A provided highest area under ROC curve compared to the other algorithms. The reasons may be from the high sensitivity and specificity of Thai EAT-10, which help to increase the test's accuracy. Futhermore, using both tests means that objective and subjective evaluations have been applied. Therefore, most of the signs and symptoms of dysphagia can be detected. Compared to previous studies[7,9], this study provided higher sensitivity and specificity, which may be due to using a standard reference tool, sample sizes, and different characteristics, resulting in various results.

It is noteworthy to mention that to use the gold-standard tool (FEES), meaning physicians have to insert parts of equipment into the body which can be dangerous or disturbing, and might not be appropriate or ethical to apply the gold standard tool in every sample. In this study we use the gold standard tools only when the case required confirmation of other clinical results. As a result, the data on the number of people with dysphagia may be less than using the gold standard in every participant. Further research may considered this dilemma during the study design.

In addition, the Thai EAT-10 or the 3-ounce water swallow test alone also provides high sensitivity and specificity. The choice of tools to assess dysphagia should take patient safety into account. The use of Thai EAT-10 alone may also be appropriate due to its being easy to use and safe in the community.

## Conclusion

The Thai EAT-10 combined with the 3-ounce water swallow test and pulse oximetry has higher reliability and validity than the Thai EAT-10 and the 3-ounce water swallow test alone. The reliability and validity of the combined test is close to the gold standard. Furthermore, the algorithm is appropriate for screening dysphagia in remote areas with a large population.

## Conflict of interest

The authors declare no conflicts of interest.

## Ethical approval

This study was approved by the institutional review board and ethically approved the study of the Faculty of Dentistry (No.3/2563) and the Faculty of Medicine, Chiang Mai University(No.054/2563).

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# Investigation of alanine dosimeter for output measurement in radiotherapy: Results from multicenter in Thailand

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**ABSTRACT**

**Background**: Alanine dosimeters are generally used in high-dose industrial applications (kGy). Later, research into employing alanine as a dosimeter in radiotherapy (1-20 Gy) has increased, since alanine may be an alternative transfer dosimeter for quality control, postal audit, and intercomparison between laboratories. However, several factors such as the dosimeter's characteristic should be investigated while utilizing alanine in radiotherapy. In addition, the optimal electron paramagnetic resonance (EPR) reader should be configured to match the absorbed dose range.

**Objectives:** This study aims to optimize the EPR setting parameters, study the characteristics of alanine dosimeters, and estimate the uncertainty of the 6MV-FFF linear accelerator in a dose ranging from 1 to 20 Gy. The output measurements from different facilities were also investigated.

**Materials and methods:** The alanine dosimeters were irradiated with a 6MV-FFF beam using linear accelerator, Varian TrueBeam (Varian Medical Systems, Inc, CA, USA), 100 cm SSD, with a field size of 10x10 cm2 at 1 to 30 Gy. The EPR operation parameter has been optimized for these dose ranges. The characteristics of alanine dosimeters were then investigated, along with the estimation of uncertainty in using alanine. Finally, the alanine dosimeter proficiency was validated using 9 distinct linear accelerator machines.

**Results:** The EPR parameters were found to be optimized at 1.589 mW of MP, 7.018 G of MA, and 40.96 ms of TC. The expanded uncertainty (k=2) was reported at 2.68% in the 1-20 Gy dose range. The alanine dosimeters' characteristics were found to have good uniformity and reproducibility, low fading, and angle-and dose-independence. Although the investigation was performed in 9 different linear accelerator machines, the difference of delivered dose output was measured and reported with difference percentages within ±1%.

**Conclusion:** This study reports the feasibility of using alanine dosimeters in radiotherapy. The important EPR parameter setting, and alanine dosimetry characteristics were

investigated, whose results suggest that alanine can be used at a dose range of 1-30 Gy. This especially benefits the SRS treatment which uses a high dose per fraction, and this dosimeter can be an alternative transfer dosimeter. Nonetheless, various factors should be explored using an appropriate phantom prior to clinical application.

## Introduction

Alanine is a non-polar neutral amino acid that is commonly used as a dosimeter in radiotherapy operated based on the radiation-induced free radical principle. The advantage of alanine is that it is more human tissue equivalent than other transfer dosimeters, such as the optically stimulated dosimeter (OSL) and the radio photoluminescence (RPL) glass dosimeter. Alanine also has a low fading rate in long-term storage (5 % over a year).[1] Dry storage should be considered as it affects the measurement findings. Alanine has two structural forms: α ($CH_3$-CH($NH_2$)-COOH) and β ($CH_2$($NH_2$) $CH_2$-COOH). α has higher stability than β, therefore the α form is popularly used for radiation detection. The alanine signal is recorded using electron paramagnetic resonance spectroscopy (EPR). Following irradiation, free radicals are created from amino acids ($NH_2$). Concentration of free radicals is proportional to the absorbed dose, which is measured by EPR. In the reading process, the microwave bridge generates and adjusts the magnetic field to supply in the cavity containing the irradiated alanine. The optimal magnetic field can be adjusted by microwave power (MP) and modulation amplitude (MA) to achieve a good sensitivity, as well as high resolution spectrum shape and amplitude, while time constant decreases noise in the system. Therefore, these three parameters are the key to approach accurate results. Although the key parameters (MA, MP, and TC) have been discovered in different samples and reported in various research,[2,3] the origin of the key parameters should be investigated in specific situations.

Alanine dosimeters are commonly used in industrial applications, such as food irradiation and medical device sterility, with doses ranging from 10-1000 kGy.[2] In fact, its sensitivity decreases when used in the dose of 1-10 Gy.[2] The alanine and EPR technique was established by several metrology institutes as a secondary standard for absorbed dose to water in the kGy dose range.[4-6] Previous studies (by IAEA, NPL) demonstrated the use of alanine to measure delivered dose to patients in radiotherapy (usually in the range of 1-30 Gy) compared to conventional dosimeters in end-to-end tests, such as TLD, OSL, and RP glass dosimeters. The findings indicate that the alanine dosimeter can be used efficiently in end-to-end tests. Since alanine has a good response at high doses, it is beneficial to use alanine in stereotactic radiosurgery (SRS), which is an intracranial treatment using radiation dose of 15-24 Gy.[7] In Thailand, there is no study on the use of alanine dosimeters for end-to-end measurement.

Accordingly, this study investigated the feasibility of using alanine dosimeters in the end-to-end test for SRS. This is the pioneer in investigating the optimal key parameters in EPR for readout process (MA, MP, and TC) for the dose range of 1-20 Gy subjected to 6 MV-FFF, followed by exploring the characteristics of alanine dosimeter which include sensitivity, linearity, energy dependence, and fading. Finally, the accuracy of delivery dose in linear accelerator using alanine dosimeter was evaluated among eight radiotherapy centers in Thailand with nine linear accelerator machines.

## Material and methods

### *Materials*

#### *Alanine dosimeter*

The Harwell alanine dosimeter batch number BY616 was used in this study. Each dosimeter was a pellet with 4.8±0.1 mm diameter, 2.8±0.1 mm height, and 60±2 mg mass. It consists of 90.9% L-alpha-alanine and 9.1% paraffin wax as a binder. Five alanine dosimeters were packed in a plastic bag during irradiation, transportation, and kept in a dry cabinet before the readout.

#### *Electron paramagnetic resonance (EPR) spectroscopy*

EPR spectroscopy is an X-Band machine, Bruker EMXmicro, installed with a standard ER 4119HS resonator (Bruker BioSpin Corporation).

#### *Linear accelerator machine*

Delivery dose was performed using nine linear accelerator machines from eight radiotherapy centers, consisting of eight linear accelerators from Varian Medical Systems, Inc. and one from Elekta.

#### *Solid water phantom*

Solid water phantom (GAMMEX RMI, Wisconsin, USA) with a density and average atomic number of 1.03 g/$cm^3$ and 5.96, respectively, was used in this study. The dimension was 30×30 $cm^2$, with a range of thicknesses from 0.2 to 5.0 cm.

### *Method*

#### *Irradiation setup*

In the study of parameters optimization, the irradiation of alanine dosimeters was performed with a 6MV-FFF (Flattening filter free) beam generated by a linear accelerator, Varian TrueBeam (Varian Medical Systems, Inc, CA, USA), 100 cm source to surface distance (SSD), with a field size of 10x10 $cm^2$ at 1 to 20 Gy. The 6 MV was chosen because it provides a good beam profile with less transmission penumbra than 10 MV, and FFF is a common technique to reduce irradiation time during patient treatment. Beam size 10x10 $cm^2$ can ensure that all dosimeters are irradiated. Five alanine dosimeters in a plastic bag represent a set of each dose. A package was placed between 1 cm bolus thickness to reduce the air gap effect in the solid water phantom as illustrated in Figure 1.

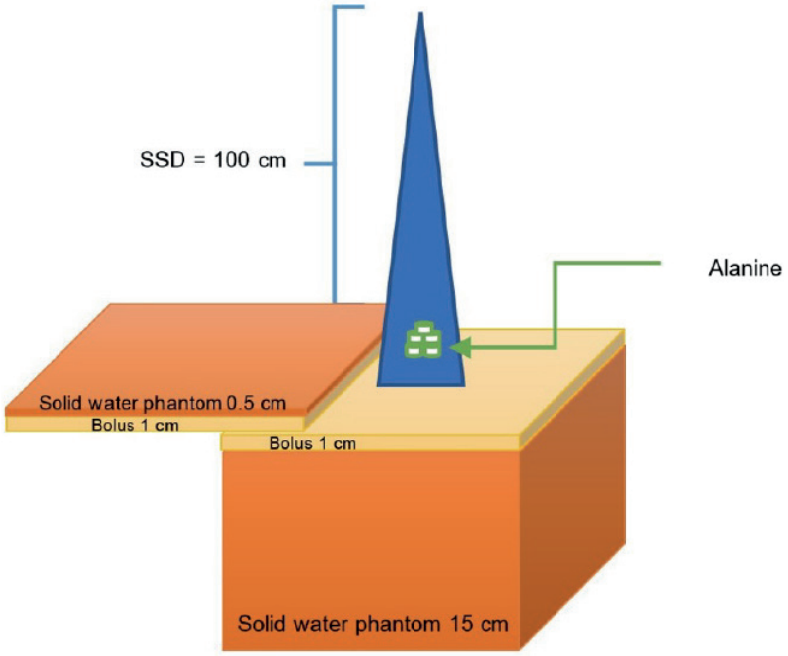


***Figure 1.*** *Alanine dosimeter irradiation setup.*

For the study of alanine characteristics to test uniformity and reproducibility, 2 packs (10 dosimeters) were irradiated at the same time using the same technique as mentioned above. Whereas the alanine for fading study, 60 dosimeters were irradiated at a time. For delivery dose measurement, 3 packs of 5 dosimeters were sent to each radiotherapy center, where set A and B were irradiated at 10 Gy (same technique as mentioned above), and set C was a control set.

***Optimization of EPR operation parameter***

The EPR operation parameter was optimized for alanine readout at dose 1, 3, 5, 10, and 20 Gy. To achieve a clear signal, microwave power (MP) was firstly investigated, followed by modulation amplitude (MA) and time constant (TC), respectively. The difference between maximum and minimum EPR spectrum for dose estimation was performed using the height method (peak-to-peak amplitude).[8]

***Microwave power (MP)***

To find MP, MA was fixed at 7.018 G following the previous study and TC was fixed at the minimum of 0.01 msec.[9] Subsequently, MP was varied from minimum to maximum (5 to 35 dB, by increasing 1 dB in each step). The spectrum from EPR signal was observed until a clear spectrum was observed, as shown in Figure 2 (red line). At this step, the optimal MP was recorded for each dose. Eventually, the MP square of all doses against the EPR intensity graph was plotted.

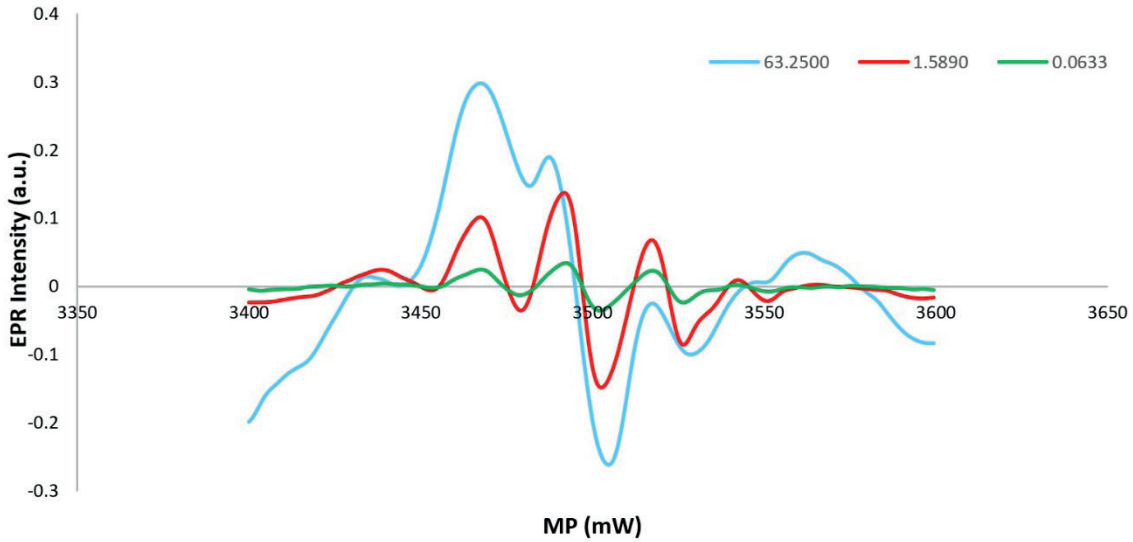


***Figure 2.*** *Spectrum from EPR signal at dose delivery of 1 Gy was set at 5 dB (0.0633 mW), 21dB (1.589 mW), and 35 dB (63.250 mW). MA and TC were fixed at 7.018 G and 0.01 ms, respectively. Clear spectrum of alanine was present in the red line.*

***Modulation amplitude (MA)***

The optimal MP found in 2.2.1.1 for each dose was used as a fixed parameter, TC was fixed at the minimum of 0.01 msec. MA was set at 1.000, 2.000, 2.999, 4.000, 5.000, 6.006, 6.579, 7.018, 7.576, 8.032, 8.584, 9.050,9.569, and 10.050 G (this is a fixed MA step of EPR machine which user cannot adjust). The spectrum from EPR signal was observed until a clear spectrum was observed as shown in Figure 3 (red line). At this step, the optimal MA was recorded for each dose. Eventually, the MA in units of G for all doses were plotted against the EPR intensity.

***Time constant (TC)***

The optimal MP and MA from 2.1.1.1 and 2.1.1.2, respectively, were used as fixed parameters to set the time constant at 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56, 5.12, 10.24, 20.48, 40.96, 81.92, 163.84, 327.68, 655.36, 1310.72, 2521.44, and 5242.88 msec (this is a fixed TC step of EPR machine which user cannot adjust). The EPR signal was observed and peak-to-peak width ($\Delta B_{PP}$) was measured as shown in Figure 4. Each $\Delta B_{PP}$ was normalized to $\Delta B_{PP}$ of TC 0.01 msec.

***Alanine characteristics***

After optimization of the EPR operation parameters possessed from the first section, this section was performed to explore the characteristics of alanine. Measurements were conducted in seven characteristics, namely uniformity, reproducibility, linearity, repetition rate, energy dependence, directional dependence, and fading. The data was employed to estimate the uncertainty of alanine.

***Uniformity***

EPR intensity was measured at each alanine pellet for readout. The average EPR intensity of 10 alanine pellets were used to determine the batch uniformity of all alanine dosimeters.

***Reproducibility***

Ten alanine pellets were read one at a time and repeated for five times each. The reproducibility was determined by the average of readout repeatability.

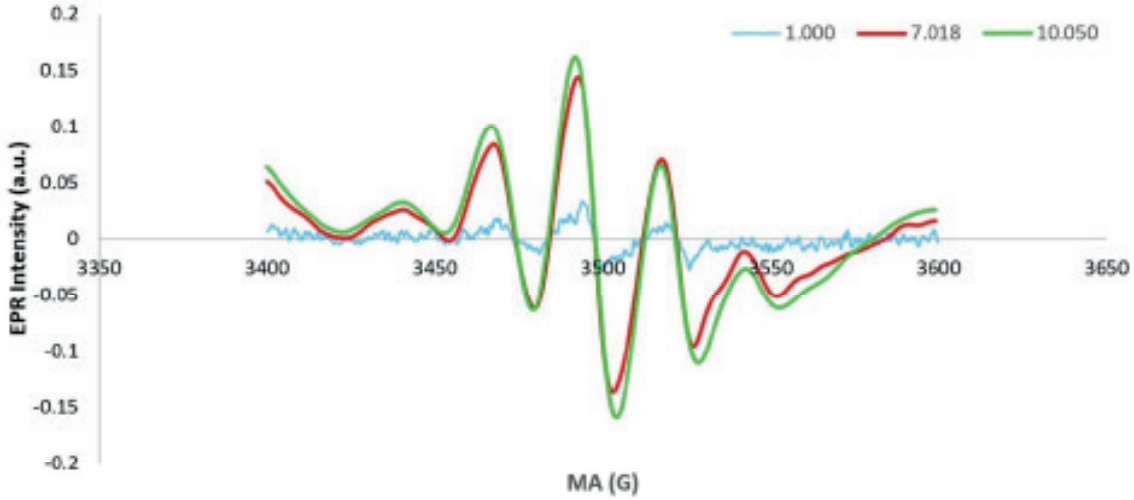


***Figure 3.*** *Spectrum from EPR signal at dose delivery of 1 Gy was set at 1.000, 7.018, and 10.050 G. Optimal MP was 1.589 mW and TC was fixed at 0.01 ms. Clear spectrum of alanine was present in the red line.*

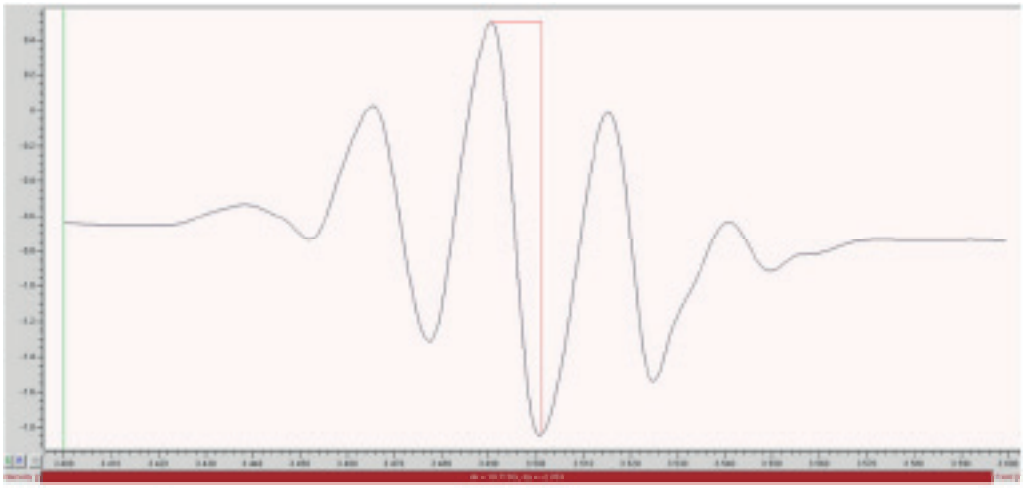

***Figure 4.*** *Measured EPR signal shown in red line for peak-to-peak width ($\Delta B_{PP}$) at TC=40.96 ms.*

***Linearity***

Ten alanine pellets were irradiated at 1, 2, 5, 10, 15, 20, and 30 Gy from 6MV-FFF, 100 cm SSD, with a 10x10 $cm^2$ field size at 1.5 cm depth. The calibration curve was established according to the International Code of Practice IAEA TRS-398. The readout was averaged from ten alanine for each dose.

***Repetition rate***

To study the response of alanine against high MU rate, five alanine pellets were irradiated with 10 Gy from 6MV-FFF, 100 cm SSD, with a 10x10 $cm^2$ field size at 1.5 cm. Repetition rates were performed at 400, 800, 1200, and 1400 MU/min. The readout of alanine pellets was averaged from EPR intensity of five alanine pellets at each repetition rate. Finally, the repetition rate in each measurement was normalized to 1400 MU/min.

***Energy dependence***

Five alanine pellets were irradiated with 10 Gy, 100 cm SSD, with a 10x10 $cm^2$ field size at 6 MV, 6 MV-FFF, 10 MV, and 10 MV-FFF. Readout of alanine pellets were averaged from EPR intensity of five alanine pellets in each measurement and was normalized to 6MV-FFF.

***Directional dependence***

Five alanine pellets were irradiated with 10 Gy, SSD 100 cm, with a 10 x 10 $cm^2$ field size at 1.5 cm depth from 6 MV-FFF for directional dependence. This was performed in two directions, perpendicular and parallel (Figure 5), where parallel measurements were normalized to perpendicular direction.

***Fading***

To examine the fading of alanine, 60 alanine pellets were exposed to 10 Gy from 6MV-FFF SSD 100 cm, with a field size of 10x10 $cm^2$. Five alanine pellets were separated for measurement readout for 12 consecutive weeks. The other alanine pellets were stored in the dry cabinet at room temperature. The fading determination was defined as the response of EPR intensity to the initial EPR intensity readout. Consecutively, the uncertainty of alanine was calculated following the "Guide to the expression of uncertainty in measurement".[10]

***Output measurement survey***

Three sets of alanine were sent to each radiotherapy center and were irradiated at 10 Gy. The delivery dose was at 10 Gy of 6 MV-FFF and the field size was 10x10 $cm^2$ at a depth of $D_{max}$. The alanine dosimeter was placed between 1 cm of bolus to reduce the air gap effect in the solid water phantom.

## Results and discussion

***Optimization of EPR operation parameter***

***Microwave power (MP) and Modulation amplitude (MA)***

Optimal MP was obtained by plotting a graph between $MP^{1/2}$ setting and EPR intensity, as shown in Figure 6. The Intensity grows linearly as $MP^{1/2}$ increases in the beginning and has the maximum value of this linear $MP^{1/2}$ of approximately 1.26. Then, the graph shows a peak shape. The maximum value of the linear $MP^{1/2}$ of 1.26 represents the optimum MP at 1.589 mW for alanine readout of 1-20 Gy subjected to 6 MV-FFF linear accelerator. Meanwhile, the optimal MA can be determined with the same condition. Figure 7 displays the relationship of MA and EPR intensity, where EPR increases as MA is being raised. The linear trend ended at 7.018, and the optimal MA is determined at 7.018 G. Therefore, for the alanine dosimeter readout at dose of 1-20 Gy subjected to 6 MV-FFF linear accelerator, the optimal MP and MA in EPR is 1.589 mW and 7.018 G, respectively. Garcia *et al.*[2] and Gago-Arias *et al.*[9] reported the optimal MP and MA parameters in the EPR setting as 1.589 mW (attenuation to 21 dB) and 7.018 G, respectively. While MA of this study coincides with the previous studies, the MP value is slightly less. This may be caused by human error when the user considers the clearest spectrum shape as previously described.

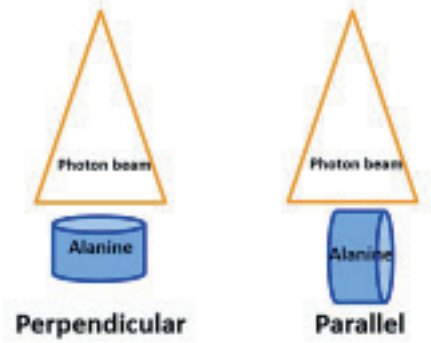


***Figure 5.*** *Measurement of alanine in two directions.*

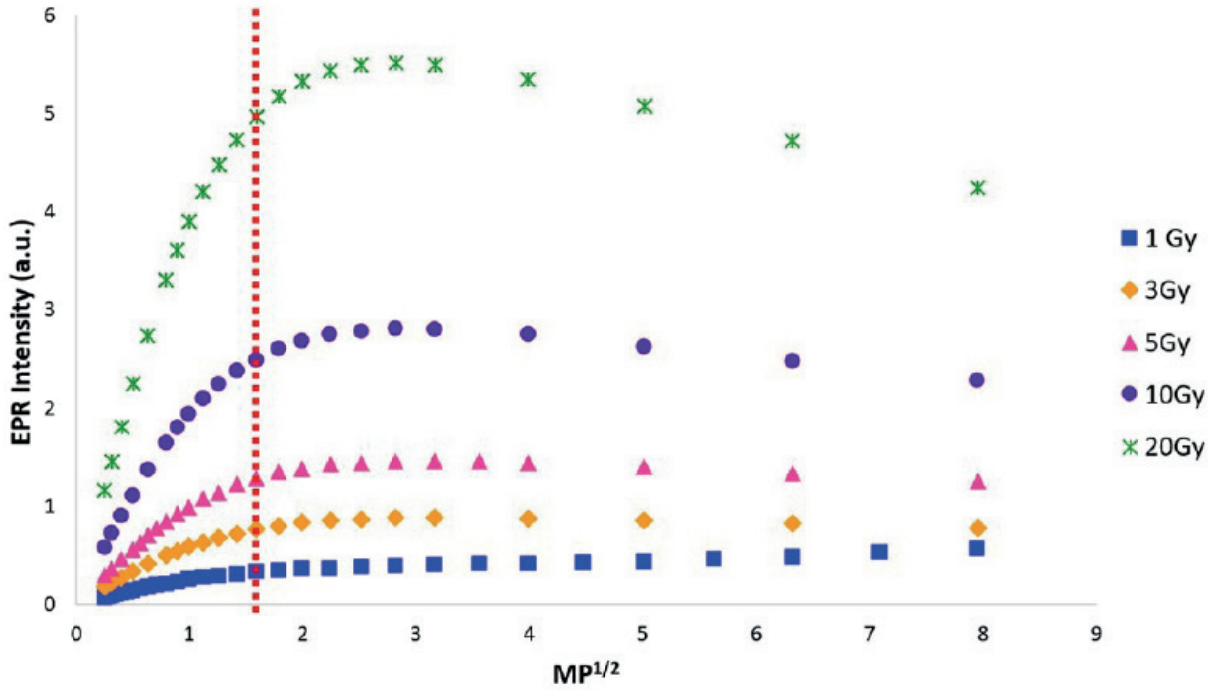


***Figure 6.*** *Relationship between $MP^{1/2}$ setting and EPR intensity. Red line represents the maximum value of the linear $MP^{1/2}$ for an alanine readout of 1-20 Gy subjected to a 6 MV-FFF linear accelerator.*

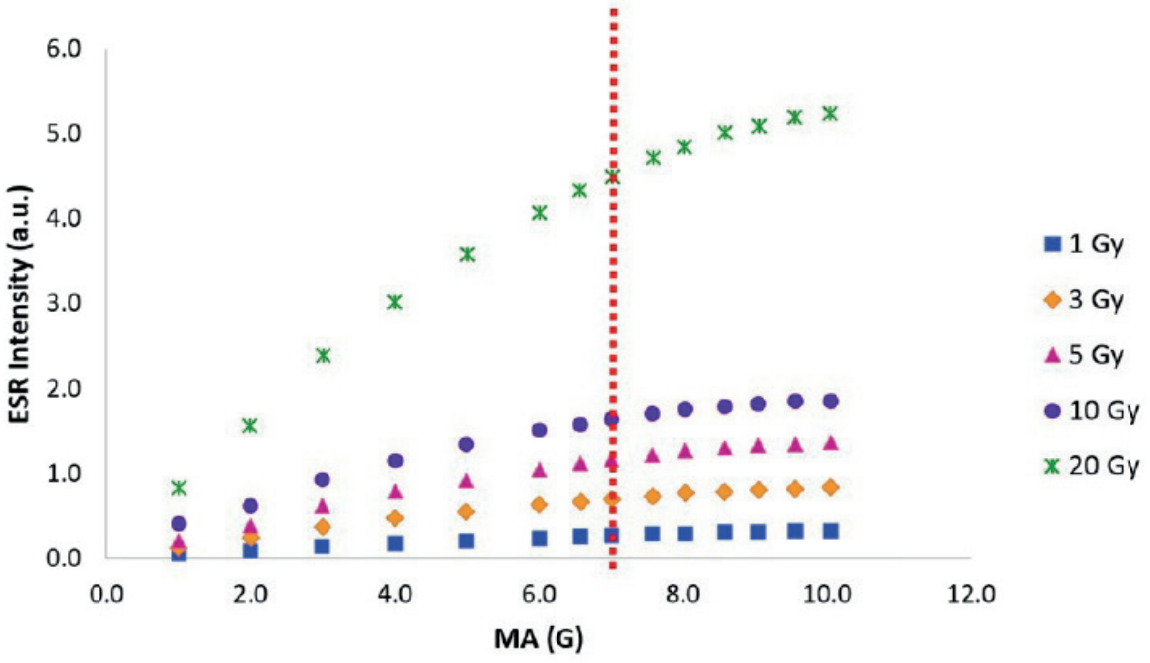


***Figure 7.*** *Relationship between MA setting and EPR intensity. Red line represents the maximum value of the linear MA for an alanine readout of 1-20 Gy subjected to a 6 MV-FFF linear accelerator.*

***Time constant or TC***

Table 1 shows peak-to-peak width or ΔBpp and relative intensity (after normalized to TC at 0.01 msec), where ΔBpp represents a high intensity of signal and relative intensity shows the resolution. Both values are not significantly different with varying TC from 0.01 to 5342.88 msec. To achieve both high intensity and better resolution, the optimal TC was determined at 40.96 msec.

***Uniformity and reproducibility of alanine dosimeter***

Uniformity of the alanine dosimeter was explored in 10 alanine dosimeters as shown in Figure 8. The uniformity of alanine was considered by the standard deviation over 10 dosimeters and reports as ±0.22%. This confirms that the alanine dosimeter has a good uniformity within the same batch.

**Table 1** Time constant on resolution and intensity of the spectra for 1-20 Gy.

| Time constant (ms) | $\Delta B_{PP}$ (G)* | Relative intensity |
|---|---|---|
| 0.01 | 10.014 | 1.000 |
| 0.02 | 10.049 | 0.998 |
| 0.04 | 10.049 | 1.002 |
| 0.08 | 10.049 | 0.997 |
| 0.16 | 10.049 | 1.000 |
| 0.32 | 10.049 | 1.003 |
| 0.64 | 10.049 | 0.999 |
| 1.28 | 10.154 | 1.001 |
| 2.56 | 10.183 | 1.001 |
| 5.12 | 10.049 | 0.996 |
| 10.24 | 10.049 | 0.998 |
| 20.48 | 10.049 | 0.999 |
| 40.96 | 10.719 | 0.996 |
| 81.92 | 10.173 | 0.998 |
| 163.84 | 10.052 | 0.988 |
| 327.68 | 10.479 | 0.974 |
| 655.36 | 11.388 | 0.908 |
| 1310.72 | 12.460 | 0.740 |
| 2521.44 | 13.815 | 0.558 |
| 5242.88 | 16.144 | 0.574 |

*Note: $\Delta B_{PP}$ (G)*: the peak-to-peak width*

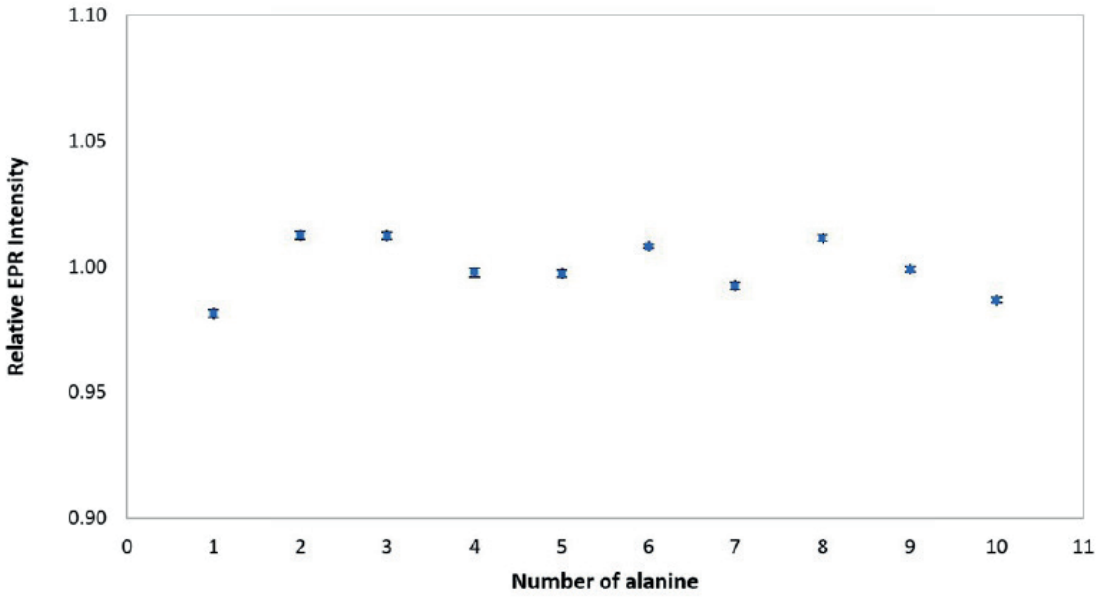


***Figure 8.*** *Uniformity of 10 alanine for 6MV-FFF. The readout of alanine dosimeters was normalized to the average of 10 alanine dosimeters. Error bars show the standard deviation for 5 times.*

***Linearity***

Figure 9 shows the dose response of alanine dosimeter with overdose of 1-30 Gy. The graph shows a linear response with $R^2$=0.9998. The result agrees with a study by Mansour (2018)[11] and Garcia *et al.*[2] This contributes to the feasibility of using alanine dosimeters in radiotherapy dose range (1-30 Gy).

***Repetition rate***

The response of alanine dosimeter subjected to a varying repetition rate delivered by linear accelerator was investigated. The result is shown in Table 2 using the relative response by normalizing all repetition rates to 1400 MU/min. The deviation of repetition rate over 400 to 1400 MU/min is 0.80%, this means that the high repetition rate did not affect the readout of the alanine dosimeter. Monitor unit (MU) is a measure of machine output from a clinical linear accelerator.

***Energy dependence***

Energy dependence of alanine dosimeter was investigated in 6 and 10 MV with and without flattening filter. Table 3 shows the relative result of beam energy with different filters, $TPR_{20,10}$ is the IAEA TRS-398 photon beam quality index for megavoltage clinical photons produced by linear accelerator and relative response to 6 MV-FFF. The maximum difference of relative response to 6MV-FFF was 0.80%, indicating that the dosimeter is energy independent.

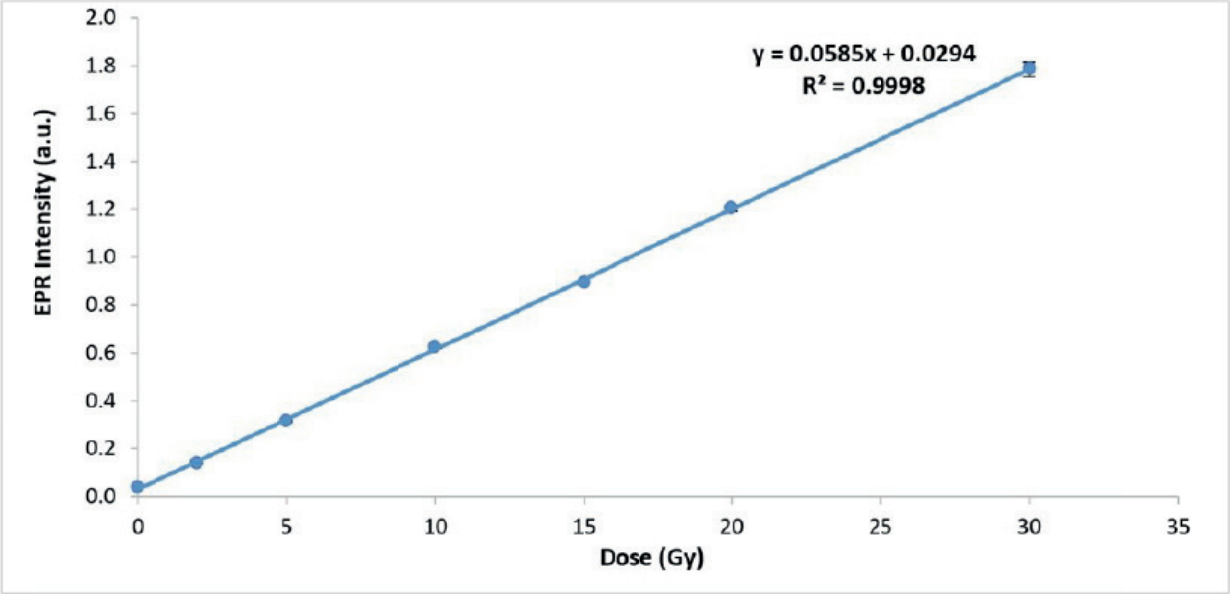


***Figure 9.*** *Linearity response of alanine dosimeter.*

**Table 2** Repetition rate of the alanine dosimeter was normalized to 1400 MU/min.

| Repetition rate (MU/min) | Relative response to 1400 MU/min |
|---|---|
| 400 | 1.008 |
| 800 | 0.999 |
| 1200 | 0.998 |

**Table 3** Energy dependence of the alanine dosimeter from varied photon beams was normalized to 6 MV-FFF.

| Photon beam | $TPR_{20,10}$ | Relative response to 6 MV-FFF |
|---|---|---|
| 6 MV-FFF | 0.63 | 1.000 |
| 6 MV | 0.67 | 1.005 |
| 10 MV-FFF | 0.71 | 0.997 |
| 10 MV | 0.74 | 0.992 |

***Directional dependence***

The directional dependence of alanine dosimeter was tested in two directions, perpendicular and parallel to the beam axis. The result after normalization to the perpendicular side shows that the difference is only 0.03% (Table 4). This confirms that the irradiation angular did not affect the alanine dosimeter.

***Fading***

The fading effect was examined for 12 consecutive weeks. As shown in Figure 10, the relative response to the first readout varies from 0.983 to 1.003. This fading over three months was 0.98% which agrees with Zeng GG *et al*.[1] This benefits in readout of alanine with low fading after long term storage. This study did not apply fading correction factors, the readout process was within two weeks after irradiation.

***Uncertainty of alanine dosimeter***

The uncertainty of alanine dosimeter is presented in Table 5. The list of uncertainty sources comes from the alanine characteristics in this study, mechanical of Varian TrueBeam, and the mass of alanine (from manufacturer). Overall, the expanded uncertainty was 2.68% at 95% confidence interval. The uncertainty from previous studies by Mansour[11] reported at 1.52% for 10 Gy, and Garcia *et al.*[2] reported at 4.9% for 2 Gy and 1.4% for 10 Gy.

**Table 4** Directional dependence of the alanine dosimeter in two directions.

| Directional | Normalization |
|---|---|
| Perpendicular | 1.000 |
| Parallel | 0.997 |

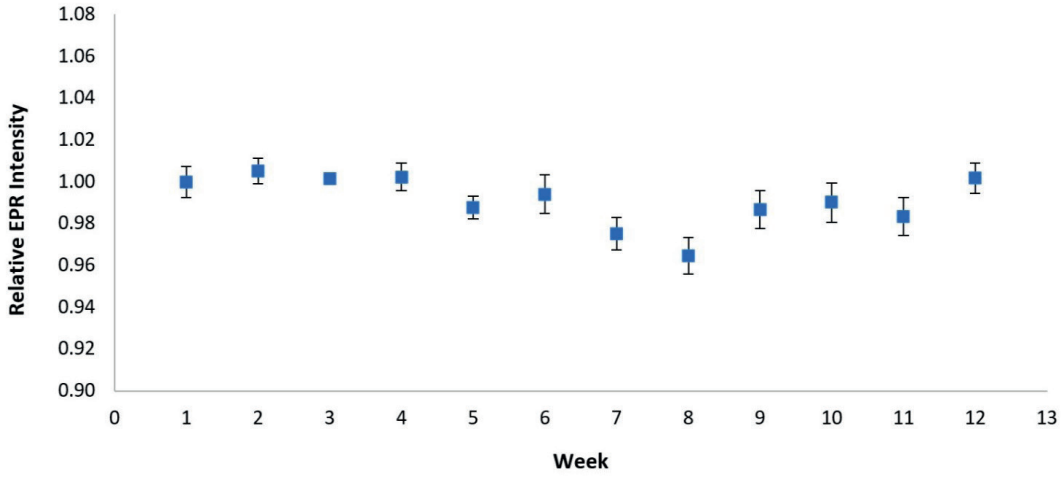


***Figure 10.*** *Fading of alanine dosimeter over 12 weeks. Error bars represent standard deviation of relative response from 5 alanine dosimeters.*

**Table 5** Uncertainty of alanine following "Guide to the expression of uncertainty in measurement"[11]

| Source of uncertainty | | Type | U (%) |
|---|---|---|---|
| Uniformity | | A | 0.3671 |
| Reproducibility | | A | 0.0315 |
| Linearity | | A | 0.4469 |
| Energy response | | A | 0.1933 |
| Repetition rate | | A | 0.1890 |
| Fading | | A | 0.0958 |
| Directional | | A | 0.3619 |
| Alanine mass | | B | 0.0144 |
| Mechanical of machine | | B | 0.5000 |
| Calibration factor ($N_{D,w}$) | | B | 1.0000 |
| Correction for irradiation | Temperature | B | 0.0004 |
| | | Combined uncertainty (Uc) | 1.3414 |
| | | Coverage factor (k) | 2.0 |
| | | **Expanded uncertainty** | **2.68** |

***Output measurement survey***

All radiotherapy centers participating in this study regularly calibrate their routine dosimeters and validate the dose output. All results can be traced to the SI unit with reliable uncertainty. This ensures that the delivered dose from the linear accelerator machines is accurate. Figure 11 shows the output measurement of 6MV-FFF using alanine dosimeters from nine different linear accelerator machines at a dose of 10 Gy. The difference of output is within ±1.0%. This contributes to feasibility of using the alanine dosimeter in quality audit programs and intercomparison between laboratories.

## Conclusion

This study determined the optimal EPR operation key parameters for alanine dosimeter readout subjected to 6 MV in dose of 1-20 Gy usually applied in radiotherapy. Both key parameters (MA and TC) agree with the previous reports, only MP is slightly different which may be due to human error. These parameters could be applied in future studies for the use of alanine dosimeters in radiotherapy. Additionally, characteristics of the alanine dosimeter have been investigated. The result showed that this dosimeter can be an alternative transfer dosimeter in quality control, postal audit, and intercomparison. The dosimeter has shown a good radiation response in dose of 1-20 Gy, which is largely lower than its usual application (kGy), good uniformity, linearity response, angle and energy independence, low fading, and the uncertainty of measurement is reported at 2.68%. The dose output comparison between nine linear accelerator machines (from 8 radiotherapy centers) revealed that the different result measured by alanine dosimeter is within ±1.0%. These results contribute to the feasibility of using alanine dosimeters in radiotherapy, especially in SRS (15-30 Gy). However, further study in SRS with an appropriate phantom should be accompanied with alanine to verify the potential of this dosimeter prior to clinical applications.

## Conflicting interests

The authors declare no conflict of interest.

## Acknowledgments

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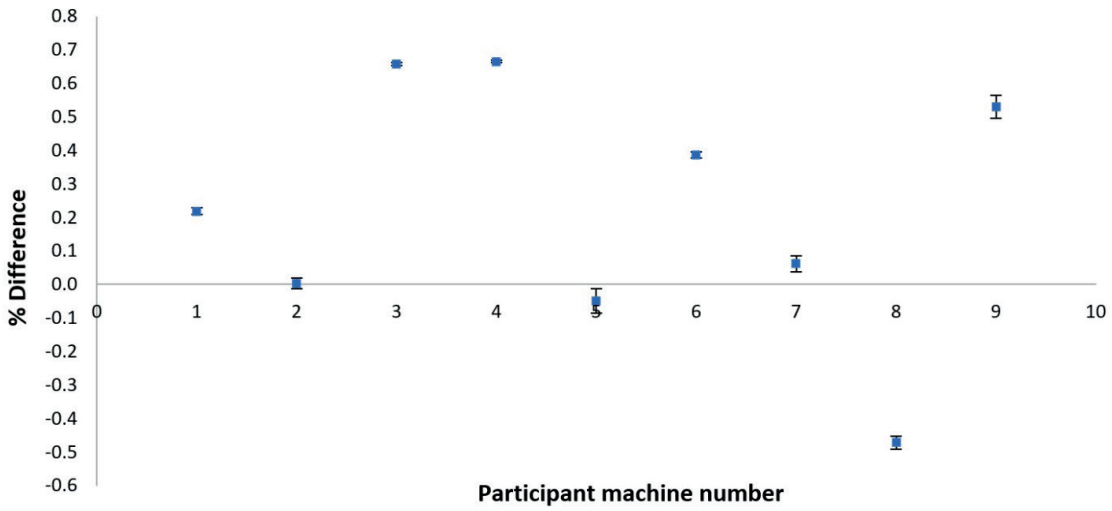


***Figure 11.*** *Percent difference of output survey in each participant.*

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# Classification of recent and long-term infection among high-risk individuals using HIV-1 rapid recency testing

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**ABSTRACT**

**Background**: HIV prevention with a significant reduction of new infections is important for epidemic control strategies. Antibody-based tests for recent infection (TRI) that can help distinguish recent from long-term HIV infection have been used since the mid-1990s to estimate population-level HIV incidence. Because the tests are done centrally, test results are not available to providers immediately. Point of care rapid testing for recent HIV infection (RTRI) was used for distinguishing recent from long-term status and estimated HIV incidence for preventing HIV infection. The usefulness of RTRI for recent HIV infection detection can be accelerated prevention of HIV infection transmission.

**Objectives:** To demonstrate RTRI assay utility to distinguish recent from long-term HIV infection among high-risk individuals.

**Materials and methods:** Between September and October 2022, HIV seropositive plasma samples of a high-risk group (N=90) and HIV-negative group (N=90) were collected from the outpatient department of Khon Kaen Hospital. All Specimens were tested by Asante™ Rapid Recency Assay (ARRA) and classified as negative, recent and long-term infection based on the presence or absence of specific lines (control line [CL], Positive verification line [PVL] and Long-term line ([TL]) by visual reading.

**Results:** Among 90 HIV seropositive plasma samples, 14.4% (13/90) were recent HIV infection and 84.4% (76/90) were long-term HIV infection. There were no significant differences between recent and long-term infections among men who have sex with men (MSM), transgender women (TG), heterosexuals and sex workers. Most of recently infected HIV patients were the MSM/TG group (53.9%, 7/13) and patients aged between 18 and 25 years old (76.9%, 10/13). CD4 count among recent cases was 396.7±278.4 cells/mm$^3$. ARRA performance has resulted in a sensitivity of 98.89% (95% CI: 93.96-99.97) and specificity of 100.00% (95% CI: 95.98-100.00).

**Conclusion:** ARRA performance showed excellent agreement with high sensitivity and specificity to comparing with a standard algorithm for HIV diagnosis. Its ability to classify new infections is crucial in HIV intervention and prevention strategies.

## Introduction

Detection and identification of HIV transmission for early treatment with prioritized prevention strategies for high-incidence populations are important for driving epidemic control.[1,2] The prevalence of HIV has been used to assess epidemic patterns and trends. The measurement of incidence can elucidate the transmission dynamic of

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new infections. Laboratory assays for the detection of recent HIV infection for HIV incidence surveillance are essential to HIV prevention worldwide because they can identify populations with a high incidence and allow the targeting of resources and monitoring of incidence trends over time. Detecting recent HIV infections can help prioritize prevention and early treatment for those at high risk of transmission. Tests that can detect recently infected individuals were the achieved goal of epidemic strategies.[3,4] Laboratory assays for the estimation of recent HIV infection were described as early as 1998, and initially were modifications of commercially available HIV antibody tests.[5] These assays indirectly measured antibody titers that are low in early infected individuals and high in chronically infected persons. However, the use of such modified commercial assays for incidence testing may not provide consistent identification of HIV incident infections in populations with diverse HIV-1 circulating subtypes since almost all commercial products use HIV-1 subtype B-derived antigens.[6,7]

For the routine diagnosis of HIV infection, the most used tests are rapid spot tests or ELISA that detect HIV antibodies; these cannot necessarily discriminate between early and chronic infections. These platforms require sophisticated laboratory infrastructure and a trained workforce.

Antibody-based tests for recent infection (TRI) that can help distinguish recent from long-term HIV infection have been used since the mid-1990s to estimate population-level HIV incidence and to evaluate the impact of large-scale HIV interventions in preventing HIV infection.[8,9] Testing for recent infection among newly diagnosed HIV-positive persons has been integrated into routine HIV surveillance worldwide.[10] TRI assays are done centrally; test results are not available to providers immediately and cannot be used to identify persons most likely to transmit the virus. Alternative methods that can expedite the results of recent infection testing through point-of-care testing (POCT) are needed.

The rapid test for recent infection (RTRI) can improve our understanding of the HIV epidemic by demonstrating shifting dynamics in the HIV epidemic over time, such as changes in the proportion of recent infections allowing public health officials and providers to trace active HIV transmission networks and deploying targeted prevention strategies.[11] While results from laboratory-based recent infection tests may take weeks to months to provide results due to sample transportation, laboratory processing, and turnaround time from the testing laboratory to the referring clinic, the results of the rapid test for recent infection can be available within minutes, facilitating more efficient and targeted responses for maximum public health impact

Asante™ Rapid Recency Assay (ARRA) is a single test device that can simultaneously diagnose HIV infection and differentiate between recent and long-term HIV-1 infection that has been developed and described previously by the CDC[12]. The performance of ARRA has been evaluated using a well-characterized panel of cross-sectional specimens with known HIV serology status and recent or long-term status based on comparative Limiting Antigen (LAg) Avidity Enzyme immunoassay (EIA) results, which has been validated to detect recent infections.[13]

The development of a rapid test for recent infection allows one to perform HIV recency testing in a routine HIV management in real time and utilize data for targeted prevention. ARRA was the rapid antibody-based test that followed the lateral flow immunoassay principle for recent infection identification. ARRA is a strip test that combines HIV diagnosis with a time indicator since infection as previously described.[12] This is accomplished by including an additional line where the antigen is striped at a limiting antigen concentration to distinguish recent from long-term infection.

Current study conducted the performance characteristics of ARRA among men who have sex with men (MSM), transgender women (TG), heterosexual people, and sex workers, demonstrating a positive verification line (PVL) of the Asante strips compared with the standard HIV diagnostic algorithm and separating recent from long-term HIV infection.

## Materials and methods

### *Blood specimens*

Leftover specimens were collected between September and October 2022 from MSM/TG and sex workers. All specimens were recruited from the outpatient department of Khon Kaen Hospital. All samples were examined in accordance with the Thailand National Guidelines on HIV/AIDS Treatment and Prevention 2021/2022. The specimens were diagnosed with HIV antibodies by the 4$^{th}$ generation Electrochemiluminescence Immunoassay (ECLIA) (Cobas e801, Roche Diagnostics), Alere™ HIV combo (Alere Medical Co., Ltd, Japan) and SD BIOLINE HIV-1/2 3.0 (Standard Diagnostics, INC, Korea). The panel consisted of HIV seropositive plasma samples of high-risk individuals (N=90) and HIV-negative patients (N=90). Following that, ARRA was used to examine all specimens.

### *ARRA procedure*

The ARRA was conducted following the manufacturer's instructions (Sedia Biosciences Corporation, Portland, USA).[14] Serum or plasma samples were warmed to room temperature before an assay. Using the collecting loop included in the test set, 5 µL of sample was transferred into and mixed in a 0.5 mL buffer tube. A test strip was inserted into the tube after the specimen collection loop was removed and incubated for 20 minutes. After 20 minutes, the strips were taken out and wiped onto filter paper to remove any excess buffer before being visually categorized as recent, long-term, or negative. To prevent false positive, the visual reading should be performed immediately. The results were recorded in a worksheet file.

### *Data collection*

Clinical data, including age, sex, sexual behavior, and

route of transmission, were collected from OPD cards and laboratory data; CD4 count, hepatitis C antibody (HCV Ab), hepatitis B antigen (HBsAg), and rapid plasma reagin (RPR) were recorded from the laboratory information system. ARRA results were recorded from the presence of all control lines (CL), positive verification lines (PVL) and long-term lines (LTL) by visual reading. The presence of all three lines (CL, PVL and LTL) indicated an HIV-positive diagnosis with long-term infection. The presence of CL and PVL indicated an HIV-positive diagnosis with recent infection while the presence of only the control line (CL) indicated an HIV-seronegative sample (Figure 1).

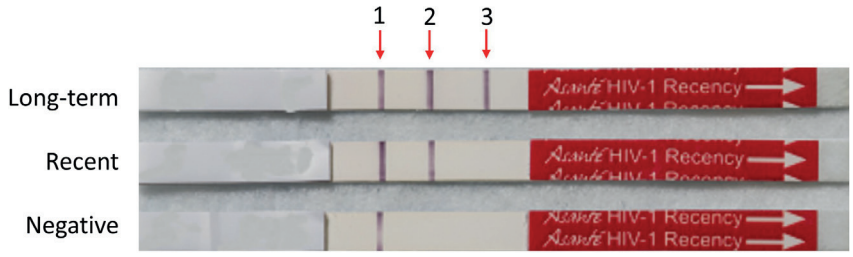


***Figure 1*** *Interpretation of the Asante Rapid Recency Assay results is based on the presence or absence of specific lines. Arrows 1, 2 and 3 represent control line (CL), positive verification line (PVL) and long-term line (LTL), respectively, by visual reading.*

***Statistical analysis***

Performance evaluation of ARRA was calculated by diagnostic statistics for sensitivity and specificity with the standard seroconversion algorithm.[15] Percentage means and standard deviation were performed using STATA version 14.0. ANOVA and chi-square were used to compare statistical significance between demographic data and recent infection or long-term infection.

***Ethical consideration***

The protocol was approved by the Ethical Committee of Khon Kaen Hospital, Ministry of Public Health (KEX65044).

## Results

Among 90 HIV seropositive samples, 14.4% (13/90) was recent HIV infection and 84.4% (76/90) was long-term HIV infections. Demographic data of this population demonstrated that most of recent HIV infection were MSM/TG (53.9%, 7/13) and patients aged between 18 and 25 years old (76.9%, 10/13). There were no significant differences in age, sex, sexual orientation, and route of transmission between recent and long-term infections among men who have sex with men (MSM), transgender women (TG), heterosexuals and sex workers ($p$>0.05). Unprotected anal activity was a higher-risk route of infection transmission compare with vaginal sex. The absolute CD4 count of recent infection cases was 396.7±278.4 cells/cu.mm. The prevalence of low CD4 count (lower than 200 cells/mm$^3$) was 28.6% (2/7) in recent infection and 16.7% (8/48) in long-term infection cases. HIV viral load among long-term infection cases demonstrated viral suppression at 73.9% (17/23), while recent infection showed only 33.3% (2/6). Other sexual transmission infection (STI) markers, including RPR, HBsAg and HCV Ab, were not different between recent and long-term HIV infection (Table 1).

The performance of ARRA was calculated by comparing the visual results with the standard HIV algorithm. The ARRA assay showed 89 HIV-positive results and 1 negative result with a sensitivity of 98.89% (95% CI=93.96-99.97), specificity of 100.00% (95% CI=95.98-100.00), agreement 99.44% (95% CI=96.94-99.99) and Kappa 0.989 (95% CI= 0.967-1.000) as shown in Table 2.

## Discussion

Diagnosis of HIV infection has spread globally due to rapid diagnostic testing, ease of performance and low cost. Recent infection identification is important for epidemic control since it has been suggested that a high proportion of new infections originates from recently infected individuals.[16] Identifying recent infections was difficult for point-of-care testing due to technical complications, specimen quality, process, and storage before testing was performed.[17,18]

The study was done to find the incidence of newly diagnosed HIV case among high-risk individuals using ARRA and to explore the demographic data in recently and long-term infected patients. Our study assessed the utility of the Asante Rapid Recency Assay to classify recent and long-term infections among high-risk seropositive cases as recommended by WHO-PQ/FDA[19] and associated results to HIV seropositive from a standard algorithm with high sensitivity and specificity (98.89% and 100.00%, respectively) (Table 2). Our study results in the classification of recent and long-term infections related to the previous study.[12,20] ARRA qualification feature has impacted recent infection surveillance surveys of recruiting new infection cases for prevention.[21-23] The advantage of this assay serves to confirm HIV seropositive status and classify recent and long-term individuals. Early identification of recent infection is important for HIV treatment and breaking off HIV transmission in the early stage, which can accelerate epidemic control. Although our data showed high sensitivity and specificity of the ARRA, this test is

**Table 1** Patient characteristics and HIV status among men who have sex with men (MSM), transgender women (TG), heterosexuals and sex workers.

| | Recent infection | | Long-term infection | | Total |
|---|---|---|---|---|---|
| | N | % | N | % | |
| **Age, years** | | | | | |
| 18-25 | 10 | 76.9 | 41 | 53.9 | 51 |
| 26-35 | 2 | 15.4 | 22 | 28.9 | 24 |
| >36 | 1 | 7.7 | 13 | 17.2 | 14 |
| **Sex** | | | | | |
| Male | 12 | 92.3 | 66 | 86.8 | 78 |
| Female | 1 | 7.7 | 10 | 13.2 | 11 |
| **Sexual orientation** | | | | | |
| MSM/TG | 7 | 53.9 | 38 | 50.0 | 45 |
| Heterosexual | 5 | 38.4 | 33 | 43.4 | 38 |
| Sex worker | 1 | 7.7 | 5 | 6.6 | 6 |
| **Route of transmission** | | | | | |
| Anal sex | 7 | 53.9 | 40 | 52.6 | 47 |
| Vaginal sex | 6 | 46.1 | 36 | 47.4 | 42 |
| **CD4 count (cell/mm$^3$)** | | | | | |
| <200 | 2 | 28.6 | 8 | 16.7 | 10 |
| 200-500 | 2 | 28.6 | 25 | 52.1 | 27 |
| >500 | 3 | 42.8 | 15 | 31.2 | 18 |
| **HIV viral load** copies/mL | | | | | |
| Suppressed (<1,000) | 2 | 33.3 | 17 | 73.9 | 19 |
| Unsuppressed (≥1,000) | 4 | 66.7 | 6 | 26.1 | 10 |
| **RPR** | | | | | |
| Reactive | 3 | 42.9 | 9 | 18.7 | 12 |
| Non-reactive | 4 | 57.1 | 39 | 81.3 | 43 |
| **HBsAg** | | | | | |
| Positive | 1 | 14.2 | 1 | 2.1 | 2 |
| Negative | 6 | 85.8 | 47 | 97.9 | 53 |
| **HCV Ab** | | | | | |
| Positive | 0 | 0.0 | 3 | 6.3 | 3 |
| Negative | 7 | 100.0 | 45 | 93.7 | 52 |

**Table 2** Two by two comparison of HIV seropositive results from the standard algorithm with visual interpretation for the positive verification line (PVL) of the Asante Rapid Recency Assay (N=180).

| | | HIV seropositive from standard algorithm | | |
|---|---|---|---|---|
| | | **HIV-positive** | **HIV-negative** | **Total** |
| **Asante PVL visual** | **HIV-positive** | 89 | 0 | 89 |
| | **HIV-negative** | 1 | 90 | 91 |
| | **Total** | 90 | 90 | 180 |

not currently used in surveillance surveys for recent HIV infection detection in the national testing algorithm of Thailand because of lack of approval. A further study of rapid tests for recent infection combined with a routine HIV diagnosis will be beneficial for recent infection surveillance. However, ARRA has not been evaluated using a large number of specimens. We describe here the utility of this test kit on classification of recent and long-term infection in patients' specimens that were examined by routine standard laboratory protocols. The purpose of this evaluation was to compare the performance of Asante strips with the standard HIV diagnostic algorithm.

However, there were some limitations to be concerned. Firstly, HIV recency testing was offered to individuals with a new HIV diagnosis. As only consenting individuals were tested for recency, our study sample did not include all individuals with a new HIV diagnosis. Secondly, some recent infections classified by ARRA may have to be reclassified as long-term infection. We could not exclude patients with CD4 counts <200 cells/mm$^3$ out of the recent infection group and cannot reclassify the recent infection group of patients who have viral loads ≤1000 copies/mL to minimize the impact of false recent infections. Because specimens for viral load detection (HIV VL) were collected after 3 months of antiretroviral therapy, which was complicated and expensive. Moreover, many patients diagnosed with HIV, especially in groups that received service in issuing proactive units, refused to enter the treatment process, resulting in a lack of CD4, HIV VL, and other baseline laboratory test results. Lastly, this study screened newly infected people in small number of samples due to short expiration date of the test kit (short study time) and fewer people used the service in the hospital during the COVID-19 outbreak.

## Conclusion

ARRA performance showed excellent agreement with high sensitivity and specificity to comparing with a standard algorithm for HIV diagnosis. Its ability to classify new infections is crucial in HIV intervention and prevention strategies.

## Conflicts of interest

In this study, the author declared no conflicts of interest in the research.

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# Profiling occupational therapists' preference for an international master's program in occupational therapy: A needs analysis study

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**ABSTRACT**

**Background**: To advance occupational therapy knowledge and practice in Southeast Asia, it is pivotal that quality master's degree programs in occupational therapy are in place. Such a program must respond to the needs of the region in terms of occupational therapy education, practice, and scholarship. By recognizing the diversity of the occupational therapy education and training in the region, it has become possible to consider alternatives in establishing a graduate program in occupational therapy through partnerships and consortia. In order to make an informed decision to decide whether co-creating a master's program in occupational therapy through an international consortium within the region is possible or not, a needs assessment is warranted.

**Objectives**: To describe the profile of occupational therapists living and working in the Southeast Asian region who would like to pursue a master's degree in occupational therapy via an international consortium.

**Materials and methods**: An online survey design was used to produce data that would be used to inform the co-creation of an international master's program in occupational therapy. The survey questionnaire was designed specifically for this study and included three sections: 1) information and consent form; 2) demographic information; and 3) information about the need for and preferences on a master's program in occupational therapy. Participants were recruited through professional organizations and various social media platforms of national and international occupational therapy groups.

**Results**: Eighty-five final-year occupational therapy students and 143 occupational therapists from the Philippines, Indonesia, Thailand, and other countries completed the survey. Majority (62%) of the respondents was interested in pursuing a master's degree in occupational therapy because they perceived that an advanced degree can enable specialization in practice, promote personal and professional development, and help generate new knowledge for the field. The characteristics of the envisaged international master's program must be: 1) completed ideally in 1.5 to 2 years, 2) taken by part-time students who are also working practicing, 3) delivered via a hybrid arrangement (i.e., online and face-to-face) with a fixed weekly schedule, and 4) matriculated for USD 1,000 per semester.

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**Conclusion**: While this cross-sectional survey cannot encapsulate the totality of occupational therapists' needs in terms of their professional development, the survey provided an empirical basis to inform decisions in the creation of a master's program in occupational therapy jointly offered by an international consortium of Southeast Asian universities.

## Introduction

Eleven culturally, economically and politically diverse countries, namely Philippines, Thailand, Malaysia, Singapore, Indonesia, Vietnam, Cambodia, Myanmar, Laos, Brunei, and Timor-Leste, compose the Southeast Asian region.[1] This diversity along with unique health and educational systems have shaped how entry-level occupational therapy education in Southeast Asia is being offered-either as a diploma course (3 or 4 years) or a bachelor's degree (4 to 5 years). In the aforementioned list, only the first six have an established occupational therapy educational program and, except for Vietnam, have programs recognized by the World Federation of Occupational Therapists (WFOT). The curricula of these programs share the same educational goal of producing qualified occupational therapists who will eventually serve the health workforce. Diploma programs, however, focus more on the technical skills to provide occupational therapy services, whereas the bachelor's program will offer the acquisition of technical skills with an opportunity to engage in research and months of fieldwork in different fields of occupational therapy practice. In recent years, the WFOT has initiated programs to develop transitory programs from diploma to bachelor's degree in occupational therapy.[2] Entering the twenty-first century entails the need to reimagine and transform how we teach and learn occupational therapy. Transformative occupational therapy education includes introducing and developing competencies on capacity building, community-based rehabilitation, social entrepreneurship, human rights, disaster preparedness and response, climate change, and global health specific to environmental, social, and economic sustainability.[3] In order to transform the curriculum of advanced studies in occupational therapy, it is important to first examine the contexts of potential students.

### Global context of occupational therapy education

WFOT's minimum standards for occupational therapy education advocates for contextually relevant occupational therapy educational programs that are sensitive to both local health and social needs and global evolving trends of the profession.[4] However, knowledge and practice generated by Anglophonic (e.g., USA, Canada, UK, and Australia) and European countries continue to dominate occupational therapy and occupational science discourses.[4,5] Efforts to *decolonize* occupational therapy and occupational science in the Global South through knowledge generation for and by countries/regions outside the Global North has been progressing, albeit slowly owing to the generally low research output from Southeast Asia, in particular, vis-à-vis other Asian regions like South and East Asia.[6-7] In addition, the World Health Organization's Rehabilitation Competency Framework provides a timely and context-specific framework wherein learning outcomes can be aligned with the workforce and population needs of Southeast Asia.[8] In the Association of Southeast Asian Nations (ASEAN) Qualification Reference Framework or AQRF, a diploma degree, bachelor's degree, and master's degree is equivalent to level 5, level 6, and level 7 in terms of knowledge, skills, and competencies.[9] Postgraduate degree (level 7) programs are important drivers of scholarship of a profession or academic discipline.[10] These postgraduate occupational therapy degree programs exist within the region particularly in Thailand (one university), the Philippines (three universities), and Malaysia (three universities). It is unclear if existing programs are meeting scholarly productivity expectations. There are also countries within the region that still perceive occupational therapy as a technical-vocational endeavor, hence the existence of diploma programs within the ASEAN region. These variations in educational aims and graduate outcomes potentially pose a challenge in creating a clear trajectory for occupational therapists in Southeast Asia to advance their education further. However, the AQRF remains a structuring mechanism to ensure that educational and work qualifications are at par across Southeast Asian nations.[11]

In some developed countries, a master's degree is now required to become a qualified occupational therapist. In the ASEAN region, having a master's degree in occupational therapy is an optional trajectory for continuing professional education akin to completing short-term certifications and self-directed learning, training and practice. The WFOT stated that post-graduate occupational therapy programs are supposed to equip occupational therapists globally in terms of knowledge, practice, and policy making to advance the profession forward.[12] Specifically, there is a need to create a community of ASEAN scholars focused on generating evidence to strengthen and propel occupational therapy practice in the region. Moreover, the skills of the next-generation of occupational therapy education leaders in Southeast Asia need to be harnessed.[13] However, it is also important to consider that the motivation to do advanced education in occupational therapy differs from person to person. Ideally, taking a master's degree is the actuation of the pursuit to lifelong learning, while for the more pragmatic occupational therapists, it can be a stepping stone to work as an occupational therapist overseas or expand professional roles.[14,15]

### Strategic use of partnership/consortium for graduate programs in occupational therapy

Offering a graduate program in any field is a challenging feat. Aside from creating a curriculum, there is a need to identify qualified educators, procure funding and resources, prepare a space for teaching and research, and recruit competent students among others. These preparations can take years before actually offering and running the

graduate program. To facilitate the development of some master's programs, creating a consortium is strategic. The European Master of Science in Occupational Therapy or "Euromasters in occupational therapy" is an example of such a consortium. Unlike a partnership of two universities, a consortium brings together three or more universities that share resources (faculty, funding, goals, research, and students among others). The Euromasters in occupational therapy is facilitated by five universities from five countries within Europe: Netherlands, Denmark, Sweden, Switzerland, and the United Kingdom. In this consortium, Amsterdam University of Applied Sciences is the host school holding the administrative seat of the Euromasters in occupational therapy. Every semester, students take a module in one of the countries belonging to the consortium until they complete the whole program. Each module offers a different subject-from advanced occupational therapy courses, research courses, to the master's thesis. Facilitated by educators from across the five universities, modules are offered in a blended teaching and learning arrangement with the goal of advancing the research competencies of students in the field of occupational therapy and occupational science.

Through international partnership (as in a consortium) institutions are able to share academic resources, ease logistical and bureaucratic challenges, facilitate educational advancement, and promote internationalization.[16] For instance, the Zurich University of Applied Sciences (ZHAW) in Switzerland used to only offer a diploma course in occupational therapy. By joining the Euromasters in occupational therapy consortium, while not without challenges, the ZHAW was able to upgrade their occupational therapy program to a bachelor's degree level. Similarly, the use of both the on-site and on-line arrangements for teaching and learning have become advantageous since the students are usually studying part-time while staying employed or tending a family. Underpinned by adult learning (*andragogy*) and self-determined learning (*heutagogy*), a blended teaching-learning arrangement allows learners to study what they need and want, learn at their own pace, and set their own learning goals that go beyond the mandated curriculum. While not always enticing to more conservative learners, the blended teaching-learning arrangement can allow adult learners to assume the role of a student and a worker at the same time, learn from home, and keep budget to essential expenses. More importantly, educational technology has made global resources more available than ever to students without having to fly to another country and spend a lot of money. While the "human touch" and interactivity are valuable in higher education, the increased use of videoconferencing applications and learning management systems in universities has significantly reduced the cost of learning without sacrificing the quality of education.

Although most graduate programs are offered by one academic institution for consistency and tradition, more universities globally are reconsidering the potentialities of creating partnerships and consortia not only for its practical implications but also to engender internationalization, collaborative learning, transformative education, and creative economies.[17] The reforms being employed in higher education in terms of technology, logistics, curriculum, and pedagogy have made graduate programs more accessible and available to more diverse groups of learners especially in occupational therapy. Such a feat has been initiated between African and European universities for a post-graduate program in health policy and systems research and analysis and among Latin American countries on a postgraduate program on human rights.[18,19]

With the hope of advancing occupational therapy knowledge and practice in the ASEAN region, it is pivotal that quality master's degree programs in occupational therapy are in place. Such a program must be facilitated by globally qualified occupational therapy educators and proposed to respond intentionally to the needs of the region in terms of occupational therapy education, practice, and scholarship. By recognizing the diversity of the occupational therapy education and training in the ASEAN region, including learner purpose and motivation in doing advanced learning, it has become possible to consider alternatives in establishing a graduate program in occupational therapy through partnerships and consortia. In order to make an informed decision to decide whether co-creating a master's program in occupational therapy through an international consortium within the ASEAN region is possible or not, a needs analysis was warranted. While a needs assessment for a master's program in occupational therapy has been conducted locally, an empirical study with a focus on gathering data on the need for a postgraduate program in occupational therapy within the ASEAN context has not yet been conducted in the past.[20]

While a needs assessment for a master's program in occupational therapy has been performed in the Philippines and Indonesia, an empirical study with a focus on gathering data on the need for a postgraduate program in occupational therapy within the ASEAN context has not yet been conducted.[20,21] In order to make an informed decision on whether co-creating a master's program in occupational therapy through an international consortium within the ASEAN region is possible or not, a needs analysis is warranted. A needs analysis is a basis for developing a curriculum based on potential learners' profile.[22]

To this end, the current study aimed to determine if there is a market of students interested in pursuing a master's degree in occupational therapy. Specifically, this needs analysis study collected data to: 1) identify the demographics of prospective students for the master's program, 2) describe their interests and motivations, and 3) identify the specific learner preferences that will help shape the co-creation of the master's program.

## Materials and methods

This study utilized a survey research design that yielded quantitative data sets analyzed using descriptive statistical analyses.

### Participants

Respondents were recruited through electronic posters

shared via social media sites like Facebook, Twitter, and LinkedIn. Researchers approached different local occupational therapy associations and occupational therapy groups to facilitate dissemination within and outside ASEAN regions (e.g., Philippine Academy of Occupational Therapy, Occupational Therapy Association of Thailand, Indonesian Occupational Therapy Association, Asia-Pacific Occupational Therapy Regional Group, World Federation of Occupational Therapists). Respondents included the following: 1) final year students in an entry-level occupational therapy program and 2) graduates of an entry-level occupational therapy program. As an online survey, all respondents provided their informed consent electronically via Research Electronic Data Capture (REDCap) e-consent framework prior to participation. Failure to electronically sign the consent form signifies withdrawal from the study.

Target sample size was 192. This was based on probability sampling formula for survey research.[23] We estimated the population size based on WFOT's Human Resource Project statistics, which reported 9,721 practicing occupational therapists and 745 graduating students per year from Southeast Asian countries with available data (i.e., Indonesia, Malaysia, the Philippines, Singapore and Thailand).[24] The number of graduating students served as an estimate for the number of final-year occupational therapy students in the region with established occupational therapy entry-level programs. We adopted a 7% margin of error and the most conservative estimate of 50% for the survey response distribution.[23] Two-tailed level of significance was set at ≤0.05.

**Survey tool**

An online survey questionnaire was designed specifically for this study and consisted of three parts. The survey extracted some information from a needs assessment study that sought to inform a local master's program in occupational therapy.[20] First is the information and consent form, which contains all the necessary information for the participants to make an informed consent. The second part gathered demographic data to address the first objective. The third part gathered information related to interests and motivation (second objective) and preferences for a master's program in occupational therapy (third objective) in relation to schedule, mode of delivery, and cost.

For the survey implementation in the Philippines, the questionnaire was in English (i.e., no Filipino translation was envisioned) considering that the language of instruction in all BS Occupational Therapy programs in the Philippines is English. Partners from Thailand (AK) and Indonesia (ES) translated the questionnaire into Thai and Bahasa Indonesian, respectively following the World Health Organization's translation guidelines including forward translation, expert panel back-translation, pretesting and cognitive interviewing, and drafting of the final version. The survey was hosted in REDCap.[25,26]

**Procedure**

Prior to running the survey, the draft survey questionnaire was piloted to thirty individuals (ten for each language option) who belong to the population of interest. The purpose of this pilot was to get an idea of how long it takes to complete the survey and to ask for feedback regarding the comprehensibility of the questions. After final revisions based on the pilot survey, the anonymous online survey was disseminated to the public and was made available for a period of three months. It took participants 15-20 minutes to complete the online survey. All collected data were organized and stored in a password-protected Excel file and only accessible to the researchers.

**Data analysis**

Survey results were summarized using descriptive statistics, mostly count frequencies and percentages. Distribution of participant's age was positively skewed and was, thus, described using median and interquartile range.

**Ethical approval**

Study protocol has been reviewed and approved by the institutional ethics board of the University of the Philippines Manila (UPMREB 2022-0127-EX), Chiang Mai University (Thailand; AMSEC-65EM-007), and Surakarta Health Polytechnic (Indonesia; LB.02.02/1.1/693.7/2021).

## Results

The respondents who completed the survey were 85 final-year occupational therapy students and 143 occupational therapists who were residing in the Philippines (48.2%), Indonesia (29.8%), Thailand (17.5%) and other countries (4.4%). Table I presents the demographic data of the survey respondents.

A majority of the respondents (62.3%) were interested in pursuing a master's degree in occupational therapy while some (22.8%) were unsure about their future plans at the time of the survey. Their most prevalent motivations for considering an advanced degree were to: 1) enable specialization in specific practice areas; 2) facilitate personal and professional growth; 3) expand body of knowledge in occupational therapy and occupational science; 4) upgrade clinical knowledge and skills; and 5) boost opportunity for work promotion (Table 1).

**Table 1** Respondent characteristics (N=228).

| | Final year students | Graduates |
|---|---|---|
| N | 85 | 143 |
| Median age, years | 22.0 (21.0-22.0) | 26.0 (24.0-28.0) |
| Sex, F/M | 70/15 | 107/36 |
| Country of residence, N | | |
| Philippines | 44 | 66 |
| Indonesia | 40 | 28 |
| Thailand | 1 | 39 |
| Others[†] | 0 | 10 |

[†]*Australia, Bangladesh, India, New Zealand, Qatar, Taiwan, USA, South Korea*

Among those who were either interested in or were unsure about pursuing a master's degree in occupational therapy, 72.2% were willing to enroll in a master's program in occupational therapy offered by a consortium of Southeast Asian universities. A program duration that is 1.5 to 2 years long was preferred by 89.2% of the respondents. Moreover, 72.3% wanted a part-time job over a full-time study schedule. Respondents were more divided in their class schedule preference, with 46.6% and 42.4% being partial towards a fixed weekly schedule throughout the semester and block schedule (i.e., 6-8 hours of daily classes for 1 week, 2-3 times a semester), respectively. A majority wanted a hybrid course delivery (i.e., blend of online and face-to-face instruction; 81.1%) and a learning option involving a combination of student- and teacher-directed learning (90.1%). As for financial cost, 86.4% selected 1,000 USD as a reasonable tuition fee per semester.

A small number of respondents (14.9%) indicated no interest in a master's degree in occupational therapy. The most common reasons given for the lack of interest were: 1) currently attending an advanced degree program or already attained an advanced degree; 2) preferred to pursue a different field of study; and 3) financial constraints. Responses to the needs assessment questionnaire are presented in Table 2.

In summary, the survey respondents mainly came from the three member countries of the envisioned consortium: Philippines, Indonesia, and Thailand. Majority of those surveyed were interested in pursuing a master's program in occupational therapy. Based on the needs analysis, the preferred master's program should be: 1) completed ideally in 1.5 to 2 years, 2) taken by part-time students who are also working occupational therapy practitioners, 3) given through a hybrid arrangement (i.e., online and face-to-face) with a fixed weekly schedule, and 4) matriculated for USD 1000 per semester.

**Table 2** Frequency count (and percentages) of respondent's responses to survey questions.

| | Students | Graduates |
|---|---|---|
| **N** | **85** | **143** |
| **Interest in pursuing a master's degree** | | |
| Yes | 62 (72.9) | 80 (55.9) |
| No | 3 (3.5) | 31 (21.7) |
| Unsure | 20 (23.5) | 32 (22.4) |
| **Top motivation for pursuing a master's degree**[*,†] | | |
| Boost opportunity for work promotion | 28 (34.1) | 33 (29.5) |
| Enable specialization in specific practice areas | 48 (58.5) | 53 (47.3) |
| Expand body of knowledge in occupational therapy and occupational science | 36 (43.9) | 43 (38.4) |
| Facilitate personal and professional growth | 36 (43.9) | 55 (49.1) |
| Improve and expand clinical practice | 29 (35.4) | 21 (18.8) |
| Improve occupational therapy education | 9 (11.0) | 22 (19.6) |
| Meet requirements for international licensure | 19 (23.2) | 17 (15.2) |
| Upgrade clinical knowledge and skills | 27 (32.9) | 36 (32.1) |
| Upgrade leadership capabilities | 1 (1.2) | 4 (3.6) |
| Upgrade research knowledge and skills | 4 (4.9) | 18 (16.1) |
| Upgrade teaching knowledge and skills | 3 (3.7) | 6 (5.4) |
| **Interest in a master's program offered by a consortium of Southeast Asian universities**[†] | | |
| Yes | 63 (76.8) | 77 (68.8) |
| No | 1 (1.2) | 2 (1.8) |
| Unsure | 18 (22.0) | 33 (29.5) |
| **Study schedule preference**[†] | | |
| Part-time | 59 (72.0) | 80 (71.4) |
| Full-time | 23 (28.0) | 32 (28.6) |
| **Program duration**[†] | | |
| 1 year | 6 (7.3) | 7 (6.3) |
| 1.5 years | 36 (43.9) | 40 (35.7) |
| 2 years | 38 (46.3) | 62 (55.4) |
| >2 years | 2 (2.4) | 3 (2.7) |

**Table 2** Frequency count (and percentages) of respondent's responses to survey questions. (continues)

| | Students | Graduates |
|---|---|---|
| **Learning option**[†] | | |
| Student-directed | 2 (2.4) | 4 (3.6) |
| Teacher-directed | 9 (11.0) | 4 (3.6) |
| Combination | 71 (86.6) | 104 (92.9) |
| **Method of delivery**[†] | | |
| Fully face-to-face | 10 (12.2) | 13 (11.6) |
| Fully remote/online | 5 (6.1) | 8 (7.1) |
| Blended (more face-to-face) | 53 (64.6) | 52 (46.4) |
| Blended (more online) | 14 (17.1) | 39 (34.8) |
| **Class schedule preference**[†] | | |
| Fixed weekly schedule throughout semester | 41 (50.0) | 49 (43.8) |
| Block class schedule | 33 (40.2) | 51 (45.5) |
| No preference | 8 (9.8) | 12 (10.7) |
| **Willingness to attend courses from multiple international higher education institutions**[†] | | |
| Yes | 61 (74.4) | 92 (82.1) |
| No | 0 (0.0) | 2 (1.8) |
| Uncertain | 21 (25.6) | 18 (16.1) |
| **Research track master degree**[†] | | |
| Yes | 26 (31.7) | 58 (51.8) |
| No | 13 (15.9) | 20 (17.9) |
| Uncertain | 43 (52.4) | 34 (30.4) |
| **Willingness to have foreign experts as thesis adviser**[†] | | |
| Yes | 59 (72.0) | 98 (87.5) |
| No | 3 (3.7) | 1 (0.9) |
| Uncertain | 20 (24.4) | 13 (11.26) |
| **Tuition fee**[†] | | |
| 1,000 USD/semester | 73 (89.0) | 94 (83.9) |
| 1,500 USD/semester | 9 (11.0) | 16 (14.3) |
| 2,000 USD/semester | 0 (0.0) | 2 (1.8) |

[*] *Respondents were permitted to choose up three options in the list*
[†] *Respondents who had no interested in pursuing a master's degree did provide answers for this item*

## Discussion

This study aimed obtain a demographic profile and learner preferences of a potential pool of students to inform the co-creation of an ASEAN master's degree program in occupational therapy. Two groups participated in the survey: final year students (at the time of data collection) and occupational therapy practitioners.

### Demographic profile

The age and sex profile of this study's respondents are mostly young adults and female. This profile is similar to other studies of students enrolled in or have graduated from entry level graduate programs (e.g. OTD) in the United States.[27,28] Although the age of this study's cohort is younger than that of the studies of Banning *et al*. and Dalomba *et al*., collectively, it is in the young adult stage that occupational therapists consider pursuing a graduate degree.[27,28] Graduate school may be viewed to be a venue to configure career directions. The majority of our cohort being female builds on

previous literature that occupational therapy is a female-dominated profession.[24-29]

**Interests and motivations in pursuing advanced degrees**

A study by Amani *et al*. revealed five motivations of why individuals pursue postgraduate studies: employment prospects, better salary, career progression or change, personal development, and prestige or self-actualization.[30] Pursuing a postgraduate degree is a challenging feat in itself and if not supported, students could drop out midway due to an incomplete thesis, insufficient achievement motivation, lacking learning strategies available, and low confidence.[31] Career opportunities appear to be a primary source of interest and motivation to pursue graduate degrees. For this study, these were expressed as practice specialization and opportunity for promotion. This finding is consistent with the study of Dos Santos who explored the motivations and career decisions of Asia-Pacific students enrolled in occupational therapy programs in Australia.[32] Dos Santos reported favorable career opportunities as motivation for enrolling in OT in Australia although the educational level of Dos Santos' participants was not stated explicitly. The participants of this study also expressed desire to contribute to the knowledge base of occupational therapy and occupational science. This reflects this group's positive attitude towards research, scholarship, and evidence-based practice. This was further expressed by their willingness to have foreign experts for thesis supervision.[32]

**Program preferences (length of program, schedule, course delivery, tuition)**

Pragmatic and course delivery factors come into play in making decisions related to graduate program enrollment. The preference for USD 1000 per semester tuition fees, hybrid learning, a fixed weekly schedule, and interest in a Southeast Asian consortium program were favored by the majority. These preferences are similar to the decision making factors that the participants in Dos Santos' study expressed.[32] These factors indicate that quality of the graduate program and reasonable tuition fees may account for the decision to pursue graduate programs. These factors may be used by university administrators in marketing graduate programs.

Technology has enabled universities to offer blended and fully online programs. The participants' preference for a blended learning environment indicates that the generation of potential enrollees for graduate programs view blended learning as a convenience, freedom and flexibility, and continued productivity in their workplaces as reported by Banning *et al*.[27] Banning et al. compared graduates of entry-level doctor of occupational therapy programs of on-campus and hybrid environments. The authors found that both groups were similar in preparedness for examinations and the workforce, skill level and sense of belonging, professional and leadership roles assumed after graduation.[27] Banning *et al*. participants expressed that while hybrid programs honed skills such as independence, autonomy, and self-discipline, the learning style of the student must be a good fit with the learning environment.[27] Other points to be explored include students' study approach, self-efficacy, and mental health.[28] These may be considerations in student recruitment and admissions of the proposed ASEAN occupational therapy master's. Bower *et al*. argued that in-person classes for hybrid programs need to be carefully planned as attendance may be challenging.[33] This is an important aspect to consider particularly after two years of online education as a response to the COVID-19 pandemic. Studies conducted among health professions educators revealed that certain aspects of health professions education cannot be replaced by online sessions. These include clinical skills and direct feedback on clinical procedures; and interactive learning with peers.[34,35] The findings may affirm the preference of our study participants for blended with more face-to-face components.

Another consideration is the type of master's program based on the study of Serrata-Malfitano and colleagues where they classified master's program into professional master's and academic master's.[36] The professional master's is characterized as a pre-registration program that enables its graduates to practice the occupational therapy profession, whereas an academic master's entails a thesis requiring students to undergo proposal presentation, ethical clearance, the research process, and a thesis defense.[36] The envisaged master's program to be developed from this study is an academic master's program, hence expecting prospective students to be practicing occupational therapists who would like to advance their competencies in occupational therapy and willing to conduct supervised research.

Changing paradigms are transforming higher education. Successful evaluation of pedagogical initiatives requires a comprehensive approach that goes beyond just measuring students' cognitive skills. It is vital for programs to assess the relationship between teaching strategies and advancements in culturally sensitive healthcare, covering everything from therapeutic relationships and systemic processes to structural impacts.[37] Future occupational therapy programs should address educational paradigms, ethics, learning outcomes, cultural respect, and opportunities for study. It is essential to prioritize talent, integrate diversity into the curriculum, and cultivate metacognitive abilities in higher education graduates who are not only ready for work but also for continuous learning.

**Study limitations**

Generalization of this study's findings must be done with caution as our participants were predominantly graduates from the Philippines, Indonesia and Thailand. Final year students of entry-level occupational therapy programs had low response rates, in general. This might be because of the online nature of the survey and limited means to follow up on students to complete the survey. We also did not explore the perspectives of potential educators, administrators, and staff, especially of potential challenges of a consortium offering advanced degrees. Also, no examination of political, economic dimensions and health needs from the Southeast Asian region that occupational therapists can contribute to were included. In the future, it is valuable that we solicit consultation with stakeholders towards determining the postgraduate topics and disciplinary areas that will shape the curriculum as well as identifying prospective faculty members who will lead, manage, and promote the program.

**Implications**

The creation of a master's program in the ASEAN region will contribute to a global understanding of ASEAN occupational therapy education, training, practice, and research galvanizing a stronger ASEAN cultural identity. There are still many countries in the ASEAN region that require occupational therapists to be part of the workforce to address population needs other than rehabilitation needs.[38,39] It is important that occupational therapy education is grounded on the realities of the context to help address these service needs including public health and community services.[39] In addition, research and programs of research are limited in lower middle income countries in which most ASEAN countries are classified under.[40] This needs analysis study provides empirical data ascertaining that there is indeed a market for a master's degree in occupational therapy from an international consortium. Specifically, our findings provide us salient information to frame the envisaged academic master's program in occupational therapy entailing a two-year duration, hybrid arrangement, underpinned by adult learning pedagogical principles, and costs USD 1000. Moving forward, it is important that the sustainability of the program and partnership will have to be addressed by the involved institutions.[41] Ilott and colleagues highlighted lessons from the experience of the European Master of Science program in occupational therapy that can be adopted by the ASEAN consortium.[42] These include soliciting support from professional and academic networks such as the ASEAN University Network, aligning the program with the ASEAN higher education policy framework (e.g., ASEAN Qualifications Reference Framework), developing a joint decision making and accountability relationship, and underpinning the program on internationalization.

## Conflict of interest

The authors declare no conflict of interest.

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# Effects of Yang-style Tai Chi on body composition and heart rate variability during COVID-19 pandemic among Chinese college students: A preliminary study

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**ABSTRACT**

**Background**: COVID-19 restrictions have further reduced college students' opportunities for physical activity (PA), and problems related to physical and physiological health of college students have become increasingly serious. Studying the effect of Tai Chi (TC) on body composition and heart rate variability can provide insights into the potential benefits of TC as a form of exercise.

**Objectives:** The aim of this study was to assess the effects of 6-week 24-forms Yang-style Tai Chi (YTC) on body composition and heart rate variability (HRV) among college students.

**Materials and methods:** This single-arm, single-blind, pilot study enrolled 6 beginners from 25 individuals. Body composition and HRV were assessed at the beginning and end of the YTC exercise intervention. The intervention was performed twice a week for 6 weeks, each session lasting 45 minutes and consisting of warming-up, practice, and cooling-down exercises during the COVID-19 pandemic.

**Results:** After 6-week TC exercise, BMI (21.00±2.61 to 21.20±2.62 kg/m$^2$) barely changed ($p$>0.05), while body fat mass, skeletal muscle mass, and basal metabolic rate showed a significant change ($p$<0.05). In addition, the high frequency (6.68±0.40 to 7.05±0.50 nu) of heart rate variability had a positive significant increase ($p$<0.05).

**Conclusion:** The 6-week TC practice had the benefit of improving HRV, such as high-frequency (HF), in college students, but further research is needed to identify the long-term effects of TC on body composition and HRV during the COVID-19 epidemic.

## Introduction

Recent years have seen a significant decrease in physical activity (PA) among college students.[1] Prior studies conducted in China found that the highest proportion of exercising time was less than the recommended 1 hour per day at 82.5% and 89.8% in 18-year-old male students and 21-year-old female students, respectively.[2] Improving PA has significant public health implications for preventing many chronic diseases. However, the COVID-19 pandemic has limited people's free access to outdoor areas, such as parks, sports fields, gymnasiums,

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and school-related activities.[3] These isolation strategies further negatively impact the cardio-metabolic health of college students, such as body composition and heart rate variability (HRV).[4] HRV is an important tool in assessing cardiovascular health, representing the variation in the interval between heartbeats that occurs naturally as a result of the interplay between the sympathetic and parasympathetic nervous systems.[5] While the sympathetic nervous system (SNS) is responsible for the "fight or flight" response and increases heart rate, the parasympathetic nervous system (PNS) plays a critical role in maintaining cardiovascular and metabolic health, as well as promoting mental well-being.[6] A lower HRV is generally associated with an increased risk of cardiovascular disease, including aging, obesity, hypertension, and diabetes, higher levels of stress and reduced overall resilience.[7]

A recent systematic review of 33 studies found that PA interventions can have a positive effect on various outcomes, including mental health, physical fitness and quality of life, among college students and other populations during the COVID-19 pandemic.[8] However, most of these studies examined traditional forms of PA, such as aerobic exercise, strength training and yoga, rather than Tai Chi (TC). TC is a unique form of exercise, being low-impact, adaptable and socially connected, that can help enhance PA and mental well-being in college students during the COVID-19 pandemic. Yang-style TC (YTC), the most popular among the Chinese population, focuses on slow, controlled movements and deep breathing, and has been shown to enhance the parasympathetic activity of HRV while improving cardiovascular and metabolic health. In addition, it can also develop mental well-being, specifically the PNS activation that TC training promotes to counteract effects on the SNS, reducing anxiety and improving mood.[9] Compared to a control group, a study found that a 6-week TC intervention significantly improved body composition, such as reductions in body mass index (BMI) and body fat.[10] Another study found that a 24-week, 2-3 times/week, TC intervention significantly improved heart rate variability.[11] Another previous study also showed the high-intensity TC intervention group had greater improvements in body composition than the low-intensity group.[12] Proposed mechanisms for the beneficial effects of TC on body composition and HRV include improved circulation, decreased inflammation and reduced stress.[13] However, it is important to note that the evidence is still limited and further research is needed to confirm the effects of TC on body composition and HRV during the COVID-19 pandemic, as the specific TC program, frequency, duration and intensity of practice may influence the observed effects.

We hypothesized that a 6-week YTC training program could positively affect both body composition and PNS during the COVID-19 pandemic, and we aimed to assess the effect of a defined amount of YTC exercise on body composition and HRV in Chinese college students.

## Materials and methods

### *Participants*

Recruited participants were taking the TC course in September 2021 at Huzhou University, China. A total of 25 young people were assessed by questionnaire assessment, of which 19 were excluded due to ineligibility (N=9), refusal to attend (N=6) and dropout (N=4). Inclusion and exclusion criteria follow.

Inclusion criteria: undergraduate students at Huzhou University; age range between 18-24 years BMI ≥18.5 kg/m$^2$; weight stable as long as 3 months beginning to the study (weight gain or loss less than 4 kg); had enough time and willingness to receive TC training. Exclusion criteria: currently engaging in other weight loss projects; secondary obesity due to medication or other diseases; suffering from hypertension, diabetes, or other cardiovascular diseases; or exercise contraindications; recent withdrawal or medication during the intervention.

### *Study design*

A single-arm, single-blind design was used in this study, and all participants consented to participate in the YTC training program. All participants were familiar with the study tests and procedures prior to baseline measurements. When it came to measuring body composition, subjects needed to step barefoot on the scale, then pull out the handle and touch the eight electrodes on the scale surface and handle respectively and clasp their hands for about 15 seconds. To measure HRV, the end of the subject's index finger needed to be clamped for 2.5 minutes while sitting with eyes closed.

Data were collected at baseline and within half an hour of the end of the 6-week TC training session. All the above group assignment was done by assistants and was hidden from principal researchers.

### *Intervention*

In this study, participants attended supervised Yang-style Tai Chi sessions twice a week for 6 successive weeks. YTC training took place from November to December 2021. It was scheduled to occur every Tuesday and Friday at 4 pm. YTC training is led by an experienced TC-trained instructor. Before the first class, the instructor explained all TC theories and procedures, providing paper materials about its principles and techniques to participants. In subsequent sessions, participants practiced Yang-style TC with an instructor. As YTC is a low-intensity aerobic exercise, popular and easy to learn,[14] every Yang-style TC session lasted approximately 45 minutes, which included a 5-minute warm-up by jogging and stretching, a 35-minute practice, and a 5-minute cooling-down by stretching. During YTC practice, the instructor demonstrated one time on the front and two sides respectively. All participants followed the instructor's motion at the same rhythm 3 times at an interval of more than 1.5 minutes, and then practiced together 5 times in a group. The researcher recorded participant session attendance and the adherence rate was 100%, and all participants' heart rates (HR) mobilized approximately 55% of their maximum HR.

***Data collection***

***Anthropometry and body composition***

Anthropometric data were performed by trained and project members according to standardized methods. Height was measured using a portable stadiometer (Height scale, Jiangsu Suhong Medical Equipment Co., Ltd., China), with participants in no shoes and in an upright position. Body composition was measured using an eight-polar tactile-electrode impedance meter (Huawei Smart Scale 3 Pro, Huawei, China), which simultaneously measured BMI, body fat ratio, fat mass, skeletal muscle mass, limb skeletal muscle Index and basal metabolic rate. They were asked to step barefoot on the scale, then pull out the handle and touch the eight electrodes on the scale surface and handle respectively, clasping their hands for about 15 seconds. The height and weight values are accurate to 1 cm and 0.1 kg, respectively. Body mass index (BMI) used a weight/height$^2$ for calculation.

***Heart rate variability***

After a 10-minute rest, HRV measurement was made in a sitting position in a quiet and temperature-controlled room (25±1°C). This study used uBioMacpa v70 (BioSense Creative, Korea) to measure stress levels in the autonomic nervous system. The uBioMacpa v70 non-invasively measures the pulse wave of the fingertip capillaries and analyzes the pulse variability of the heart. Using a pulse wave detector identified any abnormalities and measured the body's stress level in the autonomic nervous system. The uBioMacpa v70 measures stress levels according to the mean pulse variation signal analysis guidelines published by the North American Society of Pacing and Electrophysiology and the European Society of Cardiology.[15] Since the measured autonomic nervous system pulse variability could be affected by even small movements, after completing the body composition detection, the pressure level was measured for 2.5 minutes with the hand immobilized on the mat and in a seated position.

HRV main components: high-frequency (HF) means 0.15-0.40 Hz, low-frequency (LF) means 0.04-0.15 Hz, low-frequency/high-frequency ratio (LF/HF), the root means square of successive differences between normal heartbeats (RMSSD) and the standard deviation of NN intervals (SDNN). The HF power is regarded as a marker of cardiac parasympathetic activity.[5] The LF components were mediated by the interaction of sympathetic and parasympathetic nerve activities and could also indicate baroreflex function. LF/HF is a way to quantify the relationship between parasympathetic and sympathetic activity, the sympathovagal balance index.[5] We also followed the standards of the HRV index (unit: ms) measurement and its interpretation. In general, for HF and RMSSD, higher values are considered to indicate higher parasympathetic activity, which is generally beneficial for health, and an increase of the LF and SDNN component is generally considered to be a consequence of increased sympathetic activity. The LF/HF ratio indicates the autonomic nervous system balance.[16]

**Statistical analysis**

All data were analyzed by SPSS 26.0 (IBM Corp., Armonk, USA) and were checked for normality by the Shapiro-Wilk test. Since BMI, body fat mass, body fat percentage, basal metabolic rate, LF, HF, LF/HF, RMSSD and SDNN were suitable and normally distributed, a paired samples t-test was used to evaluate the differences between before and after 6 weeks of training. Statistical significance was set at $p<0.05$.

## Results

***Participant characteristics***

A total of 6 non-TC experienced participants completed the whole training intervention and were included in this analysis. The mean age of participants was 19.5 years old. Participants did not report any adverse events during the 6-week TC training, including hunger, hypoglycemia, muscle soreness, irritability, or insomnia.

***Effect of Tai Chi on BMI and body composition***

The present study aimed to investigate the effects of a 6-week TC intervention on participants' body composition and BMI. The findings, as illustrated in Table 1, revealed an increase in body fat mass from 15.63±4.11 kg to 17.77±4.80 kg, and a decrease in skeletal muscle mass from 22.05±2.81 kg to 20.93±2.23 kg. Whereas BMI remained almost unchanged from 21.00±2.61 to 21.20±2.62 kg/m$^2$.

**Table 1** Intervention effects on BMI and body composition.

| Variables | Before (mean±SD) | After (mean±SD) | t | *p* value |
|---|---|---|---|---|
| BMI (kg/m$^2$) | 21.00±2.61 | 21.20±2.62 | -1.54 | 0.185 |
| BFM (kg) | 15.63±4.11 | 17.77±4.80 | -4.12 | 0.009** |
| BFR | 26.58±4.66 | 30.07±5.50 | -4.78 | 0.005** |
| SMM (kg) | 22.05±2.81 | 20.93±2.23 | 4.04 | 0.010** |
| LSMI | 6.30±0.66 | 6.00±0.59 | 7.89 | 0.001** |
| BMR (kJ/m$^2$·hr) | 1283.83±43.75 | 1248.00±34.63 | 3.79 | 0.013* |

***Note:*** *BMI: body mass index, BFM: body fat mass, BFR: body fat ratio, SMM: skeletal muscle mass, LSMI: limb skeletal muscle index, BMR: basal metabolic rate, *significant at the 0.05 level (two-tailed), ** significant at the 0.01 level.*

Furthermore, the results indicate that after 6 weeks of TC training, body fat mass and body fat ratio exhibited a statistically significant increase ($p$<0.01). While skeletal muscle mass, limb skeletal muscle index, and the basal metabolic rate displayed a significant decrease ($p$<0.05). Notably, despite the observed changes in body composition parameters, BMI remained relatively stable throughout the intervention period ($p$>0.05), see Figure 1.

***Effect of Tai Chi on heart rate variability***

The changes in HRV of the participants are shown in Table 2. Although the SDNN increased slightly from 57.18±8.70 to 57.40±13.59 ms, it was found that the LF decreased from 7.78±1.28 to 7.60±0.94 nu. Furthermore, the RMSSD decreased slightly from 53.00±39.31 to 45.46±5.94 ms, but the HF increased significantly from 6.68±0.40 to 7.05±0.50 nu ($p$<0.05). Moreover, the LF/HF had a decrease from 1.15±0.16 to 1.08±0.14, see Figure 2.

## Discussion

We aimed to investigate the effects of 6-week TC training on body composition and HRV among college students. Our results showed that although BMI did not significantly change, other indicators related to body composition, such as body fat and skeletal muscle mass, experienced significant negative changes during the winter COVID-19 epidemic period. While the effect of TC on body composition has been inconsistent in the literature. An exploratory study on the effects of 12 weeks of TC and brisk walking training on weight loss, bone mineral density, and metabolic syndrome parameters in middle-aged adults in Hong Kong provided new information. The study found that both moderate-intensity short-term PA programs slightly reduced body weight and body fat mass and significantly improved waist circumference and fasting blood glucose.[17] Conversely, some TC studies[18-20] have shown no significant changes in body weight or

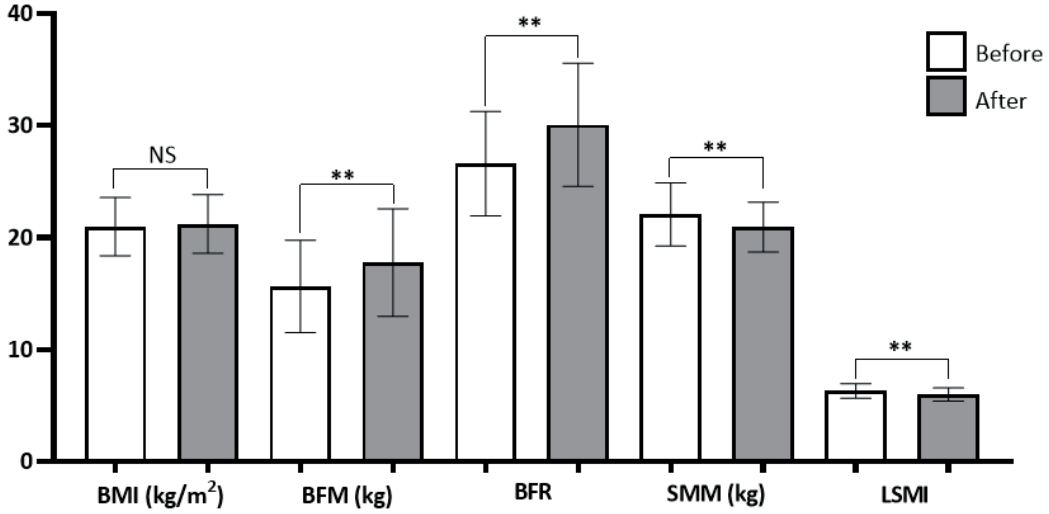


***Figure 1*** *Changes in body composition before and after the 6-week TC intervention.*
***Note:*** *BMI: body mass index,* BFM*: body fat mass, BFR: body fat ratio, SMM: skeletal muscle mass, LSMI: limb skeletal muscle index, NS: no significant, , *significant at the 0.05 level (two-tailed), ** significant at the 0.01 level.*

**Table 2** Intervention effects on heart rate variability.

| Variables | Before (mean±SD) | After (mean±SD) | t | *p* value |
|---|---|---|---|---|
| LF (nu) | 7.78±1.28 | 7.60±0.94 | 0.676 | 0.529 |
| HF (nu) | 6.68±0.40 | 7.05±0.50 | -3.202 | 0.024* |
| LF/HF | 1.15±0.16 | 1.08±0.14 | 2.000 | 0.102 |
| SDNN (ms) | 57.18±8.70 | 57.40±13.59 | -0.035 | 0.973 |
| RMSSD (ms) | 53.00±39.31 | 45.46±5.94 | 0.467 | 0.66 |
| Composite index | 30.83±8.08 | 31.66±6.02 | -0.302 | 0.758 |

***Note:*** *LF: low frequency, HF: high frequency, LF/HF: LF to HF ratio, SDNN: SD of NN intervals, RMSSD: root means square of successive differences between normal heartbeats, ms: millisecond, nu: normalized units, which are normalized by subtracting the mean value from each heartbeat interval and then dividing by the standard deviation. It is a dimensionless unit commonly used in the analysis of heart rate variability, * significant at the 0.05 level (two-tailed), ** significant at the 0.01 level.*

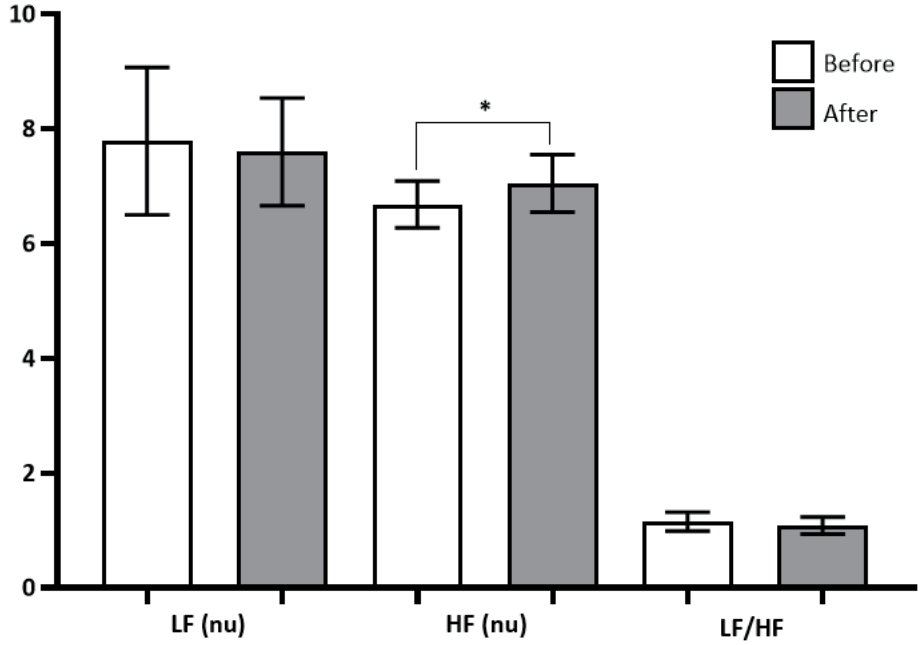


***Figure 2*** *Changes in LF, HF and LF/HF before and after the 6-week TC intervention.*
***Note:*** *LF: low frequency, HF: high frequency, LF/HF: LF to HF ratio, ms: millisecond, nu: normalized units, which are normalized by subtracting the mean value from each heartbeat interval and then dividing by the standard deviation. It is a dimensionless unit commonly used in the analysis of heart rate variability, * significant at the 0.05 level (two-tailed), ** significant at the 0.01 level.*

BMI. However, we found that 6 weeks of TC exercise slightly increased body fat mass. A possible explanation for this finding is that during the COVID-19 epidemic, the participants may not be able to involved other exercise. This can cause the body fat of college students has increased. This finding is consistent with a previous study, showed a significant rise in weight gain during COVID-19 among individuals who had a 6-week long winter vacation in the USA and UK.[21] However, it is important to note that the heterogeneous findings suggest that study designs, target population, frequency, season, and duration of TC exercise are all important factors in detecting significant clinically relevant effects. [22-24]

It is important to consider the impact of various factors on the results of this study, such as a small sample size, short duration of intervention, and differences in the participants' baseline characteristics. Due to the nature of skeletal muscle mass and basal metabolic rate adaptations, it is unlikely that significant changes would be immediately apparent after only six weeks of exercise training. Longer periods of consistent training are typically required to observe such adaptations. Although efforts were made to recruit participants with similar baseline levels, it is possible that some individuals may have had poor body composition or mental health initially. This would result in more significant improvements post-intervention compared to those with relatively good body composition at baseline. Additionally, it is important to note that some factors not considered in this study, such as exercise dosage and food intake, may have influenced changes in body composition. However, it is interesting to note that HRV indicators showed positive improvement within six weeks of TC intervention. There was consistent with the H. ChuDuc,'s study which found improvements in the HF component and a decrease in the LF.[25] This study also observed a decrease in the LF/HF after the TC intervention, but it was not statistically significant. This finding is partially consistent with the results of some studies that have observed a significant decrease in LF/HF. [26,27] It is crucial to note that HRV parameters, including HF, LF, RMSSD, and SDNN, are complex and dynamic measures that depend on individual physiological and environmental factors.

Since TC is a light-to-moderate intensity aerobic exercise that combines physical and mental exercises, it is widely believed that TC involves the control of physical tension and mental concentration, which may promote stress relief by reducing sympathetic drive and increasing parasympathetic drive, as reflected in HRV.[28] The mechanism of how slow, rhythmic breathing and focused TC training affects HRV is not fully understood, but it is thought to be related to improvements in autonomic nervous system (ANS) function and stress regulation.[29] TC may stimulate the parasympathetic branch of the ANS, reduce sympathetic activity, and enhance HRV by improving baroreflex sensitivity (BRS), reducing inflammation and oxidative stress, and improving psychological well-being. Moreover, TC has been shown to enhance parasympathetic activity, improve cardiovascular and metabolic health, and promote mental well-being.[30] Specifically, parasympathetic activity reduces heart rate and blood pressure to protect against cardiovascular disease, improves blood sugar regulation, enhances digestion and nutrient absorption, and promotes fat metabolism. TC

training activates the parasympathetic nervous system, indicating an HF increase, which counteracts the effects of the sympathetic nervous system,[31] and parasympathetic activation can reduce anxiety and improve mood by promoting relaxation and reducing stress hormones like cortisol.[30] Therefore, 6 weeks of TC training may lead to improvements in both HF and LF of HRV, indicating a shift towards a more balanced and flexible autonomic nervous system. This is consistent with previous research that has shown that TC can enhance parasympathetic activity and improve cardiovascular function, which may in turn improve HRV.

It is certainly worth noting that the COVID-19 situation has had a multifaceted impact on TC training, body composition, and HRV. [4] For example, individuals may have to adapt their training and lifestyle habits in response to challenges posed by COVID-19, such as reduced interaction opportunities during TC training due to social distancing. They can also change in dietary habits, sedentary behavior, and increased stress. Future studies with sufficient interventional duration may lead to more significant results. However, this study provides a valuable strategy for improving HRV, especially the HF of parasympathetic activity, during the winter of the COVID-19 epidemic through a 6-week TC exercise intervention. It is important to note that only 10% of the Chinese population has a good health conception and culture. Additionally, many students report spending less than one hour per day on PA which highlights the need for interventions like TC to promote physical and mental health.[2] The results of this study show that TC can not only serve as a means of promoting sports culture but can also significantly increase the HF component of HRV, indicating increased parasympathetic activity and decreased sympathetic activity.

This study presents preliminary evidence on the effects of Yang-style Tai Chi sessions twice a week for 6 weeks consecutively may promote in the parasympathetic activity of college students in the epidemic, as increases in HF. However, it should be noted with caution that the results of previous studies on the effects of TC on HRV and body composition have been inconsistent. Further research is needed to draw more reliable conclusions. This study has some limitations, including small sample size and the lack of a control group due to it being a preliminary study. We were mainly to verify whether 6 weeks of TC has an impact on body composition and HRV and to verify the operability of the protocol. Moreover, our study did not measure psychological stress directly. It is also better if future research should incorporate psychological questionnaires to provide a more comprehensive assessment of the effects of TC on stress. A well-designed study with a larger sample size and a control group is currently underway to address these limitations.

## Conclusions

This study highlights the potential benefits of TC for improving HRV, especially during challenging times like the COVID-19 epidemic. However, further research is needed to establish the long-term effects of TC on body composition, stress, and HRV, and to identify the functions for promoting physical and mental health among college students.

## Conflicts of interest

The authors declare no conflict of interest.

## Ethical approval

The experimental protocol was approved by the Center for Ethics in Human Research, Khon Kaen University, and Recorded No.4.2.01: 6/2565 (Reference No. HE652012). All participants signed written informed consent in the study.

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# Effect of the basic speech and language therapy guidebook for aphasia patients to improve language and speaking skills: A trial phase

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**ABSTRACT**

**Background**: Aphasia patients should receive intensive speech therapy in order to recover their speech and language skills for optimal results. However, there are numerous limitations. One of which is the scarcity of speech and language pathologists. Thus, this research aimed to utilize the speech and language therapy guidebook for aphasia patients which can be used by caregivers to help those with aphasia at home. This guidebook will increase the comprehension and frequency of therapy.

**Objectives**: Firstly, to develop and study the content validity of the interview form for explicit problems and the satisfaction survey of the guidebook. Secondly, to conduct a pilot study for using the basic speech and language therapy guidebook at home. Limitations and caregivers' suggestions from using this guidebook had been studied by the interview method.

**Materials and methods**: This study consisted of 2 steps: The first step was the development of the interview form for explicit problems and the satisfaction survey of the guidebook. The second step was applying the guidebook with 3 aphasic clients by their caregivers for a week. Information from the first step was analyzed for content validity. The information from the second step was analyzed to explore the problems that occurred by using descriptive statistics.

**Results**: In the first step, the Index of Item-Objective Congruence (IOC) from 5 experts for the interview form and satisfaction survey showed total scores of 0.975 and 1, respectively, revealing that the content validity was acceptable. In the second step, the information from 3 samples showed problems with a lack of understanding concerning the use of the guidebook (100%). The reflection by 2 caregivers (67%) who undertook the training as assigned found that the guidebook could help develop the speech and language skills of the samples. For the practice benefits, 3 caregivers (100%) reflected that the guidebook facilitated self-therapy and provided them and the clients with more frequent, more convenient, and easier-to-follow instructions. They were satisfied with the guidebook but offered minor suggestions, such as making the book smaller, correcting for typos, and adding more exercises.

**Conclusion**: The guidebook yielded benefits for developing speech and language skills and provided benefits for the caregivers who help practice with the samples at home. The results of this research showed problems with using the guidebook and devised precautionary measures. The feedback will be applied to guidebook development in the future.

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## Introduction

Patients with aphasia often have trouble using or/and understanding a language, including listening, speaking, reading, and writing.[1] For example, they are unable to communicate through verbal communication, comprehend speech, reach the lexicon, and speak with a lack of enunciation.[2] aphasia significantly impacts living and quality of life by increasing limitations on communication, social engagement, and social interaction, as well as risk and personal negativity towards life.[3-4]

Aphasia results from an abnormality in the central nervous system and pathology in the left brain which can be caused by stroke or other events that cause brain injury.[1, 5-6] Studies have found that roughly one-third of patients with a history of stroke suffer from aphasia.[7-9] Regarding the data on the incidence and prevalence of aphasia in the United States of America, National Institute on Deafness and Other Communication Disorders (NIDCD) reported that an estimated 180,000 people acquire aphasia each year, and approximately 1 million people had the disorder in 2016.[10] In Thailand, Nijasri Suwanwela also reported that the prevalence of stroke is estimated at 1.88% of adults 45 years and older.[11] Results of the previous study found that, when brain is damaged, the nervous system cannot spontaneously recover fully. Thus, resulting in partly recovered speech and language skills and the results differ among individuals. In addition, the first 3 months after an injury is the best duration for recovery but can be stimulated after 6 months if assessed and given proper treatment that is suitable to individual severity from a speech and language pathologist.[12-13] The previous study showed dramatic improvement in clients who received intensive therapy for at least 10 hours per week for 3 weeks.[7, 14-15]

Currently, the rehabilitation of aphasia in Thailand cannot be executed properly due to the aforementioned significant problems, as well as the lack of speech and language pathologists; it was found that Thailand only had 252 speech and language pathologists in 2022.[16] Approximately 70% of them are available in only Bangkok and the central region of Thailand.[17] Moreover, according to the criteria for the Art of Healing in the field of Communication Disorders, speech and language pathologists must have completed a Master's degree or above to be qualified to assign rehabilitation to those with aphasia.[18] Furthermore, there is a problem concerning the therapy program. Normally, aphasia patients in Thailand receive 30 minutes-1 hour of speech therapy per week with a speech and language pathologist. The program includes speech and language training activities that are appropriate for the patient's skills and short homework. However, the duration of the therapy at the speech therapy clinic cannot be considered as intensive practice. So, the caregivers must practice with the patients more frequently at home. They typically encounter the problem of assigning practices that are too complicated to achieve in the home environment, thus forgetting the stimulation techniques taught in the session due to several details in the process.[19]

In 2018, one of the researchers developed the basic speech and language therapy guidebook for aphasia patients.[20] The guidebook includes advice on aphasia, the principles of how to give practice to patients, and exercises that caregivers can do with patients at home under supervision. The guidebook helps patients to receive speech and language therapy properly and intensely, resulting in the effective improvement of speech and language skills. However, the guidebook has never been studied in terms of possible problems that can occur while using it, the results of the therapy, and the satisfaction of users. Thus, the objective of this research was a study for using the basic speech and language therapy guidebook for aphasia patients at home. The limitation of using this manual had been studied by using the interview method.

## Materials and methods

This pilot study applies the 'Small-n Research' method. The samples included patients who were diagnosed with aphasia, and are outpatients who receive therapy at the Speech Therapy Center, Faculty of Associated Medical Sciences, Chiang Mai University. A total of 3 samples were selected through purposive sampling. The criteria are as follows

*Inclusion criteria:*

1. Patients must be diagnosed by doctors as having aphasia from abnormalities in the central cervous system or stroke.[5]
2. Patients must be native Thai speakers and must have been fluent before having aphasia.
3. Patients must have caregivers who can manage and attend to the research, as well as be able to take the patients to the Speech Therapy Center, Faculty of Associated Medical Sciences, Chiang Mai University, and be able to practice with the patients at home.
4. Caregivers must be able to literate and fluent in Thai at the communicable level.

*Exclusion criteria:*

1. Patients attend other studies that involve speech therapy at the same time as the current study.
2. Patients having trouble with vision or hearing which cannot be fixed by spectacles or hearing aids.

### Research instruments

***Assessment of the thai adaptation of western aphasia battery (WAB).*** [21-22]

Thai Adaptation of WAB is a standard assessment that has been used to evaluate the speech and language skills of patients including listening, speaking, reading, and writing in Thailand. The assessment has the same calculation systems as the original WAB and can be interpreted as the aphasia quotient (AQ), language quotient (LQ), performance quotient (PQ), and cortical quotient (CQ), which can be classified into levels of severity and 8 types of aphasia. The criterion of the AQ score for normal people is 94.7 or above. This research is interested in the changes in speech and language skills. AQ was measured as it refers to the ability to comprehend and express language.

***Basic speech and language therapy guidebook for aphasia patients to improve language and speaking skills and record of results.***[20]

The guidebook was developed from studying special topics related to communication disorders that one of the researchers is interested in investigating under the supervision of an advisor and the board of the subject, which also is one of the subjects of the Department of Communication Sciences and Disorders, Faculty of Medicine Ramathibodi Hospital, Mahidol University, 2012 Edition.[23] the development aimed to help those with aphasia who lack opportunities to receive speech therapy with proper frequency. Therefore, the basic speech and language therapy guidebook for home use under the care of speech and language pathologists has been developed. The researcher read literature related to speech therapy, and then adapted and designed the guidebook from the elements of language in the Thai adaptation of WAB assessment[21] in terms of spontaneous speech, comprehension, naming and repetition, Neuroplasticity,[24,25] and the theory of distributed practice[26] by having experts in communication disorders of the nervous system to be advisors and monitor the appropriation of the content in the guidebook, such as the vocabulary that fits the Thai context, the relevance between the vocabulary and the topics of the exercises, and difficulty level categorization - in categorizing vocabulary. The entire process was created before proposing to the board of specific subjects, consisting of 5 experts who are experienced in speech therapy for those with communication disorders from the nervous system. The guidebook was registered as the Intellectual Property of the Faculty of Medicine Ramathibodi Hospital, Mahidol University in 2019. The process of developing the basic speech and language therapy guidebook for aphasia patients to improve language and speaking skills showed in Figure 1.

***Interview form for explicit problems that caregivers found during the use of the basic speech and language therapy guidebook for aphasia patients to improve language and speaking skills.***

It is a semi-structured Interview form for gathering the opinions and problems found when using the guidebook that the researchers developed and goes through the process of studying the content validity. The responses of the caregivers were recorded during the interviews.

***Satisfaction survey of the basic speech and language therapy guidebook for aphasia patients to improve language and speaking skills by caregivers.***

It is a satisfaction survey for caregivers on the guidebook, which includes satisfaction levels on the content and form design, as well as exercise practicality, overall satisfaction with the given benefits, and suggestions. The survey was developed by the researchers and put through content validity. The survey will be used along with the study of the effectiveness of the guidebook in the future.

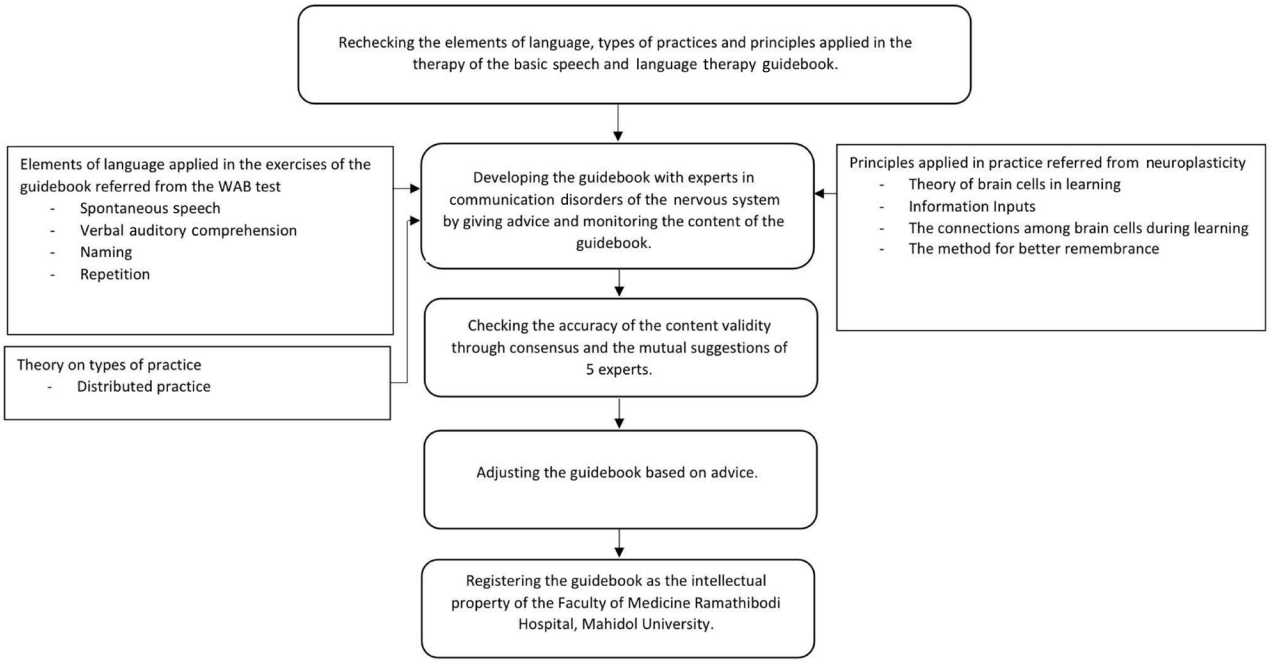


***Figure 1*** *Process of developing the basic speech and language therapy guidebook for aphasia patients to improve language and speaking skills.*

**Research methods**

The methods can be separated into 2 steps:

**Step 1.** Development of the interview form and satisfaction survey.

Firstly, the researchers developed a semi-structured interview form for the explicit problems that occurred during the use of the guidebook by the caregivers, which will be used further in Step 2. An interview consists of questions on the guidebook's instructions, difficulty in the process of use, results from use, and further suggestions. Secondly, Satisfaction Surveys of guidebook usage by caregivers, which will be conducted in the future and include questions concerning satisfaction with the design, content, practice in the guidebook, overall satisfaction, and other suggestions for guidebook improvement. After the development of both instruments, the researchers sent them to 5 experts in speech therapy for patients with aphasia for content validity and the relevance between vocabulary usage and Thai contexts to calculate the Index of Item-Objective Congruence (IOC).[27] Suggestions from experts will be used to improve the content of instruments.

**Step 2.** Applying basic speech and language therapy guidebook with aphasia patients.

The researchers selected 3 samples who passed the criteria for the research from the Speech Therapy Center, Faculty of Associated Medical Sciences, Chiang Mai University. The details are as follows:

1) Select the samples according to the criteria and evaluate the level of deficit using WAB test[21]

2) Schedule the samples and caregivers to meet the researcher at the speech therapy center for 60 minutes, once a week for a total of coupled times.

3) For the first visit, the samples receive speech therapy at the Speech Therapy Center. Afterwards, the researcher assigns the guidebook to the caregivers for home practice for 1 week, 3 sessions per day, and 30 minutes per session for a total of 90 minutes. Except for the day at the center, samples must do home practice for 1 session or 30 minutes because they already had 60 minutes of practice at the clinic.

4) The second visit, appoint the samples and caregivers when it reaches 1 week to follow the results and record the explicit problems during the use of the guidebook by applying the semi-structured interview form developed by the researchers.

5) Conclude the response from the interviews with the caregivers. This step takes approximately two weeks and has no re-evaluation.

**Data Analysis**

1) Analyze the content validity of the interview form and satisfaction survey by using IOC

2) Analyze general information about the samples including the problems that occurred, results from the guidebook use, and suggestions by applying descriptive statistics and then concluding responses from the interviews with the caregivers.

## Results

**Step 1:** Development of the interview form for explicit problems during the use and satisfaction survey of the guidebook.

The interview form consists of 4 parts: the guidebook's instructions, difficulty in using, the results, and suggestions in the guidebook. In each part, the IOC values from the experts were 0.9, 1, 1, and 1, respectively, for a total of 0.975 (Table 1). The satisfaction survey consists of 4 issues: design, content, practice, and overall satisfaction, all of which show the IOC at 1 (Table 2).

**Step 2:** The guidebook was applied by 3 samples comprised of 2 men and 1 woman. They all have different ages, duration of stroke, types of aphasia, aphasia quotient, and other details (Table 3).

The guidebook's instructions resulted that the second sample (33%) failing to use the guidebook as the researchers assigned. However, the first and third (67%) were able to use the guidebook similar to what the researchers assigned. The practice took 7 days, 2-3 times per day, for approximately 85 and 86.42 minutes or 10 and 9.9 hours per week, respectively (Table 4).

For the difficulty in the process of use, the most prevalent problem was a lack of understanding in use, which was found in all 3 samples or 100%. The second problem was the caregivers' confusion in grading the samples' responses. For example, they couldn't decide on grading when the sample answered differently from a key answer. which was 67%. Lastly, the sample lacked understanding in terms of following the instructions in the guidebook. Further, the caregivers did not regularly record the results in the record form, which was 33% (Table 5).

The results from the guidebook use, the first and third samples (67%) found that the patients had better speech and language skills, which can be observed from the scores in the record that increase when the practice is repeated the next day. Also, the patients could answer correctly more than 80% of the time, so they could increase the difficulty level on the next exercises. However, it was found that samples were more tired when practicing with the guidebook than usual practice. For the second sample (33%), there was no change in speech and language skills, but the samples collaborated with the caregivers more compared to when the guidebook was not used (Table 6).

In terms of the benefits of home practice, all caregivers (100%) reflected that they were able to practice more frequently, more conveniently, and more easily. There were 2 samples (67%) that reflected the guidebook could be adapted for further practice on their own in the future. Reflections from the caregivers can be shown in Table 6.

Afterwards, 3 caregivers (100%) were satisfied with the form, design, and content of the guidebook when asked about recommendations, there were further suggestions as follows. Concerning the design of the guidebook, they want it to be more compact (33%). In

the content, they want the typos to be corrected (33%), decrease the number of words in the description (33%), improve the clarity of the choices (33%), as well as add more topics to the practice (33%). All suggestions can be found in Table 7.

**Table 1** Index of Item-Objective Congruence (IOC) from the experts of the interview form for explicit problems during the use of the guidebook.

| Consideration list | Scores and suggestions from the experts | | | | | | |
|---|---|---|---|---|---|---|---|
| **Part 1. Questions on the guidebook's use plan** | **Expert 1** | **Expert 2** | **Expert 3** | **Expert 4** | **Expert 5** | **Total score** | **Suggestions** |
| 1. Please tell us about your experience in using the basic speech and language therapy guidebook for aphasia patients to improve language and speaking skills of patients through home pratice. | 1 | 1 | 0 | 1 | 1 | 4/5=0.8 | The third expert agrees with the question but suggests that it be moved to part 3. |
| 2. Can you practice with the patients using the guidebook as frequently as the researcher assigned or as you planned? why? | 1 | 1 | 1 | 1 | 1 | 5/5=1 | The third expert suggests that the question be changed to "Can you practice with the patients using the practice plans the researcher assigned or as you planned? why?" |
| **Part 2. Questions on difficulty in the process of using the guidebook** | | | | | | | |
| 1. During home practice using the guidebook, were there any difficulties during or from the practice? If so, how? | 1 | 1 | 1 | 1 | 1 | 5/5=1 | The first expert suggests adding more questions on the difficulty of using the record form in the same question. |
| 2. From the guidebook use, are there any parts of the content in which you lack understanding or need more explanation? If so, which part? | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | |
| 3. Do you need further help from the researcher in home practice concerning the use of the guidebook? If so, how? | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | |
| **Part 3. Questions about the results from the guidebook use** | | | | | | | |
| 1. Did you notice any changes in the speech and language skills of the patients after receiving the practice in the guidebook? If so, how? | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | |
| 2. Do you think that the basic speech and language therapy guidebook for aphasia patients yields benefits for home speech therapy? If so, how? | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | The fifth expert suggests that the question be changed or given specifications to convey the specific meaning of the question. |
| **Part 4. Questions concerning further suggestions for enhancement of the basic speech and language therapy guidebook for aphasia patients to improve language and speaking skills** | | | | | | | |
| 1. Do you have any more suggestions for improving the basic speech and language therapy guidebook for aphasia patients? If so, how? | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | The third expert suggests separating the question into suggestions and what they want to improve.<br>1. General design of the guidebook.<br>2. Content of the guidebook. |

**Table 2** Index of Item-Objective Congruence (IOC) from the experts on a satisfaction survey of guidebook usage by caregivers.

| Consideration list | Scores and suggestions from the experts | | | | | | |
|---|---|---|---|---|---|---|---|
| **Part 1. Questions on the guidebook's use plan** | **Expert 1** | **Expert 2** | **Expert 3** | **Expert 4** | **Expert 5** | **Total score** | **Suggestions** |
| 1. The size of the guidebook is proper. | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | |
| 2. The design of the guidebook is pleasant and beautiful. | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | |
| 3. The text font is easy to read and pleasant. | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | |
| **Content in the guidebook** | | | | | | | |
| 1. The content is grouped and easy to read. | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | |
| 2. The number of exercises is adequate for the duration of home practice. | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | |
| 3. The content of the guidebook will help caregivers understand therapy principles for patients with aphasia. | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | |
| **Practice in the guidebook** | | | | | | | |
| 1. The information and practice can be adapted into home speech and language therapy. | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | |
| 2. The guidebook helps increase the frequency of practice for patients. | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | |
| 3. There are better changes in the speech and language skills of the patients after using the guidebook. | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | |
| 4. The time assigned in the guidebook is sufficient for the number of practices. | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | |
| **Overall satisfaction with using this guidebook** | 1 | 1 | 1 | 1 | 1 | 5/5 = 1 | |

**Table 3** General information of the samples.

| N | Gender | Age | Duration of aphasia | Types of aphasia | Aphasia quotient | Caregiver(s) | Age of caregivers | Time with patients |
|---|---|---|---|---|---|---|---|---|
| 1 | Woman | 70 years 8 months | 2 years 2 months | Broca's aphasia | 58.5/100 | Son | 40 years 8 months | All day |
| 2 | Man | 62 years 7 months | 3 years 7 months | Global aphasia | 13.2/100 | Daughter | 29 years 11 months | 7-8 hours |
| 3 | Man | 39 years 4 months | 4 months | Global aphasia | 23.6/100 | Wife | 26 years 5 months | All day |

**Table 4** Results of the guidebook use concerning problems with the guidebook's instruction.

| N | Problems with using the guidebook | | Number of days practicing | Average time for practice | Hours/week |
|---|---|---|---|---|---|
| | Able to follow most of the principles | Unable to follow the principles | | | |
| 1 | √ | | 7/7 days | 86.42 minutes | 10 |
| 2 | | √ | 4/7 days | 37.50 minutes | 2.5 |
| 3 | √ | | 7/7 days | 85 minutes | 9.9 |

**Table 5** Results of the guidebook use concerning problems and difficulties in the process of use.

| Problems | Caregivers of patients who encountered problems | | | Number of those who encountered problems | Percentage (%) |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| Caregivers lack understanding of the process. | √ | √ | √ | 3 | 100 |
| Caregivers are confused about how to grade the response of the patients. | √ | √ | | 2 | 67 |
| Caregivers need more instructions and examples in the practice. | | √ | √ | 2 | 67 |
| Samples lack understanding in following the practice. | | √ | | 1 | 33 |
| Caregivers do not routinely record the results. | | √ | | 1 | 33 |

**Table 6** Results from the guidebook use.

| Results | Samples | | | Percentage (%) | Significant topics from interviews with the caregivers |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **Speech and language skills** | | | | | |
| The samples have better speech and language skills. | √ | | √ | 67 | *"I feel like the patient began to utter the words with longer syllables, like 2 or 3 syllables. I also noticed that, when words are repeated, the patient will be more fluent in using that word."* |
| **"The patient has better repetition after being partly wrong and right."** | | | | | |
| The practice is more frequent, more convenient, and easier. | √ | √ | √ | 100 | *"I get a lot of benefits because it would be impossible if we had to come up with our thoughts."*<br>*"There is no difficulty. It seems like the practice is even easier."* |
| It can be adapted for further use in practice on your own. | √ | √ | | 67 | *"This provides me with the topic, so I follow that. When I get used to the topic, I can adapt to the context on my own."*<br>*"This guidebook can be used as examples for the practice."* |
| The practice has more principles and systems. | √ | √ | √ | 100 | *"The guidebook instructs how to start and how to go on because most of the words the doctor has listed are daily words, and the next one will be more difficult. It is very systematic and convenient."*<br>*"It's beneficial. I don't have to come up with my own practice because there are given topics, and it makes the practice neat."* |
| **Others** | | | | | |
| The samples get more tired compared to the usual practice. | √ | | √ | 67 | *"90 minutes is quite tiring compared to what we have practiced because it usually takes only 30-40 minutes per day, but this practice takes 7 days in a row. It is quite tiring."* |
| The samples pay more attention to the practice. | | √ | | 33 | |
| The samples collaborate more with the caregivers in the practice. | | √ | | 33 | *"It's like there are guidelines and examples. It gives my father confidence to follow the guidelines because, before this, we only practiced with a piece of paper. My father really likes the story in the pictures. He loves to point out the story in the pictures."* |

**Table 7** Results of the guidebook use concerning further suggestions for improvement of the guidebook.

| Satisfaction and Suggestions | Samples | | | Percentage (%) | Significant topics from interviews with the caregivers |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| **Satisfaction with the design** | √ | √ | √ | 100 | *"It's actually already ok (laugh). It's good."*<br>*"I think content like this is ok."*<br>*"The physicals are ok. There's no problem."* |
| **Satisfaction with the content** | √ | √ | √ | 100 | |
| **Suggestions on the design of the guidebook** | | | | | |
| | | √ | | | *"The design looks like a scientific book. The picture quality is very good, but I prefer smaller ones."* |
| **Suggestions on the content of the guidebook** | | | | | |
| Correct the typos in the guidebook | √ | | | | *"If the small details are fixed, that would be good, like the typos and colors for example."* |
| Decrease the number of words in Chapter 1 to make it easier to read | | √ | | | *"The placement of the pictures is very interesting and readable, but there are too many words in the beginning. There must be an adjustment to make it more readable."* |
| Improve the clarity of the choices in the practice | | √ | | | *"When I point at the shirt, there will be a shirt and a sweater*. I'm afraid he will get confused so I have to repeat that it is the shirt, not a sweater" (in Thai, both words start with 'Sua')* |
| Add more topics | | √ | | | *"I want you to add more topics like when we started practicing, such as mouth moving, when there are new patients."* |

## Discussion

From the content validity of the interview form and satisfaction survey, the researchers improved and modified the content of the instrument based on the suggestions of the experts before applying it in this pilot study and the study of the effectiveness of the guidebook in the future. Besides, the IOC of the interview form and satisfaction survey were 0.975 and 1, respectively (Tables 1,2), which is a great number of validities that can be evaluated. It is consistent with the study of Ongiem and Vichitvejpaisal, which stated that questions that have suitable validity must have an IOC of more than 0.5.[27]

The problems occurred in the guidebook's instructions that the caregiver was unable to follow the schedule resulted from a lack of collaboration in practicing and the conditions that made the sample unprepared for practice, such as stress and fatigue. There was also a lack of readiness from the caregiver due to the workload and time management. This is consistent with the research of Gunning *et al*.,[28] which showed that intensive speech therapy yields benefits, but is often more difficult to practice compared to usual practice due to different problems such as fatigue, time management, and individual problems. The previous study also suggests the significance of sample selection, stating that patients who undertake intensive speech therapy must be eager to practice, flexible, and able to self-control, as well as have body stamina. The researchers need to consider this for the selection process in the future. For example, the samples and caregivers must be eager to practice in order to improve speech and language skills and be interested as well as patient enough to practice throughout the assigned duration in the guidebook.

Although the guidebook has detailed instructions and all caregivers come from diverse backgrounds, the most found problem was the lack of understanding from the caregivers about the guidebook, such as stimulating the samples with more exercises than the assigned plan, being unable to correctly practice the exercises, or being unable to change to the next exercise. This is in agreement with the study of Kurland, Wilkins and Stokes which examined a therapy program that could be further practiced at home.[29] The study found problems in using the instrument by the samples, such as samples being unable to match the exercises with their capability, being unable to use the instrument as assigned due to a lack of understanding and having individual problems. Thus, the suggestions for successful results include the patients having an eagerness to use the instrument and being trained accordingly. With that being said, the researchers have devised prevention plans for the aforementioned problems as well as other secondary problems for the study of the guidebook's effectiveness in the future, such as arranging training sessions, providing instructions on how to use the guidebook properly before home use, and following the results during the week.

The superiority of this guidebook is intensive practice. According to the guidebook schedule, it was found that samples who are able to practice as assigned by the instructions will improve speech and language skills, which can be observed from the grading recorded by the caregivers in the record of results. The outcome had the same direction that when the samples practiced the exercise regularly, they usually had a higher score the next time of practice. Frequently, they were also able to answer the questions correctly with 80% accuracy, so they could start the next level of exercise. The caregivers reported that the patients could think of words, interact, repeat more quickly and stutter less. This agrees with the study of Des Roches *et al*.,[30] which stated that the samples that did home practice received more frequent practice, resulting in more changes in speech and language skills than those who only practiced at the speech therapy center. This also agrees with the study of intensive speech therapy by Breitenstein *et al*.,[14] which found that at least 10 hours of intensive speech therapy per week and at least 3 weeks for patients with chronic aphasia helps improve speech and language skills at a significant level, which agrees with the intensity found in this research (Table 4). Additionally, the caregivers found that using the guidebook could facilitate home practice without having to come up with the stimulus themselves; they can adapt the guidebook on their own for home practice in the future. This aspect agrees with the study of Kurland, Wilkins and Stokes,[29] which showed that the samples are satisfied with how freely they can choose time and exercises and how conveniently they can do home practice, without going to the speech therapy center. However, intensive speech therapy in the guidebook caused more fatigue than usual practice and this condition affected the effectiveness of the exercise. To clarify, the subjects might not cooperate well in the tired condition. Thus, the caregivers were unable to train and utilize the guidebook effectively. This agrees with the study of Gunning *et al*.[28] which found that intensive practice causes more fatigue and suggests that the patients should rest and get support from family.

Although the caregivers are satisfied with the guidebook, there are a few suggestions that the researchers should take into consideration. In the design, the caregivers want the size of the guidebook to be reduced. As the current size is equal to A4, which cannot be stored in a pocket. Some caregivers want the guidebook to be smaller so it can be portable but still keep the quality of the pictures. In the content, they want the guidebook to correct the typos so that reading can be more fluent, and adjust the scientific descriptions to be more concise because there are detailed descriptions of the scientific theory in the current version, and the caregivers feel that there are too many words, making it uncomfortable to read. It also includes the suggestion to add the topics of the practice, such as practice that exercises the speech organs or the articulation for vowels, which would be useful for those who are still not fluent in speaking or unable to utter words. All of the suggestions will be collected by the researchers to improve and modify the guidebook in the future.

## Conclusion

The study of the explicit problems during the use of the basic speech and language therapy guidebook for aphasia patients to improve language and speaking skills, which the researchers studied for the development of the interview form concerning the problems found during use and satisfaction survey, found that the content validity of 2 instruments was at an impressive level. The results from the trial phase of using the guidebook by caregivers 90 minutes a day for a week with 3 Aphasic clients found that repetitive practice as assigned by the guidebook yielded benefits for the samples in terms of speech and language skills improvement. It also benefits the caregivers who do the home practice. However, it can cause more fatigue compared to usual practice. During the use of the guidebook, the caregivers reported that difficulties and problems occurred during the use process, most of which were from a lack of understanding about usage. The researchers have acknowledged the problems during the guidebook use and devised precautionary measures from the study in this research. The suggestions will be adapted for guidebook improvement in the future.

## Acknowledgments

In publishing this research, only the results from the study of the development process of the instruments are used in the research and the guidebook trial, which will aid the researcher to reveal the tendencies of the results from the guidebook and the problems that occurred during usage. This will help devise precautionary measures for the next phase of the study, which will involve the study of the effectiveness of the guidebook as well as the satisfaction of caregivers towards the guidebook.

## Conflicts of interest

The authors declare no conflicts of interest in the execution of this work.

*1.Ethics approval*

This research gained ethical approval from the Faculty of Associated Medical Sciences, Chiang Mai University (AMSEC-64EX-116) in order to study humans. All samples and participants received all necessary information related to the research and informed written consent was gathered before enrolling in the research.

*2.Recommendations for future directions*

A further study should be conducted with the satisfaction of the guidebook in a multi-category, e.g., the design, content, activity of exercise, and overall satisfaction in order to revise the better version of the guidebook in the future.

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# Effects of COVID-19 pandemic on parents of children with special needs' stress in Chiang Mai, Thailand

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**ABSTRACT**

**Background**: The coronavirus disease 2019 (COVID-19) is spreading over the world. Thailand is another country that has suffered greatly as a result of the pandemic. Recognizing the stress levels of parents of children with special needs will lead to appropriate support.

**Objectives**: The purpose of this study was to examine the stress levels of parents of children with special needs in Chiang Mai province during COVID-19.

**Materials and methods**: An exploratory study was conducted from August 2020 to March 2021. The study subjects comprised parents of 162 students with visual, auditory, intellectual, and physical dysfunctions. The Suanprung Stress Test (SPST-20) and a questionnaire were used to collect data. The data was analyzed using descriptive statistics.

**Results**: The results indicated that most parents of children with special needs had moderate to high stress levels, at 38.89% and 25.93%, respectively. Parents of children with hearing and visual impairments had the greatest severe stress levels of any group of parents, at 21.43% and 36.37%, respectively. Most parents (60.49%) perceived that their stress had changed from their everyday circumstances; however, 58.65 percent agreed that their stress in this situation did not affect their caring for their children.

**Conclusion**: Parents of children with hearing and visual impairments experienced the greatest severe stress levels due to the pandemic. Psychotherapy and counseling that focus on mental health should be considered.

## Introduction

The coronavirus disease 2019, also known as COVID-19, was named by the World Health Organization as a virus discovered in 1965.[1] The epidemic of COVID-19 started in Wuhan, Hubei Province, China. COVID-19 is spreading over the world. The WHO said that after the virus spread all over the world, it was a worldwide emergency.[2] Thailand is another country that has suffered greatly as a result of the pandemic. In July 2022, the WHO reported about 4,550,924 confirmed cases of COVID-19 and 30,907 deaths in Thailand.[3]

A sudden change in the situation affects mental health problems, particularly for parents.[4-6] Studies found that parents of children with special needs are more stressed than parents of typically developing children.[7-9] Furthermore, parents of children with special needs and children who

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show unusual patterns of development due to a disability or a persistent medical condition have been found to have high levels of psychological stress.[10-12] Recognizing the stress levels of parents of children with special needs will lead to appropriate support.

Families with children with special needs face greater difficulties. This is due to the fact that these children not only face a higher risk of getting sick but also an interruption of critically needed therapeutic services, exclusion from school, and other forms of prejudice.[13] Furthermore, since these children require one-on-one assistance, parents must be extremely cautious regarding the health of the individual who works directly with their children. This can be both emotionally and physically draining. Also, the children needed more attention and supervision from their parents than usual.[14]

High levels of parental stress may have a negative impact on the functioning of a child with developmental disabilities.[15-16] Parental distress harmed caregivers' capacity to supervise their children at home and implement appropriate actions.[4] The psychological well-being of parents is regarded as especially significant. Therefore, the child with developmental disabilities is not the only one who has an impact on their lives and the dynamics of the family. The family has an impact on the child's development, both positively and negatively. Professionals must be aware of and cope with parental stress. Therefore, the purpose of this study was to examine the stress levels of parents of children with special needs in Chiang Mai Province during COVID-19.

## Materials and methods

The current study was an exploratory study on the parents' stress among parents of four types of special-needs students, including those with visual impairments, hearing impairments, intellectual disabilities, and physical disabilities, in Chiang Mai, Thailand. These students attended a disability-specific school under the Office of Special Education Administration, including the Northern School for the Blind, Anusarn Soonthorn School, Kawila Anukul School, and Srisangwan School. All of the population enrolled from August 2020 to March 2021.

### Participants

Purposive sampling was performed. The participants included 162 parents from one province, Chiang Mai, in Thailand. The inclusion criteria were: 1) parents who have children studying in the academic year 2020; and 2) parents must be able to read and write and have a good understanding of the Thai language. See Table 1 for detailed information about the parents' demographics as well as their children with special needs.

### Measures

The research tools consisted of two parts to collect data. The general information was used for collecting children's and parents' demographic information. The Suanprung Stress Test (SPST-20) was used to collect parents' stress.[17] It consisted of 20 items in a Likert scale format of 1 to 5: 1 = no stress, 2 = mild stress, 3 = moderate stress, 4 = high stress, and 5 = severe stress. The categories of stress levels were 0-23 = mild stress, 24-41 = moderate stress, 42-61 = high stress, and 62 = severe stress. The items were divided into physical, psychological, and financial stress responses. It is a valid and reliable instrument for measuring stress levels (IOC =0.78; Cronbach's alpha =0.94).[18]

### Procedure

This study was endorsed by the Human Research Ethics Committee, Faculty of Associated Medical Sciences, Chiang Mai University, by certifying the research project Document No. 431/2020. The researcher distributed questionnaires to parents who agreed to participate in the research by bringing the questionnaire to the school administration. When parents were interested in participating in research, they signed their names on the consent form, filled out the questionnaire, and returned it to the administrative office or the class teachers. The time spent collecting data was one month. The data were analyzed with descriptive statistics.

## Results

Table 1 illustrates the demographic data of the parents and children. The majority of the parents, 68.52%, were female, with an average age of 43 years and 5 months. 67.28% had married status, and 30.24% had a high school education. Of 77.78%, there were single families, 91.98% had one child with disabilities, and 39.51% had an average family income per month of 5,000-10,000 Baht. For children, 51.23% were male and 48.77% were female, with an average age of 12 years and 7 months. Most of the children, 64.81%, can support themselves in all daily activities.

In addition, the overall stress levels of parents of children with special needs during COVID-19 were moderate (38.89%). When classified by type of child with special needs, parents of children with visual impairment, hearing impairment, and intellectual disabilities had the highest stress levels at the moderate level: 40.00, 42.86, and 41.46%, respectively. However, parents of children with physical disabilities had higher stress levels than others at the high level, at 44.74%, as shown in Table 2.

**Table 1** Demographic data of parents and children (N=162).

| | N (Percentage) |
|---|---|
| **Gender** | |
| Male | 51 (31.48) |
| Female | 111 (68.52%) |
| **Age** | |
| 20-30 | 13 (8.02%) |
| 31-40 | 50 (30.86%) |
| 41-50 | 61 (37.65%) |
| 51-60 | 29 (17.90%) |
| 61-70 and above | 9 (5.56%) |
| **Education level** | |
| Below high school | 71 (43.83%) |
| High school or diploma | 54 (33.33%) |
| Bachelor's degree | 33 (20.37%) |
| Above undergraduate | 4 (2.47%) |
| **Marital status** | |
| Single | 17 (10.50%) |
| Marriage | 109 (67.28%) |
| Widow/divorce | 36 (22.22%) |
| **Occupation** | |
| Unemployed | 8 (4.94%) |
| Housewife/husband | 17 (10.49%) |
| Civil servant/ state enterprise employee/ private company employees | 106 (65.43%) |
| Self-employed/ agriculture/ other | 31 (19.14%) |
| **Family role** | |
| Mother/father | 140 (86.41%) |
| Other (e.g., cousin, caregiver and adoptive parent) | 22 (13.59%) |
| **Family characteristic** | |
| Nuclear family | 126 (77.78%) |
| Extended family | 34 (20.99%) |
| Foundation | 2 (1.23%) |
| **Family monthly income (Baht)** | |
| Lower than 5,000 | 29 (17.91%) |
| 5,000-10,000 | 64 (39.51%) |
| 15,000-20,000 | 38 (23.45%) |
| More than 20,000 | 31 (19.13%) |
| **Number of children with disabilities** | |
| 1 | 149 (91.98%) |
| 2 | 12 (7.40%) |
| More than 2 | 1 (0.62%) |
| **Child's disability type** | |
| Intellectual disability | 41 (25.31%) |
| Visual impairment | 55 (33.96%) |
| Hearing impairment | 28 (17.28%) |

**Table 1** Demographic data of parents and children (N=162). (continued)

| | N (Percentage) |
|---|---|
| Physical impairment | 38 (23.46%) |
| **Age of Children** | |
| 5-10 | 51 (31.49%) |
| 11-15 | 65 (40.12%) |
| 16-20 | 44 (27.16%) |
| 21-25 | 2 (1.23%) |
| **Activities of daily living (self-help)** | |
| No needs assistance and constant supervision for ADL task | 105 (64.81%) |
| Constant supervision still needed for ADL's | 18 (11.11%) |
| Still needs assistance and constant supervision for ADL task | 30 (18.52%) |
| 24-hour supervision and care | 9 (5.56%) |
| **Changes in parents' activity patterns** | |
| Yes | 98 (60.49%) |
| No | 64 (39.51%) |
| **Stress during the situation affects the children in their care** | |
| Yes | 67 (41.35%) |
| No | 95 (58.65%) |

**Table 2** Frequency, percentage, and parental stress level interpretation. Classified by the type of disabilities of children with special needs.

| Interpretation of stress levels of parents | Number of children with special needs (%) | | | | |
|---|---|---|---|---|---|
| | Visual impairment | Hearing impairment | Intellectual disabilities | Physical disabilities | Total |
| Mild | 8 (14.54) | 3 (10.71) | 6 (14.63) | 5 (13.16) | 22 (13.58) |
| Moderate | 22 (40.00) | 12 (42.86) | 17 (41.46) | 12 (31.58) | 63 (38.89) |
| High | 5 (9.09) | 7 (25.00) | 13 (31.71) | 17 (44.74) | 42 (25.93) |
| Severe | 20 (36.37) | 6 (21.43) | 5 (12.20) | 4 (10.52) | 35 (21.60) |

## Discussion

The purpose of this study was to examine the stress levels of parents of children with special needs in Chiang Mai Province during COVID-19. The results indicated that most parents of children with special needs had moderate to high stress levels, at 38.89% and 25.93%, respectively, as shown in Table 2, which is consistent with the Department of Mental Health survey.[10] It also found that 60.49% of parents had changes in their activity patterns during COVID-19, which could be attributed to the fact that this leads to stress and mental illness and causes a person to adjust suddenly, which is consistent with Fofana *et al*.[19] Therefore, stress among parents of special-needs children during the COVID-19 outbreak necessitates increased attention from mental health practitioners, such as psychotherapy and counseling, to cope with difficulties.[20,21]

Interestingly, when compared to parents with intellectual disabilities and physical disabilities, parents of children with hearing and visual impairments had the greatest severe stress levels of any group, at 21.43% and 36.37%, respectively, which is higher than parents with other types of disabilities. Communication problems could arise for those who have a hearing impairment. Wearing a face mask restricts facial expressions and lip movements, as well as the high-frequency content of sound, which is necessary for speech intelligibility.[22]

The study by Tuz, Aslan, and Yucel found that there were behavioral changes in children with hearing impairment

before and during the pandemic, and parents showed high levels of anxiety.[23] One study found that parents might have anxiety associated with their child's visual condition. The anxiety levels of parents of children with visual impairments were higher than those of normal children.[24] However, it should be noted that our results did not investigate the relationship between the severity of hearing and visual impairments and their impact on parents' stress.

Our results found that the majority of parents (60.49%) reported that COVID-19 did not cause any changes in their activity patterns. Also, the majority of parents (58.65%) reported that stress during the situation did not affect the children in their care. It might be that most of them (65.43%) have job stability; they work as civil servants, state enterprise employees, or private company employees. Hobfoll's theory of the conservation of resources (COR) says that people see work insecurity as the possibility of losing an important resource, which they think would hurt their well-being.[25,26] Therefore, our study assumes that having job stability affects stress, which is one of the aspects that leads to quality of life. Alongside job stability reasons, it might be that the vast majority, 64.81%, were able to support themselves in doing all their daily activities. The study by Wanicharoen found that self-help in daily life was related to the quality of life of caregivers of children with language impairment, including specific language impairment, global developmental delay, autism, intellectual disability, and down syndrome.[27]

It should be mentioned, however, that this research was conducted from August 2020 to March 2021, during the second pandemic phase. In fact, COVID-19 constitutes a challenging period for children and parents. Parents may make some changes to the situation. Similarly, the survey indicated that 90% or more individuals have less panic and can adjust better to the COVID-19 epidemic scenario compared to the first round of the epidemic, according to the Department of Mental Health.[28]

There were several limitations that should be considered. Firstly, it was an exploratory study at the time of specific events during the critical period of the epidemic and was limited to a specific province, which might not represent all Thai parents of special needs children or might not be generalizable to other provinces. There was also no other study on the effects of the COVID-19 pandemic on the stress of parents of children with special needs in other provinces. Future studies will collect and represent data from larger and more diverse populations. Second, this study did not identify any personal or contextual characteristics that may influence the stress of parents of children with special needs. This factor can allow healthcare professionals to identify families that are at risk of adjustment problems at an early stage.

## Conclusion

The current study examined the stress levels of parents of children with special needs in Chiang Mai province during COVID-19. Most parents of children with special needs had moderate to high stress levels. Parents of children with hearing and visual impairments experienced the greatest severe stress levels due to the pandemic. For parents of children with disabilities, approaches such as psychotherapy and counseling that focus on mental health should be considered.

## Conflict of interest

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

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# Correlation of serum protein and lipid profile with OSMF severity among the rural population of Southern India

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**ABSTRACT**

**Background**: Carcinogenesis is mostly observed due to alterations in serum protein and lipid levels.

**Objectives**: In this present study, serum protein and lipid profile are to be evaluated in OSMF patients and compared with various clinical stages of OSMF.

**Materials and methods**: Out of 100 study subjects, 45 OSMF patients were selected as cases; 55 apparently healthy individuals were chosen as controls. The level of serum total protein, albumin, and globulin; high density lipoprotein (HDL), very low density lipoprotein (VLDL), total cholesterol (TC), triglycerides (TG), and low density lipoprotein (LDL) were measured by COBAS-6000 autoanalyzer. The obtained data were processed with an independent sample t-test.

**Results**: Statistically significant reduction in serum total protein and albumin, LDL, HDL, TC, and TG levels were detected in all stages of OSMF patients in comparison with controls, whereas changes in serum VLDL level were not statistically significant.

**Conclusion**: The current study showed significant alterations in serum protein and lipid profile, with advancement in the clinical stages of OSMF. In the future, the alterations in these parameters might possess a diagnostic significance and can be utilized as a biochemical indicator for the detection of neoplastic processes like OSMF.

## Introduction

Oral submucous fibrosis (OSMF) is a disabling, premalignant lesion of the buccal mucosa, with insidious onset.[1] It is predominant in India, Polynesia, Taiwan, Southern China, Sri Lanka, Pakistan, and Bangladesh.[2] In 2002, it is reported by OSMF statistics of the Indian sub-continent that near about 5 million people (a total of 0.5% of the Indian population).[3] In research, a study based on oral precancerous lesions as well as oral cancer among the rural population of India, the rate of OSMF malignant transformation, was observed to be 7.6% over 17 years.[4]

Any part of the buccal cavity, sometimes even the pharynx can be affected by OSMF. It is invariably linked with an inflammatory process of juxta epithelial cells and subsequently fibroelastic alteration of lamina propria leading to the formation of the vesicle with epithelial atrophy, causing stiffness of the buccal mucosa, which results in trismus and difficulty in chewing.[5] The significance of

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OSMF lies in the highest rate of malignant transformation and its restriction during the opening of the mouth. The prevalence of OSMF in India is found to be 0.2-0.5%, whereas the malignant transformation rate is about 7-13%.[6,7] Though the causative etiological factor of OSMF is multifactorial, areca nut possesses a pivotal role in the manifestation of the disease.[8,9] The chronic oral irritation due to chewing pan masala and gutkha causes injury, ultimately resulting in prolonged inflammation along with the production of cytokines and finally oxidative damage. Subsequent production of ROS (reactive oxygen species) along with oxidative stress can evoke cell proliferation, and cell aging and ultimately result in cell death, The whole series of events is solely dependent on the magnitude of the production of ROS.[10,11] Aside from that, protein oxidation has a crucial role in oral carcinogenesis.[12,13] Hypoproteinemia, exhibited as cachexia is often observed in oral malignancy.[14] So, the serum protein levels can be implemented as a vital prognostic, as well as, a diagnostic tool for this premalignant OSMF. Lipids are considered the cell membranes' elementary components which perform a wide spectrum of biological events such as the proliferation and growth of normal cells as well as malignant tissues.[13] Very less studies are available to date regarding the correlation of serum protein and lipid profile with the advancement of OSMF stage. Therefore, the study aims to correlate the levels of serum proteins and lipid profiles with OSMF severity.

## Materials and methods

### Source of Data

The current study was carried out at the Department of Biochemistry and Oral Medicine at Panineeya Institute of Dental Sciences and Research Centre, situated in Telangana, India. The study was sanctioned by Institutional Ethics Committee.

### Study participants

A total of 100 subjects were chosen from the Outpatient Department of Oral Medicine for this study. The study subjects were categorized into two groups, such as the case group and the control group. The case group comprises of 45 OSMF patients and the control group of 55 age and sex-matched apparently healthy individuals. The subjects with any associated comorbidities which can affect the level of aforesaid study parameters were not included in this study. The case group was further categorized into stages I, II, III, and IV, according to their initial mouth opening as measured by inter-incisal distance.

### Sample collection

After acquiring institutional ethical committee clearance, and subsequently informed written consent from every subject, a detailed clinical history was noted. After a minimum of 12 hrs fasting the study subjects were called for blood sample collection. Five mL of venous blood was collected under strict aseptic precautions. Then, the collected samples were centrifuged at 3,000 rpm for five minutes to obtain serum.

### Parameters analyzed

The serum levels of TC, HDL, LDL, TG, VLDL, total protein, albumin, and globulin were analyzed using commercially available assay kits of Roche Diagnostics using Cobas 6000 autoanalyzer. The data obtained were processed using SPSS, version 20.0. Mean with the standard deviation were determined for TC, HDL, LDL, TG, VLDL, total protein, albumin, and globulin in the cases, as well as, in the control group.

### Data processing

An Independent sample t-test was implemented to verify the statistical significance of mean differences. The collected data were entered into an Excel spreadsheet and analyzed using SPSS software v20.0.

## Results

As mentioned formerly, A total of 100 subjects were included in the study, out of which 45 were cases and 55 were controls. As far as the clinical staging is concerned, 17 cases (37.78%) were included in Stage I, 11 cases (24.45%) in Stage II, 14 cases (31.11%) in Stage III, and 3 cases (6.66%) in Stage IV. (Table 1).

**Table 1** Comparison of Baseline characteristics: number (%), age, and gender among OSMF subjects.

| | Cases (N=45) | Controls (N=55) |
|---|---|---|
| Mean±SD | 45.60±10.84 | 47.08±9.90 |
| Median (IQR) | 47.00 (36.5-55.0) | 49.00 (41.5-55.0) |
| *p* value[#] | 0.586 | |
| **Age group** | **Cases N (%)** | **Controls N (%)** |
| <30 years | 4 (8) | 3 () |
| 30-40 years | 13 (30) | 10 (18) |
| >40 years | 28 (62) | 42 (76) |
| *p value*[Ω] | 0.308 | |
| **Gender** | **Cases N (%)** | **Controls N (%)** |
| Male | 24 (53) | 26 (47) |
| Female | 21 (47) | 29 (53) |
| *p* value[*] | 0.548 | |
| **Stage of OSMF patients** | **Number** | **%** |
| Stage I | 17 (8M+9F) | 37.78 |
| Stage II | 11 (7M+4F) | 24.45 |
| Stage III | 14 (8M+6F) | 31.11 |
| Stage IV | 3 (1M+2F) | 06.66 |
| **Total** | 45 | 100 |

*IQR: inter-quartile range, M: male, F: female, [#]Mann-Whitney U test was used to calculate p value ,[Ω]Fischer exact test was used to calculate p value, *Chi-square test was used to calculate p value.*

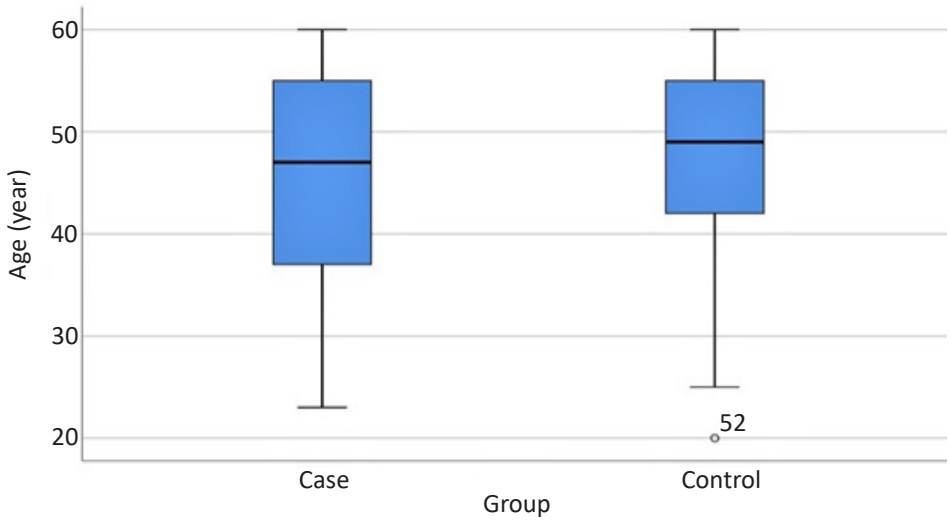


***Figure 1.*** *Box plot showing a comparison of median age between the case and the control group.*

Table 2 represents, the mean serum total protein level (5.83±0.36 g/dL) and albumin level (3.21±0.45 g/dL) among OSMF cases, which was found to be significantly less in comparison with the total protein level (7.42±0.67 g/dL) and albumin level (4.50±0.51g/dL) in the control group, at $p<0.001$ each. But the mean serum globulin level among the cases (3.87±0.54 g/dL), was found to be raised significantly, in comparison with the control group (3.20±0.43 g/dL) at $p<0.001$. Here, p values were calculated by the Mann-Whitney U test. The mean serum total protein, albumin exhibited a decreasing trend, with the advancement of the clinical staging of OSMF. In contrast, the mean serum globulin level exhibited a reverse trend among different stages of OSMF [Table 2]. The A/G ratios were found to be 0.91±0.07, 1.04±0.21, 0.88±0.56, and 0.69±0.54 in OSMF cases, belonging to Stage I, II, III, IV respectively.

**Table 2** Mean±SD of serum protein parameters (g/dL) among OSMF subjects.

| Group | Total protein | Albumin | Globulin |
|---|---|---|---|
| Controls (N=55) | 7.42±0.67 | 4.50±0.51 | 3.20±0.43 |
| Cases (N=45) | 5.83±0.36 | 3.21±0.45 | 3.87±0.54 |
| *p* value | <0.001* | <0.001* | <0.001* |
| **OSMF stage** | **Total protein** | **Albumin** | **Globulin** |
| Stage I | 5.98±0.56 | 3.42±0.57 | 4.13±0.31 |
| Stage II | 4.94±0.38 | 3.58±0.39 | 3.50±0.75 |
| Stage III | 5.01±1.16 | 3.22±1.13 | 3.76±1.47 |
| Stage IV | 4.89±1.58 | 2.90±1.46 | 4.38±0.98 |

**Significant*

Table 3 represents the mean with standard deviation (SD) and *p* value of all serum lipid parameters, those were analyzed and compared. Significantly lower levels of serum TC (156.76±20.25 mg/dL), HDL (34.16±7.06 mg/dL), and LDL (85.69±17.58 mg/dL) were observed among OSMF cases, as compared to the control group (TC=185.35±13.87 mg/dL, HDL=43.60±6.29 mg/dL, LDL=116.36±21.56 mg/dL). Statistically significant differences were observed for TC, HDL, and LDL with $p<0.001$ each. While, the mean value of serum TG (118.02±17.02 mg/dL) and VLDL (24.28±4.41mg/dL) among cases compared with controls (TG=124.44±11.01 mg/dL), and (VLDL=24.76±4.67 mg/dL), p value found to be 0.025 and 0.601 respectively, a statistically significant difference was not found in the later ($p>0.05$). Here, p values were calculated by Mann-Whitney U test (Table 3).

The mean serum Total Cholesterol, HDL and LDL levels exhibited a markedly decreasing trend along with the advancement of the clinical staging of OSMF, unlike the mean serum TG and VLDL levels. The mean serum Total Cholesterol, HDL and LDL levels exhibited a markedly decreasing trend along with the advancement of the clinical staging of OSMF, unlike the mean serum TG and VLDL levels (Table 3).

**Table 3** Mean±SD of serum lipid parameters (mg/dL) among OSMF subjects.

| Group | Total protein | Triglyceride | HDL | LDL | VLDL |
|---|---|---|---|---|---|
| Controls (N=55) | 185.35±13.87 | 124.44±11.01 | 43.60±6.29 | 116.36±21.56 | 24.76±4.67 |
| Cases (N=45) | 156.76±20.25 | 118.02±17.02 | 34.16±7.06 | 85.69±17.58 | 24.28±4.41 |
| *p* value | <0.001* | 0.025* | <0.001* | <0.001* | 0.601† |
| **OSMF stage** | **Total protein** | **Triglyceride** | **HDL** | **LDL** | **VLDL** |
| Stage I | 159.39±9.13 | 36.63±3.34 | 98.38±9.91 | 119.7±17.33 | 33.22±2.93 |
| Stage II | 160.38±6.07 | 36.94±3.89 | 98.39±4.87 | 129.26±10.64 | 25.07±1.93 |
| Stage III | 147.60±10.55 | 38.98±3.44 | 85.32±19.19 | 141.91±35.12 | 28.32±7.01 |
| Stage IV | 145.93±6.47 | 32.85±3.27 | 84.52±3.94 | 140.69±19.73 | 28.26±4.10 |

**significant, †not significant, HDL: high density lipoproteins, LDL: low density lipoproteins, VLDL: very low density lipoproteins.*

## Discussion

The fragmented molecule with one or more unpaired electrons and having the capacity of independent existence is termed a free radical. At a higher concentration, free radicals interact with macromolecules at intracellular levels like lipids, carbohydrates, proteins, and DNA which initiates carcinogenesis as well as inflammation.[15] During the pathogenesis of cancer, an important role is played by protein oxidation as seen in the case of premalignant OSMF, the protein level is decreased.[12,13] In oral cancer, free radicals play a vital role as an etiological factor that damage's the tissues and such damage is accentuated by the regular habit of areca nut and tobacco. Such habits are rampant among Asians, irrespective of age and sex.

In our present study, low serum protein level among OSMF cases is observed, which is statistically significant. Similar findings were observed by Patidar *et al*, Rajendran *et al*., and Dawood *et al*. among cases and controls, whereas Chandran *et al*. study findings differ from our result. [13,16-18] In oral malignancy, the rise in the level of serum protein levels may be due to inflammatory reactions.

Comparing on the intergroup basis for the level of serum albumin, a statistically significant ($p$>0.05) value was found and our study result was in accordance with Singh *et al*. Nayyar *et al*. Chandran *et al*. and Rajendran *et al*.[12-14, 17] While the rise in the level of serum globulin among OSMF cases can be attributed to the action of acute phase reactant and our study is in concordance with Dawood *et al*.[18]

Lipids are the major and essential components of a cell membrane that has different biological functions such as maintaining the functional and structural integrity of all biological membranes, as well as supporting in division and growth of the cell that may be a malignant or normal tissue.[19] Many studies have proved that various esophageal, as well as head and neck cancer, are directly associated with lowered blood lipid levels.[20-24,19] Researchers have observed that with a lower level of serum cholesterol, there is a high risk of cancer and mortality.[25,26] The inverse association of the incidence of cancer and cholesterol concentrations can be explained by three major competing hypotheses such hypothesis one states that before the detection or manifestation of cancer or during the process of cancer low cholesterol value is observed. Another hypothesis suggests that the association of low cholesterol value with cancer is secondary indicating that cholesterol serves as a marker for some other causal variable or set of variables, and the third hypothesis suggests cholesterol values may be decreased preceding the development of cancer and can be causally associated with its occurrence.[19,26] LDL and HDL, are those lipoproteins containing, Cholesterol is an important constituent. Most of the cholesterol is transported in the form of LDL. LDL, the fraction of lipoproteins gets restored in the human body cells as cholesterol. For circulating and metabolizing the LDL level, there is always a requirement for LDL receptors. In order to lower the serum cholesterol level, the high activity of the LDL receptors is essential. In the current study, it is observed, the patients suffering from OSMF have a significant decrease in LDL and serum cholesterol levels, this may be due to the effect of a disease that is based on cholesterol utilization for membrane biogenesis.

## Conclusion

The current study demonstrates the presence of an inverse relationship between serum proteins (total protein and albumin) and serum lipids (total cholesterol, HDL, and LDL) with the oral precancerous state of OSMF. The initial development that arises in the neoplastic cells can be studied from the status of reduced serum protein and lipid levels, which can be used as a marker or indicator. The difference in lipid and protein levels may be applied as a diagnostic or prognostic biochemical marker of malignant and premalignant oral lesions.

## Limitations

The current study findings are limited to a small-scale population, so in order to validate the study the sample size may be increased or may be conducted with a large population. An advanced and in-depth study on the alteration of serum protein and lipid levels can be carried out by taking various cancerous and precancerous lesions among a larger population. Correlation with epithelial dysplasia has not been included in the present study.

**Conflict of interest**

Nil

**Funding Source:**

Nil

**Ethical Approval:**

The study has been approved by IEC of Panineeya Institute of Dental Sciences and Research Centre with approval number PMVIDS&RC/IEC/PHD/PR/0064-15.

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# Traditional umbrella painting activity improved upper extremity function and decreased depression in stroke survivors in Northern Thailand

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**ABSTRACT**

**Background**: Upper extremity (UE) dysfunction and depression often occur after a stroke. Strategies to promote UE function and reduce depression require task-related training.

**Objectives:** This study aimed to investigate the effectiveness of conventional rehabilitation combined with traditional umbrella painting activity on UE function and depression in stroke survivors.

**Materials and methods:** Twenty-two participants from rehabilitation units in Chiang Mai province, northern Thailand, were recruited according to the inclusion criteria and divided into experimental and control groups of 11 each. The experimental group received conventional rehabilitation combined with traditional umbrella painting activity for 140 minutes a day, three days a week, for a period of eight weeks, while the control group received conventional rehabilitation alone for the same amount of time. The instruments used were: 1) the Functional Test for Hemiplegic Upper Extremity (FTHUE) - Thai version; and 2) the Patient Health Questionnaire (PHQ-9)-Thai version. Statistics used were the Wilcoxon Signed Ranks test and the Mann-Whitney U test.

**Results:** The UE function was significantly higher at the post-test than at the pre-test ($p$<0.004) with a large effect size (d=1.59), and depression was significantly reduced at the post-test compared to the pre-test ($p$<0.003) with a large effect size (d=1.62), whereas the control group did not change. However, the function of UE and depression in these participants were not significantly different between the two groups both before and after the intervention ($p$<0.05).

**Conclusion:** Findings indicate that conventional rehabilitation combined with traditional umbrella painting activity appears to be more effective for improving UE function and decreasing depression in stroke survivors than conventional rehabilitation alone. This can be an alternative treatment for occupational therapists in their clinical practices.

## Introduction

Stroke is the leading cause of death[1, 2] and long-term disability in those who survive.[1] In 2012, stroke mortality was 30.7 per 100,000 people in Thailand,[1] and this increased to 44.8, 47.8, and 52.8 in 2014, 2017, and 2020, respectively.[3,4] In 2016, the total recorded incident rate of stroke was 451.4 per 100,000 people, which increased to 467.5 in 2017, 506.2 in 2018, and 542.5 in 2019.[5] Approximately 90% of stroke victims suffer from a sequela of stroke, mainly weakness of the muscles and

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sensory deficits on the affected side, which leads to poor performance in the upper extremity (UE) function for these individuals.[6,7]

Loss of UE control is frequently found after a stroke. More than 70% of clients who have suffered a stroke have some degree of UE dysfunction[8] due to muscle weakness, spasticity, limited joint range of motion (ROM), pain, etc.[7,9] Poor UE function obstructs the ability of stroke survivors to perform activities of daily living (ADL), work, and recreational activities and therefore needs sufficient care. Brunnstrom defined the six stages of motor recovery after stroke in UE as follows: stage 1 is flaccidity; stage 2 is spasticity developing with involuntary movement; stage 3 is a voluntary movement with synergy and spasticity; stage 4 is decreased synergy and the ability to initiate joint isolation movement; stage 5 is the disappearance of basic synergy and the ability to perform good joint isolation movement, and stage 6 is voluntary movement near normal.[10] Brunnstrom also detailed six stages of hand function after stroke as follows: stage 1: flaccidity; stage 2: little or no active finger flexion; stage 3: mass grasp but no voluntary finger extension; stage 4: lateral prehension and semi-voluntary finger extension; stage 5: palmar prehension, possibly cylindrical and spherical grasp; voluntary mass extension of digits; and stage 6: complete control of all prehension types.[10] A therapist can set the milestone to see a progression of clinical trials following levels of motor recovery.

Depression is a substantial consequence of a stroke. The incidence of depression in stroke survivors is 35%.[9] Post-stroke depression (PSD) delays the effects of therapy and slows down the recovery process due to the patient's resignation from rehabilitation and a lack of motivation to regain physical fitness.[11] Depression causes neglect of self-care, physical inactivity, and substance abuse in stroke survivors.[12]

Art and craft can promote physical and mental health, self–esteem, and enjoyment gained from creating a useful or decorative item with the hands of participating clients.[13] Kim *et al*.[14] conducted a study on the effectiveness of art therapy on rehabilitation outcomes in a stroke survivor, which revealed that the subject showed improvement in emotional status and motor function after a 10-week, twice-weekly intervention program.

Morris *et al*.[15] explored opinions on participation in visual arts programs among 11 stroke survivors and three artists during inpatient rehabilitation to identify benefits and potential mechanisms of action. The results demonstrated that the creation of art facilitated social interaction and provided enjoyment and distraction from a stroke. Research on creative art therapy to improve treatment in patients with neurological diseases found that art can reduce depression and protect against isolation and hopelessness.[16,17] However, most of these studies used multiple and general arts as treatment media for the participants.

The traditional umbrella or Bo Sang umbrella (Figure 1) is a hand–made bamboo umbrella and parasol at Bo Sang Umbrella Village, Chiang Mai Province, northern Thailand. It is a long-standing tradition, a beautiful culture, and a spiritual benefit. Until it has become a traditional umbrella-making community, where Thai people in Bo Sang

***Figure 1*** *Traditional (hand-made) umbrella painting.*

Umbrella Village and the surrounding area earn a good living, a traditional umbrella is not only useful in everyday life; it is also attractive and colorful. These are attractive to tourists, and many of them buy these hand-made umbrellas as souvenirs.[18,19]

There are four steps to making an umbrella:[19]

1) Make the umbrella frame using bamboo as the umbrella strips and softwood for the cap and shaft.

2) Make the canopy by laying Sa paper, which is made of Mulberry bark, on top of the umbrella ribs and gluing it with latex.

3) Paint the background of the Sa paper canopy, and

4) Draw pictures on the painted Sa paper canopy.

Through activity analysis, steps 3 and 4 are appropriate for stroke clients whose muscle recovery stage in their arms and hands is sufficient to move and hold painting equipment. Painting the background and drawing pictures on the umbrella canopy in steps 3 and 4 can increase the number of repetitive movements, which fundamentally improves arm and hand function in stroke patients.[20]

The present study aims to examine the effect of a combined treatment of conventional therapy with traditional umbrella painting activity on UE function and depression in stroke survivors in Chiang Mai Province, Thailand.

## Materials and methods

This study was a pre-test and post-test control group research design.

### *Participants*

The sample size was calculated using G*Power 3.1, α err prob =0.05, power (1 - err prob) =0.80, and the average effect size from two previous similar studies by Song & Hwang,[21] and Pongtham *et al*.,[22] =1.125. Zones of desired or acceptable effects were the intermediate (d=0.45-0.75) and large effect sizes (d>0.75), which indicated a practically significant level of the intervention program and a good outcome of the study.[23] The larger the effect size of similar previous studies, the fewer subjects are required in the present study. The appropriate sample size, as calculated, was 18 subjects. Researchers added 20% of the participants in anticipation of some dropping out during the study. Therefore, the total number of participants was 22.

Following inclusion criteria, we recruited stroke participants who received services in eight community rehabilitation centers in Chiang Mai province. Due to the pandemic of COVID-19 during the time of the research project, we could not recruit as many participants as 22 at the same time to randomly select them into the experimental and control groups. As a result, we sequentially allocated the participants into experimental and control groups in a ratio of 1 to 1 as soon as we had the subjects that met the inclusion criteria. The diagram of the recruitment and allocation of participants to the study is shown in Figure 2.

The inclusion criteria were as follows:

1) clinical diagnosis of a first stroke.

2) age between 20 and 70 years old.

3) hemiparesis for 6-24 months after onset.

4) the ability to reach Brunnstrom stages III to V in the proximal and distal arm;[10]

5) no significant cognitive deficits, as determined by the MSE T10,[24] with scores of at least 15 for uneducated people; a score of at least 18 for persons finished with

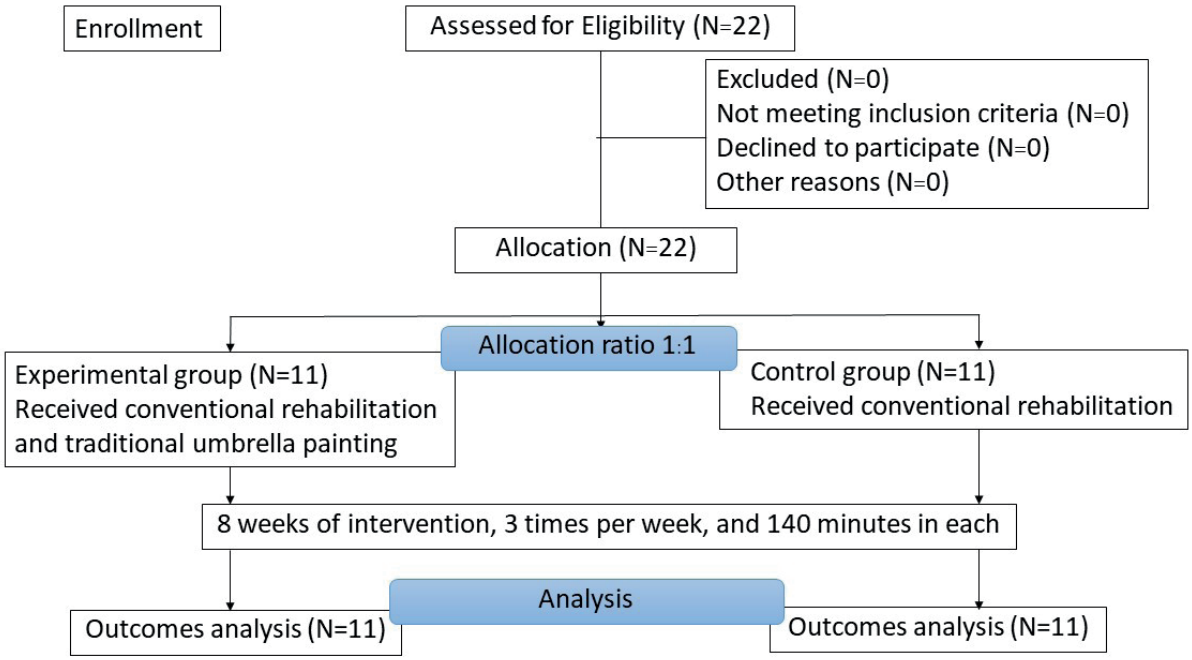


***Figure 2*** *Diagram for the recruitment and assignment of participants to the study.*

elementary school, and scores of at least 23 for individuals finished with secondary school and above.

6) no excessive spasticity in the affected UE (Modified Ashworth Scale score less than or equal to 2 in any joint)[25] that could prevent functional movements;

7) nobody unilateral neglect (assessed by giving the participants a blank page of paper and telling them to draw a clock);

8) risk of depression (measured by the Two Questions: Screening Test for Depression),[26] persons who answer "yes" to one or both questions would be classified as at risk of depression;

9) no other neuromuscular or orthopedic diseases; and

10) willing to participate in the study.

The withdrawal criteria were:

1) subjects who participated in the intervention program less than 80% of the time.

2) subjects who joined another institute's rehabilitation at the same time as receiving the intervention program in the present study.

All participants signed informed consent forms before being involved in the research project. The present study was approved by the Human Research Ethics Committee, Faculty of Associated Medical Sciences, Chiang Mai University, ethics clearance number 122/2563, and registered at the Thai Clinical Trials Registry (TCTR20220706002).

***Intervention***

All participants in the experimental and control groups received 24 treatment sessions with a duration of 140 minutes per session, three times a week for eight consecutive weeks. Stroke participants in the experimental group received conventional rehabilitation guided by an occupational therapist and physical therapist, who did not receive information regarding the research goals of the present study, for 90 minutes, combined with traditional umbrella painting activity for 50 minutes. The time for the physical therapy (PT) session was 70 minutes, as usual. The period of time for occupational therapy (OT) was also 70 minutes, with 20 minutes spent mostly on activities of daily living (ADL) and 50 minutes for umbrella painting, as this activity was considered a modality that can practice not only UE function but also improve perception and cognitive function in the clients. Participants in the control group received conventional OT and PT from therapists, who had not been informed of the research goals by the research team, for 70 minutes each. The conventional treatment protocol consisted of passive and active ROM in both upper and lower extremities on the affected side; manual therapy techniques; trunk balance training; walking on parallel bars and stairs; exercises with and without resistance; basic activities of daily living (BADL) training; and UE function training designed the movement of the arm and hand to reach out, hold, carry, and release objects.

Motor training for UE function by using traditional umbrella painting activity in the experimental group has designed the movement patterns to be like those in conventional UE training, which includes reaching out for, holding, carrying, and releasing objects.

As Morris *et al*.[15] suggested, health professionals should be concerned with patient levels of motor recovery and the appropriateness of the art provided. If the patients struggled to use their affected arm during art-making, this could reduce the positive impact of art on recovery and cause increased stress. Therefore, the research team analyzed and designed activities that suited the ability levels of participants. Individuals with UE muscle function at stage 3 of Brunnstrom could perform bilateral arm practice using the unaffected and affected hands to hold the large handle paintbrush together and simultaneously paint the colors as a background on the plain Sa paper canopy. After finishing the background painting, participants drew pictures with their unaffected hands, as this activity was quite complicated. Pictures can be large and do not require much precision.

Participants who had their muscle function of the UE at stages 4 and 5 of Brunnstrom could use their affected hands, whether dominant or not, to hold the paintbrushes and paint the background with one hand. However, they could use their unaffected hands to help the affected one draw pictures after a background painting session.

Equipment for traditional umbrella painting consisted of 1) a ready-made unpainted umbrella; 2) acrylic color; 3) paintbrushes, both round and flat types with different sizes; 4) a chair with a backrest but no armrest; and 5) paint jars, which were arranged on top of the appropriate height table. The subjects sat in chairs with hip and knee flexion at 90 degrees and both feet properly positioned on the floor. The unpainted umbrella was placed in front and the paint jars on top of the table were placed on the affected side within reachable range of the subjects. The positions of stroke participants and equipment are demonstrated in Figure 3.

Before starting the umbrella painting session, therapists allowed stroke participants to warm up for 5 minutes by using their unaffected hand to hold the affected one, raise it above the horizontal shoulder line, swing the hand to the left and right sides, and flex and extend the elbow and wrist joints. There are four major movement steps in traditional umbrella painting: 1) holding a paintbrush in hand; 2) reaching out an arm to drop a paintbrush into the paint jar; 3) moving the arm back towards the umbrella canopy; and 4) painting or drawing pictures on the umbrella canopy.

Subjects had one-on-one supervision from an occupational therapist, who was blinded to the research goals during the traditional umbrella painting activity.

***Instruments***

1. The Functional Test for Hemiplegic Upper Extremity (FTHUE)-Thai version. The FTHUE was first developed at Rancho Los Amigos Hospital, USA.[27] It aimed to measure the recovery of hemiplegic UE from nonuse to full hand function. The test comprises 18 activities sequenced in

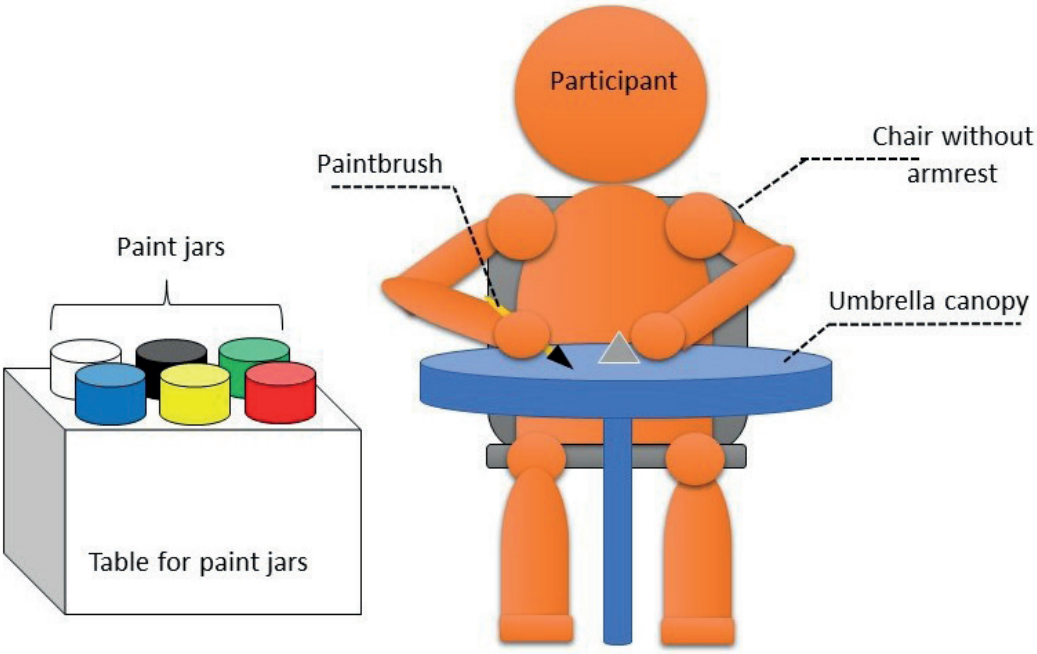


***Figure 3*** *Participant and Equipment Placement.*

a hierarchy of seven functional levels by the degree of difficulty, and the grading is based on a pass-fail basis for activities within each level. It took around 30-45 minutes to complete a single evaluation, which made the test impractical and time-consuming for bedside use. Fong *et al*.[28] has developed the FTHUE Hong Kong version to adapt the instrument to suit people in Asian culture and take less time to administer. There are 14 testing activities, which are sequenced into seven levels of difficulty. The scores are classified from 1 to 7 by difficulty levels in the FTHUE-Hong Kong version, with good psychometric properties, and it took around 10 minutes for the evaluation process.

Pingmuang *et al*.[29] has adapted the FTHUE-Hong Kong version into the Thai language in Thailand. The process involved back-translation, field testing of the pre-final version, and final adjustments. The test items and scoring were still the same, as we have a similar culture. The study of the psychometric properties of 30 stroke participants in Thailand demonstrated that the FTHUE-Thai version has very good internal consistency (r=0.83, $p$<0.01) and excellent inter-rater reliability (r=0.96, $p$<0.01).[29]

2. The Patient Health Questionnaire (PHQ-9)-Thai version: This instrument has been used as a reliable depression screening tool in primary care, demonstrating good sensitivity and specificity for depressive disorder.[30] The PHQ-9 is substantially shorter than most other depression screening measures, with only nine items. PHQ-9 is a self-reported measure based on the 9 DSM-IV criteria for major depressive episodes.[30] It refers to symptoms experienced by patients during the two weeks before answering the questionnaires. The study of the psychometric property of PHQ-9 (Thai version) in 924 patients with depression demonstrated satisfactory internal consistency (Cronbach's alpha=0.79, $p$<0.05) and moderate convergent validity with the gold standard Thai version of the Hamilton Rating Scale for Depression (HAM-D) (r=0.56; $p$<0.001).[30]

Scores for PHQ-9 range from 0 to 3, where 0 stands for "not at all", 1 stands for "several days", 2 stands for "more than half of the day", and 3 stands for "nearly every day". The summed scores for this instrument range from 0 to 27. PHQ-9 can be used as a screening tool with a recommended cut–off score of nine or higher for the diagnosis of major depression.[31]

### *Data Collection*

Two trained research assistants blinded to the objectives of the study, and to which group each participant was assigned (the experimental or control group) collected data within seven days before and after the 8-week intervention program.

### *Data Analysis*

Differences in outcome measures, UE function, and depression within each group were analyzed using the Wilcoxon Signed Ranks test (Table 2). The Mann-Whitney U test was used to analyze the difference in outcomes between the two groups, pre-test, and post-test (Table 3). The statistical significance level for all tests was set at $p$<0.05. The effect size (Cohen's d) was also calculated to demonstrate the practical significance of the study findings.[23] The intermediate or medium (d ranges from 0.45 to 0.75) and large effect sizes (d>0.75) were preferable.[23]

## Results

All participants in both groups completed the intervention programs. The results of the study were demonstrated as follows.

Data from Table 1 demonstrated that the socio-demographic characteristics of stroke participants were not significantly different between the experimental and control groups at baseline at $p$<0.05.

Data from Table 2 demonstrated that the scores of UE function in the experimental group increased significantly after intervention ($p$<0.004) with a large effect size (d=1.59). In addition, the score of depression in the experimental group decreased significantly ($p$<0.003) with a large effect size (d=1.62).

Data from Table 3 demonstrated that the UE function and depression in stroke participants were not significantly different between the two groups both before and after intervention at $p$<0.05.

## Discussion

### *Upper extremity function*

The results of the present study demonstrated that the use of traditional umbrella painting activity combined with conventional rehabilitation produced higher UE function scores for stroke participants in the experimental group after the intervention than at the pre-test significantly ($p$<0.004). This may be because traditional umbrella painting inspired participants to do activities more than the conventional routine treatment alone, as in Thailand, painting an umbrella is a symbol of wealth and joy. In addition, stroke participants saw

**Table 1** Sociodemographic characteristics of participants at baseline.

| Baseline Characteristics | Experimental | | Control | | *p* |
|---|---|---|---|---|---|
| | N | % | N | % | |
| Gender | | | | | |
| Male | 9 | 81.80 | 8 | 72.70 | 1.00[F] |
| Female | 2 | 18.20 | 3 | 27.30 | |
| Age (years) | | | | | |
| 60 and below | 9 | 81.80 | 6 | 54.50 | 0.36[F] |
| Above 60 | 2 | 18.20 | 5 | 45.50 | |
| Time since onset (months) | | | | | |
| 1-12 | 3 | 27.30 | 5 | 45.50 | 0.66[F] |
| 13-24 | 8 | 72.70 | 6 | 54.50 | |
| Education | | | | | |
| None and elementary | 8 | 72.70 | 6 | 54.50 | 0.66[F] |
| Secondary and above | 3 | 27.30 | 5 | 45.50 | |
| Stroke recovery (Brunnstrom) | | | | | |
| Spasticity with synergy (stage 3) | 4 | 36.40 | 5 | 45.50 | 1.00[F] |
| Isolate movement of muscles (stage 4-5) | 7 | 63.60 | 6 | 54.50 | |
| Risk of depression (from 2 questions) | | | | | |
| Answer “yes” to 1 question | 8 | 72.70 | 9 | 81.80 | 1.00[F] |
| Answer “yes” to 2 questions | 3 | 27.30 | 2 | 18.20 | |
| Caregiver | | | | | |
| Family members | 11 | 100 | 10 | 90.90 | 1.00[F] |
| No caregiver | 0 | 0.00 | 1 | 9.10 | |

*Note: N=22 (11 for each group), *p<0.05, [F]Fisher's Exact Test*

**Table 2** Comparisons of UE function and depression in stroke participants within the experimental and control groups between baseline and post-intervention.

| Outcomes | Median (IQR)** | | z | *p* | Effect sizes |
|---|---|---|---|---|---|
| | Baseline | Post-intervention | | | |
| UE function | | | | | |
| Experimental (N=11) | 4 (3, 4) | 5 (4, 6) | -2.92 | 0.004* | 1.59 |
| Control (N=11) | 4 (3, 5) | 4 (3, 6) | -1.41 | 0.16 | 0.63 |
| Depression | | | | | |
| Experimental (N=11) | 12 (11, 14) | 6 (5, 7) | -2.95 | 0.003* | 1.62 |
| Control (N=11) | 12 (9, 15) | 7 (5, 11) | -1.88 | 0.06 | 0.87 |

**p<0.05, **IQR: interquartile range*

**Table 3** Comparisons of UE function and depression in stroke participants between the experimental and control groups at baseline and post-intervention.

| Outcomes | Median (IQR)* | | z | *p* | Effect sizes |
|---|---|---|---|---|---|
| | Experimental (N=11) | Control (N=11) | | | |
| UE function | | | | | |
| Baseline | 4 (3, 4) | 4 (3, 5) | -0.11 | 0.92 | 0.05 |
| Post-intervention | 5 (4, 6) | 4 (3, 6) | -1.74 | 0.082 | 0.80 |
| Depression | | | | | |
| Baseline | 12 (11, 14) | 12 (9, 15) | -0.90 | 0.37 | 0.39 |
| Post-intervention | 6 (5, 7) | 7 (5, 11) | -1.03 | 0.30 | 0.45 |

**IQR: interquartile range*

the results of their productive outcomes at the end of every practice session, which motivated them to put more effort into creating artwork in the next session. The process of painting colors and drawing pictures on the umbrella canopy requires more repetition and variety of motions from the UE than conventional rehabilitation. Additionally, the authors designed the movement pattern specifically for arms and hands to be suitable for motor recovery stages. In subjects whose recovery level was at stage 3 of Brunnstrom, we designed bilateral movement using the affected and unaffected arms to paint the umbrella simultaneously. When the intact arm performs the same spatiotemporal pattern as the affected arm, this could lead to a more efficient performance of the affected arm.[32] Bilateral arm movement demonstrated a good reduction in motor impairment as measured by the Fugl Meyer Assessment.[32] Simultaneous activation of both hands may have rebalanced interhemispheric activation and inhibition, resulting in increased facilitation in the affected hemisphere and positive aftereffects in reducing motor impairment in the affected UE.[33,34] Different art modalities and task-oriented training are also suggested to help stimulate different parts of the brain and enhance the neuroplasticity process, which can help facilitate the recovery process after stroke.[35,36]

For subjects who reached recovery stages 4 and 5 of Brunnstrom, we allowed them to use mainly only their affected limbs because stroke survivors can perform isolated movements of their arms and hands at these levels. The traditional umbrella painting activity encourages the clients to move the UE more repetitively than the routine training, where patients change practice from station to station within a short time. These repetitive movements can enhance the functional ability of the UE in these individuals.[22,32] The results of the present study were per a study by Kim *et al*.,[14] investigated the effectiveness of art therapy on UE function in a stroke case study and found that the score of the UE function, as measured by the Fugl-Meyer, was higher on the post-test than at the pre-test significantly.

However, the comparison of UE function between the experimental and control groups did not show a significant statistical difference post-intervention (Table 3) because stroke survivors in the control group still received a conventional rehabilitation program that helped improve their ability. However, it was not as high as stroke participants in the experimental group.

***Depression***

The current study found that depression in stroke participants in the experimental group was significantly lower post-intervention than at the pre-test ($p$<0.003), while its score did not change in the control group. This indicated that the traditional umbrella painting activity, combined with conventional rehabilitation, could be an effective treatment modality for reducing depression in stroke participants, especially when compared with individuals who received only conventional rehabilitation. The result of the present study was consistent with Kongkasuwan *et al*.,[37] studied the effectiveness of creative art therapy on depression in 118 stroke patients, revealing that subjects who attended an artwork program twice a week for four weeks in addition to conventional therapy experienced significantly lower depression than those who received conventional rehabilitation alone. Furthermore, several studies[15,22,37-41] demonstrated that participating in art activities helped with relaxation, self-esteem, inspiration, and distraction in a stressful rehabilitation environment, which could lead to less depression in stroke patients. Gangwani *et al*.[42] identified factors affecting post-stroke recovery that self-esteem, and motivation could promote good rehabilitation outcomes. Alwledat, *et al*.[38] studied the effects of two sessions per week, 1.5 hours per session, for two weeks of creative art therapy on depression, anxiety, and stress on 85 stroke patients, which demonstrated that art activities could significantly reduce depression, anxiety, and stress in these participants.

However, the comparison of depression between the experimental and control groups did not show a significant statistical difference after intervention (Table 3). The results might be because stroke survivors in the control group still received conventional rehabilitation programs and could meet with therapists and peers who helped lessen depression, but not as much as those in the experimental group.

***Limitations***

All the participants in our study lived in the northern part of Thailand. As a result, a task such as traditional

umbrella painting may specifically inspire these people but not others. There are actually more inspired traditional task-related therapies for people in northern Thailand, for example, Thai silk weaving, wood carving, silver engraving, etc., that should be incorporated into rehabilitation in the next study, and these could add more evidence for therapists to make their decision whether or not to apply task-based treatment into their routine clinical practices.

Another limitation of the study was the small number of participants, as we needed to recruit as many subjects with similar characteristics as possible. Therefore, the present study should be considered an empirical study. Results should be considered carefully, and the findings may not be generalized for use in other regions or countries. The inclusion of a larger sample size in further studies should be considered.

## Conclusion

The findings in the present study demonstrated 2 issues involving UE function and depression in stroke participants: 1)The comparisons of outcomes variables within the group revealed that the conventional rehabilitative approach combined with the traditional umbrella painting would appear to be more effective than conventional treatment alone to improve UE function and decrease depression in stroke survivors, and 2)The comparisons of UE function and depression between the experimental and control groups did not differ both at pre-test and post-test, which indicated that the conventional rehabilitation also improved UE function and alleviated depression but was not as efficient as the combination of traditional umbrella painting activity and conventional rehabilitation.

## Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

## Ethical Approval

Ethical approval was obtained from the Human Research Ethics Committee, Faculty of Associated Medical Sciences, Chiang Mai University, Thailand; project number AMSEC-63EX-013, ethics clearance number 122/2563.

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2. In-text citation: Indicate references by number(s) in the order of appearance in the text with superscript format. Reference numbers are to be placed immediately after the punctuation (with no spacing). The actual authors can be referred to, but the reference number(s) must always be given. When multiple references are cited at a given place in the text, use a hyphen (with no spacing) to join the first and last numbers that are inclusive. Use commas (with spaces) to separate non-inclusive numbers in a multiple citation e.g. (2-5, 7, 10). Do not use a hyphen if there are no citation numbers in between inclusive statement e.g. (1-2). Use instead (1, 2).
3. References list: number the references (numbers in square brackets) in the list must be in the order in which they are mentioned in the text. In case of references source from non-English language, translate the title to English and retain "in Thai" in the parentheses.
4. Please note that if references are not cited in order the manuscript may be returned for amendment before it is passed on to the Editor for review.

## Examples of References list

***Multiple Authors:*** List up to the first 6 authors/editors, and use "et al." for any additional authors.

***Journal Articles (print):*** In case of reference source contains Digital Object Identifier (DOI), retain doi: at the end of reference. Vancouver Style does not use the full journal name, only the commonly-used abbreviation: "Physical Therapy" is cited as "Phys Ther". As an option, if a journal carries continuous pagination throughout a volume (as many medical journals do) the month and/or issue number may be omitted. Allow one space after semi-colon and colon then end each reference with full stop after page number.

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***Book / Chapter in an Edited Book References***

PLEASE be informed that references of books and chapter in edited book should not be include in the research article, but others manuscript categories.

[1] Grove SK, Cipher DJ. Statistics for nursing research: A workbook for evidence-based practice. 3rd ed. St. Louis, Missouri: Elsevier; 2019.

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