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

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A comparison of modulation transfer function of indirect digital radiography systems

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ABSTRACT

Background: There are several parameters to characterize the quality of digital image. Resolution is one of the main parameters of an image quality. Modulation transfer function (MTF) is a quantitative measurement describes image resolution properties of an imaging system as a function of the spatial frequency. Several reports compared the spatial resolution between direct and indirect digital radiography (DR) systems proved that direct DR systems had better spatial resolution. Moreover, they also compared the different phosphor detectors of indirect DRs. However, to our knowledge, there is no report that compares the same gadolinium oxysulfide (GOS) phosphor detectors of indirect DR from different DR system manufacturers.

Objectives: To compare MTF of flat panel detectors (FPDs) indirect conversion using GOS phosphor with fixed focal spot size under radiation beam condition according to International Electrotechnical Commission (IEC) RQA5 standard.

Materials and methods: Three indirect FPDs from 3 DR manufacturers i.e. detector A, detector B and detector C were used in this study. Measurement tests for spatial resolution evaluating were performed by means of a set of 30 groups of bar patterns with different spatial frequencies which vary increasing order and express as line pairs per unit distance (lp/mm). MTF can be performed from a bar pattern within the image file by elaboration software AutoPia (Auto Phantom image analysis). Frequency at the 0.1 point of MTF was applied with this limiting spatial resolution.

Results: Three FPDs had similar MTF shape. All of MTF values from detectors were decreased with increasing spatial frequency from detector A, B, and C, respectively. This can be sorted in descending order as follows. MTF showed that detector A demonstrated both the highest contrast resolution and spatial resolution. Nevertheless, detector B and C had the same contrast resolution, yet the spatial resolution of detector B was better than that of detector C. Spatial frequency reflects the limiting spatial resolution (MTF=0.1) of detector A, B, and C, at 4.40, 4.02, and 3.77 lp/mm, respectively.

Conclusion: The bar pattern method with an automatic software analysis can be simply obtained MTF result. Test of MTF in beam quality as recommended in IEC RQA5 standard of three FPDs show that spatial resolution sorted in descending order were through detector A, B and C, respectively.

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Introduction

There are several parameters for characterizing the quality of a digital image. Resolution is one of the main parameters of image quality.[1–3] In a digital radiography, resolution consists of spatial resolution and contrast resolution.[4] Spatial resolution describes the ability of medical imaging process to discriminate small objects that are close together.[5] This depends on pixel size in that the smaller the pixel size is, the higher spatial resolution becomes. Spatial resolution is affected by several image processing factors, objects and motion blurs, focal spot size, and receptor blur. Meanwhile, contrast resolution is the ability of an imaging system to discriminate an object with small density differences and/or differentiate small attenuation variety on the image.[5] This is affected by tube collimation, number of photons, noise, scatter radiation, beam filtration, detector properties, and algorithmic reconstruction used.[4]

To evaluate the resolution, both qualitative and quantitative measurement methods can be performed. Qualitative methods are based on human observations. Visibility measurement of line pair test object is one of the methods which has been used for a long time especially in film-screen imaging. Introduction to digital imaging systems allows access to image information directly via the DICOM format and this should permit a move to more quantitative measure of image quality.[6] Modulation transfer function is a quantitative measurement that describes the image resolution properties of an imaging system as a function of the spatial frequency.[7] Considering a patient's body that consists of fine objects and coarse objects, these objects can be represented as spatial frequencies (line pairs per mm or lp/mm), where fine and coarse objects generate high and low spatial frequencies, respectively. While the high spatial frequencies represent fine detail or sharpness, the low spatial frequencies represent object contrast information. The image would contain both sharpness and contrast (intensity grayscale values). MTF is a graph of the contrast plotted as a function of spatial frequency. Maximum of MTF value at 1.0 represents a perfect transfer of spatial and contrast information. All digital imaging systems have the limiting resolution whose spatial frequency limit is obtained at an MTF value of 0.1. A system with higher spatial frequency at an MTF of 0.1 will show better spatial resolution.[8]

Measurement of MTF for various detectors demonstrates decreasing MTF with increasing spatial frequency.[7] A higher MTF value at a higher spatial frequency means the detector provides better spatial resolution. Furthermore, a higher MTF value at a lower spatial frequency means that it provides better contrast resolution. MTF is the most important parameter which plays an important role to evaluate resolution for digital radiography systems.

There are three methods i.e. bar pattern, slit pattern, and edge pattern to accomplish the MTF measurement. The slit test uses the Fourier transform of a finely sampled line spread function from a slightly angulated slit to determine MTF. While, the edge test uses the edge spread function from an opaque object with a straight, sharp, and smooth edge to determine MTF. The edge test method is widely used to measure MTF of digital X-ray detector.[1] There was a report showing that MTF obtained by edge method compared to bar pattern method was not statistically different. Although, the slit test is very high precision method, but it requires a time-consuming. Moreover, slit test and edge test require a complicated alignment. Therefore, bar pattern is a simple method that can be used to the routine quality control.[1,9,10]

The traditional field of projection X-ray imaging went through a significant transformation into a digital age during the last decade. Digital radiography (DR) has become an everyday technique in a clinical practice since the beginning of this century. In DR system, the image is obtained immediately after X-ray examination of the patient has been performed. Its popularity around the world is mainly a result of the increased availability of flat panel detectors (FPDs) on the market. There are two types of FPD which have been developed i.e. direct and indirect systems; first, a direct type FPD which converts the X-ray signal into an electrical signal by a thin film transistor (TFT) covered with Selenium (Se) in a photoconductor.[11] It is a device with high resolution,[12] and second, is an indirect type FPD phosphors. For example, one of them is Thallium activated Cesium Iodide (CsI(TI)) on TFT which converts an X-ray signal into a light signal and converts it into electrical signal later. Generally, the majority of indirect FPDs available on the market employ an x-ray converter made of Gadolinium Oxysulfide (GOS) phosphor. Several reports through these DR system devices compared the spatial resolution between direct and indirect DR systems and proved that direct DR systems have better spatial resolution.[1-3,13,14] Moreover, they also compared the different phosphor detectors of indirect DRs.[12] However, there has never been any report that compares the same GOS phosphor detector of indirect DR from different DR system manufacturers. This gives rise to this research study with a specific purpose as follows.

Purpose of this research study is to compare the MTF of flat panel detectors indirect conversion using GOS phosphor with Leeds test objects medical imaging phantoms (TOR CDR).[15] The images are acquired from three detectors with the same X ray generator with a fixed focal spot size because the focal spot size also affects spatial resolution.[9] The radiation beam condition is operated according to IEC RQA5 standard because energy of X ray affects to spatial resolution as well.[9] The MTF were analyzed by software (AutoPIA from Leeds University) from bar pattern images.[16]

Materials and methods

1. Digital radiography detectors studies

Three indirect flat panel detectors (FPD) i.e. detector A, detector B, and detector C from 3 DR manufacture were used in this study. All of them are indirect conversion units that used GOS phosphor boned to a light-sensitive thin film transistor (TFT) array formed from amorphous silicon (a-Si). Basic technical parameters for the systems are shown in the table 1.

Table 1 Basic parameters for each FPD from 3 DR system manufacturers.

FPD	Image area (cm x cm)	Matrix size	Pixel size (mm x mm)	Bit depth (bits)
A	35x43	2304x2880	0.15x0.15	12
B	35x43	2560x3072	0.14x0.14	14
C	35x43	2466x3040	0.14x0.14	14

2. Test equipment

The device used for acquiring an image in this study is TOR CDR phantom from Leeds University. Measurement tests for spatial resolution evaluating were performed by means of a set of 30 groups of bar patterns with different spatial frequencies which vary an increasing order and express as line pairs per unit distance (lp/mm).[16] Spatial frequency values for all bar pattern are shown in Table 2.[17]

Table 2 Spatial frequency values for bar patterns of TOR CDR.

Group number	Spatial frequency (lp/mm)	Group number	Spatial frequency (lp/mm)
1	0.50	16	2.80
2	0.56	17	3.15
3	0.63	18	3.55
4	0.71	19	4.00
5	0.80	20	4.50
6	0.90	21	5.00
7	1.00	22	5.60
8	1.12	23	6.30
9	1.25	24	7.10
10	1.40	25	8.00
11	1.60	26	8.90
12	1.80	27	10.00
13	2.00	28	11.10
14	2.24	29	12.50
15	2.50	30	14.30

After TOR CDR phantom was imaged, recorded with DICOM file, and opened with elaboration software AutoPia (Auto Phantom image analysis),[16] the MTF could be performed from bar pattern with different spatial frequencies that varied in increasing order and expressed as line pairs per unit of distance (lp/mm) of distinguishing structure of different sizes according to Droege and Morin.[18] Then, MTF was plotted automatically. Details of characteristics of image and detector can be retrieved from DICOM header for analyzing and reporting. An output data can be saved as CSV file for analyzing later. A radiograph of bar line pair pattern from TOR CDR phantom and MTF analysis result with the frequency at MTF of 0.1 to determine the limiting spatial resolution[19] as shown in Figure 1.

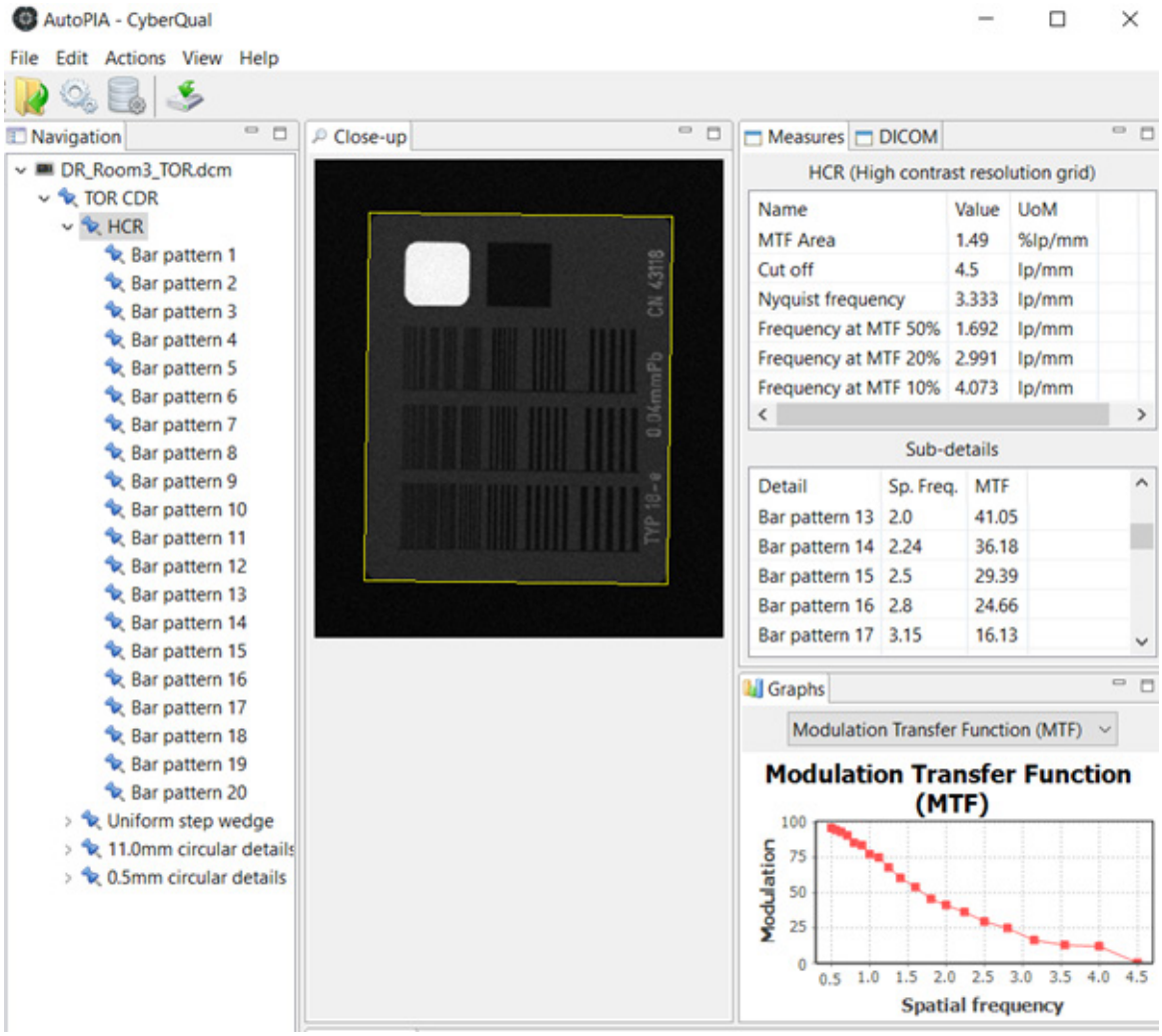

***Figure 1**. A radiograph of bar line pair pattern from TOR CDR phantom and MTF analysis result.*

3. Beam condition

The X ray beam condition is set up according to the IEC RQA5 standard which requires a 21 mm aluminum filter block in the beam. The IEC guidelines require two beam-limiting Pb apertures for MTF measurement. Based on those guidelines 2 mm thick, Pb sheets were used to construct a 5x5 cm^2 and 16x16 cm^2 apertures. The 5x5 cm^2 and 16x16 cm^2 apertures were placed along the beam axis at 39 cm from the focal spot and 12 cm from detector, respectively as in Figure 2.[20] With test phantom and collimators in place, a half value layer (HVL) of 6.88 mmAl was achieved at 70 kVp[21] and exposure measurement for HVL calculation were made by Piranha X ray testing device (RTI R 100 dose detector).

As the focal spot size affects to spatial resolution, X ray generated from mobile unit is used to fix a nominal focal spot size at 0.60 mm for all test images in this study. The most interesting range for characterizing detectors for a digital radiography with about 1 µGy to upper limit of around 10 µGy.[22] In this study the technique was fixed at 70 kVp 20 mAs that provided 10 µGy.

4. Image acquisition

TOR CDR phantom was placed in contact with the detector cover, aligned with the central axis and perpendicular to cathode and anode of the X ray tube. All images were acquired using a 180 cm source-to-detector. The images were acquired 3 times for each detector with an average data to calculate MTF. All images used in this work were acquired with non-processing.

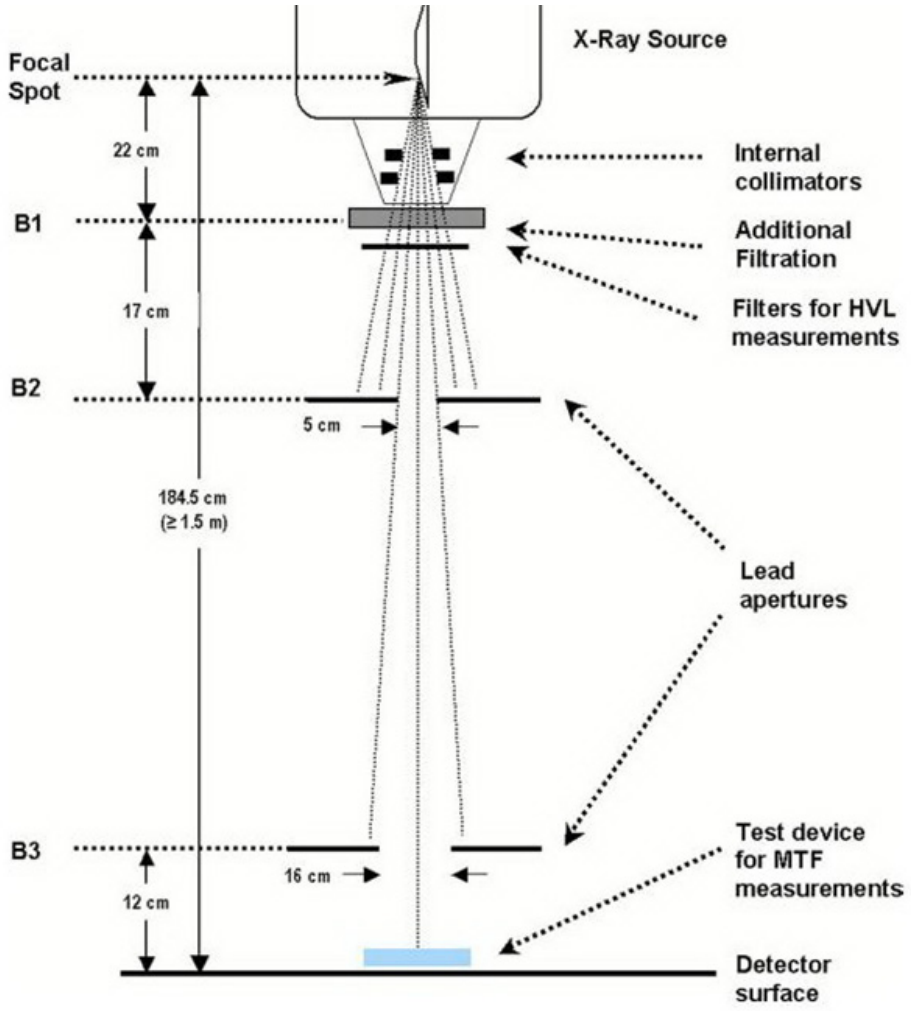

Figure 2. *X ray beam condition according to the IEC RQA5 standard.*

Results

The result shows that 3 FPDs have similar MTF shapes. MTF values from all of detectors decrease with increasing of spatial frequency as shown in figure 3. This can be sorted in descending order from A, B, to C. The MTF value of detector A at frequency 0.5 lp/mm is 0.951 while detector B and C are 0.895 and 0.891, respectively, as shown in table 3. This means that detector A is the highest contrast resolution. Although detector B and C have the similarly high contrast resolution, detector B has a better contrast resolution than detector C. The spatial frequencies of detector A, B, and C at 0.8, 0.5, 0.2, and 0.1 points of MTF had the same tendency as shown in table 4. The spatial frequency reflects limiting spatial resolution (MTF=0.1) of detector A, B, and C, at 4.40, 4.02, and 3.77 lp/mm, respectively. These confirmed that detector A has both the highest contrast resolution and spatial resolution.

Discussion

All detectors in this study were Indirect DR with the same GOS phosphor. In general, DR has better contrast resolution than conventional radiography and CR because of more dynamic range. Normally contrast resolution also depends on gray scale bit depth. Detector with high bit depth obtains high contrast resolution. However, the result of this study detector A (bit depth 12) obtained contrast resolution higher than the others (bit depth 14) as shown in table 3. There may be some factors can be improved contrast resolution. Moreover, the contrast resolution of detector can be considered from MTF curve in the low spatial frequency zone (0 to 1.5 lp/mm) as shown in Table 3 and Figure 3.

Another result showed that detector A (pixel size 0.15 mm) obtained better spatial resolution than those of the others (pixel size 0.14 mm) as shown in Table 4. As we know spatial resolution depends on pixel size of the detector especially in CR, but in DR with smaller pixel size do not necessarily obtains better spatial resolution because other factors such as light scatter within the detector contribute to degradation of spatial resolution.[11]

As a problem of indirect DR comes from spreading of light on surface of the phosphor leading to a gradation of spatial resolution,[5] this problem has been solved by performing a new scintillator design from an X ray penetration-side photo detection (PS) to an X ray incident-side photo detection (IS) by some DR system manufactures. Benefit of this configuration is a reduction of light attenuation and blurring effect. The use of IS system is applied to improve spatial

resolution. The use of IS system is also applied to improve contrast resolution by increase thickness of scintillator layer thus increase number photons of light and improving to increase sensitivity of the system.[12,23]

Thus, result from detector A which obtained much more contrast and spatial resolution can be from the same reason. Therefore, MTF can be a useful tool for comparing image quality of different radiographic systems. More importantly, the method of bar pattern phantom with automatic software has various advantages. It is simple to evaluate MTF which is useful for day to day job of applying by personnel in the health care services. The achievement of measurements in accordance to the most common standards among staff in radiology department can be developed as a new simple skill. As a result, it is important to select the best of medical imaging systems and perform the test to maintain image qualities which this newly discovered test can be achieved periodically in very short time for monitoring a system and ensuring the image quality.

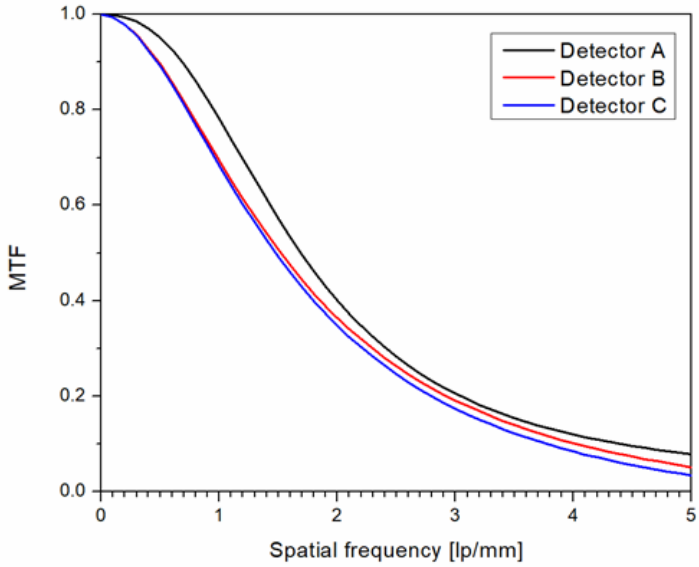

***Figure 3**. MTF values from all of detectors.*

Table 3 MTF from the three FPDs at different spatial frequency.

Spatial frequency (lp/mm)	MTF		
	Detector A	Detector B	Detector C
0.5	0.951	0.895	0.891
1.0	0.782	0.694	0.684
1.5	0.572	0.506	0.493

Table 4 Spatial frequency from the three FPDs at different values of MTF.

FPD	Spatial frequency (lp/mm)			
	MTF at 0.8	MTF at 0.5	MTF at 0.2	MTF at 0.1
A	0.96	1.69	3.04	4.40
B	0.74	1.52	2.93	4.02
C	0.73	1.48	2.80	3.77

Conclusion

This research paper confirms that MTF measurement can be simply obtained through the method of using bar pattern with automatic software analysis. To test MTF in beam quality as recommended in IEC RQA5 standard of three FPDs proves the spatial resolution in descending orders range from detector A, B to C respectively.

Conflicts of interests

The authors declare that they have no conflict of interest.

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In-house development of automatic iontophoresis current source for sweat chloride test

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ABSTRACT

Background: The standard method for cystic fibrosis screening is pilocarpine iontophoresis sweat chloride test. The iontophoresis device must supply reliable and accurate electrical current within the safety range. The researchers determined that an in-house development of reliable automatic iontophoresis current source for sweat chloride test would have an impact to cost and popularity of the test in Thailand. Furthermore, it will promote the development of a technological self-reliance in the future.

Objectives: Automatic iontophoresis current source was designed and fabricated to conform to IEC 479-1 electrical safety standard and CLSI C34-A3 Sweat Chloride Testing Standard. Performance of the device was tested by performing sweat chloride test in healthy volunteers.

Materials and methods: Design and develop automatic iontophoresis current source for sweat chloride test with electrical safety standards IEC 479-1 and conform to CLSI C34-A3 standard. The developed device will be tested by performing classic sweat chloride test (CST) in healthy adult volunteers.

Results: Seventy healthy volunteers (age between 18 to 40 years old) were recruited. No history or signs of illness or being treated for diseases including the lungs, liver, pancreas, and intestines were found. 27 males and 43 females are compared between control and test with pilocarpine iontophoresis using stimulated direct current of 1.0 mA for 4 minute (0.07 mA/cm^2) and 30 minutes for sweat collection. The electrical current used was far lower than recommendation by CLSI C34-A3 standard and other known studies, but still can give out weight of sweat (more than 0.077 gram) as recommended by CLSI C34-A3 standard. Mean weight of collected sweat in male volunteers are 0.35±0.10 gram and 0.17±0.08 gram in female volunteers. And there are significant differences in chloride concentration obtained from male and female volunteers that correlated with previous studies.

Conclusion: The in-house development of automatic iontophoresis current source device for sweat chloride test was successful and successfully used in the sweat chloride test in normal healthy adult volunteers. Sweat can be stimulated effectively with low iontophoresis current in normal subject. Moreover, compare with CLSI-C34-A3 recommendation, the device can stimulate sweat production far more than minimum sample weight of 0.077 gram with lower electric current and shorter duration of stimulated time.

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Introduction

Mutation of cystic fibrosis trans-membrane conductance regulator (CFTR) gene on chromosome 7 results in autosomal recessive disease-Cystic Fibrosis (CF). Ion transport abnormalities in various tissues especially in epithelium of lungs and pancreas are the mainstay of clinical manifestations.[1, 2] Levels of chloride in sweat of CF patient was also abnormally elevated.[3] Subsequently, classic sweat chloride test (CST) that utilized pilocarpine as a cholinergic stimulant of sweat gland on the skin via iontophoresis was also published.[4] Sweat chloride concentration in normal individuals are below 30 mmol/L but are higher in CF.[5] At present, CFTR sequencing can be performed as reliable diagnostic method in CF but are expensive, so sweat chloride test is still in use and considered the gold standard in screening and diagnosis of CF.

It is long believed that CF is rare in Thai population. But in recent years, there are many reports of CF in Thais.[6-9] The actual incidence of CF in Thailand is still unknown. Sweat chloride test might be the appropriate method for screening test for CF in Thailand because of lower expense and being relatively less complicate to perform.

In Gibson and Cooke CST, pilocarpine is used for sweat stimulation from sweat glands of forearm. Pilocarpine is a parasympathomimetic alkaloid mimicking acetylcholine to cholinergic receptors that result in stimulation of muscarinic receptors of eccrine sweat gland to produce sweat. Small amount of electric currents is used to drive pilocarpine through skin to eccrine sweat gland. The process of electromotive repulsion of substances is called iontophoresis. Collected sweat is analyzed for chloride concentration. In CST, iontophoresis device consists of batteries connected in series with potentiometer to manually set electric current. Ampere meter is used to monitor the electric current. This device is simple and not expensive to duplicate but electric current is not constant. The electric current depends on multiple factors such as decay of battery, potentiometer setting, electrodes placement, electrolytes in use and skin electrical resistance. Burn and blister at the sites of electrodes placement usually occur if inappropriate overcurrent setting or prolonged stimulation time are used.[10]

The aim of this project was to design and construct an automatic constant current iontophoresis device from locally available parts that can easily be duplicated. With this device, standardized CST can be performed because iontophoresis current and stimulation time can be set and automatically regulated.

Materials and methods

Iontophoresis voltage and current source

Versatile high precision voltage controlled programmable current source using DACs, Op Amps, and MOSFET transistors as described in Analog Devices Circuit Note CN-0151 was used as a guide in designing of constant current source (Figure 1).[11] MCP4921, 12-Bit Voltage Output Digital-to-Analog Converter[12] was used to provide controlled voltage to current source. Arduino Uno R3 microcontroller board was used to control the selected current and timing as well as all the safety monitoring. Working iontophoresis current supply circuit is shown in Figure 2. Six 1.5 volts alkaline cells were used to provide 9 volts power supply to the device. XL6009 buck and boost regulator integrate circuit[13] was used to boost 9 volts supply voltage to 16-22.5 volts which is required to generate adequate iontophoresis current. The principle circuit of XL6009 in used is shown in Figure 3. The 33uH inductor in the circuit was modified to 80uH to lower the idle current in order to preserve battery life and limit the iontophoresis current to below 10 mA. The highest skin resistance is around 6000 ohms while determined by electrodes 2 cm in diameter and placed 2 cm apart using alternating current of 1000 Hz in frequency.[14] The highest allowed skin resistance during iontophoresis with our device is set at 5000 ohms while the highest iontophoresis current is set at 4 mA. In this case, the required iontophoresis voltage would be 20 volts ($4x10^{-3}$ x 5000=20). The device will first check skin resistance with small amount of electric current, if skin resistance is more than 5000 ohms, alarm will be activated and the procedure will be aborted. If the required iontophoresis current is lower than 4 mA, then the voltage required will be lower. We plan to use low iontophoresis current of not more than 1.5 mA as in use in pilocarpine gel discs (Pilogel®).[15]

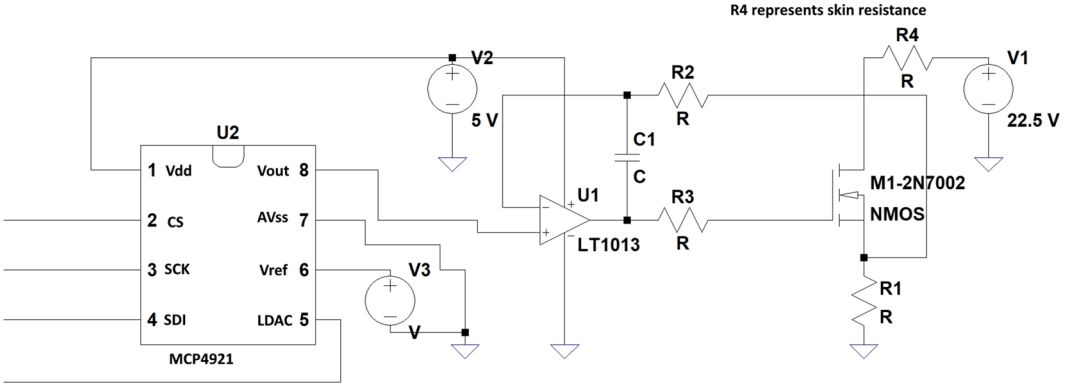

Figure 1. *Microcontroller controlled constant current source.*

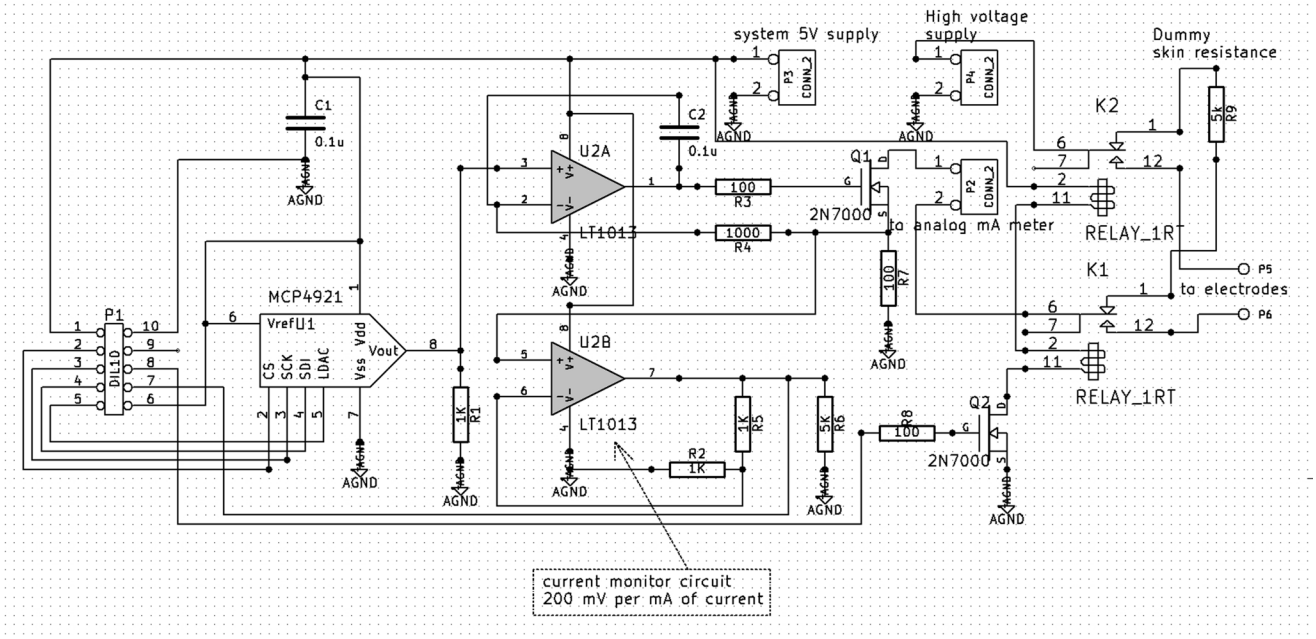

***Figure 2**. Working iontophoresis current supply circuit.*

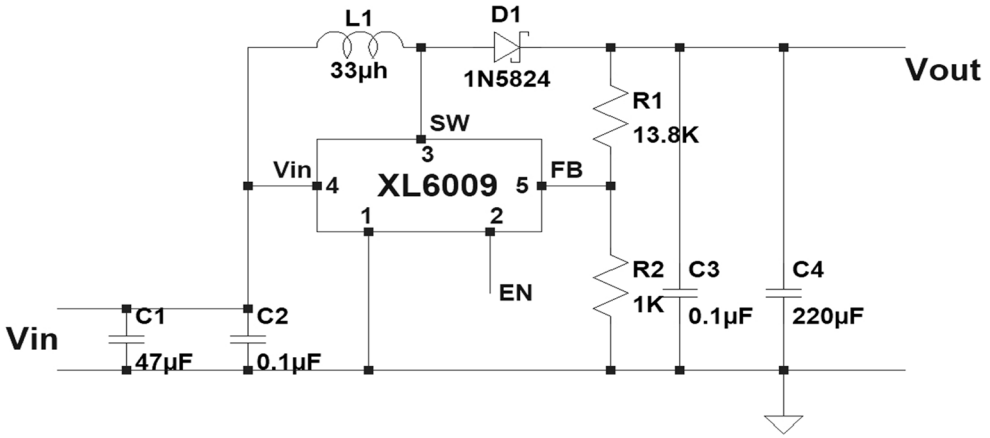

***Figure 3**. XL6009 typical application circuit (Boost converter).*

Electrodes Fabrication

Electrodes are made of 0.28 mm thick 5.1x5.1 cm square copper plate with exposed surface area of 3.75x3.75 cm as described and recommended in CLSI guideline. If iontophoresis current of 4 mA is used, current density would be 4/(3.75x3.75) = 0.28 mA/cm^2. Effective current density can be as low as 0.14-0.21 mA/cm^2. [16]

Controller Circuit

Arduino Uno R3 microcontroller board is used to control this automatic iontophoresis device. Arduino Uno board is well known in DIY hobby and very easy to program using C-style language.

Preliminary Evaluation of the Device

In order to test the performance of the iontophoresis device, a simple circuit of variable resistance was made from 0-5000 ohms potentiometer to simulate skin resistance and electrodes circuit. After the iontophoresis current was set, potentiometer was connected instead of electrodes circuit and the resistance was varied to simulate varying skin resistance while current was monitor. The current provided by iontophoresis device should be constant.

Sweat chloride test

After the project was approved by institutional ethic committee, the device was assembled and 70 healthy adult volunteers (age between 18 to 40 years old) were recruited. 27 males and 43 females were compared between control and test with pilocarpine iontophoresis. The mean (SD) average age of male and female were 29 (6), 31 (6) respectively (p=0.64). No history or signs of illness or being treated for diseases including the lungs, liver, pancreas, and intestines were found. CST was performed; sweat was collected and analyzed according to CLSI recommendation. The 1.0 mA iontophoresis current with duration of 4 minutes and 30 minutes for sweat collection was used. In each volunteer, skin of each forearm was prepared by cleaned the test area with 70 % Ethyl alcohol and Distilled water, then wipe dry

with a gauze. Electrodes with pilocarpine solution were attached according to CLSI standard while iontophoresis current was applied only to right arm site as test site. The left arm site was used as control site (Figure 4). For the test site, Gauze soak with 2% Pilocarpine at the Positive electrode place on lower arm area. And gauze soak with 0.05 mol/L $MgSO_4$ at the Negative electrode place on upper arm area. Then switch on the iontophoresis current source device at 1.0 mA for 4 minutes. After that, the electrodes were taken off and the areas were cleaned with distilled water. The areas were then wiped dry with dry gauze. Pre-weight 5.1 cm x 5.1 cm filter paper (Whatman® No.1, Whatman International Ltd, Maidstone England) were placed at the areas of positive electrodes in the tested site and also at the controlled site for 30 minutes (Figure 5.) After that, 4 digits weighing scale was used to determine the weight of the filter papers so that the amount of stimulated sweat were recorded for analysis.

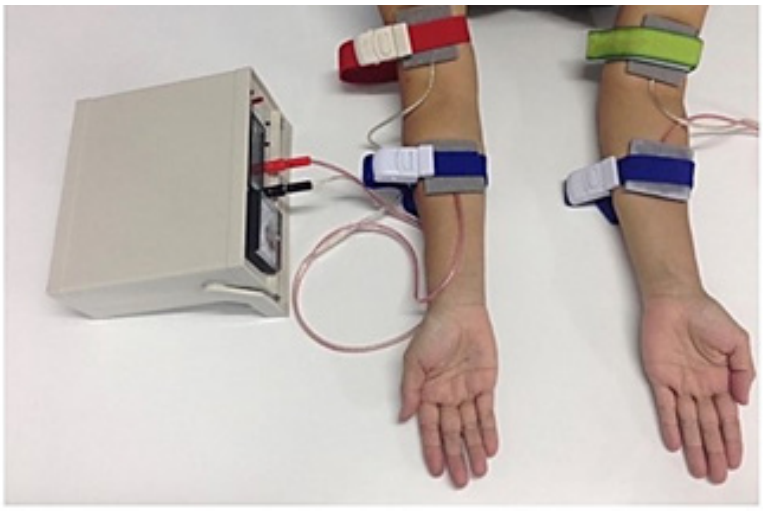

Figure 4. *Sweat stimulation with pilocarpine iontophoresis. The iontophoresis current source device is seen on the left of the picture. Positive electrodes with gauze soak with 2% pilocarpine were placed on the lower arms. Negative electrodes with gauze soak with 0.05 mol/L $MgSO_4$ were placed on the upper arms. Right arm was the tested site (near the iontophoresis device) while the control site was in the left arm.*

Figure 5. *Sweat collection. Filter paper was placed at the same area of positive electrode for 30 minutes.*

Sweat chloride test evaluation

After collected and weighted the filter papers from tested and controlled sites, 8 ml of distilled water were added and leave at room temperature for 40 minutes. Standard, control and test samples were prepared as described in table1. Then 0.002N Mercuric Nitrate was used in titration process as described in CLSI-C34-A3 guideline. The chloride level reference range for cystic fibrosis diagnostic were shown in table2.

Table 1 Preparation of Standard chloride concentration tube, Control tube and Test tube for CLSI- C34-A3 chloride determination using titration method.

	Standard Tube	Control tube	Test tube
Standard saline 10 mmol/L	0.5 mL	-	-
sample	-	2 mL	2 mL
3% Nitric acid	1 drop	1 drop	1 drop
Diphenyl carbazone	2 drops	2 drops	2 drops

Table 2 Chloride level reference range for cystic fibrosis diagnosis. (CLSI C34-A3 standard-sweat testing: sample collection and quantitative chloride analysis).

	Infant – 6 months, Cl- level	>6 months, Cl^- level
Non- cystic fibrosis	≤29 mmol/L	≤39 mmol/L
Intermediate*	30-59 mmol/L	40 – 59 mmol/L
cystic fibrosis	≥60 mmol/L	≥60 mmol/L

**Intermediate mean that Cystic fibrosis is possible. Should be repeated and confirmed with other method.*

Results

The iontophoresis device was constructed and can operate within the required specification as shown in table 3. To test the stability of the iontophoresis current, a 0-5000 ohms potentiometer was used to simulate electrodes circuit and skin resistance. After the iontophoresis current was set, potentiometer was varied to simulate varying skin resistance while current was monitored. Resistor of 200 ohms was used as serial current sensor. If current was set to 1 mA, 200 ohms resistor would provide 200 mV across its terminals. The potentiometer would provide 0 to 5 volts to represent its resistance. Despite the intense variation in simulated skin resistance, the current provided by iontophoresis device appeared to be highly constant as shown in Figure 6. Then iontophoresis device was used to perform CST in the volunteers. The test site significantly produced more sweat than control site. The sweat obtained from the test site are all farer than minimum sample weight of 0.077 gram recommended by CLSI-C34-A3 standard in every volunteer subjects. But there are significant differences in sweat weight and chloride concentration obtained from male and female volunteers (Table 4). The results were conformed to previous investigations in the literatures that male can produced more amount of sweat and chloride concentration than female subjects.[17-20] There was no blister and burn at the electrode sites in all subjects.

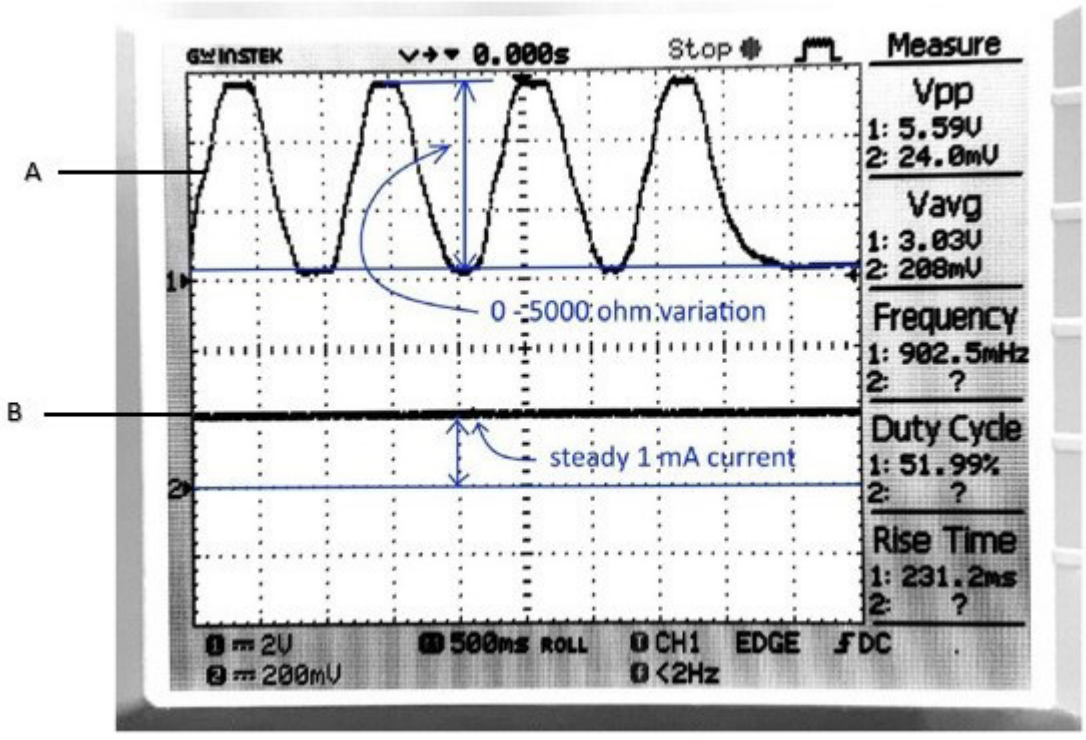

***Figure 6**. Constant iontophoresis current versus varied simulated skin resistance recorded with GW Instek GDS-1102A digital storage oscilloscope Good Will Instrument CO., LTD. Taipei, Taiwan. (A) which is seen very constant despite extensive varying of skin resistance from 0 to 5000 ohms. (B) represent set current (1 mA).*

Table 3 Specification of iontophoresis Device.

Power supply:	9 volts DC form 6 AA Alkaline cells
Electrode surface area:	3.75 cm x 3.75 cm.
Iontophoresis current:	0.5-3.0 mA in step of 0.5 mA
Iontophoresis current characteristic:	Continuous direct current
Timer:	0.5-5 minutes in step of 0.5 minutes
Skin-electrodes direct current resistance:	400-5000 ohms
Error detection:	Battery voltage below 7.8 volts: "LOW Batt Pls Replace Bat" Battery voltage below 7 volts or higher than 9.5 volts: "Error" Working voltage: below 4.7 volts or higher than 5.6 volts: "Error" Iontophoresis voltage below 12.0 volts or more than 16.5 volts: "Error" If any "Error" occurs, system will shut down.

Table 4 Age, Sweat weight and chloride concentration obtained from male and female volunteers.

	GENDER	N	MEAN±SD	*p* value
AGE (year)	Male	27	29±6	0.64
	Female	43	31±6	
SWEAT WEIGHT (gm)	Male	27	0.35±0.10	0.01*
	Female	43	0.17±0.08	
CHLORIDE (mmol/L)	Male	27	23.42±9.22	0.01*
	Female	43	14.90±6.51	

Discussion

The automatic iontophoresis device can be built using locally available parts to perform within required specification. While 0.28mA/cm^2 current density with 3.75 cm x3.75 cm exposed surface area is recommended by CLSI guideline, the developed iontophoresis device can induce sweat with only 1.0 mA current using the same stimulating 4 minutes time and 30 minutes for sweat collection. The current density is only 0.07mA/cm^2, which is far below the CLSI guideline recommendation. No blister or burn at electrode sites were found.

Conclusion

The automatic iontophoresis device can be built to perform within required specifications. While current can be set to 1 mA or higher with this device, sweat can be stimulated effectively with low iontophoresis current in normal subject. But the performance of the device in CF patient or in pediatric age group is yet to be determined.

Acknowledgements

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Gender difference on myelin content in healthy young adult brain: a quantitative magnetic resonance imaging study at 1.5 Tesla

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ABSTRACT

Background: Diffusion tensor imaging (DTI) of cerebral white matter integrity in healthy young adult is not well studied due to anisotropy variation in brain regions with complex fiber architecture. Investigation how such variation of gender differences may offer a discernible clinical benefit.

Objectives: To evaluate gender difference on white matter tissue properties in healthy young adult brain using T1 weighted and high-resolution DTI at 1.5 Tesla MRI.

Materials and methods: Twenty healthy volunteers (10 men, 10 women, age 20-24 years) underwent DTI for quantification of apparent diffusion coefficient (ADC) and fractional anisotropy (FA) values. A T1 weighted sequence was chosen to provide anatomical reference while diffusion-weighted sequence was chosen to provide DTI. Visualization of white matter fiber tracts were obtained with FiberTrak software of the manufacturer. Selected tracts were constructed along corpus callosum, cingulum-cingulate gyrus, and corticospinal tract. To determine water diffusion in certain regions, ADC and FA values were measured. Finally, the two-sample t-test was performed to evaluate the difference between genders and p-values below 0.05 were considered statistically significant.

Results: The ADC values of men ranged from 0.675 to 0.926 mm^2s^{-1} while these values ranged from 0.671 to 0.918 mm^2s^{-1} for women. In thalamus, a significant ADC difference was found (t=2.781, $p<0.05$) and otherwise no significant ADC differences were seen. For men, the FA values ranged from 0.175 to 0.832 while these values ranged from 0.163 to 0.845 for women. The highest FA value was found in corpus callosum. Two-sample t-test showed no significant FA differences between genders ($p>0.05$).

Conclusion: There was no significant FA difference between men and women's brains while the mean ADC values in thalamus was statistically significant different. However, there is still no clear correlation of ADC and FA values regarding the gender differences on white matter integrity.

Introduction

Human white matter is mainly made up of myelinated axons (also called "tracts") that coated with a fatty layer of myelin. The white matter is white in color due to the lipid content of myelin. White matter tract can be classified into three categories, namely projection fiber, association fiber, and commissural fiber.[1-3] Projection fiber provides tracts connecting the cerebral cortex with other area in the central nervous system. The association fiber connects the two halves of the cerebrum (also known as cortex-cortex connection). The commissural fiber connects the same cortical areas in the right and left hemispheres, enabling the two sides of the brain to communicate. Distinguishes brain tissues based on the freedom of water diffusion provide an important insight.

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Magnetic resonance imaging (MRI) allows the investigation of brain structures and their changes in relation to disease progression. Changes in brain myelination have shown clinically relevant related to degenerative diseases of aging.[4-6] Neither cognitive decline nor weak learning algorithm are affected by myelination breakdown.[7,8] Changes in myelin have been linked to various neurodegenerative conditions which are known to greatly impact of progression of symptoms in the cerebral hemispheres.[9-11] It means that detection of white matter damage plays a key role in screening at early stage before diseases present. Factors found to be influencing white matter fiber have been explored in several studies. Few studies have shown that cognitive decline in normal aging caused by low myelinated fiber density and volume in brain white matter.[12,13] Through these changes, advanced MRI can detect. Techniques such as diffusion tensor imaging (DTI) is useful in probing detailed of white matter fiber tracts in human brain based on the mobility of water molecules and voxel-wise map along the microstructural properties. The presence of fiber orientations obtained from DTI depends on strength of diffusion and degree of anisotropy. In 1994, Basser and Mattiello demonstrated diffusion tensor model using shape and orientation of anisotropic diffusion.[14]

Diffusion in white matter is restricted anisotropy. To measure water diffusion in the underlying tissue, fractional anisotropy (FA) is used to generate quantitative brain maps. FA value provides the degree of anisotropy as a quantitative biomarker of white matter integrity. Greater organization of white matter indicated by significantly increased FA value. These include maturation of physical characteristics and circulating hormone levels that happens during puberty. Many studies have been used to demonstrate the structure-function relationship of white matter and how it changes. In normal brain, tract-specific white matter is positively correlated with intelligence.[15] Reading ability has been correlated with the arcuate fasciculus (the temporo-parietal white matter connects Broca's area to Wernicke's areas) which is responsible for language production.[16,17] For the visual pathways, an increase of anisotropy in white matter fiber tracts has been correlated with improved reaction time. Interestingly, these tracts are not well-organized in the blind.[18] A study of patient with early Parkinson disease found that the anisotropy in substantia nigra were decreased.[19] Similarly, increased diffusivity and decreased anisotropy also found in the Alzheimer disease and those with Lewy body dementia.[20,21] DTI continues to be used in psychiatric disease, for example, the presence of auditory false perception has been correlated with decreased anisotropy, as well as patients with depression and bipolar disorders.[22,23]

However, diffusion tensor imaging of cerebral white matter integrity in healthy young adult is not well studied due to anisotropy variation in brain regions with complex fiber architecture. Investigation how such variation of gender differences may offer a discernible clinical benefit. The aim of this study was to evaluate gender difference on white matter tissue properties in healthy young adult brain using T1 weighted and high-resolution diffusion MRI at 1.5 Tesla scanner. We hypothesized that optimized T1 weighted MRI and high-resolution diffusion MRI would allow visualization of gender-related white matter architecture in vivo. To test our hypothesis, a preliminary study of healthy young adult was imaged at 1.5 Tesla MRI scanner. Analyzed sub-region of the brain together with streamline tracking (color-coding of fiber) were performed to explore normative values of DTI matric and degree of water diffusion underlying tissues in different brain regions. Knowledge of white matter architecture based on diffusion contrast such as changes in diffusivity, anisotropy, and fiber orientation may help to explain directional dependence through geometry visualization between men and women's brains.

Materials and methods

Subjects

We examined 20 healthy young adults (10 men and 10 women between 20 and 24 years of age). All participants gave informed consent approved by the ethical review committee at Naresuan University (IRB No. 0919/62). After MRI exams, all participants were interviewed and recorded experience of adverse effects.

MR imaging

All scans were acquired on the Philips Ingenia Prodiva 1.5T CS MRI scanner (software version 5.4.1.0) at King Naresuan Camp Hospital with pulse oximeter gating. The gradient coils were achieved a maximum gradient strength of 33 mT/m and 120 T/m/s maximum slew rate for each axis. A standard quadrature transmits receive head coil was used for both radiofrequency transmission and reception of signals. Figure 1 shows sequential acquisition of T1W and DWI images of white matter tissue in the brain. Survey scans were aligned to the AC-PC line. The MRI protocol consisted of a T1 weighted sequence for anatomical reference (sagittal orientation, isotropic resolution 1x1x1 mm^3), a three-dimensional turbo field echo (turbo factor 232, 3D-TFE) that delivers a faster scan and improved grey white matter contrast in the brain. The optimized sequence parameters are as follows: TR/TE = 7.2/3.3; TFE shots = 130; TFE dur. shot/acq (ms) = 1714.9/1674.8; min. TI delay = 870.9; SENSE (P reduction: AP/ S reduction: RL) = 1/2; Flip angle (deg) = 8; NSA = 1; scan time = 6.30 minutes. The diffusion-weighted MR sequence was a single shot, spin echo, echo planar imaging (SS-SE-EPI) with diffusion gradients in three orthogonal directions (b-value = 0, 800 s/mm^2); 32 diffusion-encoded images; TR/TE = 6730/65; EPI factor = 45; SENSE (P reduction: AP) = 2; half-scan = 0.6; Fat suppression = SPAIR; Flip angle (deg) = 90; NSA = 2; scan time = 7.30 minutes. A 24-cm square field of view with a 256x256 matrix size was used for TFE sequence and a 128x128 matrix size for EPI sequence. A typical approach we used to avoid susceptibility-induced distortion and eddy current are acquiring field maps for every diffusion scan. The inherent field map was calculated from the total phase accumulated at different echo times and carried out to derive artifact-free data in each DWI. Finally, the field map was added back to obtain dynamic and inherent field maps for correction of individual DWIs. We take an advantage of the modified reconstruction software implementing per-echo phase correction provided

by Philips healthcare incorporates for distortion-free images.[24,25] Each MR examination lasted about 15 minutes.

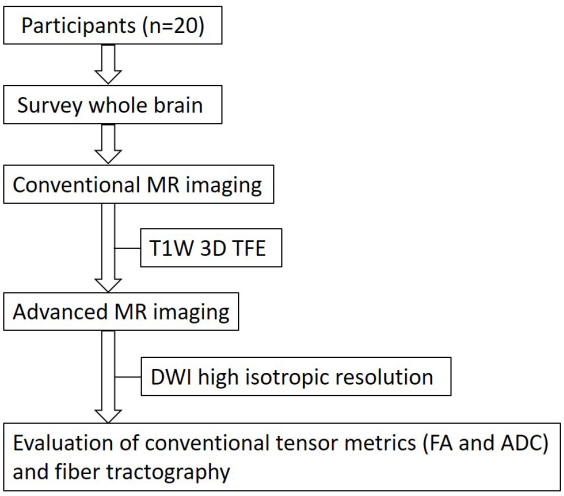

Figure 1. *Graphical representation of the research methodology.*

Regions of interest (ROIs)

Visualization of white matter fiber tracts based on diffusion tensor was obtained with FiberTrak software of the manufacturer. The DTI tensor implemented on the MR console screen. Selected tracts were constructed and analyzed along three major white matter pathways: corpus callosum (CC), cingulum-cingulate gyrus (CCG) and corticospinal tract (CST). To determine water molecular mobility in tissues, ADC and FA values were then measured for each subject in certain brain regions.

DTI contains information about the motion of water in 32 directions, describing water motion in 32 directions in more details. FA value reflects the directionality of water molecule by diffusion ranging from 0 and 1 (0 represents isotropic diffusion, 1 represents infinite anisotropic diffusion) while ADC value reflects the measurement of the free random diffusion of water molecules in tissues. Eight ROIs used for measuring ADC and FA values were chosen to determine water molecular mobility in tissues. For each ROI, all values were averaged over both left and right hemispheres to yield a mean value for each subject. The ROIs were carefully selected to avoid partial volume effect and drawn manually on the image (shaped like a square as shown in Figure 2). After processing all of the values, two-sample t-test was performed to evaluate the difference between two groups. Correlation coefficient was used to examine the effect of gender on the relationship of ADC to FA values. The level of significance was set at $p<0.05$.

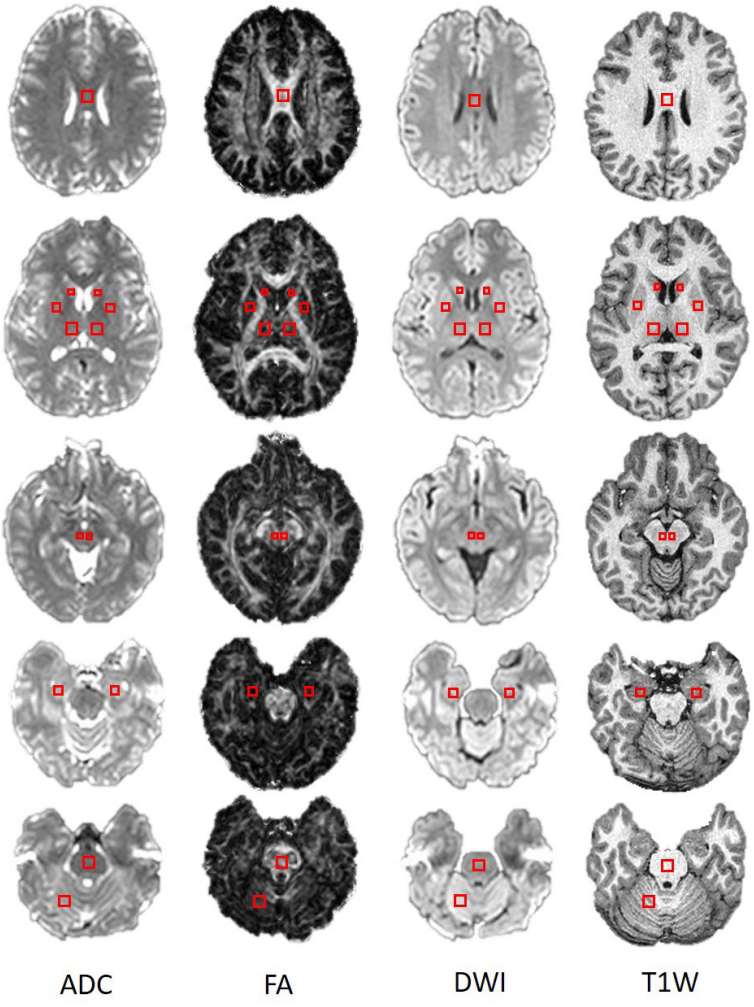

Figure 2. *Eight ROIs used for measuring ADC and FA values (named top to bottom: corpus callosum, caudate nucleus, putamen, thalamus, red nucleus, hippocampus, pons and cerebellum).*

Results

Twenty normal young adult brain (10 men and 10 women; age range from 20 to 24 with a mean of 22 years) were scanned with three-orthogonal diffusion gradient directional DWI and a 32-direction DTI. Figure 3 shows typical diffusion tensor imaging images with b-value of 0 and 800 s/mm^2 and fiber tractography of corpus callosum in pink. The image of cingulum-cingulate gyrus was depicted in yellow and the corticospinal tract was in blue. The ADC and FA values of eight ROIs measured in healthy young adult brain are given in Figure 4. The ADC values of men ranged from 0.675 to 0.926 mm^{2}s^{-1} while these values ranged from 0.671 to 0.918 mm^{2}s^{-1} for women. In thalamus, a statistically significant difference was found between gender on ADC values at 1.5 T MRI (t = 2.781, p<0.05) and otherwise no significant ADC differences were seen in the subjects. If we now turn to the FA values, in men's brains ranged from 0.175 to 0.832 while these values ranged from 0.163 to 0.845 in women. Interestingly, the highest FA value was found in corpus callosum. Two-sample t-test showed that no significant FA values differences were seen between men and women (p>0.05). We found variable degrees of correlation on the measure of ADC and FA values. As we can see in Table 1, the correlation coefficient of ADC values between genders in various brain regions ranged from -0.420 to 0.351. Among subjects, the ADC values had a weak positive correlation in corpus callosum, caudate nucleus, putamen, and otherwise showed a weak negative correlation. By contrast, a negative correlation of FA values existed in caudate nucleus, thalamus, pons, and the others had a positive correlation. The effect of gender differences on the relationship of FA values ranged from -0.209 to 0.513 (Table 1). The size of the correlation varied considerably, but no significant correlation was found between genders on ADC and FA values in young adult brain. The results obtained from the overlay scatter plot are presented in Figure 5, indicating that there was a positive trend on regional FA values between men and women's brains.

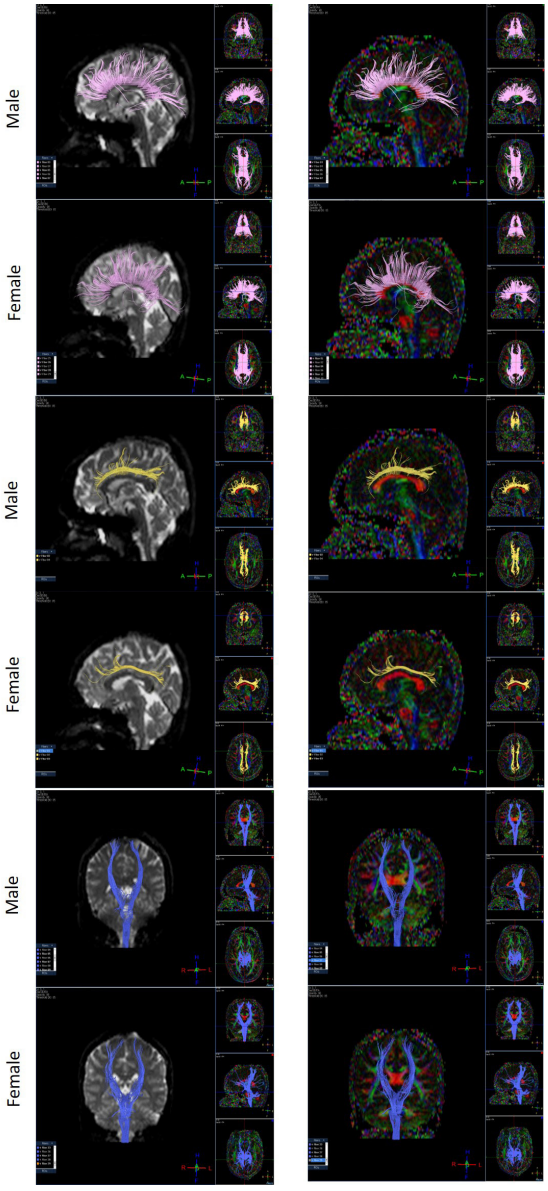

Figure 3. *Fiber tractography of corpus callosum (CC, pink), cingulum-cingulate gyrus (CCG, yellow) and corticospinal tract (CST, blue). Diffusion image (left) and FA maps (right) were conducted in sagittal and coronal plane. Color-coding was based on red-green-blue (RGB) code (red: right-left, green: anterior-posterior, blue: superior-inferior).*

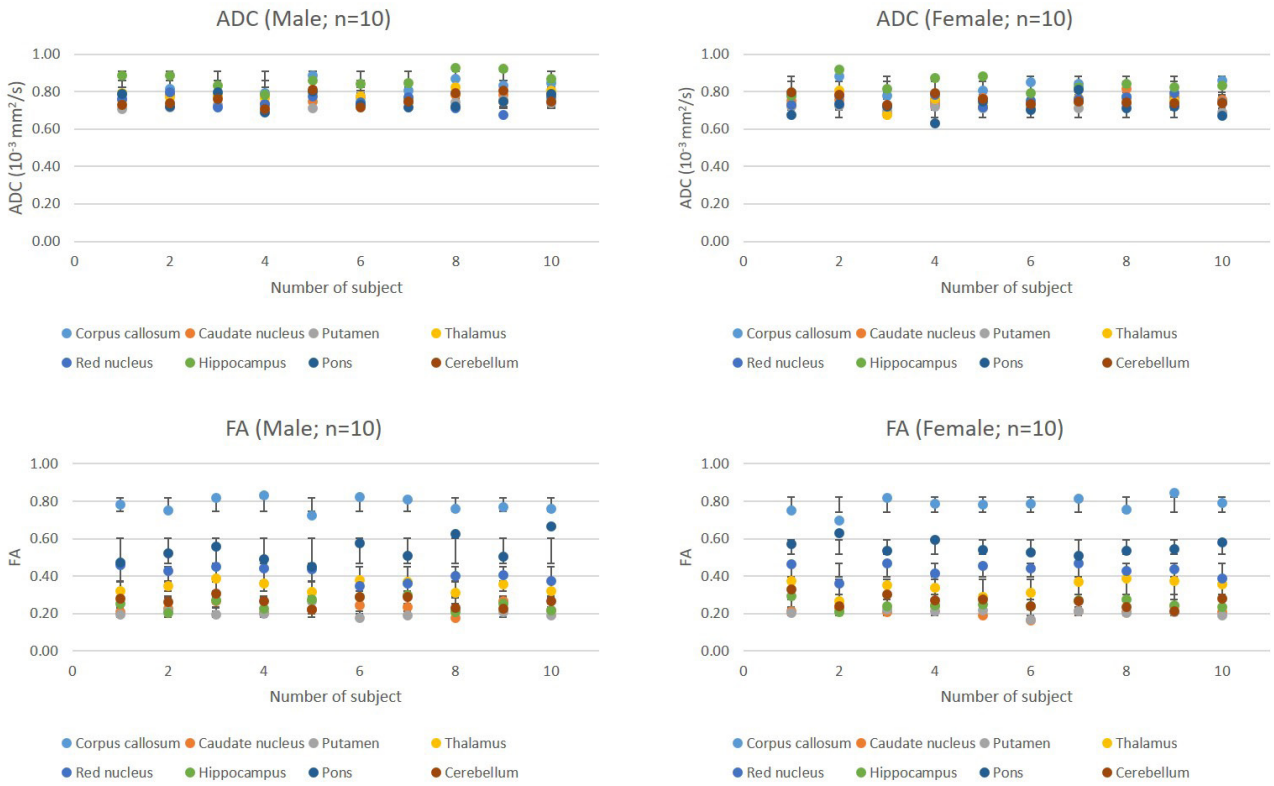

Figure 4. *ADC and FA values in eight ROIs region from 20 healthy young adults.*

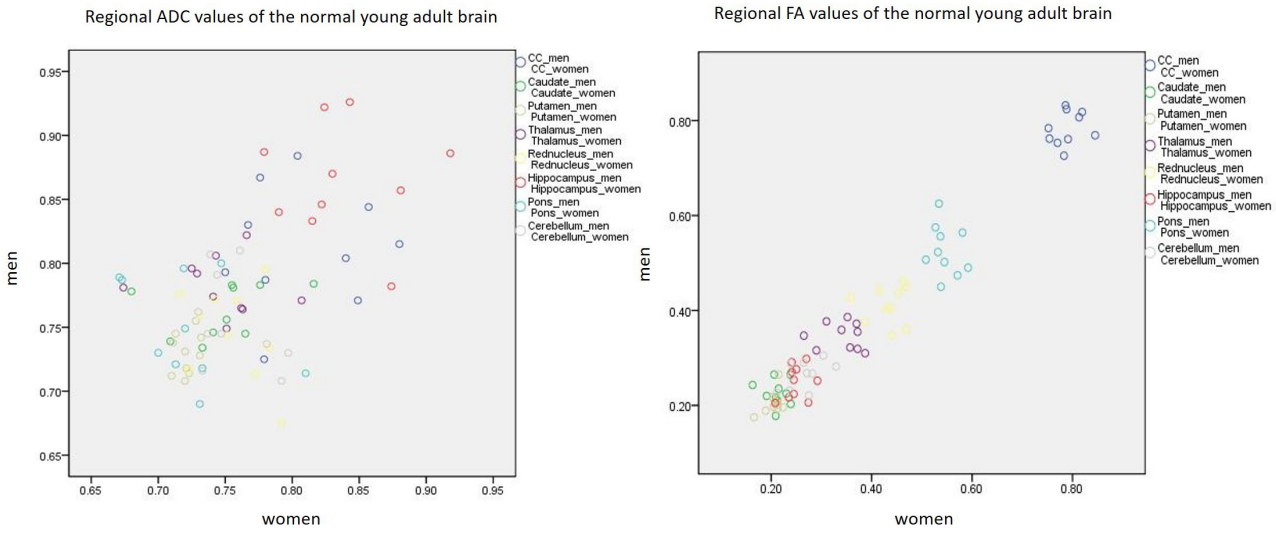

Figure 5. *Scatter plot showing the relationship of the ADC and FA values across regions known between men and women's brains.*

Table 1 Mean values and standard deviations of apparent diffusion coefficient (ADC) and FA values for all ROIs obtained at 1.5 T MR scanner. The level of significance was set at $p<0.05$.

Region of interest (ROIs)	Mean ADC					
	Men	Women	t	p value	Correlation, r	*p* value
1. Corpus callosum	0.81±0.05	0.81±0.04	0.185	0.855	0.073	0.841
2. Caudate nucleus	0.76±0.02	0.75±0.04	1.099	0.286	0.351	0.320
3. Putamen	0.73±0.02	0.72±0.01	1.874	0.077	0.242	0.500
4. Thalamus	0.78±0.02	0.75±0.03	2.781	0.012*	-0.152	0.674
5. Red nucleus	0.75±0.04	0.75±0.03	-0.660	0.518	-0.354	0.315
6. Hippocampus	0.86±0.04	0.84±0.04	1.420	0.173	-0.075	0.837
7. Pons	0.75±0.04	0.72±0.04	1.548	0.139	-0.420	0.227
8. Cerebellum	0.76±0.04	0.76±0.03	-0.071	0.944	-0.389	0.267
Region of interest (ROIs)	**Mean ADC**					
	Men	**Women**	**t**	**p value**	**Correlation, r**	***p* value**
1. Corpus callosum	0.78±0.04	0.79±0.03	0.041	0.968	0.240	0.504
2. Caudate nucleus	0.21±0.02	0.21±0.02	1.323	0.202	-0.066	0.857
3. Putamen	0.21±0.02	0.20±0.02	0.065	0.949	0.501	0.140
4. Thalamus	0.35±0.03	0.34±0.04	0.310	0.760	-0.092	0.801
5. Red nucleus	0.41±0.04	0.43±0.04	-1.256	0.225	0.079	0.828
6. Hippocampus	0.25±0.03	0.25±0.02	-0.060	0.952	0.265	0.460
7. Pons	0.53±0.05	0.55±0.03	-1.063	0.307	-0.209	0.563
8. Cerebellum	0.26±0.03	0.27±0.04	-0.069	0.946	0.513	0.130

Discussion

Diffusion tensor MR imaging and tractography are capable of utilizing information related to water molecules in the living brain. The results of the ADC and FA values obtained from healthy young adult brain are consistent with previous reports.[26-29] Our study also showed no quantitative MRI measures (both the ADC and FA values) in which the relationship between different group differs at 1.5 T MR scanner. The meaning of these ADC differences in healthy subjects is not straightforward. At least one region in the thalamus demonstrated a statistically significant difference ($p<0.05$) in ADC values when comparing with others, reflecting that degree of water diffusion across the regions have different sensitivities to myelin content. This result may be explained by the fact that gender-related morphological differences exist widely in neurobiological function. A rapid motion of water molecules depends on the direction of main flow and more slowly when it moves perpendicular to the preferred direction. At the level of thalamus, previous studies have reported that the gender-related difference may be associated with some compositional and metabolic aspects.[30-32] Factors found to be influencing a shift of water diffusion in tissue have been observed in several studies such as blood flow, glucose uptake, macromolecule-bound protons in myelin and water proton,[33-36] seems not surprising. Water diffusivity is highly dependent on brain location. Additional work would be required to concentrate the diffusion process between diffusion signal and the underlying tissue structure. Nevertheless, there are no significant differences in the tract-averaged FA values compared between genders.

In all participants, the major white matter tracts were successfully created in greater details. The FiberTrak software offers user-selected color coding of the fiber projection reconstruction by hand-selecting. Diffusion MRI-based fiber tracking opens up the capability to delineate white matter fiber pathways in human brain which can be integrated as either structural and functional paradigm between health and disease. Streamline tractography provided a clear visualization of white matter fiber architecture. The color reflects the direction of dense fiber at the selected points which may help clinician performing a diagnostic procedure towards a pathologic condition affecting the human brain. Prior studies that have noted the importance of DTI for planning neurosurgical intervention.[37,38] The use of DTI in surgical planning for epilepsy has been a slight increase to determine the location of suspects as shown by Henry et al.[39] Likewise, probabilistic fiber tracking in epilepsy has been used to determine visual field deficits after temporal lobectomy.[40,41] A broad range of possible applications of DTI tractography has become increasingly important, not only aspects for brain structure and oncological pathologies but also includes neurological and traumatological investigation.[42,43] However, the quantitative analysis of tract volume, fiber length, and fiber density between genders not now covered all of these studies.

This study has several limitations. First, a study of

twenty subjects is likely to be small for investigation that may prevent a clear generalized statement about water diffusivity underlying tissue properties in specific brain regions. With a larger sample including a greater number of subjects in different age groups and handedness would allow more findings. Second, through our samples were relatively well educated and greater than 80% of participants were right-handed. MR scanner operated at field strength at 1.5 Tesla which may affect the measured diffusion coefficient of water. However, there was slightly difference between these values as compared to 3.0 T MR systems.[44-46] Finally, three major tracts such as the corpus callosum, the cingulum-cingulate gyrus, and the corticospinal tract were chosen to visualize white matter fiber tracts in the young adult brain. We also need more works to clarify these values in clinical and neuroscience application.

Further study will consider different age groups, different types of brain disorders that cause a gradual decrease in learning and memory performance.

Conclusion

Visualization and quantification of ADC and FA values on 1.5 T MRI were achieved in healthy young adult volunteers with diffusion tensor MR imaging. There was no significant FA difference between men and women's young adult brains while the mean ADC values in thalamus was statistically significant different from each other. With regard to gender-related white matter integrity in specific brain regions, there is still no clear correlation on the map of ADC and FA values.

Acknowledgements

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Conflict of interest

The authors declare no conflicts of interest in this research.

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Reliability and internal consistency of Thai activity card sort for stroke survivors in Occupational Therapy Units

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ABSTRACT

Background: In Thailand, it is estimated that there are 250,000 new cases of stroke each year. About 50,000 patients lose their lives and around 30% of patients become paralyzed. Occupational Therapy (OT) has a key role in rehabilitation for stroke. In particular, it enables survivors to reengage in their occupations. The Activity Card Sort (ACS) is a useful tool for assisting clients to select therapeutic activities and occupations that are relevant to their needs and contexts.

Objectives: This study aims to create, test of reliability and internal consistency of Thai-ACS for stroke survivors in occupational therapy units.

Materials and methods: The development of the Thai-ACS for stroke rehabilitation was based on a survey of stroke survivors and occupational therapists (OTs) from OT clinics in 6 regions of Thailand. Cluster analyses were used to identify group and patterns of activities.

Results: The survey was completed by 120 clients and 60 OTs who then engaged with ACS in therapy. Thai-ACS was comprised of 100 activity items obtained from OTs and stroke survivors' input. Three clusters emerged for domain areas, consisting of Basic rehabilitation skills and ADL (18 items); IADL, household and education (60 items); and Leisure and socio-cultural participation (22 items). All activities were created by the need of stroke survivors and related to their occupations. This final Thai-ACS also showed a Cronbach alpha coefficient of 0.832, which reflected high reliability and inter-correlations among test activity items.

Conclusion: Thai-ACS for stroke clients is a comprehensive instrument to engage collaboratively with stroke survivors in activities based on their cultural lifestyle. It will facilitate rigorous clinical and population-based research and will direct appropriate therapeutic engagement relevant to the client's needs and contexts.

Introduction

A stroke is a brain injury that results from bleeding or a blockage in the brain. It is a leading cause of death and disability in older adults. Long-term health conditions create ongoing needs for the individual and has implications for rehabilitation that is needed after a stroke. The onset of disability after stroke can severely affect participation in meaningful occupations and life satisfaction.[1] In Thailand, there are more than 250,000 new stroke cases each year. Consequently, stroke survivors create burdens on patients and their families.[2]

The American Occupational Therapy Association defines the main focus of occupational therapy (OT) as "*Achieving health, well-being, and participation in life through engagement in occupation*".[3] Throughout OT processes, occupational therapists

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are mainly concerned with the individual's ability to participate in different contexts. Therefore, the assessment or evaluation of participation in all areas of function can be a primary aspect for implementing intervention plans and can affect outcomes for clients receiving OT services, including stroke survivors with disabilities and their families in developing countries such as Thailand.

OTs considered the activities and occupations that the stroke survivors needed to participate. Participation is defined as "engagement in work, play, or activities of daily living that are the part of one's socio-cultural context and which are necessary to individual's well-being".[4] The meaningful and purposeful activities also demonstrate a positive effect on stroke survivor's health and quality of life. The ACS is a useful tool of OT. The purpose of the assessment is to assist clients in select therapeutic activities that are relevant to their occupations.[5-7] The ACS provides therapists with valuable information to plan meaningful, client-centered interventions. A key principle involves determining the activity domains in order to generate an area of performing activities while considering the appropriate frequency of participation. Previous ACS were developed by the use of questionnaires or primary sources and subsequently are collected from target participants based on cultural considerations of everyday activities.[8-10] There are many different kinds of activities, such as engaging in instrumental activities, leisure activities, and social activities.[7] These domains could be adopted to measure participation in occupation while providing different therapeutic media for stroke survivors specific to their needs.

This occupation-focused measure is one of the few standardized and psychometrical instruments available for use with older people. In Thailand, there is a dearth of standardized assessment tools that are culturally valid and can be used for clinical and research purposes with the Thailand population. The limited number of assessment tools has a major effect on evaluation, intervention planning and intervention among occupational therapists, and is a major professional concern. Therefore, the purpose of this study is to create, test of reliability and internal consistency of Thai-ACS for stroke rehabilitation in occupational therapy units based on the opinions of stroke survivors and OTs. Therefore, the recognition of the importance of cultural relevance to enhance the integrity of the tool guided the development of the Thai-ACS for stroke clients

Materials and methods

Development of Thai-ACS for stroke survivors in occupational therapy units occurred in three stages: item generation, item reduction by Delphi procedure, and item determination. Figure 1 shows the developmental process of Thai-ACS for Stroke survivors. Ethical approval was granted from the Mahidol University Institutional Review Board (COA No. MU-IRB 2010/278.0710) and Document No. 53050 from the Institutional Review Board/Independent Ethics Committee, Department of Medical Services, Ministry of Public Health, Thailand.

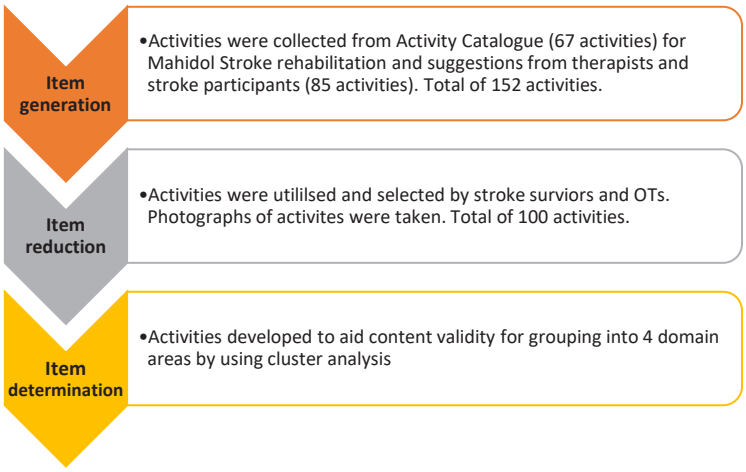

Figure 1. *The developmental process of activity card sort (ACS).*

Participants

One hundred and eighty participants were comprised of 60 occupational therapists who had experience in rehabilitation and 120 stroke survivors. OT practitioners were selected from each region (10 participants) across six different regions of Thailand (n=60) by purposeful sampling based on the following criteria 1) possessed occupational therapy national license; 2) worked full-time in public or private hospitals; 3) had more than two years' experience in occupational therapy for stroke rehabilitation; and 4) demonstrated good communication skills.

Stroke participants were also selected by purposive sampling from each region (20 participants) across six different regions of Thailand (n=120), which were eligible for inclusion in the study if they met the following criteria:

Thai citizens or residents and stroke survivors that can understand the study procedures. Individuals who had a severe vision or cognitive impairments which would impact their ability to sort photos were also excluded. Participants were recruited using purposive sampling through personal and professional networks. Each participant was required to read and sign a written consent form before participating in the study. The demographic data are shown in Table 1.

Table 1 Demographic Data of stroke survivors and OTs.

Variable	
Out-patient department (OPD) stroke survivors	n = 120
	Mean (SD)
Age,	57.45 (14.74)
Gender	**n (%)**
Male	82 (68.33)
Female	38 (31.67)
Thailand region of residence	**n (%)**
Central	20 (16.67)
Eastern	20 (16.67)
Northern	20 (16.67)
North-Eastern	20 (16.67)
Southern	20 (16.67)
Western	20 (16.67)
Ethnicity	**n (%)**
Thai	120 (100)
Affected side	**n (%)**
Right side	34 (28.33)
Left side	84 (70)
Neither/Bilateral	2 (1.67)
Occupational therapists (OTs)	n = 60
Gender	**n (%)**
Male	14 (23.33)
Female	46 (76.67)
Years of experience	
≤2 years	3 (5.00)
3-5 years	8 (13.33)
6-10 years	14 (23.33)
10-15 years	18 (30.00)
16-20 years	9 (15.00)
>20 years	8 (13.33)
Region and Types of Hospital	
Central region (Medical Institute and University hospital)	36 (60.00)
Northern region (Provincial hospital)	5 (8.33)
North-east region (University hospital)	5 (8.33)
Eastern region (Regional hospital)	4 (6.67)
Western region (Regional Hospital)	5 (8.33)
Southern region (Regional hospital)	5 (8.33)

Procedure

Stage one – Item generation

This process included 3 sources of data to generate potential activity and occupation items for the measurement of participation. The activity and occupation items were drawn from the Activity Catalogue from Mahidol Stroke Rehabilitation, the suggestions of OTs who had experience in stroke rehabilitation and stroke participants.

Stage two – Item reduction

This process reduced the number of initial items. The most common activities and occupations of the occupational therapy session were chosen based on the suggestions from OTs and stroke survivors in six regions of Thailand. The various kinds of activities and occupations were chosen by stroke participants and formed the representative classification of activity participation. Activities and occupations were divided into four domain areas comprising of 1) Basic rehabilitation skills and ADL; 2) Instrumental activities of daily living (IADL) and household activities; 3) Socio-cultural/educational activities; and 4) Leisure activities.[11] This process was used to ask participants to rate 6 Likert scales in terms of importance (0=No accepted, 1=Very low, 2=Low, 3=Moderate, 4=High, and 5=Very high). The items were selected by stroke survivors using a mean score of 2.00 to be used as the cut-off point for inclusion.[12]

To increase generalization, the Delphi method was used by doing face-to-face therapeutic interventions/ interactions, questionnaires, and interviews.[13] The survey was designed not only to gain the perspective of therapists and stroke participants, but also to focus on the potential relationships between functional performance and activity items.

Stage three – Item determination

To establish the content validity of the Thai-ACS for stroke survivors in occupational therapy units, then activity and occupation items were entered into a hierarchical cluster analysis. The classification was identified as the fundamental basis for examining the domain area in Thai stroke rehabilitation.

Data Analysis

All data were entered into and managed via the Statistical Package for the Social Sciences, PASW software for Windows version 18 (SPSS Inc., Chicago, IL., USA). For reliability, Cronbach's alpha coefficient was used to analyze internal consistency within each domain area. The final process was analyzed using a hierarchical cluster analysis[14] which grouped different activities (Determination of Domains) representing the classification of activity and participation from both therapists and users.

Results

Item generation

For this stage, 152 activity and occupation items from the Activity Catalogue (67 activities) and suggestions of OTs who had experience in stroke rehabilitation and stroke survivors (85 activities) were used. Therefore, this phase collected multiple activity items based on experience, culture, lifestyle, and local materials and resources across six different regions throughout Thailand.

Item reduction

This process reduced the number of initial items. All activities were divided into four domain areas comprising of 1) Basic rehabilitation skills and ADL; 2) Instrumental activities of daily living (IADL) and household activities; 3) Socio-cultural/educational activities; and 4) Leisure activities. The most common activities of the stroke survivors in six regions were designed based on suggestions from many OTs and stroke survivors. To increase generalization, the Delphi method was used. The survey was designed not only to gain perspective of therapists and stroke survivors, but also to focus on potential relationships between functional performance and activity items. Item reduction was undertaken by the use of the activity catalogue and various new activity items from six regions of Thailand. There were in total of 152 activities that allowed a choice in choosing appropriate therapeutic media for stroke survivors. A total of OTs and stroke survivors were asked to rate the commonalities of each activity to be done during OT sessions. However, 100 activity items were selected by stroke survivors using a mean score of 2.00[12] to be used as the cut-off point for inclusion in the final Thai-ACS for stroke survivors as shown in Table 2. Based on the activity selected these were calculated and activities were ranked from most to least in terms of utilization. Using a mean score of <2.0 eliminated some, leaving 100 out of the 152 therapeutic items. These items were then chosen for potential inclusion in an ACS for Thai stroke survivors in occupational therapy units.

Table 2 Ranking, mean score and standard deviation in the level of activity approach for 100 of 125 activities, from most utilization to least utilization.

Activities by ranking numbers	Mean±SD	Activities by ranking numbers	Mean±SD	Activities by ranking numbers	Mean±SD
1. Button skill	4.00±0.00	35. Mouse and key board skills	3.63±0.49	69. Hand cycling exercise	3.10±0.30
2. Pressing toothpaste	4.00±0.00	36. Stapler skill	3.58±.051	70. Flipping coins/ cards	3.09±0.29
3. Driving car or motor bike	4.00±0.00	37. Using scissors	3.58±0.57	71. Ball throwing at target with a bounce	3.07±0.26
4. Washing hands	4.00±0.00	38. Tearing paper	3.57±0.50	72. Golf skills	3.07±0.26
5. Weight bearing	4.00±0.00	39. Drawing skill	3.56±0.51	73. Incline board	3.06±0.42
6. Bathing with bucket and bowl	4.00±0.00	40. Drying clothes by clothes rack/ clothesline	3.55±0.52	74. Using spoon/fork to remove bead	3.04±0.58
7. Using remote control (TV)	4.00±0.00	41. Tennis ball pick and place	3.54±0.53	75. Trunk control/bilateral arm movement	3.03±.016
8. Opening and closing the door	3.98±0.13	42. Folding fabric	3.54±0.51	76. Climbing board	3.02±0.14
9. Grooming	3.96±0.18	43. Lashing rope	3.52±0.51	77. Looping curve skill	3.02±0.22
10. Taking shower	3.96±0.20	44. Writing skill	3.52±0.54	78. Abacus skill	3.00±0.00
11. Dressing with top (shirt/blouse)	3.94±0.23	45. Basketry	3.50±0.57	79. Handle a ball for wrist exercise	3.00±0.00
12. Wearing a belt	3.93±0.25	46. Folding banana leaves to carry food	3.50±0.57	80. Bowling skill	3.00±0.00
13. Watering plants	3.93±0.26	47. Using a soup ladle	3.50±0.57	81. Kick ball	3.00±0.00
14. Wearing trousers	3.93±0.26	48. Washing cloth skill	3.50±0.51	82. Playing table tennis	3.00±0.00
15. Holding and drinking water from bottle	3.92±0.27	49. Pinching peas (grain, seed)	3.50±0.51	83. Dart	3.00±0.00
16. Zipper	3.92±0.28	50. Scrubbing and sweeping the house	3.43±0.50	84. Forceps pick and place with ping pong	3.00±0.00
17. Spray nozzle for butt wiping	3.91±0.30	51. Using pencil sharpener (device)	3.40±0.54	85. Bimanual putting pin in a bead	3.00±0.00
18. Pump action of lotion bottle (shampoo)	3.90±0.30	52. Going to temple/ church/ mosque	3.40±0.49	86. Key grip skill (turning)	3.00±0.00
19. Key skill (lock-unlock)	3.83±0.37	53. Phoning skill	3.39±0.49	87. Big peg board	3.00±0.21
20. Holding and rotating bottle (control object)	3.83±0.38	54. Thai Chess game	3.22±.044	88. Bimanual holding of cone and place	2.98±0.13
21. Meal preparation and cooking meal	3.80±0.42	55. Calculation skill	3.22±.041	89. Stacking cones or rod	2.98±0.14
22. Sorting stones and crystal marbles	3.75±0.46	56. Piano and keyboard (musical instrument)	3.20±0.63	90. Putty activities	2.98±0.27
23. Knife skill (under closely super-vision)	3.75±0.50	57. Hammer/Axe skill (plastic)	3.20±0.44	91. Shape matching skill	2.98±0.15
24. Using cash machine	3.75±0.50	58. Gardening & growing plants	3.18±0.40	92. Pinch grip (pinching clothespin)	2.97±0.17
25. Opening/ closing a book	3.75±0.57	59. Pick and place ball in small cone	3.17±.0.38	93. Throwing rings (quoits) at target	2.94±0.24
26. Stereognosis skill	3.73±0.46	60. Flowering arranging	3.17±0.40	94. Playing jigsaw puzzles	2.94±0.57
27. Reading the Tripitaka/ Koran/ Bible	3.71±0.48	61. Playing card	3.16±0.37	95. Picture mosaic skill	2.93±0.26
28. Scoop and pour liquid into bottle	3.71±0.45	62. Folding paper boat or Sarus crane bird	3.14±0.37	96. Small peg board	2.92±0.33
29. Wiping the table	3.71±0.52	63. Pronation & supination task	3.14±0.35	97. Constructing chain from plastic link	2.88±0.33
30. Sorting tiddlywinks with spoon	3.69±0.46	64. Plier skill	3.13±.034	98. Placing beads on pins	2.86±0.35
31. Pinch off veggie	3.67±0.51	65. Bolt-screwing (into board)	3.12±0.41	99. Coins dropping into piggy bank	2.86±0.53
32. Picking and sorting small bead (rice, bean)	3.67±0.47	66. Fruit pole	3.11±0.32	100. Organising medicine	2.25±0.50
33. Lettering and enveloping skills	3.67±0.57	67. Sliding board	3.11±0.47		
34. Using chopstick	3.65±0.48	68. Throwing and receiving ball	3.11±0.31		

Item determination

The final Thai-ACS for stroke survivors in occupational therapy units after domain area determination using cluster analysis is described herein. When clustering therapeutic activities, cluster analysis categorized 100 activity items into three groups (clusters) by placing the same items in the same cluster. Therefore, there are three domain areas of the final Thai-ACS which comprised of Basic rehabilitation skills and ADL (18 items); IADL, household and education (60 items); and Leisure and socio-cultural participation (22 items) as shown in Table 3.

Table 3 Final Activity Card Sort for Thai Stroke Rehabilitation after item determination.

Classification of Activities Into 3 Domain Areas (100 items)		
CLUSTER 1 Basic rehabilitation skills and ADL (18 items)	CLUSTER 2 IADL, household and education (60 items)	CLUSTER 3 Leisure and socio-cultural participation (22 items)
- Looping curve skill	- Abacus skills	- Using chopstick
- Key grip skill	- Reading the Tripitaka/Bible/ Koran	- Use of spoon and fork (bimanual) to remove bead from putty
- Pronation and supination task	- Lashing rope	- Writing skills
- Bimanual holding of cone and place	- Flower arranging	- Picking and sorting small bead (like cleaning rice, bean)
- Dressing with top (shirt/blouse)	- Kick ball	- Sorting tiddlywinks with spoon
- Peg board	- Bowling skills	- Calculation skill
- Stacking cones or rod	- Folding banana or coconut leaves to carry food	- Fruit pole
- Pick and place ball in small cone	- Basketry	- Washing cloth skill
- Tennis ball pick and place	- Watering plants	- Scrubbing and sweeping the house
- Trunk control/bilateral arm movement	- Using cash machine	- Phoning skill
- Sliding board	- Driving car or driving simulation	- Hammer/Axe skill (plastic)
- Pinch grip (Pinching)	- Spray nozzle for butt wiping	- Scoop and pour liquid into bottle
- Putty activity	- Bathing with bucket and bowl	- Opening and closing door
- Bimanual putting pin in a bead	- Using a soup ladle	- Pump action of lotion bottle
- Forceps pick and place with ping pong ball	- Using remote control	- Bolt-screwing (into board)
- Incline board	- Organising medicine	- Mouse and key board skill
- Constructing chain from plastic (small) link	- Knife skills	- Shape matching skills
- Placing beads on pins	- Pinch off veggie	- Picture mosaic skills
	- Pressing toothpaste	- Dart
	- Button skills	- Piano and musical instrument
	- Wearing a belt	- Throwing rings (quoits) at target
	- Stereognosis skills	- Ball throwing at target with a bounce
	- Handle a ball for wrist exercise	
	- Pinching peas	
	- Washing hand	
	- Pliers Skills	
	- Taking shower	
	- Grooming	
	- Zipper	
	- Wearing trousers	
	- Tearing paper	
	- Weight bearing	

Table 3 Final Activity Card Sort for Thai Stroke Rehabilitation after item determination. (continues)

Classification of Activities Into 3 Domain Areas (100 items)		
CLUSTER 1 Basic rehabilitation skills and ADL (18 items)	CLUSTER 2 IADL, household and education (60 items)	CLUSTER 3 Leisure and socio-cultural participation (22 items)
	- Holding and rotating the bottle - Climbing board - Hand cycling exercise - Big peg board - Flipping coin/ card - Drying clothes with clothesline - Lettering and enveloping skills - Meal preparation and cooking - Playing table tennis - Folding fabric - Using pencil sharpener - Coins dropping into piggy bank - Sorting stone and crystal marbles - Key skills - Using scissors - Opening/Closing a book - Stapler skills - Wiping the table - Holding and drinking water from bottle or cup - Folding paper boats - Thai chess game - Gardening and growing plants - Drawing skills - Golf skills - Playing jigsaw puzzles - Playing card - Throwing and receiving ball - Going to temple	

Cronbach's alpha coefficient was used to analyze internal consistency within each domain area. Internal consistency statistics were alpha 0.866 for Basic rehabilitation skills and ADL; alpha 0.948 for IADL, household and education; and alpha 0.934 for Leisure and socio-cultural participation. When all activities for 3 domain areas were integrated as the final Thai-ACS. Moreover, this final Thai-ACS was scrutinized by using Cronbach's Alpha for a coefficient of reliability after a psychometric test score that involved questioning participants. This final Thai-ACS presented a Cronbach alpha coefficient of 0.832, which indicated high reliability in practice,[15] or a good level of internal consistency, thereby reflecting high reliability and inter-correlations among test activity items as shown in Table 4.

Table 4 The reliability of Activity Card Sort for Thai Stroke Rehabilitation.

Domain area	Cronbach's Alpha coefficient	Number of Item
Basic rehabilitation skills and ADL	0.866	18
IADL, household and education	0.948	60
Leisure and socio-cultural participation	0.934	22
Thai ACS for Stroke Clients	0.832	100

Discussion

The purpose of this study was to develop a version of Thai-ACS that may provide therapeutic activities and occupations for stroke survivors in occupational therapy units. A total of 100 items were included in the final Thai-ACS. Three categories were finalized for the tool; however, this was a difficult part of the tool development with the sorting of activities into the categories. The classification of activities was changed and modified by the cluster analysis from four to three domain areas (Basic rehabilitation and ADL; I-ADL, household and education; and Leisure and socio-cultural participation). According to Katz et al.[16] there were 88 activity items placed in four domain areas (IADL; low-physical leisure activities; high-physical leisure activities, and social-cultural activities) of ACS for adult Israeli people. Packer et al.[11] surveyed elderly Australian people in Brisbane and Adelaide when creating their Australian version of the ACS. They also determined four domains (social, leisure, work, and household activities) before the data collection. During the data collection, there were more than one hundred activities in the phase of item generation, and then the outcome of ACS showed 82 pictorial activities which were arranged into three domains of household activities; social/educational activities; and leisure activities. In the ACS Japanese version, there are seventy-two activity items that were finally included and were classified into four domains as instrumental activities of daily living, low-demand leisure activities, high-demand leisure activities, and social/cultural activities.[5]

To be consistent with universal terms and standards, the activities of daily living (ADL) are normally divided into two positions: personal ADL (P-ADL), and instrumental ADL (I-ADL).[17] However, another term generally used is self-care which represents the basic activities for caring for oneself in daily life.[18] This study used ADL and I-ADL in the domains to clarify the characteristics and forms of activities accommodated by OTs and stroke participants in their selection and performance. As revealed in the findings, we provided the stroke survivors with opportunities for meaningful and purposeful therapeutic activities based on their own needs and lifestyles. Moreover, the development of the ACS opened a new arena in which to embrace novel relevant activities for stakeholders to practice in rehabilitation sessions and apply them elsewhere. Consistent with Wottrich et al.[19] the rehabilitation team found that the key activities were unique to each client, such as shopping in a favorite deli store or convenient mall. These opportunities increased the wide range of activities meeting the patients' needs creatively linking them to trends in the societal environment. New dimensions to tackle new problems were created which bridged gaps between the desired and achievable goals of individual patients.

In this research, the Thai-ACS provided various therapeutic media, where activities and occupations were classified into domains corresponding to functional performance and the needs of stroke survivors. According to Guidetti and Tham[20], if the aim of therapeutic intervention was unclearly managed by the occupational therapists, stroke survivors found that it was hard to understand the goals and directions of treatment. In the same way, Kielhofner[21] argued that the therapeutic strategy must be coupled with a client's doing, feeling, perspective, and thoughts in ways which would influence the attainment of the desired goals. According to a systematic review of randomized trials, stroke survivors who received occupational therapy intervention focusing on the improvement of personal activities of daily living can improve skill performance and minimize the risk of deterioration in functional abilities than those who received usual care.[22]

Nevertheless, the ACS does not indicate specific techniques, but instead indicates modalities to be used in specific situations for an individual's needs. The occupational therapist positions him/herself as a supporter or facilitator. The therapists' teaching should not implement specific intervention techniques without therapeutic activities being a key element in the initiation of, and support to stroke survivors who must find ways to perform activities. They will draw on their own past experiences in similar situations. Consistent with the previous work reported that therapists should be aware and recognize what techniques individual clients experience as the most useful and powerful. Then they can encourage the clients to perform that technique and use it in both specific and diverse situations.[23]

Conclusion

The set of therapeutic occupations or activities in Thai-ACS suitable for Thai patients has been formulated. The history and evidence related to the importance of activities and frequency of participation related to various health conditions have been considered. The development of culturally appropriate ACS for stroke survivors in Thailand began with item generation; item reduction; and the item determination. The new Thai-ACS for stroke survivors contains three domain areas; Basic rehabilitation skills and ADL; IADL, household activities and education; and Leisure and socio-cultural participation. Hence, Thai-ACS is a comprehensive instrument used to collaboratively engage with stroke survivors and

their families when performing activities and facilitates the rehabilitation effectively based on local, regional, and cultural lifestyle considerations.

Limitations

These results should be considered within the context of the study's limitations. At the development phase, the stroke participants were predominantly drawn from purposive sampling which may limit to explore the activity participation and engagement in participants with various diversity, individuals experiencing health conditions, and a representative gender distribution. Additionally, the recovery stage or functional status of stroke participants should be considered during the development of ACS. For each region, the most of OTs were recruited from the same settings that may affect to activity selection. In clinical practice, the occupational therapy practitioner should consider the context and individualized stroke clients before providing activity and occupation-based intervention. Hence, a measurement tool with reliability and validity is necessary. Therefore, this Thai-ACS was only generalized at clinical based setting. Further research may also need to develop ACS that are suitable for home or community-based participation and engagement for stroke survivors and the qualitative studies from the OTs would be interpreted for the using of activity card sort in stroke rehabilitation.

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Conflict of interest

The authors state that there are no conflicts of interest.

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Validation of Thai Smartphone Addiction Scale-short version for school students between 10 to 18 years

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ABSTRACT

Background: With the dramatic increase in the number of smartphone users, concern has been raised that smartphone overuse can be hazardous to health. There is a need of smartphone addiction screening instrument that can be used for the Thai people, particularly children and adolescents.

Objectives: This study aimed to translate Smartphone Addiction Scale – Short Version (SAS-SV) into Thai for school students between 10 to 18 years and to comprehensively validate the translated version.

Materials and methods: After completing the translation according to published guidelines, Thai version of the SAS-SV (THAI-SAS-SV) for school students underwent thorough many psychometric tests. The content validity was evaluated by a panel of seven experts. Internal consistency and construct validity of the THAI-SAS-SV were then tested among 200 Thai school students between ages 10 and 18 (mean age 12.82±2.21 years). The test-retest reliability was also evaluated in half of all participants.

Results: THAI-SAS-SV for school students demonstrated an excellent validity index for scale (S-CVI = 0.97) and an item content validity index (I-CVI) ranging from 0.86 to 1.0. Cronbach's alpha for internal consistency was calculated as 0.85. The THAI-SAS-SV for school students has similar construct to the original instrument because the confirmatory factor analysis clearly revealed a single-factor structure. Intraclass correlation coefficient (ICC) value for test retest reliability was 0.73 (95% CI: 0.62-0.81).

Conclusion: The findings suggest that THAI-SAS-SV for school students between 10 to 18 years is a valid and reliable instrument for screening smartphone addiction targeted towards Thai children and adolescents.

Introduction

Smartphones are becoming ever more essential in the lives of human beings, as they can be used as a multifunction device. Despite the many benefits of using a smartphone, mental-and physical-health problems related to the cumulative effect of excessive smartphone user are increasingly raising concern among healthcare professionals. Today, smartphone overuse is an emerging object of worldwide concern, especially for teenagers, who can relatively easily develop dependency upon the device.[1] Unsurprisingly, a high prevalence of smartphone addiction has been reported in many countries in the past few years, especially among young people.[2]

The number of smartphone users has dramatically increased all over the world, as tracked through the year 2018. Globally, there are 5.11 billion unique smartphone users

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today, and increase of 100 million (2 percent) over the year 2018.[3] In the previous year, Thailand is ranked eighteenth highest in the world as to number of active smartphone users. Meanwhile, Thailand was the fourth-highest as to number of smartphone owners in Southeast Asia after Indonesia, Philippines, and Vietnam.[4] Furthermore, the digital intelligence quotient (DQ) impact report revealed that Thai children and adolescents aged between 8 and 12 years spend an average of 35 hours a week browsing the internet which is more than 3 hours worldwide average. Moreover, the Thai school students access the Internet via personal smartphone up to 73 percent. Critically, excessive use of smartphones may lead to an increased risk of online perils in Thai youths.[5] It is, however, difficult to determine whether or not spending more time with smartphone leads to the development of behavioral addiction. It is worrisome that teens were found to be highest across all age groups that use social media on smartphones.[6] Also, it is predicted that mobile internet usage by this age groups will likely continue to increase.[7] Given that there has been a rapid progress in increasing access to smartphones in Thai vulnerable persons, an effective instrument is necessary to identify Thais who are at risk of developing smartphone addiction. Although there is the Thai version of Smartphone Addiction Scale – Short Version[8], the participants in previous work were young adult university students, which different from the original version. Importantly, only two tests of the psychometric properties of the translated version were performed. It is important to conduct several types of psychometric properties to confirm validity and reliability of the newly translated questionnaire.

Smartphone Addiction Scale - SV (SAS-SV) developed by Kwon et al[9] is an acceptable questionnaire instrument for evaluation of smartphone addiction. The intention of this original questionnaire instrument was to produce a brief screening instrument to evaluate smartphone addiction for use in 2nd year of junior high school students.[9] The instrument consists of 10 items that are relatively short and suitable for use with young people subjects. SAS-SV has been shown to possess validity and reliability. There are adaptations of the SAS-SV to other languages including German[10], Spanish, French[11], Arabic[12], Turkish[13], Italian[14], Chinese[15], and Thai.[8] The good to excellent validity and reliability of all translated versions have been reported. The SAS-SV has been used in several studies that indicated its usefulness and adequate psychometric properties. So far, the prevalence of smartphone addiction in Thai school students between 10 to 18 years have been inadequate due to some limitations in the instrument's development as mentioned earlier.

As the use of smartphone increases worldwide, there is wide concern among healthcare professionals about the spreading of chronic mental and health problems of device dependence. Although the smartphone device seems to be an essential tool of modern life, it is likely to be a "double-edged sword" for users as well.[1] Previously, many self-report questionnaires were developed for determining smartphone addiction among smartphone users.[2] Among these questionnaires, the SAS-SV has fewer items than others and it has a cut-off score for each gender. It is well known that a brief questionnaire should be selected to deal with teenager. In addition to the rapid growth of smartphone penetration, it is well known that the rate of social media and Internet usage of Thailand and South Korea are quite similar. Accordingly, it is probably best suited to use this questionnaire in teens. Thus far, few valid or reliable instruments have been available to screen smartphone addiction for Thais, even though there is a fairly high rate of smartphone usage in Thailand. Currently, there are only two screening questionnaires for Thai children and adolescents, related to addiction of Facebook[16] and game.[17] It is well accepted that prevention is better than a cure. Going forward, screening instruments are needed to assist in early detection of this behavioral addiction, particularly for a group like children and adolescents with high-risk for smartphone dependence. Therefore, this study aimed to translate the SAS-SV into the Thai language for school students between 10 to 18 years and to determine the various psychometric properties of the Thai version of the SAS-SV (THAI-SAS-SV) for school students, thus establishing the psychometric properties as well as suitability of its use for further research in Thailand.

Materials and methods

Material

Smartphone Addiction Scale Short Version (SAS-SV) is a self-reporting questionnaire instrument that consists of 10 items with a six-point Likert-type scale, ranging from 1 ("strongly disagree") to 6 ("strongly agree"). The total score ranges from 10 to 60, and higher scores indicate a high risk use of the smartphone. This instrument was developed and validated in a sample of 343 boys and 197 girls in South Korea. The average age of participants was 14.5 years. The cut off score is 31 point for males and 33 point for females.[9] SAS-SV was developed from the Smartphone Addiction Scale (SAS) that have 33 items and was proven to be relatively reliable and valid, though it was validated in adults aged 18 to 53 years.[18] SAS-SV has a proven internal consistency reliability (Cronbach's alpha of 0.911) and concurrent validity which correlated with the Korean Internet Addiction Self-Assessment Tool (r=0.421) and Smartphone Addiction Proneness Scale (r=0.762).[9]

Study design

The study was a cross-sectional design conducted in two phases from December 2018 to April 2019. Phase I comprised translation and cross-cultural validation of SAS-SV for school students between 10 to 18 years. Phase II determined the psychometric properties of the THAI-SAS-SV for school students including content validity, construct validity, internal consistency reliability, and test-retest reliability.

Sample size determination

Sample size determination was divided into two phases: translation and validation of the psychometric properties of the translated questionnaire. Based on previous recommendation for testing of the prefinal version in translation phase, the sample size was 40 persons.[19] Based on previous recommendations for testing the psychometric

properties of questionnaire, the sample sizes of 200 persons for confirmatory factor analysis and internal consistency reliability. The subject-to-item ratio considered in this study was 1:10[20] and 100 persons for the test-retest reliability test. The minimum sample size required in this study was 50 persons.[21] In this phase, sample size determination was chosen to be twice the minimum requirement because we attempted to recruit the same number of primary school students and high school students. Therefore, we set out to recruit approximately 200 individuals for factor analysis as well as internal consistency reliability and 100 individuals for repeatability. For assessing repeatability of THAI-SAS-SV for school students, the 100 participants were randomly selected from 200 participants in main study by the lottery method to avoid bias the test.

Participants

For this study, participants between the ages of 10 and 18 years were recruited in both genders. This study intended to recruit a homogeneous set of participants in term of school setting. As a result, the participants consisted of school students (Grades 4 to 12). According to the Thai education system, the participants were primary school students (10 to 12 years) and high school students (13 to 18 years). The participants were recruited from five schools in large province (Chiang Mai) and small province (Phayao) in northern Thailand.

The inclusion criteria were 1) experience of smartphone usage at least three months prior to study participation and 2) the ability to read and understand Thai language.

Translation process

In this study, the translation process was carried out based on international standard regarding the translation of research instruments.[19]

Forward translation: the original SAS-SV was authorized for translation and validation as Thai version. This questionnaire was translated into Thai by two independent bilingual translators whose first language is Thai. Both translators had lived in Australia and France for more than three years.

Synthesis of the translations: the two forward translations (Thai version) and original SAS-SV (English version) were compared and synthesized by forward translators and the researcher. The synthesis was performed regarding ambiguities and discrepancies of words, sentences, and meanings of the initial Thai version of SAS-SV.

Back translation: the initial Thai version of SAS-SV was back translated accurately by two independent bilingual translators whose first language is English. Both translators had no access to the English version of SAS-SV.

Expert committee: the pre-final version was derived by the expert committee comprising translators, a lecturer in the department of Physical Therapy who had lived in the United Kingdom for more than four years, a high school English teacher who has worked with intensive English program, a child and adolescent psychologist, and a psychiatric nurse. The aim of this step was to consolidate all versions of the questionnaires and produce the appropriate pre-final Thai version of SAS-SV for testing in the field. To achieve successful adaptation, the committee's considered four aspects including semantic equivalence, idiomatic equivalence, experiential equivalence, and conceptual equivalence. The committee also were required to ensure that the pre-final version would be appropriate for understanding by a 10-year-old child.

Test of the pre-final version: forty participants were recruited to read and answer without assistance the pre-final Thai version of SAS-SV for school students between 10 to 18 years and were later questioned about its conceptual clarity. The pilot testing used an equal number of male and female participants. All participants were interviewed subsequent to pilot testing to prove their understanding of each questionnaire item as well as their choices of responses. All participants reported that they understood the translation, and the items in the translated version were relatively simple to score.

Psychometric properties testing

In the present study, the psychometric properties testing followed the previous suggestion.[22]

Content validity: the THAI-SAS-SV for school students was assessed for its content validity by a panel of seven experts from different institutions, including three psychiatrists, two psychologists, one psychiatric nurse, and one lecturer in mental health nursing. All of the experts possessed more than five years of experience in work related to psychology. Content validity was assessed by asking the experts to rate each question as a valid measure of the construct using a four-point Likert scale (1= not relevant, 2=somewhat relevant, 3=quite relevant, and 4=highly relevant). Content validity of individual items (I-CVI) was calculated from the number of experts giving the item a relevance rating of either 3 or 4, divided by the total number of experts. Content validity of the overall scale (S-CVI) was calculated from the number of items given a rating of either 3 or 4, divided by the total number of items. A value greater than 0.78 is considered satisfactory for I-CVI, and a value of at least 0.80 of S-CVI value is acceptable given also that there is congruence between the evaluation question and the objectives, or content.[23]

Construct validity: the construct validity of the THAI-SAS-SV for school students was tested by confirmatory factor analysis (CFA). In this study, we hypothesized that the THAI-SAS-SV would exhibit a single dimension similar to the original instrument. Thus, it would be said that the structural model of the THAI-SAS-SV was well overall fit to the Thai culture. Several model fit indices and their criteria were used to examine the goodness-of-fit of the model with the given dataset: Relative chi-square value (ratio of chi-square to degrees of freedom), goodness-of-fit index (GFI), adjusted goodness-of-fit index (AGFI), normed fit index (NFI), comparative fit index (CFI), root mean square error of approximation (RMSEA), and standardized root mean square residual (SRMR). The relative chi-square should be less than 3. The recommended value of RMSEA and SRMR should be less than 0.08 and 0.05, respectively. The value of GFI, AGFI, NFI, and CFI larger than 0.90 are acceptable.[24]

Internal consistency reliability: the THAI-SAS-SV for school students was assessed for internal consistency using Cronbach's alpha coefficient, corrected item-total correlation,

and Cronbach's alpha if item deleted. A result of high Cronbach's alpha indicates high correlations among the items in the instrument. An alpha value between 0.70 and 0.95 was regarded as acceptable reliability.[21] Corrected item-total scale correlation of 0.30 or higher was considered acceptable for each item in the instrument.[25] In principle, all of the alpha if item deleted values should not be greater than the overall alpha.[26]

Test-retest reliability: the THAI-SAS-SV for school students was investigated for test-retest reliability using the intraclass correlation coefficient (ICC).

In this study, all participants were asked to maintain their regular amount of smartphone usage with the same their smartphones throughout the 2-week interval. Time interval between two weeks was conducted to minimize recall bias[21] and behavior cannot become an addiction within a short period of time.[27] Based on the study by Koo et al[28], the values of ICC between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.90 are indicative of moderate, good, and excellent reliability, respectively.

Data collection

Data were collected from December 2018 to April 2019. Permission was granted to administer this questionnaire from the headmaster in each school. In the field, a paper-based questionnaire was used for collecting data. The present study was approved by the Ethic Review Board of the Faculty of Associated Medical Sciences, Chiang Mai University (Ethic Code: AMSEC-61EX-076). Written informed consent was obtained from the parents or legal guardians of the participants prior to data collection.

Statistical analysis

Statistical Package for the Social Science (SPSS) version 17.0 was used to analyze descriptive statistics such as frequency, percentage, mean, standard deviation (SD), and 95 percent confidence intervals (95% CI) and the statistical significance was set at $p<0.05$. Confirmatory factor analysis was determined using LISREL 8.8.

Results

Translation and pilot testing

In consensus with the expert team, instructional guideline and cartoon pictures representing facial expressions of agreement were provided in the THAI-SAS-SV for school students between 10 to 18 years for more understanding when using this instrument. The original SAS-SV did not provide cartoon images. Based on a pilot testing together with a consensus of the expert team, we decided to add instructional guidance and cartoon images representing facial expressions of agreement for more understanding when using this instrument. It is important to note that the youngest participants in this study were 10 years. During the pilot testing, most of the youngest participants complaint about a six-point Likert-type scale ("strongly disagree, disagree, somewhat disagree, agree, somewhat agree, and strongly agree") that is difficult to distinguish one from the other. To clarify subjective information and avoid confusion, cartoon images were then used as an appropriate solution as shown in Figure 1.

Figure 1. *Cartoon images used in this study to represent strongly disagree, disagree, somewhat disagree, agree, somewhat agree, and strongly agree, respectively.*

In this study, seven items were adapted properly for use in Thai culture and examples of events were provided for youngsters. All participants were instructed to read and answer the questions by themselves. Therefore, some situations and common nouns as such in item 7 and 10 were used to decorate for enhancing good understanding of the participants. The term "I" was provided in items 1 to 4, and in item 8 to represent the respondent. The term "activity" was added into the items 1 and 2 because term "work" might not be appropriate for activity involvement of children and early adolescents. For item 7, some situations were intended to serve as examples for better understanding. Based on the context of social media use in Thailand, the term "Line" and "Instagram" were also added into item 8 and ranked in order of popularity among Thais. Similar to item 7, the term parents, teacher, friend, brother, and sister were intended to represent "the people around me" in item 10. In addition, local language was used to expand the term "holding" in item 5. The participants during pilot testing reported that they understood the translation and that the items in the translated version were easy to score. Following the pilot study, THAI-SAS-SV for school students between 10 to 18 years was approved without further changes.

Participant characteristics

A total of 200 participants were included in the main study. Of those, 110 participants were males (55 percent). The average age of participants was 12.82±2.21 years (range 10 to 18 years). Regarding school grade level, the mean ages of primary school students (male 52, female 48) and high school students (male 58, female 42) were 10.95±0.72 years and 14.69±1.50 years, respectively. The demographics of participants are presented in Table 1.

Table 1 General characteristics of participants (N=200).

Characteristics	Mean±SD	Male (n)	Female (n)
Age (years), Overall	12.82±2.21	110	90
Primary school students (10 to 12 years)	10.95±0.72	52	48
High school students (13 to 18 years)	14.69±1.50	58	42

Reliability and validity

Content validity: the content validity results showed that THAI-SAS-SV for school students had a S-CVI score of 0.97. All of the I-CVI scores ranged from 0.86 to 1.00, as presented in Table 2. THAI-SAS-SV for school students demonstrated excellent content validity.[23]

Table 2 I-CVI and S-CVI scores of the THAI-SAS-SV for school students between 10 to 18 years.

Item	The opinion of experts							I-CVI
	Rater 1	Rater 2	Rater 3	Rater 4	Rater 5	Rater 6	Rater 7	
1	4	4	3	4	4	4	4	1.00
2	4	4	4	4	4	4	4	1.00
3	4	4	3	4	4	4	4	1.00
4	4	3	4	2	4	4	4	0.86
5	4	4	3	4	4	4	4	1.00
6	4	4	3	2	4	4	4	0.86
7	4	4	3	3	4	4	4	1.00
8	4	4	4	4	4	4	4	1.00
9	4	4	4	4	4	4	4	1.00
10	4	4	4	4	4	4	4	1.00
S-CVI	1.00	1.00	1.00	0.80	1.00	1.00	1.00	0.97

Note: *I-CVI indicates content validity index for item, S-CVI indicates content validity index for instrument.*

Construct validity: Thai version for school students was found to be consistent with the original version which included one dimension.[9] The results of CFA indicated that the model was well fitted.[24] The model fit indices were as follows: X2=82.18, df=35, GFI=0.92, AGFI=0.88, NFI=0.95, CFI=0.97, IFI=0.98, RFI=0.94, RMSEA=0.08, and SRMR=0.05). Result of the factor analysis are presented in Figure 2.

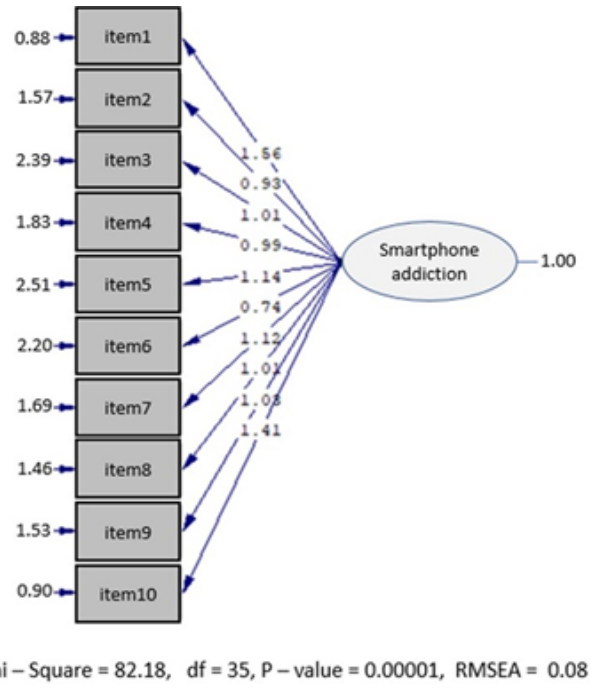

Figure 2. *Factor loadings for the THAI-SAS-SV for school students between 10 to 18 years..*

Internal consistency reliability: results of internal consistency reliability showed the overall Cronbach's alpha coefficient was 0.85 for the ten items in the Thai version of SAS-SV for school students. The corrected item total correlation coefficients ranged from 0.399 to 0.717. The deletion of any individual item would have decreased the internal consistency of the instrument. This result reflects that all the items of THAI-SAS-SV for school students had good relationships.[21,25-26] The values of corrected item-total correlation and Cronbach's alpha if item deleted are shown in Table 3.

Table 3 Item-total Correlation and Cronbach's alpha if item deleted of THAI-SAS-SV for school students between 10 to 18 years (N=200).

Item content	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
1) การใช้สมาร์ตโฟน ทำให้ฉันพลาดงาน หรือ กิจกรรม ที่วางแผนไว้	0.717	0.820
2) การใช้สมาร์ตโฟน ทำให้ฉันไม่มีสมาธิในขณะเรียน ขณะทำแบบฝึกหัด ขณะทำกิจกรรม หรือ ขณะทำงาน	0.497	0.840
3) ฉันรู้สึกปวดข้อมือหรือด้านหลังคอ ขณะใช้สมาร์ตโฟน	0.495	0.840
4) ฉันรู้สึกทนไม่ได้ เมื่อไม่มีสมาร์ตโฟน	0.527	0.837
5) ฉันรู้สึกกระวนกระวายและหงุดหงิด เมื่อไม่ได้พกสมาร์ตโฟน	0.512	0.839
6) ฉันมักนึกถึงสมาร์ตโฟนอยู่ในใจของฉัน แม้ว่าไม่ได้ใช้มันอยู่ก็ตาม	0.399	0.849
7) ฉันจะไม่ยอมเลิกใช้สมาร์ตโฟน แม้มันจะกระทบต่อการใช้ชีวิตประจำวันของฉันอย่างมากแล้วก็ตาม เช่น กิจวัตรประจำวัน หรือ การเรียน	0.546	0.836
8) ฉันเช็คสมาร์ตโฟนอยู่เสมอ เพื่อไม่ให้พลาดการพูดคุยสนทนากับคนอื่นๆ ผ่านทางไลน์ เฟซบุ๊ค อินสตาแกรม หรือ ทวิตเตอร์	0.548	0.836
9) ฉันใช้เวลากับสมาร์ตโฟน นานกว่าที่ฉันตั้งใจไว้	0.565	0.834
10) คนรอบ ๆ ตัวฉัน เช่น ผู้ปกครอง พี่น้อง ครู หรือ เพื่อน บอกฉันว่า ฉันใช้สมาร์ตโฟนมากเกินไป	0.691	0.822

Test-retest reliability: the analysis of the repeatability results demonstrated that THAI-SAS-SV for school students exhibited moderate reliability, indicated by the ICC value of 0.73 (95% CI; 0.62-0.81).[28]

Discussion

The present study determined the validity of THAI-SAS-SV instrument for use with smartphone addiction screening in Thai school students between 10 to 18 years. Due to the increasing number of smartphone users, particularly Thai youth, a brief questionnaire for screening smartphone addiction should also be developed in addition to Facebook and game addiction questionnaire. In this study, SAS-SV was selected to translate and culturally adapt to Thai language since number of items is relatively less than other instruments. It is easy for school children to complete the score with themselves in a short period of time (on average, answer all questions in 5 minutes). As youths generally have short attention spans[29], it seems suitable to use THAI-SAS-SV for screening smartphone addiction in this age group. Overall, THAI-SAS-SV for school students was shown to be reliable and valid in the Thai language. In addition, the participants during pilot testing and the main study reported that they well understood THAI-SAS-SV and the rating score of each item was fairly simple.

This study is the first attempt to cross-cultural adaptation concerning the use of this instrument with children and adolescents and investigate the usefulness of THAI-SAS-SV in primary school students together with high school students. According to a study by Kwon et al[9], SAS-SV was developed from SAS which consisted of 31 items.[18] The mean age of participants in the present study is relatively lower than that in previous studies.[8,10-12,14-15] Although some previous studies have shown that the translated versions of SAS-SV are valid and reliable, most participants were over the age of 18 years. Ideally, the principal goal of development was to produce the short form of SAS for cooperating in adolescents. The original SAS-SV was validated in second-year junior high school students from two schools, and the average age of those participants was 14.5 years. Correspondingly, the mean age of participants in the present study is similar to the previous one. In addition to using the same number of instrument items (ten) as in SAS-SV, THAI-SAS-SV school students incorporates instructional guidance and facial expressions of agreement to assist participants' comprehension. According to the findings, all components of THAI-SAS-SV for school students might offer the user an accurate understanding. This might reflect that THAI-SAS-SV for children and adolescents can be used for early detection of smartphone addiction.

In the current study, THAI-SAS-SV for school students showed excellent content validity (I-CVI 0.86 to 1.00, S-CVI 0.97). The mean I-CVI in this study was a little higher than that (mean I-CVI 0.94) in the original study.[9] Content validity helped assess whether the content was relevant to the concept of smartphone addiction defined for the study. The number of experts in this study is equal to the number of experts (expert committee of seven persons) in the study by Kwon et al.[9] No previous study examined the content validity of the translated version. As a result, our findings provide much more comprehensive testing than the previous studies with respect to all translated versions. In addition to content validity, we also investigated construct validity via factor analysis to ensure validity. Factor analysis assessed

the theoretical construct of THAI-SAS-SV for school students. The results indicated that THAI-SAS-SV in this study had only one dimension which was similar to the results for the original version.[9] The one factor was also consistent with other translated versions,[11,13,15] which supported that THAI-SAS-SV for children and adolescents can be used across cultures.

Regarding internal consistency, the Cronbach's alpha (Cronbach's α=0.85) reached the recommended level for clinical use because THAI-SAS-SV in this study showed good internal consistency. Cronbach's alpha coefficient was higher than 0.70, reflecting homogeneity of items. Importantly, Cronbach's alpha coefficient in our study was lower than 0.95, and thus redundancy was not an issue. In addition, the corrected item-total correlation for all items was higher than 0.30, which reflected their homogeneity. In previous studies, internal consistency reliability was examined to indicate the reliability of the translated version of SAS-SV. As in all other translated versions, the internal consistency reliability of THAI-SAS-SV was slightly lower than the other languages. The Cronbach's alpha coefficient for total instrument of THAI-SAS-SV for school students was high (α=0.85), and was fairly similar with the original (α=0.91)[9], THAI-SAS-SV for university students (α=0.94)[8], German (α=0.85)[10], Spanish (α=0.88), French (α=0.90)[11], Arabic (α=0.84)[12], Turkish (α=0.88)[13], Italian (α=0.79)[14], and Chinese (α=0.84).[15]

Regarding repeatability, the test-retest reliability of THAI-SAS-SV for school students was moderate (ICC=0.73). To the best of our knowledge, only one study has investigated test-retest reliability of a translation of SAS-SV. The test-retest reliability of the THAI-SAS-SV in this study was fairly comparable with the Chinese version of SAS-SV (ICC=0.76).[15] Nevertheless, the Chinese version of SAS-SV was examined for repeatability, given its set of adults participants of age 18 to 65 years. In the present study, the results are specific to participants of age 10 to 18 years. Therefore, our results indicate the stability of the responses to the items on THAI-SAS-SV over time even using the instrument with children and adolescents.

The current study has several strengths and limitations. First, the current study cooperates with experts and specialists from different locations to minimize bias. Second, experts engaged in this study comprised professionals with direct work experience with children and adolescents. Third, use of five content experts is commonly considered adequate for assessing content validity in developing clinical instruments. In this work, THAI-SAS-SV was examined for content validity by seven experts with experience in working with psychiatric patients. Finally, the study's collection of data used an adequate sample size according to the simple random sampling method. Although we attempted to establish a rigorous translation process followed by comprehensive testing of the newly translated questionnaire, we acknowledge that the present study had some limitations. To ensure the consistency of the amount of smartphone usage, screen time should be recorded. In this work, the participants were recruited from five schools; however, the data was collected from students living in two provinces in northern Thailand, thus limiting generalization to other populations. To gain a broader understanding of THAI-SAS-SV for school students between 10 to 18 years, the instrument should be tested in various settings. Multicenter collaboration that includes all regions of Thailand should be considered in future studies. Further studies are necessary to consider whether socio-demographic characteristics are related to the smartphone addiction level score such as socio-economic state and family environments. In addition, data collection was performed during the final examination period in the study participants' various schools. An improvement to the current study would be to conduct the questionnaire during a class period or semester break. Future studies might be aimed toward investigating whether THAI-SAS-SV demonstrates concurrent validity. Importantly, the cut-off scores used for Thai version of SAS-SV were based on the Korean version of the instrument that conducted Receiver Operating Characteristic (ROC) analysis using a sample of teenagers from Korea. Hence, statistically supported cut-off scores for screening smartphone severity in Thai school students between 10 to 18 years should be investigated in future studies. Separately, it might be worthwhile to develop a parent version of THAI-SAS-SV for combination with THAI-SAS-SV for children and adolescents in future research.

Conclusion

In summary, this study translated and cross-culturally adapted the-SAS-SV into Thai. This study also provides robust evidence that THAI-SAS-SV for school students between 10 to 18 years is a valid and reliable, self-administered tool that can be used for screening smartphone addiction in Thai youth. Our results suggest that the Thai version of SAS-SV may assist in early detection of problematic use of smartphones in Thai children and adolescents.

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Conflict of interest

The authors declare no conflicts of interest.

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Fully automatic left ventricular ejection fraction evaluation in magnetic resonance images using patch-based clustering technique

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Left ventricular ejection fraction (LVEF), magnetic resonance imaging (MRI), patch-based clustering, Fuzzy C-means clustering (FCM), mathematical morphology

ABSTRACT

Background: Left Ventricular Ejection Fraction (LVEF) has been used in evaluating cardiac function. It is calculated generally by the difference between end-diastolic volumes (EDV) and end-systolic volumes (ESV). In order to obtain the EDV and ESV from magnetic resonance imaging (MRI), an experienced cardiologist is required to select the smallest and largest axial images from 30 phases in each slice that represent the ESV and EDV, respectively. This process is time consuming and could have individual-dependent variability.

Objectives: To develop an algorithm that determines the EDV and ESV, and automatically calculates the LVEF as an output in MRI in order to reduce operation time and interobserver variability for the user.

Materials and methods: Fifteen thalassemia patients were recruited into this study. Image data were acquired using a 1.5 Tesla MRI scanner. Scanning protocol included field of view (FOV) of 300 mm, matrix size of 256×256, slice thickness of 8 mm, pixel spacing of 1.25 mm, and repetition time (TR) and echo time (TE) of 3.83 and 1.69 ms, respectively. The proposed algorithm included 3 steps; left ventricle (LV) segmentation, end diastolic (ED) and end systolic (ES) phase selection, and LVEF calculation. The LV region was segmented by using the patch-based clustering method. Shape of the LV was tuned by mathematical morphologies and the Gaussian filter. The processes were repeated for all phases. The ED and ES phases were selected from those that had the maximum and minimum number of pixels, respectively, in the LV area. The EDV and ESV were the sum of the number of pixels in the segmented LV areas of the ED and ES phases, which were multiplied by pixel spacing and slice thickness from all slices. Finally, the LVEF was calculated and reported.

Results: Precision and recall were used to evaluate segmentation performance, which was good in the experimental results of the proposed method. Precision and recall were on average approximately 0.9 and 0.7 for the ED and ES phase, respectively. The different percentage values of the LVEF was 3.32% between the proposed and manual segmentation method.

Conclusion: This work proposed automatic LVEF evaluation from MRI. Patch-based clustering techniques, mathematical morphologies and the Gaussian filter were used to segment the LV area automatically. Experimental results showed that the proposed method could evaluate LVEF values that were close to those from two experts who used the manual segmentation method.

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Introduction

A normal cardiac cycle includes the diastole, systole, and intervening pause. Frequency of the cardiac cycle relates to the heart rate, which is expressed regularly as beats per minute. There are many indicators for checking cardiac activities, for example, heart rate (HR), stroke volume (SV), cardiac output (CO), heart sounds, electrocardiogram (ECG), and ejection fraction (EF).[1,2]

This work focused on the EF. There are many ways to evaluate the EF such as echocardiography, multi gated acquisition scan (MUGA scan), left ventriculography, computed tomography (CT), and magnetic resonance imaging (MRI).[1,2] Patients do not need any radio activities or contrast media for MRI. Therefore, MRI was used for evaluating the EF in the left ventricle (LV), which is called the left ventricular ejection fraction (LVEF) in this research.

The main process of this work originally involved segmenting the LV manually by an expert, which was time-consuming with high interobserver variability. Therefore, automatic segmentation methods were proposed.

Segmentation of the LV in short axis MRI is popular and challenging. The endocardial and epicardial contours are segmented by many semi and fully automatic algorithms. In 2011, a survey paper proposed LV segmentation algorithms in short axis cardiac MRI.[3] Examples of segmentation methods and their improvement or hybrid methods are thresholding, region growing,[4,5] dynamic programming (DP),[6] deformable models,[7] active contour models (ACM),[8,9] level-set,[10,11] graph cuts,[7] K-nearest neighbor (KNN) classifier,[12] convex relaxed distribution matching,[13] edge modelling,[11,14] Gaussian-mixture model,[15] marker-controlled watershed,[16] and convolutional neural network (CNN) regression.[17]

Some works have tried to evaluate the LVEF automatically such as a study by Alain Lalande et al.,[18] which proposed a method for calculating the LVEF from automatically selecting and processing diastolic and systolic frames in short-axis cine-MRI. They used fuzzy set theories and dynamic programming to detect the cardiac contours on both diastolic and systolic slices. After that, the endocardial surface area on each slice was calculated in order to compute the end diastolic volume (EDV) and end systolic volume (ESV). Finally, the LVEF (%) was calculated. Ying-Li Lu et al.[19] proposed the automatic functional analysis of the LV in cardiac cine MRI such as the EDV, ESV, LVEF and Left ventricle mass (LVM) using the optimal threshold method of Otsu, watershed, and 1D fast Fourier transform (FFT) method in their segmentation algorithm. In order to evaluate the segmentation performance, they used four quantitative measures; average perpendicular distance (APD), dice metric (DM), LV location and t-test statistics. These measured the difference between the contour of endocardial and epicardial end diastolic (ED) and end systolic (ES) phase of all slices from the proposed automatic segmentation and ground truths (manual LV contour delineation).

Two previous works proposed the evaluation of automatic LVEF calculation in MRI, but they still had user interventions. In reference,[18] the user must indicate a point near the center of the LV on the first processed image. In reference,[19] the user must specify the phase in the middle slice before beginning the segmentation algorithm.

This work proposed fully automatic LVEF evaluation, in which the ED and ES phases were selected, and the LV was segmented automatically to calculate LVEF without user intervention. Performance of the proposed segmentation method was reported by precision and recall and compared to the manual segmentation method used by two experts. Finally, LVEF values from the automatic and manual methods were compared.

Materials and methods

1. Studied population and MRI Protocol

Data from fifteen thalassemia patients were analyzed retrospectively (7 females, 8 males; mean age: 14.60±3.85 yrs.). The magnetic resonance (MR) images used in this work were acquired on a 1.5 Tesla Achieva Philips MRI scanner, installed at the Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Thailand. The acquisition parameters of this sequence were: repetition time (TR) at 3.54 ms, echo times (TE) at 1.77 ms; flip angle of 60°; matrix size of 256×256; field of view (FOV) at 320 mm × 320 mm; spacing between slices of 8 mm; contiguous sections of 10-15; and number of phases, 30.

2. Manual processes by the experts

LV contours were drawn for the slices in the selected ED and ES phases by two experts. These manual contours were defined as the ground truth for evaluating performance of the proposed automatic method. After that, the LVEF was calculated, which is explained in the next section.

3. LVEF calculation from cardiac MRI

EF measurement determines how well the heart pumps out blood with each heartbeat. It can be used to diagnose and track heart failure and be calculated from both the LV and right ventricle (RV). Blood from the RV is ejected via the pulmonary valve into the pulmonary circulation, and that from the LV is ejected via the aortic valve into the cerebral and systemic circulation. In most cases, the term ejection fraction refers to the LVEF. A normal heart ought to pump little more than half of its blood volume with each heartbeat. Table 1 shows the ranges of EF measurement and their meanings.[2]

Table 1 The ranges of EF measurement.

LVEF Measurement (%)		Meaning
Male	Female	
52-70	54-74	Normal
41-51	41-53	Mildly abnormal
30-40	30-40	Moderately abnormal
<30	<30	Severely abnormal

The ejection fraction is defined by the following equation,

$$\text{LVEF}(\%) = \left(\frac{\text{EDV} - \text{ESV}}{\text{EDV}}\right)\times 100\,, \qquad (1)$$

where EDV means the volume of blood within a ventricle before immediate contraction. ESV means the volume of blood remaining in a ventricle at the end of contraction. Therefore, the difference between EDV and ESV represents the stroke volume (SV), which means the volume of blood pumped from one ventricle of the heart with each heartbeat.

This work calculated the LVEF from MR images. Therefore, the area of the LV must be selected manually by an expert in ED and ES phases. The EDV and ESV can be computed by the following equations:

$$\text{EDV} = \sum_{i=1}^{N}\left(\text{Dias\_LV pixels}_i \times \text{Pixel spacing} \times \text{Spacing between slices}\right), \qquad (2)$$

$$\text{ESV} = \sum_{i=1}^{N}\left(\text{Sys\_LV pixels}_i \times \text{Pixel spacing} \times \text{Spacing between slices}\right), \qquad (3)$$

where Dias_LV pixelsi and Sys_LV pixelsi are the number of pixels in the LV selected in ED and ES phases for slice i, respectively. Pixel spacing is the ratio between the FOV and matrix size (FOV/matrix size). Spacing between slices is the space between each slice in total N slices. Finally, the LVEF can be calculated by equation (1).

As mentioned previously, the area of the LV must be selected manually by an expert before the LVEF is calculated, which requires much time and experience. Therefore, automatic LV selection was proposed in this work. The method is described in the next section.

4. Patch-based clustering and over-segmentation

To automatically select the area of the LV, patch-based clustering, and over-segmentation[20] were used. Patch-based clustering and over-segmentation were segmentation methods suitable for the inhomogeneous area. They made more sense than pixel-based segmentation techniques (such as conventional fuzzy c-means (FCM) clustering[22]) since they mimic how human complete segmentation of complicated regions in medical images. The original image for this work was segmented into 10 clusters by conventional FCM clustering. After that, patches in the over-segmented image were combined into two clusters, objects, and background, by checking the prototypes of FCM.

4.1. Slice selection to start the automatic process

As the cardiac MR images used were a series to calculate the LVEF, the middle slice in the image series should be selected to start the automatic process. It was assumed that the LV in the images should have a clear edge in the middle slice, as shown in Figure 1.

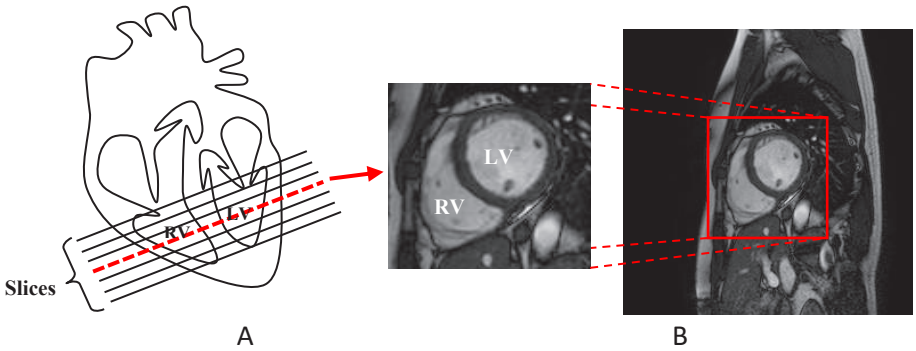


Figure 1. *Slice selection for starting the process. A: selected slice (dash line) in the 4-chamber view of heart, B: selected slice in the short axis view.*

Figure 1A shows the 4-chamber view; the red line is the middle slice selected to start the automatic process. Example of the MR image in the selected slice in the short axis view is shown in Figure 1B, which shows that the edge of the LV is clear. Therefore, it was applied easily in the segmentation process.

4.2. Human body segmentation

Body segmentation was the first segmentation procedure. If the prototypes of the patches were less than 90% of the mean of all prototypes, then the patches were labelled as background. On the other hand, if the prototypes were more than 90% of the mean of all prototypes, they were labelled as the human body. Figure 2 shows the patch-based method and over-segmentation technique applied for human body segmentation.

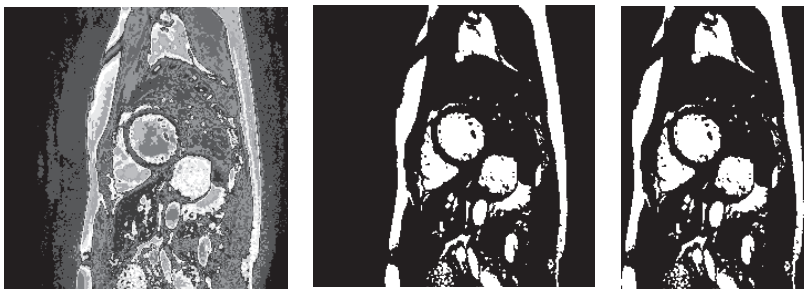


Figure 2. *Processes for human body segmentation; A: over-segmented image (10 clusters), B: binary image (2 clusters), C: Some background has been removed to reduce calculation time.*

4.3. Left ventricle (LV) segmentation

After the human body was segmented and unnecessary parts were removed, as shown in Figure 2C, the LV was selected. The processes are described below:

1) To remove the objects touching the borders of the image by assuming that the LV should not touch them. The result is shown in Figure 3A.

2) To remove over-large and over-small objects by checking the number of pixels in them. If the number of pixels in the objects were more than 2,500 or less than 500, they were removed. The specified numbers of pixels were obtained by testing in multiple images. The result of this process is shown in Figure 3B. Only 3 objects remained in the image.

3) In general, the LV should have a round shape, and the radii from the centroid to the edge of the object should be the same length. The LV was selected by considering the standard deviation (SD) of these radii, as shown in Figure 3B. The LV was chosen from the object that had the smallest SD of the radii, which meant that the object was mostly circular when compared to the others. The image that had the LV only is shown in Figure 3C.

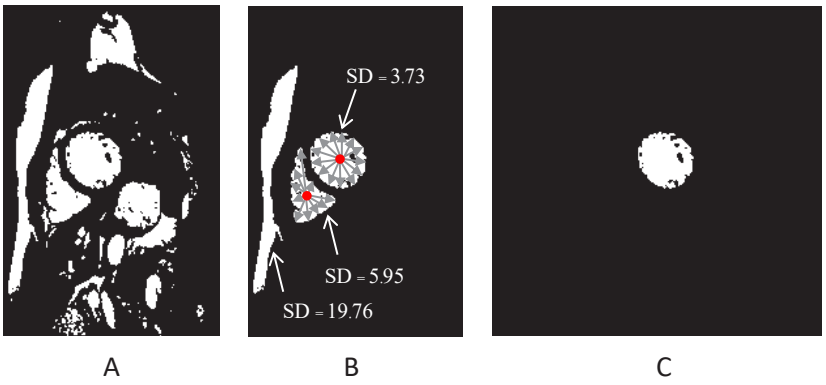


Figure 3. *Process for selecting the LV; A: image with objects touching image borders rejected, B: image with over-large and over-small object was rejected before SD of the objects was calculated, C: LV selected from the object with the smallest SD.*

4.4. End-diastolic (ED) phase and End-systolic (ES) phase selection

The selection of ED and ES phase in each slice of MR images was described in this topic. The ED phase occurred immediately before the ventricle started to contract or the phase in which the heart expanded to the extreme. Therefore, the LV area should be the largest one or have the maximum number of pixels. The ES phase occurred at the end of contraction or the phase in which the heart shrinks to the extreme. Therefore, the LV area should be the smallest one or have the minimum number of pixels.

The processes for selecting the ED and ES phase are described below:

1) In order to reduce the calculation time from the area of LV in Figure 3C, a sub window (red square) was created by expanding it from the four edges of the LV (dotted yellow square), as shown in Figure 4. Then, the sub window was used to find the LV area in the MR images in every phase.

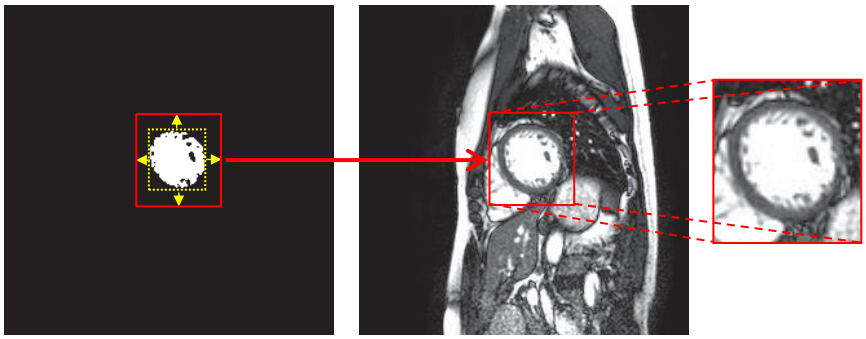


Figure 4. *Process for creating the sub window.*

2) Patch-based clustering and over-segmentation techniques were used again in this step for segmenting the area of the LV in every phase. Then, the mathematical morphologies, including opening and closing,[23] were used to fill the holes and also reject segmentation errors in every phase. The results are shown in Figure 5.

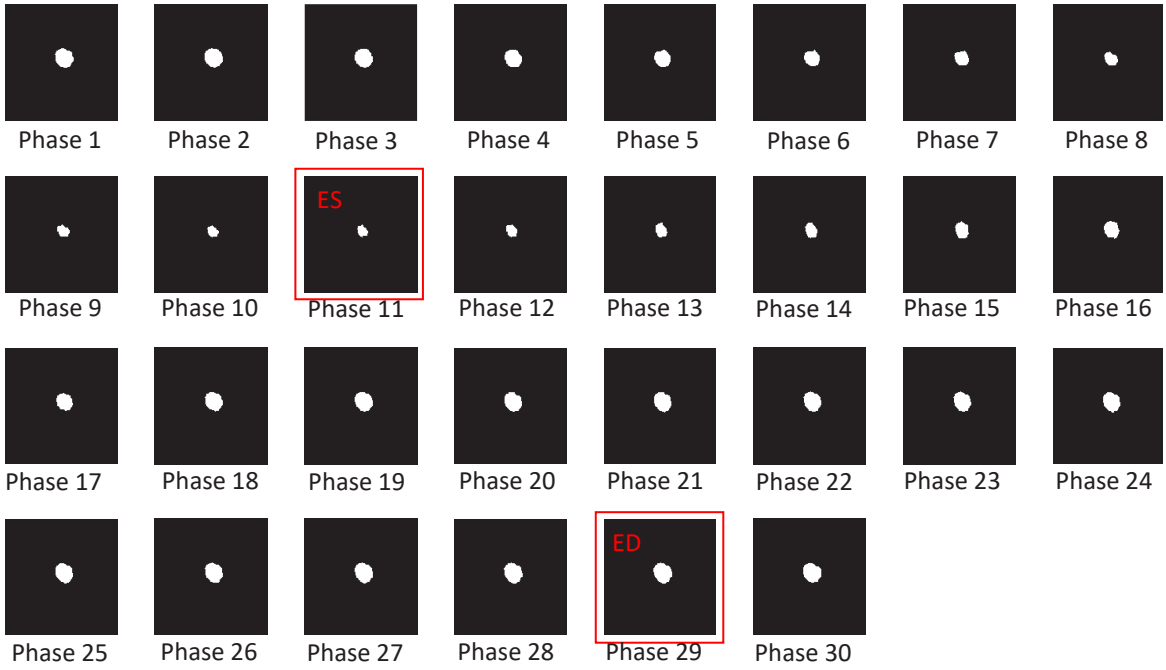


Figure 5. *Area of the LV in every phase in a slice.*

3) Then, the phase that had the maximum number of LV pixels was selected as the ED phase; and that which had the minimum number of LV pixels was selected as the ES phase. Phase 29 and 11 were selected as the ED and ES phase, respectively, as shown in Figure 5.

4.5. LV segmentation processes for the remaining slices

After the LV in the ED and ES phase was received in the slice used to start the automatic process, as described in topics 3.1-3.4 (e.g. slice number 5 from all 10 slices), its size and position was used to find it in the ED and ES phase in the remaining slices (slice numbers 1-4 and 6-10). On the assumption that the ED and ES phases should not be near to more than one slice in the remaining slices, the sub window was created from the size and position of the LV in the slice, which started to find the LV in the ED phase in the remaining slices. Over-segmentation, patch-based clustering and mathematical morphologies were used again to segment the LV. Then the phase that had maximum LV pixels was selected as the ED phase. The processes are shown in the diagram in Figure 6.

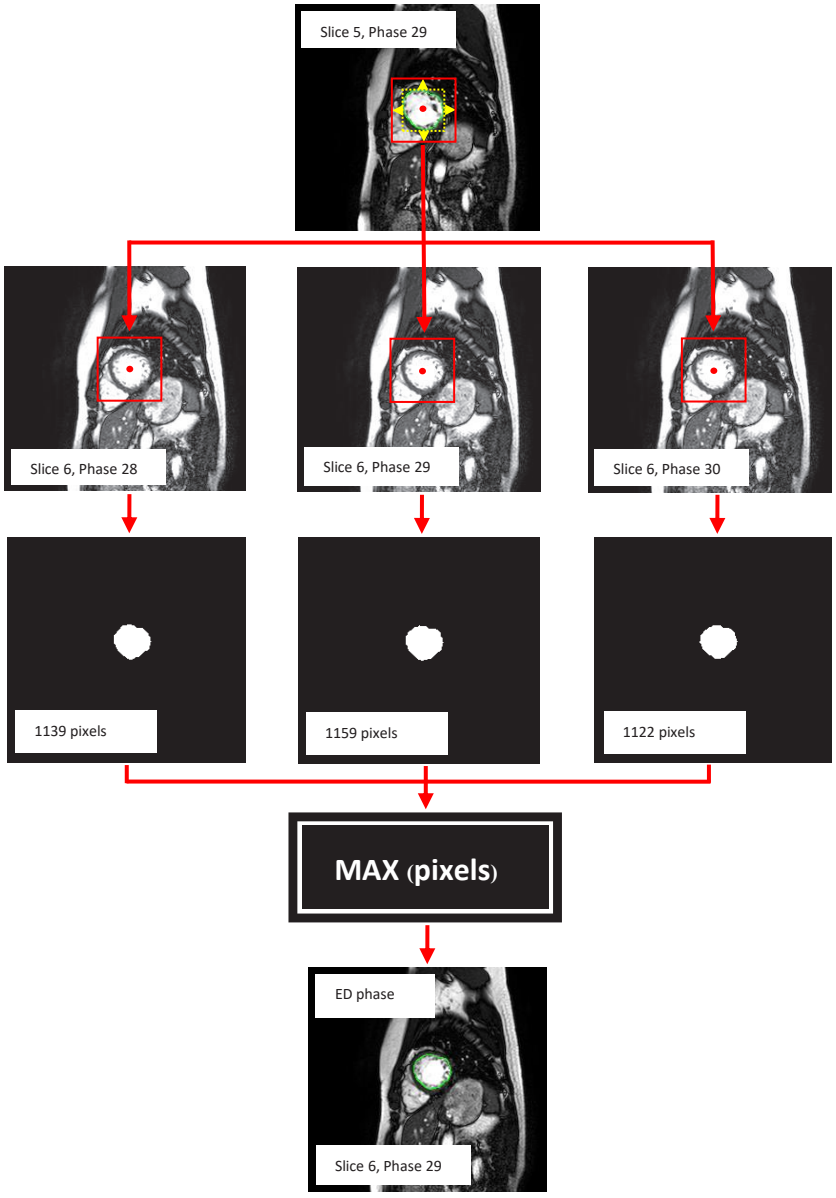


Figure 6. *Processes for finding the LV in ED phase of the remaining slices (i.e. slice number 6).*

Regarding LV segmentation in the ES phase in the remaining slices, segmented LV in the ED phase in the same slice was dilated by the structuring element or se = 5 pixels. This area was used to find the LV in the ES phase by processing in the same manner as that for finding the LV in the ED phase, as shown in the diagram in Figure 7. A small difference was apparent in finding minimum instead of maximum LV pixels.

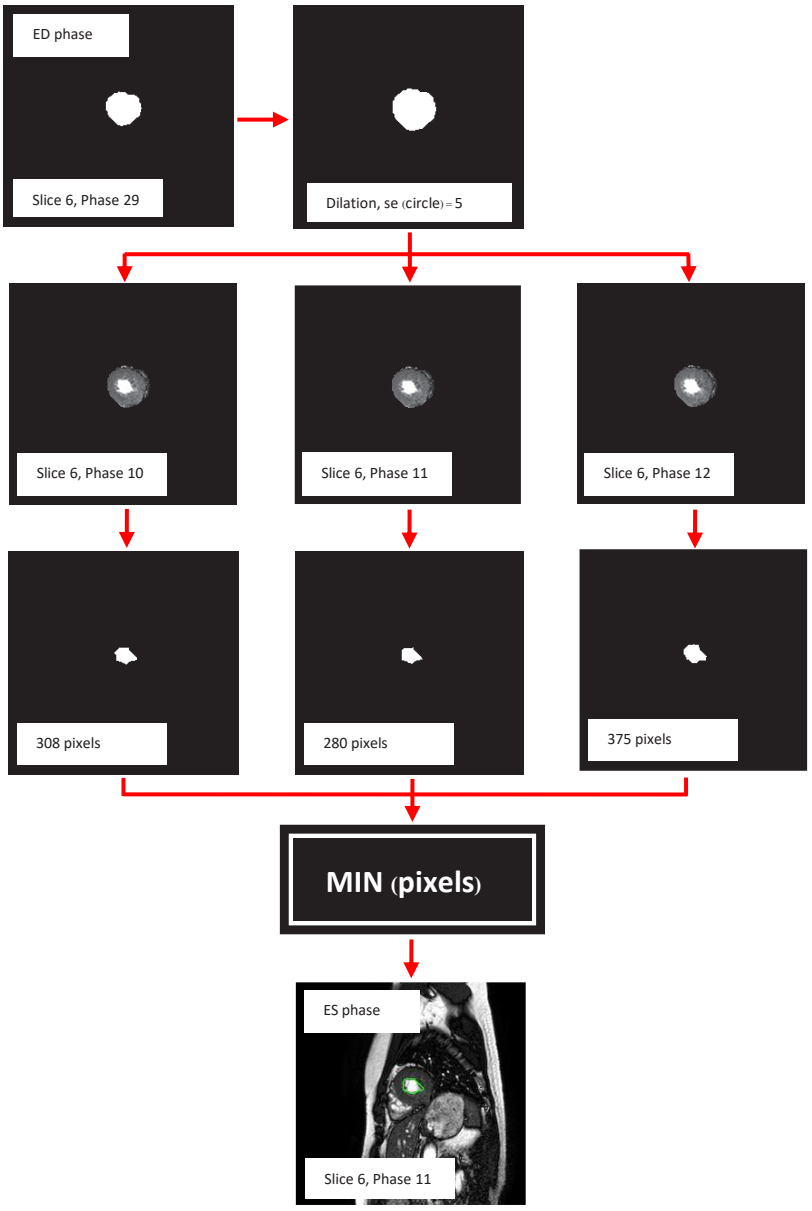


Figure 7. *Processes for finding LV in ES phase in the remaining slices.*

Moreover, this system can reject the objects in which the number of pixels is less than 20, the objects touch the edges of the sub window (for finding the LV in the ED phase) and the edges of the LV are dilated (for finding the LV in the ES phase). The objects are segmented by this method, but they are not the LV area, as shown in Figure 8A and 8B.

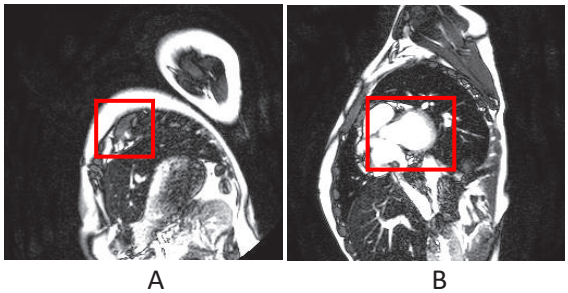


Figure 8. *Examples of segmented objects rejected by this system; A: there was no LV area in the image, B: myocardium was not clear which mean no LV in image.*

5. Endocardial Contour Smoothing by the Gaussian filter

As the boundaries of segmented LV from topic 3 were not smooth as shown in Figure 9A, the contour smoothing process was applied to solve this problem by using the Gaussian filter,[21] which is a low-pass filter applied to reject high frequency parts that do not smooth the signal.

The position of every pixel in the contour for this work was defined in order to calculate the angle from the centroid along the radius of the LV. Then the Gaussian filter was applied to smooth the plot of the Radius. Figure 9C shows the plot between angles and the position of pixels in the contour, including before and after smoothing. Finally, the smoothed contour was transformed back to LV shape, as shown in Figure 9B.

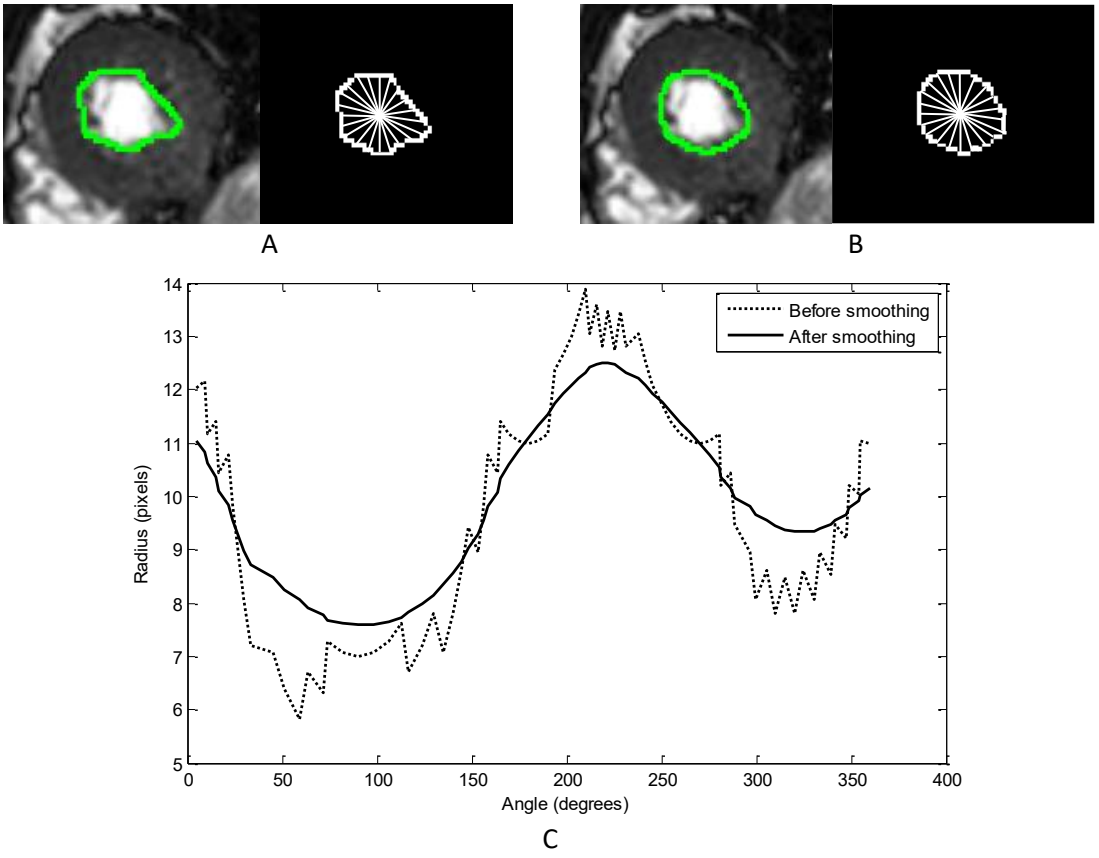


Figure 8. *Endocardial Contour Smoothing. A: unsmoothed contour, B: smoothed contour by Gaussian filter, C: plot between angles and the position of pixels in contour.*

6. Evaluation of segmentation performance

In order to measure the performance of segmentation, precision and recall were used in this work, as shown in equation (4) and (5), respectively

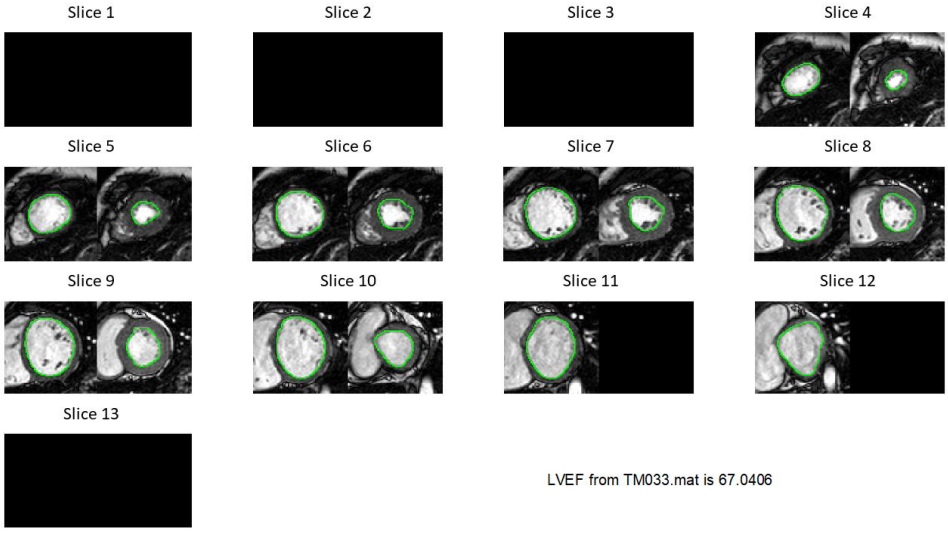
$$\text{Precision} = \frac{\text{TPs}}{\text{TPs} + \text{FPs}}, \quad (4)$$

$$\text{Recall} = \frac{\text{TPs}}{\text{TPs} + \text{FNs}}, \quad (5)$$

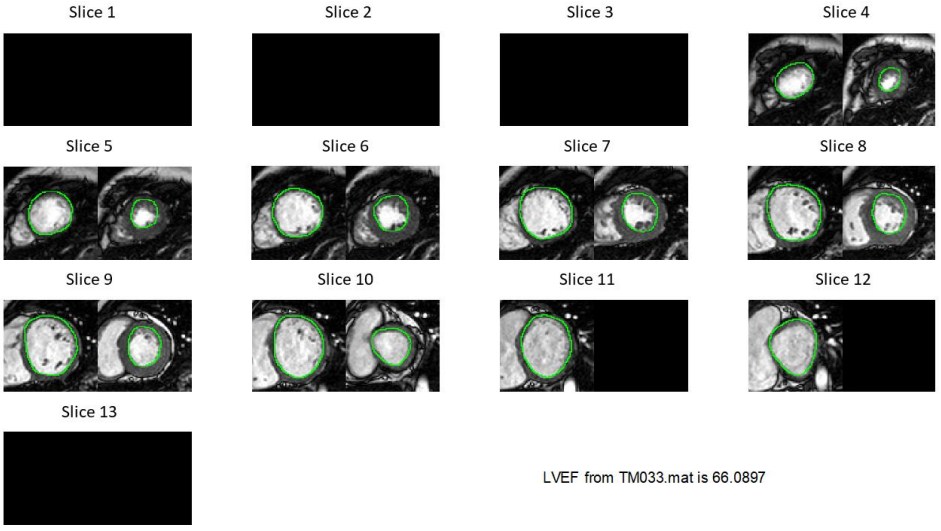
where TPs is the true positive, with the number of pixels correctly segmented by the proposed method. FPs is the false positive, where the number of segmented pixels is not in the ground truth (the pixels that were segmented manually by the experts). FNs is the false negative, where the number of pixels in the ground truth cannot be detected by the proposed method. Accurate segmentation was achieved when both the values of precision and recall were equal to one.

Results

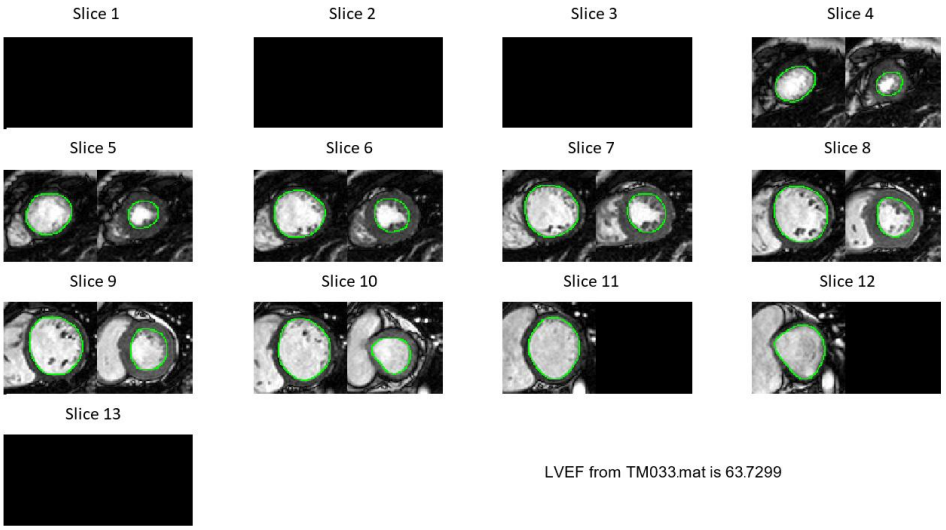
Figure 10A, 10B and 10C show examples of segmentation results from the proposed automatic method, and manual method by expert 1 and 2, respectively.



A



B



C

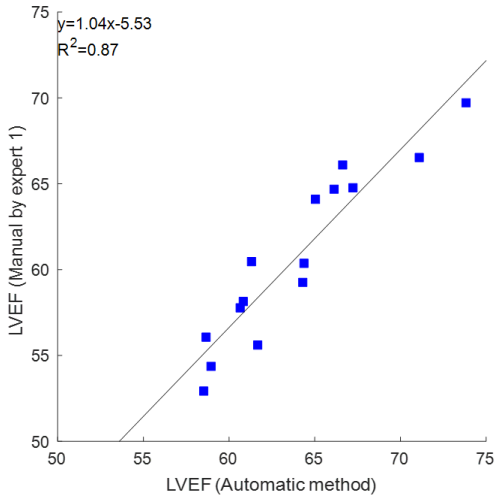
Figure 10. *Examples of the experimental result. A: automatic segmentation results and LVEF calculation by the proposed method, B: manual segmentation results and LVEF calculation by expert 1, C: manual segmentation results and LVEF calculation by expert 2.*

.

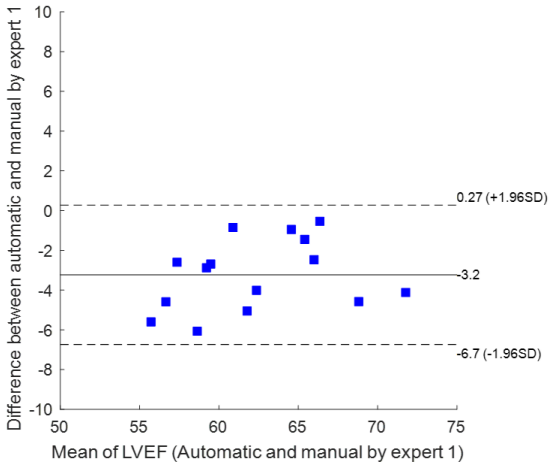
The proposed automatic method was selected the ED and ES phases, which therefore might be different to the manual method used by the experts. This work measured the performance of segmentation, although the selected phases from the proposed method, and the method of the experts, were not same, and their differences were assumed to have little effect. Most of the selected phases had no more than 2-3 differences. The experimental results are shown in Table 2 and Figure 11, which show that the proposed method gave a good segmentation performance. The average precision and recall for the ED phase were approximately 0.95 and 0.9, respectively, and 0.74 and 0.66, respectively, for the ES phase. A good correlation between the automatic and manual method is shown in the plot in Figure 11A and 11C (R2 = 0.87 for comparison with the manual method by expert 1, and R2 = 0.74 for comparison with the manual method by expert 2). The different values of the LVEF between the two experts and the proposed method were -3.23±1.73% and -1.02±2.32%, which indicated that the LVEF was underestimated slightly by the automatic method, as shown in the Bland-Altman plot in Figure 11B and 11D. Most errors between the two methods came from the difference of ED and ES phase selection, and the decision made in selecting the LV area, particularly at the first and last slices of heart volume. In comparison between the two experts, precision and recall in the ED phases was 0.99±0.01 and 0.86±0.02, respectively, and that in the ES phases was 0.78±0.08 and 0.62±0.07, respectively, and the difference in values of the LVEF was 2.21±3.07%, which represented high interobserver variability.

Table 2 Experimental results including mean of precision, recall and the difference of LVEF (Diff LVEF).

Comparison	ED phase		ES phase		Diff LVEF (%)
	Precision	Recall	Precision	Recall	
Automatic vs manual by expert 1	0.97±0.02	0.86±0.03	0.76±0.07	0.59±0.09	-3.23±1.73
Automatic vs manual by expert 2	0.93±0.02	0.94±0.05	0.72±0.06	0.67±0.19	-1.02±2.32
Manual by expert 1 vs manual by expert 2	0.99±0.01	0.86±0.02	0.78±0.08	0.62±0.07	2.21±3.07



A



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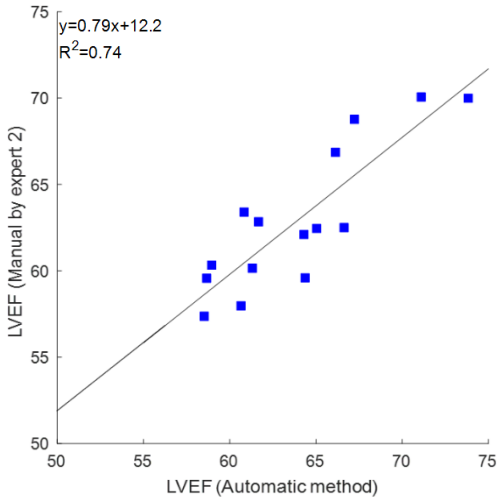

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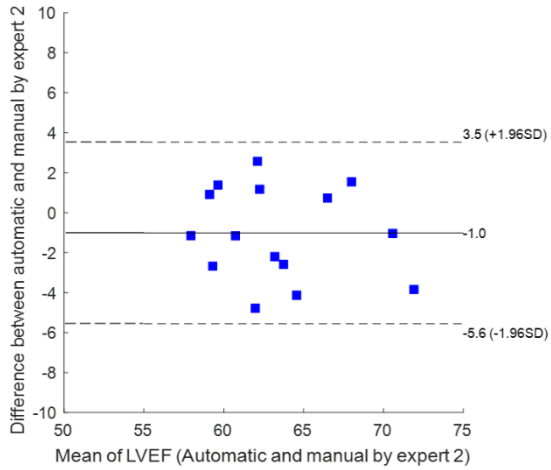

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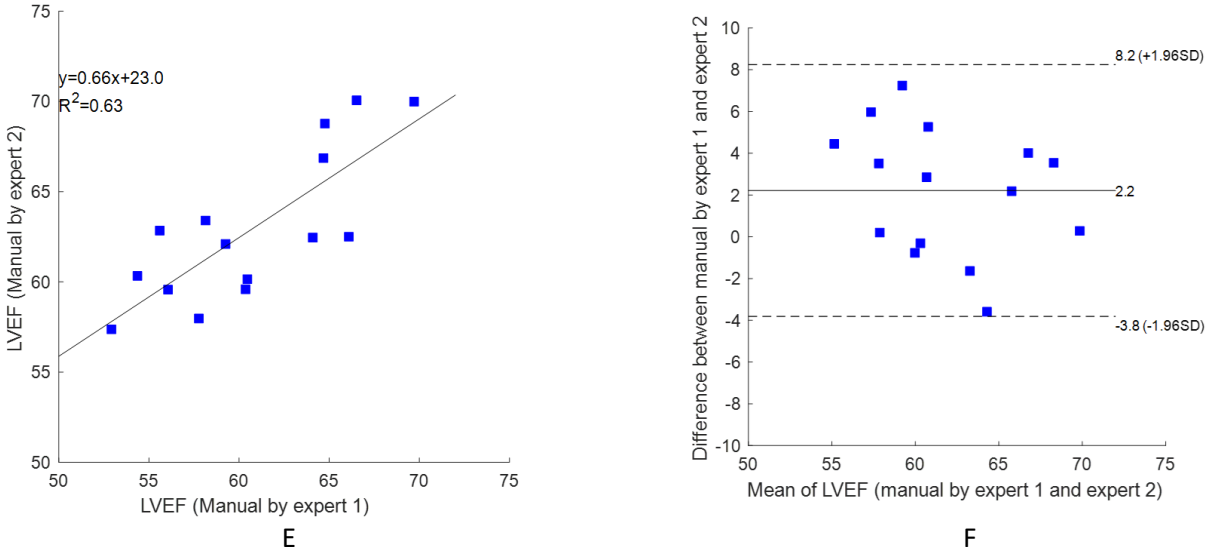

Figure 11. *Comparison of the experimental results. A and C: correlation between the LVEF obtained from automatic and manual method by expert 1 and 2, respectively, B and D: Bland-Altman plot between both techniques, E and F: correlation between the LVEF obtained from manual method by expert 1 and 2, and Bland-Altman plot between the LVEF obtained from manual method by expert 1 and 2, respectively.*

The comparison between the proposed method and previous works is shown in Table 3. Although previous works might have slightly better performance for automatic evaluation of the LVEF, they still have some manual processes, while the proposed method in this work is fully automatic and does not need user intervention.

Table 3 Difference of LVEF values between the automatic and manual method.

Method		Diff LVEF (%)	R^2
Proposed	Compared with expert 1	-3.23±1.73	0.87
	Compared with expert 2	-1.02±2.32	0.74
Alain Lalande et al[18]		2.13±4.30	0.95
Ying-Li Lu et al[19]		0.75±5.93	0.90

Conclusion

Fully automatic LVEF evaluation from MR images was proposed in this work. Over-segmented and patch-based clustering techniques were used to segment the area of LV automatically. Mathematical morphologies and the Gaussian filter were used to eliminate some noise and tune the shape of the LV. The experimental results showed that the proposed method can evaluate the LVEF values closely with the experts. Future works should improve the algorithm for segmenting the LV in the ES phase and find a better algorithm for detecting the LV in the beginning and end of the slices

Conflict of interest

The authors declare no conflict of interest.

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The radioprotective potential of *Centotheca lappacea* (L) desv. extract in human endothelial cell

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ABSTRACT

Background: Radiation-induced vascular injury in normal tissue is a common adverse effect of radiation therapy. Radioprotectors can reduce the adverse effects of radiation-induced vascular injury. However, therapeutic applications of current radioprotectors are limited due to toxicity. Natural compounds derived from medicinal plants are less toxic and favorable for developing of radioprotectors. *Centotheca lappacea* has been long-term used for medicinal purposes and contains a variety of biological active compounds. Thus, the ethanolic extract from *Centotheca lappacea* may possibly have the potential to act as radioprotector.

Objectives: To determine radioprotective property of the ethanolic extract from *Centotheca lappacea* in human endothelial cell (EA.hy 926).

Materials and methods: The effect of the ethanolic extract from *Centotheca lappacea* on cell viability was assessed by MTT assay. Cell cycle distribution was determined by flow cytometry. Nuclei morphology was determined by fluorescence microscopy. The levels of Akt and phospho-Akt were determined by Western blot analysis.

Results: Pretreatment of cells with 0.2 µg/ml *Centotheca lappacea* extract for 3 hrs prior to irradiation demonstrated a radioprotective effect on EA.hy926 cells by significantly increasing cell viability and decreasing abnormal nuclei formation. Treatment of the cells with the extract in combination with radiation clearly increased the level of Akt phosphorylation. The extract is not toxic and does not interfere with cell cycle progression of EA.hy926 cells.

Conclusion: The ethanolic extract from *Centotheca lappacea* possesses radioprotective activity. No toxic impact and no impact on cell cycle progression in EA.hy926 cells were observed. The results indicate that *Centotheca lappacea* extract is an ideal resource of radioprotector for protecting radiation-induced damage to human endothelial cells.

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Introduction

Radiotherapy is one of the most effective cancer treatment modalities. The goal of radiotherapy is to maximize the killing effect of radiation on tumor cells and to minimize radiation damage to normal surrounding tissues.[1, 2] Although advanced technologies provide a greater precision of radiation delivery to tumor lesions, radiation toxicity in normal tissues remains problematic.[2] Radiation–induced vascular injury is one of the most common adverse effects of cancer radiotherapy. Radiotherapy primarily damages endothelial cells of blood vessel within the irradiation field.[3, 4] Such damage initiates vascular events like stenosis, thrombotic or fibrotic occlusion, which leads to the reduction of blood flow to normal organs of the damaged area.[3-5] The severity of clinical manifestation from vascular injury depends on organ functions and the extent of the injury. Frequently, quality of life of patients decreases due to a partial loss of function of an effected organ. Life-threatening complications such as stroke and aortic rapture can occasionally develop.[3-6]

Radioprotective agents can be used in combination with radiation to reduce radiation damage in normal tissues. These agents are given to cancer patients before or at the time of irradiation.[7] However, therapeutic application of radioprotectors is limited due to their toxicity.[7, 8] Natural compounds derived from plants have been reported to have radioprotective activities, low toxicity and do not manifest adverse effects.[9] Therefore, it can be expected that some Thai local medicinal plants may have a radioprotective potential against radiation–induced vascular injury. *Centotheca lappacea*, a perennial tufted grass, belongs to the family of *Poaceae*.[10] It has been long term used in traditional medicine in Thailand and many Southeast Asia countries for wound healing and muscle tightening in post labor women.[11, 12] Drinking of an infusion prepared by boiling of *Centotheca lappacea* in water has been purposeful for body nourishing.[11, 12] *Centotheca lappacea* extract contains phenolic compounds, such as catechin, gallate, rosmarinic acid, epigallocatechin, gallate and gallic acid.[13] It also contains flavonoids, such as flavonoids fatty acids, triterpenes and phytosterols, which have antioxidative and anti-inflammatory properties.[14] Moreover, it contains 4-coumaric acid, which has been reported to protect rabbit corneal cells from UVB-induced DNA damage.[14, 15]

Summarizing, *Centotheca lappacea* extract contains a variety of biological active components, such as anti-oxidants and anti-inflammatory substances, which may facilitate cellular repair activity and tissue regeneration. It can be expected that it may have radioprotective activity against radiation-induced vascular injury. Therefore, the radioprotective potential of *Centotheca lappacea* extract on human endothelial EA.hy926 cells is evaluated in this study.

Materials and methods

Chemicals and antibodies

Dulbecco's Modified Eagle Medium and fetal bovine serum, penicillin G, and streptomycin were purchased from Gibco (Invitrogen, USA). RNAase A , Hoechst 33342, and Propidium Iodine were purchased from Sigma-Aldrich (St. Louis MO, USA). Amersham ECL Plus™ was purchased from GE Healthcare (Buckinghamshire, England). Antibodies were obtained from following companies: alpha tubulin (A01410) from Gen Script (New Jersey, USA), Akt1 (2967), phospho-Ser473-Akt (9271), and anti-rabbit IgG HRP-linked (7074) from Cell signaling (Beverly, MA).

Cell culture

Human endothelial cells EA.hy926 (ATCC® CRL-2922™) were kindly provided by Dr. Piyanuch Thitiwuthikiat. The cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, penicillin G (40 units/mL), streptomycin (40 µg/mL), and 0.25% Sodium bicarbonate. The cells were maintained in a humidified 5% CO_2 incubator at 37°C.

Preparation of the ethanolic extract from *Centotheca lappacea*

The dried aerial part of *Centotheca lappacea* was provided from Chaopraya Abhaiphubejhr hospital, Prachinburi, Thailand. The size was reduced by grinder and pass through the 60 mm mesh size sieve. Then the powder was macerated in 95% ethanol for one day under shaking and filtered through filtered paper (repeated two times). The filtrates were combined and evaporated under reduced pressure until dry. The extract was kept in -20 °C until analysis. The fingerprint was made and kept as a reference. The percent yield of the extract was 3.3%.

Cell treatment and irradiation

For cell treatment, fresh media containing 0.2 µg/mL of *Centotheca lappacea* extract was added to each well. The cells were incubated with the extract for 3 hrs prior to X-irradiation.

For cell irradiation, the cells were irradiated with 6 MV x-rays using linear accelerator (Varian 2100CD, Varian Medical Systems, Palo Alto, CA, USA). The x-rays was delivered with the dose rate of 600 MU/min at room temperature. The source to sample distance was 100 cm.

Cell viability assay

EA.hy926 cells were seeded into 96-well plates with a seeding density of 6x103 cells per well and cultured for 24 hrs. After that, the cells were treated with or without 0.2 µg/ml *Centotheca lappacea* extract for 3 hrs. The cells were then irradiated with a single dose of 2 Gy at room temperature. At 24 or 48 hrs after irradiation, the culture medium was replaced with 100 µl serum free medium and 30 µl MTT solution, and the plates were kept in the dark for 4 hrs. Afterwards, the medium was discarded and the formazan crystals were dissolved in 100 µl DMSO. The plates were kept in the cell culture incubator for 15 min to complete solubilization of the purple formazan crystals. The absorbance at 540 nm was then read using a reference wavelength of 630 nm on a microplate reader (EnSpire™ Multimode Plate Reader; PerkinElmer, USA). All experiments were performed in triplicate and the percentage of cell viability was calculated from three independent experiments.

Cell cycle analysis by flow cytometry

EA.hy926 cells were seeded into 6-well plate with a seeding density of $6x10^4$ cells per well and cultured for 24 hrs. After that, the cells were treated with or without 0.2 µg/mL *Centotheca lappacea* extract for 3 h prior to

X-irradiation. At the indicated time points, the cells were trypsinized and collected by centrifugation. The isolated nuclei were stained with propidium iodide (PI) as described previously.[16] Briefly, the cell pellet was resuspended in a solution containing 584 µg/mL NaCl, 1,000 µg/mL Na-citrate, 10 µg/mL RNase A, 0.3 µg/mL Nonidet P-40 and 50 µg/mL PI and incubated in the dark at room temperature for 30 min. After that, a solution containing 15 mg/mL citric acid, 0.25 mM sucrose and 50 µg/mL PI was added. The suspension of PI-stained isolated nuclei was kept at 4 °C in the dark before cell cycle distribution measurement using flow cytometry (Cytomics FC500 MCL with CXP 2.2 software; Beckman Coulter, Indianapolis, USA)

Nuclei staining and fluorescence microscopy analysis

Approximately 12×10^4 EA.hy926 cells were seeded onto sterile glass cover slips and cultured in 6-well plates overnight. Cells were pre-treated with or without 0.2 µg/mL of *Centotheca lappacea* extract for 3 h before X-irradiation with a single dose of 2 Gy. At 24 or 48 hrs after irradiation, the cells on cover slips were washed briefly with PBS and fixed in ice-cold methanol for 10 min. The cells were washed with PBS thrice and stained the cell nuclear with Hoechst 33342 for 20 min. The cells were washed thrice with PBS and mounted with anti-fading solution for fluorescence microscopy analysis (Zeiss HBO100 microscope Illuminating System Axiovision Rel 4.8; Carl Zeiss AG, Oberkochen, Germany. Abnormal nuclei were scored from cells containing condensed nucleus, fragmented nucleus, micronucleus, and multilobulated nucleus. Abnormal nuclei were expressed as a percentage of the total of nuclei.

Western blot analysis

Whole cell lysates of treated cells were prepared at the indicated time points as described previously.[16] The protein from each sample was subjected to electrophoresis on 8-15% SDS-polyacrylamide gels and electro transferred to a PVDF membrane. The membrane was blocked in TBS-T containing 2% nonfat skim milk for 1 h at room temperature prior to antibody treatment. The membrane was probed with primary antibody diluted in 2% BSA in TBS-T at 4 °C overnight. The membrane was washed thrice with TBS-T and then probed with the secondary antibody diluted in blocking buffer for 1 h at room temperature. The membrane was washed thrice with TBS-T and the protein bands were detected with chemiluminescence using a digital phosphorimager (Chemi Doc™ XRS+Image Lab™ 5.1 software, Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analysis

The data are presented as Mean±standard error of three independent experiment. The differences in each experiment group were analyzed by independent sample student's t-test at 95% confidence level. A p value less than 0.05 was considered as statistically significant.

Results

Radioprotective effect of *Centotheca lappacea* extract on human EA.hy926 endothelial cells

The radioprotective effect of *Centotheca lappacea* extract was assessed by MTT assay. EA.hy926 cells were pretreated with 0.2 µg/mL of *Centotheca lappacea* extract or left untreated. Three hrs later, the cells were irradiated with a single dose of 2 Gy of X-rays. Treatment of the cells with only *Centotheca lappacea* extract, did not affect cell viability of EA.hy926 cells (Figure 1). Cell viability of EA.hy926 cells significantly reduced to 68.0% after irradiation as compared to that of control untreated cells ($p<0.05$). Remarkably, treatment of the cells with *Centotheca lappacea* extract prior to irradiation significantly increased cell viability of EA.hy926 cells to 92.6% as compared to that of cells that were treated with only irradiation ($p<0.05$). These results clearly indicate a protective effect of *Centotheca lappacea* extract against radiation-induced toxicity in human endothelial cells EA.hy926.

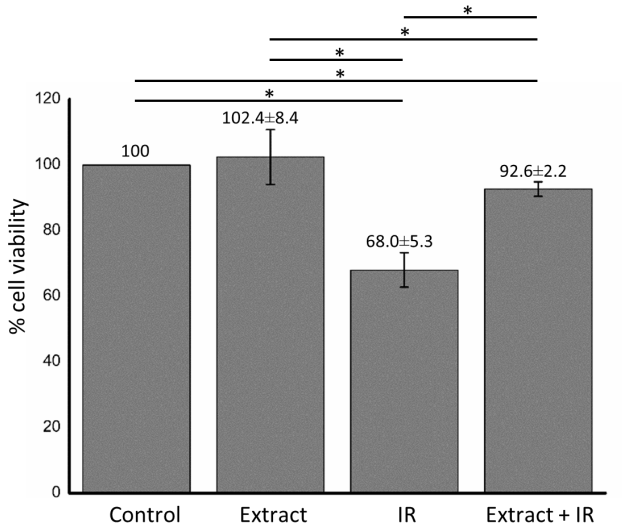

Figure 1. *Mean±standard error of cell viability at 24 hrs after irradiation of EA.hy926 cells pretreated with or without 0.2 µg/mL of Centotheca lappacea extract for 3 hrs prior to 2 Gy X-irradiation (Control: untreated cells, Extract: cells treated with only extract, IR: cells irradiated only, Extract+IR: cells treated with extract prior to irradiation).*

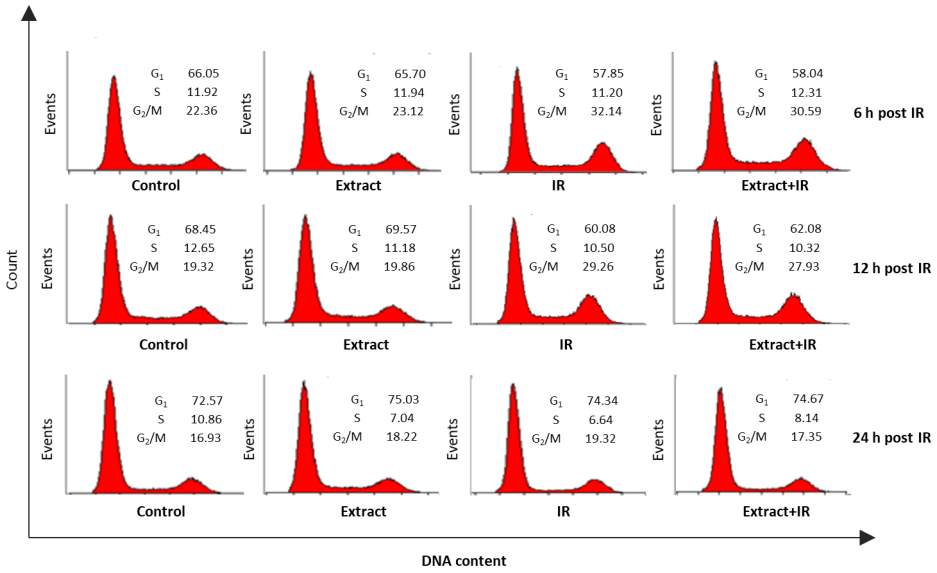

Figure 2. *Cell cycle distribution profiles of EA.hy926 cells at indicated time points after 2 Gy X-irradiation. (Control: untreated cells, Extract: cells treated with 0.2 µg/mL of Centotheca lappacea extract only, IR: cells irradiated only, Extract+IR: cells treated with 0.2 µg/mL of Centotheca lappacea extract prior to irradiation).*

Centotheca lappacea extract has no impact on cell cycle distribution of EA.hy926 cells.

The impact *Centotheca lappacea* extract on cell cycle progression might be involved in its radioprotective activity. Therefore, this hypothesis was elucidated by cell cycle analysis. Flow cytometry shows that cells treatment with 0.2 µg/mL of *Centotheca lappacea* extract did not alter cell cycle distribution as compared to that of control untreated cells (Figure 2). It is widely known that radiation can induce cell cycle arrest of mammalian cells at G2/M phase. Therefore, cell cycle distribution of EA.hy926 cells pretreated with or without *Centotheca lappacea* extract was determined at 6, 12 or 24 hrs after irradiation. As expected, radiation induced G2/M arrested was demonstrated in EA.hy926 cells that were not treated with the extract by an increase of the G2/M population from 22.36% to 32.14% at 6 hrs after irradiation. After that, the G2/M population of EA.hy926 cells slightly decreased to 29.26% at 12 hrs after irradiation, which remained evidently higher than that of control untreated cells. Similar pattern of radiation induced G2/M arrested was demonstrated in EA.hy926 cells that were pretreated with the extract prior to irradiation. The G2/M population of *Centotheca lappacea* extract pretreated cells increased from 23.12% to 30.59% at 6 hrs after irradiation. Afterward, the G2/M population slightly declined to 27.93% at 12 hrs after irradiation. Remarkably, radiation induced G2/M arrested in EA.hy926 cells completely abolished within 24 hrs after irradiation. Taken together, these results suggest that radioprotective mechanism of *Centotheca lappacea* extracts does not involve in the cell cycle progression of EA.hy926 cells.

Centotheca lappacea extract lessens radiation-induced abnormal nuclei formation of human EA.hy926 endothelial cells

To investigate whether *Centotheca lappacea* extract can reduce radiation-induced abnormal nuclei formation, nuclear morphology of EA.hy926 cells was analyzed. Cells pretreated with or without 0.2 µg/mL of *Centotheca lappacea* extract were irradiated with a single dose of 2 Gy. Twenty-four or 48 hrs later, cells were stained with Hoechst 33342 and nuclei were visualized by fluorescence microscopy. The percentages of abnormal nuclei in control untreated cells were 7.75+0.09% and 8.80+0.49% as determined at 24 and 48 hrs after mock irradiation, respectively (Figure 3). While percentages of abnormal nuclei in *Centotheca lappacea* extract treatment cells were 8.35+0.23% and 9.93+0.49% as determined at 24 and 48 hrs after mock irradiation, respectively. There were no statistically significant differences in the proportions of abnormal nuclei between *Centotheca lappacea* extract treatment and control untreated cells (p>0.05). In contrast, treatment of cells with 2 Gy of X-rays strikingly increased abnormal nuclei formation to 22.77+0.91% and 25.35+0.80% as determined at 24 and 48 hrs after irradiation, respectively. The portions of abnormal nuclei were 15.18+1.22% and 18.25+0.38% as determined at 24 and 48 hrs after irradiation, respectively, in cells that were treated with the extract in combination with radiation. Irradiation significantly increased the percentages of abnormal nuclei in cells that were pretreated with plant extract and in cells that were left untreated before irradiation as compared to control untreated cells (p<0.05). Yet, a significant lower percentage of abnormal nuclei was observed in cells that were pre-treated with extract before irradiation as compared to cells that were left untreated before irradiation (p<0.05). These observations indicate radioprotective effect of *Centotheca lappacea* extract against radiation-induced abnormal nuclei formation in EA.hy926 cells.

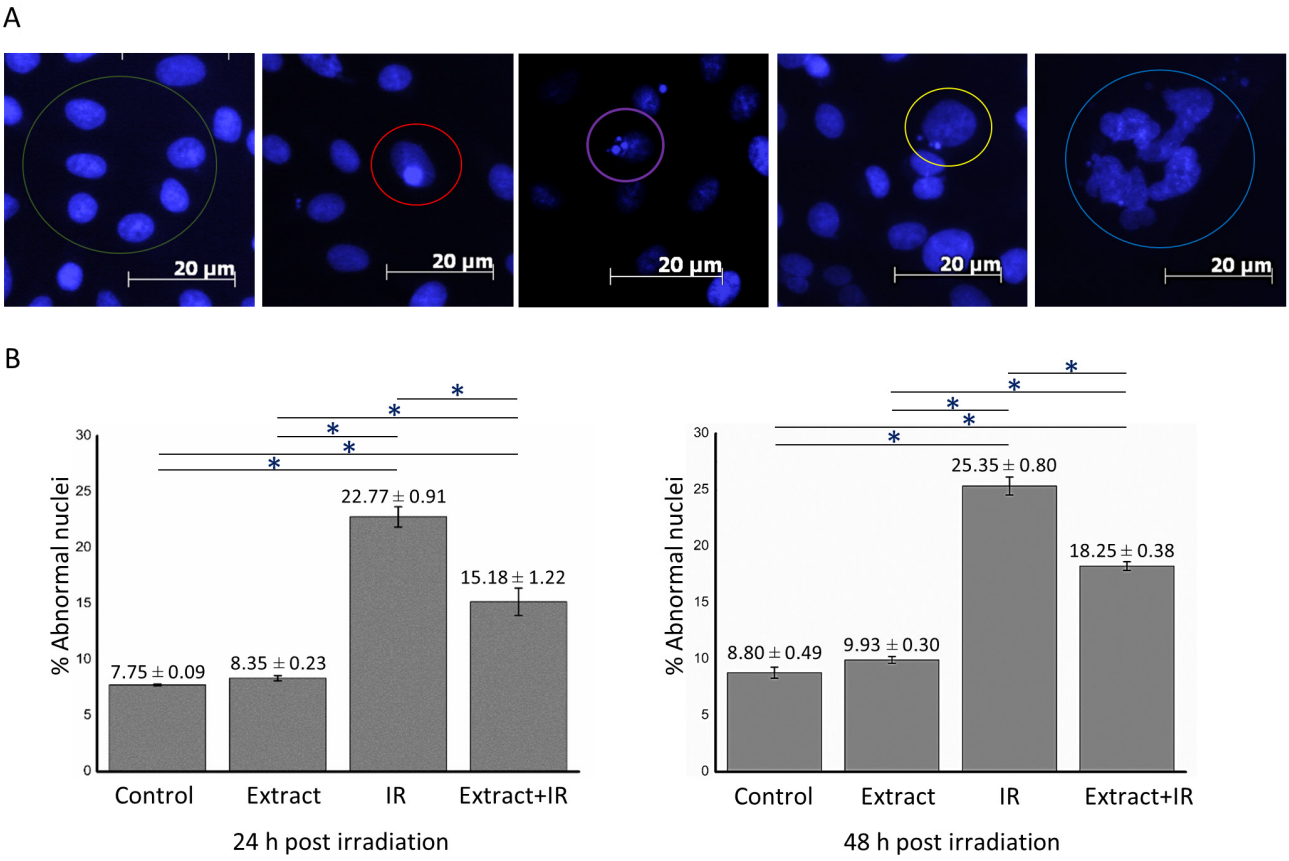

Figure 3. *Nuclei staining and fluorescence microscopic images of EA.hy926 cells. Cells were left untreated or treated with 0.2 µg/mL of Centotheca lappacea extract for 3 hrs. Subsequently, they were irradiated with a single dose of 2 Gy or left unirradiated. Hoechst staining was performed at 24 and 48 hrs after irradiation or mock irradiation. A) Representative fluorescence microscopic images of normal nuclei (green circle), condensed nucleus (red circle), fragmented nucleus (pink circle), micronuclei (yellow circle), and multilobulated nuclei (blue circle) are shown. B) Abnormal nuclei frequencies quantified from fluorescence microscopy of Hoechst 33342 nuclear stained cells. The percentages of abnormal nuclei are presented as the mean ± standard error of three independent experiments. More than 500 cells were counted for each determination. (Control: untreated cells, Extract: cells treated with 0.2 µg/mL of Centotheca lappacea extract only, IR: cells irradiated only, Extract+IR: cells treated with 0.2 µg/mL of Centotheca lappacea extract prior to irradiation).*

Centotheca lappacea extract effects Akt activity in EA.hy926 cells

Akt is a key protein in the PI3K-Akt pathway. It plays a role in controlling cell survival in response to DNA damage.[17-18] Immunoblotting of total lysates of EA.hy926 cells was performed to observe the effect of *Centotheca lappacea* extract on the activity of Akt. The level of Akt before and after irradiation did not alter in cells that were treated or untreated with the extract as compared to that of control untreated cells (Figure 4A). In contrast, the level of Akt phosphorylation (pAkt) was noticeably lower in cells that were treated with the extract as compared to untreated cell (Figure 4A and 4B). One h after irradiation, the level of pAkt clearly increased in cells that were pre-treated with plant extract and in cells that were left untreated before irradiation as compared to control untreated cells. Yet, a remarkably higher of pAkt level was observed in cells that were pretreated with extract before irradiation as compared to cells that were left untreated before irradiation. Three hours after irradiation, the level of pAkt was found to decline in cells that were treated with extract or were left untreated before irradiation. These results indicate an impact of *Centotheca lappacea* extract on Akt activity in EA.hy926 cells.

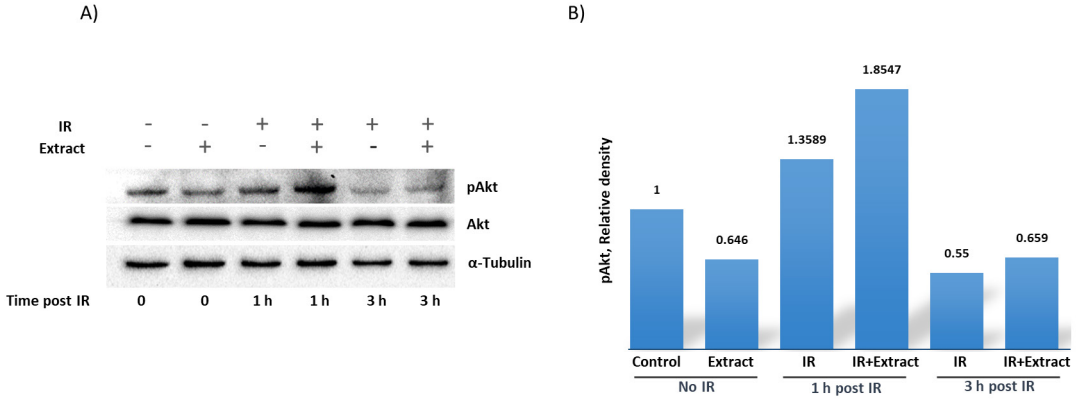

Figure 4. *EA.hy926 cells were collected at indicated time points for protein extraction. The levels of Akt and pAk were determined by Western blot analysis. The detection of tubulin was used as a loading control. (Control: untreated cells, Extract: cells treated with 0.2 µg/mL of Centotheca lappacea extract only, IR: cells irradiated only, Extract+IR: cells treated with 0.2 µg/mL of Centotheca lappacea extract prior to irradiation).*

Discussion

Over the past decades, investigators have been attempting to search for novel radioprotectors. Nevertheless, only very few compounds have been approved for clinical application due to high toxicity and side effects.[7, 19] In the present study, we clearly demonstrate that *Centotheca lappacea* extract decreases radiation-induced cytoxicity and abnormal nuclei formation in human endothelial cells EA.hy926. This finding reveals the radioprotective potential of *Centotheca lappacea* extract. Importantly, treatment of EA.hy926 cells with the extract prior to irradiation markedly increases the percentage of cell viability and lower the percentage of abnormal nuclei as compared to cells that were left untreated before irradiation. Whereas, treatment with the extract alone is not toxic to EA.hy926 cells. These findings indicate that *Centotheca lappacea* extract is a safe and potential candidate for the development of radioprotector reducing endothelial cell damage.

The protective effects of radioprotectors occur via different mechanisms, such as free radical scavenging, anti-inflammation, and facilitation of cellular repair activities.[8] *Centotheca lappacea* extract contains a variety of phenolic compounds and flavonoids, which have antioxidant and anti-inflammatory properties.[15-17] In addition, it contains p-coumaric acid, which has been reported to protect the rabbit corneal cells from UVB induced DNA damage.[15] Therefore, it is highly likely that radioprotective activity of *Centotheca lappacea* extract mediates its radioprotective effect via its antioxidant and anti-inflammatory properties. However, the precise mechanisms require further investigation.

Radiosensitivities of cells in each cell cycle phase are somewhat different. Cells are most radiosensitive in the G2/M phase, less sensitive in the G1 phase, and least sensitive in the S phase.[20] It has been reported that some plant extracts can alter radiosensitivity of the cells by interfering cell cycle regulation.[11, 12] However, we found no different in the pattern of cell cycle distribution before and after irradiation in cells that were treated or not with the extract. Therefore, the mechanism by which *Centotheca lappacea* extract mediates radioprotective effect does not involve cell cycle regulation.

Ionizing radiation has been shown to rapidly activate several cellular survival signaling cascades including PI3K-Akt pathway.[21] Activation of Akt is an intracellular stress response which has been found to promote cellular survival and inhibit radiation-induced cell death in several studies.[17,18, 21] In this experiment, we found that the level of phosphorylated Akt in EA.hy926 cells that were treated with *Centotheca lappacea* extract in combination with radiation was remarkably higher than that of irradiated cells and control untreated cells, as observed at 1 h after irradiation. This finding indicates that the radioprotective activity of *Centotheca lappacea* extract might mediate via the PI3K-Akt pathway. The extent of Akt activation depends on the magnitude of the damage caused by radiation.[22,23] In this study, we irradiated EA.hy926 cells with 2 Gy of x-ray. We found that, the level of phosphorylated Akt in irradiated cells strikingly declined as observed at 3 hrs after irradiation. It can be speculated that cellular stress induced by 2 Gy of x-ray in EA.hy926 cells was effectively eliminated within 3 hrs after irradiation.

Conclusion

Centotheca lappacea extract promoted cellular survival and inhibited radiation-induced abnormal nuclei formation with no toxic effect, no alteration of cell cycle regulation, and increase in Akt phosphorylation. The results of this study indicate that Centotheca lappacea extract is a promising candidate for radioprotector development to defend radiation-induced damage to endothelial cells.

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Conflict of interest

All authors declare no conflict of interest.

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Effects of an exercise program on cervical posture and sensorimotor function in persons with forward head posture

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ABSTRACT

Background: Forward head posture is associated with impaired musculoskeletal function and sensorimotor system. However, the effects of an exercise program as part of routine activities in persons with forward head posture remain unknown.

Objectives: To investigate the effects of an exercise program on cervical posture and sensorimotor function in persons with forward head posture.

Materials and methods: Sixty university students with forward head posture (aged between 19 and 24 years old) were recruited. Participants were allocated to either experimental or control group. The experimental group received a specific exercise program including postural correction, co-contraction of the cervical muscles and suboccipital muscle stretching, 7 days a week for 4 weeks. The control group continued with their normal daily routine. The outcome measures were the craniocervical (CV) angle, sagittal head tilt (SH) angle, cervical joint position error (JPE), and postural sway during narrow stance with eyes open and closed. The outcomes were measured at baseline and 4 weeks after intervention.

Results: The results showed that the CV angle and JPEs in rotation to the left and right sides were improved significantly in the experimental group compared to the control group after the exercise program (p<0.05). The CV angle, SH angle and JPEs before and after the exercise program were significantly found in the experimental group (p<0.05). There were no significant differences in the total sway area between groups and within group (p>0.05).

Conclusion: The results of this study suggested the 4-week exercise program (postural correction, co-contraction of the cervical muscles and suboccipital muscles stretching) was effective for improving the head and cervical posture and joint position sense.

Introduction

Forward head posture is one of the most common postural problems. It is often described when resting head posture held in front of the line of gravity with the eyes level or angled downwards in upright position.[1] The incidence of forward head posture varies from 66% to 85% of healthy populations.[2] Forward head posture is proposed as a risk factor of neck pain,[3, 4] although its relationship is still controversial,[5, 6] and is often caused by imbalance between the deep and superficial cervical muscles.[7] Deep cervical flexor muscles (i.e. longus colli and capitis muscles) providing dynamic cervical stability and support of cervical spine were demonstrated to be impaired[8, 9] while cervical extensor muscles, in particular suboccipital muscles were shortened.[2, 10, 11]

In addition to the musculoskeletal system, forward

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head posture has also been proposed to be associated with the sensorimotor system.[12, 13] This may be explained by sensorimotor integration of the cervical proprioception and other sensory information from the visual, vestibular and somatosensory systems required for the postural control system.[14] Evidence suggests that the deep cervical muscles, in particular the longus coli and suboccipital muscles have high density of muscle spindles, which provide proprioceptive information for the sensorimotor system.[15, 16] Previous studies demonstrated that forward head posture and static neck flexion led to changes in the cervical proprioception [12, 13] and impaired postural control.[17] In contrast, a study reported that forward head posture did not affect postural control.[18] Although the association of forward head posture and the sensorimotor control is modest, its potential to impaired cervical proprioception contributing to postural control deficit is still promising.

A recent systematic review and meta-analysis study by Sheikhhoseini et al.[19] suggested that therapeutic exercises consisting of strengthening and stretching exercises for the cervical muscles might result in large changes in craniovertebral (CV) angle and moderate improvement in neck pain in participants with forward head posture. Exercise intervention involving craniocervical flexor muscles is mostly attributed to forward head posture. The head and cervical posture (CV and head tilt angles) was found to improve after craniocervical exercise program.[20-22] Whereas it is evident that exercise interventions can improve forward head posture, the exercise programs designed in most studies were conducted in non-functional position and a center-based program,[19, 20, 22-24] which pose limits to manage as part of activity in daily life. Given that the correct upright posture is necessary for forward head posture and the deep cervical flexor muscles play an important role for stabilizing and controlling the cervical spine, a specific exercise program involving the craniocervical exercise and posture correction should be encouraged as part of routine activities. This would partially help unload the cervical spine. Additionally, stretching exercise for shortened muscles, particularly the suboccipital muscles could be added as its impairment and effectiveness on forward head posture have also been revealed.[21] As yet there is no research addressing the effects of an exercise program as part of routine activities. Thus, the present study aimed to determine the effects of an exercise program on head and cervical posture as well as sensorimotor function (cervical proprioception and standing balance) in healthy persons with forward head posture. Knowledge gained would reinforce preventive and promotive programs in persons with forward head posture.

Materials and methods

Study design

This study was a randomized, controlled, assessor-blinded clinical trial (allocation ratio 1:1). Ethical approval was gained from the ethical review committee for research in humans, Faculty of Associated Medical Sciences, Chiang Mai University (AMSEC-61EX-085). The study was registered on the Thai Clinical Trials Registry (No: TCTR20190211005). All participants provided written informed consent before the commencement of the study.

Participants

Participants were volunteer university students, aged between 19 and 24 years old. University students were chosen as they were a relatively controlled setting and comparatively homogenous population. All participants were recruited through an advertisement on Facebook, and flyers on university campuses located in Chiang Mai. Inclusion criteria were no history of neck and shoulder pain for the past year, no any musculoskeletal problems/ conditions that could affect outcomes (e.g. torticollis, scoliosis, back pain, ankle pain, and myofascial pain) and non-athletes.

Participants were made an appointment with an independent assessor to receive initial physical assessment. All eligible participants must have forward head posture measured by the CV angle with a digital camera (Canon EOS 600D) in a natural sitting position.[25] Forward head posture was defined as the CV angle was lower than 50°.[26, 27] Manual examination was also performed on the cervical spine (C0-C7) to confirm no symptomatic cervical joint dysfunction.

Randomization and masking

Randomization was undertaken by an independent researcher who was not involved in the trial. Randomization was performed by computer-generated permuted blocks with a block size of four, stratified by gender and severity of forward head posture (<43.5° or ≥43.5°).[28] Allocation was concealed in sequentially numbered, sealed, opaque envelopes. The envelopes were opened by a researcher allocating participants to each group. The examiner was blinded to group allocation throughout the trial. The therapist was not blinded to treatment.

Interventions

Participants in the experimental group received the exercise program delivered by a physiotherapist with over 3 years of experience. The exercise program included postural correction, co-contraction of the cervical muscles, and suboccipital muscle stretching, according to the previous studies.[29-31] The postural correction consisted of gently rolling of pelvis forward to an upright neutral sitting position, followed by scapular correction and occipital lift. The participants were actively performed and maintained the position for 10 seconds with 5 repetitions, every hour at least 7 times throughout the day (Figure 1A). The co-contraction of the cervical muscles consisted of self-resistance isometric craniocervical flexion, and cervical rotation (right and left). For the isometric craniocervical flexion, the participants were asked to push the chin inferiorly against the fist of one hand while used the other hand wrapping around the front of the neck to monitor overuse of the sternocleidomastoid and scalene muscles. For the isometric cervical rotation, the participants performed with eye movement by pressing their palms against each side of their head and looking into the elbow (left or right) (Figure 1B).[30] Each isometric exercise was performed 10 repetitions with a 10-second hold a day. For the suboccipital muscles stretching, the participants sat upright, placed both hands under the back of the top

portion of the head, and pushed the top of the head down with the chin tucked into the front of the neck (Figure 1C).[31] The participants were asked to hold the position for 10 seconds, with 5 repetitions a day. The exercise program was commenced the day after baseline assessment. The participants were asked to perform the exercise program daily for 4 weeks and complete an exercise diary to monitor compliance and record adverse events.

Figure 1*. Exercise program for experimental group. (A) postural correction, (B) co-contraction of the cervical muscles, (C) suboccipital muscle stretching.*

Participants in the control group were asked to carry on with their regular activities with no additional exercise and record if any particular discomfort or injury occurred during the study period.

Outcomes

Head and neck posture

The CV and sagittal head tilt (SH) angles were measured using a digital camera (Canon EOS 600D). The CV angle was defined as the intersection of a line drawn from the tragus of the ear to the C7 spinous process, and a horizontal line passing through the C7 spinous process[32, 33] (Figure 2). The SH angle was an angle between a line from the canthus of the eye to the tragus of the ear, and a horizontal line pass through the tragus of the ear. Three reflective adhesive markers were placed over anatomical landmarks (i.e. canthus of eye, tragus, and C7 spinous process). The participants were asked to assume their natural sitting position with

barefoot. The camera was set at 80 centimeters away from the participants in sagittal standing posture and the camera lens was adjusted at the level of external auditory meatus by adjusting the height of the camera tripod.[6] The head and neck posture was taken two times in a lateral view with the right side of the participant photographed. The photographic data was exported into a computer and the CV and SH angles of each photograph were measured twice in degrees using Image J program.

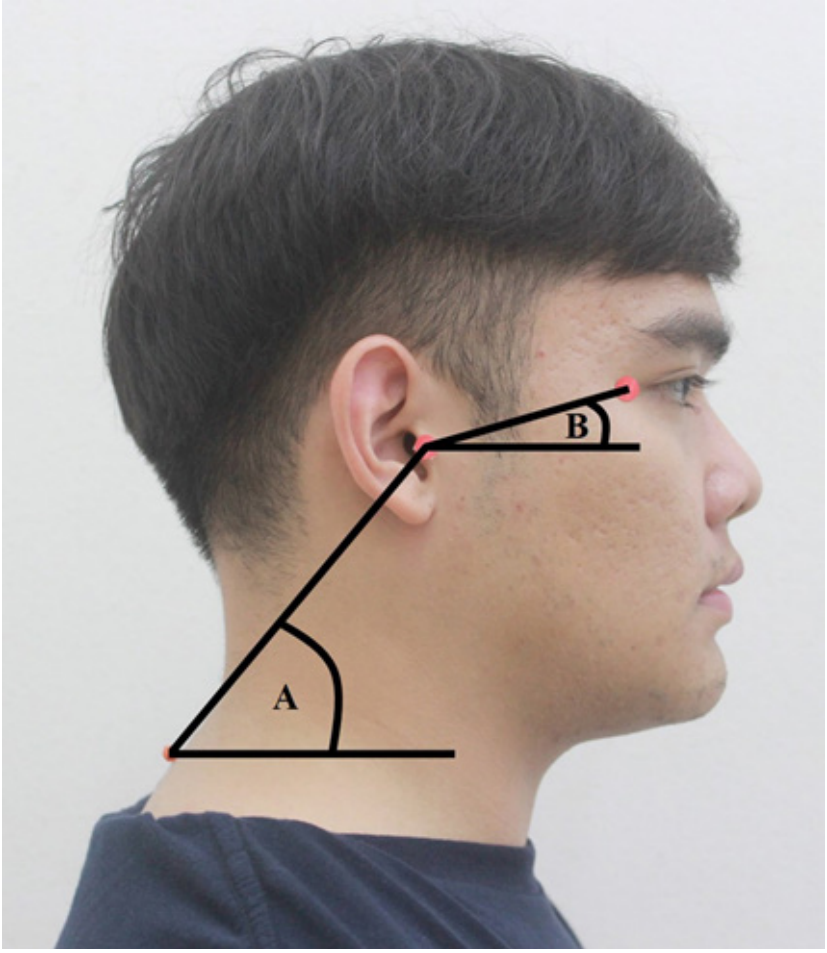

Figure 2*. (A) Craniovertebral angle, (B) sagittal head tilt.*

Intra-rater reliability was conducted in 15 university students, aged between 19 and 24 years old. The measurement of CV and SH angles were performed twice with an interval of 24-48 hours by a blinded examiner. The reliability results for both CV and SH angles were excellent ($ICC_{3,1}$ = 0.91 and 0.85, respectively).

Cervical proprioception

Cervical proprioception was measured by joint position sense (JPS) test using a laser pointer attached to the head as described by Revel et al.[34] The participants sat upright with the head in the neutral resting position, 90 centimeters away from the center of a target attached on a wall. They were asked to perform active cervical movements with eyes closed and then return to the starting position as accurately as possible. JPS was tested in cervical extension and rotation to the right and left directions in random order and each direction was measured three times. The participant's head was repositioned back to the starting position by the examiner before each trial. No verbal feedbacks were given during the measurement. An absolute joint position error (JPE) was calculated from the final laser position and the starting position for each trial in millimeters and then calculated to degrees using equation:

$$\text{Degree} = \tan^{-1}\left[\frac{error\ distance}{900(mm)}\right].$$

Degree of JPE of each movement was used for further analysis.

Static standing balance test

Sway meter was used to measure static standing balance.[35, 36] It comprised a 40-cm-long rod with a vertically mounted pen at its end attached to a belt. The sway meter was attached to the participants at their waist levels with rod extending posteriorly. Standing balance was tested under two different conditions: narrow stance (feet together) with eyes open and closed.[18] For each condition, the participants were instructed to stand as still as possible for 30 seconds. The pen was participant's postural sway on a millimeter graph paper fastened to the top of an adjustable-height table. The postural sway was calculated by displacements of center of pressure in the maximum anterior-posterior

(APmax) and medial-lateral (MLmax) directions and total sway area was summed by number of square millimeter squares (mm^2) traversed by the pen and then converted to square centimeter (cm^2).

Study procedure

All eligible participants completed a consent form and randomly assigned to either the experimental or control group. The outcome variables (CV angle, SH angle, JPEs, and postural sway) were measured at baseline and 4 weeks after intervention by a blinded examiner. The participants in the experimental group were trained to facilitate correct performance of each exercise program (postural correction, co-contraction of the cervical muscles and suboccipital muscle stretching). After the participants were able to do the exercise correctly and independently, they were asked to practice the exercise programs daily for 4 weeks. An appointment was made within the first week to ensure that the participants could perform the exercises correctly by themselves. A telephone reminder was scheduled to remind the participants to exercise every hour of the day. To increase compliance rate, the participants were also called by a researcher every week. The control group continued with their normal daily routine. The participants in both groups were asked not to change their normal daily routine with no additional exercise.

Sample size calculation

Sample size estimation was computed based on the CV angle data of a previous study by Gupta et al.,[23] using G*Power 3.1.9.2. The effect size for the difference between two independent means was 0.7. With a power of 0.8 and a significant level of 0.05, a total sample size of 52 participants was required for the study. To allow for a 15% dropout rate, 60 participants were recruited for the study.

Statistical analysis

To be included in the analysis, all participants had to complete at least 80% of the prescribed exercise program. A per-protocol analysis was used to determine the treatment effect. Descriptive statistic and independent t-test were used to determine demographic characteristics of the participants. Shapiro-Wilk test was used to analyze normality of the outcome variables. An analysis of covariance (ANCOVA) was used to examine differences in the outcomes between groups, using the baseline values as covariates. A mixed-model ANOVA was used to analyze differences in the outcomes between times for each group. All statistical analyses were performed using SPSS software. Level of significance was set at 0.05.

Results

Participants

The study commenced in February 2019 and was completed in July 2019. A flow diagram of participant recruitment and retention is presented in Figure 3. Sixty participants were enrolled into the study and none of those lost to follow-up. Seven participants in the experimental group (23.33%) did not achieve at least 80% of the exercise program and their data were excluded from the analysis. No additional exercise and no adverse effects were reported. Demographic characteristics for the participants are presented in Table 1. There were no between-group differences in the participant characteristics (p>0.05).

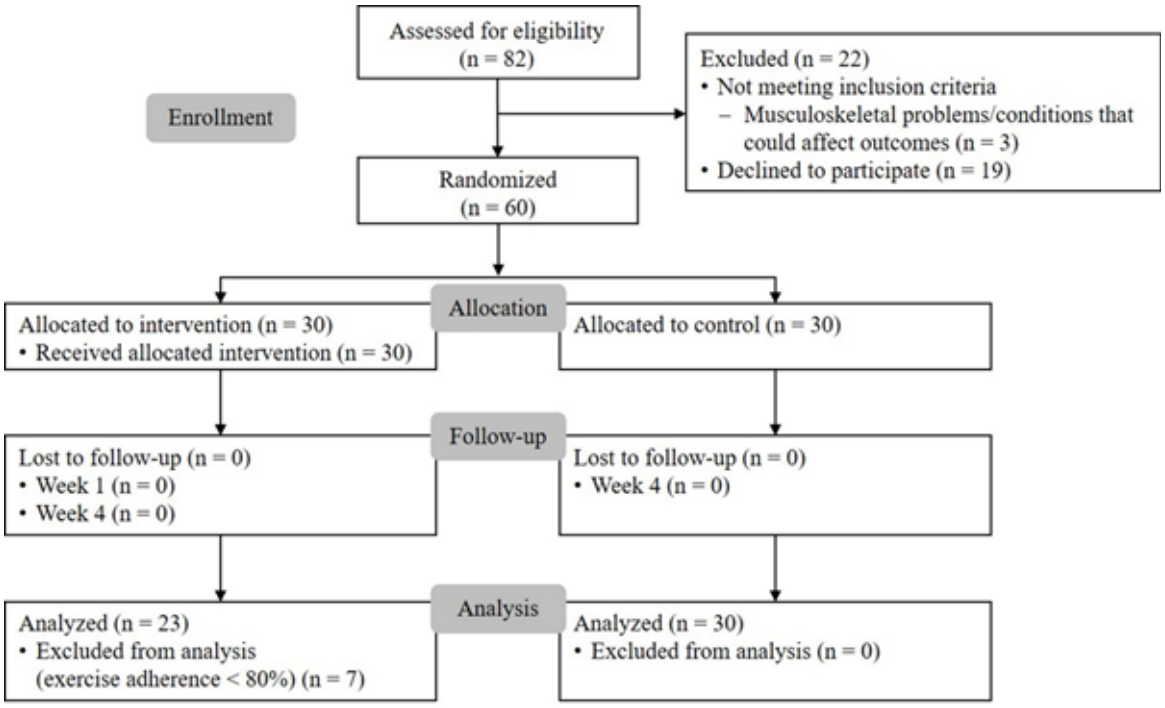

Figure 3. *Flow diagram of the trial.*

Table 1 Demographic characteristics for the participants.

Variables	Experimental group (n=23)	Control group (n=30)	*p* value
Age (yrs)	22.00±1.28	21.67±1.54	0.41
Gender (% female)	65.22	66.67	0.91
Height (cm)	164.00±9.17	164.18±9.21	0.94
Weight (kg)	61.00±12.55	60.34±11.26	0.84
Duration of study in sitting position (hrs)	5.74±1.78	6.75±2.23	0.08
Duration of smartphone use (hrs)	7.63±3.41	8.07±3.92	0.97

***Note**: Data are presented with mean±SD, otherwise as indicated. yrs: years, cm: centimeters, kg: kilograms, hrs: hours.*

Outcomes

Head and neck posture

The CV and SH angles for the experimental and control groups are provided in Table 2. The CV angle was significantly improved in the experimental group compared to the control group after the 4 weeks exercise program ($p<0.05$) (Table 2). There was no difference in the SH angle between groups ($p>0.05$). The CV and SH angles before and after the exercise program were significantly found in the experimental group ($p<0.05$), but not found in the control group ($p>0.05$) (Table 3).

Table 2 All outcome variables for baseline (pre-test) and after intervention (post-test) between the experimental and control groups**.**

Variables	Experimental group (n=23)		Control group (n=30)		Mean difference	*p* value	Effect size
	Pre-test	Post-test	Pre-test	Post-test	95% confidence interval		
Head and neck posture (degrees) - CV angle	46.23±2.74	49.27±2.99	45.07±3.13	45.93±3.47	-2.47 (-3.82 to -1.11)	0.01	0.21
- SH angle	20.53±4.82	18.03±5.63	18.57±4.33	17.76±5.49	0.67 (-2.31 to 3.64)	0.66	0.01
JPE (degrees)							
- extension	3.39±2.39	3.08±2.38	4.13±1.99	4.53±2.15	1.04 (-0.05 to 2.13)	0.06	0.07
- rotation to the left	3.83±1.34	2.94±1.47	5.16±2.78	5.04±3.13	1.20 (0.01 to 2.39)	0.05	0.08
- rotation to the right	3.58±1.32	2.49±1.19	4.06±2.48	4.83±2.65	1.41 (0.41 to 2.42)	0.01	0.24
Sway area (cm^2)							
- eyes open	0.83±0.39	0.74±0.34	0.77±0.38	0.78±0.36	0.06 (-0.11 to 0.24)	0.45	0.01
- eyes closed	0.96±0.49	0.83±0.38	0.85±0.39	0.90±0.52	0.13 (-0.11 to 0.37)	0.29	0.02

***Note**: Data are presented with mean±SD, otherwise as indicated. yrs: years, cm: centimeters, kg: kilograms, hrs: hours.*

Cervical proprioception

The JPE values for the participants in both groups are provided in Table 2. The JPE values in rotation to the left and right sides were decreased in the experimental group compared with the control group after exercise ($p<0.05$) (Table 2). There was no significant difference between groups in the JPE value in extension ($p>0.05$). The JPE values in rotation to the right and left sides, but not in extension, before and after the exercise program were significantly found in the experimental group ($p<0.05$) (Table 3). The JPE values for all directions were not found before and after intervention in the control group ($p>0.05$).

Static standing balance

The total sway area during standing with eyes open and closed for both groups is provided in Table 2. There were no significant differences in the total sway area between groups and within group ($p>0.05$) (Table 2 and 3).

Table 3 Mean differences (95% confidence interval) within-group for all outcome variables.

Variables	Experimental group (n=23)		Effect size	Control group (n=30)		Effect size
	Mean difference (95% CI)	*p* value		Mean difference (95% CI)	*p* value	
Head and neck posture (degrees)						
- CV angle	3.04 (2.00 to 4.08)	0.01	0.41	0.85 (-0.06 to 1.76)	0.07	0.07
- SH angle	-2.50 (-4.91 to -0.89)	0.04	0.08	-0.81 (-2.87 to 1.25)	0.43	0.01
JPE (degrees)						
- extension	-0.31(-1.21 to 0.59)	0.49	0.01	0.39 (-0.39 to 1.18)	0.32	0.02
- rotation to the left	-0.89 (-1.80 to -0.01)	0.05	0.07	-0.13 (-0.92 to 0.67)	0.75	0.01
- rotation to the right	-1.09 (-1.99 to -0.19)	0.02	0.10	0.10 (-0.69 to 0.89)	0.06	0.07
Sway area (cm^2)						
- eyes open	-0.09 (-0.24 to 0.06)	0.25	0.03	0.01 (-0.13 to 0.14)	0.98	0.01
- eyes closed	-0.14 (-0.34 to 0.06)	0.17	0.04	0.05 (-0.13 to 0.22)	0.59	0.01

Note: *CV angle: craniovertebral angle, SH angle: sagittal head tilt angle, JPE: joint position error.*

Discussion

This study demonstrated that the CV angle and JPEs were significantly improved in the experiment group compared to the non-exercising control group. Additionally, the CV angle, SH angle and JPEs were significantly improved at 4 weeks of training in comparison with the baseline data. However, there was no change in standing balance after the exercise program. The results suggested that the 4-week exercise program consisting of postural correction, co-contraction of the cervical muscles, and suboccipital muscle stretching could improve forward head posture and joint position sense in healthy individuals.

The results of this study support a systematic review suggesting that therapeutic exercise might result in large changes in CV angle and moderate improvement in neck pain in participants with forward head posture.[19] Notably, the CV value in the experimental group was closed to normal value as suggested in previous studies.[26, 27] In addition, the results of this study are consistent with previous findings,[20-22] although the exercise program in this study was given as part of routine activities. Camitsis et al.[22] reported that the CV angle was greater in both standing (about 11%) and sitting (about 15%) after a home-based craniocervical exercise (10 times/set, 3 sets, 3-5 weeks) in asymptomatic subjects. Kim et al.[21] also demonstrated that the craniocervical exercise combined with suboccipital release could improve the CV angle in subjects with forward head posture. Similarly, Falla[20] found that the craniocervical flexor exercise improved the ability to maintain an upright cervical posture (CV angle) during the computer task in patients with neck pain.

Forward head posture is often associated with increased compressive loading of the cervical spine as well as a creep response in connective tissues.[3] There is also a contribution of prolonged static posture to risk for the development of symptoms in the neck and upper body.[37, 38] Evidence suggests that deep cervical flexor muscles play an important role for providing support and stability for the cervical spine and the weight of the head against gravity.[39] Particularly, a study found that logus colli counteracts the lordosis increment related to the weight of the head and to the contraction of the dorsal neck muscles.[8] This supports our results. Postural correction facilitates the activity of the deep neck flexor muscles, scapular muscles and lumbar multifidus, which are key postural muscles for improving the pattern of cervical, scapula-thoracic and lumbo-pelvic movement.[29] Co-contraction of the cervical muscles increases the activity of the deep neck flexors incorporated with superficial neck flexors, neck extensors and neck rotators.[40] Suboccipital muscle stretching decreases tightness of the suboccipital muscles, leading to reduced chin out posture. Nonetheless, the findings of this study found no significant difference in the SH angle between groups but between pre- and post-test in the experimental group. No differences (pre-post) in the SH angle were observed for the control group. One reason may be associated with the correctness of the exercise performance. Although all participants in the experimental group were properly trained by the physiotherapist, the head may not be well stretched into the stretch position when they performed independently at home. Thus the cervical extensors might be rather more stretched than the suboccipital muscles. Alternatively, no difference may be due to small sample size. The sample size in this study was calculated based on the CV angle. Also, the exercise duration may be not enough. This should be addressed in further research.

The results of this study revealed that JPEs in the left and right rotation were significantly decreased after exercise program 4 weeks, when compared with the control group. A trend was also observed in cervical extension for the experimental group, which no statistical significance may be due to a large variabilty in the experimental group. Additionally, there were significant differences between pre- and post-test for the JPEs in the left and right rotation in the experimental group, but not in the control group. Our results are supported by a previous study demonstrating that forward head posture was correlated with greater repositioning error than a more upright posture and could lead to disruption of afferent input from the muscle spindles.[41] The JPEs may also be related to alteration in cervical proprioception. Deep cervical and suboccipital muscles

have the highest density of muscle spindles, which contain mechanoreceptors providing cervical proprioceptive information for the sensorimotor system.[15, 16] There is also evidence that high spindle density in the muscles is relevant to movement control of the cervical spine and kinesthetic information.[42] Thus, it is possible that the specific exercise intervention focusing the deep cervical muscles may rebuild cervical proprioception in persons with forward head posture.

Unexpectedly, there were no significant differences in the static standing balance after the exercise intervention. It is well-known that postural control requires sensory input from the visual, vestibular, somatosensory and cervical proprioception systems.[14, 43] One study found that forward head postures during computer-based work might contribute to some disturbance in the balance of healthy adults[17] whereas another study found that induced forward head posture did not affect postural control.[18] In this study, the change of postural sway in the experimental group seems to be greater than that in the control group, but the difference did not reach statistical significance. Knowingly, the deep cervical muscles providing proprioception which is important for the sensorimotor system.[15, 16] However, the sensorimotor system also comprises other sensory input from the visual, vestibular, and somatosensory subsystems for controlling postural stability. Thus although forward head posture can alter cervical proprioception and disturb balance,[17] it may be compromised by the other sensory systems. On the other hand, it is possible that the exercise may not be sufficient to improve standing balance. A specific balance training may be needed. Also, as we studied in young adults, their balance may be less impaired or affected by forward head posture. Yet, there is no normative data of postural sway using the sway meter for comparison with. Besides, the nonsignificant results may be due to small sample size. As this study is the first study investigating the effectiveness of a specific exercise on static standing balance, it can therefore be challenged to conclude whether static standing balance in those with forward head posture can be improved from the exercise intervention including postural correction, co-contraction of the cervical muscles, and suboccipital muscle stretching. Further research in this area is still required.

There are some limitations to this study. Participants recruited into the study were narrow age range (19-24 years) and university students. This potentially limits the generalizability of the study's results to other populations and in particular to older adults who are susceptible to age-related changes in musculoskeletal and sensorimotor systems. Additionally, the sample size in this study was calculated from one variable (CV angle). This may lead to non-significant results for the other outcomes. Additionally, it is difficult to control whether the participants in the experimental group could perform the exercise program correctly thoughout the study periods, in particular the suboccipital muscle stretching. Although the sway meter is reliable and valid to measure postural sway, forceplates or force platforms are ideal for measuring static standing balance. There was quite a number of the participants who did not complete the exercise program. This study is expected to support existing data and further emphasize the importance of good head and neck posture and a routine specific exercise during daily life activities. However, there remains a need for further research in order to reinforce the effects of a specific exercise intervention in persons with forward head posture. Further research is still requried to confirm the study findings in variety of ages (e.g. middle-aged and older adults) and populations (e.g. persons with neck pain and office workers). Additionally, further research should investigate whether the co-contraction of the cervical muscles can improve balance in those with forward head posture or a specific balance training is needed. The effects of the exercise program during performing functional or specific tasks such as computer typing should also be investigated in persons with forward head posture. Furthermore, sophisticated laboratory tests should be considered in further research to better understand the effects of exercise on balance in persons with forward head posture.

Conclusion

The results of this study suggested that an exercise program including postural correction, co-contraction of the cervical muscles, and suboccipital muscle stretching for 4 weeks was effective in improving the head and cervical posture (CV angle) and joint position sense (cervical rotation) in persons with forward head posture. Improvement in static standing balance was not found. The study emphasizes the importance of a routine specific exercise during daily life activities for university students with forward head posture.

Conflict of interest

No conflict of interest in this study

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Preliminary Report: Modified and innovative workstation and tools in a male wood carver at Chiang Mai Province, Thailand

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ABSTRACT

Background: Wood carving is a very well-known occupation in Chiang Mai Province, Thailand. Traditional posture during wood carving with conventional tools has been demonstrated by sitting on the floor. However, work-musculoskeletal disorders (WMSDs) during carving had not been assessed and whether workstation modification could be involved in posture, pain and satisfaction.

Objectives: To evaluate posture and pain during wood carving at before and after workstation modification and to develop an innovative workstation and tools for a wood carver.

Materials and methods: This study was performed preliminarily in a male wood carver aged 28 years, with ten years of carving experience. Before and after 4-weeks when the workstation and tools were modified, the ergonomic risk was evaluated by using the Rapid Upper Limb Assessment (RULA), and pain area and intensity as well as satisfaction were interviewed. Then, the innovative workstation was designed and developed after face to face discussions.

Results: The results of posture in a conventional workstation showed a slumping position on a low stool and use of various unsafe steel carving tools, and the total RULA score was 7, whereas an intense pain located at the neck and lower back regions. When the workstation was modified with marble and wooden chairs, and tools with hammer with rubber wrapped heads, the total RULA score decreased to 6. Additionally, pain intensity at the neck and lower back decreased and the satisfaction improved. Finally, when an innovative workstation was designed and developed, the result showed looked convenient, comfortable and usable for wood carving.

Conclusion: Modification of the workstation and tools for wood carver can reduce pain. Furthermore, an innovative workstation should have developed for wood carver for protecting the WMSD.

Introduction

Current work-related musculoskeletal disorders (WMSDs), especially pain, can occur during work in many occupations, such as cooks, storage laborers, transport laborers and building structure cleaners.[1] Contact stress, forceful contraction and awkward postures can induce pain at various regions of the body such as the knees, back and shoulders.[2] WMSDs also present in various occupations in Thailand, for example, construction-related work,[3] and frozen food manufacturing.[4] Wood carving is a famous and specific occupation that requires vast experience gained from predecessors, who were reputable craftsmen producing famous products worldwide, like ceramics, bronzeware, and Buddhist sculptures and dragons. Unfortunately, traditional wood carving had to be performed on the floor, so poor posture such as a slumped

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posture commonly developed when carving for a long time. Previous data claimed that slumping and prolonged static posture tends to increase WMSDs or pain.[5] Thus, appropriate postures, working environment and workstation adaptation are very important factors for improving tasks in the workplace[6] and preventing injury while working.[7] Interesting evidence showed that an adjustable workstation for carpet weavers, with height and seat adaptation, could reduce discomfort while working,[8] the same as tool adaptation for hairstylists, who reduced fatigue during hairdressing. [9, 10] Therefore, evaluating on WMSDs and modification of table, stool, and tools in the workstation for wood carving are very challenge that should improve work performance and productivity and can be prevented the WMSDs among of wood carvers as same as other occupations. As a consequence, the aim of this preliminary study was to survey the workstation and tools for wood carving and evaluate the benefits of their modification on posture, pain and satisfaction. Finally, the present study also aimed to develop an innovative workstation with a pilot design after face to face discussion.

Materials and methods

This case study was performed with a male wood carver aged 28 years, with ten years' experience in wood carving. He worked for seven hours daily at Baan Tawai, Khun Kong sub-district, Hang Dong district, Chiang Mai Province, Thailand. The protocol in this preliminary study was approved under exception review from the Ethical Human Committee at the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand. The data was permitted from wood carver by a personal contact. Then, a survey of a conventional workstation and tools was performed before modification of both the table, stool, and tools for a short-term trial. Ergonomic risks and pain were evaluated before and after the wood carving trial. Finally, both the table and stool were designed and developed for an innovative workstation that worked after face to face discussions was completed.

Ergonomic risk and pain assessment

Baseline data of the ergonomic risk while working was assessed according to the Rapid Upper Limb Assessment (RULA) guideline,[11] which was permitted by Professor Alan Hedge of Cornel University (November 2000). RULA is a standardized tool showing the reliably good evaluation of posture,[12] and popularly used to evaluate musculoskeletal disorder relating to overall posture imbalance as the previous standardized protocol.[13] RULA score was determined by viewed the best videos captures which were adjusted to slow the speed of movement for more precise and accuracy during wood carving for 20 minutes at least and summarized the final scores both individual parts and total scores. RULA scores was conducted by a researcher by training and reliability test with two expert ergonomists. Then, the acceptable both intra-rater (ICC 3,2) and high inter tester (ICC 2,2) reliabilities (0.77 & 0.75) was confirmed before data collection. The RULA score can be categorized into four classes depending the number of movements; static muscle work, force, work posture and time worked without a break, and the total score ranges from 1 to 7 points. Total RULA score deals mainly with parts of the body; upper arms, lower arms, wrists, wrist twist, neck, trunk and leg. The priority level of exposure and investigation is classified as; acceptable posture if not maintained or repeated for long periods (1 or 2 points), more investigations are needed and changes may be required (3 or 4 points), investigation and changes are required (5 or 6 points), and investigation and changes are required immediately (7 points).[14] Finally, the areas of most pain intensity were evaluated using the numerical rating scale (NRS) of no pain (scale at 0) to the most intense pain imaginable (score at 10).[15]

Modified workstation and tool protocols

The workstation was modified as previously suggested.[16] Appropriate or good posture should help the muscles in the body to optimal load and relax, and it prevents unnecessary strain and fatigue. In this study, the workstation was modified by using slightly tilted and higher marble chairs in the workplace, in order to keep the body alignment on a vertical plane. The tools for wood carving were modified in order to reduce the risk of occupational injuries to the upper limbs and hands, especially swollen finger joints or neuralgia, as previously suggested,[17] and the handle of hand tools was remodeled with finger grooves for better grip as shown in a previous study.[10] When the modified workstation and tools had been used for 4 weeks without any contact stress, the ergonomic risk, pain intensity and areas of pain were reassessed. Finally, satisfaction of the modified workstation was discussed.

Innovative workstation development

After 4-week modification of the workstation and tools were completed, advantages or disadvantages were performed by the face to face interviewing method[18] for one day meeting in order to design and develop an innovative workstation, with both a table and stool for wood carving. The design of the workstation was followed a previous recommendation, with a tilting adjustable stool and high seat pan that sloped forward by approximately 10-25 degrees, which could provoke anterior pelvic tilt.[8, 16] Moreover, the surface of the table was inclined slightly[5] in order to adjust to 20 cm above elbow height during the carving process.[8] Furthermore, the table was made with spaces in front and by each side for hand tools that could be kept within reach while the wood carver was working. This possibly helped safe body movement. When the innovative workstation was constructed completely, possible ideas for wood carving were discussed.

Statistical analysis

Data of all the parameters were presented with descriptive results.

Results

Conventional and modified workstation and tools

This study was studied in a male wood carver who carving specific the Buddha image on the plate wood for 2 days. Initial survey results showed that conventional wood carving tools were constructed from traditional culture and wisdom with a selection of tough and forced absorbed wood such as that from trees. Furthermore, chisel handles, iron hammer heads, sew Ngon (curved chisel), and iron stamps were unsafe with rough and hard surface. Wood carvers worked in stationary and prolong sitting positions on a low seat (Figure 1.A), that caused a humpback, affected neck and knee flexion and induced dominate pain at the neck and lower back regions.

Figure 1. *Conventional (A) and modified workstation (B) during wood carving. (Photos were permitted by wood carver for academic publication without conflict of interest).*

The workstation was modified by using wooden and marble chairs with a higher level (Figure 1.B). In addition, hammers were adapted for safe handling with grooves in the handle for fingers to fit and rubber wrapped around the hammer head and handle (Figure 2). The total RULA scores during use of the conventional and modified workstation are shown in Table 1, the total RULA scores were 7 and 6, respectively. The RULA scores for each part of the body did not change in the upper arm position, wrist position, wrist twist or legs, except the lower arm, neck, and trunk position. Results for areas of pain and intensity at the neck and lower back regions decreased from intense (scale=6) to comfortable (scale=2) sensation. Moreover, the satisfaction of wood carver on the modified workstation was excellence.

Figure 2. *Conventional (Left) and modified tools (right). Hammer adapted for safe handling with grooves in the handle for fingers to fit and rubber wrapped around the hammer head and handle.*

Table 1 Rapid Upper Limb Assessment (RULA) scores between carving with conventional and modified workstations

RULA part	Conventional workstation	Modified workstation
Individual body part;		
- Upper arm postition	3	3
- Lower arm position	2	3
- Wrist postition	3	3
- Wrist twist	1	1
- Neck position	2	3
- Trunk position	4	3
- Legs	1	1
Total score	7	6

Innovative workstation design and development

From the face to face discussions on the modified workstation with the fourteen-wood carvers, an innovative workstation with table and stool was designed and developed, comprising a wooden tilted table and stool. The general characteristics of the innovated workstation were agreed upon, with their low cost, and tailor-made and small flat designs. Figure 3 shows the innovative workstation with adjustable sloping surface was made at an appropriate height at elbow level in a sitting position. Moreover, the table was developed with spaces in the front and at both sides for hand tools and free space for legs under the table. The height stool was designed appropriate to popliteal level and a width of seat pan with a round ridge can be adjustable tilt, in order to avoid contact stress. Finally, the result of discussions claimed that the innovative workstation was convenient, comfortable and usable for wood carving.

Figure 3. *The innovative workstation with both table and stool for wood carving. Table is wooden and tilted with hand tool spaces in the front and on both sides. Stool was designed with an appropriate popliteal height and seat pan of appropriate width with a round ridge, and special adjustable tilt, in order to avoided contact stress.*

Discussion

This preliminary study showed that a wood carver in a conventional workstation worked traditionally in a slumping posture on a low, flat seat. A previous report showed that prolonged slumping in a sitting position for one hour reduced abdominal muscle strength and induced muscle fatigue.[19] In addition, pressure was loaded at the cervico-thoracic and lumbosacral joints.[20] Therefore, slumping posture directly affects the cervico-thoracic and lower back regions that reflect WMSDs.

In this study, ergonomic risk during wood carving was assessed with a standardized tool and Rapid Upper Limb Assessment (RULA), which has good reliability in evaluating posture[12] or overall posture imbalance.[13] RULA has been a tool for evaluation in many previous occupations, such as carpet hand-weaving,[8, 16] the ceramic industry,[21] warehouse working,[22] etc. A high RULA score reflects the high risk of WMSDs, which requires immediate change in posture.[14] The total RULA scores when wood carving as the figure 1 (A) before modification of the workstation was 7, which can be interpreted as action level 4, meaning that the wood carver has the worst posture with the risk of sudden injury and the need to change posture immediately.[23] Although the total RULA score of the modified workstation as the figure 1 (B) was 6, it can be interpreted as action level 3, which means that the wood carver has an awkward posture and risk of injury, and needs to investigate the posture and change it quickly.[23] Although the total RULA scores were not dominantly different, either before or after modification of the workstation, scores for many parts of the body part were not reduced, but increased, however, the score of trunk position decreased from 4 to 3. These results can be explained by modification of the workstation with marble chairs that can correct lower back alignment. Sitting on a low seat involves the posterior pelvic tilt position and lumbar flexion or reversed lumbar curve.

It is possible that non-dominantly changed RULA scores on other parts of the body, especially the upper and middle thoracic position, can refer to loss of voluntary trunk control from prolonged muscle fatigue, characterized by an extended cervical spine, a long C-shaped kyphotic thoracolumbar spine and posteriorly tilted pelvic region, which is consistent with a previous report.[24] In addition, pain was located at the lower back in this study, which is similar to previous evidence that reported on either crossed-leg or heel sitting posture affecting the lumbar multifidus muscles during work, and the center of gravity shifted anteriorly, thus, muscle fatigue and pain could be presented.[25] The results of RULA scores of the upper arm position, wrist position, wrist twist, and legs did not change, whereas those of the lower arm and neck position increased because of higher of table height when compared to before modification. This can be explained possibly by the height of the workstation being insufficient or unadaptable for wood carving. Moreover, strength of the back and neck muscles were not evaluated, therefore, posture analysis with the RULA sheet at the back region from muscle weakness, including work efficacy after modification of the workstation during different wood carvings, should be studied in the future.

Not only the workstation was modified in this study, but also the hand tools because they are very important for ergonomic risk factors in all of the wood carvers. Previous data showed a strong relationship between occupational musculoskeletal disorders (MSDs) and the use of hand tools[26] that may result in traumatic events.[27] In addition, inappropriate hand tools have influence on musculoskeletal disorder during work, for example, those in the study of hairstylists.[9, 10] Carving tools were modified by rubber wrapping in order to reduce stress on the palm of the hand and improve grip. Unfortunately, this study did not evaluate the efficiency of tool modification, while the RULA scores of wrist position and twist did not improve. However, it has been suggested that a tool handle should be coated with soft material and a non-slip surface.[10] Thus, the force and safety of hand tools during wood carving should be studied and confirmed in the future. In addition, the size or cross-sectional shapes of the tools may affect contact pressures. A previous study found that cross-sectional shapes of tools caused variations in distribution of contact-pressure, especially in the palm region close to the index finger, which induces a comfortable grip during work.[28]

When the face to face discussions and RULA scores were completed and after the 4-week working trial with the modified workstation, many weak points needed to be corrected. An innovative workstation was designed and developed from the ideas discussed and some evidence in a previous report that evaluated the appropriate posture for weaving.[8] A good workstation should correct the posture of the wood carver to a neutral one (sitting upright), with symmetry according to the sagittal plane, looking straight ahead horizontally, arms hanging down beside the trunk, and forearms perpendicular to the upper arms. In theory, good posture during work has been recommended as neck flexion of less than 20 degrees, shoulder flexion or abduction of less than 30 degrees, shoulder extension and internal rotation, slight elbow flexion, supination and pronation with grasp, the same as wrist flexion or extension, and ulnar or radial deviation while grasping tools, which can prevent musculoskeletal injury.[29]

In addition, the innovative workstation was finally developed with an adjustable tilting stool for the individual wood carver. It had a high seat pan that sloped forward by approximately 10 degrees, thus provoking an anterior pelvic tilt as previously suggested[8, 16] for industrial carpenters.[10] Furthermore, the design of a table with a tilted surface and adjustable stool is very important for wood carving and consistent with a previous recommendation 5 and previous study on carpet weavers, which found that postural discomfort reduced when the weaving height was adjusted to 20 cm above elbow height and a high seat pan sloping forward was used.[8] It is interesting that the wood carvers suggested design of the hand tool spaces in front and at both sides of the table, so that tools can be kept within reach while working with no need to drag, push or pull heavy hammers during carving. A previous study showed that exposure to both 'fatigue-inducing or painful posture' and 'dragging, pushing, or moving heavy objects' related to work-related low back pain.[30] Thus, the development of spaces for keeping

tools reachable possibly helps safe body movement and protects from muscle overload.

Therefore, the innovative workstation in this study should have more benefits for wood carvers such as allowing changes in leg position and helping to decrease neck bending forward when carving. Furthermore, the seat pan of the stool can be tilted together with the wood carver's body movement, possibly promoting a straight low back (lumbar lordosis) and avoiding a slumping position. Moreover, the good posture with neutrality alignment of head, neck, back, trunk, and lower extremities is not only direct clinical effect on neuromuscular function, but it also promotes the diaphragmatic function and breathing efficiency that possibly prevents the respiratory dyspnea during working. Whether an innovative workstation can correct the posture or characteristics of all parts of the body should be studied in the future. Finally, satisfaction with the innovative workstation and adjustable tilted table was discussed completely and immediately considered excellent for wood carving. However, efficiency of wood carving and ergonomic risk should be studied and confirmed in the future.

Weakness, limitation and suggestion

The results of this preliminary study were specific to the wood carver, represented with descriptive data without a statistical method, which possibly makes the data weakness. Moreover, modification and development of the innovative workstation had specificity that limited this study from other specific types of carving and possibly all wood carvers or different occupations. However, studying larger sample sizes, with adjustable seats and workstations for preventing musculoskeletal injury or disorder in various wood carvers, is very interesting for future research. Moreover, other factors in wood carving, especially environments, size of tools, and duration of carving, should be concerned and evaluated in the future. Because previous evidence proposed that risk factors came from inappropriate posture, either prolonged static or dynamic posture, and working environment such as lighting, dust and noise level related to WMSDs[29] should be studied in the future. In addition, multifactorial ergonomic intervention and exercise training should be introduced into the program as recommended in a previous report.[31]

Conclusion

Modification of the workstation and seat for wood carver can correct posture and reduce pain. Furthermore, the innovative workstation with reachable spaces for hand tools in front and at both sides and adjustable tilted table, which possibly could correct low back alignment. This workstation should have more potential benefits for wood carvers, especially protecting the musculoskeletal disorder when compared to original workstation.

Conflict of interest

No potential conflict of interest was reported by the authors.

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A simple method for Isolation of serine protease inhibitor from Siamese land snail Cryptozona (*Cryptozona siamensis*) mucus

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ABSTRACT

Background: Siamese land snail is the single shell snail which has become widespread very quickly in Thailand and other parts of the world. It is considered to be a pest by most farmers since it destroys parts of plants such as leaves, vegetables and fruit by consuming them and leaving behind the line of sticky mucus over the plants. This mucus is believed to be the snail's defense mechanism, such as lubricant, for wound healing as well as a response against the snail's predators. That the land snail releases mucus in response to the predator has been hypothesized, however, screening for protease inhibitor has still not substantiated this. On the basis of the prevailing assumptions, through biochemical, and biophysical isolation we investigated the proteinase inhibitor functions of the protein from the mucus of the Siamese land snail.

Objectives: This study aimed to find a potential protease inhibitor from the mucus of the local Northern Thailand land snail (*Cryptozona siamensis*) together with the biochemical characterization of its general properties.

Materials and methods: Land snail extract collected from Phayao Province, Thailand, was initially subjected to trypsin inhibitory activity assay using alzoalbumin as substrates. The detection of its inhibition activity was assessed by spectrophotometry. Additionally, the molecular size was observed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

Results: Land snail (*Cryptozona siamensis*) mucus contained serine protease inhibitory activity. The molecular size of the inhibitor was suspected to be about 10.5 kD. Interestingly, its inhibitory property was retained after heat inactivation at 100 °C for 20 min.

Conclusion: A novel heat-tolerant serine protease inhibitor from acetone extraction of land snail mucus was characterized as a small peptide with tolerance to organic solvent (acetone) and high temperature, which could be used as a new peptide protease inhibitor targeting serine protease that may benefit to scientific and medical fields.

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Introduction

Protease inhibitors are substances that bind to and inactivate target proteases activities. They play an important role in many biological processes, such as regulation of proteolysis reaction in blood coagulation, and tumor suppression.[1] There have been reports of their application in the medical field, agriculture, and biotechnology.[2] Several protease inhibitors were discovered in a wide range of living organisms, including animals, plants, and microorganisms.[3] However, only a few protease inhibitors have been reported in snail mucus.[4]

Land snail (*Siamese cryptozona*) in Thailand, known in Thai as "hoi-dak-daan", is a small invertebrate animal in the Mollusca phylum with a circular single shell and brown to reddish-brown color. The shell is built up by an open circular Tubular coiled flat with a mature size of up to 30 mm in width. This snail is found throughout Thailand and other parts of the world. It habit is to hide itself during the day in dark and humid places, such as under the log, tree, and rotting tree. At night or dawn, it moves around on the ground, climbing the tree to find food, feeding on leaves, and vegetable.[5, 6] Along with moving around, the land snail also produces mucus. Different substances in land snail mucus have been studied[4, 7] Land snail mucus has been investigated with reports of several beneficial properties, such as antioxidant[8], anti-inflammation[9], antimicrobial[10], anti-cancer[11], and wound healing.[12] As for the commercial applications, land snail mucus has been developed, distributed and sold in the market such as cosmetic beauty products.[13] Snail mucus contains various valuable substances that are waiting to be discovered. In this study, we are interested in isolating and characterizing the protease inhibitor from *Siamese cryptozona* which may be the defense mechanism against microorganisms or predators.

The rapid mucus extraction procedure was introduced to be simple and less equipment consuming. To harvest the mucus from *Cryptozona siamensis*, a simple plastic bag was used as a moving pathway while it moved along and produce mucus in a critical environment as occurred in the nature. This adaptive procedure was capable of producing the large amount of snail mucus for the research as compared with other method of extraction.[14] Acetone precipitation was performed to isolate proteins instead of salt- induced protein precipitation as acetone could effectively solubilize sticky glutinous mucus from the protein pellet, avoiding clumping phenomenal that usually occur when perform salt- induced protein precipitation. Molecular weight of the protein was determined by SDS-PAGE as well as its activity by inhibition of trypsin catalyzing azoalbumin.[15]

It was found that the peptide showed the trypsin inhibition activity with heat-stable property while the molecular weight was approximately 10.5 kD.

Materials and methods

Materials

Land snails were collected from the ground area of the University of Phayao, Phayao Province, Thailand. Trypsin, Azoalbumin, Bovine serum albumin (BSA), and Phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma, USA. all the other chemicals were analytical grade.

Methods

Snail mucus extraction

In this study, 10-20 mature snails were cleaned by wet cloth and put in a clean 6*8" plastic bag, wrapped around and applied to stress at 60 °C for 5 min. The bag was then open in room temperature around 10 min. The snail will climb up toward the open site of plastic bag in company with mucus secretion. The mucus which flew down to the bottom of plastic bags was collected into test tube and centrifuged at 10,000 x g for 10 min to collected supernatant as a crude extract. (Figure.1A,B,C)

Protein precipitation from the mucus of snail with 20-80% serial acetone precipitation

Crude snail mucus was subject to a 20-80% serial acetone precipitation. Start with 4 ml of mucus with 0.8 ml 100% acetone, mixed and kept in -20 °C for 1 hr. then centrifuged to collected pellet and supernatant. Pellet was re-suspended and kept for protein and activity assay. The supernatant was further precipitated with 40, 60, and 80% acetone concentration, respectively.

Determination of protein concentration

Protein content in the sample was determined by a colorimetric method using Bio-Rad protein assay kit I (Bio-Rad, USA). Briefly, pipetting 20 µl of sample or distil water into 1.5 ml micro centrifuge tube following by 1 ml of Bradford reagent (1X), mix well and leave at room temperature for 5 min. Then measured the absorbance at 595 nm. The concentration of the protein sample was calculated according to standard curve of BSA. The protein was express as a unit of milligrams.

Determination of protease inhibitor molecular weight using SDS-PAGE

The molecular weights of inhibitors were estimated using SDS-PAGE. A reaction mixture contained 5 µg of fractionate protein fraction was mix with 5x SDS buffer, immediately heated at 100 ºC for 5 min and then centrifuged at 10,000 x g for 5 min. The supernatant was subjected to 13% polyacrylamide, SDS-PAGE. The proteins were visualized by Coomassie blue staining.

Protease inhibitor assay by spectrophotometric method

Method was modified from Tomarelli 15 as follows, 1000 µL reaction composed of 858 µL of 0.5% (w/v) sodium bicarbonate, pH7.4, 100 µL of 1.25% (w/v) Azoalbumin, 40 µL of distilled water or testing sample (snail mucus extract) or PMSF as positive control and 2 µL of 1 mg/mL of trypsin solution. The reaction was started by adding the trypsin solution and incubate 37 °C for 20 min. Then 300 µL of the reaction mixture was taken, immediately mixed with 600 µl of 5% TCA, and 450 µL of 0.1 M sodium hydroxide was then added. After mixing, the solution mixture was centrifuged at 10,000 x g for 5 min. The supernatant was measured for absorption at 440 nm. To determine the effect of heat, the snail mucus extract was incubated at 100 °C for 10, 20, 40, and 80 min. before adding to the reaction

mixture. The triplicate reaction was carried out in all experiments and the results were evaluated by the mean standard.

Results

Protease inhibition activity of the crude extract from Siamese land snail mucus

Snail 100 gm. was initially used to induce mucus secretion and obtained 40 mg protein crude extract which was then assessed for the protease inhibition activity by trypsin catalyzing azoalbumin.

Partial purification of the protease inhibitor

From crude extract 40 mg protein follow serial concentration 20, 40, 60, and 80% acetone protein precipitation, the recovery protein was 3, 10, 17, and 1mg respectively. All collected sample, were tested for their inhibitions to trypsin. The fraction of 60% acetone precipitation (P60) showed the strongest activity with 57% inhibition (Figure 2).

Determination of protease inhibitor activity using SDS-PAGE.

Following the analysis of fractionated protein by SDS-PAGE. Fraction P60 showed the single major bands of protein approximately 10.5 kD. (Figure. 3).

Thermo stability of the protease inhibitor

Protein from P60 fraction was incubated separately at 100 °C for 10, 20, 40, or 80 min before performing protease inhibition assay by the spectrophotometric method. The protein retaining protease inhibition activity (43% inhibition) after incubation at 100 °C for 20 min. (Figure. 4).

A B C

Figure 1. *Snail mucus extraction. A, Cryptonas siamese snail, B, and C, Cryptonas siamese snail were forced to secrete mucus on a clean plastic bag.*

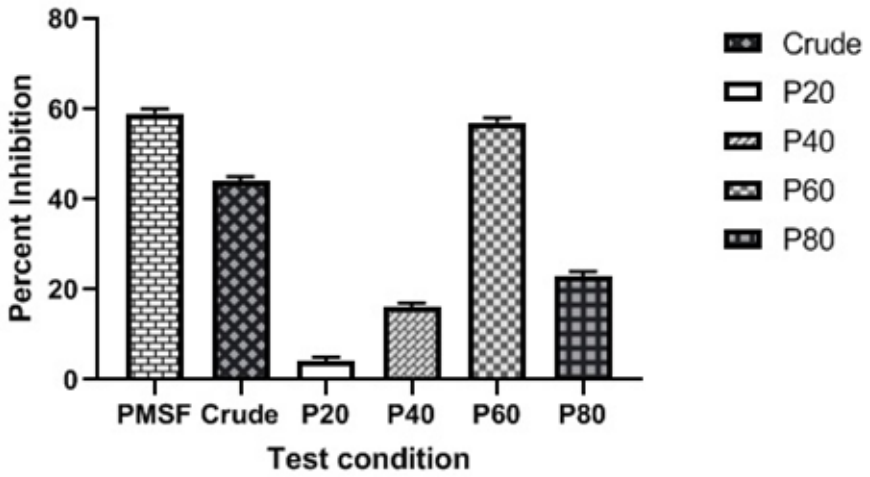

Figure 2. *Inhibition activity of the purified fractions. Crude, 20% (P20), 40 % (P40), 60% (P60), 80 % (P80) acetone precipitation respectively, were tested for their inhibition of trypsin activity.*

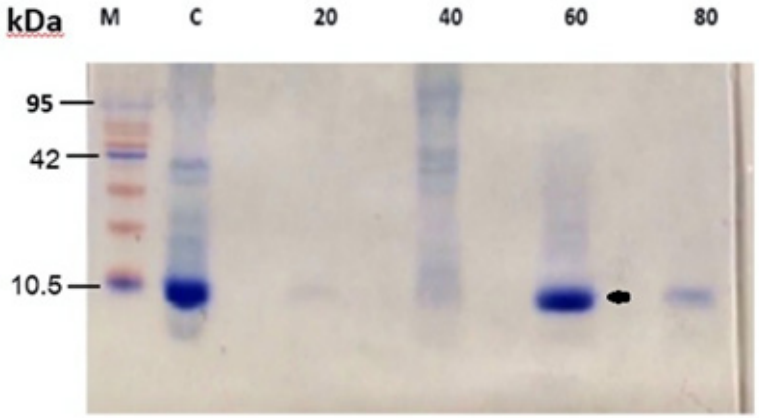

Figure 3. *SDS-PAGE of Cryptonas siamese mucus fractionations. C: Crude, 20, 40, 60, 80 are protein fraction which serial precipitation with 20, 40, 60 and 80% acetone, respectively. Arrow indicated a band of protease inhibitors.*

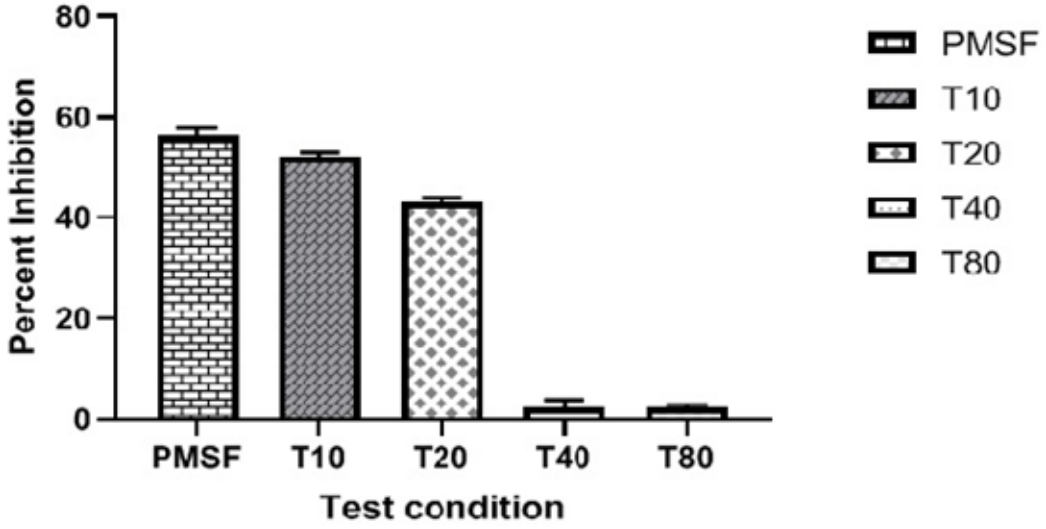

Figure 4. *Thermo stability of the protease inhibition factor. P60 snail extract was divided into four parts and then incubated at 100 °C for 10, 20, 40, or 80 min. (T10, T20, T40, and T80) respectively, before using for the protease inhibition assay.*

Discussion

The mucus of *Cryptonas siamese* contains several worthy substances to investigated and provide necessity to human, for instance; development to gel for wound healing[12], cosmetic cream[13] and other clinical used for human life.[9, 16] In this study, we provided information with a simple method for verify serine protease inhibitor from *Cryptonas siamese*. Including, an easy way to get a mucus from snail in plastic bag, this condition may cause lands snail consider for survival by moving and secreting a lot of mucus to defense itself.[7,17] Protein precipitation from mucus by acetone effectively removed undesirable proteins leaving only clear pellet from a viscous supernatant. This result clearly showed better procedure of protein isolation than salting-out method [10], since high salt usually causes clumping of glutinous mucus and then interfere protein separation. The procedure for evaluating the serine protease inhibition activities was performed simply by colorimetric spectrophotometric method and that for determining molecular size by SDS-PAGE. Following those simple procedures, the serine protease inhibitor in snail mucus was observed. Since several curative products have been made from protease inhibitor especially raised against the virus, this include: NS3; anti-hepatitis C[18], NS3 and NS5 proteins, for anti-dengue drug design.[19] Recently, a potential anti-viral drug discovery against SAR CoV was reported.[20] The present study protein with high protease inhibitor activity was pelleted in the fraction of 40-60% acetone precipitation (Figure. 2) which correlates to Crowell AM work that uses acetone 50-80% to precipitate protein.[21] SDS-PAGE analysis the protein was found the single band of about 10.5 kD (Figure. 3) which is correlated to serine protease inhibitor that reported in *Antheraea mylitta* with the molecular size of 10.4 kD.[22] The present protease inhibitor show high stability toward heat inactivation at 100 °C for 20 min (Figure.4) which is correlated to an evaluated of a serine protease in Death cap mushroom that stability at 100 °C for 10 min[23] and that of small pinto beans which tolerate at 100°C for half an

hour.[24]

This study informs the uncomplicated method to isolate and characterize of protease inhibitor protein from land snail mucus. Next work, the amino acid and DNA sequence, and deep qualifications needed to be verified. This protease inhibitor is plausibly applied to various applications in area of medical, scientific, and industries.

Conclusion

This study highlighted the simple procedure to harvest mucus of *Cryptonas siamese* and also characterized some biochemical properties of isolated protein. The protein contains serine protease inhibitor with molecular size approximately 10.5 kD. The inhibitor is shown to be tolerated to solvent (acetone) and high temperature.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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Content validity and psychometric characteristics of the Thai translated version of the physical activity questionnaire for children (PAQ-C) and adolescents (PAQ-A)

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ABSTRACT

Background: Physical inactivity in young people which increases with age have been linked to increased risks of non-communicable diseases. Therefore, assessment and monitoring of physical activity (PA) in young populations is needed. However, a valid and feasible self-report measure for large-scale PA for Thai children and adolescents is limited.

Objectives: To determine the content validity and psychometric characteristics of the Thai translated version of the Physical Activity Questionnaire for Children (PAQ-C) and Adolescents (PAQ-A).

Materials and methods: The 10-item of PAQ-C and 9-item of PAQ-A were translated into a Thai version and the cross-cultural adaptations were included. These questionnaires were conducted with children aged 8-<14 years and adolescents aged 14-<20 years that were recruited from one large private and two public schools in Amphur Muang, Chiang Mai Province. Each group of children and adolescents was classified by age, gender, and school type, equally. After that, the content validity was assessed using a content validity index. Furthermore, psychometric characteristics, including the scale's internal consistency, test-retest reliability, and agreement of measurements were also determined.

Results: Both PAQs had an excellent content validity index for scale (S-CVI was 0.91 for PAQ-C and 0.96 for PAQ-A) with an acceptable content validity index for item (I-CVI) ranging from 0.83-1.00. The internal consistency and the reproducibility of measurements of the PAQs were acceptable (α = 0.71, ICC = 0.67 for PAQ-C and α=0.84, ICC=0.78 for PAQ-A; P<0.001). The standard error of measurement (SEM) between the two trials for administration of both PAQs were smaller than the smallest detectable change (SDC) (SEM=0.36, SDC=0.99 for PAQ-C; SEM=0.35, SDC=0.96 for PAQ-A).

Conclusion: Having an excellent content validity and acceptable psychometric properties, both PAQ-C and PAQ-A have the potential to be applied for research purposes and surveillance of PA in Thai children and adolescents.

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Introduction

Lack of physical activity (PA) in young people has become a public health concern worldwide. It has been reported that insufficient PA generally occurs with aging, and might consequently lead to increased risk of morbidity and mortality.[1] In Thailand, a national survey on PA in children and adolescents found that 23% of them were insufficiently active according to the global PA guidelines.[2] Their PA levels were also associated with several factors including sex, age, body mass index (BMI), geographical regions, and participation in sports and recreation.[3] However, a systematic review[4] of PA and sedentary behavior have shown that PA studies among children and adolescents remained scarce and there was a lack of the specified and validated instruments for surveillance and monitoring of PA in Thai children and adolescents. A valid instrument could be used to understand the health behaviors of young people and identify the effectiveness of interventions designed to enhance PA.

The use of a self-report questionnaires for measuring PA among young people has raised interest because it is practical, economical, time-efficient, and feasible for use in a large-scale study. A systematic review[5] of PA instruments reported that the Physical Activity Questionnaire for Children and Adolescents (PAQ-C/PAQ-A, PAQs) is one of the most suitable tools for assessing levels of PA for young population. The PAQ has acceptable reliability (ICC=0.82-0.96 for PAQ-C and 0.71-0.78 for PAQ-A; kappa=0.51-0.68 for PAQ-A) and validity (α=0.72-0.82 for PAQ-C and 0.67-0.85 for PAQ-A) for measuring PA.[6-13] Both PAQS have shown the association with health-related fitness. For example, the total PAQ-A score showed a moderate validity with peak oxygen uptake[12] and the activity monitor.[10] However, PAQ-C showed a low to moderate association with accelerometer,[6, 9, 13, 14] activity monitor,[10] body fat percentage,[7, 15] and cardiovascular fitness.[7, 8, 15] The PAQ was translated and is used in many countries including Italy,[6] Japan,[7] United Kingdom (UK),[8] Hong Kong,[9] United States,[10, 15] Belgium,[12] Spain,[13] and Canada.[14] Prior to using the PAQ in the Thai population, cross-cultural adaptation and psychometric properties must be evaluated. The aim of this study was to examine the content validity and psychometric characteristics of the Thai version of both PAQs.

Materials and methods

Participants

A school-based survey was conducted on healthy children aged 8-<14 years and adolescents aged 14-<20 years who reside in Amphur Muang, Chiang Mai Province. These purposing samples were recruited from three schools including a large private school, a public primary school, and a public high school which contacted participants for permission prior to the beginning of the study. Participants with illness or other limitations to regular activity occurring one week prior to data collection were excluded from the study. A sample of 48 children and 48 adolescents were recruited for the content validity process, and 120 children and 110 adolescents were enrolled for the reliability process in accordance with previous guidelines.[16] In each process, participants in each group were randomly assigned according to the allocation orders by school type, age, and sex. All children and their parents provided written inform consent. Data was collected in February-March of 2018. Ethical approval was granted by the Research Ethics Committee, Faculty of Associated Medical Sciences, Chiang Mai University.

Instruments

The original Canadian PAQ was developed in 2004 by Kowalski et al.[17] It was structured to evaluate the level and the frequency of moderate to vigorous physical activity (MVPA). This questionnaire has two versions: one for children aged 8-<14 years and one for adolescents aged 14-<20 years. PAQ-C has demonstrated good internal consistency and test-retest reliability (α=0.79-0.89 and ICC=0.75-0.82). Both versions of the PAQ moderately correlated with the objective activity measures such as Caltrac motion sensor (r=0.39 for PAQ-C and r = 0.33 for PAQ-A).[18] Both PAQs are a self-measuring seven-day recall of PA during leisure time, school time, and the weekends. The PAQ has ten question items designed for children (C) and nine for adolescents (A). Both PAQs have similar question items, except that the question item asking for MVPA in morning break was excluded for PAQ-A. The first question item of the PAQ is comprised of a checklist of 22 common leisure and sport activities and two other fill-ins using 33 choices. This question is scored as the average of all activities. The remaining PAQ question items were distinguished by time of day and time during the weekends. Each activity item was scored on a five-point Likert scale, with higher scores indicating higher levels of PA. The summary score is the average of the nine question items for PAQ-C and eight for PAQ-A. The last item of each PAQ, asking for other reasons that prevented the participant from engaging in regular PA, was not used to calculate the summary score.

Procedure

The study was divided into two phases. Phase one was a qualitative method designed to determine the test content and response process validity of both PAQs. Phase two assessed the psychometric characteristics of the PAQs. These procedures were administered to participants during school hours. In phase one, all translation processes were comprised of five steps and were conducted based on previous guidelines.[16] Initially, the original English PAQ-C/PAQ-A was translated to produce two forward-translated Thai versions by two independent bilingual and bicultural Thai translators. Both translators are Ph.D. lecturers in the Departments of English and Physical Therapy, Chiang Mai University. The two forward-translated Thai versions and the original version were then compared by the same translators along with a third bilingual and bicultural Thai translator regarding ambiguities and discrepancies of words, sentences, and meanings generated in the preliminary translated version (PI-TL). The third translator is a Ph.D. lecturer in the Department of Occupational Therapy, Chiang Mai University. The PI-TL was then back-translated into the English by two additional independent translators. They are Ph.D. lecturers in the Departments of English and Physical Therapy, Chiang Mai University. The semi-final Thai version of PAQ-C/PAQ-A was synthesized by a seven-member expert committee including

the five translators involved in generation of the preliminary translated version of the PAQ and two researchers. The original English version of PAQ-C/PAQ-A, the PI-TL, and two back-translated versions were reviewed, revised, and verified to achieve the cross-cultural equivalence based on sport and exercise disciplines. Finally, a pilot testing of the final version of Thai PAQ-C/PAQ-A was performed using a focus group interview to determine the response process validity of the PAQs in order to evaluate the instructions, response format, and the clarity of items of the PAQ. A small group of three to five participants were assigned to a well-trained staff that sought to obtain evidence for test content and response process validity. Testing lasted for 15-20 minutes per session. For primary school students, the content of PAQ was read and explained by the staff. A six-member expert committee including two senior physical education lecturers, two physical education teachers, and two senior physical therapy lecturers who had the background in sports science and/or exercise physiology were asked to rate each item of PAQ-C and PAQ-A to determine content validity.

In Phase two, participants completed the final version of Thai PAQ-C/PAQ-A twice with a two-week interval scheduled between tests to determine reproducibility of measurements. Of those, the second responses to the modified PAQ-C and PAQ-A were used to explore the internal consistency of the questionnaire according to the methods of Gauthier et al.[19] Also, the measurement error and the degree of test-retest agreement were determined using the smallest detectable change (SDC) and Bland-Altman plot, respectively. Data collection for each age group developed according to the children's capabilities and maturity was done during school class time. Participants aged 8-<11 years were asked to complete the PAQ-C in the presence of a research staff in a proportion of 5 to 1. Participants aged older than 10 years completed the questionnaire with a class teacher. A total of eighteen class teachers from both private and public schools were provided explanations regarding the study and were assigned to distribute the PAQ questionnaires to their children aged 11-<14 years (n=6) and adolescents aged 14-<20 years (n=12).

Statistical analysis

Data distribution was examined using the Shapiro Wilk test. After that, descriptive statistics were generated to describe sample characteristics. The content validity index for items (I-CVI) was determined by asking the expert committee to rate each question item of both PAQs in terms of its relevance to the underlying construct based on a 4-point ordinal scale (i.e. 1: not relevant; 2: somewhat relevant; 3: quite relevant; and 4: highly relevant). Subsequently, the I-CVI was calculated for each item as the number of experts who rated the scale of 3 or 4 divided by the total number of experts. The content validity index for scale (S-CVI) was computed for each PAQ as the average of the I-CVIs for all items on the scale. The I-CVIs of 0.78 or higher and an S-CVI of 0.90 or higher were considered as excellent content validity.[20] The intraclass correlation coefficient (ICC) was used to examine the test-retest reliability. ICC values of >0.90 are considered excellent, 0.75-0.90 good, 0.50-0.75 moderate, and <0.50 poor.[21] The internal consistency of the questionnaire was analyzed using Cronbach's alpha coefficient (α), with a value of 0.70 or greater deemed acceptable.[22] Every single question item was removed to confirm redundancy of the individual items, using Cronbach's alpha. The agreement between two measurements of the PAQ was determined by two methods based on previous guidelines.[23] First, the Bland-Altman analysis which indicate random error and bias was graphically plotted. The 95% limits of agreement were calculated as mean difference +1.96 SD of the differences. Second, the standard error of measurement (SEM) which depicts the within-subject variability and the smallest detectable change (SDC) were both determined in order to indicate a real change was calculated as SEM=SD x [square root (1-ICC)] and SDC=SEM x 1.96 x square root(2), respectively. For interpretation, the measurement error should be smaller than SDC to ascertain that a real change has occurred. Statistical analyses were carried out using SPAA version 19.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Table 1 Descriptive characteristics.

	Children			Adolescents		
	Male (n=57)	Female (n=58)	Total (n=115)	Male (n=49)	Female (n=47)	Total (n=96)
Age (yrs)	10.36 (1.87)	10.29 (1.87)	10.32 (1.87)	16.10 (1.62)	16.04 (1.63)	16.07 (1.62)
Stature (m)	1.44 (1.34)	1.42 (1.11)	1.43 (0.12)	1.54 (0.48)	1.51 (0.24)	1.53 (0.37)
Body mass (kg)	42.30 (16.09)	36.60 (9.96)	39.38 (13.54)	64.41 (17.52)	51.97 (10.31)	58.05 (15.53)
BMI ($kg.m^{-2}$)	19.88 (4.57)	17.95(3.16)	18.89 (4.01)	22.69 (5.70)	21.79 (4.08)	22.23 (4.93)
A summary PAQ score	2.80 (0.56)	2.53 (0.63)	2.67 (0.61)	2.61 (0.81)	2.20 (0.61)	2.41 (0.74)

Note: *Values are presented as mean±SD, BMI: body mass index, PAQ: physical activity questionnaire.*

Sample characteristics

Data for five children who had an ankle sprain or illness during the second set of reproducibility testing were excluded. Fourteen adolescents aged 17-19 years (6 male and 8 female) refused to participate in the study and there were no adolescents with age ranges of 19-<20 years in the private school. This resulted in a final sample size of 115 children and 96 adolescents. Participants' characteristics are shown in Table 1.

Table 2 I-CVI and S-CVI scores for the Thai PAQ-C.

Item	Expert 1	Expert 2	Expert 3	Expert 4	Expert 5	Expert 6	I-CVI
Q 1. Activity checklist	4	3	4	4	3	4	1.00
Q 2. Physical education	4	3	3	3	4	4	1.00
Q 3. Recess	3	3	4	4	2	4	0.83
Q 4. Lunch	3	3	4	3	1	4	0.83
Q 5. After school	4	2	4	4	4	4	0.83
Q 6. Evenings	4	4	4	4	4	4	1.00
Q 7. Last weekend	4	4	4	4	4	4	1.00
Q 8. Self-description	4	2	3	3	3	4	0.83
Q 9. Weekly activity	4	2	4	4	4	4	0.83
						S-CVI	0.91

Note: *I-CVI: the content validity index for items, Q: question, S-CVI: the content validity index for scale.*

Phase one: Translation and Adaptation processes

Response validity process

Approximately 94.8% of children (n=48) and adolescents (n=43) participated in this process. The remaining 5.2% (n=5) were adolescents aged 19-20 years that could not be found during data collection. According to participants' feedback, several changes were made to the questionnaire including removing the name of classroom teacher, adding the school name, and changing the format for sex from an underline to a tick box. The instructions were added to clarify the way participants should answer questions, such as "please fill out the following how you usually do". In terms of cultural adaptation, four uncommon activities in Question One were removed: football, street hockey, cross-country-skiing, and ice hockey/ringette. Six activities commonly conducted by Thai children and adolescents including table tennis, sepak takraw, tennis, chair ball, petanque, and the hula hoop were added. The definition for "tag" was defined. Question Two involved the frequency of performing activities was clarified by adding a percentage to each written frequency: don't do (0%), hardly ever (20%), sometimes (<50%), quite often (>50%). and always (80%). Question Ten for the PAQ-C and Question Nine for the PAQ-A were the last questions and were concerned with participant illness. These questions were modified for clarity.

Table 3 I-CVI and S-CVI scores for the Thai PAQ-A.

Item	Expert 1	Expert 2	Expert 3	Expert 4	Expert 5	Expert 6	I-CVI
Q 1. Activity checklist	4	3	4	4	3	4	1.00
Q 2. Physical education	4	3	4	3	4	4	1.00
Q 3. Lunch	3	3	4	3	2	4	0.83
Q 4. After school	4	2	3	3	4	4	0.83
Q 5. Evenings	4	3	4	4	4	4	1.00
Q 6. Last weekend	4	3	3	4	4	4	1.00
Q 7. Self-description	4	4	4	3	4	4	1.00
Q 8. Weekly activity	4	3	4	4	3	4	1.00
						S-CVI	0.96

Note: *I-CVI: the content validity index for items, Q: question, S-CVI: the content validity index for scale.*

Content validity

Both PAQs had I-CVIs ranging from 0.83-1.00. For PAQ-C, I-CVI was highest for Questions One, Two, Six, and Seven and were rated as average for the remaining questions. For PAQ-A, I-CVI was highest for Questions One, Two, Five, Six, Seven, and Eight. The remaining two questions were rated as average. Both PAQs showed a high S-CVI (0.91 for PAQ-C and 0.96 for PAQ-A) (Tables 2 and 3).

Table 4 Test-retest reliability and internal consistency of the Thai PAQ.

Activity	PAQ-C (n=115)		PAQ-A (n=96)	
	ICC (95%CI)	α if each item deleted	ICC (95%CI)	α if each item deleted
Total PAQ-score	0.67 (0.54-0.77)	0.71	0.78 (0.68-0.85)	0.84
Q 1. Activity checklist	0.70 (0.46-0.83)	0.75	0.55 (0.37-0.68)	0.88
Q 2. Physical education	0.57 (0.43-0.68)	0.78	0.53 (0.32-0.68)	0.90
Q 3. Recess	0.61 (0.47-0.71)	0.76	NA	NA
Q 4. Lunch	0.44 (0.28-0.58)	0.76	0.65 (0.52-0.76)	0.87
Q 5. After school	0.46 (0.30-0.59)	0.75	0.62 (0.47-0.73)	0.85
Q 6. Evenings	0.40 (0.23-0.54)	0.73	0.47 (0.29-0.61)	0.85
Q 7. Last weekend	0.44 (0.28-0.58)	0.75	0.42 (0.24-0.57)	0.87
Q 8. Self-description	0.37 (0.20-0.52)	0.75	0.59 (0.44-0.71)	0.85
Q 9. Weekly activity	0.57 (0.42-0.69)	0.73	0.45 (0.28-0.60)	0.85

Note: *: ICC of all items have p value <0.001, α: Cronbach's alpha, A: adolescents, C: children, CI: confidence interval, ICC: Intraclass correlation coefficient, NA: not applicable, PAQ: physical activity questionnaire, Q: question.*

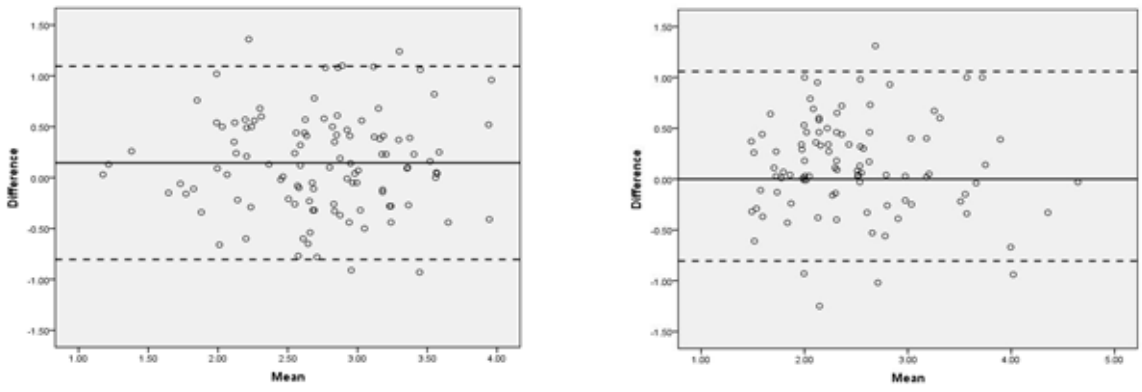
Phase two: Reliability process

Test-retest reliability

The ICC values for the total score of the PAQ-C and PAQ-A were 0.67 and 0.78, respectively. The reliability values of Questions Four to Eight of the PAQ-C were less than 0.50. The reliability values of Question Five, Question Six, and Question Eight of the PAQ-A were less than 0.50 (Table 4).

Internal consistency

PAQ-C and PAQ-A generated a Cronbach's alpha of 0.71 and 0.84, respectively. Both PAQs showed that the internal consistency had slightly increased after deleting every single question item, compared to that of the total PAQ score. This indicates that no redundant items are included in the questionnaire. PAQ-A obtained higher values for internal consistency than the PAQ-C for all questions and the total score (Table 4).



(A) PAQ-C ***(B) PAQ-A***

Figure 1. *Bland-Altman plots showing the agreement between two trials for PAQs measurement. Solid line: mean difference between test and retest, dashed lines: limits of agreements (± 1.96 * standard deviation (SD)).*

Agreement of measurements

The mean difference of PAQ-C scores for two measurements was 2.73. The SEMs and SDC for PAQ-C activity were 0.36 and 0.99, respectively. The Bland-Altman analysis showed that the mean difference (±SD) among two trials for PAQ-C measurement was 0.15±0.48 (95% CI=0.054-0.237) (Figure 1A). The mean difference of PAQ-A scores for two measurements was 2.47. The SEMs and SDC for PAQ-A activity were 0.35 and 0.96, respectively. The Bland-Altman analysis showed that the mean difference (±SD) among two trials for PAQ-A measurement was 0.13±0.48 (95% CI = 0.304-0.223) (Figure 1B). There were four children (3.48%) and five adolescents (5.21%) whose results were not within the limit of agreement.

Discussion

In this study, we translated the English version of the PAQ-C and PAQ-A into Thai. We also performed cross-cultural adaptation and examined its psychometric characteristics. An excellent content validity and an acceptable internal consistency and test-retest reliability suggests that both PAQs are reliable tools for measuring physical activity in Thai children and adolescents. The contextual and cultural–specific modifications of the Thai PAQs including the instructions, questions, response format, and physical activities checklist were done to derive a validated PAQs that is appropriate for young Thai people. Additionally, the agreement of measurement between two trials observed for both PAQs indicate the PAQs abilities to detect changes in PA between time periods.

Three types of the reliability results of the Thai PAQs were reported. Firstly, we found that the Cronbach's alpha values of both PAQ-C and PAQ-A were within acceptable range, indicating good internal consistency of results across questionnaire items. These results were similar to results obtained from previous studies.[8-12, 15] Secondly, the test-retest reliability which denote the stability of results across time was moderate for PAQ-C and its ICC value was lower than that of children in other countries.[8-10, 15] Furthermore, the observed PAQ-A showed good reliability similar to a study done on English youth. Thirdly, the present study showed a mean difference (±SD) between the first and second trials of the PAQ-C and PAQ-A measurements that was greater than the original English version (-0.08±2.21 and -0.02±0.07, respectively).[17] These measurement errors in both PAQs were small, whereby the value of SDC remained lower than the mean score of the PAQs. The Bland and Altman plot also showed minimal range in the limit of agreement compared to the mean score of both PAQs. Taken together, this data confirms the stability of the PAQs scores over time. However, these reliability results of children seemed inferior to that of adolescents. This might be explained by their cognitive limitation in recall ability or comprehension.[5, 24, 25] Thus, the measurement of children's PA should be carefully handled. It should be noted that a research assistant and images using picture illustrations of each activity in Question One might be required for better validation of results.

We found that all Thai children and adolescents except boys failed to meet the criteria for PAQ-score which corresponds to 60 minutes of MVPA as obtained in the study done by Benetez et al[26] (a cut-point of 2.75 for PAQ-A and 2.73 for PAQ-C). However, different results were obtained when using the Voss et al[27] cut points (2.9 for boys and 2.7 for girls). Both boys and girls in this study failed to meet that criteria. Likewise, the observed summary PAQ score of Thai children was lower than that of children from western countries such as the UK or Canada, but were higher than that observed in Hong Kong children.[8, 9, 15, 18] In addition, a lower level of PA in adolescents was observed compared to children and males engaged in PA rather than for females in both groups. These results indicate that PA of Thai children seems to decrease with age and also has sex disparities, which was consistent with previous studies.[10, 12, 27] Therefore, PAQs score cut-off values should be established at a national and/or international level for monitoring PA of both children and adolescents. The individual PA type and patterns based on information obtained from the PAQs might be analyzed and used for compliance and as interventions to increase their level of physical activity.

This study's strengths included the rigorous protocols that were employed during the translation and sampling processes and made use of a broad sample range of samples that was distinguished by sex and age that improves the generalizability of the findings. The validity and reliability of the PAQ for children and adolescents were established, as well. However, some limitations should be considered in this study. First, the PAQ is a subjective measure of PA and could not be used to provide measurements of time or intensity relative to PA guidelines. Further study is needed to validate the PAQ against the objective measures of PA to confirm its feasibility as an effective tool. Secondly, data collection in classes was performed at the teacher's discretion even after permission had been granted from the school principal. Additionally, the research staff was not allowed to meet participants in some classes. This might interpose error into measurement. Lastly, participants in the study were recruited from urban areas, so these results do not imply that similar results could be obtained from other areas. Likewise, seasonality should be considered when determining physical activity in children. A previous study[28] has shown that seasonal differences in physical activities were observed among healthy children, but not for adolescents. Children had significantly higher mean physical activity levels in Spring than in Winter and Fall.

Conclusion

Excellent content validity and acceptable reliability suggests that the Thai version of PAQs could be used in local studies measuring the overall level of PA in children and adolescents and for monitoring change in PA over time.

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Conflict of interest

There are no conflicts of interest.

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Effects of cognitive training on fall risk and cognitive performance in individuals with mild cognitive impairment

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ABSTRACT

Background: Accumulating evidence suggests that older adults with mild cognitive impairment (MCI) not only had cognitive impairment but also fall risk. Cognitive training has been shown to either improve cognitive function or reduce fall risk among older adults with and without cognitive impairment. Only a limited number of studies have investigated the beneficial effects of cognitive training on both cognitive performance and fall risk in older adults with MCI and the findings have been inconclusive.

Objectives: To examine the effectiveness of cognitive training program on fall risk and cognitive performance in older adults with mild cognitive impairment.

Materials and methods: Forty older adults with MCI (mean age 68.91±4.25) were randomized into the intervention group (n=20) and control group (n=20). Participants in the intervention group underwent cognitive training program for 60-70 minutes per session, 3 times per week for 12 consecutive weeks. The control group received educational tutorial covering cognitive enhancement and fall prevention strategies. Outcome measures were fall risk including Timed Up and Go test (TUG) single and dual task, Physiological Profile Assessment (PPA), and cognitive performance including, Alzheimer's disease Assessment Scale (ADAS-cog), Verbal Paired Associated (VPA)-immediate recall, and Trail Making Tests (TMT). All outcomes were assessed at baseline and the end of the 12-week training.

Results: At the end of 12-week training, participants in the intervention group had significantly better performance on ADAS-cog, VPA-immediate recall, and TUG dual task compared to the control group (p=0.007, p=0.033, and p=0.006 respectively). They also demonstrated significant improvement on TUG single and dual task, ADAS-cog, VPA-immediate recall, and TMT B-A from baseline (p=0.006, p=0.002, p=0.001, p=0.012, and p=0.019 respectively), whereas these improvements were not observed in the control group.

Conclusion: The 12-week cognitive training program has a beneficial effect in improving global cognitive function, memory, and decreasing fall risk while performing functional mobility in concurrent with cognitive task in older adults with MCI. The findings suggest that cognitive training should be considered when designing a fall prevention program for older adults with MCI.

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Introduction

Cognitive impairment such as dementia and Alzheimer's disease (AD) is a common geriatric syndrome that leads to functional decline and loss of independence in older adults.[1] Mild cognitive impairment (MCI) is the transitional state between early dementia and normal cognitive function.[2] Rate of progression to dementia is about 3 to 5 times greater for older adults with MCI as compared to cognitively intact older adults.[3] However, unlike Alzheimer's disease and dementia, MCI is potentially reversible.[4] Previous study found that approximately 44 percent of older adults with MCI return to normal cognition one year after the first assessment.[5] Therefore, MCI has been regarded as the target population for preventive interventions of dementia.

Executive function, episodic memory, and attention are neurocognitive domains often impaired in persons with MCI.[6, 7] Emerging evidence has demonstrated that cognition particularly, attention and executive function which consisted of mental flexibility, updating working memory, and inhibition is associated with gait, balance, and falls.[8, 9] Moreover, a series of dual-task studies have demonstrated that persons with cognitive impairment showed decreased gait speed and increased gait variability when walked under dual-task condition.[10, 11] These findings suggest that cognitive impairment, balance and gait impairment are linked. Furthermore, previous studies revealed that older adults with MCI not only demonstrate cognitive impairment, but also balance and gait impairment.[12, 13] In line with these findings, it has been reported that the incidence of falls in older adults with MCI was twice that of cognitively intact peers.[14] Thus, older adults with MCI are at increased risk of dementia and falls. Therefore, interventions that could improve both cognitive function and reduce risk of falls in this population are imperative.

Systematic review and meta-analysis have concluded that cognitive interventions are an effective method to delay cognitive decline in MCI. The training duration of 12 weeks is recommended as this period has been shown to be sufficient to demonstrate the sustained effects of training on cognition without inducing the attrition risk.[15, 16] While extensive studies have demonstrated benefits of cognitive training on cognitive outcomes, only a limited number of studies have examined their effects on gait, balance, and fall risk and findings have been inconclusive. Li et al.[17] found that healthy older adults who received computer-based cognitive intervention involved executive function training skills over 10 weeks improved significantly in body sway compared with baseline, while the control group showed no improvement. In addition, Smith-Ray et al.[18] found that cognitively intact older adults who received computer-based cognitive training program which focused on executive function demonstrated significantly better performance on the Timed Up and Go (TUG) and 10 meters walk test (10MWT) than the control group. However, in their later study, they only found an improvement trend for TUG and other 4 of 5 standing balance outcomes when implemented the cognitive training program to older adults with cognitive impairment.[19] The authors attributed the discrepancies in findings to methodological limitations in the study including the lack of information on confounding factors (i.e. polypharmacy and change in social engagement) and the use of pre-post, within-subject design. Taken together, further research is warranted to confirm the beneficial effects of cognitive training on gait, balance, and falls. Furthermore, studies that concurrently explore the effects of cognitive training on both cognitive and physical outcomes are scarce. This information would provide better understanding regarding the link between cognitive and physical functions.

Therefore, the present study aimed to examine the effects of cognitive training on fall risk and cognitive performance in older adults with MCI and hypothesized that cognitive training would significantly reduce fall risk and improve cognitive performance compared to controls.

Materials and methods

Design and Participants

A cluster randomized controlled trial design was used in the present study to avoid contamination between the intervention and control groups. The sample size calculation was based on the following assumptions: a general linear model, medium effect size (0.24), a power of 0.80, and an alpha level of 0.05. The effect size of 0.24 was obtained from the PPA fall risk score from the pilot study. With this, a total sample size of 34 participants was required. To accommodate 15% drop-out, a total sample size of 40 participants (20 participants per group) was recruited in the study.

Forty older adults aged 65 years or older with MCI were recruited from the local communities in Chiang Mai. All participants met the following criteria for MCI:[20] (a) subjective concern on declining cognitive function or changing in cognition by the participants and/or informant, (b) objective cognitive impairment in one or more cognitive domains, (c) generally independence in everyday functioning, (d) absence of clinical dementia which determined by Mental State Examination T10 (MSET10).[21] Other inclusion criteria were presence of mild impairment based on the Montreal Cognitive Assessment (MoCA) score of <23[22], able to walk without an assistive device at least 10 meters, and able to follow the study procedures.

Participants were excluded if they had neurological conditions (e.g. cerebrovascular Disease, multiple sclerosis, and Parkinson's disease, etc), chronic diseases (e.g. severe cardiovascular disease, poorly controlled hypertension, and crippling arthritis) which affect gait and balance, depressive symptoms (Thai Geriatric Depression Scale (TGDS) ≥6), uncorrected visual or hearing impairment, participated in other cognitive training programs, treated with cognitive enhancing drugs, or exercised regularly (≥30 min/d, ≥3 d/wk). The study protocol was approved by the Human Ethical Review Board of the primary investigator's institution (COA No. AMSEC60EX024). All participants signed the written informed consent form and were asked for demographic data including age, gender, weight, height, medicine, education, and fall history prior to participation. A fall is defined as "an unexpected event in which a person comes to rest on the ground or lower level".[23]

Cognitive training protocol

Participants in the intervention group received cognitive training program. The training protocol was based on previous studies which shown to be effective for older adults with MCI.[24-26] Specifically, it covered three neurocognitive subdomains (i.e. memory, executive function, and attention) often impaired in people with MCI[6, 7] and associated with gait and balance.[8, 9] The training protocol included the following training components:

Memory training included the practice of using Method of Loci (MOL) technique, visual and auditory memory training. For the MOL technique, participants practiced remembering words by forming an association between the words and participant's familiar environment.[25] For visual memory training, they practiced remembering the objects and its location presented on the screen. They were asked to listen to a short story and remember its content in auditory memory training.[24]

Attention training included the practice of both visual and auditory attention. For the visual attention training, participants were asked to find the pair of cards which had the same letter/picture sequences.[26] They practiced detecting specific words in a song in auditory attention training.[24]

Executive function training included the practice of visuospatial skill through visual perception, inhibition training, and simulated tasks in activity of daily living that require executive function. For visuospatial skill training, participants practiced visual perception by associating body positions with object positions or the relationship between objects.[26] For inhibition training, they were asked to respond or not respond to certain conditions predetermined in the game's rule. Finally, complex tasks in activity of daily living such as creating a recipe from the ingredients available, describing a cooking process, calculating the total cost of goods.[24]

The cognitive training was implemented in a group-based setting (3-5 persons per group) by one physiotherapist researcher. Participants attended 60-70 minutes per session, 3 days per week for 12 consecutive weeks (36 sessions). The training program was classified into 3 phases based on the level of cognitive demand. The training program was progressed every 4 weeks in a group based format.

Participants in the control group received educational tutorial covering cognitive enhancement and fall prevention strategies. A weekly phone call was delivered to each participant to monitor their general health and activities. Furthermore, participants in both groups were asked to maintain their routine lifestyle throughout the study period and to inform the research team if any unexpected events occurred. Participants who missed the class ≥20% of the total training sessions or unable to complete both pre- and post- assessment were withdrawn from the study.

Outcome measurement

The outcome measurement were fall risk (Timed Up and Go test and Physiological Profile Assessment) and cognitive performance (Alzheimer's Disease Assessment Scale, Verbal Paired Associated-mmediate recall, and Trail Making Tests). Structured instructions and demonstration of each test were given to the participants prior to testing. All outcomes were assessed at baseline and the end of the 12-week training by blinded assessors. Each outcome measure (i.e. PPA, TUG, cognitive outcomes) was administered by one assessor, therefore, baseline and post-training assessment for each test was performed by the same assessor. The assessors were physiotherapists trained by experienced geriatric physiotherapist or neuropsychologist to deliver the assessment using a standard protocol. All assessors practiced administering the test to ensure that they provided standardized assessment prior to data collection.

Timed Up and Go test (TUG)

TUG is extensively used as a routine screening test for falls in older adults. TUG is a functional mobility test that involves activities in daily life through an aspect of balance and gait assessment. The participants were asked to rise from a chair, walk as fast and safely as possible to a 3-meter line marked on the floor, then turn 180 degrees and walk back to the chair and sit down.[27] Furthermore, the TUG dual task was assessed in the study. The participants were instructed to perform the TUG test in concurrent with the naming test in each trial (i.e. animal and fruit naming tests).[28] Two trials were undertaken and the average time to complete the trials was recorded.

Physiological Profile Assessment (PPA)

The PPA is used to evaluate risk of falls through directly assessing an individual's physiological abilities. The short version of PPA was used in the study. It involves five simple tests of vision (edge contrast sensitivity), knee proprioception, knee extension force, hand reaction time, and body sway.[29] Edge contrast sensitivity was assessed by using the Melbourne Edge Test, which includes 15 circular patterns containing edges with reducing contrast. The lowest contrast patch that the participant can identify correctly was recorded in decibel units. Knee proprioception was assessed by using lower limb matching test. The participants were asked to align their lower limbs on either side of a vertical clear acrylic sheet inscribed with a protractor which placed between their legs. The average of five differences in aligning the lower limbs was measured in degrees. A spring gauge was used to measure force of knee extensor muscle. The participant was asked to extend the dominant knee against the spring gauge with maximal force. The maximal force of the three trials was recorded in kilograms. For hand reaction time, the participant was asked to press the modified computer mouse with their finger as fast as possible in response to the light stimulus. An average of 10 testing trials was recorded in milliseconds. Finally, the sway meter was used to measure a displacement of body sway. The participants were asked to stand on medium density foam with eyes open for 30 seconds. The sway path was recorded in millimeter. PPA composite score which calculated from the five subdomains through the NeuRA FallScreen® software was used to indicate fall risk. The higher composite PPA scores indicate higher risk of falling.[29]

Alzheimer's Disease Assessment Scale (ADAS-cog)

The ADAS-cog was used to measure global cognitive function in the study. The ADAS-cog is the most commonly

used test to measure cognitive changes in persons with Alzheimer's disease (AD).[30] It has also been verified to be useful for assessing cognitive function among individuals with MCI.[31, 32] The ADAS-cog consists of 11 test items as follows; word recall, naming, commands, constructional praxis, ideational praxis, orientation, word recognition and 4 questions for the assessor to rate the participant's ability on remembering test instructions, language, comprehension of spoken language and word finding difficulty. The higher the ADAS-cog score indicates the greater severity of cognitive impairment.[30]

Verbal Paired Associated-immediate recall (VPA-immediate recall)

The VPA-immediate recall was used to assess memory in the study. It is a subtest in the Wechsler Memory Scale-III (WMS-III). The participants were asked to remember eight pairs of related and unrelated words which the assessor read. Then, the assessor read the first word of each pair and the participants were asked to provide the corresponding word. The higher score of average three testing trials indicates a greater memory performance.[33]

Trail Making Tests (TMT)

The TMT is one of the most commonly used tests to measure executive function. The TMT consists of 2 parts, A and B. In the TMT Part A, the participants were asked to draw a line to connect the consecutive numbers as fast and correctly as possible (e.g. 1–2–3). For TMT Part B, they were asked to draw a line to connect consecutive numbers and Thai letters alternately (e.g. 1–ก–2–ข–3) as quickly and correctly as possible. The difference between the time taken to complete part B and A (B-A) was used as a measure of task switching ability, a subdomain of executive function. A smaller difference score indicates better shifting ability.[34]

Statistical analysis

All statistical analyses were performed using SPSS software (version 21.0, IBM Corporation, Chicago, IL). Tests of data normality were performed using the Shapiro-Wilk test. The demographic data of the two participant groups were compared by using independent student t-test and Chi-Square test. Two-way mixed model repeated measures ANOVA was used to compare the outcome measures across the two different assessment intervals (at baseline and the end of 12-week training) and between the two groups. The significance level was set at $p<0.05$, 2-sided. Partial eta-squared (η_p^2) was used to estimate effect size in the present study. The equation of partial eta-squared is

$$\eta_p^2 = \frac{SS_{\text{effect}}}{SS_{\text{effect}} + SS_{\text{error}}}$$

; where SS is sums of squares. Partial eta-squared values are interpreted as follows: 0.01 or more are small effect size, 0.06 or more are medium effect size, and 0.14 or more are large effect size.[35]

Results

Participants

Forty older adults with MCI aged between 65-80 years old (mean 68.91±4.25 yr.) participated in this study. There were no significant differences in any demographic characteristics between the two groups ($p>0.05$). Participant demographic characteristics are shown in Table 1. Participants in the intervention group attended the class with an average of 33.95±.39 sessions (range 30-36 sessions). No participant was withdrawn from the study.

Table 1 Baseline characteristics of participants (N=40).

Characteristic	Intervention Group, n = 20	Control Group, n = 20
Age, yr	68.78±4.52	69.03±4.07
Sex, female, n (%)	17 (85)	17 (85)
Weight, Kg	55.29±11.92	57.46 ±7.93
Height, cm	153.40±5.47	154.00±4.77
Education, yr	5.05±2.63	5.05±2.16
Number of types of medicine	0.85±0.99	0.85±1.44
Falls of the past year, n (%)	5 (12.5)	1 (2.5)
MSET10 score (0-29 points)	24.55±2.24	23.70±2.39
MoCA score (0-30 points)	17.55±2.48	18.9±2.67
TGDS score (0-15 points)	2.60±1.50	2.10±1.65

Note: *All values are means±SD except for gender and falls of the past year, MSET10: Mental State Examination T10, MoCA: Montreal Cognitive Assessment, TGDS: Thai Geriatric Depression Scale.*

Cognitive Performance

There was no significant difference between the intervention and control groups for baseline cognitive outcomes. The significant group x time interaction was found in ADAS-cog and VPA-immediate recall test (Table 2). At the end of 12-week, the intervention group had significantly better performance on ADAS-cog score and VPA-immediate recall than the control group (p=0.007 and p=0.033, respectively). In addition, participants in the intervention group demonstrated significant improvement in ADAS-cog and VPA-immediate recall from their baseline (p=0.001 and 0.012, respectively). The time taken to complete TMT B-A for the intervention group significantly decreased from baseline (p=0.019) while this was not observed in the control group.

Table 2 Cognitive performance between the intervention and control group at baseline and the end of 12-week.

Cognitive performance	Intervention Group (n=20)		Control Group (n=20)		Group x Time Interaction		
	Baseline	Post-assessment	Baseline	Post-assessment	F (1,38)	*P*-value	η_p^2
ADAS-cog (points)	7.68±2.75	4.93±2.07 [a, b]	7.42±3.19	7.72±3.82	10.664	0.002	0.219
VPA-immediate recall (points)	4.55±1.38	5.27±1.31 [a, b]	4.25±1.25	4.12±0.77	4.821	0.034	0.113
TMT part B-A (sec)	238.49±179.65	150.06±89.31 [b]	211.16±135.61	183.20±88.57	1.626	0.210	0.041

Note: *All values are means±SD, ADAS-cog: Alzheimer's Disease Assessment Scale-cognitive section (total score = 70 points), VPA – immediate recall: Verbal Paired Associated-immediate recall (total score = 8 points), TMT: Trail Making Test, a Significant difference between two groups; b Significant difference between baseline and post-assessment.*

Fall risk

There was no significant difference in baseline of fall risk between the intervention and control groups. The significant group x time interaction was found in TUG-single and dual task (Table 3). At the end of 12-week, participants in the intervention group took significant lesser time to perform TUG-dual task than the control group (p=0.006). Their performance on TUG for both single and dual task significantly improved from baseline (p=0.006 and 0.002, respectively) while it did not change for controls. PPA composite score and its components were comparable between the two groups both at baseline and post-intervention.

Table 3 Fall risk outcomes between the intervention and control group at baseline and the end of 12-week.

Fall risk outcomes	Intervention Group (n=20)		Control Group (n=20)		Group x Time Interaction		
	Baseline	Post-assessment	Baseline	Post-assessment	F (1,38)	*P*-value	η_p^2
PPA composite score	1.51±1.07	1.21±1.29	1.35±0.69	1.26±0.72	0.459	0.502	0.012
• Vision (dB)	20.15±1.73	19.40±3.45	20.55±1.76	20.65±2.58	1.011	0.321	0.026
• Knee Proprioception (deg)	2.65±1.14	2.34±1.27	2.08±0.97	2.25±1.29	0.788	0.380	0.020
• Knee strength (kg)	17.70±8.24	20.75±5.43	19.60±5.83	19.95±5.58	1.261	0.269	0.032
• Hand reaction (msec)	291.82±45.31	273.33±29.13	304.18±54.56	292.85±50.91	0.171	0.682	0.004
• Sway path (mm)	196.70±108.37	179.95±124.64	167.15±87.89	163.70±63.58	0.239	0.628	0.006
TUG single task (sec)	8.69±0.97	8.09±0.97 [b]	8.22±1.31	8.59±1.13	10.641	0.002	0.219
TUG dual task (sec)	10.56±1.48	9.12±1.33 [a, b]	10.52±1.82	10.58±1.83	5.953	0.019	0.135

Note: *All values are means±SD, PPA: Physiological Profile Assessment, TUG: Timed Up and Go, a Significant difference between two groups, b Significant difference between baseline and post-assessment.*

Discussion

This study aimed to investigate the effectiveness of the cognitive training program on fall risk and cognitive performance in older adults with MCI. The results partly support the study hypothesis in that the 12-week cognitive training was effective in improving certain aspects of cognitive function and fall risk. After 12-week training, participants in the intervention group demonstrated significantly better performance on global cognitive function and memory as assessed by ADAS-cog and VPA-immediate recall, respectively, and significantly decrease fall risk as assessed by TUG with dual task as compared to controls.

The finding that the intervention group significantly improved performance on ADAS-cog after cognitive training suggests the beneficial effect of the program on global cognition. This finding is consistent with previous studies which found that persons with MCI had a significant improvement in ADAS-cog score after receiving cognitive intervention.[31, 32] The average magnitude of change in ADAS-cog score after cognitive training in the present study was slightly greater than that reported in previous studies[31, 32]; however, still considered in the same range. Such comparable gain observed might be due to the similarity in cognitive domains (i.e. memory, attention, and executive function) covered in the training protocol across studies. Further analysis of the ADAS-cog's subparts revealed that only improvement of word recognition test reached statistical significance. This finding suggests that the improvement of ADAS-cog was due mainly to memory improvement.

Similar to ADAS-cog, participants in the intervention

group showed significantly greater VPA-immediate recall scores when compared to the control group and their baseline. This finding is consistent with previous studies that reported the beneficial effect of cognitive training in improving memory of persons with MCI.[24, 36] The VPA-immediate recall test required the participants to remember related and unrelated word list. It is likely that the Method of Loci (MOL), a memory enhancement strategy, included in the cognitive training protocol is responsible for the gain in the VPA-immediate recall scores. Specifically, the Method of loci technique activated their sequential retrieval ability by recall unrelated list item via a self-generated mental association between the new information and the information that already known. In addition, repetitive practice might be another factor contributing to memory improvement as repetition has been proven to be one effective strategy that persons with cognitive impairment use to gain new information.[37]

On the whole, the present study revealed the potential benefit of the cognitive training program in improving cognition in part of global cognitive function and memory of older adults with MCI. Previous study suggested that cognitive training induces neural plasticity, stimulates neuron activity at bilateral temporal poles, insular cortices and hippocampus cortices.[38] Increasing in neural volume and changing in neural activity occurs when face with novel and challenging task.[39] Thus, it may be possible that the cognitive training protocol in this study which included practice of new tasks with progression of difficulty might induce changes at the neuronal level, consequently resulting in memory improvement observed at the behavioural level. Further study of this aspect would provide insight into this issue.

For executive function, there was a significant difference in TMT B-A performance between baseline and post-cognitive training. A similar result has been demonstrated in a study by Boripuntakul et al.[24] which found that MCI participants who received 18-session cognitive training significantly improved their TMT B-A performance from baseline. However, contrary to the study hypothesis, TMT B-A performance did not differ between the two groups at the end of the trial. Fiatarone Singh et al.[40] also failed to demonstrate difference in executive function between the cognitive training and sham training group. The authors did not discuss why their results were not as expected. In the present study, we postulated that the large variability observed in TMT B-A data may account for the non-significant differences. In addition, the statistical analysis indicated small effect size of TMT part B-A as represented by the partial eta squared (n_p^2=0.041) (Table 2). Given a small effect size and large variability of the data, further research with larger sample size is required to confirm this finding.

The present finding demonstrated that the intervention group significantly improved in TUG single task performance from their baseline but there was no difference between the two groups after training. It is noteworthy that the time to complete TUG single task at baseline was quite short, suggesting that this test might not challenge enough for MCI participants in the present study. Therefore, there might be only a small room of improvement after training, not sufficient to reveal the difference between groups. A previous systematic review suggested that balance impairment in older adults with MCI were more pronounced when adding the cognitive load to the balance test (i.e. dual task).[41]

Dual-task paradigm has been widely used to examine the interaction between cognition and mobility function by having an individual to simultaneously perform another activity while walking.[11] The finding of significantly better performance in TUG dual task (motor-cognitive dual task) in the intervention group compared to the control group supports the existing knowledge that cognitive process is associated with gait, balance, and falls.[8, 9] Previous studies reported that not only executive function and attention are associated with gait and balance, but global cognitive function and memory are also linked to gait and balance performance.[9, 42-43] Thus, the improvement of TUG dual task performance in the intervention group might be explained by the improvement of memory and global cognitive function after 12-week cognitive training. The result of this study was in line with findings from previous studies which demonstrated improvement of gait and balance performance after implemented the cognitive training program in cognitively intact older adults.[18, 44] Based on our knowledge, this study is among a few studies that investigated the impact of cognitive training on fall risk in older adults with MCI.

The finding of PPA outcome did not support the study hypothesis. The PPA evaluates risk of falls through directly assessing an individual's sensorimotor abilities including vision, proprioception, knee extension force, hand reaction time, and body sway. It is expected that reaction time and postural sway would improve after cognitive training as these tasks related to cognitive function, consequently resulting in improving the PPA composite score. However, the results showed no significant improvement in both subtests. It may be possible that the simple reaction time test and static postural sway used in the PPA were not sufficiently challenged to detect changes in individuals with MCI.

Although the results of this study provide the potential benefits of the cognitive training program in improving cognitive function and reducing fall risk in older adults with MCI, there are some limitations to this study. Given the large variability and small effect size observed in some outcome measures, the study may not have sufficient power to detect changes. Additionally, the effects of cognitive training were examined immediately after the training cessation. Thus, it is unknown whether the training effects would retain for the longer term. A study with larger sample size and long term follow up is required to confirm these findings. Moreover, future research that investigates neuronal responses would provide better understanding about the underlying mechanisms of cognitive training in improving cognitive function and reducing risk of falls.

Conclusion

The present study demonstrated that 12-week cognitive training for 3 times per week could improve global cognitive function, memory and decrease fall risk while performing functional mobility in concurrent with cognitive task in older adults with MCI. Overall findings suggest that cognitive training had beneficial effects on certain aspects of cognitive function and fall risk. Therefore, cognitive training may be considered when designing a fall prevention program for older adults with MCI.

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Conflict of interest

The authors declare no conflict of interest.

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