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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

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- **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).
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Innovative utilization of compact disc for measuring fast neutron generated from a 10 MV medical linear accelerator

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ABSTRACT

Background: High-energy photons produced from a medical linear accelerator (LINAC) have long been used as one of the most effective ways for treating cancers. During the treatment process, some photo neutrons are unavoidably created by [γ,n] reactions, imposing additional and undesirable dose on a patient. This amount of unplanned dose from photo neutrons can potentially harm the patient as well as medical personnel during the treatment.

Objectives: To develop a methodology for measuring fast neutron dose generated from 10 MV LINAC by employing polycarbonate from base material of compact disk (CDs) and a fast neutron converter.

Materials and methods: The polycarbonate base layer of CDs has been applied to fast neutron dosimetry with nuclear track method by combining with polymethyl methacrylate (PMMA) converter for fast neutrons. A number of CDs badges were irradiated with high energy photon from 10 MV Elekta Synergy LINAC in the solid water phantom at depth of 0, 2.5, 5, 10, 15 and 20 cm then etched with potassium hydroxide ethanol water (PEW) solution that containing with potassium hydroxide, ethanol and water with ratio of 15:45:40. The optimal condition for the chemical etching were found at 60±2 °C, for 14 hr.

Results: Comparison of neutron equivalent doses from measurement of CD track detector and CR-39 track detector has shown that the maximum fast neutron dose equivalent was at depth of 5 cm of phantom. This agreement has confirmed that the CD track detector can be employed to measure fast neutron doses produced from LINAC in an accurate and affordable fashion.

Conclusion: It is confirmed that the CD track detector can be employed to measure fast neutron doses produced from LINAC in an accurate and affordable fashion.

Introduction

Medical linear accelerator (LINAC) is widely considered as one of many standards for cancer treatment. LINAC relies on producing high-energy photon and electron radiations and delivering them to targets. However, photon with energy higher than 8 MeV may activate the target materials by a (γ,n) reaction. Examples of such materials are ^{56}Fe, ^{184}W and ^{208}Pb. These produced photo-neutrons contaminate the LINAC beams and potentially become a cause of second cancer due to high linear energy transfer (LET) which allows photo neutrons to penetrate through medium and to indirectly generate free ions increasing cancer risks.[1-3] Cheol-Soo Park et al. also observed additional radiation dose from fast and thermal photo neutrons generated from a 10 MV LINAC using CR-39 method. It was found that neutron generation increased when a wedge filter was used.[4]

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However, there is no evidence that excessive dose from contaminating photo neutrons is taken into consideration during the radiation treatment planning. It is partly because of complex nature of neutron interactions, suitable neutron dosimeter and radiation measurements for high-energy and fast neutrons.

There were various methods used to measure photo neutron dose generated during radiation treatment with LINAC.[5] Hassan Ali Nedaie et al. used thermo-luminescent dosimeter (TLD) to measure neutron generated from Varian and Elekta LINAC and compared TLD600 and TLD700 measurements with MCNP calculations. The comparison showed that TLD600 and TLD700 were not suitable dosimeters for neutron dosimetry inside LINAC due to extremely high photon flux. They concluded that MCNP was more suitable.[6] MCNP, Monte Carlo N-Particle Transport Code System, is a three-dimension computational transport code that can be used practically for all particles and all energies with all reactions given in a particular cross-section evaluation such as ENDF/B-VI. Applications for the code are quite broad, including neutron dosimetry. MCNP5 development has begun in 1994 as a code merger of MCNP4B[7] and LAHET 2.8[8] and constantly been developed. In addition, the most common neutron measurement method is using neutron detectors, BF_3 or ^{3}He gas-filled proportional counter, ^{6}Li glass scintillator. Most of neutron detectors are only applicable in the thermal region. However, they are made possible for fast neutron detection by incorporating hydrogenous materials in Bonner sphere systems. Activation foil has an advantage that it is transparent to radiation in the treatment field. However, using activation foil comes with an expense for a need of a gamma spectrometry system which is usually not available on site. Solid-state nuclear track detectors (SSNTDs) are a passive method for neutron measurement, which can register charged particles by the neutron-induced damage caused along their interaction path. SSNTDs in the market are called differently, depending on their types and manufacturers. Their backbone materials are made of a variation of plastic polymers such as Cellulose nitrate, Allyl diglycol carbonate, Diethylene glycol bis (allyl carbonate) and Polycarbonate.[9] These plastic polymers are electrical insulators that can readily register ion tracks. However, neutrons cannot leave ion tracks on these plastic polymers as they are neutral particles. The plastic polymers must then be doped with converters before being used as a neutron detector. One of the most common SSNTDs is CR-39 detector due to its ability to measure both thermal and fast neutrons with appropriate neutron converter and chemical etching method. CR-39 detector is responsive to a wide range of neutron energy while it is insensitive to gamma, beta, ultraviolet (UV) and x-ray. In addition, neutron tracks produced in CR-39 detector are easily assessed by a microscope.[9] A need to import CR-39 detector makes it less economical for domestic use. Makrofol-polycarbonate has been a frequently used base material for neutron detectors.[10, 11] and that Makrofol and equivalent polycarbonates are widely used as a basic structural material for compact discs (CDs). An attempt to reprocess used CDs as SSTNDs would make neutron dose assessment more readily available and more economical.

This study was aimed to develop a methodology (or technique) for measuring fast neutron dose generated from 10 MV LINAC, which is the most common model of medical LINACs in THAILAND, by using polycarbonate from base material of CDs and a particle converter. Equivalent doses obtained from the prototype are compared with results from CR-39 neutron detector for evaluating the accuracy and applicability of this technique in the actual cancer treatment and planning.

Materials and methods

Preparation of CDs fast neutron track detector (CDs detector)

The new CDs (Princo, CD-R 700MB 56X) were used in this study. CDs were scraped out of lacquer, acrylic and metal layers and were left with only a polycarbonate layer. CDs were cut into rectangular badges with 2-cm wide and 7-cm long. The rectangular pieces are smeared with neutron converter composing of boron powder. Boron can capture thermal neutrons and release alpha particles through $^{10}B(n,\alpha)^7Li$ reactions and polymethyl methacrylate or acrylic (PMMA), a hydrogenous material, can convert fast neutrons into recoil proton by elastic scattering. Each badge is segmented into four regions with different material modification. The first region is the original CD material without any modification. The second region only has aluminium tape applied on its surface. The first two regions are designed to control regions for this study. The third region has boron converter attached to its surface by aluminium tape. The fourth region has PMMA converter attached to its surface. Cadmium sheet is applied across all regions to make sure that only fast neutrons interact with the badge. This experimental setup is demonstrated in Figure 1.

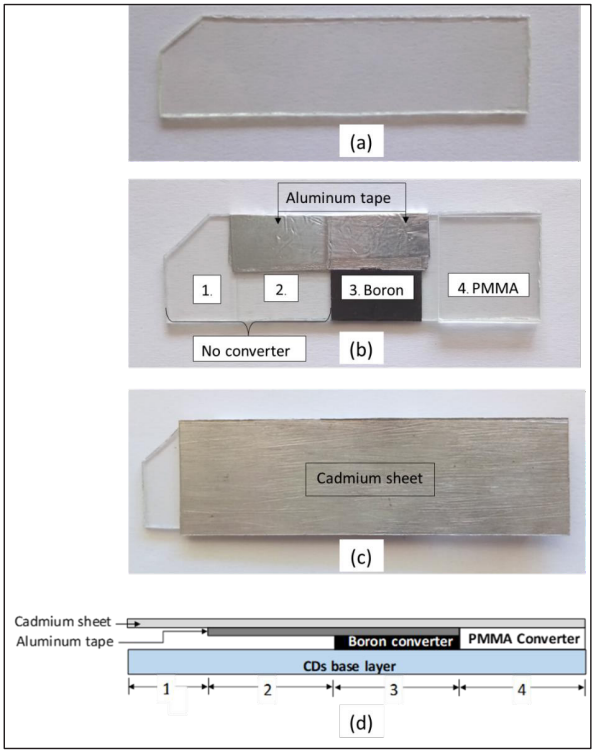

Figure 1*. CDs track detector composed of first layer of a: CDs base (polycarbonate), b: second layer of various neutron convertor, c: covered with cadmium sheet, and d: side view illustrating diagram of CDs track detector.*

Irradiation

The 10 MV Elekta Synergy LINAC (Elekta AB, Stockholm, Sweden) at Lampang Cancer Hospital was used to irradiate a number of CDs badges. The irradiating conditions were set on the LINAC to deliver an irradiation dose of 200 Monitor Unit (MU) or 2 Gy prescribed dose. Note that the gantry and collimator angle were positioned at 0° vertically oriented, pointing down at the couch table and the distance between source and phantom surfaces (SSD) was equal to 80 cm. A 30 cm x 30 cm x 20 cm rectangular cuboid water phantom (GAMMEX RMI®, Middleton, WI, U.S.A) was used in this study and was placed in an irradiation area at position of isocenter with radiation field size as 10 cm x 10 cm. The CD badges were placed at five different depths: 0, 2.5, 5, 10 and 20 cm from the phantom surface as shown in Figure 2. The phantom was exposed to the radiation for three times.

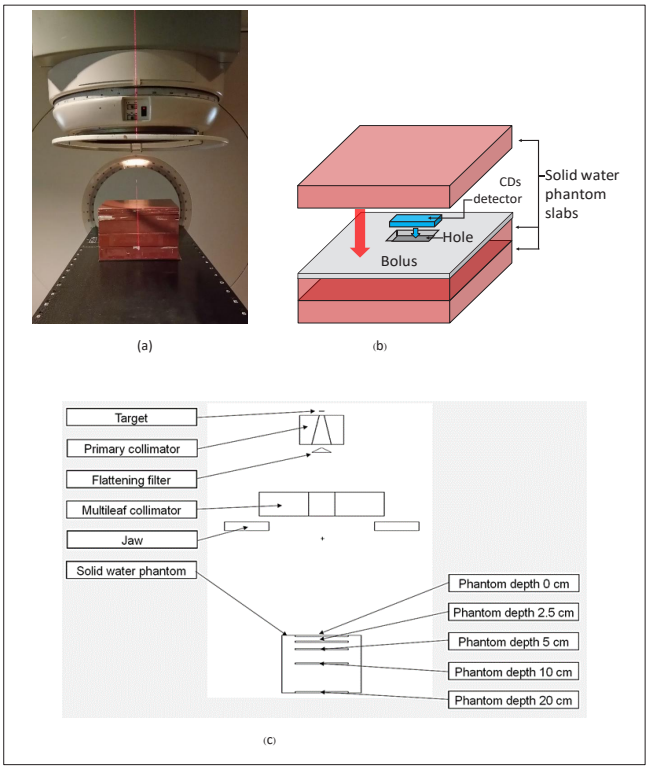

Figure 2. *Irradiation set up (a) in room with (b) hole of bolus, and (c) side view illustrating diagram of irradiation set up.*

Chemical etching on CDs detector

An etching chemical for polycarbonate track detectors used in this study was a mixed solution called PEW[12, 13] which was composed of 15%, 40% and 45% of potassium hydroxide (KOH), ethanol (C_2H_5OH) and water (H_2O), respectively. After irradiations, the CDs track detector badges were etched in PEW solution at 60±2 °C, 70±2 °C and 80±2 °C. The etching time was set from 0 to 24 hours. After etching, the badges were washed with 56 % ethanol and deionized (DI) water, and subsequently dried in dry (or dehumidified) air at room temperature. Then the etched track (etch pit) images generated on CDs track detector at position of 4. Five randomly chosen areas of 2.65 mm^2 from PMMA converter were counted for several tracks using a digital microscope with 100X magnification. Subsequently, the track density was calculated to find the most optimal chemical etching conditions.

Fast neutron dose calibration

The CDs track detector badges were irradiated with the neutron irradiation facility equipped with a 50 Ci ^{241}AmBe neutron source at Thailand Institute of Nuclear Technology or TINT. The irradiation times were adjusted to achieve neutron doses ranging from 500 µSv to 100 mSv. The experimental setup was shown in Figure 3. After irradiation the badges were etched with optimal conditions and track densities were subsequently calculated.

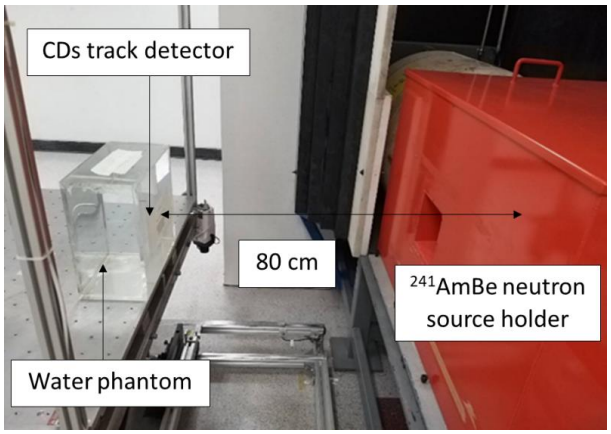

Figure 3. *Fast neutron dose calibration set up.*

Comparison of fast neutron equivalent dose from CDs track with CR-39

The statistical method of independent t-test with *p* value equal to 0.05 was used to compare calculated neutron equivalent dose of CDs detector and CR-39 detector.

Results

Optimization of chemical etching parameters of CDs detector

Track densities as a function of chemical etching time at various PEW solution temperatures are shown in Figure 4. At temperature of 60 °C, the track densities gradually increase with increasing etching time and reach the maximum at about 14 hours. Similar behaviors were observed in case of 70 °C and 80 °C when the maximums were found at 10 hours and 20 hours, respectively. While longer etching time is needed to retrieve the tracks formed at greater depth, the tracks formed near the surface are worn away in the process. As a result, the track densities start to decline as the etching times increase. It is important to point out the effect of increasing PEW solution temperature. At temperature of 80 °C, the corroding reaction takes place at a faster rate and directly contributes to a number of observed tracks which are less than a number of tracks actually score. Based on these preliminary results, the chemical etching parameters for this study are the PEW solution temperature of 60 °C and the etching time of 14 hours. The 100X magnification microscopic images of tracks attributed from the CDs track detector at various etching time in PEW solution at 60±2 °C as shown in Figure 5. Nevertheless, etching condition at temperature of 50 °C should be done to consider the optimum parameter.

The CD track detector badges, which were previously irradiated at TINT to receive a number of different neutron doses were etched at specified conditions to study the relationship between track densities and equivalent doses. As expected, the track density is proportional to equivalent dose as shown in Figure 6. and their relationship can mathematically be explained by a power equation: $y=5E\text{-}08x^{2.2988}$ ($R^2=0.9618$). This was found to be in contrast to a number of studies[14, 15] which indicate linear relationship between track density and neutron equivalent dose. A reason for this discrepancy was thought to be a wide range of neutron energy, from 0.048 to 96.4 mSv, considered in this study. The CD track detectors are likely to have different response functions across this wide range of neutron energy. As a result, a power relationship is obtained when attempting to fit all data with one function. The dose calibration had been performed at both low and high dose, the result would be more conformed with other mentioned studies. However, large standard deviation of track densities was observed that at high dose due to an increasing likelihood of counting errors. A number of repetitions can be increased to reduce statistical errors of track density observed in this study.

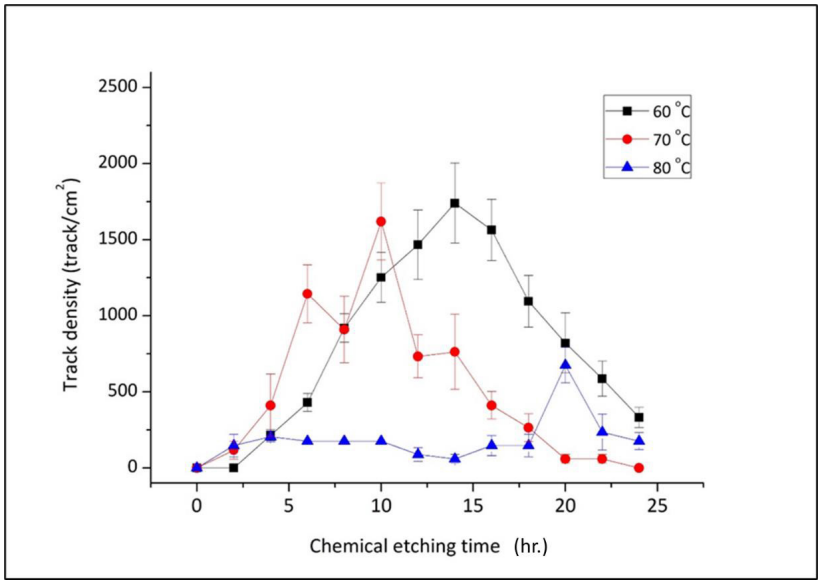

Figure 4. *Average track density (Track/cm^2) as a function of chemical etching time in CDs track detectors etched in PEW solution at 60°C, 70°C and 80°C.*

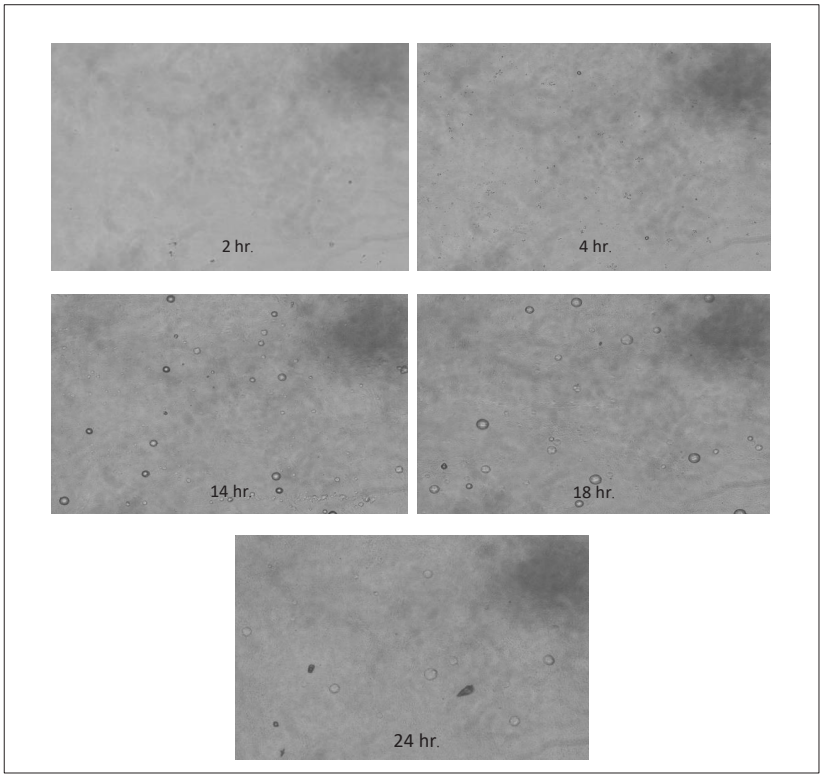

Figure 5. *The 100X magnification microscopic images of tracks obtained from the CDs track detector at various etching time in PEW solution at 60±2 °C.*

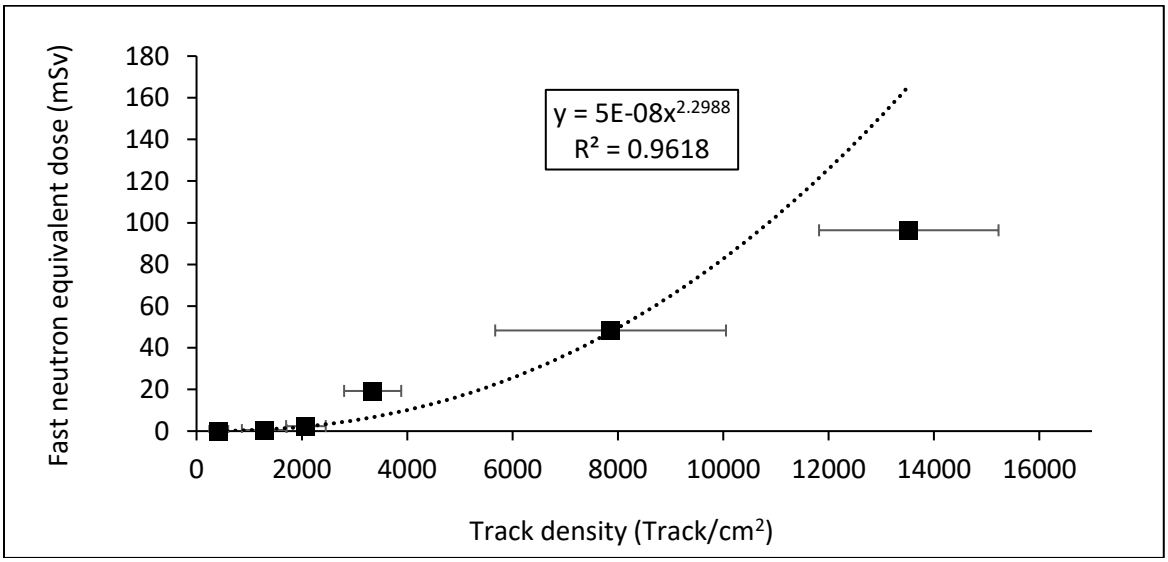

Figure 6. *Relationship between the track density and the fast neutron equivalent dose, obtained from 241AmBe neutron source.*

Comparison of fast neutron equivalent dose from CDs track with CR-39

Table 1 shows equivalent doses from fast neutrons as a function of depths in the water phantom using the CD track detector and CR-39 detector. The equivalent doses have high values at the surface, decrease at the depth of 2.5 cm and reach their maximums at the depth of 5 cm.

However, results from this study are different from those from other studies[16] because different LINACs in all studies have different configurations which may lead to different neutron spectrums and intensities. The maximum equivalent doFses at the surface for the CD track detector and CR-39 detector can be explained by the fact that the fast neutron converters in both cases can effectively produce protons which can tally permanent tracks on the phantom surface. As the fast neutrons continue their journey deeper into the water phantom, they are expected to lose energy due to collision or to get absorbed by hydrogen. These phenomenons dominate fast neutrons in the region from the surface to the depth of 5 cm, causing equivalent doses to decrease. Nonetheless, some LINACs are able to produce fast photo neutrons which can penetrate deeper into the water phantom. These photo neutrons play an important role in making the equivalent doses at 5 cm the highest. Statistically, independent t-test between the CD track detector and CR-39 detector reveals that equivalent doses at the phantom depths of 0, 2.5, 5 and 20 cm are not significantly different with a p value equal to 0.05. However, the results between the two cases are significantly different at the phantom depth of 10 cm.

Mean free path of neutrons from a 10 MV LINAC Model Elekta Synergy with an average energy of 2 MeV in water is about 4 to 5.5 cm.[17] Since the neutrons are expected to travel approximately 4 to 5.5 cm before undergoing any reaction with the water medium, both CD track detector and CR-39 detector register maximum equivalent doses at the phantom depth of 5 cm and score lower equivalent doses as it is getting deeper into the water phantom.

Shagholi N *et.al*.[18] evaluated equivalent doses on a tissue-like material from neutrons generated from LINAC Elekta at two distinct energies of 10 and 18 MV. The study measured equivalent doses at various depths: 0, 1, 2, 2.5, 3.3, 4, 5 and 6 cm, using TLD600 and TLD700 and compared them to equivalent doses calculated by MCNP code. The study by Shagholi N *et.al*. also exhibited a similar behavior of equivalent doses which increase from the surface, reach maximum at the depth of 5 cm and decrease at further depths. Therefore, this early study of using the CD track as a fast neutron detector has shown a promising sign to an innovative and economical approach for measuring collateral equivalent doses in cancer treatment.

Table 1 Fast neutron equivalent dose as a function of depth (d) in the solid water phantom using CD track detector and CR-39 detector.

Phantom depth (cm)	Fast neutron equivalent dose (mSv/Gy)	
	CDs track detector	CR-39 detector
0	(1.32±0.02) E-01	(1.28±0. 77) E-01
2.5	(1.14±0.01) E-01	(1.10±0.05) E-01
5	(1.32±0.05) E-01	(1.63±0.85) E-01
10	(3.70±0.2) E-02	(1.28±0.44) E-01
20	(2.50±0.4) E-02	(5.70±5.70) E-02

Conclusion

The CD track, whose foundation material is polycarbonate, together with PMMA and cadmium sheet, which etched with PEW solution at 60±2 °C, is proved to be reasonably accurate and affordable for measuring equivalent doses from fast neutrons generated from LINAC. In addition, the technique helps address a problem with electronic waste to some degree. An immediate future work to further develop the technique will include an investigation of the efficiency of PMMA sheet as a fast neutron converter. Neutron dose calibration should be done seperately for low and high neutron energy range for better precision and accuracy.

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The local diagnostic reference levels for breast screening using digital mammography at Songklanagarind Hospital

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ABSTRACT

Introduction: This work aimed to determine local diagnostic reference levels (local DRLs) for screening mammography at Songklanagarind Hospital on digital mammography.

Materials and methods: Retrospective data of screening mammography were collected from 400 patients from Songklanagarind Hospital, 200 patients from Tanyawej Breast Center and 200 patients from Premium Diagnostic Imaging Center. The patients were women aged between 40-75 years old with compressed breast thickness between 41-65 mm. and undertaken screening mammogram during 1st January 2019 – 31st October 2020. Patient data and exposure parameters collected were as follows: average glandular dose (AGD), entrance surface air kerma (ESAK), compressed breast thickness (CBT), compression force (CF), peak kilovoltage (kVp), tube current-time (mAs), target and filter (W/Rh, W/Al), and patient age.

Results: The result showed that the average glandular dose of FFDM (2D mode) and DBT (3D mode) for compressed breast thickness 41-65 mm were 1.41±0.43 and 1.68±0.39 mGy, respectively and the 75th percentile of FFDM (2D mode) and DBT (3D mode) were 1.65 mGy and 1.89 mGy, respectively. The average ESAK were 4.93±1.96 mGy in FFDM (2D mode) and 5.31±1.55 mGy in DBT (3D mode), respectively. The 75th percentile of ESAK in FFDM (2D mode) and DBT (3D mode) were 6.03 and 6.17 mGy, respectively. There were 24.88 % and 24.63% received the average glandular dose over the 75th percentile in FFDM (2D mode) and DBT (3D mode).

Conclusion: Local Diagnostic Reference Levels for FFDM (2D mode) and DBT (3D mode) at Songklanagarind Hospital were 1.65 mGy and 1.89 mGy. The Local DRLs in our study was safe and lower than the standard reference levels reported by the International Atomic Energy Agency at 3 mGy/view.

Introduction

Breast cancer accounts for 1.7 million deaths per year worldwide and many people are badly suffering from such type of cancer. Breast cancer accounts for 32% of cancer incidence and 18% of cancer deaths in women in the United States. Presently, breast cancer is the most common type of cancer in Thai women and has the second highest rate of mortality.[1] Mammography is the x-ray machine that is recommended for breast screening program because it is a low-cost, low-radiation-dose procedure and has the sensitivity for early detection and improved treatment.

The international Atomic Energy Agency (IAEA)[2] proposed diagnostic reference levels (DRLs), defined as investigational levels applied to easily measured quantity

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using standard phantom or representative patient, expressed mean glandular dose (MGD), Entrance surface air kerma (Ka,e) to help optimize radiation doses and identify unjustified doses. IAEA emphasized that DRLs should be derived from national or local data and the "As Low as Reasonably Achievable" or ALARA principle should be adhered to minimize potential hazards of ionizing radiation. Mammography dosimetry is a complex issue. The mean glandular dose depends on breast size, compressed breast thickness (CBT), kVp, mAs and compression force (CF). IAEA recommended MGD as a DRL quantity, even though it is a measure of organ dose rather than the amount of ionization radiation used to perform a medical imaging task.

In mammography, the recommended DRLs quantity is one or more of incident air kerma, entrance surface air kerma and MGD with the choice of quantity depending on local practices and regular requirement. A study by Singkavongsay A. et al reported the national diagnostic references levels of mammography in Thailand. They reported that the third quartile of the MGD in 2D was 2.04 mGy, the median was 1.59 mGy and the third quartile of ESAK was 9.74 mGy. NDRL on mammogram in 2D of Thailand was closed to Australia at 2.06 mGy and lower than Japan (DRLs). MGD was depended on breast density, age, and its composition. Digital Breast Tomosynthesis, age and breast density were not included in this study. Nguyen *et al*.[3] assessed the relationship between breast density and radiation dose by retrospective screening mammography data and found that breast thickness was primary determinant of dose. They stated that compressed breast thickness was a major factor in received AGD, breast density was a minor factor, and body mass index as well as patient's age had minimal impacts on dose levels. As Asian woman have denser breast tissue than Western women, it might affect the accuracy of radiation dose evaluation in this group of patients.

The aim of the study was to determine the local diagnostic reference levels (local DRLs) and the parameters for screening mammography in both FFDM and DBT modes at Songklanagarind Hospital, Thailand.

Materials and methods

The study was approved by the Human Research and Ethics Committee of the Faculty of Medicine, Prince of Songkla University, REC.64-010-7-2.

A retrospective descriptive study included 400 women aged between 40-75 years old with compressed breast thickness between 41-65 mm and undertaken screening mammogram during 1st January 2019 – 31st October 2020 at Tanyawej Breast Center and Premium Diagnostic Imaging Center, Songklanagarind Hospital. Women with breast implant, breast mastectomy, and breast conserving therapy were excluded.

Quality control of digital mammography system employed guideline from European Guidelines.[4] Mammographic studies were performed using Selenia Dimension Hologic and Fuji Amulet Innovality machine. Patient data and exposure parameters collected were as follows: average glandular dose (AGD), entrance surface air kerma (ESAK), compressed breast thickness (CBT), compression force (CF), peak kilovoltage (kVp), tube current-time (mAs), and target and filter (W/Rh, W/Ag, W/Al). Patient age on monitor screen was also recorded for verification. Each patient received 8 exposures in two modes (FFDM and DBT). The first 4 exposures were performed in FFDM to produce images in the RCC, RMLO, LCC and LMLO views. The second 4 exposures were performed in the DBT mode in the same view and compression. Patients with breast implants or who were imaged in special added positions were excluded from the study.

To determine DRLs, the 75th and 95th percentiles were calculated across the median AGD. Values for each mammography were then categorized according to their compressed breast thickness to range between 41-65 mm thicknesses. The R statistical analysis program was used to analyze the 75th and 95th percentiles, median, and histogram in this study.

Results

Four hundred women aged between 40-75 years old with compressed breast thickness between 41-65 mm were included in this study. The histogram shows the compressed breast thicknesses ranged between 41-65 mm. The result showed a normal distribution with mean and median of 55.22 and 55 mm. (Figure. 1), while image AGD showed a normal distribution that ranged from 0.9 mGy to 3.4 mGy with mean and median of 1.79 and 1.7 mGy, respectively (Figure 2).

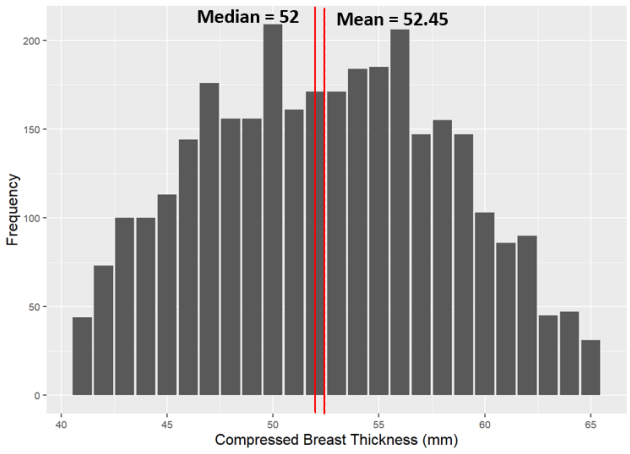

Figure 1. *Distribution of compressed breast thickness in FFDM (2D mode) and DBT (3D mode) in 1600 mammography images with median 52 mm. and mean 52.45 mm.*

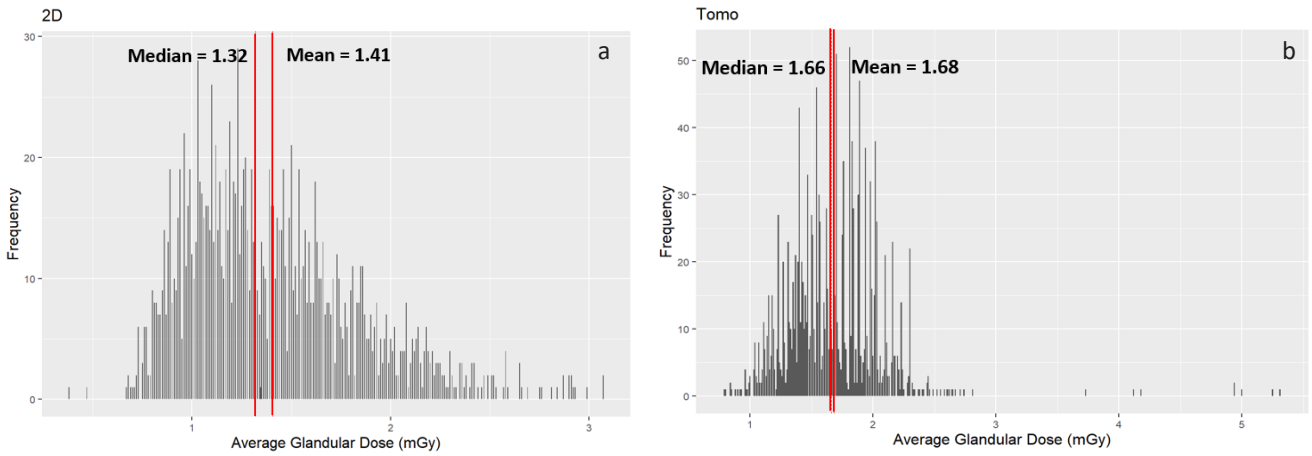

Figure 2. *Distribution of average glandular in 1600 mammography images for compressed breast thickness 41-65 mm. a: distribution of average glandular dose in FFDM (2D mode) with median 1.32 and mean 1.41 mGy, b: distribution of average glandular dose in DBT (3D mode) with median 1.66 and mean 1.68.*

Table 1 shows the parameters used in FFDM (2D) and DBT (3D) modes. Average kVp of FFDM (2D) performed in RCC, LCC, RMLO, LMLO view were 29.13±1.03, 29.16±1.04, 29.46±1.06, and 29.58±1.12, respectively. Average kVp of DBT (3D) performed in RCC, LCC, RMLO, LMLO view were 31±1.24, 31±1.31, 31±1.34, and 32±1.37, respectively. kVp used in DBT mode was significantly higher than that of kVp used in FFDM mode. The mAs values of DBT in RCC, LCC, RMLO, LMLO view were 52.84±7.89, 51.97±7.73, 53.73±7.27, and 54.71±7.33, respectively. These values were significantly lower than that of FFDM mode of 97±10.5 in RCC view, 96±10.72 in LCC view, 99±8.49 in RMLO view, and 101±8.03 in LMLO view, respectively. Mean CF was slightly different in the four views. The average CF of FFDM (2D) performed in RCC, LCC, RMLO, LMLO view were 66.03±20.58, 67.33±20.73, 87.41±24.21, and 96.24±26.83, respectively. Average CF of DBT (3D) performed in RCC, LCC, RMLO, LMLO view were 66.08±20.58, 66.99±20.65, 87.83±23.57, and 95.16±28.21, respectively. Mean CBTs were slightly different in the four views. The mean CBT in RCC and LCC projection mode was 52 mm and in RMLO and LMLO projection was 54 mm.

Table 1 Parameters used in full field digital mammography (2D mode) vs digital breast tomosynthesis (3D mode) in women aged between 40-75 years old at Songklanagarind Hospital.

View	kVp		mAs		CF (N.)		CBT (mm)	
Mode	2D	3D	2D	3D	2D	3D	2D	3D
RCC	29.13±1.03	31.00±1.31	97.00±10.5	52.84±7.89	66.03±20.58	66.08±20.58	52.00±5.37	52.00±5.39
LCC	29.16±1.04	31.00±1.31	96.00±10.72	51.97±7.73	67.33±20.73	66.99±20.65	52.00±5.54	52.00±5.55
RMLO	29.46±1.06	31.00±1.34	99.00±8.49	53.73±7.27	87.41±24.21	87.83±23.57	55.00±5.81	54.5±5.82
LMLO	29.58±1.12	32.00±1.37	101.00±8.03	54.71±7.33	96.24±26.83	95.16±28.21	55.00±6.06	55.00±6.06

Note: *kVp: kilovoltage peak, RCC: right-craniocaudal, LCC: left-craniocaudal, RMLO: right-mediolateral oblique, LMLO: left-mediolateral oblique, mAs: milliampere-seconds, CF: compression force, N.: newton, CBT: compressed breast thickness, mm: millimeters, 2D: two-dimension, 3D: three-dimension.*

The results of radiation dose are shown in Table 2. AGDs were 1.41±0.43 and 1.68±0.39 mGy in FFDM (2D mode) and DBT (3D mode). The average ESAKs were 4.93±1.96 mGy in FFDM mode and 5.31±1.55 mGy in DBT mode, respectively. The third quartiles of AGD and ESAK in FFDM were 1.65 and 6.03 mGy, respectively. In DBT mode, the third quartiles were 1.89 and 6.17 mGy, respectively, which were higher than that of FFDM mode. The 95 percentiles of AGD and ESAK in FFDM were 2.14 and 8.60 mGy, respectively. In DBT mode, the 95 percentiles were 2.23 and 7.56 mGy, respectively, which were higher than that of FFDM mode.

Table 2 Average glandular dose in full field digital mammography (2D mode) compared to digital breast tomosynthesis (3D mode) in women aged between 40-75 years old at Songklanagarind Hospital.

	AGD (mGy)		ESAK (mGy)	
	2D	3D	2D	3D
1st Q	1.08	1.42	3.46	4.25
Median	1.32	1.66	4.45	5.05
Mean	1.41	1.68	4.93	5.31
75th Q	1.65	1.89	6.03	6.17
95th Q	2.14	2.23	8.60	7.56

Note: *AGD: average glandular dose, ESAK: entrance surface air kerma, 2D: two-dimension, 3D: three-dimension.*

Figure 3 shows the third quartiles and 95 percentiles of AGD in FFDM (2D mode) and DBT (3D mode) in 4 conventional views. The third quartiles and 95 percentiles of AGD in FFDM (2D mode) were 1.65 and 1.89 mGy, respectively. In DBT (3D mode), the third quartiles and 95 percentiles of AGD were 2.14 and 2.23 mGy, respectively, which were higher than that of FFDM (2D mode).

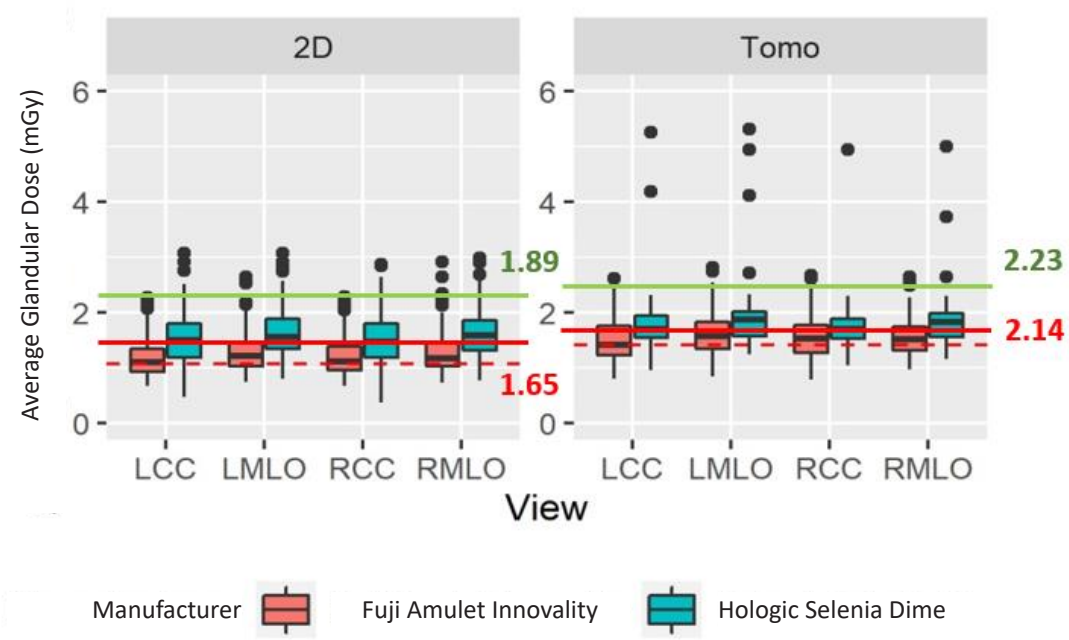

Figure 3. *Box and whisker plot of the average glandular dose in FFDM (2D mode) and DBT (3D mode) in RCC, LCC, RMLO, LMLO views.*

Discussion

The histogram of compressed breast thickness and the average glandular dose indicated normal distribution in Figure 1 and 2 were similar to the distribution reported in Suleiman et al. study.[5] Overall, it was found that our reported median compressed breast thickness and median average glandular dose were less than Suleiman et al study in 2016.

According to average glandular dose in Table 1 and Figure 3, there were 24.88% received average glandular dose over the 75th percentile in 2D technique. There were 24.63% received average glandular dose over the 75th percentile in tomosynthesis technique. There were 4.88% and 4.10% received average glandular dose over the 95th percentile in FFDM (2D mode) and DBT (3D mode), respectively. The protocol setting at Songklanagarind Hospital was effective and suitable for diagnostic breast screening.

Table 3 Mean, median, 75th and 95th percentile of digital mammography in FFDM (2D mode) and DBT (3D mode).

	Mode	Mean (mGy)	Median	75th Percentile	95th Percentile
Songklanagarind Hospital	2D	1.41	1.32	1.65	2.14
	Tomosynthesis	1.68	1.66	1.89	2.33
NDRLs Thailand[6]	2D	1.72	1.59	2.04	-
Ritlumlert N.[7]	2D	1.36	-	1.67	-
	Tomosynthesis	1.63	-	1.81	-
NDRLs Japan [8]	2D	1.84	-	-	2.40
	Tomosynthesis		-	-	-
IAEA recommended [2]	2D	3	-	-	-
	Tomosynthesis	-	-	-	-

As shown in Table 3, median AGD and 75th percentile of FFDM (2D mode) for median compressed breast thickness of 52 mm were 1.32 and 1.65 mGy, respectively. These values were lower than 1.59 and 2.04 mGy reported in NDRLs Thailand[6] in 2020 which employed similar methods to estimate the dose with median compressed breast thickness of 52.3 mm. The 75th percentile of FFDM (2D mode) and DBT (3D mode) for mean compressed breast thickness of 52.45 mm were 1.65 and 1.89 mGy., respectively. Comparing to the study of Ritlumlert N.,[7] the 75th percentile for mean compressed breast thickness of 52.83 mm were 1.67 and 1.81 mGy. Result from this study was lower than Ritlumlert N. study in FFDM (2D mode) and higher than Ritlumlert N. study in DBT (3D mode). Regarding to Ritlumlert N. study, they used the different mammography machine compared to this study. The 75th percentile of DBT (3D mode) in this study that higher than Ritlumlert N. study received by symptomatic women could be explained by the inclusion of younger women with denser breasts.

More recent study in NDRLs Japan [8] reported mean AGD and 95th percentile of FFDM (2D) of 1.84 and 2.4 mGy, respectively. These values were higher than 1.41 and 2.14 mGy reported in this study. Local DRLs in this study was safe and lower than the standard reference levels reported by the International Atomic Energy Agency at 3 mGy/view.

Moreover, the differences compressed breast thickness ranges and the symptomatic women different image detector technology might contribute to higher doses. The results suggested some potential for optimization of the protocol setting (kVp, mAs, CF) included in this study. DRL values in mammography should be specific to breast thickness and image detector technology as large variations between compressed breast thickness ranges and image detector technologies were found.

Conclusion

The Local Diagnostic Reference Levels for FFDM (2D mode) and DBT (3D mode) at Songklanagarind Hospital were 1.65 mGy and 1.89 mGy. The Local DRLs in this study are safe and lower than the standard Reference Levels reported by the International Atomic Energy Agency at 3 mGy/view.

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Conflict of interest

There are no conflicts of interest to disclose.

Ethic approval

Human Research Ethics Committee under project number REC.64-010-7-2

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Development of a computer-based cognitive training game and usage feasibility with Thai stroke patients

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ABSTRACT

Background: Cognitive impairments are common sequelae found in patients after a stroke event, leading them to necessitate long-term care. Currently, there are two common cognitive approaches, namely conventional intervention, and technology-based intervention, with the latter having become extensively used, especially in developed countries. In a developing country like Thailand, there is still a clear lack of usage of computer-based cognitive training and there is still a lack of research focus on investigating the feasibility of its usage with Thai stroke patients.

Objectives: This study aimed to develop a computer-based cognitive training game and investigate its feasibility for being used with Thai stroke patients

Materials and methods: This study was a developmental research design consisting of two phases. Phase one involved the development, content validity, and pilot use of our computer-based cognitive training game. The game contents were examined by three experts, who are occupational therapists with more than 5 years of experience with cognitive rehabilitation for stroke patients, to assure content validity. Phase two instead, involved the process of investigating the feasibility of using the newly developed computer-based cognitive training games with Thai stroke patients. Participants in this study were stroke patients with cognitive impairments identified with the Mental State Examination (MSET10) and who were familiar with the technology. Fourteen participants were asked to rate their overall experience with the newly developed game, the design, the convenience aspects, and the portability of the training material, by using The Test of Satisfaction on Computer-based Cognitive Game. Demographic characteristics and user experiences were analyzed by descriptive statistics.

Results: The newly developed computer-based cognitive training game, called CoWMeG, is a game-like training using simulated real-life activities whose design is based on the Thai ecosystem, and it consists of ten games. The game's contents involve working memory tasks, such as verbal, visuospatial, executive, and process speed skills-related tasks. Each game was designed to have different difficulty levels with each level consisting of three subsequential screen pages (i.e., instruction, playing, and scoring screen pages). Most of the participants rated their user experiences with CoWMeG as very satisfied, corresponding to 84.2 percent of the total answers on satisfaction.

Conclusion: In accordance with the obtained results, the newly developed computer-based cognitive training game, named CoWMeG, may represent a feasible tool to be used with Thai stroke patients who have cognitive impairments.

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Introduction

Stroke is the highest leading cause of morbidity and mortality among non-communicable diseases in Thailand[1] and one of the major causes of death and disability worldwide.[2] Wolfe estimated that by 2023, 30 percent of the world population will be affected for the first time by a stroke, with approximately 65 percent of stroke patients being functional dependent after one year.[3] Adults and elderly people are those in the segment of the population more affected by stroke.[4, 5] The study of Yi et al,[5] highlighted a higher number of stroke cases occurring in people aged 40 and above. In addition, considering the segment of the population aged 55 years old and older, the number of stroke cases is currently on the rise, with the number of registered stroke events doubling every 10 years.[4] These previous findings were confirmed also in Thailand where the average age for stroke patients was found to be 65 years old.[6] In general, stroke can cause physical dysfunctions, language communication issues, and cognitive impairments.[7] Cognitive impairments after a stroke had been confirmed happening with a probability that ranges between 20 percent to 70 percent depending on patient status.[8] In addition, research studies have shown cognitive impairments as a significant predictor for occupational limitations.[9, 10] In fact, stroke patients usually are required to undergo cognitive rehabilitation and long-term care due to their issues related to the stroke event.[11]

Regarding cognitive rehabilitation, it may be divided into two techniques: conventional and computer-based.[12] Conventional training usually includes manual exercises like paper-pencil tasks whereas computer-based training includes computerized cognitive rehabilitation and game-life programs. In the research field, paper-pencil and table-top tasks are currently the most common cognitive training in Thailand.[13-15] However, in recent years, many research works have employed computer-based training for cognitive rehabilitation, especially in developed countries.1[2, 16-18] Prior to the adoption of computer-based intervention, computer -based training was only considered an option for cognitive training, and it was not extensively used.[11, 19] The increasing usage of computer-based intervention in the healthcare sector may be explained by the digital transition that is currently happening all around us, including in many professional settings, like the health one. The portability and easiness of interaction with touchscreen devices have opened new doors of opportunity for deploying touch-screen solutions also in the segments of the population usually less tech-savvy, like the older generation. Even though elderly people are less likely to use technology than younger people,[20] they have become more open to accepting it and more familiar with it,[21] also due to the exposure and interaction with relatives and family members more tech-savvy. However, it is important to keep in mind that not every technology-based intervention would be suitable for being used indistinctly, especially with elderly people, due to a wide array of age-related issues.[20] In this regard, the study of Fager and Burnfield offered interesting insights on patients' experience with using technology for their inpatients' rehabilitation.[22] In the specific, the participants in their study stated that using games and mobile technologies motivated and supported them throughout the therapeutic intervention they underwent. In general, it can be said that games are usually related to fun and enjoyable experiences and thus they motivate people in desiring to play, without realizing that they are training.

Considering Thailand, based on our literature review, there is only one prior research study related to the occupational therapy field currently present in the body of literature,[23] where the researchers adopted technology-driven solutions to create computer-based cognitive training for stroke patients: CogTA-Tab. This application focused on the training of different cognition spheres such as attention, memory, and executive functions. CogTA-Tab consists of a total of fifteen cognitive games with different difficulty levels. In the aforementioned study, fifteen stroke patients underwent a CogTA-Tab training for 6 consecutive weeks, 3 times per week. The results of the study showed a significant improvement in cognitive functions (p<0.05). However, regarding its design, even though the researchers tried to introduce sensory information such as sounds, pictures, numbers, and symbols into the training application, few elements such as score or sound feedback represent an area of improvement for CogTA-Tab. Specifically, sensory information like feedback represents an important factor in a game-like activity. According to the definition of a game by Juul,[24] games usually require players to put in their efforts for earning an outcome like a score, which represents feedback of performance. Using feedback, such as a visual score and verbal feedback may help improve a person's self-awareness, functional task completion, and fulfill individual satisfaction on performance, as stated in Schmidt et al.[25] Thus, aiming to fill the gap in previous research work,[23] we designed a computer-based cognitive training game by adding some elements of gamification such as verbal and score feedback and by creating three subsequential screen pages for each level (e.g., instruction, playing, and outcome pages). Additionally, we designed a computer-based cognitive game tailored specifically around the Thai ecosystem (i.e., Thai language, culture, and common activities in Thailand) capable to be user-friendly also for elderly people. In summary, the aims of this research study were to develop a computer-based cognitive training game for stroke patients with cognitive impairments and to investigate its usage feasibility with Thai patients.

Materials and methods

This research adopted a developmental research design and it consists of two phases: development and pilot use phase, and user experience phase. In the development and pilot use phase, we adopted the modified development process guideline of Benson and Clark.[26] In the user experience phase, we investigated the feasibility of our computer-based cognitive training game for being used with Thai stroke patients with cognitive impairments. More details on each phase can be found following.

Phase 1: Development of computer-based cognitive training game and pilot use

The objective of this phase was to develop the computer-based cognitive training game following specific

steps, namely planning, construction, and quantitative evaluation. In addition, during the development and pilot use phase, we also adopted a co-design approach, an approach that encourages to add in the process stakeholders who could offer holistic perspectives.[27, 28] In our case, the group of stakeholders involved in the study included the research team, occupational therapists, academics staff, one information technology engineer, a team of game developers, and stroke patients.

Planning step: In this step, we reviewed the related research studies, theories, and frameworks. This research study adopted the Dynamic Interaction Model for framing dimensions related to the cognition sphere (i.e., person, environment, and activities) and the Model of Human Occupation (MOHO) as a framework for understanding the desire of a person in engaging in activities.[29, 30] We decided to focus on the working memory sphere because it is commonly affected by stroke events and, additionally, there are many research works currently present in literature that highlighted the effectiveness of working memory training.[11, 18, 31-33] The game contents of the computer-based cognitive training game were identified based on the previous research work and theory and included verbal tasks, visuospatial tasks, executive tasks, and processing speed tasks.[34-38] Finally, we identified a set of simulated real-life activities related to the Thai culture, language, and in general, the Thai ecosystem. The deliverable of this step was the game contents of the computer-based cognitive training game created by using simulated real-life activities tailored to the Thai ecosystem.

Construction step: In this step, three experts examined the game contents included in the computer-based cognitive training games. These three experts are occupational therapists with more than 5 years of experience with cognitive rehabilitation for stroke patients, both in the practical field (2 experts) and in the academic field (1 expert). The Index of Item-Objective Congruence (IOC) from the content validity was equal to 0.67-1.00, which indicated that the game contents were well-congruent with its objectives.[39] Next, we revised the game contents following the experts' advice. Before developing the computer-based cognitive training game into its application, the different stakeholders, including the research team, information technology engineer, and game developers, met for providing their perspectives and offering their suggestions regarding the user interface design. At the end of this step, we had the computer-based cognitive training game application. We developed the game for being used on an Android Samsung Galaxy Tab S6 Lite tablet (screen-wide 10.4 inches). We opted for a tablet as a therapy tool due to its portability, touch screen solution, relatively low cost, and large availability on the Thai market. These characteristics made a tablet solution suitable for this research work's goals. For developing the computer-based training game application, the cross-platform game engine 'Unity' (version 2020.1.0) was used. Unity is a well-known engine for game development.[40]

Quantitative evaluation step: In this step, we conducted a pilot use of the computer-based cognitive training game application on a tablet with five stroke patients who were familiar or had previous related experience with using technology tools (e.g., laptop, tablet, and smartphone). Participants were recruited from Buriram Hospital, Thailand, during the month of December 2020. Participants were first-time stroke patients and had cognitive impairments identified by the MSET10, with cut-off scores at ≤14 for no education; at ≤17 for primary school level; and at ≤22 for a higher level of education. Additionally, participants with visual and communication impairments were not included. Five stroke participants, who had cognitive MSET10 scores ranging between 12 and 22, were asked to play the computer-based cognitive training game 3 times, 30-45 minutes for 1 week. After finishing to play in each level of each game, participants were asked to rate the difficulty of every level in terms of time countdown, gameplay, and overall graphic interface including clarity of objects adopted in the levels. The questions were presented in the form of a Likert scale ranging from 1 (very easy) to 5 (very hard). During the playing sessions observation phase, notes and comments were recorded as guidelines for the final minor revision of the computer-based cognitive training game. The deliverable of this step was the fully developed computer-based cognitive game (CoWMeG). The flowchart of phase 1 can be seen in Figure 1.

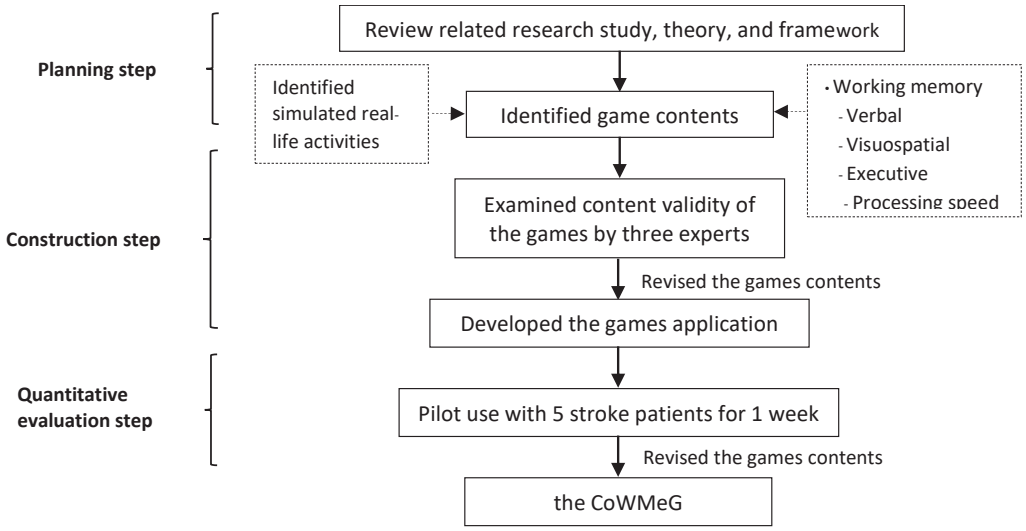


***Figure 1**. Flow chart of the development process of the computer-based cognitive training game (CoWMeG).*

Phase 2: Investigation of user experience

This phase aimed to investigate the usage feasibility of CoWMeG with Thai stroke patients. The chosen sample size of this phase was based on the previous research work of Muskens et al,[41] where the authors developed an entertainment application for elderly people with physical and cognitive impairments. The fourteen participants were recruited from Buriram Hospital, Thailand, between February and April 2021. The selection criteria of the participants were the same as in the pilot use process. The participants were asked to play CoWMeG 3 times per week, 30-45 minutes, for 2 weeks following the guidelines for using CoWMeG. Then, the participants were asked to rate their user experience with playing CoWMeG by using the Test of Satisfaction on Computer-based Cognitive Game which was developed by the authors.

Instruments

There were two assessment instruments used in this study; the Mental State Examination (MSET10) and the Test of Satisfaction on Computer-based Cognitive Game. The MSET10 was used to screen patients who had cognitive impairments. It consists of 10 questions whose scores range from 0 to 29 which the lower the score indicates the higher cognitive impairments. The cut-off scores depend on the level of education: at ≤14 with no education, at ≤17 in the education level of primary school, and ≤22 in the education level of higher than primary school. The psychometric properties of the MSET10 showed high sensitivity and specificity.[42]

The Test of Satisfaction on Computer-based Cognitive Game was used to investigate the user experience with the computer-based cognitive training game. The test was developed and modified based on previous research works.[15] The test includes questions related to 4 different aspects: 1) overall experience: including enjoyment, quality, and satisfaction; 2) design of the games: including satisfaction with score and verbal feedback, clarity of pictures, clarity of sounds, and clarity of text; 3) portability of training material: including size, shape, weight, and durability of the tool used (tablet); and 4) convenience aspects: including setting up, laying aside, and maintenance. The questions were provided in the form of a Likert scale ranging from 1 (very unsatisfied) to 5 (very satisfied).

Statistical analysis

Demographic characteristics and user experience of participants were analyzed using descriptive statistics. Demographic characteristics of participants included gender, age, education, diagnosis, affected side, stroke onset, and the MSET10 scores. These characteristics were analyzed to obtain quantities such as frequency, percentage, mean, standard deviation, and maximum and minimum. The user experience was reported as a percentage.

Results

In this section, we present the results of the computer-based cognitive training game and user experience. Further details can be found following.

The computer-based cognitive training games: CoWMeG

CoWMeG is a computer-based intervention that consists of a total of ten working memory games involving verbal, visuospatial, executive, and processing speed tasks. Each game may involve more than one task. For example, in game 10, the gameplay involves both visuospatial and processing speed tasks (see Table 1). In addition, each game contains different difficulty levels, ranging from easy to hard, with an increasing number of stimuli (to remember) according to a higher difficulty level. CoWMeG is considered as a game-like training, created on the definition of the game from Juul, [24] where players need to put their efforts into earning an outcome like a score. In addition, CoWMeG adopts simulated real-life activity games, where the type of activities or stimuli included in the games were chosen based on Thai culture, language, and environment. Specifically, each of the games was created relying on common real-life activities, such as cooking, using a phone, or interacting with a home-like environment. Each activity was created recalling elements commonly found in Thailand, such as typical Thai foods and ingredients, animals common in Southeast Asia, in addition to using Thai language and alphabet across the game. These choices aimed to offer a virtual experience that could resemble a Thai user's familiar environments.

Table 1 Ten games of the computer-based cognitive training game and its representative working memory skills.

The computer-based cognitive training games: CoWMeG	Number of levels	Working memory skills			
		Verbal	Visuospatial	Executive	Processing Speed
Game 1 Shopping	5		/		/
Game 2 Measure your speed	3				/
Game 3 Making omelet with pork menu	3	/			
Game 4 Making fried rice menu	4	/			
Game 5 Making clear soup with tofu, glass noodle, and minced pork menu	3	/			
Game 6 Remembering the objects in a bedroom scene	3		/		
Game 7 Catching animals	3			/	/
Game 8 Remembering by eyes and listening by ears	3	/	/		/
Game 9 Telephone	3		/		
Game 10 Selling food by customer's order	3		/		/

Regarding working memory tasks of the CoWMeG (i.e., visuospatial, verbal, executive, and processing speed tasks), participants were asked to engage in different tasks, described following.

Working memory tasks:

Visuospatial tasks involve visual memorization. For example, in game 6, the player was asked to remember some bedroom-related objects (e.g., bed, curtain, clock, frame) that were shown in a specific sequence and in a specific position in the presented bedroom scene. After this initial phase, the player was presented with the same scene, filled with many objects, included those previously visualized. Thus, the player had to select only the previously presented objects, following the correct order, touching them on the screen accordingly.

Verbal tasks involve verbal memorization. For example, in game 3, the player was asked to remember some ingredients (e.g., oil, eggs, sauce), listed verbally, for preparing a specific meal. Sequentially, the player had to select, following the right order, each ingredient shown as an icon on a table in the center of the screen presented together with other ingredients, for then dragging them into a cooking pan (visualized on the screen).

Executive tasks, like in game 7, require a player to select only the animals (e.g., fish, shell, octopus) belonging to the group of those living in the ecosystem represented with the background scene (e.g., underwater scene).

Processing speed tasks require participants to complete a specific task within a specific time. For example, in game 2, the player was presented with a scene (e.g., sky, jungle, or nature scene). The player was then required to memorize a specific sequence of two or more items (e.g., star-moon-star-moon) that appeared in the same scene (e.g., many stars and many moons). During the selection, the player was required to select all the items (disappearing on touch) on the screen following the right sequence previously presented, and within a specific time, until all the items (e.g., moons and stars) disappeared from the scene. Once the countdown reached zero, all the items collectively disappeared from the scene, indicating the end of the game.

User interface design:

Regarding the user interface design of the CoWMeG, it consists of three pages: the main page, the level page, and the subsequential screen page (see Figure2). On the main page, the player can choose the game to play (between game 1 and game 10). On the level page, the player can choose the levels of difficulty of each game. The subsequential screen page consists of the instruction page, the playing page, and the scoring page. The first screen page presents the instructions (for each level) presented to the player. Additionally, on this screen page, the stimuli, presented on the screen after a vocal instruction, need to be remembered by the player. The second screen page represents the actual playing phase, where the player needs to play and thus perform the level-required task. The last screen page refers to the outcome part, where the score of the player's performance on the task is shown. In addition to the visual score, sound feedback is provided, in the form of a sound such as 'well done', 'almost got the highest score', or 'please try again'. This overall user interface design of CoWMeG was based on the research team's needs and on the information technology engineer and game developers' suggestions. An example of the level page and the subsequential screen page can be seen in Figure 3.

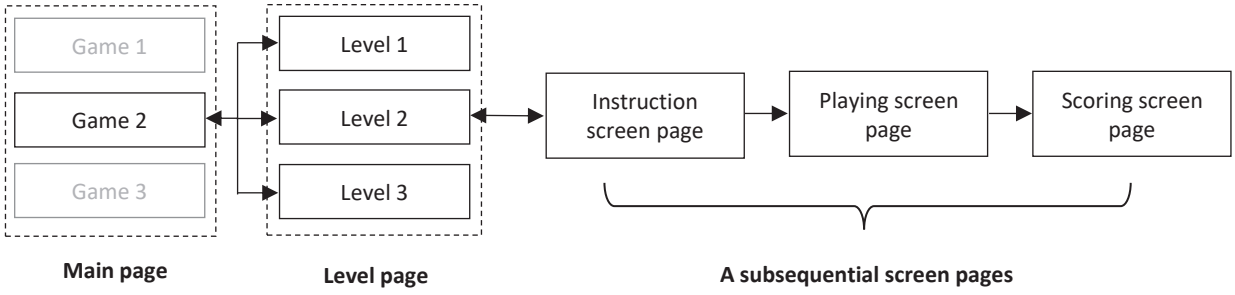
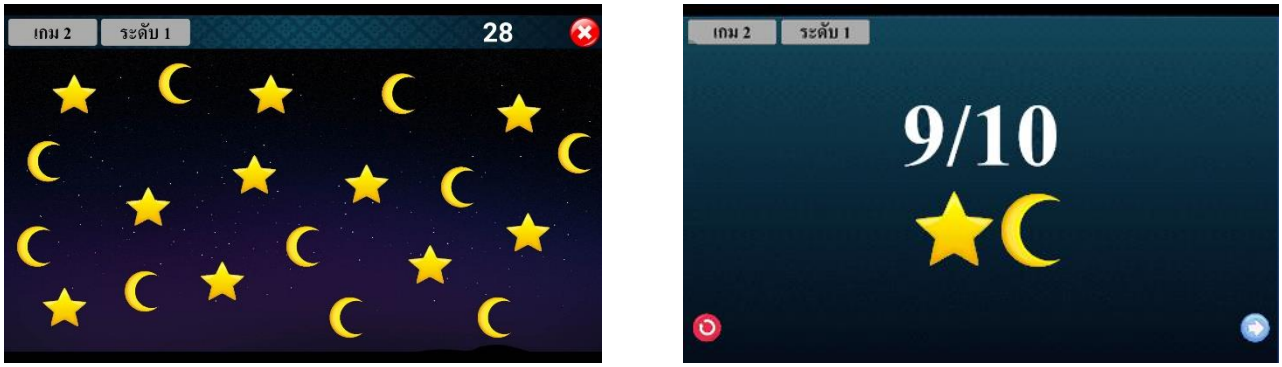


Figure 2. *User interface design of the computer-based cognitive training game (CoWMeG).*

A

B



C D

Figure 3. *Example of sequential screen appearance for CoWMeG games. A: different difficulty levels page, B: Instruction page, C: Playing page, and D: Scoring page.*

User experience with CoWMeG

For the user experience phase, we included fourteen first stroke patients. Most of the participants were male. The age of participants ranged from 44 to 70 years old, with a mean of 60.57. The majority of the participants reported to have elementary school education (50%, n=7), 5 participants (35.7%) reported a secondary school education level, and 2 participants (14.3%) a high school one. The participants had a cognitive MSET10 score ranging between 13 to 22. Further information can be seen in Table 2.

Table 2 Demographic characteristics of participants in user experience phase.

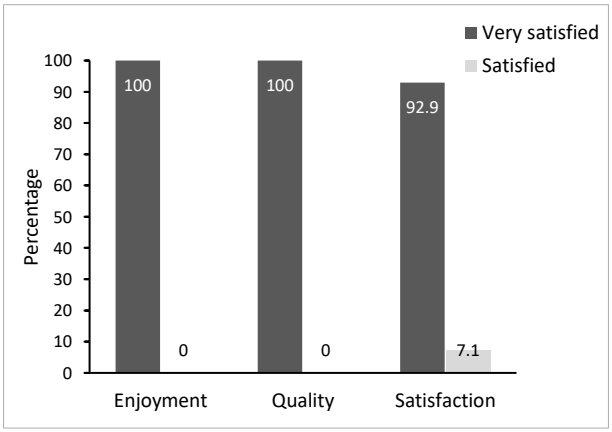
Demographics		The samples (n=14)			
		n	%	(Mean±SD)	Min-Max
Gender:	Male	10	71.4		
	Female	4	28.6		
Age:				60.57 (±8.30)	44-70
	35-49 years old	2	14.3		
	50-60 years old	4	28.6		
	61-70 years old	8	57.1		
Diagnosis:	Ischemic stroke	9	64.3		
	Hemorrhage stroke	5	35.7		
Affected side:	Right hemisphere	7	50		
	Left hemisphere	7	50		
Stroke onset (month)				4.71 (±1.73)	3-8
The scores of MSET10				17.29 (±3.29)	13-22

Note: *MSET10: the Mental State Examination, Max: Maximum, Min: Minimum, SD: Standard deviation*

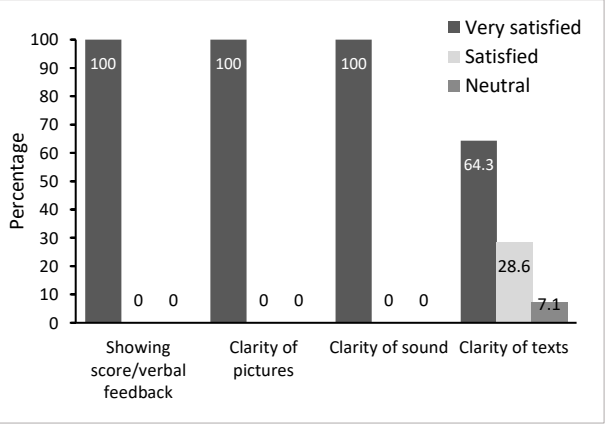
After playing with CoWMeG for 2 weeks, fourteen participants were asked to rate their user experience in four different areas. According to the results, most participants rated their total experience in the four different areas equal to 'very satisfied', corresponding to 84.2 percent of the total answers. The results for some of the questions in the areas of overall experience and design of CoWMeG received a 100 percent 'very satisfied' rate (in the question of enjoyment, quality, showing score/verbal feedback, clarity of pictures, and clarity of sound), as can be seen in Graph 1 and 2.

Most of the participants rated their experience on the convenience aspect as 'very satisfied' with the same percentage (85.7%) in all three questions (see Graph 3). The results in the area of portability of training material depict slightly different rates, with most of the participants rating their experience equal to 'very satisfied', ranging from 42.9 to 85.7 percent (of the total answers for each question within the portability category) depending on the question (see Graph 4). However, it is important to highlight the fact that all participants rated their experience with CoWMeG as ranging from neutral to positive experience (represented as 3 to 5 in the Likert scale) and no participant rated their experience as negative (represented as 1 to 2 in the Likert scale).

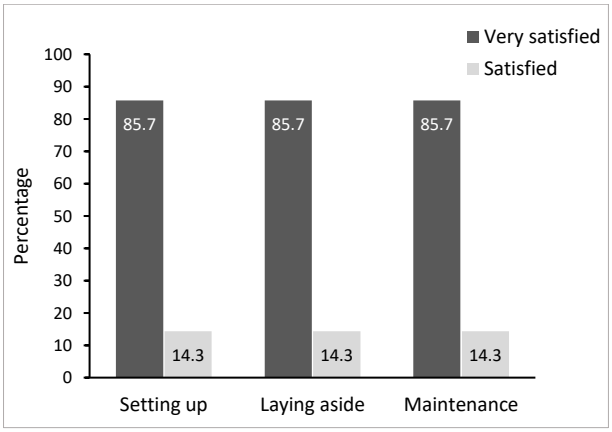
Graph 1 The percentage of user experience on overall experience with CoWMeG



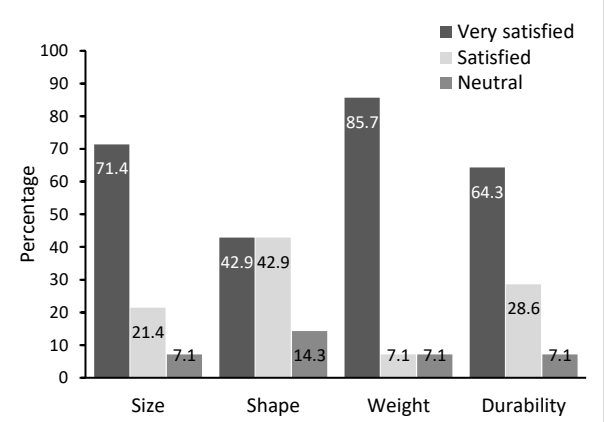
Graph 2 The percentage of user experience on the design of CoWMeG



Graph 3 The percentage of user experience on convenience aspect with CoWMeG



Graph 4 The percentage of user experience on a portable training material (tablet) with CoWMeG



Discussion

In the discussion section, we are discussing two parts: the computer-based cognitive training game and the user experience with the computer-based cognitive training game (CoWMeG).

The computer-based cognitive training game

Computer-based interventions for cognitive impairments have become popular in recent years, and they have been extensively used for cognitive rehabilitation, especially in developed countries.[12, 16-18] The increase in popularity of these computer-based interventions may present several advantages compared to conventional interventions like paper-pencil tasks or table-top tasks. Specifically, in the first place, a computer-based solution offers flexibility and adaptability in terms of task difficulty. In this regard, in fact, a specific task can automatically increase its difficulty level, ranging from easy to hard, and thus adapting to the user's individual cognitive abilities level. This approach of adaptability in the level of difficulty is also commonly found in commercial computer-based cognitive training such as PSSCogRehab software, CoTras program, Parrot software, and Cogmed QM.[32, 43-45] Secondly, a computer-based solution supports easy implementation of game-like functionalities like a countdown timer or processing speed during training. In general, in fact, working memory training is characterized by a storage time equal to 1 minute or less, with an input speed of stimuli of 20 bits per second, requiring the player who trains with it to memorize or to respond to the stimuli within a specific time.46 Due to this characteristic of working memory training and its requirement of countdown timer features, processing speed training may be less convenient to implement in a paper-pencil task or a table-top task.

In addition to the aforementioned convenient aspects of using computer-based interventions for cognitive training, there are some additional advantages that we would like to further discuss. Specifically, a computer-based solution allows a training task to provide automatic feedback. For example, in CoWMeG, we used immediate feedback such as auditory and visual feedback on the player's actions as in game 2, where the beeping sound accompanies the disappearance of the visual stimulus after the player touches it on the screen. Additionally, verbal feedback and score assignments can be easily supported by a computer-based solution, which is also capable to guarantee an error-free and consistent environment over time, typical characteristics of digital solutions. Moreover, Schmidt et al.,[25] stated that the usage of feedback may help to improve a patient's

self-awareness, functional task completion, and fulfill an individual's satisfaction with their performance. An additional potential advantage of computer-based training consists in its ability to enhance the accessibility to the training. Specifically, patients or players can easily install an application on their own technology devices, thus being offered the ability to train and play by themselves in any convenient setting, besides the healthcare ecosystem (like in a clinic, or in a hospital). However, we need to remind the reader that CoWMeG is not yet appropriate for being played by a player alone unless they are already familiar with its user interface design. It is also important to highlight the fact that computer-based cognitive interventions have been shown to have better effects on cognitive functions compared to conventional interventions.[47-49] The reasons for this have not yet been fully investigated, but a partial explanation could reside in the advantages offered by computer-based solutions, such as increased motivation and engagement through the use of game elements or gamification (e.g., number of levels, sound, leader board, scores).[50, 51] Factors like immediate feedback and gamification may motivate players to put more effort into repetitive training tasks with the goal of reaching better outcomes (scores) or rewards, and thus, paving the path for their own improvement.

Furthermore, the development of computer-based cognitive training created to be appropriate for a specific target group needs to keep in consideration an important factor such as the user interface. For instance, the interface design may results very important during the development of computer-based solutions tailored for elderly people, who may be less likely to be tech-savvy, and affected by common related-age issues like dexterity impairments and sensory impairments (e.g., blurred eyesight, color vision loss, hearing impairment).[20, 41] Regarding the user interface, Muskens et al.[41] stated that elderly people prefer a lower deep hierarchy, few icons, large buttons or objects, and immediate feedback. In accordance with these suggestions, the user interface of CoWMeG has been designed with only three deep hierarchies (i.e., main page, level page, and a subsequential screen page), and with few icons for each page (e.g., next-back, try again, and close icons) - see in figure 2 and 3. Another element to keep in consideration is the button size. In this regard, the recommended minimum size of a button is 11.43 mm where 17 mm is considered a large size.[52, 53] In alignment with these recommendations, the size of the adopted buttons in CoWMeG ranges from 12 to 60 mm, dimensions that can be considered large and appropriate for elderly people and could address their common problems, such as hand dexterity. Additionally, we used clear pictures and clear sound/texts within a color-contrasting background in order to overcome vision and hearing issues .[20, 54] Thus, we believe that the user interface design of CoWMeG accounts for possible age-related characteristics of the average stroke patient-user, and thus it might be said to be characterized by an 'elderly-friendly' design.

User experience

In our research work, we demonstrated that the newly developed computer-based cognitive training games (CoWMeG) may be feasible to use for training with Thai stroke patients. Regarding this, the majority of the participants in this study expressed their positive user experience with CoWMeG, rating their total overall satisfaction as 'very satisfied' (84.2 percent) and no participants expressed a negative user experience. Specifically, CoWMeG may be identified as an elderly-friendly training game, characterized by user-friendly features like the absence of deep hierarchy, few icons adoption, and large buttons.[41, 52, 53] We also included sensory-information input (i.e., immediate auditory and visual effects, score feedback, and using simulated real-life activities) to maximize the overall understanding, enhance motivation and engagement with the games.[25, 51, 55] Thus, we believe that these characteristics of CoWMeG positively contribute to the user experience outcome. This statement could be also confirmed in Graph 2, where it can be seen that all the participants were very satisfied with the usage of features such as showing the score and verbal feedback, clarity of pictures, and sound. Regarding the clarity of texts (i.e., instruction texts), the 'very satisfied' level recorded a figure of 64.3 percent, lower compared to the 100 percent figures in the other categories of Graph 2. Yet, it is important to stress the fact that even within the clarity of texts category, all the respondents reported a positive experience with CoWMeG, since all the users rated their experience in the range of 3-5 in the Likert scale, thus, positive overall. Finally, we would also like to highlight the fact that game instructions for each level were not offered only in the textual format, but they were additionally presented to the player in alternative ways such as auditory instructions, to facilitate and maximize the overall understanding of the tasks and to support also those players who may be not proficient or even not understand the Thai alphabet.

Furthermore, positive ratings for enjoyment, quality, and satisfaction with CoWMeG were reported as very high, with all participants agreeing that CoWMeG offered them a feeling of enjoyment accompanied by a perception of quality. In this regard, there was only one participant who rated their satisfaction as 'satisfied' (4 out of 5 on the Likert scale) with the rest of the participants who reported a 'very satisfied' level of experience (5 on the Likert scale - refer to Graph1). These positive user experiences could be related to the use of a computer-based solution with game-like training, where tasks are usually designed to be fun and engaging. Specifically, video games have been proven to support engagement and motivation,[56, 57] and besides being useful tools for the training of cognitive processes, numerous studies have demonstrated the ability of video games to offer a variety of positive emotion-triggering situations with positive effects on individuals well-being, and with a specific focus on cognitive and emotive enhancement.[51, 58] Moreover, video games, and gamification in activities, 'produce an emotional state induced by several factors, most important of which is fun',[56] supporting both engagement and motivation. Motivation is that driving force that makes people want to invest all their efforts into whatever they do and it is an essential element for having the player engaged with the game-like activities.[59] In general, it can be said that motivation is a fundamental element necessary for every activity someone is involved in.[60] Additionally, Ryan, Rigby,

and Przybylski,[61] showed that the need for satisfaction during playing a game leads to short-term improvements in well-being, and in general, playing videogames has positive effects on the cognitive, motivational, emotional, and social aspects of well-being.[62]

Furthermore, our choice to develop CoWMeG for tablets appears to be a feasible solution for being adopted by Thai stroke patients. This could be confirmed in Graphs 3 and 4, where most of the participants perceived this solution as convenient (i.e., setting up, laying aside, and maintenance) expressing a high level of satisfaction (very satisfied) with the physical characteristics of the used tablet (i.e., size, shape, weight, and durability). In fact, a tablet is usually characterized by a wider screen compared to smartphones, thus providing more space for action and wider vision while playing games. Additionally, a touch-screen solution allows players to directly act on the objects with a simple movement and touch of their fingers, thus removing the need to pass through pointing devices, like a mouse, or cumbersome interactions with a keyboard, common requirements found in laptop or table-desktop solutions. Importantly, a tablet solution enhances accessibility, allowing a player to engage with the game-like training anytime and anywhere, due to its portability nature. Moreover, computer-based cognitive training games like CoWMeG may be a solution for supporting occupational therapists in their professional activities with Thai stroke patients, especially in a clinical setting, where there may be a lack of dedicated performing areas.

Finally, it should be acknowledged that the participants in this study were stroke patients who have cognitive MSET10 scores between 13 and 22. Thus, using CoWMeG with stroke patients who have cognitive MSET10 scores lower than 13 may show different results on user experience because the game tasks might be too hard for the players' cognitive abilities. Investigation of CoWMeG for being used with those who have cognitive MSET10 lower than 13 may be needed. However, we assume that CoWMeG may be feasible to use also with those patients who have MSET 10 scores lower than 13 due to the fact that our game was designed based on a number of stimuli (that needs to be remembered by the players) ranging between a minimum of 1 and a maximum of 7±2.[63] Additionally, we do believe that cognitive abilities are not the only element to account, but therapists should also keep in consideration the user's previous technology-related experience, especially during the first time playing. However, Kueider et al.,[64] stated that most computer-based cognitive training does not require tech-savviness for completing the tasks. This might also be seen in CoWMeG where the player needs to perform relatively simple actions like touching, moving, or dragging target objects on the screen in accordance with the game instructions. On the last note, it is important to remind that skin dryness (commonly found in elderly people) could lead to unresponsiveness when touching the screen. This problem could be easily overcome with the usage of a tablet pen.

Limitation and future research

The computer-based cognitive training game (CoWMeG) developed in this study was used with Thai stroke patients who were familiar or had previous related experience with using technology tools (e.g., laptop, tablet, and smartphone), and had cognitive MSET scores between 13 and 22. Different results may be obtained with users who are not familiar with technology, and with stroke patients who have cognitive MSET10 scores lower than 13. Future research could focus on testing the usage of CoWMeG with patients with different cognitive function impairments, and different technology-based experience backgrounds. In addition, future investigation on the effectiveness of CoWMeG is needed mainly on its effects on working memory and occupational performance.

Conclusion

In this research work, we focused on the development of a computer-based cognitive training game and on measuring its feasibility for being used with Thai stroke patients who have cognitive impairments. The newly computer-based cognitive training game, named CoWMeG, consists of a total of ten working memory games. Each game has different difficulty levels with each level consisting of three subsequential screen pages (e.g., instruction, playing, and scoring screen pages). CoWMeG is considered as a game-like training that uses simulated real-life activities whose design is based on the Thai ecosystems (e.g., language and activities). The results on the user experience indicate that CoWMeG may be feasible for being used with Thai stroke patients who have cognitive impairments.

Conflict of interest

All authors declare no personal or professional conflicts of interest relating to any aspect of this study.

Ethic approval

This research project was approved by the Research Ethics Committee of Faculty of Associated Medical Science at Chiang Mai University, Thailand with study code: AMSEC-63EX-041, ethic clearance number:371/2020, and by the Human Ethics Committee of Buriram Hospital, with ethic clearance number 6/2563.

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Comparison of three monocyte depletion techniques for lymphocyte isolation from peripheral blood mononuclear cell

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ABSTRACT

Background: Lymphocytes are crucial cells in the immune system. Studying lymphocyte function can lead to better understanding of the immune system. Essentially, lymphocyte isolation technique is required for studying lymphocyte function. Several techniques were developed to prepare lymphocytes, including depletion of monocytes from peripheral blood mononuclear cells (PBMCs).

Objectives: To compare utilization of three different techniques for lymphocyte isolation by monocyte depletion from PBMCs.

Materials and methods: Lymphocytes were isolated from PBMCs by depletion of monocytes using (i) magnetic beads phagocytosis, (ii) Percoll density gradient centrifugation, and (iii) anti-FITC antibody conjugated micro-magnetic beads. The number of cells collected was counted using Turk's solution. The cellular profiles of PBMCs and monocyte-depleted PBMCs were determined by immunofluorescence and flow cytometry.

Results: The highest yield and purity of monocyte-depleted PBMCs were achieved using the anti-FITC antibody conjugated microbeads depletion method. However, this method consumed the longest time and had the highest cost. Magnetic beads phagocytosis depletion method required the shortest time; however, the wide range of collected yield was a concern. Percoll gradient centrifugation method was the cheapest, but the percentage yield was the lowest among the three methods.

Conclusion: The utilization of three different methods was able to deplete monocytes from PBMCs. However, each technique had some advantages and disadvantages. The information obtained from this study might give some guidance for selecting a suitable method for isolation of lymphocytes based on the monocyte-depleted PBMC strategy.

Introduction

The immune system is an important host defense system that relates to several types of cells and soluble molecules, which collaborate systematically to protect our body from infectious microorganisms and tumors.[1, 2] In the immune system, lymphocytes are one of the crucial cells that play several roles in both innate and adaptive immunity. Lymphocytes are divided into three major groups that play distinct roles, including B cells, T cells, and natural killer (NK) cells.[1-3] B cells, upon antigen stimulation, differentiate into plasma cells to secrete antibody and pro-inflammatory cytokines to fight pathogens. Moreover, B cells also act as antigen presenting cells (APCs), which recognize antigens and present peptide-major histocompatibility complex (MHC) class II complex to induce T cell activation.[4, 5] T cells

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play an important function in cell-mediated immunity, including directly killing infected cells, producing cytokines to activate and regulate other immune cells.[6] NK cells are innate lymphocyte subsets, which rapidly respond and kill virus-infected and tumor cells via direct killing or the ADCC mechanism.[7] Since lymphocytes play an important role in immune responses, studying lymphocyte function will build a path toward better understanding the immune system.

Venous blood contains several types of unstimulated leukocytes, including lymphocytes, monocytes, and granulocytes. These leukocytes are different in cell density and surface molecule markers.[8, 9] In order to study the function of lymphocytes, a method for lymphocyte preparation is necessary. The rapid and non-altering processing of cell function is an essential approach in clinical and basic research.[9, 10] Nowadays, several strategies are employed to prepare lymphocytes from peripheral blood, such as immune precipitation, flow cytometry sorting, magnetic particle sorting, and sorting by microfluidic chips blood.[9, 11, 12] However, lymphocyte isolation from whole blood using the available methods is difficult. Each technique has different advantages and disadvantages.[11, 13] In this study, we compare three different techniques for lymphocyte preparation by depletion of monocytes from peripheral blood mononuclear cells (PBMCs). The methods conducted in this study include Percoll density gradient centrifugation, anti-FITC mAb-conjugated microbeads, and magnetic beads-phagocytosis. The technique selected was based on physical properties and functions of cells, i.e. cell density, specific surface molecules, and phagocytosis ability.[11, 14] For Percoll density gradient centrifugation, monocytes were separated from lymphocytes by their density using isosmotic Percoll density gradient.[15, 16] In magnetic beads-phagocytosis method, monocytes were depleted by their phagocytosis function.[11] PBMCs were incubated with magnetic beads and then placed on a magnetic stand to remove beads-phagocytosed monocytes. For anti-FITC mAb-conjugated microbeads, monocyte-depleted PBMCs were prepared by depletion of monocytes (negative selection) from PBMCs using FITC-conjugated anti-CD14 mAb. Anti-FITC micro magnetic beads were added and monocyte-beads complexes were trapped in a magnetic column placed in a magnetic field. We demonstrated that each technique showed different advantage and limitations. Thus, the information obtained from this study could provide guidance for the selection of the appropriate method of lymphocyte preparation.

Materials and methods

Antibodies and reagents

PE-conjugated anti-CD3 mAb was purchased from ImmunoTools (Friesoythe, Germany). PE-conjugated anti-CD19 mAb was obtained from BD Bioscience (San Jose, CA, USA). FITC-conjugated anti-CD14 mAb, PerCP-conjugated anti-CD45 and Alexa Fluor647-conjugated anti-CD56 mAb were purchased from BioLegend (San Diego, CA, USA). Percoll reagent was ordered from Amersham Biosciences (Uppsala, Sweden). Magnetic beads sized 1.23 µm and anti-FITC microbeads were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany).

Peripheral blood mononuclear cells

Heparinized whole blood was obtained from healthy donors. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized whole blood using Ficoll-Hypaque gradient centrifugation. In brief, heparinized blood was diluted with phosphate buffer saline (PBS), pH 7.2, at a ratio of 1:1. Then, diluted blood was overlaid on Ficoll-hypaque solution and centrifuged at 25°C, 400 ×g for 30 minutes. After centrifugation, the mononuclear cells were collected and washed 3 times with PBS. The number of cells was counted using Turk's solution. Cells were resuspended in the appropriated buffer for further experimentation. It was noted that PBMCs used in this study were chosen in various ranges of monocytes to determine each technique's monocyte depletion ability.

Monocyte-depleted PBMC preparation using phagocytosis of magnetic beads

PBMCs ($1x10^7$ cells) and magnetic beads were resuspended in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). Then, magnetic beads were added into cells to obtain cell-to-bead ratios of 1:1, 1:2 and 1:5 in total volume of 250 µL. The cell and beads mixtures were rotated at room temperature for 30 minutes. After incubation, cells were placed in a magnetic stand for 5 minutes to remove phagocytosed and free magnetic beads. The unbound solution containing monocyte-depleted PBMCs was collected and counted using Turk's solution. Cells were stained with either a cocktail of PerCP-conjugated anti-CD45 mAb and PE-conjugated anti-CD14 mAb or a cocktail of FITC-conjugated anti-CD3 mAb, Alexa Fluor 647-conjugated anti-CD56 mAb, and PE-conjugated anti-CD19 mAb at 4°C for 30 minutes. The percentages of monocyte contamination and remaining T cells, B cells, and NK cells were analyzed by flow cytometer.

Monocyte-depleted PBMC preparation using Percoll density gradient centrifugation

PBMCs ($1x10^7$ cells) were resuspended in PBS. PBMCs were then overlaid on Percoll solution (48.5% Percoll, 0.16 M sodium chloride in ddH_2O) and centrifugated at 865 ×g at room temperature for 40 minutes. After centrifugation, monocyte-depleted PBMCs were pelleted at the bottom of the tube. The enriched monocyte layer and Percoll solution was discarded. Monocyte-depleted PBMCs were washed three times with PBS by centrifugation at 1000 xg for 5 minutes. After depletion, cells were stained with either FITC-conjugated anti-CD14 mAb, FITC-conjugated anti-CD3 mAb, PE-conjugated anti-CD19 mAb, or Alexa Fluor 647-conjugated anti-CD56 mAb at 4°C for 30 minutes. The percentages of monocyte contamination and T cells, B cells, and NK cells remaining were analyzed by flow cytometer.

Monocyte-depleted PBMC preparation using anti-FITC mAb-conjugated microbeads

PBMCs ($1x10^7$ cells) were stained with 10 µg/mL of FITC-conjugated anti-CD14 mAb at 4°C for 30 minutes. After incubation, PBMCs were washed to remove unbound antibodies. Anti-FITC mAb microbeads were then added and incubated in a refrigerator for 15 minutes. The stained cells were washed with PBS containing 0.5% bovine serum

albumin (BSA) and 2 mM EDTA. Then, magnetic separation process was performed by placing an LD column in the magnetic field. The column was prepared by rinsing with 1 mL of PBS containing 0.5% BSA and 2 mM EDTA 3 times. After rinsing, cell suspension was applied into the column. The anti-CD14 antibody binding cells were trapped in the column by the magnetic force. The column was washed again with 1 mL of PBS containing 0.5% BSA and 2 mM EDTA 3 times. The unbound solution containing monocyte-depleted PBMCs was then collected. Percentages of monocyte contamination and T cells, B cells, and NK cells remaining were analyzed by flow cytometer.

Results

Monocyte-depleted PBMC preparation using phagocytosis of magnetic beads

Monocytes are phagocytic cells which could engulf the pathogens as well as particles.11 Therefore, lymphocytes could be prepared by depleted monocytes from PBMCs via the phagocytosis function of monocytes. For optimal cell-to-bead ratio titration, the magnetic beads were incubated with PBMCs at cell-to-bead ratios of 1:1, 1:2 and 1:5 for 30 minutes to allow phagocytosis of monocytes. By this method, after gating cell population by forward size scatter (FSC) and side scatter (SSC) plot, CD14 positive monocytes remaining in PBMCs were decreased from 18.5% to 9.42%, 6.99%, 1.65% at cell-to-bead ratios of 1:1, 1:2 and 1:5, respectively, (Figure 1A). Moreover, anti-CD45 mAb was used to gate white blood cells and analyze the percentage of monocytes. It was found that monocytes in PBMCs were decreased from 18.5% to 9.45%, 7.01% and 1.6% at cell-to-bead ratios of 1:1, 1:2 and 1:5, respectively (Figure 1A). FSC and SSC plot demonstrates that there were no beads remaining after depletion. According to the results, the cell-to-bead ratio of 1:5 was selected for further monocyte depletion experiments. As shown in Figure 1B, the percentages of each cell subpopulations in PBMCs compared with monocyte-depleted PBMCs were determined. After depletion, B cells were decreased from 10.9% to 4.20% but T cells and NK cells were not altered. Based on our findings, this method was able to deplete monocytes in PBMCs from 8.52-19.3% to 0.98-1.49%, as shown in Figure 1C, and the required time for the depletion step was around 60 minutes. The percentage yield of monocyte-depleted PBMCs was obtained in a range of 30.3-60% of PBMCs, which is unstable when compared with other methods, as shown in Table 1. The isolation cost to obtain $1x10^7$ cells of lymphocytes was approximately 1.56 USD (Table 1).

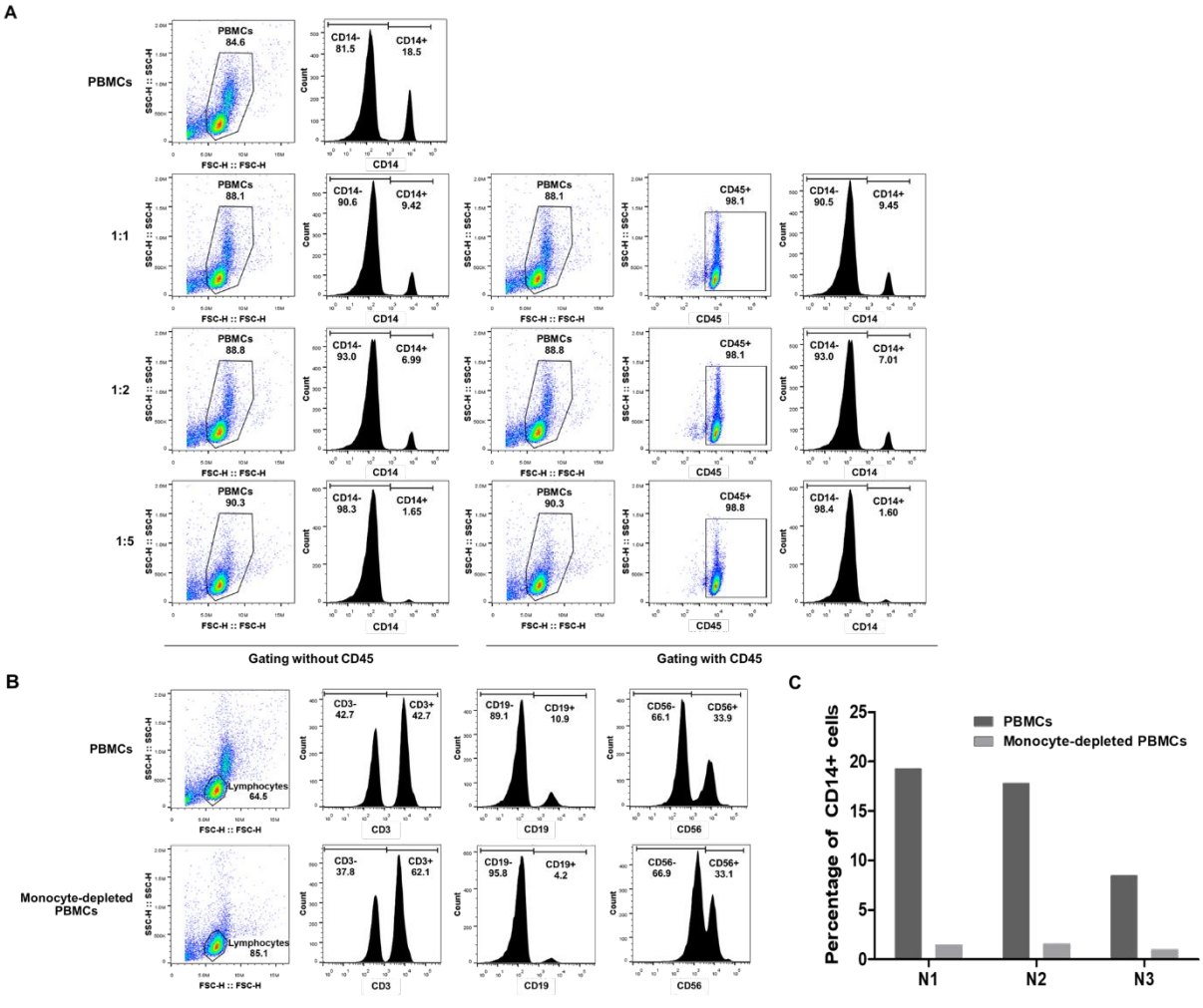

Figure 1. *Percentage of monocytes, T cells, B cells and NK cells after monocyte depletion by phagocytosis of magnetic beads.*

PBMCs were incubated with magnetic beads at cell-to-bead ratios of 1:1, 1:2 and 1:5. The beads-phagocytosed monocytes and free beads were removed. Percentages of each cell subpopulations in PBMCs and monocyte-depleted PBMCs were analyzed by flow cytometry. (A) Percentages of CD14+ cells were compared by gating with forward scatter and side scatter plot in combination with or without PerCP-conjugated anti-CD45. (B) Percentages of indicated cell subpopulations after monocyte depletion at cell-to-bead ratio of 1:5 is shown in histograms. CD3+, CD19+ and CD56+ cells were gated from CD45+ in lymphocyte gate. (C) Three individual representative data of percentage of CD14+ cells in PBMCs and monocyte-depleted PBMCs are shown (n=6).

Table 1 Summary comparison of the lymphocyte isolation using three different monocyte depletion methods.

Monocyte depletion methods	Depletable range of Monocyte (%)	Monocyte contamination (%)	Estimated time used for lymphocyte isolation (minutes)	Estimated cost for preparation of $1x10^7$ lymphocyte (USD)	Yield of isolated lymphocyte from PBMCs (%)*
Magnetic beads	8.52-19.3	0.98-1.49	60	5.13	30.3-60
Percoll solution	8.52-26.8	0.4-1.67	120	1.56	35.5-44.1
Anti-FITC microbeads	10.4-19.3	0.65-1.47	150	25.1	55-66.9

** % Yield of isolated lymphocyte from PBMCs = (total number of isolated lymphocyte – total number of monocyte in isolated lymphocyte) / (total number of PBMCs – total number of monocytes in PBMCs) x 100*

Monocyte-depleted PBMC preparation using Percoll solution

Lymphocytes can be separated from monocytes based on their different density using a low viscosity density gradient medium. In this experiment, we prepared lymphocytes from PBMCs by using Percoll gradient centrifugation method. As shown in PBMC gate, monocyte contamination in monocyte-depleted PBMCs was 1.67%, which was reduced from 20.4% of monocytes in PBMCs (Figure 2A). B cells were also reduced in monocyte-depleted PBMCs from 7.98% to 3.77%, while T cells and NK cells were changed only slightly. In Figure 2B, data shows that monocytes were reduced from a range of 8.52-26.8% to 0.4-1.67% using this method. This method can achieve a 35.5-44.1% yield of monocyte-depleted PBMCs in around 120 minutes (Table 1). The isolation cost to obtain 1x107 cell of monocyte-depleted PBMCs was approximately 5.13 USD (Table 1).

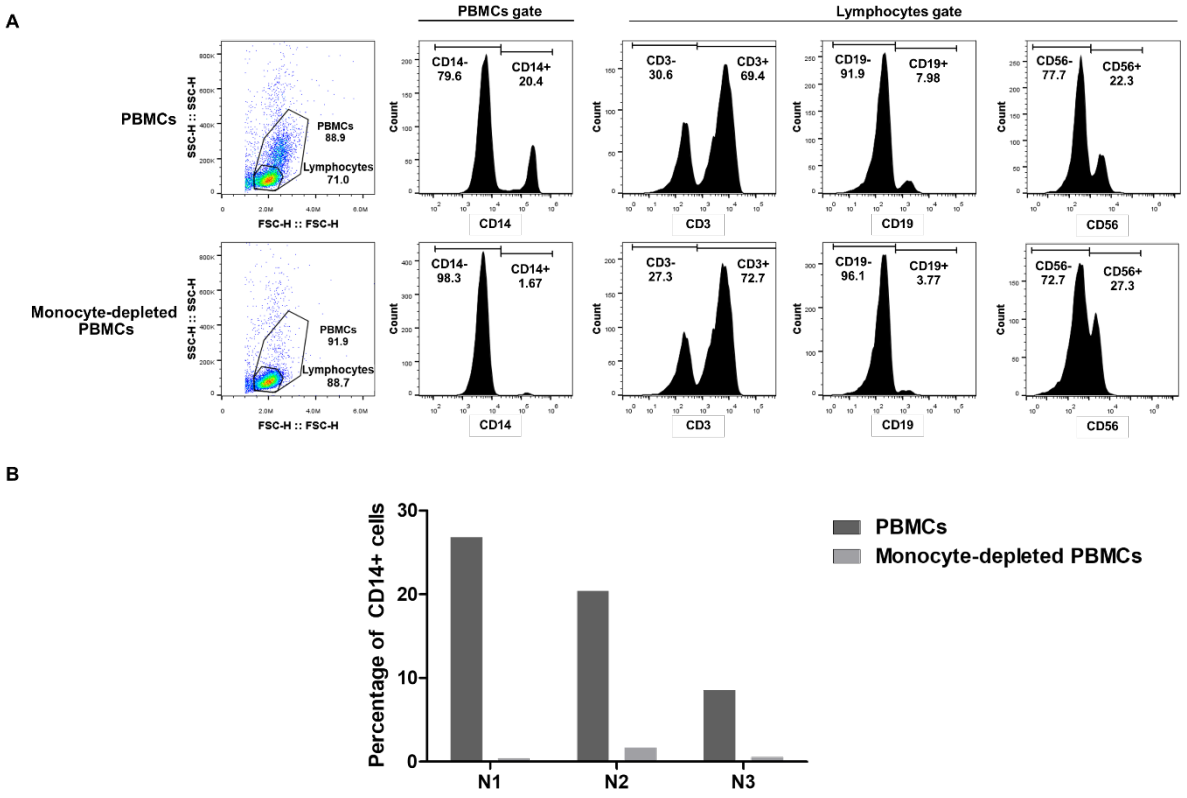

***Figure 2**. Comparison of the percentage of monocytes, T cells, B cells and NK cells after lymphocyte isolating using Percoll gradient centrifugation method.*

Monocytes were depleted from PBMCs using Percoll solution. (A) Percentage of each cell subpopulation in PBMCs compared with monocyte-depleted PBMCs from representative data are shown in histograms. (B) Three individual representative data of percentage of CD14+ cells in PBMCs and monocyte-depleted PBMCs are shown (n=6). CD14+ cells were gated from PBMCs while CD3+, CD19+ and CD56+ cells were gated from lymphocytes.

Monocyte-depleted PBMC preparation using anti-FITC antibody conjugated micro-magnetic beads

Immunomagnetic selection method is a technique used for the isolation of cells from the blood using high-affinity antibodies and magnetic bead technology.[9, 10,17] In this experiment, monocyte-depleted PBMCs were prepared using anti-FITC magnetic beads based on negative selection method. CD14+ cells were bound with anti-CD14 FITC mAb and trapped into a column with anti-FITC micro-magnetic beads under a magnetic field. As shown in Figure 3A, monocytes in PBMCs were depleted from 15.1% to 0.65%, whereas T cells and NK cells were altered only slightly. In addition, B cells were also depleted from 10.6% to 5.96%. After depletion by anti-FITC microbeads, the monocyte contamination was 1.47-0.65%, which was reduced from monocytes in PBMC ranging from 19.3-10.4%, as shown in Figure 3B. The percentage yield of monocyte-depleted PBMCs prepared by this method was 55-66.9%. Estimated time for depletion in this method was around 150 minutes (Table 1). The isolation cost to obtain $1x10^7$ cell of lymphocytes was approximately 25.1 USD, as shown in Table 1.

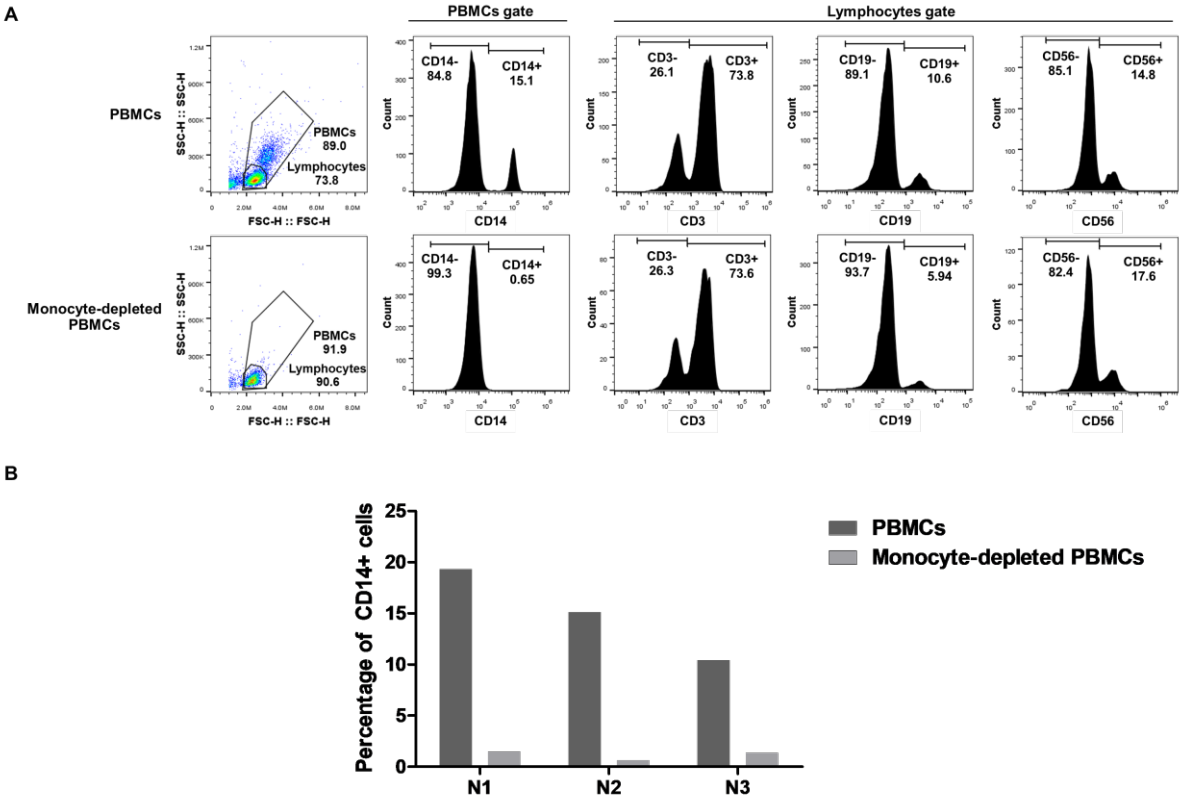

***Figure 3**. Comparison of the percentage of monocytes, T cells, B cells, and NK cells after monocyte depletion using anti-FITC microbeads.*

PBMCs were stained with FITC-conjugated anti-CD14 mAb. Anti-FITC microbeads were added to deplete monocytes from PBMCs using LD column. (A) Percentage of cell subpopulation in PBMCs compared with monocyte-depleted PBMCs is expressed from representative data in histograms. (B) Three individual representative data of percentage of CD14+ cells in PBMCs and monocyte-depleted PBMCs are shown (n=6). CD14+ cells were gated from PBMCs, whereas CD3+, CD19+ and CD56+ cells were gated from lymphocytes.

Discussion

Lymphocytes are crucial cells that play several roles in immune systems. In order to study the function of lymphocytes, a method for cell preparation from peripheral blood without altering cellular function is important.[10] Conventionally, a density gradient medium, such as Ficoll-Hypaque, is used to isolate PBMCs, in which the majority of cells are lymphocytes and monocytes, from granulocytes, erythrocytes and platelets. In this method, diluted blood with PBS is placed in Ficoll-Hypaque solution. After centrifugation, layers of cells are separated depending on the cell density. The layer in the middle between Ficoll-Hypaque solution and plasma contains PBMCs.[18,19] However, the densities of lymphocytes and monocytes overlap so that efficient separation proves difficult on the basis of density difference alone, since PBMCs consist of two major cell populations, including lymphocytes and monocytes.[20, 21] The responses of monocytes to stimuli can induce cytokine production, which affects the survival, proliferation, and immune deviation of other cell types.[22,23] Moreover, the contact between monocytes and lymphocytes stimulates the signaling triggering pro-inflammatory

mechanisms.[24, 25] The previous study reported that T cell stimulation with anti-CD3/CD28 antibody-coated beads is less effective due to non-specific binding of contaminated monocytes with beads.[26] Furthermore, the efficiency of gene transduction on lymphocytes can be decreased by monocytes that compete in the gene vector.[26] Therefore, the study of lymphocyte function can be interfered with by monocyte contamination.[27] The appropriate lymphocyte isolation techniques are required for studying roles of lymphocytes. In this study, we demonstrated 3 different methods for the isolation of lymphocytes by depletion of monocytes from PBMCs, using naked magnetic beads, Percoll density gradient centrifugation and anti-FITC mAb-conjugated microbeads.

Monocytes are classified as phagocytes, which engulf and eliminate invading pathogens, foreign particles, and cell debris.[1, 2] According to their function, PBMCs were incubated with magnetic beads and then monocytes were depleted by bead phagocytosis in a magnetic stand. The naked magnetic beads, sized 1.23 µm, were used to induce monocyte phagocytosis. Ben *et. al*. reported that beads sized 1.0–3.0 µm are taken up to monocytes via phagocytosis and 1.0 µm beads were optimal targets for monocytes.[28] In this study, after optimization, bead-to-cell ratio of 1:5 was optimal for monocyte depletion. Monocytes in the range of 19.3-8.52% in PBMC were almost completely depleted. This method takes the shortest times when compared with other methods. However, the percentage of lymphocyte yield obtained had a wide range of 30-60%. The difference in phagocytic activity of each individual was reported.[29] Monocytes from each individual might engulf different numbers of beads, resulting in different magnetic forces required to trap phagocytosed monocytes in the magnetic stand. The phagocytosed monocytes might bring non-phagocytosed cells trapped together with the magnetic stand in different levels for each individual. This might be one possible reason that a wide range of lymphocytes was obtained after monocyte depletion by magnetic beads phagocytosis method. B cells were also depleted because this cell can also phagocytose beads.[30] Study of B cell might not be appropriate, however, due to the limited number of B cells obtained after cell preparation using this method. The lymphocyte preparation using this method was able to study T cell proliferation induced by mIgG2b and anti-CD3 mAb.[31] For Percoll density gradient centrifugation, monocytes were depleted from lymphocytes using isosmotic Percoll density gradient. Percoll consists of various sizes of silica particles (15 to 30 nm diameter) coated with non-dialyzable PVP. These particles have a specific density to form density gradient in the range of 1.0-1.3 g/mL during centrifugation.[32] After centrifugation, monocytes in the middle layer were separated from lymphocytes, which were present in the bottom of the tube. Therefore, the step that discards monocytes and collects lymphocytes by pipette should consider monocyte contamination. By this method, monocytes in the range of 26.5.4-8.52% in PBMCs were almost depleted. This method takes moderate time and uses inexpensive reagents when compared with other methods. However, the yield of cells obtained by this method is lower than other methods. Besides monocytes, platelets and granulocytes that interfere with lymphocyte function can also be depleted by Percoll technique.[27] A previous study reported that the functional activity of cells is not harmed by polyvinyl-coated colloidal silica particles. The lymphocytes obtained from Percoll isolation responded to mitogens, including pokeweed mitogen and phytohemagglutinin in a close level with the lymphocytes obtained from a Ficoll-Hypaque gradient.[32, 33] Moreover, T cell proliferation assay with immobilized anti-CD3 mAb plus anti-CD28 mAb stimulation could be performed by monocyte-depleted PBMCs obtained from Percoll.[25] However, this method was not suitable for the study of B cell function due to the loss of cells during the lymphocyte isolation process.

In the last two decades, the isolation of cells from blood has been prepared by using high-affinity antibodies and magnetic beads technology.[10] Processes involved in cell separation including positive selection and negative selection have been developed. Positive selection enriches cells by antibodies, which directly bind to target cells and allow the labeled cells to be retained in the isolated fraction, whereas negative selection enriches cells by using antibodies specific to the non-target cells to deplete unwanted cells in the cell fraction.[9-11] Importantly, the study of cell function should use negative selection method because the binding of antibody to target cell could alter their features and functions. Antibody pull down is a well-known method for isolating lymphocytes using the specific antibody to bind to the cell surface.[9] For monocyte-depleted PBMCs preparation using anti-FITC mAb-conjugated microbeads, monocytes were stained with FITC-conjugated anti-CD14 mAb. Then, anti-FITC mAb-conjugated microbeads were added to bind with labeled monocytes. In order to deplete monocytes, monocyte-beads complexes were subjected to a magnetic LD column. Then, the unbound solution containing monocyte-depleted PBMCs was collected. This column is appropriate to deplete cells because the matrix of columns is composed of ferromagnetic spheres, which amplify the magnetic field by 10,000-fold when the column is placed in a magnetic stand. Moreover, the unique specifications of LD columns have a specific shape and matrix, resulting in a slow flow rate. This is crucial for the efficient isolation of the cells, which are minimally magnetically labeled. The space between the sphere matrix is larger than primary cells to allows the cells to freely flow through the column. Furthermore, the MACs magnetic beads do not activate or alter the status of target cells isolated.[34] In this study, when compared with other methods, the yield of cells obtained by this method is the highest and has the lowest monocyte contamination. Monocytes in the range of 19.3-10.4% in PBMC were depleted. However, this method takes a long time because several steps are required, including staining cells with FITC-conjugated anti-CD14 mAb, incubating cells with anti-FITC beads and subjecting cells in an LD column with a low flow rate. Moreover, this method is quite expensive because the reagent and column are a commercial kit. In our laboratory, lymphocyte isolation by anti-FITC beads was used for studying T cell proliferation assay using immobilized anti-CD3 mAb plus anti-CD28 mAb stimulation. Moreover, T cell proliferation

and cytokine production in Toll-like receptor 2 agonist were determined in monocyte-depleted PBMC preparation by anti-CD14-conjugated magnetic microbeads.[37] The interaction between monocytes and NK cells in IFN-γ and CD107a production during Zika virus infection was also studied by monocyte-depleted PBMCs. In addition, isolating lymphocytes by immunomagnetic separation column was previously reported to be used for studying quantitative and qualitative cell viability assay.[34]

In this study, we demonstrated the utilization of three different methods for lymphocyte isolation from PBMCs by monocyte depletion. All of them were able to remove the monocytes from PBMCs. The isolated lymphocytes can be used for studying their function. However, each technique has some advantages and disadvantages. Therefore, the lymphocyte isolation method should be considered and appropriately selected for each experiment.

Conflict of interest

The authors declare that they have no conflict of interests.

Human ethics approval

The human ethics of this study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand (AMSEC-61EX-080).

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Comparison of entrance surface air kerma to eye lens in head computed tomography protocols between 32-MDCT and 64-MDCT on an anthropomorphic phantom

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ABSTRACT

Background: Brain multi-detector computed tomography (MDCT) is commonly performed for diagnosis of traumatic or non-traumatic brain injury cases. During brain CT scan, the eye lens is highly sensitive to radiation and may cause radiation-induced cataracts irradiated by CT primary beam.

Objectives: This study aimed to determine and compare entrance surface air kerma (ESAK) to the eye lens in clinical routine head protocols between 32-MDCT and 64-MDCT using an anthropomorphic phantom.

Materials and methods: A PBU-60 head phantom was scanned by 32-MDCT and 64-MDCT in helical, axial, and tilted axial modes used in clinical routine head protocols with tube voltage of 120 kVp, tube current of 108-150 mAs for 32-MDCT, and 200-310 mAs for 64-MDCT. The Nanodot™ optically stimulated luminescent dosimeters (OSLDs) was used to measure ESAK to eye lens. Dose length product (DLP), normalized volume CT dose index ($_nCTDI_{vol}$), and normalized mean ESAK were compared between two CT scanners.

Results: The ranges of mean normalized ESAK to the eye lens in each scanning mode was found from 0.41±0.01 to 0.51±0.01 mGy/100 mAs for 32-MDCT and 0.30±0.01 to 0.40±0.01 mGy/100 mAs for 64-MDCT. The normalized ESAKs obtained from 64-MDCT were lower than 32-MDCT by 21.57-37.50%. The lowest normalized ESAK of 0.30±0.01 mGy/100 mAs was obtained in tilted axial scanning mode in 64-MDCT with the difference of 37.50% compared to 32-MDCT of using identical scanning mode.

Conclusion: This study demonstrated that normalized mean ESAK to the eye lens for 64-MDCT in all brain scanning protocols was lower compared to 32-MDCT. In addition, using tilting gantry in axial scanning mode as well as using an automatic tube current modulation system could be beneficial for reducing radiation dose to eye lens during brain CT in clinical routine.

Introduction

Computed tomography (CT) plays an important role as a powerful imaging modality in diagnostic imaging. In the past few decades, the use of CT has increased tremendously, particularly in the emergency department.[1] Multi-detector computed tomography (MDCT) has multiple rows of X-ray detectors, results in faster image acquisition that would be useful for several advance CT applications.[2] However, radiation exposure received from ionizing radiation during CT scans is a point of concern. Since the eye lens is highly sensitive to CT primary beam associated with radiation-induced cataracts[3], the International Commission on Radiological Protection (ICRP) Publication 103 recommended that the threshold dose for preventing radiation-induced cataracts should not be exceeded 0.5 Gy for acute and fractionated

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exposures.[4] Typically, CT scanning parameters and number of detector rows are factors affecting radiation dose to the patients.[5] MDCT scanners from different manufacturers have different included numbers of detector channels. Although the detector configurations can also vary widely, it should be determined based on a type of study performed and a small width of X-ray beam can increase the radiation dose due to increased scanning time.[6]

Most MDCT scanners have similar scanning modes such as helical and axial mode. However, the scanning parameters of these scanners are not definitely identical. Among several techniques for dose reduction in CT, automatic tube current modulation (ATCM) is one of the most effective methods to reduce the radiation dose based on size and attenuation coefficient of the patient's body parts.[7,8] Moreover, utilizing gantry tilt is the another approach that can avoid the primary beam irradiated to the orbit during head CT scans, and could be reduced the radiation dose for the eye lens approximately 75%.[8,9] CT examinations should be performed on a basis of the optimization by balancing radiation dose and adequate image quality for diagnosis in each scanning mode in clinical practice. Therefore, radiological technologists should be concerned in this issue in order to determine optimal scanning protocol for reducing the radiation dose to high sensitivity organs.

To our best knowledge, there were no studies relevant to radiation dose delivered to the eye lens in a routine head CT protocol by comparing between 32-MDCT and 64-MDCT in Thailand. Therefore, in this study, the entrance surface air kerma (ESAK) to the eye lens in clinical routine head protocols was measured using a head anthropomorphic phantom in both 32-MDCT and 64-MDCT.

Materials and methods

Brain phantom

A multipurpose anthropomorphic head phantom-PBU-60 (Kyoto Kagaku, Japan) was employed for this study. The phantom consists of a synthetic skull, cervical spines, and brain with contrast media through arteries in the left side, to simulate a standard human head. This phantom is 30 cm long, measuring from the skull vertex and to the seventh cervical spine. The measurement of the phantom's eye lens dose represents ESAK to the eye lens of a patient who underwent a brain CT scan.

MDCT scanners and scanning parameters

Two MDCT scanners, Canon Aquilion Lightning 32-MDCT at the Department of Radiology, Mettapracharak Hospital and Philips Incisive 64-MDCT at the CT Unit, King Chulalongkorn Memorial Hospital (KCMH) were used for measuring ESAK to the eye lens. Automatic tube current modulation (ATCM) on both MDCT scanners enabled automatic adjustment of tube current in longitudinal (z-axis) and angular modulation (x-y axis) based on size and attenuation coefficient of the patient's body part. ACTM can be estimated through the scan projection radiograph (SPR).

For 32-MDCT, "SureExposure3D" was used for the software of ATCM z-axis modulation. SureExposure3D can be adjusted in order to obtain a preferred image quality for a patient-specific scan. This allows desired standard deviation (SD) for image quality (IQ) reference parameter to maintain the noise level in the image.[10] The SD of 2.61 was set for routine CT head protocol on 32-MDCT. The IQ reference parameter in terms of dose right index (DRI) was utilized for ATCM z-axis tube current modulation in case of 64-MDCT. It was estimated from SPR at the reference standard patient size of 29 cm in diameter with adjustable mA.[10] DRI values can be varied based on patient size and image noise level. The DRI of 34.4 was set for routine CT head protocol on 64-MDCT in this study. The scout protocols of both MDCT scanners were performed with 120 kVp, 20 mA and 300 mm scan length. CT parameters used in clinical routine head examination are listed in Table 1.

Table 1 CT parameters used in clinical routine head examination.

CT protocol	MDCT	ATCM	Setting mAs	Effective mAs	Tube Voltage (kV)	Section collimation (mm)	Beam width (mm)	Rotation time (s)	Reconstructed slice thickness (mm)	Pitch	Gantry tilt (degree)
Brain (*helical mode*)	32	On	108-150*	N/A	120	0.5x1.6	8	0.6	2.0	0.688	0
	64	On	N/A	288	120	64x0.625	40	0.5	3.0	0.600	0
	32	Off	150	--	120	0.5x1.6	8	0.6	2.0	0.688	0
	64	Off	310	--	120	64x0.625	40	0.5	3.0	0.600	0
Brain (*axial mode*)	32	Off	150	--	120	0.5x1.6	8	0.6	2.0	N/A	0
	64	Off	200	--	120	64x0.625	40	1	2.5	N/A	0
Brain (*tilted axial mode*)	32	Off	150	--	120	0.5x1.6	8	0.6	2.0	N/A	10
	64	Off	200	--	120	64x0.625	40	1	2.5	N/A	10

**min-max tube current was set up at 180-250 mA; N/A indicates not applicable.*

Optically Stimulated Luminescence Dosimeter

NanoDot™ (Landauer, Inc., IL, USA), a small-type optically stimulated luminescence dosimeter (OSLD), was used to measure ESAK to the eye lens during head CT scan procedures. As shown in Figure 1, OSLD detector (Al_2O_3:C) consists of a small round crystal with a 0.2 mm layer and 5 mm diameter sealed in 10x10 mm plastic cassettes. NanoDot™ has a wide energy range from 5 keV to 20 MeV with accuracy of ±10%. The calibration and correction factors of NanoDot™ OSLDs for this study were obtained from the reference calibration set of CT dosimeter response.[11] Scarboro et al. found that the signal fading over time had consistency

with dose linearity of less than 3%.[12] The angular response of OSL dosimeters with horizontal and vertical rotations are factors affecting the value of ESAK measurement for the eye lens. At the incidence angle of 60 degrees from the normal (relative to 1), variations of dose measurement should be within 10%.[13]

Irradiated OSLDs were read using a microStar Reader (Landauer, Inc., IL, USA). To optically stimulate the dosimeters, an array of light-emitting diodes was utilized. The luminescence emission signal is proportional to the amount of radiation exposure absorbed by OSLDs. To reduce the measurement uncertainty, each dosimeter was read three times consecutively. OSLD signal was corrected for signal depletion for multiple readouts and individual sensitivities. Since the energy response was different between high and low energy, the average of readings was corrected using a correction factor according to the energy dependence after reading out.[11]

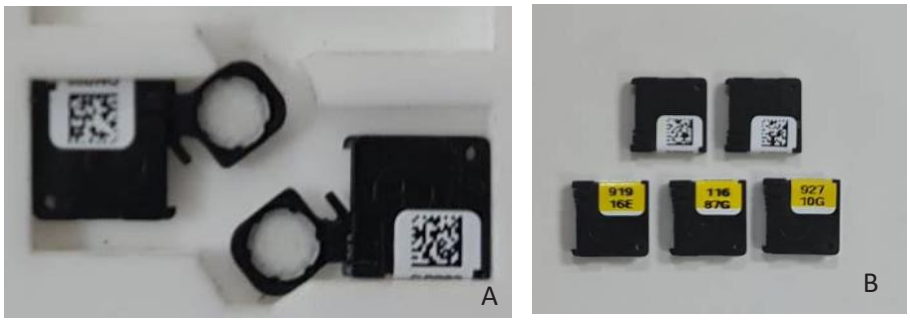

***Figure 1**. NanoDot™ dosimeter. A: an open crystal detector with 2D barcode, B: closed dosimeter showing front and back sides.*

Experimental setup

For accuracy and reproducibility of the measurement, the volume CT dose index ($CTDI_{vol}$) was verified. A 100-mm pencil-ionization chamber model Unfors RaySafe X2 (Billdal, Sweden) was inserted at the center and peripheral holes of polymethylmethacrylate (PMMA) 16 cm diameter head CTDI phantom. The PMMA head phantom was scanned three times with tube voltage of 80-135 kVp. The real-time $CTDI_{vol}$ values displayed on the CT monitor were recorded and compared with the measured values. The percentage differences between measured and displayed values on both CT scanners were then calculated and compared.

To measure ESAK to the eye lens for each scanning protocol, a head phantom was placed in supine position with the midline position located at the center of head support as shown in Figure 2A. To maintain the consistency of measurement for helical and axial scanning modes, the table height was adjusted to be a center of gantry. As a result, the external acoustic meatus (EAM) was at the center of gantry rotation.[14] For tilted axial scanning mode, the gantry was tilted 10 degrees backward parallel to the supraorbital line.[8] Two OSLDs were randomly selected and placed at the center of phantom's eyes surface as shown in Figure 2B. Each imaging protocol was scanned twice to reduce random error (8 protocols x 2 times). The scanning range was set according to a routine head examination and varied from 174 to 180 mm from base of skull to vertex. The field of view (FOV) of 230 mm was fixed for all scanning modes on both MDCT scanners. After scanning, the $CTDI_{vol}$ and the dose length product (DLP) were recorded from the CT monitor. For tube current comparison, mA per slice and effective mAs were collected from the DICOM header. For 64-MDCT, the iterative reconstruction was used for helical with ATCM, while the filtered back projection was used for helical, axial, and tilted axial scanning modes without ATCM. For 32-MDCT, the iterative reconstruction was used for all scanning modes (with and without ATCM). In order to eliminate the bias for comparison of radiation dose between two MDCT scanners, mean ESAK to the eye lens was normalized by 100 mAs.[15] Percent difference of normalized mean ESAK between 32-MDCT and 64-MDCT can be calculated using Equation (1) as follows:

$$\%Difference=\left(\frac{ESAK_{32\text{-}MDCT}-ESAK_{64\text{-}MDCT}}{ESAK_{32\text{-}MDCT}}\right)\times 100,$$

where $ESAK_{32\text{-}MDCT}$ refers to normalized mean ESAK of 32-MDCT, and $ESAK_{64\text{-}MDCT}$ refers to normalized mean ESAK to eye lens of 64-MDCT.

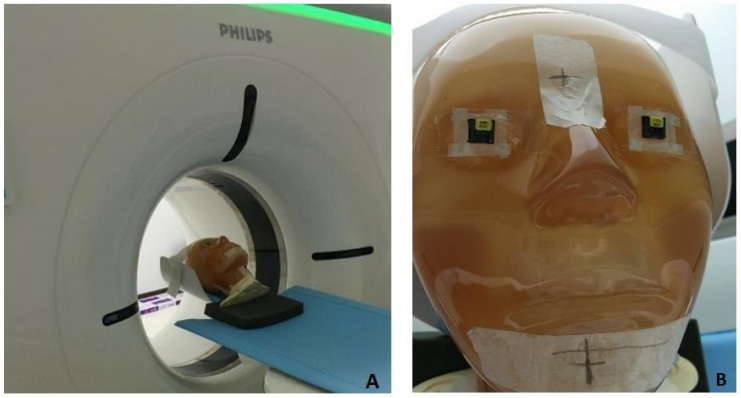

***Figure 2**. A head phantom on the head support (A) and the locations of OSLDs for the measurement of ESAK to eye lens (B).*

Results

For $CTDI_{vol}$ verification, the differences of $CTDI_{vol}$ between measured and displayed values for 32-MDCT and 64-MDCT were within 10% acceptable criteria. Table 2 demonstrates the results of DLP, normalized $CTDI_{vol}$, and normalized mean ESAK to the eye lens measured for each scanning mode on 32-MDCT and 64-MDCT. It was found that the DLP, normalized $CTDI_{vol}$, and normalized mean ESAK obtained from 64-MDCT were lower than those values obtained from 32-MDCT for all scanning modes. However, the DLP values of helical mode with ATCM on 64-MDCT were slightly higher than those from 32-MDCT. Among scanning modes, the minimum and maximum values of normalized mean ESAK to the eye lens varied from 0.41±0.01 to 0.51±0.01 mGy/100 mAs for 32-MDCT and 0.30±0.01 to 0.40±0.01 mGy/100 mAs for 64-MDCT. In Table 2, it can be observed that the tilted axial mode provided the lowest normalized mean ESAK to eye lens of 0.30±0.01 mGy/mAs for 64-MDCT, while helical mode with ATCM offered the lowest normalized mean ESAK to eye lens of 0.41±0.01 mGy/mAs for 32-MDCT. The differences of normalized mean ESAK to eye lens between 32-MDCT and 64-MDCT varied from 21.57% to 37.50% for various scanning modes. Figure 3 depicts the comparison of normalized mean ESAK to eye lens on 32-MDCT and 64-MDCT for each scanning mode. It was seen that tilted axial mode resulted in the highest percentage difference of normalized mean ESAK to the eye lens between two MDCT scanners.

Table 2 Normalized CTDIvol and mean ESAK to the eye lens for each scanning protocol.

CT Protocol	MDCT	ATCM	Normalized $CTDI_{vol}$ (mGy/100 mAs)	DLP (mGy.cm)	Normalized mean ESAK (mGy/100 mAs)	%Difference
Brain (*Helical mode*)	32	On	0.44	859.50	0.41±0.01	N/A
	64	On	0.41	952.95	0.38±0.01	
	32	Off	0.54	1046.80	0.51±0.01	21.57%
	64	Off	0.44	1023.62	0.40±0.01	
Brain (*Axial mode*)	32	Off	0.56	976.80	0.50±0.01	34.00%
	64	Off	0.38	675.85	0.33±0.01	
Brain (*Tilted axial mode*)	32	Off	0.56	976.80	0.48±0.01	37.50%
	64	Off	0.38	675.85	0.30±0.01	

N/A: not applicable.

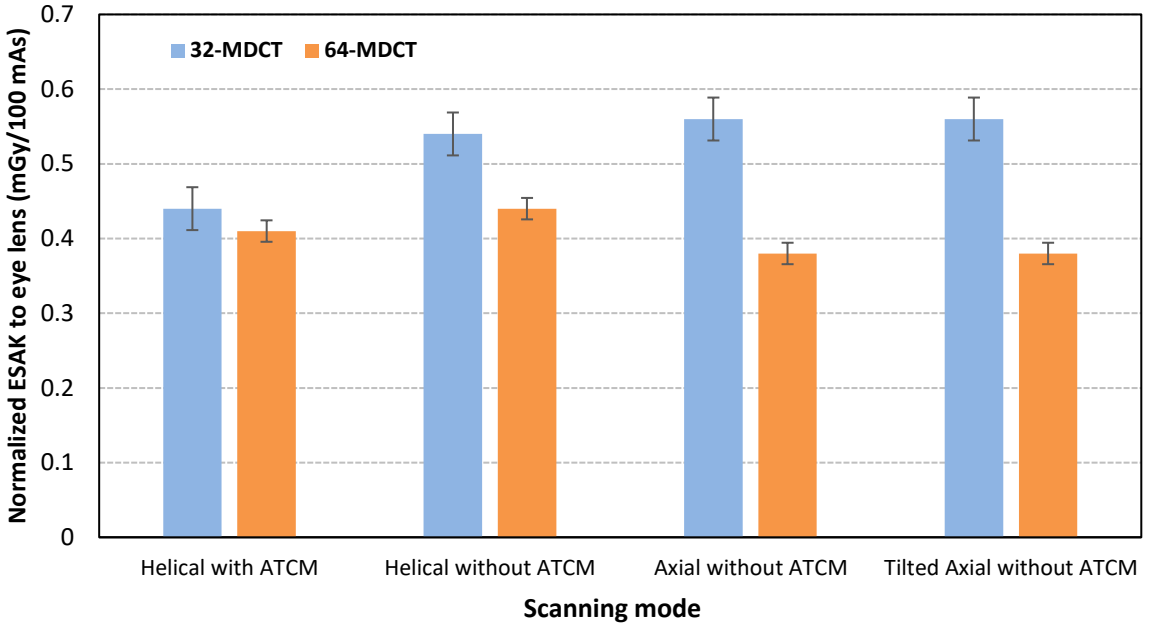

***Figure 3**. Comparison of eye lens dose between 32-MDCT and 64-MDCT for each scanning mode.*

Discussion

According to previous studies, ATCM is one of the most effective methods for radiation dose reduction.[16,17] In this study, the results showed that ATCM could reduce radiation dose to the eye lens by using helical scanning mode. However, the efficiency of using ATCM for radiation dose reduction also depends on tube current used for fixed mAs technique in clinical practice.[14]

The mean normalized ESAK to eye lens of helical mode with ATCM of two MDCT scanners were not comparable

due to different IQ reference parameter settings. Each vendor has a different index for IQ reference parameter that directly affects the radiation dose.[10,17] The DLP of helical mode with ATCM of 64-MDCT was slightly higher than the DLP of 32-MDCT due to small difference of scan length related to irradiated range.[18,19] The mean normalized ESAK of 64-MDCT was lower for all scanning modes compared to 32-MDCT as the beam width 40 mm was used, while the beam width of 8 mm was set for 32-MDCT. In addition, 32-MDCT used a gantry rotation time to complete the scan length longer than the 64-MDCT. Beam width and gantry rotation time are factors related to scattered radiation and penumbra of the radiation dose profile distribution.[19,20]

For axial scanning mode, the slice interval was set to zero without overlapping for data acquisition. This setting was slightly affected by eye lens dose when compared to helical mode in order to complete the coverage scan range. Thus, the mean normalized ESAK to eye lens of 64-MDCT was decreased when compared to helical mode. Comparing the axial mode to helical mode without ATCM on 32-MDCT, it could be noticed that the mean normalized ESAK of axial mode was not different from the helical mode without ATCM. Nevertheless, the mean normalized ESAK to eye lens of axial mode was increased when compared to helical mode with ATCM because the tube current setting was different. Moreover, tilted axial scanning mode showed the lowest mean normalized ESAK to eye lens and provided the highest percent dose difference between two scanners accordingly. As a result, the value of mean normalized ESAK was decreased by 5.88% and 25% for 32-MDCT and 64- MDCT respectively, when compared to helical mode without ATCM. In addition, tilting of the gantry at +10 degree along to the supraorbital line is recommended for eye lens dose reduction since the eye lens is completely out of the CT primary beam.[8,14] Although there was variation between the scanners, mean normalized ESAK to eye lens on both scanners was well below the threshold dose of 0.5 Gy recommended by the ICRP Publication 103.[4]

To demonstrate the radiation doses for the eye lens obtained from MDCT in clinical routine, the results obtained from this study were compared only the existing head routine protocols for both CT scanners without any modifications. Although the CT protocols were slightly different from each other, the head brain phantom images acquired from these protocols can provide an adequate image quality as shown in Figure 4. The noise values (SD) at corona radiata and lateral ventricle in each scanning mode ranged from 3.92 to 5.54 HU for 32-MDCT and 2.87 to 4.81 HU for 64-MDCT. Nevertheless, comparison of image quality on different scanners can be used to analyze the impact of eye lens dose reduction and to determine the optimal protocol for further studies.

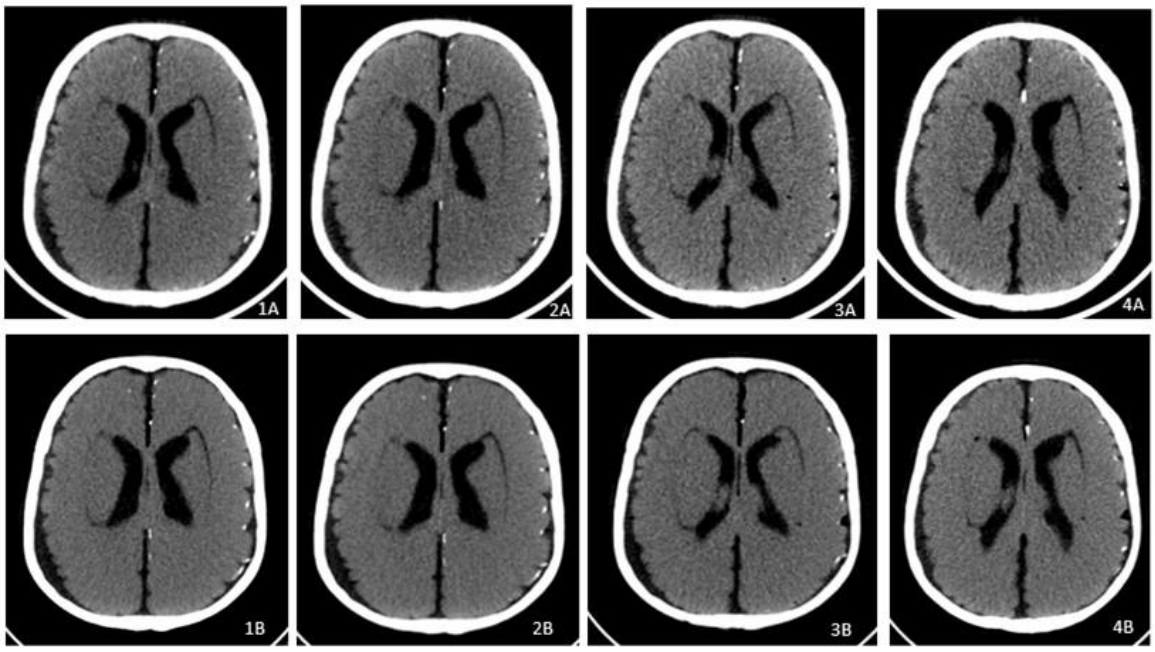

Figure 4. *CT transaxial brain image of 32-MDCT (A) and 64-MDCT (B) in each scanning mode. 1: helical with ATCM, 2: helical without ATCM, 3: axial without ATCM, 4: tilted axial without ATCM.*

Regarding factors affecting OSLD measurement such as geometry and angular dependence, Perks et al. reported the errors of measurement at a specific incidence angle of gantry rotation.[13] For 60-degree incidence angle, the variation of OSLD measurement could be increased to 10% (relative to 1 at normal incidence). In this study, a 10-degree incidence angle was chosen. As a result, the variation of measurement was relatively low at close to 1% from normal incidence. This study has some limitations. First, the scanning protocols were not exact identical between two scanners resulting in slightly different radiation dose measurement. Second, the eye lens dose obtained from this study was not generalized to the other CT scanners due to different characteristic scanner output. Finally, only a standard size head phantom of 16 cm was used. Therefore, different sizes of head phantom should be examined for further study.

Conclusion

The number of detector rows, scanning mode, and parameter settings are factors affecting eye lens dose in head CT examinations. Comparing the eye lens dose between 32-MDCT and 64-MDCT, normalized mean ESAK of 64-MDCT were lower than 32-MDCT in all scanning modes. The eye lens dose in routine brain CT scan obtained from this study was still below 0.5 Gy. Using tilting gantry in axial scanning mode and ATCM in helical mode could reduce eye lens dose during brain CT. Thus, these scanning techniques should be applied for dose reduction in clinical practice to provide benefit to a patient.

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Conflict of interest

There are no conflicts of interest to disclose.

Ethic approval

The study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University.

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Rapid detection of *FLT3*-ITD (exon14-15) gene mutations analysis in acute myelogenous leukemia patients by High Resolution Melting analysis

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ABSTRACT

Background: Molecular genetic characterization allows prognostic stratification and can potentially alter treatment choices in acute myeloid leukemia (AML). Activating mutations of the *Fsm-like tyrosine kinase*3 (*FLT3*) gene, especially *FLT3*-ITD mutations, have been associated with an adverse prognosis in AML. Therefore, *FLT3* mutation detection becomes essential for AML patients. High Resolution Melting (HRM) analysis is an alternative method for the rapid and affordable detection of gene mutations.

Objectives: To establish and evaluate a rapid and affordable method for detecting mutations of *FLT3*-ITD (exon14-15) gene in acute myeloid leukemia by High Resolution Melting analysis.

Materials and methods: Thirty-five patients with newly diagnosed AML from Maharaj Nakorn Chiang Mai Hospital were included in this study. *FLT3*-ITD mutation screening was performed by HRM analysis, and the results were compared with the data obtained using conventional PCR with gel electrophoresis and direct sequencing.

Results: Among the 35 AML patients studied, 6 patients were scored positively for *FLT3*-ITD mutation in the conventional PCR, whereas HRM analysis identified 7 out of 35 patients who were positive for *FLT3*-ITD mutation, which was concordant with direct sequencing results. Interestingly, one sample that was positive by HRM analysis was scored by conventional PCR as negative. Therefore, HRM analysis is more sensitive than conventional PCR.

Conclusion: HRM analysis is a rapid and promising screening method for *FLT3*-ITD mutation, enabling the real-time evaluation of AML progression, which is significant for decision-making regarding treatment. Our results showed that HRM analysis could be a useful clinical tool for the rapid and affordable screening of *FLT3*-ITD mutation in AML patients.

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Introduction

Acute myelogenous leukemia (AML), a hematological malignancy with a high mortality rate, is characterized by the overproduction and dysregulation of white blood cell proliferation.[1] In Thailand, AML is the major cause of death from hematological malignancy. According to current diagnostic criteria, the World Health Organization (WHO) classified AML by clinical features, morphology, immunophenotyping, cytogenetics, and molecular genetics.[2] The risk stratification into favorable, intermediate, and unfavorable is based on chromosomal abnormalities and genetic alterations such as *FLT3*, *KIT*, *NPM1*, and *CEPBPA*.[3] Among these, mutations of the *FMS-like tyrosine kinase* 3 (*FLT3*) are the most common genomic alteration in AML. *FLT3* mutations can be found in approximately 30% of AML patients and correlate with a poor prognosis. *FLT3* mutations can be subdivided into internal tandem duplicates (ITD), present in approximately 25% of patients, and point mutations in the tyrosine kinase domain (TKD), present in approximately 5%. Both *FLT3*-ITD and *FLT3*-TKD mutations are constitutively activating, leading to ligand-independent *FLT3* signaling and cellular proliferation. *FLT3*-ITD mutations result from a 3 bp repeated sequence varying in size from a 6 to 180 bp insertion in the juxtamembrane (JM) region (exons 14 to 15) of the *FLT3* wild-type gene. In wild-type (WT) *FLT3*, the *FLT3* JM domain inhibits receptor activation; the presence of ITDs disrupts this inhibitory effect, resulting in autophosphorylation. The constant activation of the *FLT3* receptor led to the uncontrolled proliferation of blast cells. *FLT3*-ITD mutations are the majority of *FLT3* mutations detected in AML patients.[4-8] Therefore, the identification of *FLT3* mutations by molecular analysis is of great importance for the prognostic information and the determination of appropriate therapeutic interventions in AML patients. Traditionally, *FLT3*-ITD mutations can be detected by using conventional polymerase chain reaction (PCR)-based methods followed by agarose gel electrophoresis, but they have limited to low sensitivity and also the inability to detect a small insertion of less than 20 bp. In response to the demand for rapid and sensitive methods to detect *FLT3* mutations, we established and evaluated a High Resolution Melting analysis for detecting mutations of *FLT3* gene in clinically sample from acute myeloid leukemia patients.

High Resolution Melting (HRM) analysis was first established in 2003, and has since been developed for the high-throughput and convenient genotyping of individual polymorphic loci. The key to this technology is the use of saturating fluorescence dyes, which intercalate into double-stranded DNA during amplification of the DNA without inhibiting the PCR reaction. The dye fluoresces strongly when intercalated into the double-stranded DNA, but as the temperature increases during the HRM analysis, so the DNA melts and the intercalating dyes are released without fluorescence. The changes in fluorescence are sequence specific and can be recorded and analyzed by the designed program.[9, 10] HRM is a closed-tube system that prevents contaminations and has increased sensitivity when compared to conventional PCR followed by agarose gel electrophoresis.

Therefore, the present study aimed to establish and evaluate a rapid and affordable method for detecting mutations of *FLT3* gene in acute myeloid leukemia using HRM analysis.

Materials and methods

Sample collection

A total of 35 blood samples were collected from patients newly diagnosed with acute myelogenous leukemia at Maharaj Nakorn Chiang Mai Hospital from January 2017 to December 2018. This research was approved by the Research Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand (AMSEC-61EM-006).

Cell line and culture

The MV4-11 cell line (*FLT3*-ITD) and KG-1a cell line (*FLT3*-wild type) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and were cultured in Roswell Park Memorial Institute 1640 Medium (RPMI1640, Gibco, Grand Island, NY, USA) supplemented with 10% Fetal Bovine Serum (FBS, Gibco, Grand Island, NY, USA) and 1% penicillin streptomycin (Gibco, Grand Island, NY, USA) at 37°C in a 5% CO_2 incubator.

DNA extraction

Blood samples and cell lines were extracted using DNA extraction kit (NucleoSpin® Blood, Macherey-Nagel). Briefly, whole blood 200 µL was mixed with 25 µL of proteinase K and 200 µL of buffer B3 and then vortex mixed vigorously for the lysis of cells. After that, the mixture was incubated at 70°C for 10-15 minutes and 210 µL of absolute ethanol was added to the mixture before being vortexed again. Then, the mixture was transferred into a Nucleospin Blood Column from the DNA extraction kit and centrifuged at 12,000 rpm for 2 minutes. After that, the silica membrane was washed 2 times and was dried by centrifuging the column at 12,000 rpm for 2 minutes. Then, 100 µL of elution buffer was added, and the column was incubated for 1 minute and centrifuged at 12,000 rpm for 2 minutes to elute pure DNA.

Conventional PCR assay

A primer pair was designed to specifically amplify both of *FLT3*-ITD mutant and *FLT3*-wild type at exon 14-15 (F 5'-GCAATTTAGGTATGAAAGCCAGC-3' and R 5'-CTTTCAG-CATTTTGACGGCAAC-3') yielding a 300 bp wild-type PCR product. Any patient with an additional higher molecular weight band was considered to be *FLT3*-ITD mutant. The conventional PCR for *FLT3* mutation was performed on the MyCycler Thermal Cycler machine (BIO-RAD). Positive and negative controls, as well as a blank control with distilled water, were included in each run of unknown samples. Here, 50 ng/µL of DNA samples were amplified in a total volume of 20 µL containing 0.2 µM of each primer and 10 µL of 2X Quick Taq Hs DyeMix (Toyobo, Japan). PCR was performed at 95°C for 3 minutes followed by 40 cycles at 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, with a final extension step at 72°C for 3 minutes. The PCR products were separated by electrophoresis through 3% agarose gel electrophoresis and the PCR product bands were viewed under UV illumination. Cases in which an additional higher molecular weight band was identified

were considered *FLT3*-ITD–positive.

PCR and HRM analysis

PCR and HRM for *FLT3* mutation were performed on a Corbett Rotor-Gene 6000 HRM Real-Time PCR Machine, a real-time PCR machine with HRM capability. All samples were tested in triplicate. The PCR reaction mixture contained 50 ng/µL of DNA, 0.3 µM of each primer (F 5'-GCAATTTAG-GTATGAAAGCCAGC-3' and R 5'- CTTTCAGCATTTTGACGG-CAAC-3'), and 10 µL of THUNDERBIRD SYBR® qPCR Mix (Toyobo, Japan). Reaction conditions consisted of an activation step at 95°C for 3 minutes followed by 40 cycles amplification of 5 seconds at 95°C and 30 seconds at 60°C. Subsequently, the products were heated to 95°C and then cooled to 4°C. HRM was performed from 75°C to 95°C, increasing by 2°C/second with 25 acquisitions per degree. Upon completion of the run, analysis was performed using the software supplied with the Corbett Rotor-Gene 6000 HRM Real-Time PCR Machine. Melting curves were generated, normalized and temperature-shifted to allow samples to be directly compared by Rotor Gene 6000 Series Software 17 (Qiagen). The HRM analysis was validated by direct sequencing.

DNA sequencing

The PCR products of the samples were further submitted for sequencing in both directions using fluorescent dye-terminator sequencing on the ABI3730xl DNA Sequencer.

By using HRM analysis to detect *FLT3*-ITD mutations in 35 AML patients, 7 cases were positive for *FLT3*-ITD mutation (Tm 78.8±1.1°C) and the rest of the samples were negative (Tm 80.4±0.2°C). HRM analysis was represented as a well-differentiated normalized melting curve with two clear separate clusters corresponding to the different nucleotide sequences from wild-type, while selecting only a sample from the same cluster to compare to a wild-type control for analysis, which guaranteed a clear sample clustering (Figure 2). All PCR products were confirmed by direct sequencing in both directions (Figure 3). The results were 100% concordant with HRM analysis. The mutations were inserted by repeated sequence size ranging from 24 to 138 bp in exons 14-15 of the *FLT3* gene (Table 1).

Results

Thirty-five AML patients were included in this study. The MV4-11 cell line and KG-1a cell line were homoduplex mutated (positive) control and wild-type (negative) controls, respectively. Six patients were *FLT3*-ITD mutated, and 29 patients were wild-type, as determined by conventional PCR followed by 3% agarose gel electrophoresis. Patients with an additional higher molecular weight band were identified as *FLT3*-ITD–positive. As shown in Figure 1, mutated PCR products showed two distinct bands on agarose gel electrophoresis, probably based on heteroduplex formation. In contrast, mutated PCR products showed an upper band product of 300 bp (Figure1, lane 6), which represents homoduplex formation.

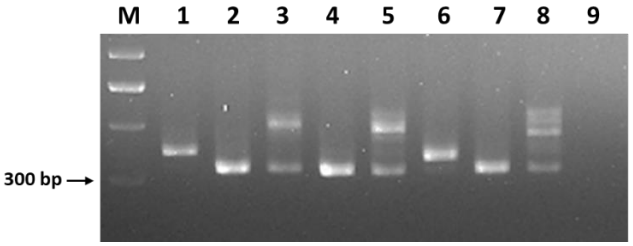

Figure 1. *Conventional PCR products on a 3% agarose gel. Double band and upper product of 300 bp: heteroduplex and homoduplex mutation, Single band at 300 bp: wildtype sequence, Lane 1: mutated control MV4-11 cell line, Lane 2: wild-type control KG-1a cell line, Lane 3, 5, 6, and 8: samples with mutated FLT3-ITD, Lane 4 and 7: samples with wild-type FLT3, Lane 9: no template control.*

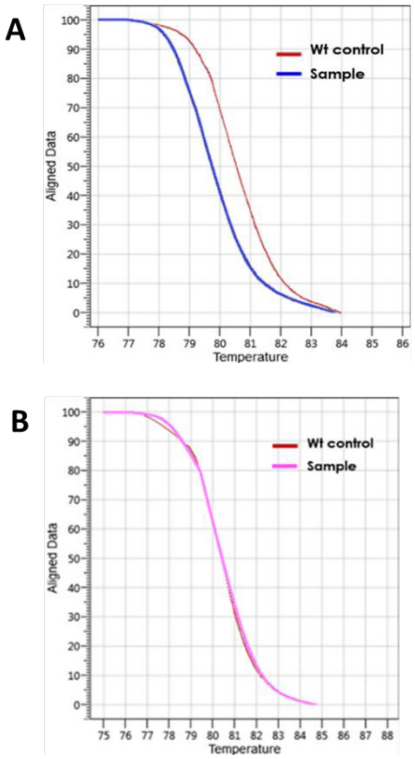

Figure 2. *HRM analysis presented on normalized melting curve plot. A: mutation cluster compared to the wild-type control, B: wild-type cluster compared to the wild-type control, Red indicate wild-type FLT3-ITD controls, Blue indicates a sample with mutated FLT3-ITD, while Pink indicates a sample with wild-type FLT3-ITD.*

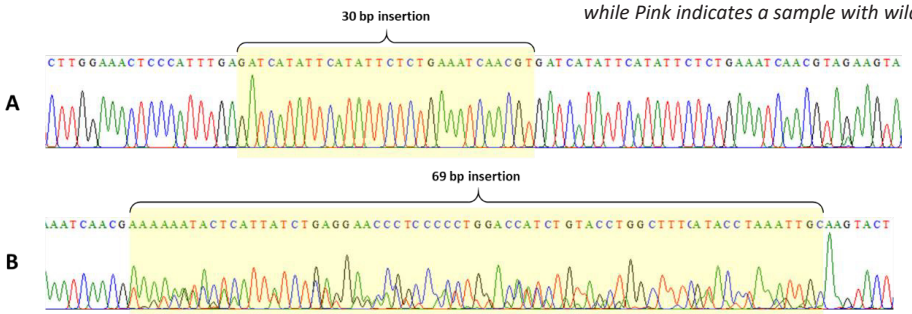

Figure 3. *Direct sequencing in the reverse direction. A: FLT3-ITD–positive sample, B: FLT3-ITD–positive sample with a discrepant result between conventional PCR and HRM analysis.*

Discussion

Genomic investigations of acute myeloid leukemia (AML) have demonstrated that the mutation of several genes is involved in AML development, leading to new genomic classifications, predictive biomarkers, therapeutic targets and individual therapeutic optimization. Mutations of the *FLT3* gene have been found in approximately 30% of all AML cases. The most common type present in AML patients is *FLT3*-ITD mutations.[8, 11] Although *FLT3*-ITD mutation has been associated with a poor prognosis in AML patients, the identification of *FLT3*-ITD has become an important indicator for determining treatment modalities, especially first-generation *FLT3* inhibitors.[7] Since *FLT3*-ITD mutations result from base pair insertion, it was shown that *FLT3*-ITD could be analyzed by traditional PCR with agarose gel electrophoresis.[12]

Table 1 The results of *FLT3*-ITD detections in patient samples.

Sample No.	Traditional PCR with agarose gel electrophoresis	PCR with HRM analysis	Sequencing
1	*FLT3*-ITD mutation	*FLT3*-ITD mutation	Mutation with 30 bp insertion
2	Wild-type	Wild-type	Wild-type
3	Wild-type	Wild-type	Wild-type
4	Wild-type	Wild-type	Wild-type
5	Wild-type	Wild-type	Wild-type
6	Wild-type	Wild-type	Wild-type
7	Wild-type	Wild-type	Wild-type
8	Wild-type	Wild-type	Wild-type
9	*FLT3*-ITD mutation	*FLT3*-ITD mutation	Mutation with 138 bp insertion
10	Wild-type	Wild-type	Wild-type
11	Wild-type	Wild-type	Wild-type
12	-ITD mutation	*FLT3*-ITD mutation	Mutation with 84 bp insertion
13	Wild-type	Wild-type	Wild-type
14	*FLT3*-ITD mutation	*FLT3*-ITD mutation	Mutation with 78 bp insertion
15	*FLT3*-ITD mutation	*FLT3*-ITD mutation	Mutation with 24 bp insertion
16	Wild-type	*FLT3*-ITD mutation	Mutation with 69 bp insertion
17	Wild-type	Wild-type	Wild-type
18	Wild-type	Wild-type	Wild-type
19	Wild-type	Wild-type	Wild-type
20	Wild-type	Wild-type	Wild-type
21	Wild-type	Wild-type	Wild-type
22	Wild-type	Wild-type	Wild-type
23	Wild-type	Wild-type	Wild-type
24	Wild-type	Wild-type	Wild-type
25	Wild-type	Wild-type	Wild-type
26	Wild-type	Wild-type	Wild-type
27	Wild-type	Wild-type	Wild-type
28	Wild-type	Wild-type	Wild-type
29	Wild-type	Wild-type	Wild-type
30	Wild-type	Wild-type	Wild-type
31	Wild-type	Wild-type	Wild-type
32	Wild-type	Wild-type	Wild-type
33	Wild-type	Wild-type	Wild-type
34	Wild-type	Wild-type	Wild-type
35	*FLT3*-ITD mutation	*FLT3*-ITD mutation	Mutation with 51 bp insertion

However, It was unable to detect mutated DNA at levels lower than 6.7% in a mutant- wild-type mixture.[7] Moreover, gel electrophoresis will not be able to clearly differentiate mutant from wild-type when inserted fragments are shorter than 20 bp such as in the case of *FLT3*-ITD with a 3 bp insertion.[13]

Real-time PCR with HRM analysis is easy to set up and the turnaround time is about 2-3 hours; several studies have employed the HRM assay to develop a molecular approach for the detection of gene mutation in various diseases including leukemia.[14-17] The major advantage of HRM is preventing contamination due to the closed tube system. HRM is a simple method: after PCR, carried out in the presence of a suitable dye, the product is heated while the level of fluorescence is measured. As the temperature rises and the duplex passes through its melting transition, dye is released, and fluorescence intensity is reduced. Although several instruments capable of performing the fluorescence acquisition exist, they vary in performance, with those designed for HRM giving a more satisfactory outcome. In a previous study, it was shown that the HRM method is capable of detecting up to 1% of mutated DNA which clearly differed from the wild-type template.[18]

Our analysis included 35 patients with AML. We found that 6 of the 35 AML patients were positive for the *FLT3*-ITD mutation in the conventional PCR, whereas HRM analysis could detect 7 out of 35 patients who were positive for the *FLT3*-ITD mutation. The mutations involved an inserted sequence with a size ranging from 24 to 138 bp. Interestingly, one patient (sample no. 16) who was positive by HRM analysis was scored as negative by conventional PCR, probably due to the low percentage of mutant allele in the *FLT3* wild-type background, as this case is lower than the limitations of detection using conventional PCR with agarose gel electrophoresis. It could not be detected by conventional PCR with agarose gel electrophoresis. Therefore, HRM analysis is more sensitive than conventional PCR and is a suitable method for the daily routine of a molecular laboratory. Once a mutation is detected, DNA sequencing could be used for confirmation if necessary. Overall, it can be considered a rapid and cost-effective method.

HRM analysis is a rapid, inexpensive method that does not involve opening the PCR tube, which has the advantage of preventing contamination. Furthermore, HRM analysis improves sensitivity when screening for *FLT3*-ITD mutation and reduces the complications of post-PCR methods such as agarose gel electrophoresis.

Conclusion

HRM analysis is a rapid, promising screening method for *FLT3*-ITD mutation and enables the real-time evaluation of AML progression, which is of great importance for decision-making regarding treatment. It is more sensitive than conventional PCR, is inexpensive and can be integrated into the routine molecular diagnosis of AML.

Conflict of interest

There are no conflicts of interest associated with this publication.

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Survey of photoneutron emitted from 6MV, 10MV, and 15MV medical LINAC using nuclear track detection

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ABSTRACT

Background: Medical linear accelerators (LINAC) can produce unwanted photoneutrons that might cause tissue damage or cancer in other organs. The efficient techniques and detectors are importantly required to detect these harmful neutrons.

Objectives: This study aimed to measure photoneutrons produced from medical LINAC of 6, 10, and 15 megavolts (MV).

Materials and methods: Nuclear track detector (CR-39 detectors) were employed to quantify the number of neutrons from LINACs. The X-ray energies, doses, and radiation techniques were varied to compare the number of neutrons. The photoneutron productions inside the LINAC room were also examined.

Results: The results showed that there were no neutrons from medical LINAC 6 MV, whereas photoneutrons could be detected from 10 and 15 MV LINAC. Radiotherapeutic techniques with moving multi-leaf collimator (MLC) produced higher photoneutron than techniques without using MLC. The neutrons were detected on the walls of the LINAC room.

Conclusion: X-ray energy greater than 10 MeV generated undesired photoneutrons that can penetrate the shielding and increase the patient dose. For the safety of staff to re-enter the treatment room, delaying time should be considered for the neutron decay process.

Introduction

In 2020, the International Agency for Research on Cancer (IARC) reported the incidence of cancer of 19.3 million cases globally and around 10.0 million cancer deaths.[1] The cancer burden in Asia accounted for 49.3% of global cases.[1] Radiotherapy has been recognized as one of the effective cancer treatment methods. This treatment employs high-energy ionizing radiation such as X-rays, gamma rays, and other particles to kill cancer cells. The radiation dose given to patients must be correctly calculated to destroy cancer cells with minimum damage to nearby normal cells. Thus, radiotherapy must be carefully planned for ensuring safety of the patients. Radiotherapy can be combined with surgery or chemotherapy, depending on characteristics, staging and location of cancer, and patients' health. There are two types of radiation therapy categorized by radioactive sources. First is brachytherapy, which is a treatment where a radioactive source is temporarily placed inside the body near the cancer cells. The radioactive sources might also be swallowed by the patient or injected into the patient's body. Second is teletherapy, which can be given by placing a radioactive material at 10 to 100 centimeters (cm) from the patient and using a collimator to control the radiation

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beam to the desired direction. Cobalt-60 machines and medical linear accelerators (LINAC) that generate photon beams with high energy ranging from 1 to 25 mega electron volts (MeV) are currently used for the teletherapy.[2] Recently, medical LINACs have been substituting for cobalt-60 machines because they do not require radioactive sources, more secure and easy for waste management. Consequently, LINACs are more practical to use. Integrating computer systems to control collimator leaf position according to the tumor shape helps alleviate radiation damage on cells surrounding the tumor and enhance the treatment efficacy to eradicate tumors by the high-energy photons. However, a photon beam with energy more than 10 MeV can interact with high atomic number materials, such as tungsten and lead. These elements are commonly used as components of the target and collimation system in LINAC's treatment head. As a result, high-energy neutron particles have been undesirably produced and they potentially pass through the protection device to the patient. Thus, the radiation dose to the patient is increased.[3] Similarly, the high-energy neutrons contaminating the radiotherapeutic room and its wall can have an impact on operators.[4] Hence, neutron protection needs to be considered while designing room structure. Besides, care should be taken of photon-induced radioactive material as it can capture neutron and produce undesirable high energy photons.[5]

Medical LINAC can produce photoneutrons that are generated by the interaction between high-energy X-rays and high atomic number substances such as tungsten and lead. The threshold energy of X-rays and lead interaction in producing photoneutrons is 7.4 MeV.[6] Neutron particles have no electric charge and can move deeper through medium. It can directly interact with nuclei of the medium, then transfers most of its energy to nuclei of an element that has a similar mass to the neutron. The human body contains abundant hydrogen that has comparable mass with neutrons. Neutrons interact with human tissues and transfer the energy to hydrogen nuclei. This effect potentially causes genetic deterioration leading to cancer.

Apart from being one of the most effective cancer treatments, LINAC itself produces unwanted photoneutrons that might cause tissue damage or cancer in other organs. Therefore, the efficient techniques and detectors are importantly required to detect these harmful neutrons. Nuclear track detector has been used for measuring radiation dose and energy of photoneutrons from LINAC.[3, 7, 8] CR-39 plastic nuclear track detector (CR-39 PNTD) is Polyallyldiglycol Carbonate ($C_{12}H_{18}O_7$) polymer. The material contains hydrogen and oxygen atoms, similar to human tissue. This property allows the study of interaction of neutrons and tissue. Neutron energy with the range of 1-20 MeV can be indirectly measured by the interaction of neutron and hydrogen atoms of CR-39. The size of the CR-39 detector is small and requires no electricity, so it is easy to place on patient's skin. It is sensitive to charged particles, but it does not respond to any photon.

This study aimed to examine the number of neutrons produced by 6, 10, and 15 MV LINAC and measure neutrons in the radiotherapy room using the CR-39 radiation detector.

Materials and methods

Two medical LINACs; 6 and 10 MV (LINAC#1) and 10 and 15 MV (LINAC#2) were used in this study. CR-39 PNTD is composed of boron nitride (BN) and polyethylene sheet (TASTRAK™ PADC, Track Analysis Systems Ltd (TASL), Bristol, UK). The former allows neutron-alpha (n, α) interaction for measuring neutron energy less than 1 MeV. The latter interacts with neutron energy of 1-10 MeV (or called fast neutron) via elastic scattering producing protons.

Etching process started with immersing the CR-39 plastic detector into NaOH aqueous solution of 6.25% concentration at 70 °C in a water bath. After 15 hours, CR-39 were rinsed in distilled water and dried at room temperature. The nuclear tracks were magnified and visualized under 100x-400x microscope as dark circles (Figure 1). The etched CR-39 detectors were manually counted under optical microscope with 400x to obtain the number of nuclear tracks.

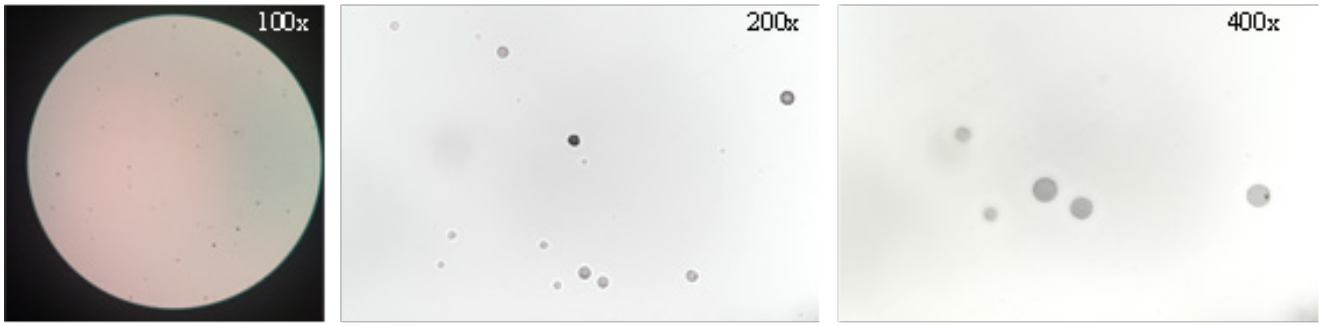


Figure 1. *Nuclear tracks on CR-39 detectors.*

Calculation of neutron equivalent dose

The dose conversion graph and equation for the calculation of neutron equivalent dose were provide by the secondary standards dosimetry laboratory (SSDL), the office of atomic for peace (OAP). CR-39 detectors were irradiated with (^{241}AmBe neutron source) neutrons equivalent dose of 0.5, 1, 5 and 10 mSv. These neutrons equivalent doses were traceable to Primary Standard Dosimetry Laboratory at Korea Research Institute of Standard and Science (KRISS). After etching process, the nuclear tracks were automatically counted. The relationship between the number of tracks and radiation equivalent dose were plotted (Figure 2) and the neutron equivalent dose was calculated from equation (1) as follows:

$$y = 0.0199x + 0.7862 \ldots (1)$$

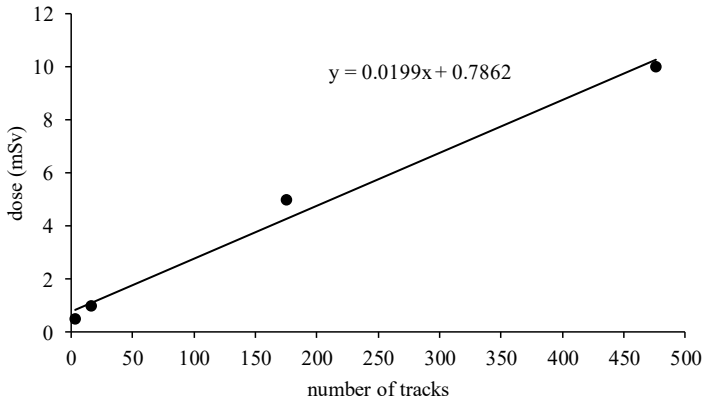


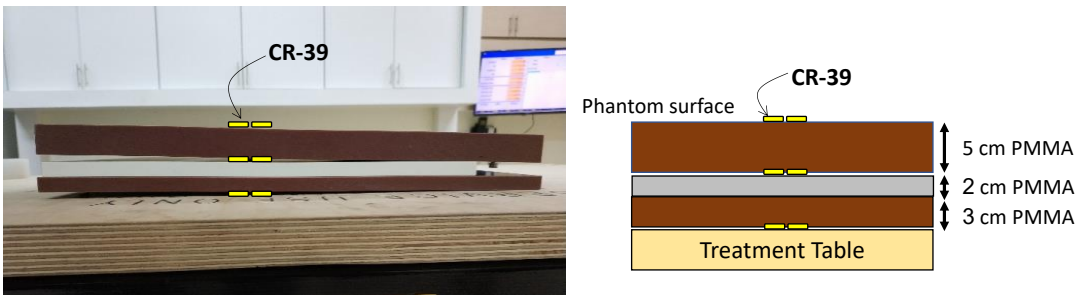
Figure 2. *Dose conversion graph and equation for the calculation of neutron equivalent dose.*

Measurement of neutron using phantom

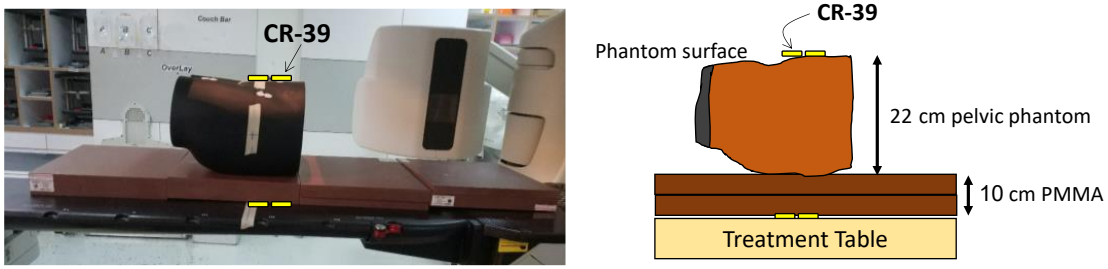
The amount of neutron equivalent dose produced by two different LINACs with different energies were compared. LINAC#1 produced X-ray beams from accelerating electrons with 6 and 10 MV and LINAC#2 with 10 and 15 MV. The radiation dose of 200 cGy and 990 cGy were set to irradiated CR-39 detectors. This study also compared the number of neutron equivalent dose from different irradiation techniques, which were intensity-modulated radiation therapy (IMRT), volumetric modulated arc therapy (VMAT), 3-dimensional radiotherapy (3D-RT), and 2-dimensional radiotherapy (2D-RT).

The irradiation conditions for LINAC#1 were to compare the potential difference of 6MV and 10MV, assigning a dose of 200 cGy and 990 cGy (calibrated at 1 Monitor unit = 1 cGy), using two radiation techniques; 3-Dimensional radiotherapy (3D) and Intensity-modulated radiation therapy (IMRT). Two of 5-centimeter polymethylmethacrylate (PMMA) phantoms were placed on the LINAC's table at 100 cm source-to-surface distance (SSD) with 15 cm^2 field size. Six of CR-39 detectors were used to measure neutron particles. Two of CR-39 detectors were placed isocenter at the top of the phantom, while another two were inserted between the phantom, and another two were underneath (Figure 3A).

Whereas LINAC#2 has a higher energy range of 10MV and 15MV. The phantom thickness was increased by adding two of 5-cm PMMA sheets under the Anthropomorphic pelvic phantom. The total thickness became 32 cm mimicking a large patient. Two CR-39 detectors were placed at isocenter on top of the phantom, and another two were underneath (Figure 3B). The radiation dose was 200 cGy using 2-Dimensional radiotherapy (2D) technique and volumetric modulated arc therapy (VMAT) technique.



(A) Medical LINAC 6MV and 10MV



(B) Medical LINAC 10MV and 15MV

Figure 3. *CR-39 detectors were placed on phantom to measure neutrons from the two LINACs.*

Measurement of neutron around the LINAC room

Background radiation was measured by keeping the two CR-39 detectors in a non-radiation environment area. Then the number of radiation nuclear tracks from the background will be subtracted from the experiments.

CR-39 detectors were placed inside and outside both LINAC rooms. Two detectors were used to survey and measure neutrons at each position. In LINAC#1 (6MV and 10MV) room, CR-39 detectors were placed on the wall of three locations 1) the junction between LINAC vault and maze, 2) the maze way, and 3) the entrance door inside the LINAC room (Figure 4A). In LINAC#2 (10MV and 15MV) room, CR-39 detectors were placed on the wall of six locations 1) the bunker inside LINAC vault and maze, 2) the bunker between LINAC vault and maze, 3) the maze way, 4) the entrance door inside the LINAC room, 5) the entrance door outside the LINAC room, and 6) the operation room (Figure 4B).

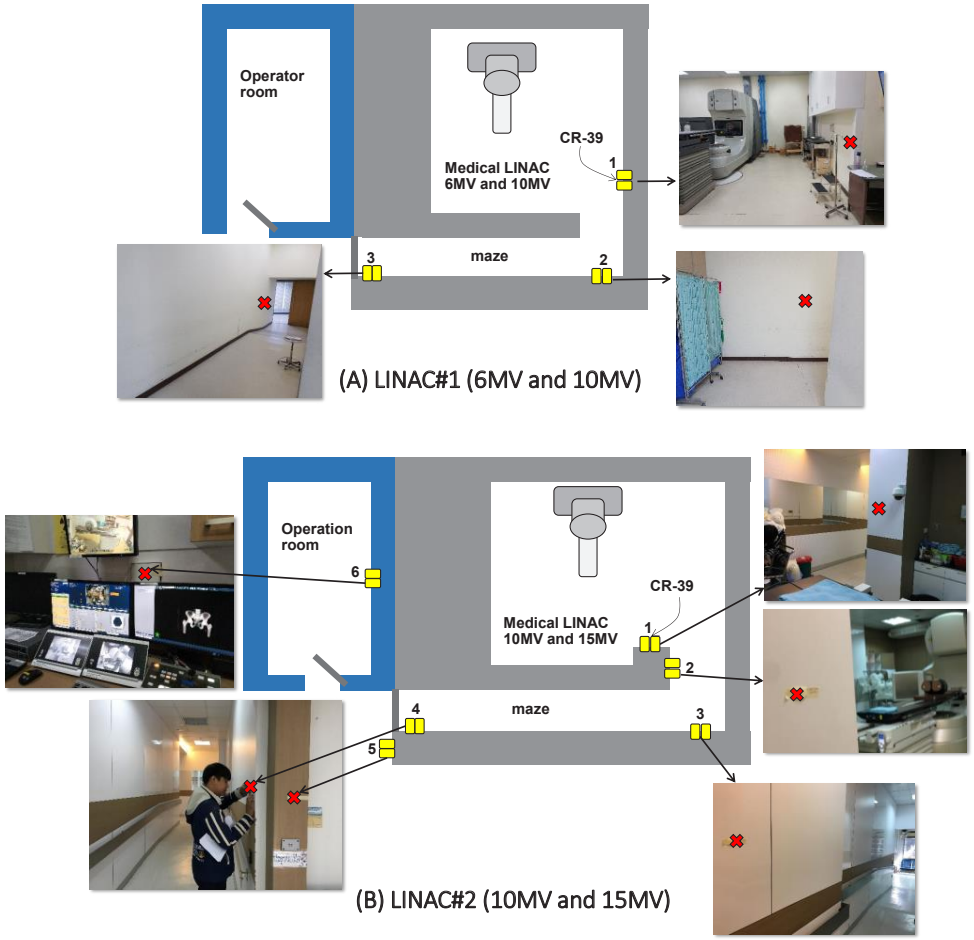


***Figure 4**. CR-39 detectors located in both LINAC's room to survey neutron particles.*

Results

Neutron particles produced from medical LINACs

Table 1 shows the number of neutrons produced when varying energies, radiation doses, and radiation techniques. For 6 MV LINAC#1, the number of nuclear tracks is similar to that of the background radiation, implying that no neutron particles from the 6MV acceleration were found. Increasing the radiation dose and changing the radiation techniques did not enhance the number of tracks. However, when increasing the acceleration potential to 10 MV, the number of tracks was higher than background radiation. The results also showed that the number of nuclear tracks increased with the radiation doses. The center of phantom presented a higher number of tracks than those at the top and bottom. The IMRT technique, using a multileaf collimator (MLC), produced more radiation tracks than 3D techniques without MLC.

For 10 and 15 MV LINAC#2, it was found that both energy levels generated the number of neutron tracks. The higher the energy was used; the more tracks were produced. The VMAT technique, using a multileaf collimator, produced more radiation tracks than 2D techniques without MLC. The bottom of phantom presented a higher number of tracks than those at the top.

Radiotherapeutic parameters are generally used at 6, 10, or 15 MV depending on patient thickness with a dose of 200 MU. The mean neutron radiation doses were calculated using equation (1). The results revealed that the radiation dose that used 6 MV LINAC was about 0.8 mSv. The use of 10 MV LINAC associated with MLC yielded a radiation dose range of 1.4 - 4.6 mSv. The highest neutron equivalent dose of 7.7 mSv was from 15 MV LINAC with VMAT (Table 2).

Neutron around the radiotherapy room

The survey results found neutron particles in the interior of 10 MV, 900 MU LINAC room. The highest radiation dose was 1.2 mSv at the junction between LINAC vault and maze. The farther the accelerator, the lesser number of nuclear tracks produced. At the door, the amount of neutron radiation was similar to the amount of natural background radiation.

The survey results inside LINAC rooms showed that the location near the accelerator had the highest number of tracks. The calculation of radiation dose inside 10 MV, 200 MU LINAC room was 1.2 mSv. Increasing the voltage to 15 MV, 200 MU, the radiation dose inside the LINAC room was increased to 8.3 mSv. The CR-39 detectors, that were placed at the front door outside the irradiated room, can detect the nuclear tracks and the calculated dose was 1.3 mSv. Nuclear tracks on the detectors placed at the junction point between the control room and the LINAC room were also found. (Table 3)

Table 1 Number of nuclear tracks produced from neutron particles.

No.	Parameters		200MU			990MU		
			top	middle	bottom	top	middle	bottom
LINAC#1	6MV	3D	2	1	2	0	0	0
		IMRT	6	0	1	8	5	1
	10MV	3D	27	129	72	168	379	324
		IMRT	47	193	176	330	666	610
LINAC#2	10MV	2D	21	n/a	32	n/a	n/a	n/a
		VMAT	31	n/a	56	n/a	n/a	n/a
	15MV	2D	37	n/a	78	n/a	n/a	n/a
		VMAT	327	n/a	348	n/a	n/a	n/a

n/a: not available

Table 2 Neutron equivalent dose in mSv.

No.	Parameters		200MU		
			top	middle	bottom
LINAC#1	6MV	3D	0.8	0.8	0.8
		IMRT	0.9	0.8	0.8
	10MV	3D	1.3	3.3	2.2
		IMRT	1.7	4.6	4.3
LINAC#2	10MV	2D	1.2	n/a	1.4
		VMAT	1.4	n/a	1.9
	15MV	2D	1.5	n/a	2.3
		VMAT	7.3	n/a	7.7

n/a: not available

Table 3 Number of nuclear tracks and the number in parenthesis is neutron equivalent dose in mSv.

		Location					
		#1	#2	#3	#4	#5	#6
LINAC#1 (6MV,10MV)	10MV 900MU	22	1	0	n/a	n/a	n/a
		(1.2)	(0.8)	(0.7)	n/a	n/a	n/a
LINAC#2 (10MV,15MV)	10MV 200MU	20	21	4	0	6	3
		(1.2)	(1.2)	(0.9)	(0.8)	(0.9)	(0.8)
	15MV 200MU	376	278	117	38	24	22
		(8.3)	(6.3)	(3.1)	(1.5)	(1.3)	(1.2)

n/a: not available

Discussion

The high-energy medical LINAC has been widely used to generate X-rays and electrons for treating cancer patients. However, the machine also produces neutron particles. This unintentional byproduct increases unnecessary exposure to radiation of patients. Besides, the neutron particles can disperse beyond tumor cells, making it difficult to protect the harmful effect of neutron radiation.[9] This study aimed to determine neutrons generated from the LINAC by varying X-rays energy levels, radiotherapy techniques, and radiation doses. In terms of X-rays energy levels, the 6, 10, and 15 MV accelerations are commonly used to treat cancer patients. The results showed that none of the neutron particles occurred when using the X-rays energy lower than 6 MeV. The neutron particles were detected from the 10 and 15 MeV X-rays and the increase in X-rays energy led to the increase in the number of neutron particles. Our findings were in line with various theories and studies that neutron particles are generated from the interaction between high-energy photons and materials of the LINAC head, air, and patient's body. Since, the LINAC head contains high-atomic number metals such as lead, tungsten, copper, and iron, these metals have threshold energy for neutron emission at 7.37, 7.41, 10.85, and 11.19 MeV, respectively.[7, 9] This real-world evidence has proven the potential risk from detectable photoneutrons occurring from routine clinical practice. Thus, thorough measures for neutron radiation protection should be developed and strictly implemented in hospital radiology departments to prevent overexposure of patients and healthcare personnel.

Various advanced radiotherapeutic techniques were developed with the aims of improving target volume coverage and minimizing the effect on normal cells, such as intensity modulated radiation therapy (IMRT) and volumetric modulated arc therapy (VMAT) that can control a multileaf collimator (MLC) at the LINAC head. However, moving the collimator increases the chance of its high-atomic number metals to interact with the high-energy X-rays. Thus, IMRT and VMAT can produce a higher level of neutron particles compared to radiation techniques without the collimator movement such as 3-dimensional radiotherapy (3D-RT) and 2-dimensional radiotherapy (2D-RT).10 Additionally, the highest average neutron equivalent dose was detected in the middle of the phantom when using 3D-RT and IMRT techniques. It might be a result of the increasing number of neutron particles due to X-rays emission through the phantom and some particles interacted with CR-39 detector.

This study examined neutron contamination inside and outside the radiotherapy room. At above 10 MeV X-rays, the neutron particles were detected near the LINAC more than other areas, while the 15 MeV X-rays directly affected the increasing number of neutron particles. The results highlighted that room size, devices, medical supplies, shelves, furniture, and materials for walls and floors should be considered when designing a LINAC radiotherapy room.[11]

Besides, medical staff must avoid the dangers of the high-energy neutron decay inside the irradiation room. Staff should delay room entry times after previous treatment. A study proposed that the waiting time of 7 to 11 minutes should be appropriate for allowing the neutron decay based on the employed techniques.[12] Moreover, the waiting time depends on the energy level of X-rays, radiation techniques, components of the LINAC head, and materials of the collimator.[12, 13]

Conclusion

The neutron particles were detected from X-rays of above 10 MeV. The number of neutron particles in the radiotherapy room and their effect on people in such an area should be further examined. The decay of contaminated neutrons in the treatment room from various radiation techniques could be another matter of investigation. This evidence can raise radiation safety awareness and be used to develop related preventive procedures.

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Conflict of interest

There are no conflicts of interest to disclose.

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Improving upper extremity function in chronic stroke using occupational therapy task-oriented approach

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ABSTRACT

Background: Recovery of upper extremity function in chronic stroke requires functional rehabilitation.

Objectives: The objective of this study was to investigate the effects of task-oriented occupational therapy on the upper extremity function of chronic stroke clients.

Materials and methods: Twenty participants were selected for the study, which was carried out through purposeful sampling process. The participants were then divided into two groups: the intervention and the control groups. Both groups were given a conventional rehabilitation program. The intervention group was also provided with occupational therapy task-oriented training within the duration of 6 weeks, with the consistency of 3 sessions a week, and 1 hour each session. The training program was individually designed based on an analysis of the three most important activities identified by the participants from the Canadian Occupational Performance Measure (COPM). Each training session consisted of task-oriented training (75%) and supplementary exercise (25%). The Functional Test of the Hemiparetic Upper Extremity-Thai version (FTHUE-Thai Version) and COPM were used as outcome measurements before and after the training programs.

Results: The FTHUE-Thai version study showed that the intervention group had significant improvement in the function of paretic upper extremity (p<0.05). The COPM's report found a dramatical change in performance and satisfaction of the intervention group after the training program (p<0.05). These changes were also significantly different when compared between groups (p<0.05).

Conclusion: This study indicated the effect of occupational therapy task-oriented training based on client-centered approach on improving functions of paretic upper extremities, occupational performance, and satisfaction in people with chronic stroke.

Introduction

A stroke is a major health problem that can cause disability and deaths globally.[1-3] The effects of stroke vary depending on the size of the brain and the parts of the body that are affected. It can cause various types of disability. In most cases, one of these disabilities is paralysis of the upper extremity (UE).[4-5] Weakness of UE is particularly found in acute stroke patients (85%).[6] This condition is remained in chronic stroke (more than 40%).[5,7] This impacts on a person's ability to perform their daily activities and community participation.

There are two recovery methods after a stroke: spontaneous neurological recovery and functional recovery. Neurological recovery can be accelerated up to 3 months after a stroke.[8] Meanwhile, functional recovery can happen several years after a stroke.[7,9] It is the vital recovery for stroke clients in improving their ability to perform daily functions within the limitations of their physical impairment.[10] For better

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improvement of functional recovery of UE in clients with chronic stroke, rehabilitation focusing on motor impairment and disability was necessary.[11] Some neurorehabilitation techniques are useful in promoting upper extremity motor recovery; for example, stretching, constraint-induced movement therapy, task-specific training, mirror therapy, mental practice with motor imagery, etc.[8,12]

Task-oriented (TO) approach is a neurorehabilitation technique that involves practicing real-life tasks. It is based on motor control, motor learning, and motor behavior model.[13] This approach is wildly used in stroke rehabilitation by multidisciplinary team as well as in research.[14-18] A great number of evidence is focused on the effectiveness of TO training for UE after stroke. TO approach alone or in combination with other treatment approaches can help restore UE function after stroke.[12,19] A systematic review in 2014 suggested that high intensity TO practice may improve UE function after stroke, though further investigation is needed.[20] However, the recommended intensity of TO practice is performing occupational tasks taking up 70% of therapy time. The remaining time is spent on other components enhancing motor performance.[21] A study conducted by Timmermans, Spooren, Kingma, and Seelen[22] revealed that the number of training components used in TO training was not associated with the treatment effect size. This meant that the numbers of training used in TO training was not relate with UE functional recovery. However, the components of TO training that had the great impact on the outcomes were distributed practice and feedback. Also, random practice and using of clear functional goals had an influence on the training outcomes.

The concept of occupational therapy is focused on the notion of human occupations. Occupation itself is used as therapeutic occupation and occupational outcome.[23] To improve UE function in clients with stroke, TO approach concurs with the core concept of occupational therapy. The goal of occupational therapy task-oriented (OT-TO) approach is to improve the client's performance in specific tasks. Apart from having the same principles as the TO approach, it is also based on client-centered approach by emphasizing the relationship between clients, tasks, and environments.[24] Therefore, clients' role and occupational performance task are required for designing various and specific training that fits to the individual needs.[24] The study of a randomized clinical trial aiming to evaluate functional and impairment efficacies of OT-TO approach on the affected UE of persons after stroke 3 months onset showed that impairment outcomes of the OT-TO approach were not significantly larger than the control ones. In contrast, OT-TO approach seems to be an effective post-stroke rehabilitation strategy that can improve functional status and reduce chronicity and severity of stroke. Further studies are needed to analyze the effects of OT-TO approach on stroke patients.[17] In Thailand, there is no research evidence on OT-TO approach even though Thai occupational therapists have provided stroke clients with intervention specific to their real-life activities so far. Therefore, this study aimed to investigate the effect of using OT-TO approach on UE function in clients with chronic stroke.

Materials and methods

This study was a quasi-experimental research with a two-group pretest-posttest design. Participants, outcome measures, intervention, procedure, and data analysis were described accordingly.

Participants

Sample size was calculated by using G*power 3.1 based on effect sizes of Almhdawi's study[17] (α=0.05, power test=0.80). Eighteen participants were the calculated number of participants. Ten percentage of drop out is added to calculated number of sample size. Total number of participants in this study will be 20 participants: intervention group (N=10) and control group (N=10). Research project was approved by the Ethics Committee, Faculty of Associated Medical Sciences, Chiang Mai University, Thailand. The ethics clearance number was 341/2561.

To recruit participants for the study, they had to meet the following inclusion criteria: first stroke at least 6 months onset, age range between 17-80 years, no sign of dementia (Mini- Mental State Examination-Thai version; MSET-10>17 for participants whose education level were primary school; and >22 for participants whose education level were higher than primary school), Modified Ashworth Scale <3, Fugl-Meyer Assessment Upper Extremity (FMA-UE) score 32-52. They were excluded if they had at least one other condition that affected training including seizer, UE fracture and deformity, visual field deficit, body neglect, sensory loss, and aphasia. All participants were asked to postpone other treatments affecting muscle tone e.g., botulinum toxin injections, splinting, and acupuncture.

Outcome measures

The functional outcome measures included the Canadian Occupational Performance Measure (COPM) and the Functional Test for Hemiplegia Upper Extremity in Persons with Hemiplegia Thai version (FTHUE-Thai version). The COPM serves as a self-perceived measure aiming to assesses the level of occupational performance and satisfaction with performance for each of the five problems identified by stroke clients.[25] The COPM has shown good psychometric properties in Thai stroke patients.[26]

The FTHUE-Thai version is a performance test to measure the functional limitations of UE impairments. It was adapted from the FTHUE-Hong Kong version, of which activities were closer to Thai culture than the original FTHUE.[27] The test consists of 14 tasks, from the total of 7 functional levels. The tasks are arranged from less to more complex motion. Each activity is scored on pass/fail basis. A "pass" (score=1 point) means that the participant successfully completes the task within three minutes whilst a "fail" (score=0 point) means that the participant could not complete the activity or exceeded the allowed time. The score is reported as the number of passed activities. The score of functional levels could be counted when all the activities in each level could be achieved. Also, spending time for completing each activity is measured in the 7 out of 14 activities. The FTHUE-Thai version has shown good content validity and strong inter-rater reliability.[28]

Intervention

Both groups received the usual rehabilitation training, which usually consists of occupational and physical therapy. The intervention group also trained with the OT-TO approach. A video about OT-TO training was used to gain a good understanding about OT-TO approach to generate collaboration throughout the program in the intervention group. Training tasks were identified by participants from the COPM and analyzed to fit with individual performance. The intensive training consisted of 3 significant activities that were designed to fit with the client's needs. The intervention took one hour to complete which included OT-TO training taking up 75% of therapy time. The remaining 25% of the time was spent on supplementary training due to the client's impairments and interests such as range of motion exercise, spasticity management, strengthening exercise, and grasp-carry-release objects. The training program was undergone 3 times a week for 6 weeks consecutively. Objects and materials used in the training program were real. Furthermore, environments where occupations occurred were set to be as real as possible. To improve problem solving and learning skills in motor function, physical guidance and verbal feedback was provided at the beginning of each task which included controlling and correcting movement direction. The guidance and feedback were faded out when participants got used to the task. Admiration and encouragement were also provided for promoting positive reinforcements and motivations.

Procedure

Twenty participants who met the inclusion criteria were invited from 6 hospitals and rehabilitation centers in Chiang Mai Province, Thailand. Participants were divided into two groups; intervention (n=10) and control (n=10) groups, based on their preferences. Furthermore, age, chronicity, MSET-10 score, FMA-UE score, and the center where they normally received rehabilitation were concurred between groups. All of them were asked to identify their occupational problems which resulted from stroke. They were asked to rate the importance of those occupational problems, as well as performance and satisfaction (COPM-Pretest). Then, FTHUE-Thai version was used to evaluate their UE motor functions (FTHUE-Pretest). After 6 weeks, their performances and satisfactions on those 3 activities (COPM-Posttest), and their UE motor functions (FTHUE-Posttest) were assessed. The assessments were carried out by the same blinded OTs assessor.

Data Analysis

The data was analyzed using a SPSS software version 26. Descriptive statistic was used to summarize demographic data. Multivariate analysis of variance (MNOVA) was used to test the homogeneity of the variables of age, chronicity, MSET-10 score, and FMA-UE score. Man-Whitney U test was used to compare the differences between the different group. Wilcoxon signed-rank test was also used to investigate the differences between pre-intervention and post-intervention within each group.

Results

In this section, the characteristics of the participants and the effect of OT-TO training on UE function were presented as follows.

Demographic and characteristics of the participants

Demographic and characteristics of the participants were presented in Table 1. The comparison of controlling factors between 2 groups were not significant difference as shown in Table 2.

Table 1 Demographic and characteristics of the participants.

Variable	Intervention group	Control group
Sample size *(N)*	10	10
Gender: *N (%)*		
Male	9 (90)	9 (90)
Female	1 (10)	1 (10)
Dominant side: *N (%)*		
Right hand	10 (100)	8 (80)
Left hand	0 (0)	2 (20)
Type of stroke: *N (%)*		
Ischemic	1 (10)	2 (20)
Hemorrhage	9 (90)	8 (80)
Affected side: *N (%)*		
Right hemiparesis	7 (70)	6 (60)
Left hemiparesis	3 (30)	4 (40)
Consciousness level: *N (%)*		
Alert	10 (100)	10 (100)
Muscle tone impairment		
MAS: *N (%)*		
- level 1	5 (50)	5 (50)
- level 1+	2 (30)	2 (20)
- level 2	3 (20)	3 (30)

Values are expressed as numbers (%), MAS: Modified Ashworth Scale (level 0, 1, 1+, 2, 3, 4)

Table 2 Univariate MANOVAs analysis demographic and characteristics of the participants.

Variable	Intervention group	Control group	F	*p* value
Chronicity (month):	36.60±6.56 (6-78)	43.50±8.07 (6-84)	0.440	0.515
Age (year):	48.70±2.96 (37-61)	56.50±2.38 (46-70)	4.223	0.055
Cognitive level				
MSET-10:	26.60±0.50 (23-28)	25.50±0.64 (22-29)	1.849	0.191
Sensorimotor function impairment				
FMA-UE:	40.40±1.92 (33-48)	39.90±2.11 (33-50)	0.031	0.863

Values are expressed as Mean±SD (min-max), MSET-10: Mental State Examination T10, FMA-UE: Fugl-Meyer Assessment Upper Extremity

Effect of OT-TO training on UE function

The comparison between pre and post-test was shown in Table 3. The results of the study found that there were significant differences (p<0.05) between pre and post-test of FTHUE and COPM scores in the intervention group whilst there were only significant differences (p<0.05) between pre and post-test of COPM scores in the control group.

Table 3 Results for functional outcome measures within group comparisons.

Independent variable	Pre-test Mean±SD	Post-test Mean±SD	Mean rank	Sum of rank	Z	Wilcoxon signed rank	*p* value
Intervention group							
FTHUE							
Function level	3.80±0.42	4.20±0.42	2.50	10.00	-2.00	10.00	0.046*
Activity level	6.50±0.85	7.40±0.84	3.00	15.00	-2.12	15.00	0.034*
COPM							
Performance	3.57±0.96	6.88±0.72	5.50	55.00	-2.81	55.00	0.005*
Satisfaction	3.27±1.16	7.5±1.13	5.50	55.00	-2.81	55.00	0.005*
Control group							
FTHUE							
Function level	3.90±0.32	3.90±0.32	0.00	0.00	0.000	0.000	1.000
Activity level	6.20±0.92	6.50±0.53	1.50	3.00	-1.34	3.000	0.180
COPM							
Performance	2.67±0.94	3.23±0.80	4.00	28.00	-2.410	28.000	0.016*
Satisfaction	2.53±0.76)	3.67±0.99	5.50	55.00	-2.814	55.000	0.005*

**Significant (p<0.05) for each analysis, FTHUE: Functional Test for Hemiplegia Upper Extremity in Persons with Hemiplegia, COPM: Canadian Occupational Performance Measure*

When comparing between intervention and control groups, there was no significant difference between both groups in pre-test scores either in FTHUE nor COPM. On the other hand, post-test scores were found to have significant differences (p<0.05) in activity level of FTHUE and COPM. (Table 4) In addition, there were significant differences in the change scores of performances and satisfaction of COPM between the intervention and control group (Table 5).

Table 4 Results for functional outcome measures between group comparisons.

Independent variable	Mean rank (Sum of rank)		Z	Mann-Whitney U	*p* value
	Intervention group	Control group			
Pre-test					
FTHUE					
Function level	10.00 (100.00)	11.00 (111.00)	- 0.610	45.000	0.739
Activity level	11.65 (116.65)	9.35 (93.50)	- 0.967	38.500	0.393
COPM					
Performance	12.85 (128.50)	8.15 (81.50)	-1.792	26.500	0.075
Satisfaction	12.65 (126.50)	8.35 (83.50)	-1.636	28.500	0.105
Post-test					
FTHUE					
Function level	11.90 (119.00)	9.10 (91.00)	-1.724	36.000	0.315
Activity level	13.50 (135.00)	7.50 (75.00)	-2.690	20.000	0.023*
COPM					
Performance	15.50 (155.00)	5.50 (55.00)	-3.807	0.000	0.000*
Satisfaction	15.50 (155.00)	5.50 (55.00)	-3.807	0.000	0.000*

**Significant (p<0.05) for each analysis, FTHUE: Functional Test for Hemiplegia Upper Extremity in Persons with Hemiplegia, COPM: Canadian Occupational Performance Measure*

Table 5 Changes score differences for functional outcome measures.

Independent variable	Changes score differences	Across categories of group		
	Mean±SD	Z	Mann-Whitney U	*p* value
COPM				
Performance				
Intervention group	3.32±0.94	-3.804	0.000	0.000*
Control group	0.57±0.45			
Satisfaction				
Intervention group	4.13±1.27			
Control group	1.13±0.36	-3.791	0.000	0.000*

**Significant (p<0.05) for each analysis, Changes score differences: mean treatment change score–mean control change score, COPM: Canadian Occupational Performance Measure*

Discussion

The objective of this study was to investigate if OT-TO training could improve the UE function of clients with chronic stroke. In this study, a higher-dosage of OT-TO training was provided to the participants with chronic stroke in the intervention group. The OT-TO training was provided 3 times/week for 6 weeks on top of the usual conventional rehabilitation whilst the control group received only the conventional rehabilitation. The result of the study indicated that OT-TO training could enhance UE functions which its effect was greater than the control group who received only the conventional rehabilitation. (Table 3 and 4) This highlighted the benefit of using OT-TO approach in improving UE functions in chronic strokes. However, the effect of

OT-TO training did not have the significant difference on the functional level of FTHUE-Thai version when comparing between intervention and control group. (Table 4) This might be the result of the FTHUE-Thai version criteria that could be counted as "pass" when all tasks in each functional level were accomplished. It might be difficult for the participants who had a chronic stroke to pass all tasks in each functional level.

Similar to other studies,[29-30] this study proved that UE function could be recovered after having acquired stroke for many years. The recovery in chronic stroke depended on learning adaptation strategies.[31] This study was in line with Almhdawi et al.[17], that OT-TO intervention had a positive effect on UE functions both objective and subjective performance outcomes (FTHUE and COPM scores). In this study, an intensive training with various activities could provide an opportunity for motor learning in client with chronic stroke with repeated multiple movement planes.[22,24] In addition, the intervention is based on a client centered approach whereas the participants' three most significant problems from COPM were used as training activities. Those activities were a goal-directed training that were carefully designed and graded to suit with participants' abilities. All these encouraged participants to repeat attempts in performing those activities. Furthermore, this study utilized real materials and instruments to provide participants with sensorimotor demands. Feedback was also provided throughout the intervention period to help performance improvement. All these enhanced behavioral experiences that directly replicated the sensorimotor demands requiring on an execution of the motor skill successfully.[32]

Interestingly, there were significant differences in performance and satisfaction scores of COPM in the control group after receiving the conventional rehabilitation for 6 weeks (Table 3) even though there were not much change in the FTHUE score of the control group. The evidence of systematic reviews supported the use of rehabilitation intervention for improving motor function and activities of daily living.[12,33] Hence, it was possible that participants in the control group would perceive better performance and satisfaction than prior conventional rehabilitation for 6 weeks. However, the changes on performance and satisfaction scores of COPM in the control group were not greater than those changes in the intervention group. (Table 5) Therefore, it demonstrated that the OT-TO training had the positive effect on performance and satisfaction higher than the conventional rehabilitation. This is because OT-TO approach is an individual directed intensive training for helping people to function as best as they can within the limitation of their conditions.[34]

Limitation and future research

Although this study supported the evidence that OT-TO training was a useful intervention for improving UE functions in chronic stroke. However, there were key limitations of this study. To organize efficient strategies for performance training, context-specific environment (supporting surface, people, room, etc.) was recommended to be equal or mimic the natural environment for a specific task execution.[24] The limitation of the study was that the training activities were performed at facilities that the participants received services from. Therefore, the environment where those activities occurred was set closely to the natural environment. It was difficult for the study to set specific environments for the training activities. The second limitation was distributed practice, which schedules the training program to relate with periods of time during the day of participants.[22,24] In this study, each research site had its own schedule. This means that it was difficult to set training activities to correlate with a certain period during the days.

Further research should be carried out to deliver training in the participants' home environment. This method would be applicable if the training is done in the community setting. A large group of participants is needed for the effective testing of the method. In addition, other outcomes should be added to confirm the improvement of UE functions such as magnetic resonance imaging and electromyography.

Conclusion

The impairment of UE functions in clients with chronic stroke is a priority to concerned for abilities of UE in performing activities. OT-TO training can promote the recovery of UE motor function in chronic stroke patients. It is a useful training that can encourage participants to participate in meaningful activity which relate to mental health and quality of life. The findings of this study provide guideline of using OT-TO practice for improving UE functions in clients with chronic stroke in Thailand.

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Conflict of interest

The authors declare that there is no conflict of interests.

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Psychometric properties of the apraxia of speech rating scale Thai version

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ABSTRACT

Background: Thai speech-language pathologists lack appropriate tools to describe apraxia of speech (AOS) characteristics in Thai patients.

Objectives: This study aimed to translate and adapt the most recent version of the Apraxia of Speech Rating Scale (ASRS) 3.3, the ASRS 3.3, into a Thai version and evaluate the psychometric properties of the Thai version (ASRS-Thai).

Materials and methods: The original ASRS 3.3 has been translated into Thai using the backward-translation approach. The original developer was also included in the translation process to improve the translation accuracy. The resulting tool, ASRS-Thai, was administered to 28 adults with neurological speech or language disorders, along with another AOS test available in Thai, Apraxia Test for Thai Adults (ATTA). The recordings were rated independently by 5 experienced speech-language pathologists at different hospitals. The clinical assessment of patients' performance on the ATTA was used as the reference standard to measure the sensitivity and specificity of ASRS-Thai for AOS diagnosis. Concurrent validity and reliability measures were also examined. Reliability was examined by evaluating intra-rater and inter-rater reliability.

Results: Moderate-to-strong negative correlations were found between the ATTA and the ASRS-Thai (-0.575 to -0.900). Additionally, the sensitivity and specificity of the ASRS-Thai at a cut-off score of 16 were 100% and 86.7%, respectively. Reliability was computed by measuring the intraclass correlation (ICC) values. The intra-rater ICCs were 0.96, 0.968, and 0.976, and the inter-rater ICC was 0.927 for the total score.

Conclusion: The ASRS-Thai is a reliable, valid instrument to describe the presence and severity of AOS characteristics in clinical settings and research. Additional data collection by testing a larger sample size with diverse severities, including cases of pure AOS, is warranted in future studies.

Introduction

Apraxia of speech (AOS) is a neurological speech disorder that indicates an impaired ability to plan or program the sensorimotor commands necessary to guide movements that result in phonetically, prosodically normal speech.[1] AOS can occur in the absence of language disturbances or physiological disturbances associated with dysarthria. It occurs frequently in individuals with aphasia, dysarthria, or other neurological communication disorders.[2] Additionally, the most common cause of AOS is stroke.[3] According to a study of the incidence of stroke in Thailand, the estimated prevalence of stroke is 1.88% among adults aged 45 years and older.[4] With a prevalence of 122 patients per 100,000 individuals in the population.[5] Approximately 4-20% of stroke patients have language and speech problems[2], and AOS may account for

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6.9% of motor speech disorders.[1] Of further note, AOS tends to co-occur with Broca's aphasia[6] and rarely presents without other speech-language deficits.[7]

The primary method used in the differential diagnosis of AOS is auditory perceptual assessment.[1, 8] Challenges in the differential diagnosis of AOS include its probable co-occurrence with aphasia or dysarthria and controversies regarding its diagnostic criteria.[9] The clinical diagnosis of AOS requires a task that can reveal a patient's apraxic performance.[8]

In Thailand, based on Darley's definition of AOS[10], Akamanon developed the Apraxia Test for Thai Adults (ATTA) to be administrated by speech-language pathologists (SLPs).[11] The key points of Darley's definition are that (a) AOS affects articulation, (b) AOS compromises the positioning and sequencing of the articulators, (c) AOS is not caused by muscular weakness and is unrelated neuromuscular deficits, and (d) AOS affects prosody.[10] ATTA consists of seven subtests that require nonspeech or speech tasks: the former includes automatic control of articulators, imitation of the articulatory movement, oral apraxia, and limb apraxia; while the latter includes repetition, vowel prolongation, and diadochokinetic rate, and spontaneous and automatic speech. All the subtests are used to identify the characteristics of AOS, and each subtest has a scoring system ranging from a 3-point scoring scale to a 12-point scoring scale, with no cut-off score.[11]

Sarankawin studied the test-retest reliability of ATTA and compared the performance of 100 healthy Thai participants aged 20-40 years and 41-65 years. Ranging from 0.71 to 0.97, the test-retest reliability coefficient of each subtest was high. However, the validity of ATTA in determining the level of severity of speech impairments exhibited by the tested patients has not been studied. Additionally, ATTA's scoring criteria only evaluate articulatory errors without providing details about specific AOS characteristics and their frequencies (the scoring criteria of ATTA are presented in Table 3). ATTA's scoring criteria contain a mix of numerous target behaviors and symptoms, rather than measuring one symptom along a meaningful continuum of severity. For example, a score value of 3 for the spontaneous speech and automatic speech subtest, incorporates word length, correct word use, and appropriate grammar. The limitations inherent in the variability of these scoring descriptors decrease their potential utility. Decreasing ATTA scores may not reflect an increasing severity of AOS. Clinicians who employ ATTA must interpret the results without a cut-off score, and extra examinations may be required for differential diagnosis. Furthermore, some clinicians used words and pictures from ATTA in their clinical AOS diagnosis without employing the scoring criteria.[12]

Published tests for the diagnosis of AOS include the Apraxia Battery for Adults second edition (ABA-2)[13] and Quick Assessment for Apraxia of Speech.[14] However, these tests contain words and sentences in English, which cannot be translated for assessing Thai speech defects because of cultural and phonological differences. Although the translated words and sentences for collecting speech samples would have the same content as the original test, they cannot be used for assessment within the Thai phonetic context. Only the translated scoring criteria can be used across languages. For example, "cat, catnip, catapult, catastrophe[15]" would be translated into Thai (pronunciation): "แมว (mɛ:w), กัญชาแมว (kan-t͡ɕʰa:-mɛ:w), เครื่องยิงก้อนหิน (kʰrɯ̂ạŋ-jiŋ-kɔ̂:n-hǐn), ภัยพิบัติ (pʰaj-pʰí-bàt)." The translated stimuli do not share the same first syllable and do not successively increase in length. Furthermore, the word "กัญชา (kan-t͡ɕʰa:)" is not appropriate in Thai culture because it also means cannabis (a narcotic), and Thai patients may not be familiar with the word "เครื่องยิงก้อนหิน (kʰrɯ̂ạŋ-jiŋ-kɔ̂:n-hǐn)." Therefore, any assessment in the Thai language would require developing a set of words and sentences suitable for Thai culture. Alternatively, it could be possible to use existing feasible and valid Thai words and sentences combined with translated scoring criteria from another language.

Apraxia of Speech Rating Scale (ASRS) 1.0[16] was developed at the Mayo Clinic to describe the characteristics frequently associated with AOS by using speaking tasks from the Western Aphasia Battery-Revised (WAB-R)[17], alternate motion rate (AMR), sequential motion rate (SMR), and supplementary motor speech tasks as speech samples for scoring. The validity and reliability of ASRS 1.0 were measured in 133 adults with neurodegenerative speech or language disorders. Inter-rater reliability was strong: the intraclass correlation (ICC) was 0.94 for the total score and 0.91 for the number of AOS characteristics identified to be present. Intra-rater ICC ranged from 0.91 to 0.98. Validity was strong based on correlations with clinical diagnoses and assessments of severity. Specificity was 100% when the cut-off ASRS score was set at 8. Sensitivity was 75% with a cut-off score of 14, 90.5% with the cut-off of 10, and 96% with the cut-off of 8. These results suggested that the ASRS 1.0 was a potentially useful instrument for measuring the presence and severity of AOS characteristics. However, some of the items were not easily scored, and there was some redundancy across items that required a revision of the scale.[16] The ASRS 1.0 was also translated into Spanish.[18]

In the process of validation, ASRS has undergone multiple revisions. The most recently published version is ASRS 3.0.[19] ASRS 3.0 rates 13 speech features, while ASRS 1.0 rates 16 speech features. In terms of the overall organization, ASRS 3.0 was reduced from four to three categories: articulatory features, prosodic features, and others. ASRS 3.0 also eliminates some features: a) increased distortions or distorted substitutions with an increased speech rate, b) lengthened intersegment durations, and c) sound prolongations (beyond lengthened segments). Some features were reorganized: The audible or visible group is now a single feature, and false starts are grouped with repetitions. Several features have been clarified (e.g., overall speech rate, AMRs, SMRs). Like ASRS 1.0, ASRS 3.0 uses a 5-point scale with operationalized descriptors for each rating level. ASRS 3.0 was studied in 28 adults with chronic aphasia and suspected AOS from stroke and brain injury.[20] Inter-rater ICC was 0.954 for the total ASRS score and ranged from 0.034 to 0.789 for individual items. The concurrent validity of ASRS 3.0 ranged from 0.593 to 0.991. These results suggested that the ASRS 3.0 may be a reliable measurement of AOS characteristics, and additional operationalization of rating procedures may be required to improve the inter-rater reliability of a few items.[20]

The developers' unpublished update of ASRS 3.3[21] is

similar to ASRS 3.0, but there was a wording change in item 13: from "score on a maximum number of syllables/repetitions per breath group" to "score on an average number of syllables/repetitions per breath group across tasks." Since some patients perform differently in different contexts, this feature reflects the respiratory coordination of AOS using the average number of performances when perceiving a reduction. The validity or reliability of the ASRS 3.3 has not been established.[21]

Considering the lack of a test to describe AOS characteristics in Thai patients, the present study sought to translate the ASRS, which provides details of the presence and severity of speech impairments associated with AOS and is suitable for assessing speech across languages by collecting speaking tasks from the WAB-Thai, AMR, and SMR as speech samples for scoring. Furthermore, the sensitivity and specificity of the ASRS in assessing stroke- or brain injury-induced AOS has never been studied before.

Thus, the aims of this study were as follows: Translate ASRS 3.3 into Thai language, adapt ASRS 3.3 for Thai language, and evaluate the concurrent validity, sensitivity, specificity, and reliability of ASRS Thai in assessing neurological communication disorders to achieve clinical diagnostic accuracy for acquired AOS in Thai adults. The achievement of these objectives would facilitate appropriate treatment planning by Thai clinicians and provide information regarding the additional refinement of the original ASRS.

Materials and methods

This study includes 2 phases; Phase 1 is to translate and adapt ASRS 3.3 into Thai language, and Phase 2 is to evaluate the psychometric properties of ASRS-Thai. The flow diagram of the study procedure is provided in Appendix 1.

Phase 1: Translation and cross-cultural adaptation of ASRS 3.3

ASRS 3.3,[21] the most recent version of ASRS, was translated and adapted into Thai by following a standard forward- and backward-translation process[22] with permission from the original developers.

The first stage involved the production of two translations by two independent translators with different backgrounds: an SLP and a professor (linguistic). In the second stage, the two translators synthesized the results of their respective translations to produce one translation. In the third stage, two back-translations based on the synthesized translation were produced by two other independent translators with different backgrounds who were blinded to the original version. The fourth stage involved an expert committee meeting where comparisons were conducted between all the versions of translations and back-translations and the original ASRS 3.3; adjustments were subsequently made to yield a pre-final version. The fifth stage was a cognitive debriefing to test alternative wording and verify the understandability and interpretation of the pre-final Thai ASRS 3.3 among experienced clinicians (SLPs) who were blinded to the original or any English versions. The pre-final version was revised based on the feedback obtained in this stage. The revisions modified and eliminated irrelevancies and generated word substitutes to fit the target's cultural situation while maintaining the general concepts of the items; two back-translations were produced and sent to the developer of the original ASRS 3.3 along with the previous two back-translations for review. Corrections were conducted after reviewing the developer's comments to ensure that the final version would maintain content validity. This step yielded two more back-translations that were sent to the developer for further review. The final version of the ASRS-Thai was finalized after the developer completed the reviewing process.

Adjustments to ASRS-Thai were made when the researcher sent questions seeking explanations from the developer, who then provided clarifications and some examples to be adapted into Thai. Experienced Thai clinicians (SLPs) suggested that the researcher add the evaluation method and rearrange the sequence of items to ensure consistency in the evaluation and scoring method. Thus, items 1-10 use the same scoring criteria, but items 11-13 have different scoring criteria that are specific to each item. The evaluation methods of the ASRS-Thai are presented in Table 1. The brief descriptions of items 1-13 are provided in Table 6.

Table 1 Evaluation methods and scoring criteria of ASRS-Thai.

Evaluation methods of ASRS-Thai
1) Determine speech characteristics from spontaneous speech, repetition, and naming subtests of the Western Aphasia Battery Thai version.
2) Determine AMR, SMR, and duration of the longest vowel prolongation for items 11-13.
Criteria considered for items 1-10
0 = not observable or occurs not more than 1 time.
1 = not observable often or occurs more than 1 time but less than 20% of the whole utterance.
2 = observable often or for 20%-50% of the whole utterance; score not more than 2 if occurs only in the repetition section.
3 = observable almost all the time, but not sufficiently severe to affect overall speech intelligibility.
4 = observable almost all the time or observable all the time with a severe degree that affects speech intelligibility.

Table 1 Evaluation methods and scoring criteria of ASRS-Thai. (continues)

Criteria for item 11	
0 = normal repetition rate	
1 = mild distortion (of placement - manner and/or voiced–voiceless, easily perceived as the target sounds) and occurs a few times	
2 = mild distortion (of placement - manner and/or voiced–voiceless, easily perceived as the target sounds) but occurs often	
3 = moderate distortion (misses the target sound for more than one feature of placement, manner, voiced, voiceless)	
4 = severe distortion (not perceived as the target sounds)	
Criteria for item 12	
0 = normal sequencing phonation rate	
1 = slow (SMR repetitions)	
2 = mild pause between words and/or mild distortion (easily perceived as the target sounds)	
3 = moderate pause between words and/or moderate distortion	
4 = severe pause between words and/or severe distortion (not perceived as the target sounds)	
Criteria for item 13	
Reduced words per breath group	Reduced # of AMR repetitions per breath group
0 = more than 7 syllables	0 = more than 7 times
1 = 6-7 syllables	1 = 6-7 times
2 = 4-5 syllables	2 = 4-5 times
3 = 3-4 syllables	3 = 3-4 times
4 = 2 syllables or less	4 = 2 times or less

At first, ASRS-Thai produced a confusing and less comprehensible result due to an attempt to retain the entire sentence structure and literal meaning of the original version. The suggestions from experienced clinicians matched the suggestions provided by Brislin *et al*. for writing in a short, concise form; providing context; and minimizing the use of colloquialisms, subjunctives, multiple verbs, and vague words in the formulation of the instruments.[23] The ASRS-Thai provides context by adding a few examples: e.g., in item 1, "Speech distortion but target sound still perceived, e.g., distortion of the /s/ of /sà-bùː/ (meaning soap in Thai)"; in item 2, "Substitution with a distorted sound. e.g., substitution with distorted /t/ for /s/ of /sà-bùː/ (meaning soap in Thai)"; in item 10, "use of other words with related meanings," and "/sh../ (shirt) for /kaːŋ-keːŋ/ (pants)" was added due to the confusion caused by translation of the original example into Thai (i.e., "/t͡ɕʰɔ̀ːn/ (spoon) for /sɔ̂ːm/ (fork)") that may be interpreted as unclear speech instead of semantic paraphasias.

Other concerns from experienced Thai clinicians were related to clarifications of scoring criteria, such as those in Strand *et al*.[16] and Wambaugh *et al*.[20] for the specification of the criteria and provision of particular scorings and subtle behaviors (e.g., mildly segmented SMRs; slight voicing error detected in AMRs; and slow, segmented speech in conversation) that could be helpful for ratings among clinicians and researchers. Therefore, the criteria of items 11 and 12 were revised in the ASRS-Thai based on the references from the developer (Table 1). Item 13 was divided into two categories, and quantifying nouns were added to the scorings: 0 = more than seven syllables, 0 = more than seven times. An example of how to count was also added: "count /p/ = 1 time." The final version of ASRS - Thai is presented in Appendix 2.

Phase 2: Psychometric properties evaluation of ASRS-Thai

ASRS-Thai was administered to 30 adults with neurological speech or language disorders in conjunction with ATTA and the Western Aphasia Battery Thai version (WAB-Thai), to determine concurrent validity, sensitivity, specificity, intra-rater reliability, and inter-rater reliability.

Participants

Thirty adults were recruited for this study by following these inclusion criteria:

1. a history of cerebrovascular accidents or acquired brain injury and in a stable condition (vital signs are stable and within normal limits[24]).
2. a rehabilitation physician's or SLP's diagnosis of neurological speech or language problems
3. age greater than 20 years
4. native Thai speakers
5. apparent normal hearing: the hearing was analyzed while the patient was conversing with the researcher at a normal level of loudness; if the researcher had to speak louder or repeat questions or information several times, the patient was not selected.
6. apparent normal vision or having appropriate visual aids: patients were asked if they wore glasses and whether they had visual problems that interfered

with their daily activities.
7. could repeat /pə-tə-kə/ 0-8 times within 5 s[12]
8. a score of 4 and above out of a total score of 10 on the comprehension subtest in the Western Aphasia Battery Thai version[25, 26]

Exclusion criteria were as follows:
1. patients with any condition that would limit their ability to participate in the study, such as unstable vital signs, unconsciousness, discomfort, dizziness, drowsiness, inattention, confusion, slow responses to stimulation, unresponsiveness, lack of co-operation, or on a respirator.
2. patients comorbid with Parkinson's disease or other neurodegenerative diseases.
3. refusal to provide informed consent

All participants were considered to have neurological speech or language problems with or without AOS. Written consent was obtained from all participants according to the committee's guidelines. Two participants were excluded due to their having Parkinsonism. Hence, the data of 28 participants were used in this study. Table 2 presents the demographic information of the participants.

Table 2 Demographic information of the participants.

Gender	
Male	21 (75%)
Female	7 (25%)
Age	
Mean (SD)	52.14 (15.59)
Range	26-84 years
Etiology	
1. Cerebrovascular accident	23 (82%)
1.1 Hemorrhagic	8 (28.5%)
1.2 Ischemic	15 (53.5%)
2. Acquired brain injury	5 (18%)
Post-onset (all participants are in stable condition)	
Mean (SD)	2.06 years (2.41)
Range	7 days-10 years 5 months
WAB comprehension score (AQ)	
Mean (SD)	8.16 points (2.02)
Range	4.05–10 points
Diagnosis (the AOS diagnosis is from the agreement of diagnosis among raters, whether presence or absence of AOS)	
1. Dysarthria without AOS	1 (4%)
2. Dysarthria with Aphasia	7 (25%)
3. Aphasia without AOS	7 (25%)
4. Aphasia with AOS	13 (46%)
5. Dysarthria with AOS	0 (0%)
6. AOS alone	0 (0%)

Procedures

Each participant was evaluated by using WAB-Thai (only for the spontaneous speech, repetition, naming, and word-finding subtests), and the ATTA (only for the vowel prolongation, diadochokinetic rate, repetition, spontaneous speech, and automatic speech activities). A video recording was made of the evaluation by the researcher, who used an iPhone XR (Rater #1).

Performances on WAB-Thai, vowel prolongation, and diadochokinetic rate (both AMR and SMR) were used for collecting speech samples to observe the speech performance of each participant and provide a score for ASRS-Thai. ASRS-Thai consisted of 13 items rated on a 5-point scale to determine speech characteristics from the spontaneous speech, repetition, and naming sections of the WAB-Thai. The criteria for scoring are presented in Table 1.

ATTA has words and sentences in Thai language that could be used as supplementary motor speech tasks to reveal patients' speech performances for the clinical diagnosis of AOS. Performance in ATTA was used for the clinical diagnosis of AOS through perceptual judgment. The inter-rater agreement of clinical diagnosis results from the ATTA regarding the presence of AOS was used to analyze the sensitivity and specificity of the ASRS-Thai. Each subtest of ATTA had a scoring system. Vowel prolongation and the diadochokinetic rate subtest were assessed by asking participants to sustain a vowel sound (/a/, /u/, and /i/) as long as possible two times and to produce the syllables /pə/, /tə/, /kə/, and /pə-tə-kə/ as quickly as possible within 15 s. Raters reported the results of vowel prolongation or maximum phonation time (MPT) in s and AMR and SMR by the frequency of the repetition of syllables. The repetition subtest consisted of 34 words or sentences for the participant to imitate after the examiner, which was rated on a 3-point scoring scale. The subtest for spontaneous speech and automatic speech consisted of two tasks that included 6 items. The first task was to produce spontaneous speech by describing the picture; the second task was to assess automatic speech and used a 4-point scoring scale. The criteria for scoring are presented in Table 3.

Table 3 Scoring criteria of the Apraxia Test for Thai Adults (ATTA) in repetition, spontaneous speech, and automatic speech subtest.

Criteria for repetition subtest
2 = correct, prompt, no struggle, no articulatory error
1 = self-correction, significant delay, visible or audible searching, one or more articulatory errors
0 = no response or failed attempts by not producing a word or producing a word by using the wrong number of syllables
Criteria for spontaneous speech and automatic speech subtest
3 = the characteristics of a two-word phrase or four-word sentence, all of which are appropriate and with correct grammar
2 = a partially correct or trial-and-error response
1 = defective speech, visible or audible search, articulatory error
0 = no response

All raters were instructed by the researcher on scoring ATTA for clinical diagnosis and on scoring ASRS-Thai (Rater#1). They were all experienced SLPs: Rater#1 had 7 years of experience as an SLP at the Sirindhorn National Medical Rehabilitation Institute; Rater#2 had 9 years of experience as an SLP at the Rehabilitation Unit, Vejjarak Lampang Hospital; Rater#3 had 21 years of experience as an SLP at the Department of Rehabilitation, Chulalongkorn Hospital; Rater#4 had 23 years of experience as an SLP at the Department of Rehabilitation, Somdejprapinklao Hospital; and Rater#5 had 26 years of experience as an SLP at the Department of Ear Nose Throat, Bhumibol Adulyadej Hospital. All the raters watched the video recordings and independently scored the participants while at their hospitals (in quiet rooms), i.e., independent from the other raters and the researcher (Rater#1). No rater was involved with the original ASRS development or had seen the original version. Raters 2-5 were provided with limited information-age, etiology, onset, and WAB-Thai comprehension scores-without any diagnosis. The etiological information provided to raters was regarding cerebrovascular accidents and hemorrhagic, ischemic, or acquired brain injury, with no information regarding lesions in the brain or MRI or CT scan results. All video recordings were named by using an alphanumeric code instead of the participants' real names. Raters 1-3 were selected for evaluation of intra-rater reliability based on the number of clinical experiences for comparison. Raters 2-3 (the clinicians participating in the research) received new randomly coded video recordings 14 days after the first scoring; the first-score results were collected immediately after the scoring was finished. Rater #1 (the researcher) rescored the video recordings 20 months after the first scoring to avoid the bias from remembering the first scoring since Rater#1 was both the evaluator and recorder of the videos.

Data analysis

Validity of ASRS-Thai was assessed by obtaining the results of all the samples from ATTA and ASRS-Thai to calculate concurrent validity, sensitivity, specificity, and the cut-off point.

Concurrent validity is the correlation between ASRS-Thai and ATTA, an existing well-established scale, calculated by using the Pearson correlation coefficient with IBM SPSS 26. Values near 0 indicate no correlation, and values near ±1 indicate a very strong correlation. A negative sign indicates that the 2 variables are inversely related, that is, as one variable increases, the other variable decreases. A value less than 0.3 indicates a poor correlation; values between 0.3 and 0.5 indicate a fair correlation; values between 0.6 and 0.8 indicate a moderately strong correlation, and values of at least 0.8 indicate a very strong correlation.[27]

Sensitivity refers to the proportion of individuals with AOS who are shown to have AOS on ASRS-Thai, based on the reference standard (clinical judgment of patient's performance on ATTA). Specificity indicates the proportion of individuals without AOS who are shown not to have AOS on ASRS-Thai. The majority diagnosis among the five raters - i.e., at least three of five raters agreed to the presence or absence of AOS -was used for the AOS diagnosis of the reference standard. The diagnosis results were not retrieved from the consensus evaluation to prevent bias; as senior raters might affect the evaluations of junior raters. As five raters (R#1-5) scored the ASRS-Thai of 28 participants, all data scores were collapsed using the most frequently rated score or a majority rating of each item. The scores of three raters who re-scored the ASRS-Thai were used only for the initial scoring. If the raters did not rate the same score or had two frequently rated values, the median score would be used (the average score featuring a decimal would not be applicable for a cut-off value). All selected scores for the 13 items were compiled into each participant's total score to calculate the cut-off score. The maximum value of the Youden index (Sensitivity+Specificity-1) was used to determine the most appropriate cut-off value.[28] The receiver operating characteristic (ROC) curve was plotted using SPSS version 26 (IBM Corp, Armonk, NY, USA) based on the sensitivity versus 1 - specificity of ASRS-Thai. The area under the ROC curve (AUC) indicates the diagnostic accuracy of the test. An AUC value lower than 0.7 indicates low accuracy, values of 0.7 to 0.9 indicate moderate accuracy, and value more than 0.9 indicate high accuracy.[29]

The reliability of ASRS-Thai was assessed by analyzing the results obtained by independent scoring of ASRS-Thai by multiple raters and measuring the inter-rater and intra-rater reliability. To assess inter-rater reliability, five raters with at least 5 years of experience watched the video recordings of all the participants independently and scored the ASRS-Thai without viewing the scoring results obtained by others. To assess intra-rater reliability, three raters rescored ASRS-Thai independently after the first scoring (Rater#2 and Rater#3 rescored after 14 days; Rater#1 rescored 20 months after the first scoring). The percentage of the sample re-measured to assess intra-rater reliability was 100% (28 videos of 28 participants).

Reliability was computed by using ICCs and IBM SPSS 26, based on a two-way mixed-effects model with absolute agreement type. Values less than 0.5 indicated poor reliability; values between 0.5 and 0.75 indicated moderate reliability; values between 0.75 and 0.9 indicated good reliability, and values greater than 0.90 indicated excellent reliability.[30]

Results

The psychometric properties of ASRS-Thai were assessed in all 28 participants aged 26-84 years with neurological speech or language disorders from cerebrovascular accidents or acquired brain injuries. Demographic information of the participants is provided in Table 2.

Concurrent validity of ASRS-Thai total score was estimated by comparing ASRS-Thai average total scores of all raters with the average score of all raters for each part of ATTA by using Pearson correlations. These data are provided in Table 4.

Table 4 Pearson correlations between the Apraxia of Speech Rating Scale Thai version (ASRS-Thai) and Apraxia Test for Thai Adults (ATTA).

	ATTA					
	MPT	AMR	SMR	Repetition	Automatic	Spontaneous
ASRS-Thai total scores	-0.089	-0.323	-0.575**	-0.900**	-0.711**	-0.775**

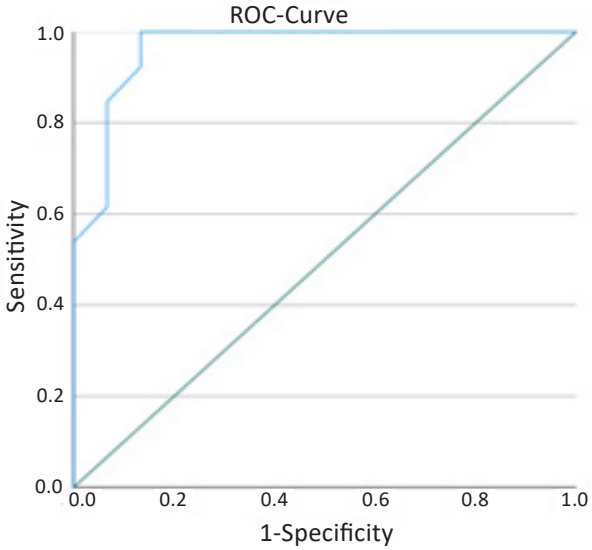
*** Correlation is significant at the 0.01 level (two-tailed).*

The optimal cut-off value for ASRS-Thai according to Youden's index was 16. The sensitivity and specificity of the ASRS-Thai using a cut-off value of 16 were 100% and 86.7%, respectively. In contrast, a cut-off score of 32 yielded a specificity of 100% with a sensitivity of 53.8%. The AUC was 0.964, indicating high diagnostic accuracy (Table 5).

Table 5 Cut-off scores, sensitivities, specificities, crosstabulation between ASRS-Thai and the reference standard, and receiver operating characteristic (ROC) curve.

Cut-off	Sensitivity	Specificity	Youden (Se+Sp-1)
1	100.0%	0.0%	0.000
5	100.0%	6.7%	0.670
6	100.0%	13.3%	0.133
7	100.0%	20.0%	0.200
9	100.0%	26.7%	0.267
10	100.0%	33.3%	0.333
11	100.0%	53.3%	0.533
12	100.0%	60.0%	0.600
13	100.0%	73.3%	0.733
14	100.0%	80.0%	0.800
16	100.0%	86.7%	0.867
19	92.3%	86.7%	0.790
20	84.6%	93.3%	0.779
27	76.9%	93.3%	0.702
31	69.2%	93.3%	0.625
32	53.8%	100.0%	0.538
33	38.5%	100.0%	0.385
48	23.1%	100.0%	0.231
52	15.4%	100.0%	0.154

Crosstabulation		Reference standard		Total
		Yes	No	
ASRS-Thai	Yes	13 (100%)	2 (13.3%)	15
	No	0 (0%)	13 (86.7%)	13
Total		13	15	28



Area under curve (95% CI) = 0.964 (0.901, 1.000)

Reliability was computed by using ICC values. The intra-rater ICCs were 0.96, 0.968, and 0.976, and the inter-rater ICC was 0.927 for the total score (Table 6).

Table 6 Intra-raters and inter-rater intraclass correlations (ICC).

ASRS-Thai (brief description)	Intra-rater Rater#1			Intra-rater Rater#2			Intra-rater Rater#3			Inter-rater Rater 1-5		
	ICC	95% CI		ICC	95% CI		ICC	95% CI		ICC	95% CI	
		Lower	Upper		Lower	Upper		Lower	Upper		Lower	Upper
Phonetic Features												
Item 1 Sound distortions	**0.884**	0.748	0.946	**0.742**	0.44	0.881	**0.663**	0.196	0.852	**0.858**	0.749	0.927
Item 2 Distorted sound substitution	**0.876**	0.732	0.942	**0.799**	0.562	0.907	**0.74**	0.315	0.89	**0.8**	0.633	0.9

Table 6 Intra-raters and inter-rater intraclass correlations (ICC). (continues)

ASRS-Thai (brief description)	Intra-rater Rater#1			Intra-rater Rater#2			Intra-rater Rater#3			Inter-rater Rater 1-5		
	ICC	95% CI		ICC	95% CI		ICC	95% CI		ICC	95% CI	
		Lower	Upper		Lower	Upper		Lower	Upper		Lower	Upper
Item 3 Distorted sound additions	**0.723**	0.402	0.872	**0.788**	0.548	0.901	**0.832**	0.634	0.922	**0.718**	0.518	0.853
Item 4 Increased distortions with increased length or complexity	**0.765**	0.489	0.892	**0.875**	0.731	0.942	**0.746**	0.426	0.885	**0.917**	0.857	0.957
Prosodic Features												
Item 5 Syllable segmentation within words	**0.941**	0.874	0.973	**0.82**	0.609	0.917	**0.937**	0.864	0.971	**0.774**	0.593	0.885
Item 6 Syllable segmentation across words in phrases/sentences	**0.862**	0.7	0.936	**0.832**	0.634	0.922	**0.953**	0.898	0.979	**0.742**	0.543	0.868
Item 7 Slow overall speech rate	**0.866**	0.71	0.938	**0.973**	0.941	0.987	**0.855**	0.688	0.933	**0.869**	0.775	0.932
Item 8 Lengthen segments independent of overall speaking rate	**0.782**	0.533	0.898	**0.75**	0.468	0.883	**0.903**	0.792	0.955	**0.638**	0.385	0.809
Other												
Item 9 Groping	**0.926**	0.808	0.969	**0.896**	0.762	0.953	**0.979**	0.956	0.99	**0.875**	0.777	0.936
Item 10 False starts/restarts	**0.82**	0.606	0.917	**0.936**	0.861	0.97	**0.969**	0.933	0.985	**0.858**	0.753	0.926
Item 11 Off-target speech AMRs	**0.826**	0.616	0.92	**0.868**	0.716	0.939	**0.889**	0.759	0.949	**0.869**	0.769	0.933
Item 12 Slow/segmented/distorted SMRs	**0.836**	0.644	0.924	**0.906**	0.799	0.956	**0.877**	0.736	0.943	**0.854**	0.745	0.924
Item 13 Reduced words/AMRs per breath group	**0.912**	0.811	0.959	**0.99**	0.978	0.995	**0.782**	0.526	0.899	**0.794**	0.616	0.897
Total score	**0.96**	0.914	0.982	**0.968**	0.932	0.985	**0.976**	0.945	0.989	**0.927**	0.867	0.963
Diagnosis	**0.884**	0.749	0.946	**0.964**	0.923	0.983	**0.926**	0.84	0.965	**0.901**	0.829	0.949

Discussion

The current translation is based on the developers' unpublished update of ASRS 3.3.[21] It can be assumed that the newest version of ASRS-English, the ASRS-3.3, inherits the high validity of the previous version. However, the validity of ASRS-English version 1.0 was evaluated in a group of adults with neurodegenerative and language disorders (progressive aphasia and/or progressive apraxia of speech). In contrast, ASRS-Thai was studied in adults with neurological speech or language disorders resulting from cerebrovascular accidents and acquired brain injuries in conjunction with ATTA, another AOS test available in Thai. Thus, the translation and the differences in the study population, including the types of diseases, ethnicities, and language-related contexts, may affect the validity. ASRS-Thai underwent systematic forward-translation and back-translation processes with culturally relevant alterations. The alterations included the addition of the evaluation method, rearrangement of the items' sequence, adoption of simpler words to improve understanding, maximization of the clarity of wordings, familiarization of word usage for the target users, and creation of appropriate Thai examples. The back translations were sent to the original developer and expert SLPs to verify content validity. The results indicated that the ASRS-Thai is consistent with the original ASRS-3.3 and evaluates the same aspects.

The results of correlational analyses demonstrated moderate-to-strong negative correlations between the

three ATTA subtest scores and the total ASRS-Thai scores. ASRS-Thai total score increased, reflecting an increased prevalence of the symptoms observed. However, in the three subtests of ATTA, the score increased, reflecting the absence of a struggle or articulatory error. A strong correlation was observed between the repetition subtest and ASRS-Thai: -0.900, with significance at 0.01. Because both ATTA subtests and ASRS-Thai focus on speech performance, moderate correlations were observed between the automatic speech and spontaneous speech subtests and ASRS-Thai: -0.711 and -0.775, respectively (p=0.01 for both). A poor correlation of -0.089 was observed between MPT and ASRS-Thai. The MPT is used to evaluate breath function and increased or decreased MPT does not reflect the symptoms of AOS but instead the symptoms of dysarthria. Individuals with dysarthria may have short MPT because of reduced breath support. Individuals with AOS may not have short MPT but do have short phrases even though they show no evidence of breathing difficulty.[31] A poor correlation of -0.323 was also observed between AMR and the ASRS-Thai. The AMR subtest in ATTA counts the frequency of syllable repetition without considering articulation errors. ASRS-Thai includes two AMR-associated items: off-target speech AMRs as item 11 and reduced words/AMRs per breath group as item 13, for which the scoring criteria of articulation errors were included. Fair correlations were observed between SMR and ASRS-Thai: -0.575, with significance at 0.01. Increased or decreased frequency of sequenced syllable repetition may be prompt suspicion of AOS. Individuals with AOS are unable or show difficulty in maintaining the correct sequence at a normal rate, as evidenced by, for example, item 12 (Slow/segmented/distorted SMRs). Individuals with dysarthria may produce a correct sequence at a slow rate or an incorrect sequence due to misarticulation.[8] Considering these findings, ASRS-Thai is comparable with the currently used test, ATTA. ASRS-Thai is also superior to the AMR subtest and the MPT subtest of ATTA in scoring criteria specific to AOS characteristics.

The results of the sensitivity and specificity analyses suggest that the most appropriate cut-off value of the ASRS-Thai according to Youden's index is 16 (100% sensitivity and 86.7% specificity). The scores used in the cut-off analysis were obtained from the total score of the 28 participants, which combined scores from the most frequent rated score of each item (majority rating) - not the consensus rating. Although the consensus rating was more accurate than the individual ratings, senior raters or persuasive raters might affect junior or passive raters. The consensus rating did not differ significantly from the majority rating.[32] As the inter-rater reliability in this study based on the agreement of scoring ASRS-Thai among five raters, yielded moderate-to-excellent inter-rater reliability for all 13 items, basing the score on the majority rating was appropriate.

A cut-off score of 16 on the ASRS-Thai is higher than the cut-off value of 8 in the original ASRS 1.0. The deviation in cut-off values may be attributed to many factors, including the number of items, scoring criteria, and population. The number of items and scoring criteria of ASRS 1.0 were revised to yield the ASRS 3.3, which was translated and adapted into the ASRS-Thai in this study. The original developers reported that ASRS is best suited to describe the nature and severity of AOS when present. It is not yet validated as a tool for discriminating AOS from dysarthria or aphasia, and a cut-off score for the ASRS 3.3 has yet to be established. The developers have had some success in using it to detect AOS in patients with degenerative conditions with or without aphasia.[21] The participants in this study were adults with neurological speech or language disorders, mostly from cerebrovascular accidents (82%), without pure AOS (Table 2). The AOS of all 13 participants co-occurred with aphasia. Nonetheless, the clinicians should employ discretion when using this cut-off in rendering a diagnosis of AOS and include diagnostic criteria and related variables that may not be reflected in the rating scale.

The reliability was excellent. The intra-rater ICC for Rater #1 was 0.96 for the total score and ranged from 0.723 to 0.941 for individual items. The intra-rater ICC for Rater #2 was 0.968 for the total score and ranged from 0.742 to 0.99 for individual items. The intra-rater ICC for Rater #3 was 0.976 for the total score and ranged from 0.663 to 0.979 for individual items. The inter-rater ICC for Raters 1-5 was 0.927 for the total ASRS score and ranged from 0.638 to 0.917 for individual items.

Comparable with the ASRS 1.0 and the ASRS 3.0, the inter-rater reliability for the total score of the ASRS-Thai was excellent. Although the inter-rater ICC for individual items of ASRS 3.0 ranged from 0.034 to 0.789, that of the ASRS-Thai ranged from 0.638 to 0.917. The highest levels of agreement for both versions differ. The feature of slow overall speech rate (item 7, ICC=0.789) showed the highest ICC in ASRS 3.0, but the feature of increased distortions with increased length or complexity (item 4, ICC=0.917) was the highest in the ASRS-Thai.

However, some features showed similar ICC values for ASRS 3.0 and ASRS-Thai, such as syllable segmentation within words (ICC=0.737 for ASRS 3.0 and ICC=0.774 for ASRS-Thai), syllable segmentation across words (ICC=0.646 for ASRS 3.0 and ICC=0.742 for ASRS-Thai), and slow overall speech rate (ICC=0.789 for ASRS 3.0 and ICC=0.869 for ASRS-Thai). Features that showed poor rating agreement for both ASRS 3.0 and ASRS-Thai were lengthened vowel and/or consonant segments independent of overall speaking rate (ICC=0.355 for ASRS 3.0 and ICC=0.638 for ASRS-Thai) and distorted sound additions (ICC=0.368 for ASRS 3.0 and ICC=0.718 for ASRS-Thai).

Additional explanations of item 3 (distorted sound additions) may improve agreement among the raters. Furthermore, differentiating the guidelines of prosodic features (items 5-8), modification of scoring, and training to identify each feature's characteristics through the use of video examples could also help achieve rater score agreement, as suggested by Strand *et al*. in ASRS 1.0.[16]

Although the ASRS-Thai was carefully translated from the most recent version of the ASRS by using the backward-translation approach and included the original developer in the translation process to improve the translation accuracy, recommendations for the use of the ASRS-Thai will include further revisions and improvements, the use of an instruction manual, and identification of severity judgment indicators.

Furthermore, the ASRS-Thai is only available for stroke patients and needs to be used in conjunction with the WAB-Thai. The AOS group in this study was small and featured no cases of pure AOS; all AOS participants also presented with aphasia due to cerebrovascular accidents. Additional data collection in future studies will require testing in a larger sample size with a wide range of severities, including pure AOS if possible.

Conclusion

In conclusion, the ASRS-Thai is a reliable and valid tool to describe the characteristics of speech features and rate the severity of AOS in clinical use. These features are anticipated to benefit Thai clinicians since existing AOS-related data are lacking in contexts specific to Thai language.

Ethical approval

This study was approved by the Research Ethics Committee of Faculty of Associated Medical Sciences, Chiang Mai University (Approval ID: AMSEC-61EX-038); the Research Ethics Committee of Faculty of Medicine, Chiang Mai University (Approval ID: NONE-2561-05641); and the Research Ethics Committee of Chiang Mai Neurological Hospital (Approval ID: EC 004-62).

Conflict of interest

The authors have declared that no competing interests existed at the time of publication.

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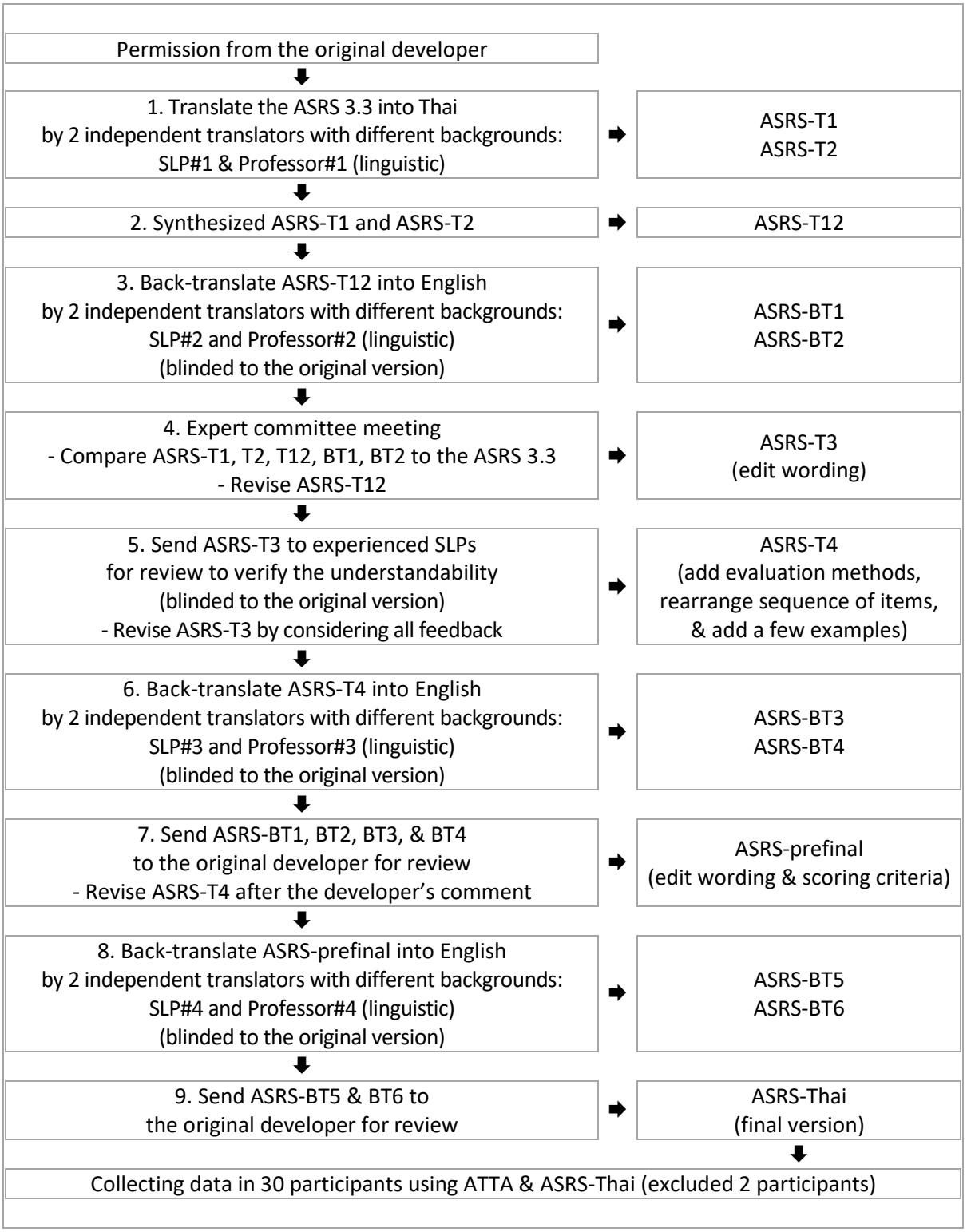
Appendix 1. Flow diagram of the study procedure



Appendix 2. The Apraxia of Speech Rating Scale Thai Version

ชื่อผู้ป่วย:_____เลขประจำตัวโรงพยาบาล:_____วันที่:_____ ผู้ประเมิน:_____

มาตรวัดภาวะเสียการรู้ปฏิบัติด้านการพูด

วิธีการประเมิน: 1) พิจารณาลักษณะการพูดจากการประเมิน Western Aphasia Battery ด้านการพูดเอง การพูดตาม และการเรียกชื่อ
2) พิจารณาอัตราการออกเสียงซ้ำ ๆ อัตราการออกเสียงตามลำดับ และระยะเวลาการออกเสียงสระที่นานที่สุด เฉพาะข้อ 11-13

เกณฑ์การให้คะแนนสำหรับข้อ 1-10

0 = ไม่สังเกตเห็น หรือ เกิดขึ้นไม่เกิน 1 ครั้ง
1 = สังเกตเห็นไม่บ่อย หรือ เกิดขึ้นมากกว่า 1 ครั้ง แต่น้อยกว่าร้อยละ 20 ของถ้อยความทั้งหมด
2 = สังเกตเห็นได้บ่อยครั้ง หรือ ร้อยละ 20-50 ของถ้อยความทั้งหมด โดย*ให้คะแนนไม่เกิน 2 คะแนน หากปรากฏเฉพาะในการพูดตาม*
3 = สังเกตเห็นเกือบตลอดเวลา แต่ไม่รุนแรงมากพอที่จะกระทบต่อการฟังเข้าใจคำพูดโดยรวม
4 = สังเกตเห็นเกือบตลอดเวลา หรือสังเกตเห็นได้ตลอดเวลา และรุนแรงมากพอที่จะกระทบต่อการฟังเข้าใจคำพูด

สัทลักษณ์		
1.	มีเสียงผิดเพี้ยนที่ยังฟังออกว่าเป็นเสียงเป้าหมาย เช่น “สบู่” โดยเสียง /ส/ เพี้ยน	
2.	มีการใช้เสียงอื่นแทนที่ผิดเพี้ยน เช่น “ตบู่” (สบู่) โดยเสียง /ต/ เพี้ยน	
3.	มีการเติมเสียงที่ผิดเพี้ยน เช่น “ขก้าว” (ข้าว) โดยเสียง /ก/ เพี้ยน (รวมทั้งการแทรกเสียงสระที่ไม่เน้นเสียง เช่น กะวาง (กวาง))	
4.	เมื่อพูดถ้อยความที่ยาวขึ้นหรือเคลื่อนไหวฐานกรณ์ซับซ้อนมากขึ้น จะมีเสียงผิดเพี้ยนหรือการใช้เสียงอื่นแทนที่ผิดเพี้ยนเพิ่มขึ้น	
ลักษณะสัทสัมพันธ์		
5.	มีการหยุดระหว่างพยางค์ในคำ (มีช่วงเงียบสั้น ๆ และ/หรือ มีการเน้นเสียงทุกพยางค์เท่ากันอย่างไม่เหมาะสม) เช่น จะ-ตุ-จักร	
6.	มีการหยุดระหว่างพยางค์ในวลีหรือประโยค (มีช่องว่างระหว่างคำ และ/หรือ มีการเน้นเสียงทุกคำเท่ากันอย่างไม่เหมาะสม)	
7.	อัตราการพูดโดยรวมช้า (ไม่รวมการหยุดเพื่อนึกคำ และ/หรือ การเรียบเรียงคำพูด)	
8.	มีการลากเสียงสระและ/หรือพยัญชนะ โดยไม่ขึ้นกับอัตราการพูดช้าโดยรวม	
อื่น ๆ		
9.	มีความพยายามในการจัดรูปปากแบบไม่มีเสียง	
10.	มีการเริ่มออกเสียงผิด หรือมีการเริ่มออกเสียงใหม่อีกครั้ง รวมถึงมีการพูดซ้ำเสียง ยกเว้น การแทรกคำตอนเริ่มประโยค (เช่น อืม คือว่า) และการใช้คำอื่นที่ใกล้เคียงมาพูดแทน (เช่น เสี้...กางเกง ช้อ..ส้อม)	
11.	**ให้คะแนนอัตราการออกเสียงซ้ำ ๆ** (เช่น ออกเสียง เพอะ เพอะ เพอะ ซ้ำเร็ว ๆ) ตามความรุนแรงของการออกเสียงผิดเพี้ยน 0 = อัตราการออกเสียงซ้ำ ๆ ปกติ 1 = ออกเสียงผิดเพี้ยนเล็กน้อย (ใน ฐาน กรณ์ และ/หรือ โฆษะ/อโฆษะ ที่ฟังออกว่าเป็นเสียงเป้าหมายได้ง่าย) และเกิดน้อยครั้ง 2 = ออกเสียงผิดเพี้ยนเล็กน้อย (ใน ฐาน กรณ์ และ/หรือ โฆษะ/อโฆษะ ที่ฟังออกว่าเป็นเสียงเป้าหมายได้ง่าย) แต่เกิดบ่อยครั้ง 3 = ออกเสียงผิดเพี้ยนปานกลาง (ผิดจากเป้าหมายมากกว่า 1 ด้านใน ฐาน กรณ์ โฆษะ/อโฆษะ) 4 = ออกเสียงผิดเพี้ยนรุนแรง (ไม่สามารถฟังเป็นเสียงเป้าหมายได้)	
12.	**ให้คะแนนอัตราการออกเสียงตามลำดับ** (เช่น ออกเสียง เพอะ เทอะ เคอะ ซ้ำเร็ว ๆ) เทียบกับอัตราการออกเสียงซ้ำ ๆ โดยให้คะแนนครั้งที่ทำได้ดีที่สุด ตามความรุนแรงของการหยุดระหว่างคำ และ/หรือ การออกเสียงผิดเพี้ยน 0 = อัตราการออกเสียงตามลำดับปกติ 1 = ช้า 2 = มีการหยุดระหว่างคำเล็กน้อย และ/หรือ ออกเสียงผิดเพี้ยนเล็กน้อย (ฟังออกว่าเป็นเสียงเป้าหมายได้ง่าย) 3 = มีการหยุดระหว่างคำปานกลาง และ/หรือ ออกเสียงผิดเพี้ยนปานกลาง 4 = มีการหยุดระหว่างคำรุนแรง และ/หรือ ออกเสียงผิดเพี้ยนรุนแรง (ไม่สามารถฟังเป็นเสียงเป้าหมายได้)	
13.	ประเมินข้อใดข้อหนึ่ง หรือ ทั้ง 2 ข้อต่อไปนี้: 1) มีจำนวนคำพูดใน 1 ช่วงลมหายใจลดลง ซึ่งสัมพันธ์กับระยะเวลาการออกเสียงสระที่นานที่สุด 2) โดยให้คะแนนจากจำนวนพยางค์ใน 1 ช่วงลมหายใจโดยเฉลี่ย 0 = มากกว่า 7 พยางค์ 1= 6-7 พยางค์ 2 = 4-5 พยางค์ 3 = 3-4 พยางค์ 4 = 2 พยางค์หรือน้อยกว่า 3) มีอัตราการออกเสียงซ้ำ ๆ ใน 1 ช่วงลมหายใจลดลง โดยไม่มีความสามารถในการหายใจที่ลดลง 4) ให้คะแนนจากจำนวนครั้งของการพูดซ้ำใน 1 ช่วงลมหายใจโดยเฉลี่ย (เช่น นับ “เพอะ” = 1 ครั้ง) 0 = มากกว่า 7 ครั้ง 1= 6-7 ครั้ง 2 = 4-5 ครั้ง 3 = 3-4 ครั้ง 4 = 2 ครั้งหรือน้อยกว่า	
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