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

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2. Physical Therapy
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Comparison of conventional and through glass portable chest computed radiography: A Phantom study

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ABSTRACT

Background: Since the outbreak of COVID-19, modified hospital unit or area for chest radiography of positive cases have become necessary. To date, relatively few studies have been investigated on the effects of portable chest radiography through glass barrier.

Objectives: Our goal was to evaluate exposure technique and radiation dose between conventional and through glass portable chest computed radiography.

Materials and methods: Experiments using an anthropomorphic phantom were performed for acquired portable chest PA radiography at SID 180 cm with glass door being open and closed. The EI and DI values were optimized to provide the appropriate exposure technique for glass barrier. Entrance surface air kerma and scatter survey were made to assess the radiation dose both inside and outside the room. Finally, HVL measurement of primary X-ray beam and after transmission through glass were determined.

Results: Based on the fixed kVp and mAs technique, the EI value with glass barrier was less than the EI without the glass. Imaging through glass barrier showed the average EI reduction of 10.4% for Carestream and 37% for Konica. The average entrance surface air kerma reduction was 56.6% over a range of 90-120 kVp. The appropriate exposure technique for conventional portable chest PA using computed radiography was 100 kVp 2.5 mAs. With the same kVp setting, doubling the mAs is required for imaging through glass barrier to produce good diagnostic image quality (100 kVp and 4.0 mAs). The acceptable EI and DI ranges for CR used were EI=1742, DI=-0.02 (without glass) and EI=1795, DI=0.11 (with glass) for Carestream and EI=352, DI=-0.03 (without glass) and EI=373, DI=0.22 (with glass) for Konica respectively. The primary beam after transmission through the glass thickness 5 mm was 36%. The measured scatter of inside room compared to outside was very low at 1-2 meters. Increasing od HVL from 3.9 to 6.1 mm Al indicates the effect of beam hardening by glass.

Conclusion: These experiments confirmed that through glass portable chest computed radiography are feasible and safe. The findings of this study have several practical implications which minimizes risk to radiographers during their work.

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Introduction

Chest radiography plays a key role in monitoring disease progression in patients infected with coronavirus disease 2019 (COVID-19).[1, 2] In the first week of illness, patients with no symptom or mild symptom may have normal baseline chest radiograph while the patients with moderate or severe symptom have abnormal finding on baseline chest radiograph. Imaging features of COVID-19 pneumonia on chest radiograph has a pattern of increased density within the lung. Typical radiographic features include ground glass opacities, peripheral opacities, consolidation, multifocal and diffuse air space opacities.[3-5] The most common chest radiographic finding of COVID-19 pneumonia is bilateral multifocal air space opacities or (consolidation) in the peripheral and lower lung zones. Additional follow-up chest X-ray of all patients are preferred due to evidence of COVID-19 on chest radiograph help understanding how individual disease progress and worsening clinical status.

Radiographers worldwide have reported an increase in workload during the pandemic.[6] At the first wave of COVID-19, the major problem are limited availability of vaccine for frontline workers and a shortage of personal protective equipment (PPE). Likewise, lack of experience in working with infectious patients showed that medical staffs had been infected.[7] One way to prevent the spread of infections is to educate them regarding infection control practices. Modified hospital unit, isolation room, or area for chest radiography of positive cases have become necessary.

Many hospitals applied portable chest radiography through glass technique (TG-CXR) of the confirmed positive patients to limit staffs direct contact with patients.[8] This technique can reduce the risk of infection due to keep the patients in place and limit transmission of the virus.[9-11] The glass door attenuates and filters some X-ray photon in the beam. At higher X-ray beam energy, transmission of primary beam is increased and become more penetrating. Optimization exposure technique is one that provide the lowest possible patient dose for sufficient image quality. A high kilovoltage peak (kVp) and low miliampere-seconds (mAs) are needed to produce the posteroanterior chest radiography (CXR). These techniques reduce radiation dose to patient while preserving good contrast detectability with infectious lung disease. However, there have been no controlled studies which compare the radiation exposure at the detector and radiation dose between conventional and through glass portable chest computed radiography. Here, we became interested in the idea of distance imaging and modified through glass portable radiography. The aim of this study was to evaluate exposure technique and radiation dose between conventional and through glass portable chest computed radiography.

Materials and methods

An anthropomorphic thorax phantom of an adult male (Model RS-111 Opaque, United States) was used to simulate the clinical experiments. The phantom positioned for an erect postero-anterior (PA) chest radiography. A Toshiba mobile x-ray model IME-100L was set up outside the isolation room at a SID of 180 cm (Figure 1). The image receptors were all computed radiography system, one by Carestream (DirectView Vita CR) and one by Konica Minolta (Regius Sigma II). The imaging plate had an image capture area of 35x43 cm. The quality control (QC) tests of equipment were monitored in accordance with the recommendation of AAPM Report No. 74 and 93 before reporting the measurement results.[12, 13] We performed portable chest radiography by setting up equipment with door open (conventional technique) and through the glass door of an isolation room (TG-CXR technique). The glass door we use was a regular glass in standard thickness of 5 mm. The experiment was tested with door open and with door closed (through glass imaging) as shown in Figure 2. All radiation measurements were obtained with a RaySafe X2 radiation meter (Unfors RaySafe, Sweden). We measured at selected point as described in a previous work.[9, 10] Dosimeter was positioned at the center of the thorax phantom to measure radiation dose. Entrance surface air kerma (ESAK) in microgray (μGy) were made and normalized to mAs (by dividing mAs to give μGy/mAs). In the presence of scatter radiation inside the room, the X2 survey probe positioned in the forward, side, and backscatter with 0 and 45 degree direction angles and at 1 m and 2 m from the center of phantom (A-F, Figure 1) while the scatter radiation outside the room we observed at the distance of 30 cm, 1 m, and 2 m from glass door (G-I, Figure 1). All measurements with X2 survey probe were taken at 150 cm above the floor. Three dose reading were recorded to minimize random errors and then we calculated the average value of all the measurement.

The initial exposure technique was the clinical setting for the PA chest examination. The exposure index (EI) and deviation index (DI) values were used as an indicator of the amount of ionizing radiation on digital image receptor. The EI values are proportional to the signal to noise ratio squared and can be referred to image quality. These values provided useful feedback to radiographers when a digital image is overexposed or underexposed image.[14, 15] In a study conducted by Lorusso showed that using a high tube voltage in range of 100-120 kVp is recommended to perform conventional portable chest radiography.[16] Exposure settings we used in PA projection of the thorax phantom ranging from 90 to 120 kVp with mAs adjustments for the glass barrier at 2.0-8.0 mAs. The appropriate exposure technique was selected from the scenario that getting closer to the target exposure index (EI_T) diagnostic reference levels (Carestream EI_T=1751, Konica EI_T=355, using exposure at 5 μGy) and to achieve a zero or minimize deviations from the index. The deviation index value was calculated by the following equation: $DI=10 \log_{10} (EI/EI_T)$. In conventional technique (leave the door open), the exposure range used 90-120 kVp and 2.0-3.2 mAs while imaging through the glass door was made for 90-120 kVp with adjusted mAs at 3.2-8.0 mAs. In this study, we have not considered in term of perceptual image quality, to only focus on optimizing technique parameters that reached image receptor and radiation dose in conventional portable chest radiography compared with through glass technique.

Lastly, we examined the effect of glass attenuation and changes in X-ray beam quality. The lower energy photons in the beam are generally absorbed or filtered out by the

glass. Therefore, we assessed the penetrating ability of X-ray beam before and after transmission through glass by measuring half-value layer (HVL). HVL measurements in term of equivalent thickness of aluminum in millimeters (mm Al) at different X-ray tube voltages from 80 to 120 kVp. All data and statistical analysis were performed in Microsoft Excel for calculating descriptive statistics.

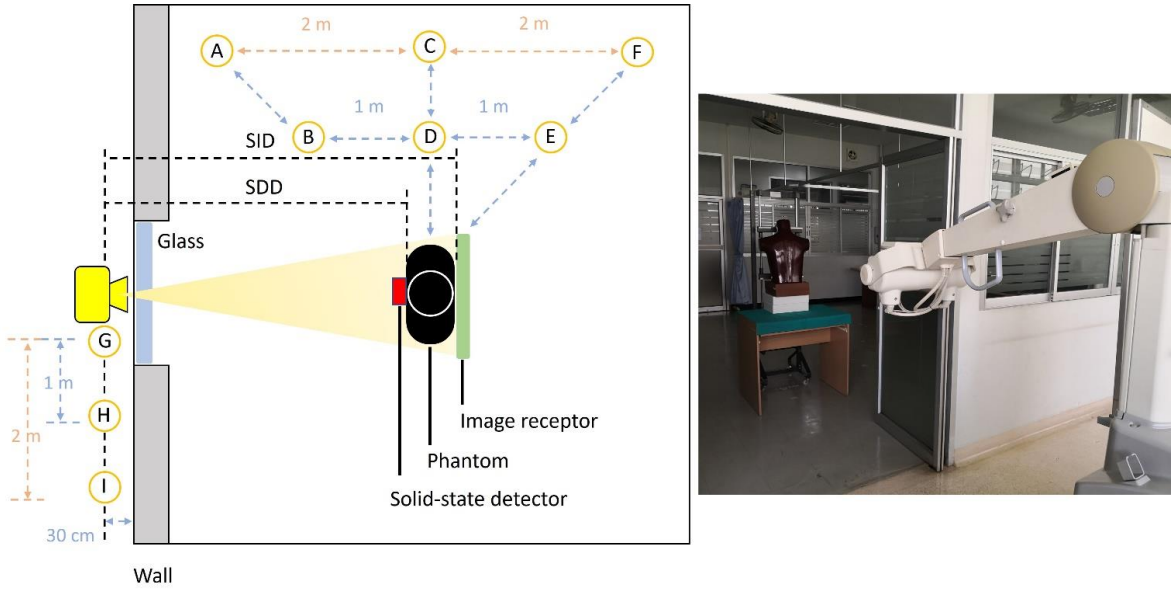

Figure 1. *Experimental set-up with the locations marked. The anthropomorphic phantom was placed to acquire portable chest PA radiography at the source to image distance (SID) of 180 cm.*

Figure 2. *Positioning of the radiographic unit. Mobile x-ray machine was operated outside the room with door open (conventional technique) and through the glass barrier (TG-CXR technique) during image acquisition.*

Figure 3. *Setting up of the anthropomorphic phantom and the radiation dosimeter. (Left) A 152 cm-height of the anthropomorphic phantom was used for this experiment, and (Right) scatter measurement with X2 survey probe was taken at 150 cm above the floor.*

Results

The results from the performance of each QC test showed that all are in the acceptable range within the AAPM Report No 93 and 74 recommendation. A total of 42 images were acquired using several exposure techniques without glass and through the glass barrier. Acquisition parameters are shown in Table 1 including the EI, DI, ESAK, % ESAK reduction, and %EI reduction. The range of EI and DI values were in the acceptable range for two CR imaging systems that provided a good image quality. Based on the fixed technique, the EI value with glass barrier was less than the EI value without glass. We found the average EI reduction on Carestream CR system was 10.4% while Konica Minolta was 37% with the glass barrier. ESAK reduction dropped by 56.6% in average (Table 1). In the conventional portable chest radiography (leave the door open), the appropriate exposure technique to generate a DI value closer to zero was 100 kVp with 2.5 mAs. This resulted in an average EI was 1742 for Carestream, 352.27 for Konica Minolta, and average ESAK was 46.73 µGy, respectively. To maintain a similar DI value to the conventional imaging with the glass barrier in the primary beam, the mAs adjustments were required to increase to 4.0 mAs. An increase of tube output needed by a factor of two (Figure 4-5). Overall, through glass portable chest radiography (TG-CXR technique) gave the average EI of 1795 for Carestream, 373.48 for Konica Minolta, and average ESAK was 30.52 µGy, respectively. Despite the increase of the mAs, the ESAK also decreased from 46.73 µGy without glass to 30.52 µGy with glass barrier. It was observed by the glass attenuates or filters some X-ray photon in the primary beam. Most of the beam hardening occurred when X-ray passed through the glass at 90 kVp.

Table 2 shows transmission measurement of the primary X-ray beam using normalized air kerma before and after transmission through glass. It can be seen from the data that the transmission of the primary X-ray beam ranging from 35 to 44% at the range of 90-120 kVp. At higher tube voltage increased the average photon energy as the X-ray beam can penetrate more easily through matter. In other words, the intensity of the primary beam decreased with their ability to penetrate matter. For the transmission of the X-ray through glass barrier was decreased by nearly half of the initial beam intensity. A mean transmission of primary through glass barrier ranged from 35% to 44% at 90-120 kVp. Transmission measurement is consistent with the others reports.[9]

The results of scatter radiation measurement at the selected position for the same technique are shown in Table 3. Regarding to the position inside the room as shown in Figure 1, the side scatter and 45 degrees direction were observed over 1 or 2 meters away from the anthropomorphic phantom while the position outside the room corresponded to the radiographers stand when operating portable x-ray machine (standing close and standing far away). At the appropriate exposure technique of 100 kVp 2.5 mAs, the measured scatter air kerma were 0.08, 0.04, 0.03, 0.03 and 0.02 µGy/mAs at point A, B, C, D and E, respectively. Dosimeter recorded the scatter radiation at 30 cm from the glass door was 0.15 µGy/mAs. Scattered radiation dose reduced to 0.02 µGy/mAs at the radiographer standing 1-2 m away from the glass depending on the kVp used.

The HVL measurements of beam quality before and after transmission through glass are shown in Figure 6. At the X-ray tube voltage from 80 to 120 kVp, the HVL values of primary X-ray beam were 3.2 mm Al, 3.5 mm Al, 3.9 mm Al and 4.7 mm Al. The X-ray beam after transmission through glass resulted in the HVL of 5 mm Al at 80 kVp, 5.5 mm Al at 90 kVp, 6.1 mm Al at 100 kVp and 7.1 mm Al at 120 kVp respectively. The HVL increased from 3.9 to 6.1 mm Al indicating the effect of beam hardening by plain glass.

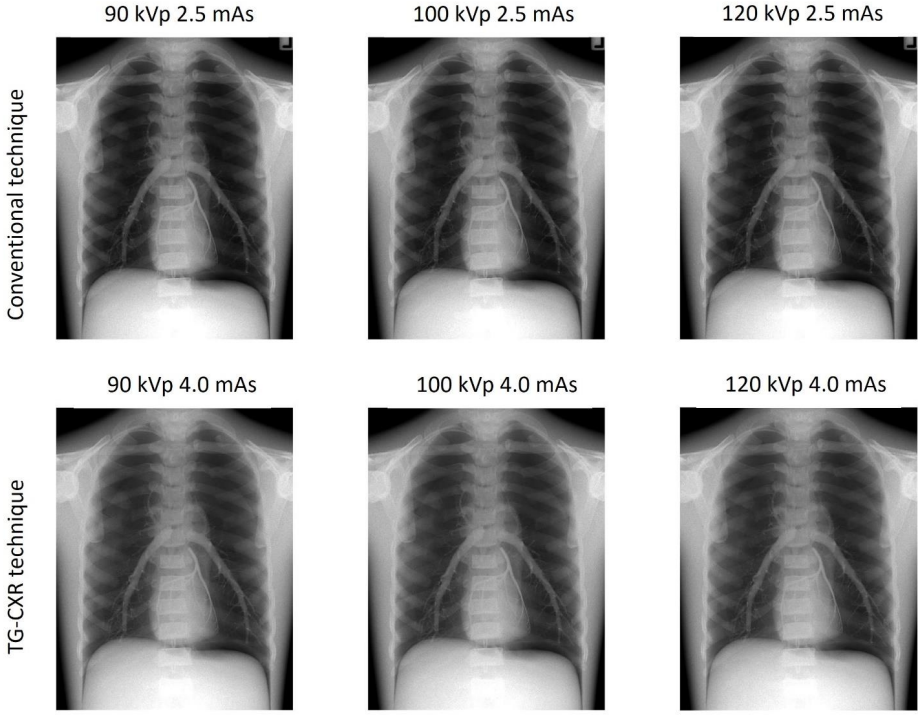

Figure 4. *Examples of chest radiographs from Konica Minolta CR system. Images obtained by conventional radiography and through the glass technique using the fixed mAs at the range of 90, 100, and 120 kVp, respectively (from left to right).*

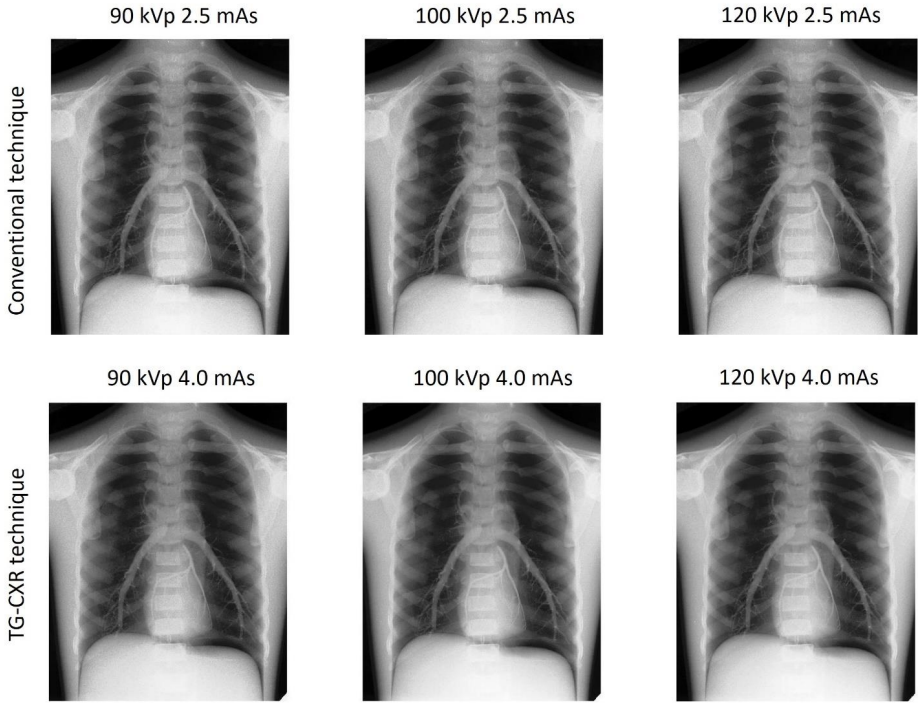

Figure 5. *Examples of chest radiographs from Carestream CR system. Images obtained by conventional radiography and through the glass technique using the fixed mAs at the range of 90, 100, and 120 kVp, respectively (from left to right).*

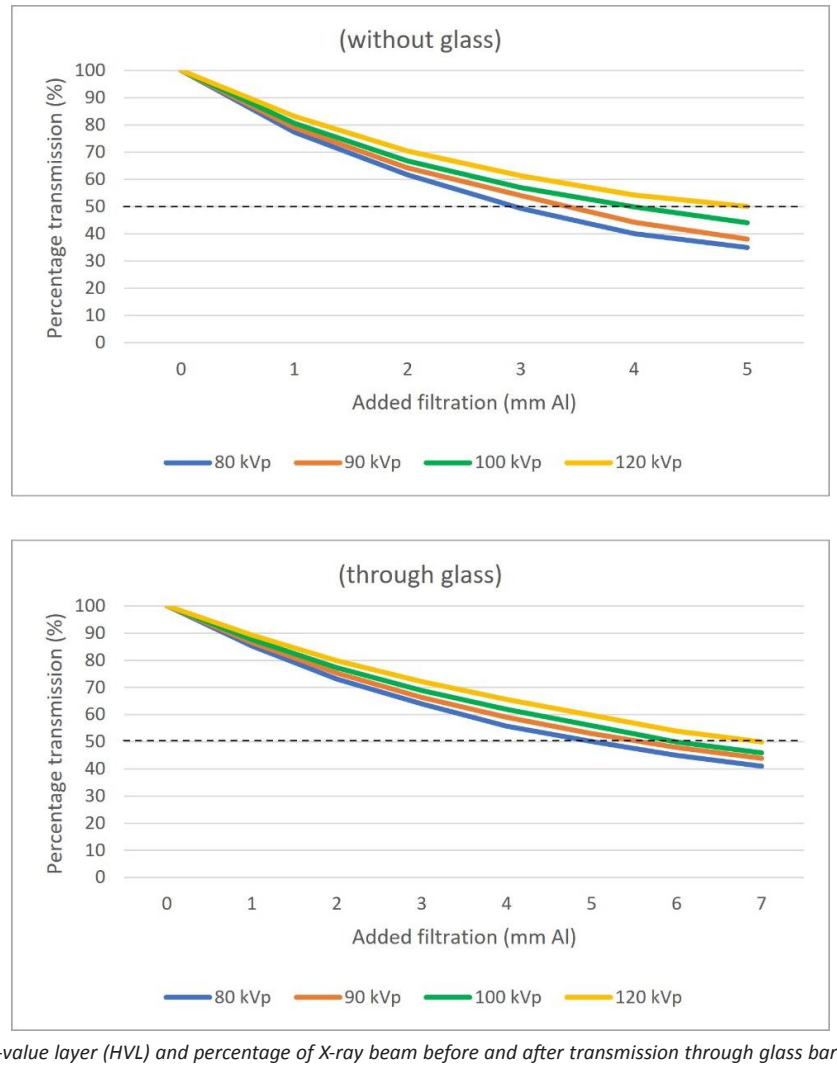

Figure 6. *Comparison of half-value layer (HVL) and percentage of X-ray beam before and after transmission through glass barrier. One mm of aluminum sheet was used for the X-ray tube voltage 80-120 kVp. Dotted line represents the amount or thickness of aluminum required to reduce the x-ray beam to half of its original intensity.*

Table 1 Summary of the exposure technique setting for portable chest radiography, the range of EI and DI values from two imaging CR systems, and entrance surface air kerma measurements with and without the glass barrier.

Chest PA	kVp	mAs	EI	EI	DI	DI	ESAK (µGy)	% ESAK reduction	% EI reduction	
			Carestream	Konica	Carestream	Konica			Carestream	Konica
Conventional technique	90	2.0	1391	187.27	-0.99	-2.78	29.30			
		2.5	1568	251.41	-0.47	-1.50	38.03			
		3.2	1724	330.03	-0.07	-0.31	49.75			
	100	2.0	1668	279.44	-0.06	-1.04	36.49			
		2.5	1742	352.27	-0.02	-0.03	46.73			
		3.2	1884	474.01	0.32	1.25	60.52			
	120	2.0	1852	452.14	0.24	1.05	50.48			
		2.5	1987	567.43	0.55	2.03	65.07			
		3.2	2100	784.40	0.79	3.44	84.45			
TG-CXR Technique	90	3.2	1541	208.61	-0.55	-2.31	19.67	60.5	10.6	36.8
		4.0	1612	257.71	-0.36	-1.39	23.75			
		6.3	1775	376.01	0.06	0.25	27.80			
		8.0	1923	510.52	0.41	1.58	36.10			
	100	3.2	1680	290.15	-0.18	-0.88	26.02	57.0	10.8	38.8
		4.0	1795	373.48	0.11	0.22	30.52			
		6.3	1928	537.62	0.41	1.80	35.98			
		8.0	2072	739.85	0.73	3.19	41.80			
	120	3.2	1896	507.09	0.34	1.55	40.30	52.3	9.7	35.4
		4.0	2065	761.80	0.72	3.32	47.11			
		5.0	2107	814.97	0.80	3.61	55.72			
		6.3	2178	945.02	0.94	4.25	64.30			

kVp, peak kilovoltage; mAs, milliampere seconds; EI, Exposure index; DI, Deviation index; ESAK, Entrance surface air kerma. A positive or negative DI indicates the amount of exposure greater or lesser than the target EI.

Table 2 Transmission measurement of normalized air kerma before and after glass barrier.

kVp	Air kerma (µGy/mAs)		Percentage transmission (%)
	Before glass barrier	After glass barrier	
90	15.14±0.45	5.25±0.92	35
100	18.62±0.34	6.67±1.42	36
120	25.89±0.59	11.43±1.01	44

Table 3 Normalized scatter air kerma measurement with tube voltages ranging from 90 to 120 kVp, in 10 kVp increments, for through glass technique at the selected position during image acquisition inside the room (from phantom) and outside the room (from glass barrier).

Tube voltage	Scatter air kerma (µGy /mAs) inside the room								
	A	B	C	D	E	F	G	H	I
90	0.07±0.02	0.03±0.02	0.02±0.02	0.02±0.01	0.02±0.02	0.01±0.01	0.11±0.01	0.01±0.01	0.01±0.01
100	0.08±0.03	0.04±0.01	0.03±0.01	0.03±0.02	0.03±0.01	0.02±0.02	0.15±0.02	0.02±0.02	0.02±0.01
120	0.10±0.03	0.04±0.01	0.03±0.01	0.04±0.03	0.03±0.01	0.03±0.03	0.22±0.02	0.02±0.01	0.02±0.01

Discussion

With an increase in radiology workload for staff during the COVID-19 Pandemic, through glass portable chest radiography technique is useful to radiographers and help to reduce the risk of infection. TG-CXR technique has allowed to prevent infection in the hospital setting, less personal protective equipment (PPE) use, decreased radiography equipment sanitization, and limit staffs' exposure to the virus that make them feel safe. Moreover, it notably allows for net operating costs per annum to be reduced for several reasons with those observed by Liu *et al*.[17] This is compared with standard portable or bedside radiography by a team of two radiographers for these patients in the hospital ward.

As to the results obtained in this study, images obtained by through glass portable radiography were satisfied with high kVp technique and variable mAs to control the image quality and patient dose. These results seem to be consistent with earlier studies which found the radiographic exposure technique of TG-CXR ranging from 100-125 kVp at 4.5-12.0 mAs. These values vary depending on source to image distance and patient variables of small, medium, and large body size.[8-11] However, the EI values from two CR systems were found within the range recommended by the manufacturer. Using the fixed kVp and mAs exposure settings, images obtained with TG-CXR technique showed lower EI value than conventional technique. This resulted in a slightly grainy appearance (quantum mottle) on the image due to low signal-to-noise ratio (SNR). Low SNR reduced spatial resolution and thus provided of image quality degradation. This implies that selection of appropriate exposure technique is still needed to produce a clinically acceptable image and safety of patients undergoing radiographic examinations. Our findings suggest that double mAs is also needed to produce good diagnostic image for modified through glass imaging. The attenuation and HVL with and without glass were similar to those observed by others.[9, 18, 19]

Based on clinical setting, the higher kVp used in radiological procedure, the better penetrating of X-ray beam is provided. This resulted in lower radiation dose to patient. For portable radiography, or bedside chest radiograph, at the radiographer standing distance of 1-2 m away from the glass, scattered radiation dose reduced to 0.02 µGy/mAs. It is apparent that scatter radiation from X-ray transmission through the glass barrier was low. Similar findings were noted by Brady *et al*.[9] who suggested the minimum distance of 1 metre from radiation source was equal to the dose from natural background radiation around three hours. In fact, radiographers stand when operating the exam more than a metre away could be even lower. This low-level exposure to radiation was generally considered safe to use.[18, 20, 21]

As we known the COVID-19 pandemic has a global impact across the world. In support of Cho et al. observed that the number of radiology examinations decreased during the pandemic, longer turnaround time required for portable radiography in COVID-19 patients, extended period of time for donning personal protective equipment in the examination of these patients.[21] One the other hand, we found that many attempts have been made in order to enhance portable chest radiography in response to the pandemic. Le *et al*.[22] have developed deep neuron network to enhance the speed and diagnostic accuracy for COVID-19 patient. They found the performance of the proposed method improved image contrast between masses and normal lung, better costophrenic angles perception, and improved conspicuity of opacities in lower lobes which is a feature of COVID-19. Comparison of these finding with those of other studies confirms deep learning artificial intelligence methods have a significant opportunity to contribute diagnostic accuracy and efficiency.[23-26]

Our experiments were based on anthropomorphic phantom exposure and one type of mobile X-ray unit. In the clinical setting they are much more situations will be involved rather than simulated clinical scenario we made. These techniques will likely be applicable to other airborne infectious diseases and applied to any parts of the body in radiographic examinations. Only one type of glass and 5 mm thickness were tested. Considerably more work in different type of glass, different glass materials, different size and thicknesses will need to be developed. However, it is also important to note that the isolation room of COVID-19 patient needs to have a clear glass door or window size which is large enough to set-up mobile X-ray unit and equipment without any obstruction in the path of the primary X-ray beam. TG-CXR technique required specific room design or modified room construction that will not be available for all hospitals. Again, this study did not compare image quality between conventional and through glass portable radiography in term of image quality metrics (e.g., SNR, CNR, spatial resolution, sharpness, and the noise level). Further study of image quality assessment between these two are warranted.

Conclusion

Through glass portable radiography technique are feasible and safe. Radiographers can avoid prolonged close contact with COVID-19 patients during their work. The appropriate exposure technique for conventional portable chest computed radiography of anthropomorphic phantom was 100 kVp and 2.5 mAs at SID of 180 cm. When modified X-ray imaging through glass barrier, double mAs is also needed to produce good diagnostic image (100 kVp and 4.0 mAs). X-ray beam after transmission through glass thickness 5 mm was 36% and the HVL of beam increased from 3.9 to 6.1 mm Al indicating the effect of beam hardening by glass. Scatter inside and outside the room could be observed at low dose levels.

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Conflict of interest

The author declares no conflicts of interest in this research.

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Evaluation of optimal kilovoltage-cone beam technique on image quality, registration accuracy, time of imaging and relative dose for head radiotherapy: A phantom study

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ABSTRACT

Background: Image-guided radiation therapy (IGRT) has improved geometry accuracy of patient positioning in radiotherapy. Nowadays, a kilo-voltage cone-beam computed tomography (kV-CBCT) is widely applied in IGRT to confirm daily patient positioning. However, one problem with IGRT is the increased dose to normal tissue outside the target area. Reduction in the dose of kV-CBCT verification imaging leads to deterioration in image quality and registration results.

Objectives: The objective of this study was to evaluate an optimal kV cone-beam technique on image quality, registration accuracy, relative dose, and imaging time using different combinations of angular range, angular separation, and mA (in total eight protocols) in the head phantom.

Materials and methods: Catphan® 503 phantom was used to evaluate image quality and a PIXY Anthropomorphic Training/Teaching Phantom was used to verify image registration accuracy. The kV-CBCT imaging was performed using an X-ray volumetric imaging (XVI) system mounted on an Elekta Versa HD linear accelerator. The absorbed dose of kV-CBCT imaging was determined using the head phantom with an ionization chamber. The eight protocols were analyzed for image quality, image registration, relative dose change, and imaging delivery time.

Results: Image quality parameter results showed that maximum contrast to noise ratio (CNR) increased by approximately 65% at 100 kV, 20 mA, θ=360°, and Δθ=0.54°, while uniformity compared with 100 kV, 10 mA, θ=200°, Δθ=0.54° (default protocol) varied within 7%. The spatial resolution showed no change (0.167 cm), with geometric distortions of less than 0.2 mm. Image registration errors were within 0.2 cm, with the highest magnitude of error seen in the vertical direction. Imaging dose can be reduced using 100 kV, 10 mA, θ=200°, Δθ=1.09° about 50% and 100 kV, 10 mA, θ=360°, Δθ=1.09° about 15% with similar CNR of default protocol. Imaging time decreased by approximately 2-folds, while Δθ increased by 2-folds.

Conclusion: The suggested protocols suitable for optimal kV-CBCT image quality, accuracy of image registration, decreased imaging time, and reduced image dose in the head region were 1) 10 mA, θ=200°, Δθ=1.09° 2) 10 mA, θ=360°, Δθ=1.09°, and 3) 20 mA, θ=360°, Δθ=1.09°. These protocols decreased imaging dose about 50%, 15%, and 2%, respectively. The developing kV-CBCT technique should be counterbalanced by careful consideration of imaging dose, image quality, imaging time, and accuracy of image registration.

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Introduction

The goal of radiotherapy is to deliver a high therapeutic prescribed dose to the tumor target while limiting treatment to the organ at risk (OAR). Radiotherapy techniques deliver doses to patients via a certain number of treatment fractions. This can cause deviations between planning dose distribution and delivered dose distribution, especially for high-precision techniques such as intensity-modulated radiation therapy (IMRT) and volumetric-modulated arc therapy (VMAT). Image-guided radiotherapy (IGRT) has recently been developed to detect target shifts relative to the target position in an approved radiotherapy treatment plan, such as kV-cone beam computed tomography (kV-CBCT). This generates 3D volumetric images of patient anatomy directly at the treatment site and uses the images to correct the set-up error during each treatment fraction.[1] Nowadays, kV-CBCT is used worldwide for patient positioning verification. However, the use of kV-CBCT imaging often results in additional patient radiation exposure to radiosensitive organs. Scandurra *et al*.[2] reported that an absolute dose of the head protocol was 0.9 cGy, while previous research indicated that image-guided kV-CBCT was effective for the evaluation of set-up accuracy.[3-6] Delishaj *et al*.[7] suggested that kV-CBCT should first be administered as three fractions and then followed by weekly doses to significantly reduce set-up errors in head and neck cancer treatment using the IMRT technique. Adequate management of the imaging dose is necessary for IGRT according to ICRP-60[8] although the dose delivered from kV-CBCT is small compared to the therapeutic dose. The AAPM task group 180[9] recommended that balancing the as low as reasonably achievable (ALARA) principle with the requirement for effective target localization required that imaging dose be managed by considering both the risks and benefits to the patient. Methods for reducing the kV-CBCT imaging dose include reduction of the tube current-exposure time (mAs), numbers of projections, and changing the imaging angle, filter, and scan length. However, reduction in the imaging dose leads to deterioration in image quality, and registration results may not be accurate.

The degrading effects on image quality and imaging dose of kV-CBCT have been studied by several investigators.[10-19] Image quality and imaging dose depend on the imaging system, while imaging dose depends on the frequency of the set-up related to the number of cancer patients at each treatment site. A continuous increase in patient numbers has impacted workloads at many treatment sites. Efforts have been made to decrease the time and frequency of kV-CBCT treatments. A 200 ° scan (180 ° plus the fan angle) has become the standard imaging configuration for head-and-neck cancers of all on broad imager (OBI) imaging. Short scan data contains high noise that impacts the registration matching process. The effect of reducing the image quality, while ensuring optimization of the imaging dose and registration accuracy, including time of imaging requires evaluation.

Purpose of this study was to evaluate an optimal kV-cone-beam technique on image quality, registration accuracy, relative dose, and imaging time using different combinations of angular range, angular separation, and mA in the head phantom

Materials and methods

CT simulation and planning

A CT simulator (Philips), Catphan® 503 phantom[20] was used to evaluate image quality, while a PIXY Anthropomorphic Training/Teaching Phantom (head phantom) was used to verify image registration accuracy. The scanning setting parameters were 100 kV and 350 mAs, with a slice thickness of 2 mm. The RayStation treatment planning system (RaySearch Laboratories, Stockholm, Sweden) was used for 3D-CRT planning of 6 MV photons in the brain and planning data was sent to the linac machine.

kV-CBCT imaging

kV-CBCT imaging was performed using an X-ray volumetric imaging (XVI) system mounted on an Elekta Versa HD linear accelerator (Elekta, Stockholm, Sweden). XVI software uses a cone-beam reconstruction process based on the Feldkamp-Davis-Kress algorithm.[21] The acquisition angle started from -90° in all protocols. The angular range (θ) used default settings for the head as 200° (half gantry rotation) and 360° (full rotation). The largest angular separation between views (Δθ) that can be used in XVI is 1.09°, while a default setting of 0.54° was used in this study. The θ and Δθ angles affect the number of projections about θ/Δθ. Therefore, the number of projections can be changed by modifying the gantry speed.

The default preset protocol of Lampang Cancer Hospital for head kV-CBCT is 100kV, 10 mA, θ=200°, and Δθ=0.54°. The absorbed doses were reduced by changing mA, θ, and Δθ. The current was tested at 10 and 20 mA, while angular ranges used in this study were θ=200° and 360° of gantry rotation. The angular separations of 0.54° and 1.09° were used in Δθ setting. Therefore, in total, eight protocols with different combinations were analyzed for image quality, image registration, relative dose change, and imaging delivery time.

Image quality analysis

The Catphan® 503 phantom is cylindrical with a diameter of 200 mm and length of 200 mm. It consists of three modules as CTP-404, CTP-486, and CTP-528 that were used to evaluate contrast-to-noise ratio (CNR), uniformity, and spatial resolution, respectively. The center of the Catphan® 503 phantom was positioned at the isocenter.

1. *CNR*. The CTP-404 module has sensitometry targets made from Teflon®, Delrin®, acrylic, polystyrene (PS), low-density polyethylene (LDPE), polymethylpentene (PMP), and air. The Hounsfield unit (HU) values in regions of interest (ROIs) of size 5×5 mm^2 within polystyrene and LDPE were calculated as follows:

$$CNR=\frac{Mean_{PS}-Mean_{LDPE}}{SD_{PS}+SD_{LDPE}}\times 2$$

where $Mean_{PS}$ and $Mean_{LDPE}$ are the mean voxel values in PS and LDPE, respectively and SD_{PS} and SD_{LDPE} are the standard deviation in voxel values in PS and LDPE, respectively. CNR values of five

slices were averaged and calculated for standard deviation.

2. *Uniformity*. The CTP-486 module as uniform water equivalent with a CT number of 20 HU was used to measure uniformity. Average values of 10 slices at the center of the phantom, with ROI sizes 10x10 cm^2 at the center and four peripheral positions were used to measure the HU values. Uniformity was calculated as follows:

$$\text{Uniformity}=1-\frac{Mean_{max}-Mean_{min}}{Mean_{max}+Mean_{min}}$$

A value close to 1 represents better image uniformity. The uniformity values of 10 slices were calculated for mean and standard deviation.

3. *Spatial resolution*. The CTP-528 module, composed of an epoxy background including a section of 21-line pair (LP) groups of 2 mm thick aluminum and two beads in the Z direction was used to measure spatial resolution. The 21 LP groups in the image were evaluated by the visual method.
4. *Geometric distortion*. This was evaluated as the maximum deviation from 50 mm of four spaced air holes drilled at 50 mm intervals in the CTP-404 module. The measurements were repeated 10 times to calculated the mean and standard deviation of geometric distortion.

For the statistical analysis, one-way ANOVA was used with SPSS version 17 (FB7E105EFD8A514130CC).

Image registration analysis

Image registration was analyzed using the head phantom. The planning CT images of the head were obtained by CT simulation (Philips) and transferred to the RayStation computer treatment planning system (RaySearch Laboratories, Stockholm, Sweden) to act as the reference image. The phantom was positioned correctly on the treatment couch of the Elekta Versa HD linear accelerator and then deliberately shifted 2.0 mm in the lateral, longitudinal, and vertical directions, respectively. The registration algorithm used an automatic registration based on bone matching in "Gray value mode", followed by a manual correction by the same technician. Deviation values of translation error from the table shifted 2.0 mm along the three axes were recorded.

Dose measurement

The absorbed dose in each protocol was determined using the head phantom (Fluke Biomedical, RMS) with an ionization chamber type IC RAD Model 6000-528 (Victoreen). The absorbed dose was measured three times for each protocol at the center of the phantom. The relative dose of each protocol was calculated and compared to protocol 100 kV, 10 mA, θ=200°, and Δθ=0.54° that was considered as the default protocol for the head in XVI.

Results

Image quality

Image quality parameters are listed in Table 1. At the fixed default protocol 10 mA, θ=200°, and Δθ=0.54°, the CNR decreased while Δθ increased. By contrast, CNR increased when θ and mA increased. Maximum CNR value increased by approximately 65% at 20 mA, θ=360°, and Δθ=0.54°. Uniformity compared with the default protocol varied within 7%, while CNR and uniformity of all protocols were not statistically different (p>0.05). All the protocols showed the same values of spatial resolution (0.167 cm), with geometric distortions of less than 0.2 mm.

Image registration

Translation vectors of image registration errors are shown in Table 2. The magnitude of error was within 0.2 cm for the vertical direction, with similar error was within 0.1 cm in both lateral and longitudinal directions. When parameters of θ and mA increased, the registration accuracy also increased.

Relative dose and imaging time

Relative dose variations of each protocol compared with the standard protocol and kV-CBCT imaging time are shown in Table 3. The protocol of 20 mA, θ=200°, and Δθ=1.09° gave a similar dose to the standard protocol. Maximum and minimum doses were 20 mA, θ=360°, Δθ=0.54°, and 10 mA, θ=360°, and Δθ=1.09°, respectively. With a fixed θ, imaging time decreased by approximately 2-folds while Δθ increased by 2-folds.

Table 1 Image quality parameters.

mA	θ	Δθ	CNR	Uniformity	Spatial resolution (cm)	Geometric distortion (mm)
10	200°	0.54°	3.97±0.66	0.86±0.01	0.167	0.17±0.32
		1.09°	3.19±0.54	0.87±0.03	0.167	0.15±0.17
	360°	0.54°	4.61±0.61	0.92±0.01	0.167	0.13±0.20
		1.09°	3.99±0.52	0.92±0.02	0.167	0.10±0.24
20	200°	0.54°	6.11±0.40	0.82±0.03	0.167	0.03±0.28
		1.09°	5.87±0.48	0.85±0.01	0.167	0.03±0.20
	360°	0.54°	6.54±0.54	0.89±0.02	0.167	0.03±0.30
		1.09°	5.95±0.42	0.90±0.02	0.167	0.03±0.26

Table 2 Image registration errors (cm) of the head phantom.

mA	θ	Δθ	Translation deviation (cm)		
			Lateral (x)	Longitudinal (y)	Vertical (z)
10	200°	0.54°	0.09±0.01	0.07±0.01	0.19±0.01
		1.09°	0.08±0.01	0.07±0.01	0.10±0.01
	360°	0.54°	0.03±0.00	0.08±0.01	0.10±0.01
		1.09°	0.03±0.00	0.08±0.01	0.08±0.01
20	200°	0.54°	0.08±0.01	0.07±0.01	0.10±0.01
		1.09°	0.08±0.00	0.07±0.00	0.10±0.01
	360°	0.54°	0.03±0.00	0.07±0.00	0.08±0.01
		1.09°	0.03±0.00	0.07±0.00	0.08±0.00

Table 3 Absorbed dose at the center of the head phantom and imaging time of kV-CBCT.

mA	θ	Δθ	Absorb dose (mGy)	Relative dose variation at isocenter (%)	Imaging time (sec)
10	200°	0.54°	12.87	default	72
		1.09°	6.47	-49.73	39
	360°	0.54°	21.70	68.61	121
		1.09°	10.90	-15.31	64
20	200°	0.54°	25.60	98.91	72
		1.09°	12.67	-1.55	39
	360°	0.54°	42.93	233.57	121
		1.09°	20.13	56.41	64

Discussion

This study evaluated the kV-cone beam technique. To achieve high image quality, high-level registration accuracy, optimized relative dose, and low imaging time were required using a combination of angular range and angular separation with decreased mA in the patient positioning set-up technique. Results will be useful to guide protocol selection in kV-CBCT for the head. kV-CBCT is an effective modern linear accelerator tool to ensure the accuracy and precision of patient set-up. However, how kV-CBCT imaging affects the dose in patients at about 2-3 cGy per fraction should not be ignored.[9] Parameters for increasing the angular separation and/or reducing the angular range, including the mAs reduction, can impact the result of image quality degradation and dose reduction. High CNR, high uniformity and small geometric distortions are useful indicators that generate high accuracy of image registration. This study showed high CNR at 20 mA, while using high mA increased the dose. CNR was increased using the protocol 20 mA, θ=200°, and Δθ=1.09° instead of using the default protocol with a similar absorbed dose. Changing from full scan (θ=360°) to half scan (θ=200°) altered CNR by about 7%, with the same angular separation in 20 mA, Δθ=0.54° and by about 25% in 10 mA, Δθ=1.09° protocol. For the same angular range, CNR was high when using angular separation at Δθ=0.54°. Maximum percentage change between using Δθ=0.54° and 1.09° was 10 mA, θ=200° at about 20%. Results showed that angular range impacted CNR more than the angular separation in protocol of 10 mA, concurring with Men and Dai[10] who found that CNR increased by about 2 times from θ=260° to θ=360°, while Δθ was little change. For protocol of 20 mA, the high change was recorded in angular separation of θ=360° about 9%, while angular separation had slightly more impact than angular range. The angular range, angular separation, and mA affected CNR and uniformity but changes in values were not significant, while spatial resolution showed no change. Geometric distortion changed in protocol of 10 mA (within 0.2 mm) but with no significant change in different mA values. However, Takei *et al*.[17] reported that a geometric distortion was within 0.1 mm of low dose head protocol. The kV-CBCT image quality is sensitive to many factors[22] including scattering and beam hardening effects.

Registration results using automatic and manual gray value mode matching methods compared between CT simulation images and kV-CBCT images showed deviations within 0.2 cm of all protocols, although the CNR value of image quality decreased by 19% in 10 mA, θ=200°, Δθ=1.09° from default protocol. The kV-CBCT image of the head phantom had high contrast details in the cranial bone and brain tissue, including spatial resolution of kV-CBCT did not change. Therefore, automatic image registration software can be used to accurately determine the anatomical structure. Hardware and software related to OBI imaging have recently been improved by manufacturers. Changes in the kV-CBCT

scan settings have increased image quality and can be used to reduce doses. One limitation of this study was the use of a solid phantom to perform accurate image registration more than in patients because of varied patient contour and anatomy structure. The imaging dose measurement was also influenced by the position of the isocenter and the dimensions of the patient. Different physiques of patients cause variation of both image quality and imaging dose. The evaluation of accurate registration and image dose of actual patients is very difficult. Therefore, phantom should first be studied before application in patients. Nowadays, image guidance has become an integral part of the radiotherapy treatment process. The undoubted advantages should be counterbalanced by careful consideration of imaging dose, image quality, accuracy of image registration, and time of imaging. The relative dose compared with the default technique can be reduced by increasing the angular separation. The suggested protocols suitable for optimal kV-CBCT image quality with accurate image registration in the head region were 10 mA, θ=200°, and Δθ=1.09° or 10 mA, θ=360°, and Δθ=1.09°. These protocols also reduced dose and imaging time. The 20 mA, θ=200°, and Δθ=1.09° protocol is recommended for increasing image quality and reducing imaging time, while maintaining the imaging dose of the default protocol.

Several studies have evaluated reductions to the imaging dose of kV-CBCT.[23-28] Daily doses to the soft tissue resulting from current kV-CBCT imaging were less than results from using an electronic portal imaging device (EPID). However, a few reports have reported on kV-CBCT imaging protocol as suitable for reducing imaging dose using optimal image quality, image registration accuracy, and imaging time. This study assessed radiation exposure to imaging quality and image registration from kV-CBCT and also offered the technician alternative suitable techniques. Lu *et al*.[23] revealed that using CBCT decreased the imaging dose by reducing the number of projections, while Men and Dai[10] evaluated angular range and separation on image quality, image registration, and imaging dose. Takei *et al*.[17] reported registration accuracy at low dose kV-CBCT. However, an evaluation of the correlation with imaging time was not performed. The time required for image verification is important in institutes that have large patient workloads and limited numbers of linac machines. Small numbers of projections by reducing the angular range and increasing angular separation can decrease imaging time. A half scan can reduce imaging time by 2-folds using half angular separation. High efficiency of position set-up to verify imaging can be achieved by choosing a suitable procedure to balance image quality and patient dose. The development of protocols using image-guidance procedures is urgently required.

Conclusion

The kV-CBCT imaging protocol can be used different combinations of angular range and angular separation with decreased mA to setting suitable protocol for patient verification. Results suggested protocols suitable for optimal kV-CBCT image quality similar to the default protocol at 10 mA, θ=200°, and Δθ=0.54°, and accuracy of image registration within 0.2 cm in the head region as 10 mA, θ=200°, and Δθ=1.09° to reduce imaging dose and imaging time by about 50% or using protocol of 10 mA, θ=360°, and Δθ=1.09° to decrease imaging dose and imaging time by about 15%. To increase the CNR value with slight change of imaging dose, protocol of 20 mA, θ=200°, and Δθ=1.09° is recommended. The developing optimize kV-CBCT imaging protocol should be optimize imaging dose, image quality, accuracy of image registration, and imaging time.

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Cognitive assessment and intervention in occupational therapy for Thai older adults with neurocognitive disorders

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ABSTRACT

Background: Occupational therapists (OTs) have a significant role in maintaining clients' well-being. Understanding the current occupational therapy (OT) practice for the elderly with neurocognitive disorders (NCDs) brings effective service.

Objectives: This study aimed to explore current OT practice for cognitive assessment and intervention for the elderly with NCDs in Thailand.

Materials and methods: This study explored OT practices via questionnaire. Questionnaires were distributed to one hundred and ninety-one OTs throughout Thailand.

Results: One hundred and fifty-two occupational therapists (79.87%) responded to the survey. Most worked full-time (94.08%), and 74.34% worked at general hospitals. Participants were more likely to employ standardized cognitive tests (45.33%) than non-standardized assessments (38.00%). Typical standardized tests were screening tests rather than comprehensive tests. The most reported cognitive problem was basic cognition (77.63% to 98.08%). The main cognitive intervention focused on basic cognition (80.92% to 94.74%). Typical interventions were caregiver education (83.89%), physical activity (73.15%), and perceptual retaining (68.46%). Challenges to OT intervention were therapists had poor evaluation skills, unclear intervention guidelines, and an insufficient number of therapists.

Conclusion: OTs should participate in further education and develop a guideline and appropriate comprehensive cognitive assessment tools.

Introduction

Due to the Thai older population increasing,[1] the proportion of healthcare-related to health decline and problems associated with aging will continue to rise. Neurocognitive disorders (NCDs), both 'mild NCD' and 'major NCD', are cognitive decline symptoms that commonly occur in the elderly.[2, 3] In the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-5), mild NCD and major NCD are explicit diagnostic terms that explain the underlying continuum of cognitive impairment from normality to severe.[4] NCDs can significantly impact an individual's quality of life (QOL) such as loss of independent life and functional performance.[5, 6] An occupational therapist (OT) has a significant role in encouraging and supporting clients to maintain health and well-being.[7] This role is played based on standard practice which includes four standards. The first standard is professional standing and responsibility. The second standard involves the screening, evaluation, and re-evaluation process. The third standard is the intervention process. Finally, the fourth standard includes transition, discharge, and outcome measurement.[8, 9] According to these four standards, the occupational therapy practice can be divided into three main processes; evaluation, intervention, and outcome.

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There have been past studies about occupational therapy (OT) practice done on patients with NCDs worldwide. In Australia, OTs spent their time on cognitive and functional assessment more than on intervention.[10] Later, Irish OTs also indicated that their assessments were primarily focused on cognitive screening and functional performance.[11] Moreover, in Canada, OTs were more concerned with cognition than functional performance. Assessment practices more often involved the use of cognitive screening tests than domain-specific cognitive assessments tools in all phases because they lacked consensus on cognitive assessment. The study also suggests that guidelines about assessment in NCDs would bring about successful OT interventions.[12] In conclusion, OT practice in those countries is focused on the evaluation process and cognitive screening rather than on intervention and outcome processes.

In Thailand, the Department of Older Persons[13] divided clinical practice for NCDs into five stages: screening, treatment, education, rehabilitation, and long-term care. However, virtually no data is demonstrating OT practice for the client, especially in terms of cognitive assessment and intervention. Therefore, this study aims to explore current OT practices for the client in Thailand. Hopefully, disclosures of the practice can be evidence-based information and used for improving the quality of OT services and will promote initiatives that support OT's role in older clients with NCDs

Materials and methods

Participants

The participants were recruited using multistage sampling. First, workplaces were randomly selected. OT workplaces in the list of the Occupational Therapist Association of Thailand (OTAT) were contacted by telephone based on two criteria: (1) There was accessible contact information; and (2) there was OT service available for the elderly with NCDs. Second, OTs in the selected workplaces were purposively sampled using two criteria: (1) they had direct experience in serving cases with NCDs at least one year; and (2) they were willing to participate in this study. One hundred and ninety-one therapists were recruited.

Instrumentation

The questionnaire was modified from Bennett *et al*.[10] The contents were composed of three domains: assessment, intervention, and outcome. It was presented through four parts: general information, work experiment information, assessment, and intervention. After that, it was examined for content validity by three experts. The first expert is an OTs who has serviced older adults with NCDs for more than ten years and had experience in using standardized cognitive measurements. The other two experts were OT lecturers who had worked for at least five years and had experience in providing both cognitive training programs and using standardized cognitive assessments. The questionnaire was then revised and was piloted by five OTs who were not participants in this study.

Procedures

This study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, with the study code of AMSEC-61FB-001. This study is a cross-sectional research design. The questionnaires were distributed to all participants. This survey was kept open for four weeks. The participants were reminded through OT's social media networking sites to complete the questionnaire by one week and three days before the closing date to get the maximum response rate. The acceptable minimum response rate is 75.8% based on the study of McGrath and O'Callaghan.[11] The data were analyzed using descriptive statistics.

Results

One hundred and fifty-two occupational therapists (79.87%) responded to the survey. Table 1 illustrates that most of the participants were female (74.34%), worked full-time (94.08%), and worked at general hospitals (74.34%). Their main caseload was elderly with 'stroke and TBI' and 'NCDs', at 96.71% and 76.97%, respectively. Most cases with NCDs were classified as mild symptoms. The vast majority of the participants (76.16%) treated the elderly with NCDs at 1-5 cases per week, each case was served for 1-2 sessions a week (69.54%), and each session took 30-60 minutes (84.11%). Moreover, most of the participants cared for both the clients and their caregivers (84.00%).

Table 1 Demographic and working information (n=152).

	n	(%)
Gender		
Female	113	(74.34)
Main practice setting		
General hospital	113	(74.34)
Private hospital	20	(13.16)
Nursing home	6	(3.95)
Employment status		
Fulltime	143	(94.08)
Number of Caseloads		
Elderly with Stroke and TBI	147	(96.71)
Elderly with NCDs	117	(76.97)
Stage of the symptom of the case with NCDs		
Mild NCD	127	(83.55)
Major NCD in the mild stage	121	(79.61)
Major NCD in the moderate stage	86	(56.58)
Major NCD in late stage	53	(34.87)
Number of NCDs case per week		
1-5 cases	115	(76.16)
6-10 cases	15	(9.93)
More than 10 cases	13	(8.61)

Table 1 Demographic and working information (n=152). (continues)

	n	(%)
Number of services for NCDs case per week		
1-2 session(s)	105	(69.54)
3-5 sessions	23	(15.23)
Everyday	13	(8.61)
Length of service per session		
Less than 30 minutes	14	(9.27)
30-60 minutes	127	(84.11)
More than 60 minutes	10	(6.62)
Service pattern		
Only elderly	22	(14.67)
Only caregiver	2	(1.33)
Together	126	(84.00)

Table 2 Number of using cognitive standardized tests used (one or more options) (n=151).

	n	(%)
Screening tests		
MMSE	75	(49.70)
MoCA	59	(39.10)
TMSE	52	(34.40)
Comprehensive tests		
Thai-CPT	40	(25.82)
LOTCA	12	(7.95)
Both screening and comprehensive tests		
MMSE and Thai-CPT	14	(10.50)
MoCA and Thai-CPT	13	(8.61)

Note: *MMSE = Mini Mental State Examination-Thai version; MoCA = Montreal Cognitive Assessment Thai version; TMSE = Thai Mental State Examination, Thai-CPT = Thai Cognitive-Perceptual Test, LOTCA = Loewenstein Occupational Therapy Cognitive Assessment*

Cognitive assessment practice

Figure 1 shows sixty-eight participants (45.53%) employed standardized cognitive tests while fifty-seven (38.00%) used non-standardized cognitive assessment. Twenty-five participants (16.67%) used both assessment types. Table 2 represents that the participants used standardized cognitive screening tests such as the Mini Mental State Examination (MMSE)-Thai version (49.70%), the Montreal Cognitive Assessment (MoCA)-Thai version (39.10%). Only two comprehensive cognitive tests mostly used were the Thai Cognitive-Perceptual Test (Thai-CPT) (25.82%) and the Lowenstein Occupational Therapy Cognitive Assessment (LOTCA) (7.95%).

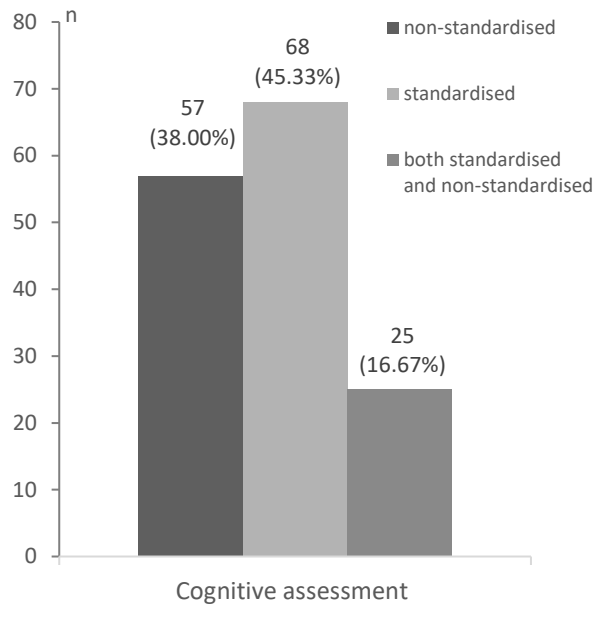

Figure 1*. Type of assessment tool (n=150).*

Cognitive intervention practice

Three main domains; memory, orientation, and attention, were intervened at 94.74%, 89.47%, and 80.92%, respectively (Figure 2). The most frequent cognitive intervention forms were individual treatment (49.67%) and individual mixed with group activity 46.98%). Typical cognitive interventions were caregiver education (83.89%), perceptual retaining (73.15%), and physical activity (68.46%) (Table 3). In addition, the participants reported that lacking knowledge, skills, intervention guidelines, and insufficient number of OTs were therapist's challenges. Clients' barriers included not understanding the role of OT, but also accessing OT services. Additionally, a lack of resources was the main institute's barrier. Furthermore, the participants also indicated that cognitive intervention would be effective if the client understood and corresponded with OT, therapists updated their knowledge and skills, and institute supported resources. In addition, they also recorded the need of further education about cognitive problems, standardized OT cognitive assessment tools, and cognitive intervention guidelines to care for the cases at each stage.

Discussion

The findings of this study that most occupational therapists worked full-time in hospitals and the majority of cases with NCDs were in early stages, are similar to findings in Australia and Ireland.[10, 11] This might be because the main duty of general hospitals is not specific to the NCDs cases but instead is focused on managing critical cases such as stroke and TBI.[11, 14] In addition, NCDs cases in later stages, who had more ADL's impairment, were typically cared for in nursing homes or communities rather than in hospitals.[15] Therefore, it might be noted that Thai OTs had a lower chance of receiving the NCDs cases in later stages. A difference issue that found from this study was that most of the occupational therapy services for the elderly with NCDs

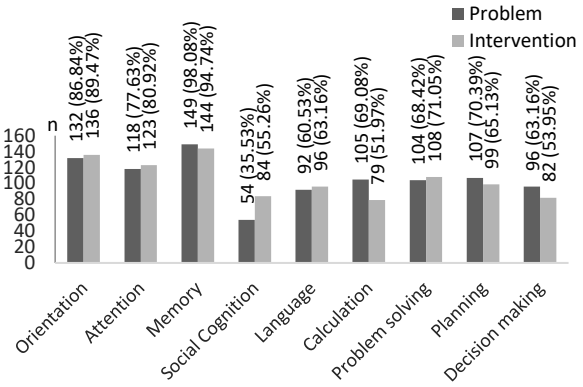

Figure 2. *Cognitive problem which has been found and intervened (n=152).*

Table 3 Cognitive intervention (n=149).

	n	(%)
Intervention forms		
Only individual	74	(49.67)
Only group	3	(2.01)
Both individual and group	70	(46.98)
Common intervention (one or more options)		
Caregiver education	125	(83.89)
Perceptual retraining	109	(73.15)
Physical activities	102	(68.46)
Patient education	91	(61.07)
Environmental modification advice	91	(61.07)
Compensatory techniques	81	(54.36)
Card games	75	(50.34)

of the other countries were serviced outside general hospitals. As findings of McGrath & O'Callaghan[11] and Gately &Trudeau[16] that large numbers of OTs worked in communities and nursing homes, moreover, greater amounts of their NCDs cases were in later stages. This indicates that Thai society might need more occupational therapists who worked in nursing homes or geriatric hospitals.

A finding that the participants often cared for the cases together with their caregivers might be because caregivers were an important part of the multidisciplinary team. According to the biopsychosocial model proposed by Grand, Caspar, and Macdonald,[17] the role of caregivers is to understand ways to improve life quality and to reduce additional disabilities. Caregivers are individuals who take the elderly to health care professionals, facilitate home treatment, and are key observers of progressive symptoms. Moreover, they are invisible patients who might become ill from providing full-time care. Therefore, they also need care and support.

It was surprising that participants in this study used standardized tests slightly more than non-standardized assessments even though there is a lack of standardized cognitive tests in Thailand. Standardized cognitive tests that were typically used were the MMSE-Thai version, the MoCA-Thai version, and the Thai-CPT. This may be because these tests are available in Thai language with no cultural bias. McGrath and O'Callaghan[11] suggested that therapists should employ standardized assessments to encourage accuracy of result discrimination and to ease communication with a multidisciplinary team. However, the screening tests were used more often than domain-specific comprehensive tests. These results are similar to the findings of Belchior, Korner-Bitensky, Holmes, and Robert[12] which indicated a lack of consensus on assessment practice. Additionally, this might result from good properties of the screening test; simple, readily available, user-friendly in the limited care time, and appropriate for the elderly.[18] However, screening tests cannot be used to gain detailed information about the severity of cognitive difficulties, and have limitations in detecting cognitive change over time.[19] Therefore, domain-specific comprehensive tests are necessary for cognitive intervention.

Problems in basic cognition such as memory, orientation, and attention were found in the cases. Some participants reported in open-end questions that they could not find overall cognitive problems regarding cases with NCDs by

using only the screening tests or a comprehensive tests. In the same way, they reported that the Thai-CPT was not suitable for the cases because it was developed to assess cognitive functions of Thai brain injury patients.[20] Consequently, participants have to use more than one assessment tool to find the client's cognitive status. Therefore, to assess cognition of the clients in OT, a test that can report comprehensive information and is appropriate to the case is required.

According to most of the participants working in general hospitals, they could service the client for just only an hour or less for 1-2 sessions weekly. However, with regards to a systematic review by Butler *et al*,[21] treatment one session a week for 12 weeks cannot make any difference but 2 sessions a week for 12 weeks can improve some cognitive functions. A systematic review by Möllers *et al*.[22] revealed that the longest length of stay in general hospitals of people with major NCD was less than six weeks. It is interesting to note that intervention programs of the participants in this study might not reach the optimal sessions to effectively delay symptoms. Therefore, it is recommended that the optimal frequency of cognitive intervention should be at least 2 sessions a week for 12 weeks, and those might be done in the form of a home program or follow-up sessions in outpatient units.

One important finding was that more cognitive interventions focused on basic cognitions such as memory, orientation, and attention, compared with executive functions. This might be because participants did not have enough time to give service for higher cognitive function in their clinical practice at hospitals due to fast discharge policies. Due to time limitations, the participants could focus only on the distinct cognitive impairments such as memory, orientation, and attention. Furthermore, executive functions are far more complicated to understand and to treat than basic cognitions.[23] The participants reported that they had insufficient skills in treating higher cognitive functions. In addition, guidelines for cognitive intervention are still lacking.

Another interesting feature was the intervention format. The reason why the participants chose individual formats was that they needed a one-on-one environment. Cognitive intervention requires a specific, quiet, and intense environment.[24] Furthermore, the reason why they combined individual and group intervention was that some group activities were necessary for the treatment. Sometimes the clients were interested in participating in group activities, and they needed a group climate to enhance their cognition. Haslam and colleges[25] explained that group activities could improve and maintain subsequent cognitive function in older adults and can slow cognitive decline. Furthermore, in a situation where the participant has limited resources and service time, group intervention is a potential approach to provide services more efficiently.[26]

The top three cognitive interventions were caregiver education, perceptual retraining, and physical activity. Since the caregiver is important in the multidisciplinary team, they should be educated in every aspect of care.[21] Perceptual retraining was frequently used because improving perception could benefit cognition. Allen and Roberts[27] concluded that both auditory and visual perception training could make positive changes in cognition. Additionally, physical activity was shown in evidence-based studies to enhance cognitive functions. For example, the study of Gheysen *et al*.[28] found that integrating physical activities such as dancing or tai-chi with cognitive training programs could enhance cognition in an older adult.

Significant findings of this study were that some participants reported insufficient knowledge and skills. In addition, they stressed the need for further education and more up-to-date knowledge attainment about cognitive intervention for NCDs. These findings are similar to the findings of Bennett *et al*.[10] It can be noted that not only OTs in other countries, but Thai OTs also should gain overall skills and knowledge to clarify the role of OT for cognition in older adults with NCDs.

Conclusion

From this study, Thai OTs paid attention on increasing numbers of older clients with NCDs, although they were not the main caseload in the hospital. Cognitive outcome assessment was rarely used because there were no available outcome measures that could provide clear information to be an official document. Therapists reported insufficient skills, lack appropriate guidelines and resources, and clients did not understand the role of OT were challenges of intervention. It is suggested that Thai OTs should heighten their awareness of their roles through further education and should develop appropriate cognitive assessment tools and intervention guidelines.

Conflicts of Interest

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

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Production of a common epitope specific anti-ankyrin monoclonal antibody

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ABSTRACT

Background: Ankyrin (Ank) is a protein family with crucial roles in retaining normal cellular physiology. In addition, ankyrin offers the potential as a non-antibody binder against various biomolecules. The designed ankyrin repeat protein (DARPin) selected from phage display libraries is useful for molecular detection and therapy. Monoclonal antibodies (mAbs) specific to the common epitope of DARPin are required to detect protein-protein interaction.

Objectives: This study aimed to establish mAbs against common antigenic determinant of ankyrins for further application in immunological techniques.

Materials and methods: Ank1D4 monomer and dimer were generated in the *Escherichia coli* expression system for immunogen preparation and validation of established mAbs. The binding activity of anti-Ank mAb obtained from different hybridoma clones was characterized using Ank1D4 by indirect ELISA. Candidate anti-Ank mAbs were validated for their cross-reactivity against irrelevant ankyrin (Ank2D3). The binding kinetic of mAbs from three candidate hybridoma clones (Ank-54, Ank-59, and Ank-94) was evaluated using bio-layer interferometry (BLI). The highest affinity clone (Ank-94 mAb) was further validated for its specificity against Ank1D4 and dimeric Ank1D4 using indirect ELISA. The interaction of three anti-Ank mAbs and ankyrins was compared by western immunoblotting analysis. The specificity of Ank-94 mAb was determined using a closely related scaffold, i.e., alpha-helicoidal HEAT-like repeat protein scaffold (αRep) by indirect ELISA. Ankyrins were detected by sandwich ELISA using Ank-94 mAb.

Results: The culture supernatant from hybridoma clones were characterized for their anti-ankyrin binding properties. Using indirect ELISA, three clones exhibited positive reactivity against the immunized ankyrin antigen (Ank1D4). The interactive epitope was found to rely on common antigenic determinants found in Ank1D4, dimeric Ank1D4, and an irrelevant ankyrin, Ank2D3. The immunoblotting results suggest that all mAbs interact with the sequential epitope of ankyrins. The cross-reactivity of Ank-94 mAb was not observed with αRep. Ank-94 mAb was selected for further purification and evaluation of binding properties due to its highest degree of binding affinity against Ank1D4.

Conclusion: The establishment of a novel Ank-94 mAb could be a valuable research tool in tracing the target of DARPins or developing immunoassays. Ank-94 mAb is superior over formerly produced Ank mAbs since it recognizes a common epitope on DARPins and relies on sequential epitope. Ank-94 mAb has no cross-reactivity with another scaffold, αRep.

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Introduction

Cellular scaffolds, including fibronectin type III domain (FN3) scaffolds, Kunitz domains, and ankyrins, are involved in maintaining the normal physiology of cells. The level of these scaffold proteins has been significantly associated with certain pathogenesis.[1, 2] FN3 scaffold expression, a component of the extracellular matrix, has been observed to decrease lung carcinomas and renal disease incidences.[3] High expressions of Kunitz domains (protease inhibitors) have been implicated in the pathogenesis of chronic pancreatitis and Alzheimer's disease; whereas, a reduced degree of expression was observed in cases of cervical cancer.[4–7] Apart from the extracellular scaffolds, an absence of intracellular-associated ankyrin-R can impact the depletion of protein 4.2, Rh, and RhAG in hereditary spherocytosis patients.[8] To monitor the expression of scaffold levels, monoclonal antibodies specific to individual scaffolds have been developed to be applied in flow cytometry, immunohistochemistry, immunoblotting, or immunocyto-chemistry.[8–10] The monoclonal anti-Kunitz protease inhibitor (KPI) captured with the human beta-Amyloid protein precursor (APP) is useful in the early diagnosis of Alzheimer's Disease detected by sandwich ELISA[5,11] Mouse ankyrin B monoclonal antibody was generated to recognize the spectrin-binding domain, which corresponded to a subset of olfactory receptor neuron (ORN) axons.[9] The monoclonal antibody against ankyrin-R (ankyrin-1) and erythrocyte ankyrin was produced to quantify ankyrin expression via flow cytometry.[8]

Designed ankyrin repeat proteins (DARPins) are small, high-affinity proteins that mediate specific protein-protein interactions.[12,13] The ankyrin structure comprises 33-amino acid residues in which conserved and variable residues are built from stacked. Each repeat forms a structural unit containing a β-turn followed by two antiparallel α-helices and a loop that connects it with the next repeat.[14,15] The structural compatibility of the natural repeat domains consists of a continuous hydrophobic core and a hydrophilic conserved molecular surface, both of which are stabilized from the capping repeats.[16] The distinct capability of ankyrin function involves the membrane-binding domain, which involves binding many unrelated proteins.[17] Various DARPins have been investigated to discover alternative protein therapeutics that rely on variable residues to repeat.[18,19]

We established a novel ankyrin monoclonal antibody (mAb) with broad immunoreactivity against the conserved region in the present study. In previously published reports, Ank1D4 was used to immunize BALB/c mouse for standard hybridoma production. Ank1D4 contains three internal repeats and is flanked by N- and C- terminus caps with certain variable residues that determine the specific recognition of the HIV-1 Gag domain.[13,20] An irrelevant Ank2D313 with different variable residues, along with a newly generated Ank1D4 dimer, was co-examined for the immunological activity and specificity of selected monoclonal antibodies.

Materials and methods

Construction and expression of ankyrin

Recombinant monomeric Ank1D4 and Ank2D3 were constructed and produced according to the previously described method.[13] Dimeric Ank1D4 was subsequently generated by relying on the monomeric Ank1D4 sequence; wherein each module was connected by a flexible $(G_4S)_4$ linker peptide. Accordingly, 6×HIS dimeric Ank1D4 was incorporated into the pQE-30 expression vector (Qiagen, Germany) (Figure 1) and transformed into *Escherichia coli* strain XL1-blue (Stratagene, San Diego, CA, USA) for plasmid propagation. *E. coli* XL1-blue harboring plasmid pQE-30-6×His dimeric Ank1D4 was confirmed using a pair of primers: Fw_NAnk1 (5'-TCC GCG GCC GCA GAC CTG GGT AAG-3') and Rev_Ank23C (5'-GCT AAT TAA GCT TTG CAG GAT TTC AGC-3'). After the purification process with QuickGene Plasmid kit S II (Kurabo, Germany), the plasmids were effectively sequenced (Agentide Inc, USA). In addition, pQE-30-6×His dimeric Ank1D4 was transformed into the *E. coli* strain M15[pREP4] (Qiagen, Germany) for protein expression.

***Figure 1**. Amino acid sequence of ankyrin repeats consists of conserved (black) and variable residues (red), flanking by N- and C- terminal capping repeats.*

Animals and immunization

Two 6-8-week-old BALB/c mice were obtained from M-CLEA Nomura Siam (Bangkok, Thailand). Accordingly, the Animal Care and Use Committee of Chiang Mai University approved all animal experiments. To produce anti-Ank mAb, a standard hybridoma technique was performed. Mice were intraperitoneally immunized with recombinant 6×HIS tagged Ank1D4 (100 µg) using Freund's adjuvant at two-week intervals for three immunizations. Complete Freund's adjuvant was used in the first immunization procedure, and incomplete Freund's adjuvant was used in the second and third immunizations. Seven days after the immunizations

were administered, blood was collected from the immunized mice by superficial temporal vein venipuncture. The presence of a specific antibody was determined by indirect ELISA. The mouse receiving the higher antibody titer was boosted with 6×HIS Ank1D4 (100 µg) in PBS. Five days after this boosting, splenocytes were harvested and fused with Sp2/mIL6 myeloma cells (ATCC, USA) using 50% polyethylene glycol. After cultivation in hypoxanthine, aminopterin, and thymidine (HAT) selective medium (GIBCO, UK), culture supernatants obtained from hybridoma were examined to determine the degree of antibody reactivity through interactions with 6×HIS Ank1D4 using indirect ELISA. The hybridomas that produced the 6×HIS Ank1D4 specific antibody were subjected to single cell cloning with limited dilutions. All mouse anti-ankyrin monoclonal antibodies produced by the clones were isotyped using an IsoStrip Mouse Monoclonal Antibody Isotyping Kit (Roche Applied Science, Germany). For simplicity, anti-ankyrin monoclonal antibodies will be denoted as anti-Ank mAb(s) and Ank - (number of clone) mAb throughout the paper.

Binding activity of anti-Ank mAb

To characterize anti-Ank mAb from different hybridoma clones, indirect ELISA was performed. Ank1D4 (1 µg/mL) was immobilized in a microtiter plate (Greiner, Germany) and left overnight at 4 °C in a moisture chamber. The coated wells were washed four times with PBST (PBS containing 0.05% Tween 20) and were blocked with 2% w/v bovine serum albumin (BSA) and phosphate buffer saline, pH 7.4 (PBS) for 1 hr at RT. After being washed with PBST, Ank1D4 was incubated with the culture supernatant form of mouse anti-Ank mAb (1:100 dilution) for 1 hr at RT. After being washed with PBST, a 1:3,000 dilution of goat anti-mouse immunoglobulins conjugated with horseradish peroxidase (HRP) (KPL, USA) in 2% w/v BSA in PBS was added. The resulting specimen was subsequently incubated for 1 hr at RT. The binding of ankyrin was detected by adding 3, 3', 5, 5'-tetramethylbenzidine (TMB) membrane peroxidase substrate (KPL, USA). Subsequently, the degree of absorbance was monitored at 450 nm.

Examining the specificity of mAbs against irrelevant ankyrin

Indirect ELISA was applied to determine the cross-reactivity of the candidate anti-Ank mAbs against an irrelevant ankyrin, i.e., Ank2D3. Ank1D4 or Ank2D3 (1 µg/mL) was immobilized in a 96-well plate and left overnight at 4 °C in a moisture chamber. The coated wells were washed four times with PBST and blocked with 2% w/v BSA in PBS for 1 hr at RT. Each protein was dissolved in PBS with 2% w/v BSA. After being washed with PBST, ankyrins were incubated with the culture supernatant form of mouse anti-Ank mAb (Ank-54, Ank-59, and Ank-94 mAbs) (1:100 dilution) for 1 hr at RT. After being washed with PBST, a 1:3,000 dilution of goat anti-mouse Igs conjugated with HRP was added. The resulting specimen was incubated for 1 hr at RT. The binding of anti-Ank mAb was detected by adding a TMB substrate. Subsequently, the degree of absorbance was monitored at 450 nm.

Comparison of binding activity against mono and dimeric ankyrin

The immunoreactivity of a selected mAb clone Ank-94 against monomeric and dimeric Ank1D4 was determined using indirect ELISA. The equimolar of monomeric (0.05 µg/mL) and dimeric (0.1 µg/mL) Ank1D4 was prepared and immobilized in a microtiter plate. The resulting specimen was subsequently incubated at 4 °C overnight. The coated wells were washed four times with PBST and incubated in PBS with 2% w/v BSA for 1 hr at RT. Each protein was dissolved in 2% w/v BSA in PBS. After being washed with PBST, ankyrins were incubated with a culture supernatant form of Ank-94 mAb (1:200 dilution) for 1 hr at RT. After being washed with PBST, a 1:3,000 dilution of goat anti-mouse Igs conjugate was added, and the resulting specimen was subsequently incubated for 1 hr at RT. The binding of ankyrin was detected by adding a TMB substrate. The degree of absorbance was then monitored at 450 nm.

Western immunoblotting analysis against ankyrins

His-tagged ankyrin proteins were purified from *E. coli* strain M15[pREP4] using affinity chromatography on an HisTrap column with ÄKTA pure™ (Cytiva, Germany). Recombinant Ank2D3, monomeric Ank1D4, and dimeric Ank1D4 were separated by 15% SDS-PAGE and were subjected to immunoblotting analysis. After treatment with a blocking solution (2% w/v skim milk in PBS), the nitrocellulose membranes (Cytiva, Germany) were incubated with a specific blocking solution containing mouse anti-Ank mAb. Ank-54, Ank-59, or Ank-94 mAbs at 1:3000 dilution for 1 hr followed by goat anti-mouse Igs conjugated HRP (1:3000 dilution). The membrane was treated with an enhanced chemiluminescent reagent (Rockford, IL, USA). The resulting reactive protein bands were visualized using a ChemiDoc™ MP Imaging System (Bio-Rad, USA).

Determination of specificity of anti-Ank mAb

To investigate whether Ank-94 mAb specifically recognizes the ankyrin structure, indirect ELISA was performed using a closely related scaffold, i.e., αRep 4E3 and 9A8.[21] Monomeric Ank1D4, αRep 4E3 and αRep 9A8 (1 µg/mL) were immobilized in a 96-well plate and incubated overnight at 4 °C in a moisture chamber. Wells were washed four times with PBST and blocked with 2% w/v BSA in PBS for 1 hr at RT. After being washed with PBST, proteins were incubated with the culture supernatant form of Ank-94 mAb (1:10 dilution) for 1 hr at RT. After being washed with PBST, a 1:3,000 dilution of goat anti-mouse Igs conjugated with HRP in 2% w/v BSA in PBS was added. The resulting specimen was subsequently incubated for 1 hr at RT. The binding activity was detected by adding a TMB substrate. The Degree of absorbance was then monitored at 450 nm.

Binding kinetics of anti-Ank mAb

The binding kinetics of Ank-54, Ank-59, and Ank-94 mAbs with monomeric and dimeric Ank1D4 were evaluated using bio-layer interferometry (BLI) with the BLItz™ system (FortéBio, Menlo Park, CA). Anti-Penta-HIS biosensors were pre-wetted for 15 min in buffer (2% w/v BSA in PBST) immediately before use. Recombinant H6-Ank1D4 (loading) at 5 µg/mL was immobilized to anti-Penta-HIS biosensors for 2 min. After being washed with buffer for 30 sec, the Ank1D4-loaded biosensor was dipped into a solution containing culture supernatant form of anti-Ank mAbs (association

step), followed by dipping in 2% BSA in PBST (dissociation step). The association (k_{on}) and dissociation rate (k_{off}) and the equilibrium dissociation constant (K_D) were calculated from a local fit to a 1:1 binding model of the data between anti-Ank mAbs and Ank1D4 using the BLItz Pro 1.1 software.

Purification of Ank-94 mAb using protein L affinity chromatography

Ank-94 mAb was seeded and expanded in Iscove's Modified Dulbecco's Medium (IMDM; Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco) under standard conditions (5% CO_2, at 37°C). The culture supernatant form of Ank-94 mAb was harvested and purified using affinity chromatography on the protein L matrix (GE Healthcare). The Ank-94 mAb supernatant was loaded onto a column and then washed with 20 ml of buffer (20 mM sodium phosphate buffer, 150 mM sodium chloride, pH 7.0). Neutralizing buffer (1 M Tris-HCl, pH 9.0) was prepared in collection tubes to be mixed with 1 mL of the eluted fractions. The antibody was eluted with 0.1 M glycine at a pH of 2.7, and fractions were selected at A280 nm. The eluted sample was eventually exchanged for the buffer to PBS.

Sandwich ELISA for ankyrin detection using Ank-94 mAb

96-well plates were immobilized with 1 µg/mL purified Ank-94 mAb and incubated at 4 °C overnight. After being washed with PBST, coated wells were incubated with 2% w/v BSA in PBS for 1 hr at RT. Recombinant monomeric or dimeric Ank1D4 (2 µg/mL) was added, followed by anti-His-HRP (1:3000 dilution, BioLegend) in 2% w/v BSA in PBS for 1 hr at RT. Ankyrin binding was detected by monitoring the ELISA plate reader at an absorbance of 450 nm after the TMB substrate was added.

Results

Isotyping of anti-Ank mAbs

All immunoglobulins were identified as IgG using IsoStrip. The Ank-54 and Ank-59 mAbs are IgG_1 kappa isotype, whereas Ank-94 mAb is IgG_{2a} kappa.

Binding activity of ankyrin antibody obtained from different hybridoma clones

Hybridoma culture supernatants containing anti-Ank mAb were performed to determine the binding activity against ankyrin immunogen. Indirect ELISA was then used to determine whether Ank-54, Ank-59, and Ank-94 mAbs could interact with the immunized Ank1D4 antigen. Among the three clones of anti-Ank mAb, the degree of binding activity of Ank-94 mAb was higher than for Ank-54 and Ank-59 mAbs (Figure 2).

Specific interactions of mAbs against ankyrin scaffold

The binding activity of mAbs was determined against irrelevant Ank2D3 and Ank1D4 scaffolds using indirect ELISA. Ank1D4 and Ank2D3 were targeted by Ank-54, Ank-59, and Ank-94 mAbs, which implied that the common antigenic determinant was specific (Figure 3).

Ank-94 mAb binding activity against dimeric Ank1D4

At mole equivalence of monomeric and dimeric Ank1D4, Ank-94 mAb demonstrated slightly higher signals in dimeric Ank1D4 (Figure 4). This result suggests that Ank-94 mAb could interact with both ankyrin forms.

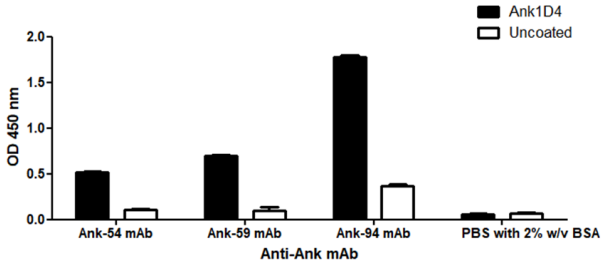

Figure 2. *Binding activity of mouse anti-Ank mAb. Different clones of mouse anti-Ank mAb interacted with immobilized Ank1D4. The binding activity of the three clones (Ank-54, Ank-59, and Ank-94 mAbs) of anti-Ank mAb and Ank1D4 antigen was determined with the use of HRP-conjugated goat anti-mouse Igs. Data were obtained from triplicate experiments and expressed as mean±SD values.*

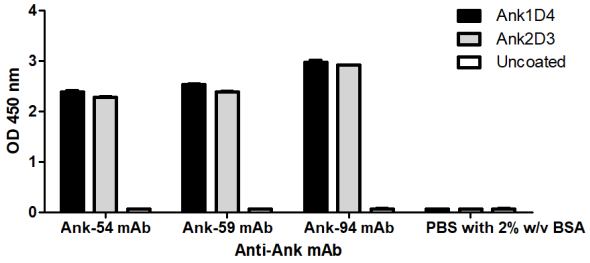

Figure 3. *An evaluation of binding characteristics of anti-Ank mAb. Ank-54, Ank-59, and Ank-94 mAbs was determined using indirect ELISA. Ank1D4 and Ank2D3 were immobilized in a 96-well plate following by anti-Ank mAb (Ank-54, Ank-59, and Ank-94 mAbs). Interactions with HRP-conjugated goat anti-mouse immunoglobulin were then detected. Data were obtained from triplicate experiments and expressed as mean±SD values.*

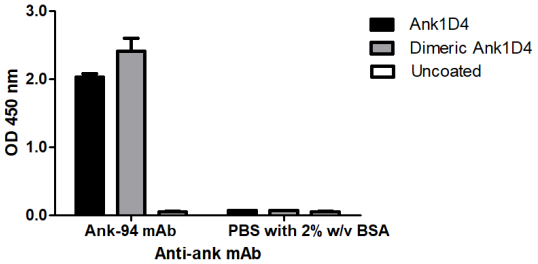

Figure 4. *Characterization of mouse anti-ankyrin monoclonal antibody clone 94. Ank-94 mAb against ankyrin was applied to detect monomeric and dimeric Ank1D4. Monomeric and dimeric Ank1D4 were immobilized on ELISA wells. After incubation, culture supernatant form of Ank-94 mAb were added and detected with HRP-conjugated goat anti-mouse immunoglobulin. Data were obtained from triplicate experiments and expressed as mean±SD values.*

Epitopic structure of ankyrins recognized by mAbs

Immunoblotting was performed to investigate whether anti-Ank mAb could interact with other ankyrin clones apart from Ank1D4. Ank2D3 was used for irrelevant proteins in lieu of Ank1D4. Ank-54, Ank-59, and Ank-94 mAbs demonstrated that the linearized recombinant Ank1D4 and Ank2D3 structures could be detected by all mAbs (Figure 5A). According to the amino acid alignment of Ank1D4 and Ank2D3 using Clustal Omega web server[22], conserved and variable regions of ankyrin scaffolds were depicted (Figure 5B). In addition, Ank-94 mAb recognized monomeric Ank1D4 (Figure 5C) and dimeric Ank1D4 (Figure 5D) in their denatured forms.

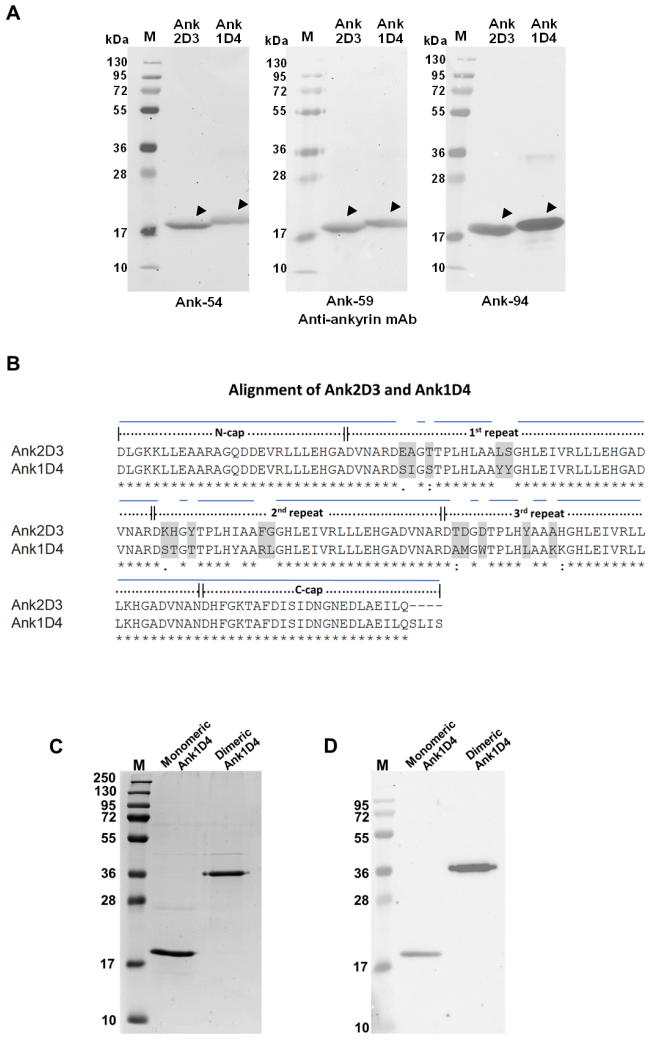

Figure 5. *Detection of anti-Ank mAb clones against ankyrin. (A) Ank-54, Ank-59, and Ank-94 mAbs detected both Ank2D3 and Ank1D4 using immunoblotting. (B) Schematic of alignment between Ank2D3 and Ank1D4. Amino acid sequences of Ank2D3 and Ank1D4 (Escherichia coli) were represented using the single letter code. Ankyrin repeats consisted of three repeat proteins flanked by the N- and C- terminus caps. Conserved and different variable residues (five positions) of ankyrins are represented in the blue line and highlighted in grey, respectively. Monomeric and dimeric Ank1D4 were separated using (C) SDS-PAGE and visualized by (D) immunoblotting using Ank-94 mAb as the primary antibody. Arrows indicate the major bands of ankyrin with M as a protein marker.*

Cross-reactivity testing of anti-ankyrin mAb with ankyrin and irrelevant protein

Indirect ELISA was performed to assess cross-reactivity between ankyrin and alpha-repeat proteins (αReps). When analyzing Ank-94 mAb, only the Ank1D4 protein was positive, while neither αRep 4E3 nor αRep 9A8 were detected in the anti-Ank mAb interaction (Figure 6). This finding suggests that anti-Ank mAb specifically interacted with ankyrin.

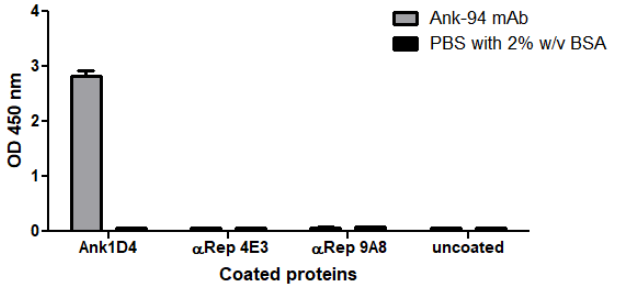

Figure 6. *Investigation of specificity of anti-Ank mAb. Cross-reactivity of Ank1D4 and two αReps was determined and detected by Ank-94 mAb using indirect ELISA. Ankyrin and αReps were immobilized in a 96-well plate followed by Ank-94 mAb. They were then detected with HRP-conjugated goat anti-mouse immunoglobulin. Data were obtained from triplicate experiments and expressed as mean±SD values.*

Binding kinetic properties of anti-Ank mAb clones against ankyrin

The binding kinetics of Ank-54 mAb, Ank-59 mAb, and Ank-94 mAb were displayed in the sensorgram (Figure 7). The equilibrium dissociation constant (KD) of the binding reaction between Ank-94 mAb (3.1×10^{-7} M) and Ank1D4 is stronger than Ank-54 mAb (4.8×10^{-8} M) and Ank-59 mAb (7.2×10^{-8} M).

Evaluation of binding preference of mAb against ankyrins

Of the three mAbs we prepared against ankyrin; Ank-94 mAb culture supernatant was purified via protein L affinity chromatography. Antibody sandwich ELISA was performed for comparisons between ankyrin monomer and dimer. The results indicated that immobilized Ank-94 mAb was able to capture monomeric and dimeric Ank1D4 following anti-His-HRP detection (Figure 8).

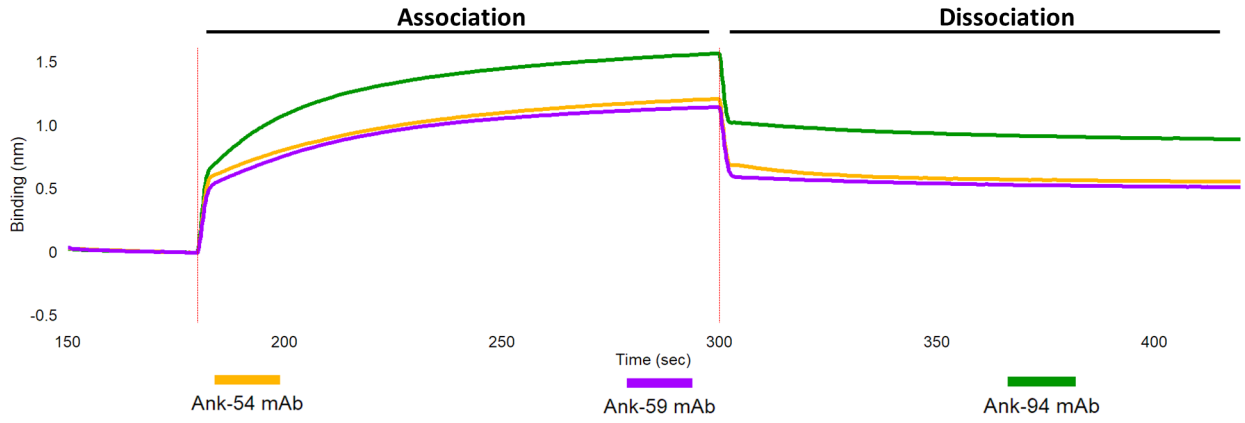

Figure 7. *The binding kinetics of anti-Ank mAbs displayed in the sensorgram. H_6-Ank1D4 was immobilized on anti-Penta-HIS biosensors and subsequently reacted with culture supernatant of Ank-54 (orange) or Ank-59 (purple) or Ank-94 (green) mAb.*

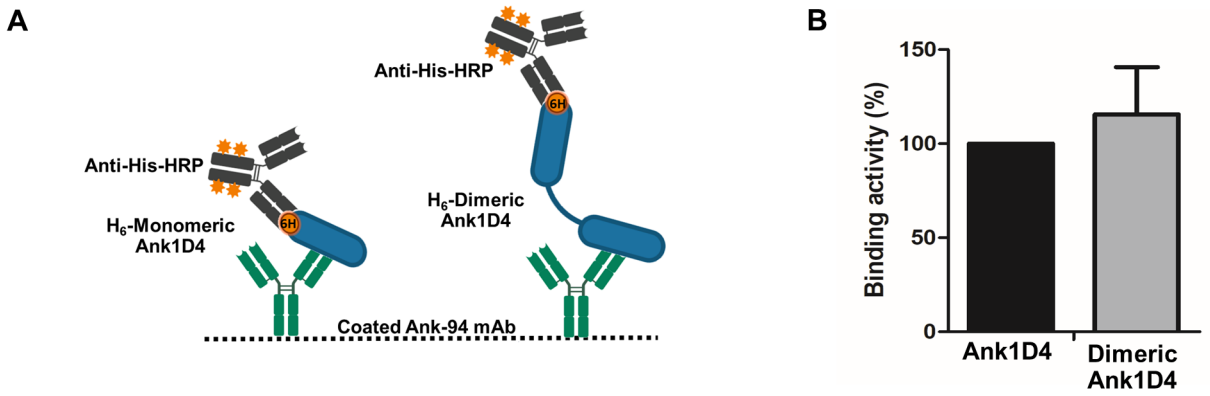

Figure 8. *Relative binding activity of purified Ank-94 mAb. (A) Schematic and (B) the percentage of antibody sandwich ELISA binding assay. Data were normalized with monomeric Ank1D4 levels to evaluate the binding activity using mathematical equation ($O_{Ddimer}/O_{Dmonomer}$)×100. This graph was produced from triplicate experiments. The results are expressed as mean±SD values.*

Discussion

The crucial role of scaffold proteins in cellular signaling pathways is required for accurate coordination.[23] Overexpression or deficiency of scaffolds can influence the development of certain diseases, such as the defects associated with tubulointerstitial fibrosis in renal failure.[3] Since a number of scaffold proteins are associated with pathogenesis, certain anti-scaffold antibodies have been established for immunological assays.[24–26] Among these, monoclonal antibodies specific to human ankyrins, i.e., ANK1, ANK2, and ANK3, have been developed.[8, 9, 27]

In our study, Ank-94 mAb was generated with the highest reactivity against the Ank1D4 immunogen. In addition, Ank-94 mAb demonstrated cross-specific reactivity with irrelevant Ank2D3 by indirect ELISA. This would suggest that anti-Ank mAb could interact with the conserved residues of DARPins in the pairwise alignment in Figure 4. The binding activity of Ank-94 mAb was further investigated for recognition with Ank1D4 dimer. The result indicates that Ank-94 mAb had reserved its binding function with Ank1D4 dimer. MAbs from three hybridoma clones, including Ank-94 mAb, were subsequently analyzed by immunoblotting. Their immunoreactivity demonstrated that the mAbs recognized the sequential epitope of either monomeric or dimeric Ank1D4 and Ank2D3. Notably, the alignment of Ank1D4 and Ank2D3 sequences indicate the presence of conserved residues of DARPins. Concerning the artificial structural proteins, conserved positions were defined for all the frameworks. Whereas non-conserved positions reflected an introduction of diversity.[16] Apart from specificity to the common epitope on DARPins, Ank-94 mAb did not exhibit non-specific activity with any other scaffold protein, i.e., αRep.

Apart from the specificity, Ank-94 mAb exhibited the highest binding affinity against Ank1D4. The binding kinetic of Ank-94 mAb (K_D=3.1 x 10^{-7} M) is stronger than Ank-54 and Ank-59 mAbs. It was therefore considered a good candidate for conferring to DARPins interaction. Generally, anti-Ank mAbs were generated to identify the natural ankyrins

(ankyrin-R, -B, and -G) expression that localized intracellular compartment for evaluation of ankyrin levels.[8,9,10] Antibodies selectively interacting with specific human ankyrins were generally established to directly evaluate ankyrin expression using immunoassays, i.e., immunoblotting or flow cytometry. Whereas Ank-94 mAb could be applied to trace protein-protein interactions to identify the specific target of DARPins in indirect ELISA. Ank-94 mAb recognized that the conserved residues occurred more than once in repeat modules. Thus, Ank-94 mAb provides an advantage of detection sensitivity compared to anti-His, which is generally used in tracing bound DARPins.[28] With regard to the consistency of mAb production and performance, Ank-94 mAb should be substituted for rabbit anti-ankyrin polyclonal antibodies to develop an immunochromatographic assay.[29] In addition, we established a sandwich ELISA to simulate the detection of DARPins. The result suggests that Ank-94 mAb captured His-tagged monomeric and dimeric Ank1D4 after being traced with anti-His-HRP. This system can be adapted to quantify other DARPins or native ankyrins that contain similar conserved residues.

Conclusion

Among the three clones of mouse monoclonal anti-ankyrin antibodies that could recognize DARPin, Ank-94 mAb demonstrated the highest degree of immunoreactivity. In addition to Ank1D4, Ank-54, Ank-59, and Ank-94 mAbs were bound with irrelevant Ank2D3 indicating that anti-Ank mAbs can interact with common residues. The binding characteristic of Ank-94 mAb relies on the linearized structure of ankyrins. No cross-reactivity with another scaffold, i.e., αRep, was observed. Notably, the specificity of Ank1D4 can assist future applications in discovering novel targets for candidate DARPins.

Conflicts of Interest

The authors declare no conflict of interest.

Ethical approval

Animal experiments were conducted following the animal use protocol approved by the Laboratory Animal Center, Office of Research Administration at Chiang Mai University. The Ethics Review Committee of the Laboratory Animal Center at Chiang Mai approved all animal experiments (Project number 2564/MC-002).

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Shoulder-abduction force steadiness in individuals with neck pain with scapular dyskinesis

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ABSTRACT

Background: Neck pain is associated with scapular dyskinesis and impaired axio-scapular and shoulder muscle activity and function. However, there is little research on force steadiness, specifically during shoulder motion in patients with neck pain with scapular dyskinesis. Its relationship with characteristics of neck pain is also unknown.

Objectives: To investigate force steadiness at 20% and 50% of MVC of shoulder abduction (30 degrees) in persons with neck pain with scapular dyskinesis compared to asymptomatic controls and to determine its relationships with characteristics of neck pain.

Materials and methods: Fifty-two women and men (26 neck pain with scapular dyskinesis and 26 asymptomatic controls) were recruited to the study. Force steadiness of 30 degrees of shoulder abduction was measured at 20% and 50% of maximal voluntary contraction (MVC) and coefficient of variation (CV) of force values were calculated. Characteristics of neck pain included neck pain intensity, duration and disability and upper limb disability.

Results: There was no interaction between group and target force level (p=0.45). Main effects were found for group (p=0.003) and target force level (p<0.001). Participants in neck pain had significantly reduced force steadiness of isometric shoulder abduction at 20% and 50% of MVC compared to the control group (p<0.05, η^2_p =0.10 and p<0.001, η^2_p =0.26, respectively). There were no correlations between force steadiness and characteristics of neck pain (p>0.05).

Conclusion: Patients with neck pain with scapular dyskinesis had reduced shoulder abduction force steadiness at 20% and 50% of MVC. The relationships between force steadiness and characteristics of neck pain are still needed to be explored in further studies.

Introduction

Force steadiness, the ability to maintain a steady and precise force with specific target muscles involved has been suggested to be associated with pain.[1-3] Reduced force steadiness has also been considered as an independent predictor of functional performance.[4,5] Previous studies demonstrated that patients with neck pain had reduced force steadiness of the cervical muscles.[2,6] O'leary *et al*.[6] demonstrated that patients with neck pain had significantly decreased ability of maintain isometric contraction of the craniocervical muscles at 20% and 50% of maximal voluntary contraction (MVC) compared to healthy controls. Similarly, Muceli *et al*.[2] showed that women with neck pain exhibited significantly decreased force steadiness of cervical flexion. Poorer contraction accuracy in maintaining a steady contraction might reflect altered cervical afferent input in neck pain, such as direct damage to mechanoreceptors, sensitization

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of mechanoreceptors from neck pain, and/or sympathetic effects on muscle spindle sensitivity.[7]

Functions of the scapula and upper limb can potentially be affected by neck pain.[8,9] Patients with neck pain up to 80% had upper limb activities aggravating their neck pain and the neck and upper limb disabilities were also related to neck pain severity.[9] Altered scapular position and movement, termed scapular dyskinesis is often related to neck pain.[10] A recent study has reported a high percentage (90%) of scapular dyskinesis in office workers with neck and scapular complaints.[11] Patients with neck pain had reduced clavicular retraction and increased scapular downward rotation and protraction during elevation of arm movement compared to a healthy population.[12-14] The scapula shares common muscle attachments with the neck, through axio-scapular muscles, in particular upper trapezius and levator scapulae.[15] Thus altered scapular position and control may be associated with dysfunction of the axio-scapular muscles and may increase mechanical force to cervical structures.[10,16] Additionally, the scapula connects to the upper limb, so increased loads placed through the upper limb may alter scapular position and control and subsequently transmit to cervical structures, resulting in neck pain.[7,16]

According to mechanistic links between neck pain and scapular and upper limb dysfunction, altered force steadiness of the axio-scapular muscles to control the scapula during submaximal contraction of the shoulder can be expected. However, one clinical study found at baseline no differences in force steadiness during 30-second shoulder elevation at a target force of 30% of MVC between F-16 pilots with and without neck pain.[17] Yet, there is little research on force steadiness, specifically in patients with neck pain with scapular dyskinesis. It is also unknown if force steadiness is correlated with neck pain features (e.g., neck pain intensity, duration and neck and shoulder disabilities). The purposes of this study were 1) to investigate force steadiness at 20% and 50% of MVC of shoulder abduction (30 degrees) in persons with neck pain with scapular dyskinesis compared to asymptomatic controls and 2) to determine the relationships of force and neck pain features. The measure of 30 degrees of shoulder abduction was chosen for the study as it was considered to play an important role in upper limb function and activities of daily living, which requires a good scapular stability and control.[18] It was hypothesized that participants with neck pain with scapular dyskinesis would have reduced force steadiness (20% and 50% of MVC) compared to asymptomatic controls and there would be some relationships between force steadiness and neck pain features.

Materials and methods

Participants

Sample size for this cross-sectional study was calculated using G*Power 3.1.9.4 based on our pilot study (10 neck pain participants and 10 healthy controls). Effect sizes were 0.35 and 1.20 for coefficient of variation (CV) for force at 20% and 50% of MVC, respectively. The smaller effect size was chosen for sample size calculation in this study. With a power of 0.80 and a significant level of 0.05, a total sample size required for the study was 52.

Fifty-two women and men (26 neck pain with scapular dyskinesis and 26 controls) aged between 18-55 years old, were recruited from local hospitals, physical therapy clinics, community, and university through social media (e.g., Facebook and Instagram). Age, gender, and body mass index were similar for both groups. Inclusion criteria for the neck pain group were a history of nonspecific neck pain for ≥3 months, an average pain intensity over the past week ≥30 mm on a Visual Analogue Scale (VAS),[19] and having scapular dyskinesis on the side ipsilateral to neck pain and the dominant arm. Participants in the control group had no history of neck pain for the past year and no clinical signs of scapular dyskinesis. The dominant arm was determined based on at least 2/3 activities: writing, grasping, and throwing.[20] Assessment of scapular dyskinesis was performed according to previous studies by an experienced physical therapist who was one of examiners in published reliability studies.[21,22] In brief, the scapular dyskinesis was observed during performed 5 repetitions of arm elevation in the scapular plane with a 1 kg weight for women and a 2 kg for men. Scapula dyskinesis was defined as the presence of either winging (prominence of any portion of the medial border or inferior angle away from the thorax) or dysrhythmia (premature, or excessive, or stuttering motion during elevation and lowering) for at least 3/5 trials.

Participants in both groups were excluded if they had a history of head and neck injury or surgery, shoulder problems, any musculoskeletal or neurological problems that could affect the scapular position and movement (e.g., scoliosis, torticollis, myofascial pain, and long thoracic nerve palsy), and/or any specific training or treatment of neck or shoulder girdle muscles over the past 12 months. The study was approved by the ethical review committee for research in humans, Faculty of Associated Medical Sciences, Chiang Mai University (AMSEC-64EX-030) and was conducted in accordance with the Declaration of Helsinki. All eligible participants signed an informed consent prior to commencement of the study. They were also asked to refrain from taking pain-relief medication at least 6 hours before testing.

Questionnaires

A general questionnaire was used to collect demographic data. Participants with neck pain were also asked to complete a 0-10 cm Visual Analogue Scale (VAS),[23] the Neck Disability Index-Thai version (NDI-TH),[24] and the disabilities of the arm, shoulder, and hand questionnaire-Thai version (DASH-TH).[25] The VAS was used to measure neck pain intensity with 0 indicating “no pain” and 10 indicating “as worst as imaginable pain”.[23] The NDI-TH was used to assess a patient's self-reported neck pain related disability.[24] It includes 10 items concerning pain intensity, headache, concentration, reading, sleeping, driving, work, personal care, lifting, and recreation. The possible total score ranges from 0 to 50, which can be expressed as a percentage. The DASH-TH was used to measure disability and symptoms related to upper extremity.[25] It consists of 30 items concerning physical function, symptom severity, and social or role function, with the possible total score ranging from 0 (no disability) to 100 (most severe disability). A higher score of the NDI-TH and DASH-TH indicates greater disability.

Experimental procedure

A dynamometer (ML003/D, Power Lab, ADInstrument, Bella Vista, Australia) were used to measure MVC and force steadiness of shoulder abduction. The raw force and position signals from a dynamometer were collected at a sampling frequency of 1,000 Hz and low-pass filter of 100 Hz. The measures of MVC and force steadiness were tested on the ipsilateral (more painful) side of neck pain or the dominant side for the control group. Participants sat upright with knees and hips at 90 degrees and feet flat on the floor. The arm being tested was attached to a resistance application pad of the dynamometer, approximately 3 cm above the lateral epicondyle with the shoulder abducted 30 degrees (using a universal goniometer), the elbow flexed 90 degrees and the forearm in neutral position (Figure 1). The other arm was placed on their side with the hand placed on their thigh. Participants were asked to perform MVC at 30 degrees of shoulder abduction three times and each time was hold for 5 seconds. A 60-second rest was provided between each trial. The highest value of MVCs was used to calculate relative target forces corresponding to 20% and 50% of MVC. Two practices were given for familiarization prior to testing.

Figure 1. *Participant position during maximal voluntary contraction (MVC) and force steadiness tests of 30 degrees of shoulder abduction.*

For the force steadiness test, a computer monitor was positioned approximately 1 meter in front of participants. The target force (20% or 50% of MVC) was displayed on the monitor with a horizontal line showing changes in the exerted force. Participants were given standard instructions to "attempt to stay as close as possible to the target force". Each target force was tested randomly three times with an interval of 10 minutes between each target force. Each trial was held for 15 seconds with a 60-second rest between trials.[26] Participants were asked to maintain an upright position in order to avoid any compensation. Participants were also asked to rate any pain occurring during the test on a 0 - 10 numerical rating scale (NRS). All measures were assessed by an independent examiner who was blinded to the participants' condition.

Data management

All force data were analyzed using LabChart v8.1.5 software (ADInstrument, Bella Vista, Australia). Force steadiness was measured for the intermediate 10 seconds to avoid range of the ramping up and down of force production.[26] Force steadiness was calculated as the coefficient of variation (CV) of the exerted force and expressed as a percentage (CV=standard deviation (SD)/mean forcex100).[27] Increased CV of force represents reduced ability to maintain a steady muscle contraction (steadiness of force).

Statistical analysis

Descriptive statistics, independent t-test, and Chi-square were used to determine differences in participants' characteristics between groups. Shapiro-Wilk test was used to test for normality of outcome variables. Mann-Whitney U test was used to analyze a difference in the MVC between groups. A mixed model analysis of variance with the Bonferroni's correction was used to analyze differences between groups for the CV values at 20% and 50% of MVC. Effect size was calculated using partial eta squared (η_p^2): small ≥0.01, moderate ≥0.06, and large ≥0.14.[28] Pearson's correlation coefficient was used to determine the relationships between the CV values and characteristics of neck pain. A significance level was set at 0.05. All statistical analysis were conducted using SPSS package.

Results

Participants

Table 1 presents demographic data and neck pain characteristics of participants. There were no significant differences between groups with respect to age, gender, and body mass index (all p>0.05). All participants in both groups were right-handed.

Table 1 Demographic data for the neck pain and control groups.

	Neck pain (n=26)	Controls (n=26)	*p* value
Age (year)	30.69±8.25	33.04±10.86	0.39
Gender (male/female, n)	7/19	7/19	1.00
Body mass index (kg/m^2)	22.67±2.39	22.50±2.47	0.80
Neck pain intensity (0-10 VAS, cm.)			
Over the past week	5.38±1.23	-	-
On testing day	4.96±1.34	-	-
Neck pain duration (months)	32.27±23.82	-	-
Neck disability (% NDI-TH)	19.23±8.54	-	-
Upper extremity disability (% DASH-TH)	16.54±9.83	-	-

Data are presented with mean±SD, otherwise as indicated. VAS: visual analogue scale, NDI-TH: neck disability index-Thai version, DASH-TH: the disabilities of the arm, shoulder, and hand questionnaire-Thai version.

Maximal force

Mean and standard deviation (SD) values of MVC for the neck pain and control groups were 130.58±51.73 newtons and 142.24±52.70 newtons, respectively. No significant difference in the MVC value was found between groups (p=0.26). Thirteen participants (50.0%) in the neck pain group reported neck pain during performing the MVC test (NRS=5.25±1.59). None of asymptomatic controls reported pain.

Force steadiness

There was no significant interaction between group and target force level [F (1, 50) =0.57, p=0.45]. There were significant main effects of group [F (1, 50) =9.90, p=0.003] and target force level [F (1, 50) = 174.56, p<0.001]. When comparing between groups, the mean CV values at 20% of and 50% of MVC were significantly higher in the neck pain group compared to the control group (p<0.05, η^2_p =0.10 and p<0.001, η^2_p =0.26, respectively) (Figure 2). Four participants in the neck pain group (15.4%) reported neck pain during testing at 20% of MVC (NRS=3.42±0.50) and 12 participants (46.2%) at 50% of MVC (NRS=4.75±1.38). None of asymptomatic controls reported neck pain.

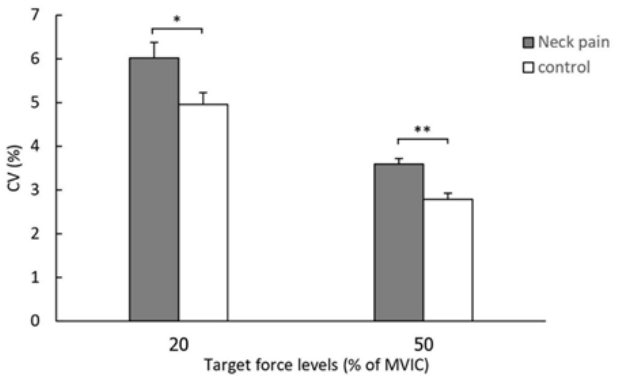

***Figure 2**. Means and standard deviations of 30 degrees of shoulder abduction force steadiness at 20% and 50% of maximal voluntary contraction (MVC) between the neck pain and control groups. (* p<0.05, ** p<0.001).*

Relationships between force steadiness and characteristics of neck pain

There were no correlations between the CV values at 20% and 50% of MVC and characteristics of neck pain (VAS, NDI-TH, DASH-TH, pain duration) (all p>0.05) (Table 2).

Table 2 Correlations between the CV values at 20% and 50% of MVC and characteristics of neck pain.

	CV at 20% MVC		CV at 50% MVC	
	r	*p* value	r	*p* value
Pain intensity (0-10 VAS, cm.)	-0.08	0.72	0.00	0.99
Pain duration (months)	0.29	0.15	0.03	0.90
NDI-TH (%)	0.04	0.83	0.04	0.84
DASH-TH (%)	0.00	1.00	-0.09	0.67

VAS: visual analogue scale, NDI-TH: neck disability index-Thai version, DASH-TH: the disabilities of the arm, shoulder, and hand questionnaire-Thai version.

Discussion

The results of this study demonstrated that patients with neck pain with scapular dyskinesis had reduced force steadiness at submaximal contractions (20% and 50% of MVC) of 30 degrees of shoulder abduction compared to asymptomatic controls. This may suggest that the steadiness of muscle contractions during shoulder abduction is associated with scapular dysfunction in neck pain. Additionally, it may suggest impaired shoulder/scapular sensory-motor control associated with neck pain.[29,30] To our knowledge, this is the first study investigating neuromuscular function (quantified as submaximal force steadiness) during shoulder abduction in patients with neck pain with scapular dyskinesis. Nonetheless, the results of this study are in accordance with previous findings of changes in sensory manifestations and motor performance during low load, repetitive work simulation in chronic neck-shoulder pain,[29] and deficits in upper limb coordination and position sense acuity in patients with neck pain.[30] Additionally, the results of this study are in line with findings of a previous study demonstrating reduced steadiness of shoulder abduction in patients with subacromial impingement syndrome (SIS).[3] It was noted that no difference in the MVC were observed between the neck pain and control groups, which contradicting the pain-adaptation model.[31] However, this result is consistent with a previous study demonstrating no difference in the MVC between patients with neck pain with scapular dysfunction and controls.[32] As neck pain intensity and disability in our participants with neck pain are mild to moderate, thus it is possible that ability to generate a maximum contraction of shoulder movement is less likely to be influenced by pain in the neck.

The axio-scapular muscles are required to stabilize and control the scapula during the submaximal contraction of shoulder abduction.[33,34] Previous studies found altered muscle recruitment patterns of the axio-scapular muscles in individuals with neck pain compared to asymptomatic controls.[32,35,36] A recent study has also shown relationships between chronic non-specific neck pain and delayed activation of the shoulder and axio-scapular muscles (i.e., anterior and middle deltoid, upper and lower trapezius).[37] Additionally, there is evidence suggesting impaired axio-scapular muscles in neck pain patients with altered scapular control/function.[32,36] Zakharova-Luneva *et al*.[32] found that neck pain patients with scapular dysfunction had changes in the lower trapezius activity during performing isometric contraction of shoulder abduction and external rotation compared with healthy controls. Likewise, Szeto *et al*.[36] demonstrated that office workers with work-related neck and upper limb disorders had altered muscle recruitment patterns of the axio-scapular muscles compared to asymptomatic controls. Thus, the reduced force steadiness may be associated with impaired scapular control and function, in particular 30 degrees of shoulder abduction, which requires neuromuscular control to stabilize the scapular and shoulder joint.[33,34] On the other hand, the reduced force steadiness in neck pain may be as a consequence of a disturbance in cervical afferent input from the neck joint and muscle receptors.[2,6,7] Impaired mechanoreceptors due to articular damage, chronic pain, and impaired muscle functions may lead to a decrease in

proprioceptive.[38] Additionally, there may be sensitization of mechanoreceptors or muscle spindles from pain and altered motor unit control strategy within painful muscles.[39] It was noted that some participants with neck pain in this study reported increased neck pain during performing the test, in particular at a higher level whereas none of asymptomatic controls reported pain. Submaximal isometric contraction of shoulder abduction may impart significant mechanical stress to the cervical structures through muscle attachments that extend into the cervical spine.[10,16]

There were no relationships between the force steadiness and any characteristics of neck pain (i.e., neck pain intensity, duration, and neck and upper limb disabilities). The results may imply that force steadiness of shoulder abduction is independent of neck pain features. A high variation of neck pain duration was observed in our participants with neck pain, but its relationship with force steadiness was not found. However, it was noted that variations of pain intensity and neck and upper limb disabilities were small. Thus, no relationships of these parameters and force steadiness may alternatively be due to such small variations in the sample characteristics. Regardless of population and methodology, a previous study of patients with hand osteoarthritis found a small positive correlation between grip force steadiness and the DASH score.[40] A study of patellofemoral pain also found a positive correlation between knee extensor force steadiness and self-reported pain (VAS) during the force-matching task.[41] Given no available evidence of relationships between force steadiness of shoulder abduction and characteristics of neck pain, conclusion on this matter cannot be drawn and further research is still needed to confirm the results.

There are some limitations of this study. Sample size was estimated from group comparisons of the CV values, but not for the relationships of force steadiness and characteristics of neck pain. More female participants were recruited into the study, although there was no difference in gender between the neck pain and control groups. Use of upper limb in daily activities may affect force steadiness, but it was not recorded in the study. Additionally, there were small variations in the characteristics of the sample, in particular pain intensity and disability. Further research with a larger sample size may help confirm the correlation results. Future research should investigate if reduced force steadiness is associated with any specific tasks of upper limb activities in neck pain patients with scapular dyskinesis. Activity of the axio-scapular muscles would also provide further information about the contribution of the muscle impairment and reduced force steadiness.

Conclusion

This study demonstrated reduced force steadiness at 20% and 50% of MVC of 30 degrees of shoulder abduction in patients with neck pain with scapular dyskinesis. The results of this study may be beneficial in developing rehabilitation of axio-scapular muscle controls for patients with neck pain with scapular dyskinesis. However, no relationships of force steadiness and neck pain characteristics were demonstrated.

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The study of postmortem blood gamma-hydroxybutyric acid (GHB) concentrations in Thai dead bodies unrelated to GHB use

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ABSTRACT

Background: Gamma-hydroxybutyric acid (GHB) is classified as a category I psychotropic substance in Thai legislation. It can be exogenously consumed or endogenously produced and cut-off concentrations are required. Baseline GHB concentration ranges in Thai postmortem cases are still not available.

Objectives: To determine baseline GHB concentrations and factors influencing GHB concentrations in Thai postmortem cases.

Materials and methods: Postmortem femoral and cardiac blood samples were collected from Thai cadavers aged 18 years old or older sent for medico-legal autopsies at the Department of Forensic Medicine, Siriraj Hospital, Mahidol University between 18th August 2021 and 25th January 2022 with postmortem interval (PMI) not greater than 24 hours. Case data including sex, age, PMI, sites of collection and causes of death were recorded. Blood GHB concentrations were extracted using protein precipitation and analyzed by gas chromatography-mass spectrometry (GC-MS). Descriptive statistics, paired samples Wilcoxon signed-rank test, Spearman's correlation, Mann-Whitney U test and Kruskal-Wallis H test were analyzed where appropriate.

Results: A total of 150 subjects were recruited; 63 (42%) were female with mean age 44.59 years old (range 18-75 years old). Femoral and cardiac GHB concentration ranges were <0.5-20.81 µg/mL and 1.12-39.04 µg/mL, respectively while median GHB concentrations in femoral and cardiac blood were 6.38 and 8.41 µg/mL. Femoral GHB concentrations were significantly lower than cardiac GHB concentrations (p<0.001). Both femoral and cardiac blood showed a positive correlation between GHB concentrations and PMI (Spearman's correlation =0.52 and 0.32, p<0.001). Most GHB concentrations in femoral blood were less than 10 µg/mL and almost all samples from both sources had GHB concentrations less than 30 µg/mL.

Conclusion: GHB concentrations in Thai postmortem cases were <0.5-20.81 µg/mL in femoral blood and 1.12-39.04 µg/mL in cardiac blood, respectively. GHB concentrations in femoral blood were significantly lower than in cardiac blood. GHB concentrations significantly increased with longer PMI.

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Introduction

Gamma-hydroxybutyric acid (GHB) is categorized as a central nervous system depressant and is currently known as a drug for recreational use and drug-facilitated sexual assault.[1] In Thailand, GHB is classified as a category I psychotropic substance in the Narcotics Act B.E.2564 (2021). In forensic casework, GHB concentrations both in living patients and dead bodies should be carefully interpreted because GHB can be generated endogenously in the human body.[1] Thus, cut-off GHB concentrations in blood and urine are required for differentiating exogenous consumption from endogenous production.[1] GHB is rapidly and extensively eliminated from the blood with terminal elimination half-life of around 30-60 minutes.[1,2] These two issues present critical analytical limitations and interpretations in medico-legal cases.

Several previous studies attempted to determine cut-off GHB concentrations both in living people and postmortem cases. Andresen H *et al*. suggested that endogenous GHB concentration range in serum from living people was 0.62-3.24 mg/L (mean=1.14 mg/L; median=0.97 mg/L) and 0.64-4.20 mg/L (mean=1.21 mg/L; median=0.96 mg/L) in urine.[3] Previous studies suggested that cut-off GHB concentrations in blood and urine from living patients should be 5 mg/L and 5-10 mg/L, respectively.[4,5] However, the determination of cut-off GHB concentrations in dead bodies is more complicated because types of specimens, sites of sample collection and postmortem intervals have significant impacts on GHB concentrations in postmortem specimens.[6-11] Kintz P *et al*. reported that most GHB concentrations in cardiac blood ranged from 10 mg/L to 40 mg/L while GHB concentrations in femoral blood were generally lower than those in cardiac blood.[6] However, Moriya F and Hashimoto Y reported that GHB concentrations in cardiac blood were not significantly different from those in femoral blood.[7] Korb AS and Cooper G presented that GHB concentrations in femoral blood ranged from 0 mg/L to 193 mg/L (mean and median concentrations were 28 mg/L and 24 mg/L) while 68.5% and 90.7% of GHB concentrations were ≤30 and 50 mg/L, respectively.[8] Ha HH *et al*. reported that GHB concentrations in heart blood ranged from 10.4 mg/L to 62.16 mg/L (median concentration was 22.45 mg/L).[11] Most published papers recommended 30-50 mg/L for cut-off concentrations of GHB in postmortem blood.[6,7,9,10] Busardo F and Jones A also suggested that 50 mg/L was suitable for postmortem blood from decomposed bodies or bodies with long postmortem intervals whereas 30 mg/L was proper for femoral blood with short postmortem intervals.[10] Elliott S *et al*. suggested that possible sources of increased GHB concentrations in postmortem cases could be attributed to microbial activities.[12] This finding was consistent with findings that GHB concentrations increased in bodies with long postmortem intervals that had begun to decay.

Previous studies mainly derived cut-off concentrations for GHB in postmortem cases from Caucasian populations. However, postmortem changes in Asian countries like Thailand differs from European countries and the USA due to tropical climatic conditions that lead to diverse alterations in dead bodies particularly those with long postmortem intervals. Thus, this study aims to investigate the baseline ranges of GHB concentrations in Thai postmortem cases that are not related to GHB abuse to determine baseline GHB concentrations. In addition, the effects of sample site collection and postmortem intervals are also studied to determine the variations of baseline GHB concentrations in the Thai population.

Materials and methods

Subject recruitment and data recording

This study included medico-legal cases sent to the Department of Forensic Medicine, Siriraj Hospital, Mahidol University, Thailand for autopsy between 18th August 2021 and 25th January 2022. The bodies were kept in the refrigerator before transportation to the mortuary for autopsy. Inclusion criteria were Thai citizens aged 18 years old or over with postmortem intervals from death to time of blood collection (PMI) not greater than 24 hours. All subjects did not have any histories of drug uses except for drugs used in underlying medical conditions, for example, diabetes mellitus and hypertension. These histories were obtained from police records and subjects' relatives performed during death investigation. If the subjects were declared dead in hospital, the length of hospitalization would be less than 3 hours because the maximal time for cardio-pulmonary resuscitation was generally less than 3 hours.[13] Exclusion criteria were subjects who died from severe sepsis or bacterial infection in specific organs and subjects who died from drug-related deaths.

Subject data including sex, age, PMI, cause of death and blood collection site were recorded for statistical analysis. PMI was also categorized into three groups as 0-8, 8-16 and 16-24 hours. Causes of death were divided into four groups as coronary artery disease and/or acute myocardial infarction (CAD & AMI), non-cardiac diseases (including cerebrovascular disease, viral pneumonia, liver cirrhosis, gastro-intestinal disease and acute pancreatitis), traffic accidents and asphyxia (hanging and drowning). The classification of the cause of death in this study was adapted from the previous study in the utilization of cardiac troponin-T for diagnosis of acute myocardial infarction (AMI) in postmortem cases.[14]

This study was approved by the Siriraj Institutional Review Board, Faculty of Medicine, Siriraj Hospital, Mahidol University (Certificate of Approval No. Si 088/2021, Research Project Number 1086/2563).

Chemicals and reagents

GHB and GHB-d_6 at a concentration of 1 mg/mL and 100 µg/mL were purchased from Sigma-Aldrich USA and supplied by U&V Holding (Thailand) Co., Ltd. Acetonitrile LC/MS grade was sourced from Duksan Pure Chemicals and also supplied by U&V Holding (Thailand) Co., Ltd. N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% of trimethylchlorosilane (TMCS) was obtained from Sigma-Aldrich USA and supplied by S.M. Chemical Supplies (Thailand) Co., Ltd.

Instrumentation

Gas chromatography-mass spectrometry (GC-MS) was performed with a Shimadzu GC-2010 coupled with a Shimadzu GCMS QP-2010 single quadrupole MS. The GC capillary

column was a DB-5MS (30 m x 0.25 mm ID coated with a 0.25 µm film). The GC conditions were carried out by programmed temperature. Initial temperature was 60 °C and held for 2 minutes. Then, the temperature was increased to 180°C at a rate of 20°C/minute. Finally, the temperature was increased to 250°C at a rate of 30°C/minute and held for 5 minutes. Helium gas was used as the carrier gas with a flow rate of 1 mL/minute. The injection port and transfer line temperature was set as 250 °C. The mass analyzer was performed in electron impact mode at 70 eV. The selected ion monitoring (SIM) mode was performed and the ions monitored were m/z 233, 234, and 204 for GHB and m/z 239, 240, and 206 for GHB-d_6. The underlined ions were applied for quantitation.

Sample collection and extraction

Blood samples were collected from both the femoral vein and the left side of the heart during the autopsy. Blood samples from the left side of the heart were drawn from the left atrium. Then, 3 mL of blood from each site was placed into a sodium fluoride (NaF) tube. All blood tubes were transferred for storage at 4°C and analyzed for GHB on the next day using GC-MS. This study only concentrated on the determination of blood GHB concentration because the baseline GHB concentrations should assist the diagnosis in sudden death conditions that were common situations in forensic setting. Thus, determination of GHB in urine was not included in this study.

Sample preparation was carried out using protein precipitation. 10 µL of GHB-d_6 (50 µg/mL) and 500 µL of acetonitrile were added to 250 µL of NaF blood sample. Then, the sample was vortexed and centrifuged at 3000 rpm for 15 minutes. The supernatant (300 µL) was transferred to a vial and evaporated under nitrogen stream at 40 °C. After the sample was completely evaporated, 75 µL BSTFA+1%TMCS was added for chemical derivatization and the sample was heated at 90 °C for 10 minutes. After derivatization, the sample was transferred into a GC vial for GC-MS analysis. All analyses were performed in duplicate with mean values calculated as the final results.

Method validation

Method validation was performed following US Food and Drug Administration (FDA) guidelines.[15] Method validation protocol was performed using expired whole blood from the Department of Transfusion Medicine, Siriraj Hospital, Mahidol University. Linearity was evaluated using six GHB calibrators at 1, 2, 5, 10, 20 and 50 µg/mL prepared as five replicates and run on five separate days. Calibration curves were generated using Shimadzu GCMS Solution Software® Version 2.50. Curve weighting factors were adjusted to obtain the best linear regression fit that achieved r^2 ≥0.99 and accuracy of each calibrator within ±15%.

Limit of detection (LOD) and lower limit of quantitation (LLOQ) were tested by gradually spiking decreasing concentrations of GHB into expired whole blood. LOD and LLOQ were considered at the lowest concentration which produced a signal-to-noise ratio (S/N) greater than 3 times and 10 times, respectively. Selectivity and interference studies were performed to achieve complete separation between GHB and beta-hydroxybutyric acid (BHB) which was a GHB analogue.

Accuracy and precision were assessed by injection of five replicates of blank expired whole blood spiked with low, medium and high concentrations of quality control (QC) samples (3, 12 and 40 µg/mL) on five separate days. Acceptance criteria included accuracy for each QC within ±15% and intra-day and inter-day precision for each QC ≤15% coefficient of variation (%CV). Carryover was determined by injection of the extracted blank blood samples after extracted spiked blood samples with GHB at 100 and 200 µg/mL and the GHB peak at specific retention time was observed.

Statistical analysis

Statistical analyses were carried out using IBM SPSS® Statistics for Windows version 21. Descriptive statistics including mean, median and standard deviation (SD) were analyzed. The Kolmogorov-Smirnov test and Levene's test for equality of variance were applied for continuous variables. Because GHB data from femoral and heart blood were not normally distributed, non-parametric analyses including the paired samples Wilcoxon signed-rank test, Spearman's correlation coefficient, Mann-Whitney U test and Kruskal-Wallis H test were analyzed where appropriate. The statistical significance was set at $p<0.05$.

Results

Method validation results

Linearity was evaluated for GHB over 1 to 50 µg/mL using back-calculated calibrator concentrations with a suitable weighting factor selected for the linear regression curve based on method accuracy. Curve weighting at 1/x produced acceptable accuracy for all calibrators with r^2 ≥0.99. Thus, linear regression with weighting 1/x was selected as the best fit model for acceptable linearity and calibrator accuracy for this method. Calibration curve parameters were shown in **Table 1**.

Table 1 Calibration curve parameters for GHB analysis.

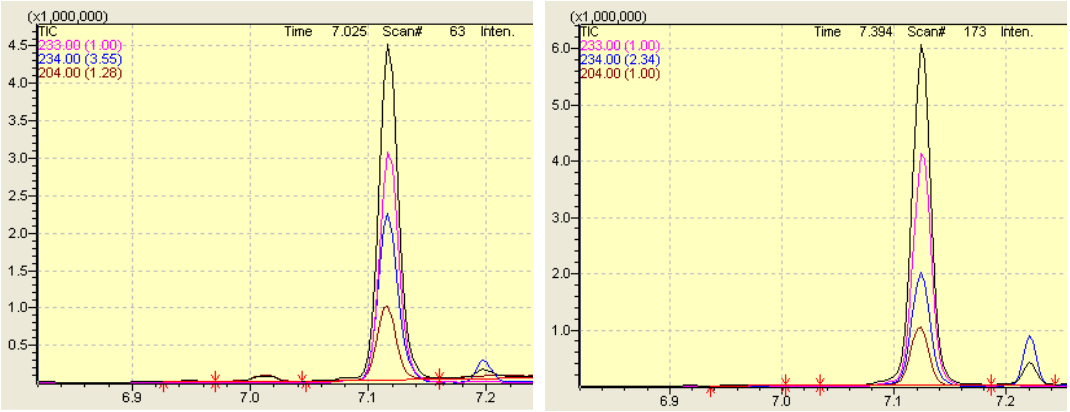
Calibration range (µg/mL)	Linear regression equation		r^2 (n=5)	Weighting factor
	Slope	Intercept		
1-50	0.634669	-0.008669	≥0.992	1/x

LOD and LLOQ were determined as 0.5 µg/mL and 1 µg/mL, respectively. Results of GHB accuracy and intra-day and inter-day precision were shown in **Table 2**. All values passed the acceptance criteria for accuracy and precision.

No interference peak was observed at the retention time of interest (approximately 7.12 minutes). GHB and GHB-d_6 did not interfere with BHB which was the isomer of GHB because BHB was eluted at 6.54 minutes and completely separated from GHB even though they produced similar m/z. No carryover was observed in blank whole blood samples following injections of spiked GHB samples at concentrations of 100 µg/mL and 200 µg/mL. This method was applied to blood samples from Thai postmortem cases to analyze blood GHB concentrations. Selected chromatograms from a Thai cadaver were shown at **Figure 1**.

Table 2 Accuracy and precision of three QC concentrations of GHB.

QC concentrations (µg/mL)	Accuracy (%) (n=5)	Precision (n=5)	
		Intra-day (%)	Inter-day (%)
3	88.67-108.33	≤4.97	≤8.27
12	89.83-102.67	≤5.15	≤6.29
40	93.42-111.08	≤6.62	≤7.57

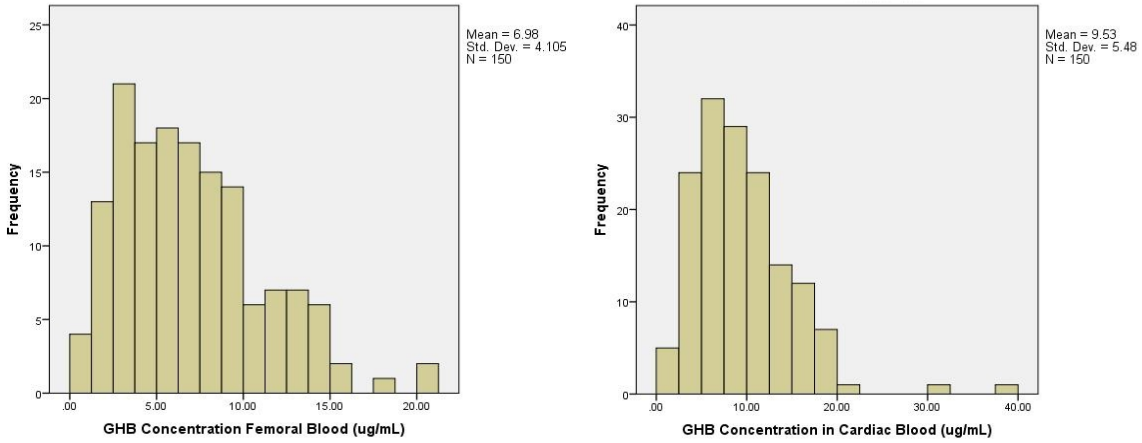


(A) (B)

Figure 1. *Selected chromatograms in GHB windows for SIM mode from a Thai cadaver containing (A) femoral GHB concentration of 7.58 µg/mL and (B) cardiac GHB concentration of 9.05 µg/mL.*

Results of GHB analyses in Thai postmortem cases

A total of 150 subjects were recruited in this study as 63 females (42%) and 87 males (58%). The sample size was obtained from sample size calculation based on the study of Kintz P *et al*. using nQuery Advisor program version 6.0.[6] Mean age was 44.59 years old (range 18-75 years old). GHB concentrations in femoral blood ranged from <0.5 to 20.81 µg/mL (mean±SD=6.98±4.11 µg/mL, median=6.38 µg/mL). GHB concentrations in cardiac blood ranged from 1.12 µg/mL to 39.04 µg/mL (mean±SD=9.53±5.48 µg/mL, median=8.41 µg/mL). Data distributions of GHB from both femoral and cardiac blood were not normally distributed, as shown by the histograms in **Figure 2**.



(A) (B)

Figure 2. *Histograms showing GHB concentrations in femoral blood (A) and cardiac blood (B).*

All blood samples were analyzed by the paired samples Wilcoxon signed-rank test. GHB concentrations in femoral blood were significantly lower than those in cardiac blood (p<0.001). Female and male subjects were analyzed separately. Results showed that GHB concentrations in femoral blood were significantly lower than those in cardiac blood both in female (p=0.004) and male (p<0.001) subjects, as shown in **Figure 3**. Female subjects also had significantly higher GHB concentrations in femoral blood than male subjects (p=0.026). However, this comparison was not significantly different when the analysis was performed in cardiac blood (p=0.965). When age was analyzed against GHB concentrations, no significant correlation was found between age and GHB concentrations both in femoral and cardiac blood (p=0.476 for femoral blood and p=0.941 for cardiac blood).

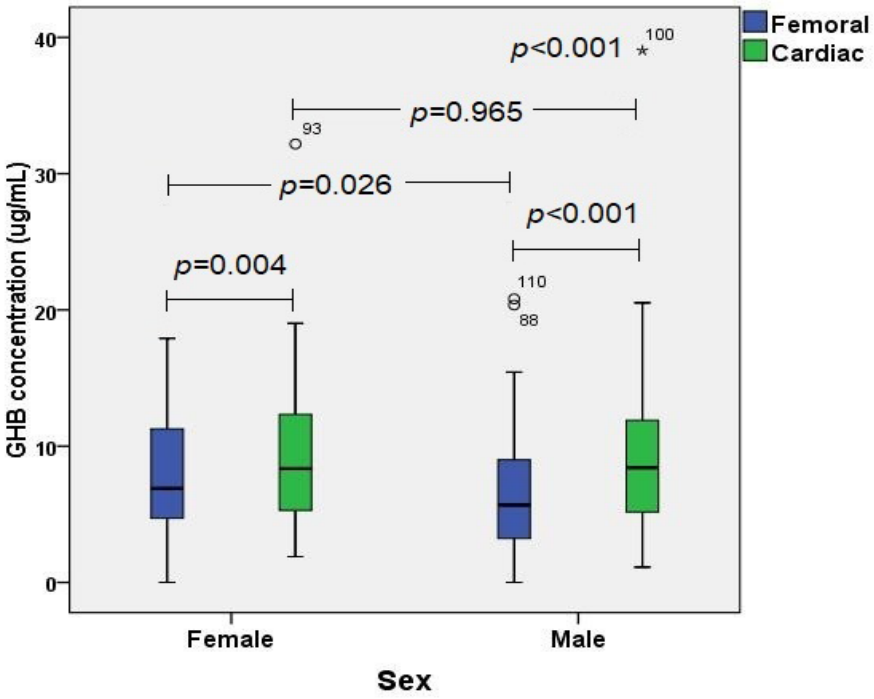
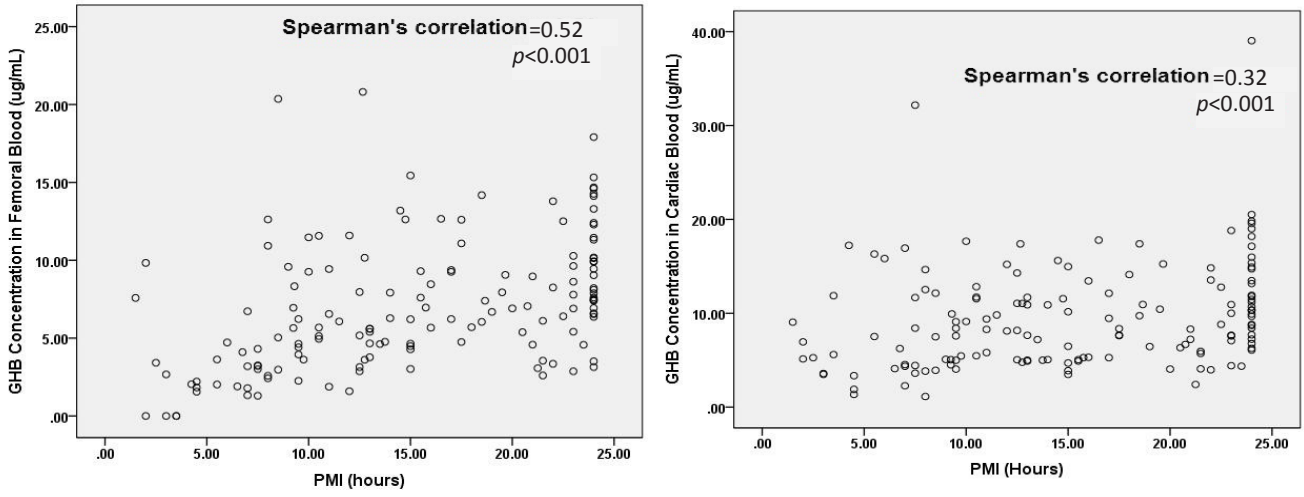


Figure 3. *Comparison of GHB concentrations in femoral blood and cardiac blood between female and male subjects.*

Correlations between PMI and GHB concentrations both in femoral and cardiac blood showed statistical significance (p<0.001), as shown in **Figure 4**. Positive correlations were shown between GHB concentrations from both sources and PMI while femoral GHB concentrations were more associated with PMI than cardiac GHB concentrations.



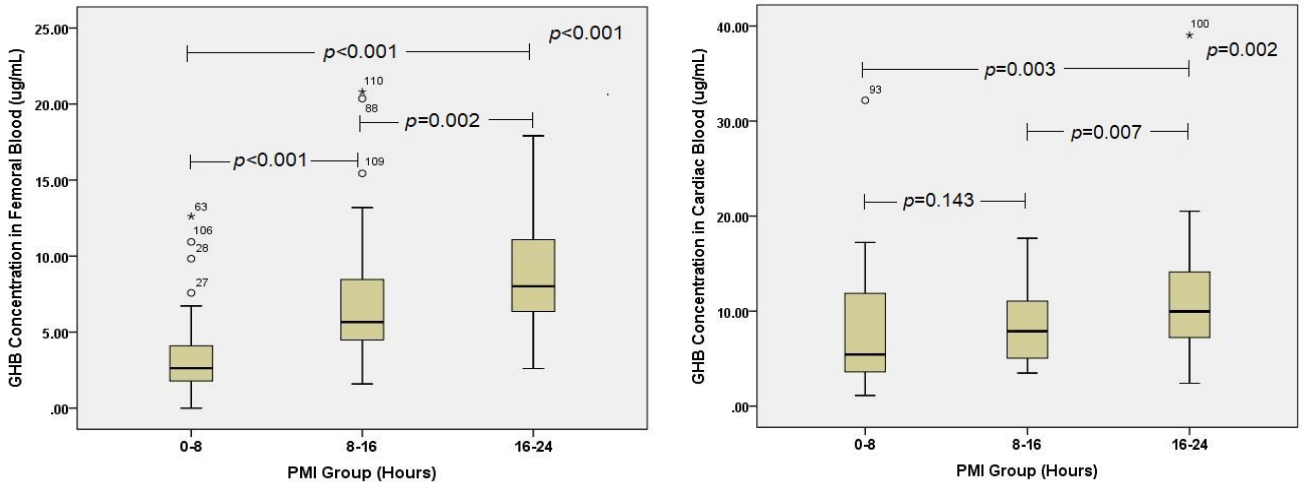
(A) (B)

Figure 4. *Correlations between PMI and GHB concentrations in femoral blood (A) and cardiac blood (B).*

When PMI was divided into the three groups, GHB concentrations in each group were elevated following increased PMI, especially in femoral blood as demonstrated in **Table 3** and **Figure 5**. GHB concentrations of 93.33% and 83.33% in femoral blood from PMI groups 0-8 hours and 8-16 hours were less than 10 µg/mL whereas only 70% and 68.52% of GHB concentrations in cardiac blood from PMI groups 0-8 hours and 8-16 hours were less than 10 µg/mL. GHB concentrations in two samples (1.33%) from cardiac blood were over 30 µg/mL while no femoral blood sample gave GHB concentration above 30 µg/mL. These findings showed that femoral GHB concentrations were mainly less than 10 µg/mL, particularly when PMI was not greater than 16 hours while most GHB concentrations from both sources were less than 30 µg/mL.

Table 3 GHB concentrations in femoral and cardiac blood for the three PMI groups.

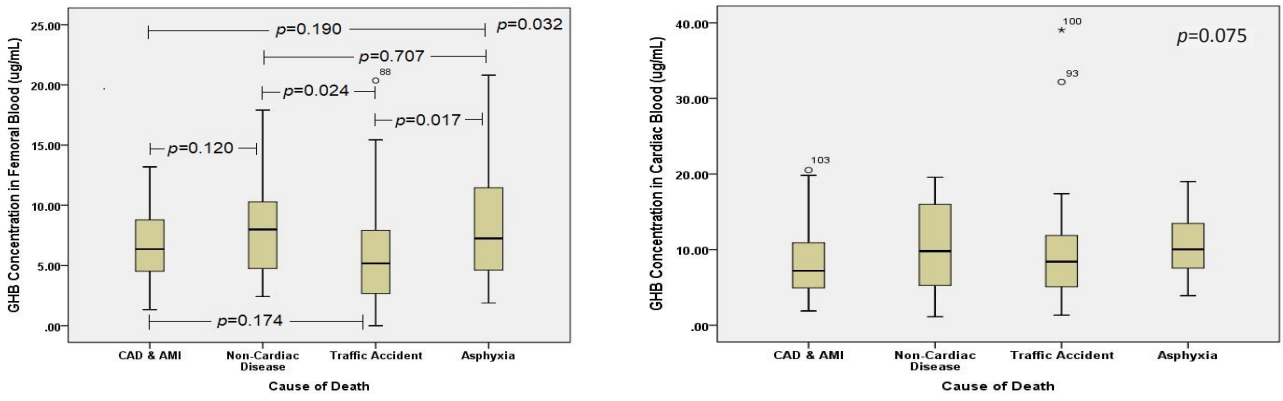
PMI (hours)	N	GHB concentrations (µg/mL) (mean±SD, range)	
		Femoral blood	Cardiac blood
0-8	30	3.47±3.15 (<0.5-12.62)	8.18±6.73 (1.12-32.18)
8-16	54	6.91±4.06 (1.59-20.81)	8.47±3.81 (3.48-17.67)
16-24	66	8.62±3.52 (2.60-17.91)	11.01±5.73 (2.41-39.04)
Total	150	6.98±4.11 (<0.5-20.81)	9.53±5.48 (1.12-39.04)



(A) (B)

Figure 5. *Comparison of GHB concentrations between the three PMI groups for femoral blood (A) and cardiac blood (B).*

GHB concentrations were analyzed against causes of death in the four groups. Significant differences in GHB concentrations were only found in femoral blood among these four groups (p=0.032), as shown in **Figure 6**. Traffic accidents as the cause of death had significantly lower GHB concentrations, particularly when compared with non-cardiac diseases and asphyxia.



(A) (B)

Figure 6. *Comparison of GHB concentrations among the four groups of causes of death in femoral blood (A) and cardiac blood (B).*

When blood GHB concentrations were compared between death at scene group and death in hospital group, it was found that death in hospital group had significantly lower blood GHB concentrations compared with death at scene group both in femoral and cardiac blood as shown in **Table 4**.

Table 4 The comparison of blood GHB concentrations between death at scene group and death in hospital group.

Blood type	Group	N	Mean±SD (range) (µg/mL)	*p* value
Femoral blood	Death at scene	102	7.65±4.33 (<0.5-20.81)	0.005
	Death in hospital	48	5.55±3.19 (<0.5-12.51)	
Cardiac blood	Death at scene	102	9.98±5.35 (1.34-39.04)	0.04
	Death in hospital	48	8.56±5.68 (1.12-32.18)	

Discussion

GHB concentrations from femoral blood and cardiac blood in Thai postmortem cases were <0.5-20.81 µg/mL and 1.12-39.04 µg/mL, respectively. These concentration ranges were different from postmortem femoral blood GHB concentrations in GHB-related death cases and GHB-exposure cases in the previous review that indicated as 30-9200 ug/mL (median 280 ug/mL) and 30-210 ug/mL (median 72 ug/mL), respectively.[10] The GHB concentrations in femoral blood were significantly lower than those in cardiac blood (median GHB concentrations in femoral blood and cardiac blood were 6.38 µg/mL and 8.41 µg/mL). It was hypothesized that the effect of bacterial contamination in the central blood compartment played an important role in this finding. The central blood compartment, like cardiac blood, was prone to bacterial translocation after death because of the proximity of the heart to the small and large intestine.[16] A previous study suggested that some bacterial strains like *Pseudomonas spp*. could be potential sources of GHB production as a possible explanation for lower GHB concentrations in femoral blood compared to cardiac blood.[12] Our findings were consistent with several previous studies and supported that cut-off concentrations in femoral and cardiac blood in Thai postmortem cases should be different.[6,9,10] According to cut-off GHB concentrations proposed in review articles, 30 µg/mL and 50 µg/mL were suitable for Thai postmortem cases.[9,10] However, further studies should be conducted to determine differences in positive and normal GHB concentrations in Thai postmortem cases to indicate the proper cut-off concentrations.

GHB concentrations increased following increased PMI in both femoral and cardiac blood. This finding could be explained by two hypotheses. Firstly, cessation of Kreb's cycle in dead bodies led to the accumulation of succinic semialdehyde which transformed to GHB.[17] Secondly, Elliott S *et al*. proposed that some bacterial strains like *Pseudomonas spp*. potentially produced GHB although the amount of GHB produced was relatively lower than GHB concentrations in postmortem cases.[12] Although the body storage in the refrigerator could theoretically decelerate the bacterial overgrowth, the previous study showed that GHB concentrations in femoral blood collected from dead bodies stored at 4 °C were still significantly higher than those collected from dead bodies at the initial time of body examination at the crime scene.[18] In addition, GHB concentrations in postmortem blood samples kept at 4 °C could even increase with some extent after storage.[19] These findings implied that GHB concentrations could increase even under the storage in the refrigerator. Bacterial translocation into bloodstream occurred when PMI increased and some bacterial strains might be responsible for the increase of GHB concentrations following increased PMI.[16]

Gender also had an impact on GHB concentrations in Thai postmortem cases. However, this effect was only significantly in femoral blood. Cardiac blood showed no significant difference between Thai male and female subjects. This finding contrasted with previous studies demonstrating no significant difference in GHB concentrations between males and females.[9,10] This impact of gender should be further studied on GHB concentrations in Thai postmortem cases.

Interestingly, our results indicated that cause of death as traffic accidents produced significantly lower GHB concentrations in femoral blood compared with non-cardiac diseases and asphyxia whereas this finding was not observed in cardiac blood. No previous publications have recorded the influence of cause of death on GHB concentrations. One issue relevant to this finding was the impact of hospitalization on GHB concentrations. This study recruited subjects with known PMI and the interval of hospitalization not greater than 3 hours; however, fluid administration within these 3 hours might affect GHB concentrations, especially fluid resuscitation in trauma patients. In addition, this study showed that blood GHB concentrations from death in hospital group were significantly lower than those from death at scene group and this finding might support the effect of management during hospitalization. However, further study should be conducted to confirm this result in Thai postmortem cases.

This study had some limitations. Firstly, sample size was relatively small and the number of female subjects was less than males. Therefore, our findings concerning significantly different GHB concentrations between female and male Thai subjects should be interpreted with caution. Secondly, fewer subjects had PMI 0-8 hours compared with the other two groups of PMI, possibly affecting statistical analyses and variations of GHB concentrations, particularly in the early PMI period. Furthermore, PMI in this study included the time that cadavers spent at ambient temperature before placement in the refrigerator. These two time intervals

were not equal in every case. Thus, GHB concentrations in this study related to the summation of GHB in ambient temperature and under refrigeration. These two conditions had different effects on GHB concentrations. Further studies should be conducted to determine the effect of different body temperatures and different time intervals before refrigeration on blood GHB concentrations. Lastly, causes of death as non-cardiac diseases and traffic accidents were collectively analyzed and each cause of death might have different mechanisms of death, for example, exsanguination and head injury. Thus, each cause of death should be analyzed separately, especially for traumatic cases to verify the effect of cause of death on GHB concentrations

Conclusion

GHB concentrations in Thai postmortem cases were <0.5-20.81 µg/mL (median=6.38 µg/mL) in femoral blood and 1.12-39.04 µg/mL (median=8.41 µg/mL) in cardiac blood, respectively. GHB concentrations in femoral blood were significantly lower than those in cardiac blood. GHB concentrations increased following increased PMI and this correlation was more significantly observed in femoral blood. GHB concentrations in femoral blood were generally less than 10 µg/mL, with PMI not greater than 16 hours while most GHB concentrations from both blood sources were less than 30 µg/mL within 24 hours

Conflict of interest

The authors declare no conflict of interest.

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A survey on functional disabilities and perceived need for allied health and complementary therapies for Thai individuals with Parkinson's disease

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ABSTRACT

Background: Evidence regarding functional disability and the need for alternative health care services to improve abilities among Thai individuals with Parkinson's disease (PD) is limited.

Objectives: The study aims to survey PD patients on their functional difficulties and determined the correlation between personal- and disease-related factors on the difficulties they experience. Additionally, we asked about the need for, utilization of, and satisfaction with allied health care (AHC) and alternative complementary therapy (ACT) to minimize these difficulties in PD.

Materials and methods: Researchers conducted one-on-one interviews in a movement disorder clinic in a Thai university hospital. The survey consisted of interviews for personal and disease data at first, followed by the self-rating of functional disabilities and the need for and experience of AHC and ACT.

Results: Patients with severe disease had regressive disability in some activities. There were 4 factors related to the level of functional disability: stage of severity, duration of disease, cognitive performance, and depression score. Half of the participants needed treatment via AHC and ACT. Of the patients, 37%, 50% and 38.9% had experience with physical therapy, massage, and acupuncture, respectively. Almost all of them were satisfied with massage and physical therapy, while a small number of patients were satisfied with acupuncture.

Conclusion: These results indicate that functional disability worsens as the disease progresses. A small number of patients have experience with AHC and ACT; however, they need alternative treatment. Further research regarding related factors for utilizing AHC and ACT in Thai PD patients' needs to be conducted.

Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative diseases worldwide. A recent study reported an increasing prevalence of PD from 290,000 to 550,000 cases, or an 89.9% increase, across the US from 1990 to 2017.[1] Moreover, a survey in Thailand reported that the age-standardized prevalence of PD was 706 per 100,000 people.[2] Both motor and nonmotor symptoms of PD result in functional disabilities involving walking, lifting, dressing, fine hand use, personal care, driving, using transportation, doing housework and eating.[3, 4] More problems with activities of daily living (ADLs) and later disease stage were related to poor quality of life.[5] When the disease progresses, patients become more dependent on others. Moreover, the disease stage also impacted the patients'

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concerns. A survey study in 2020 showed that late-stage PD patients were more concerned about their motor symptoms, while the opposite was true for early-stage PD patients.[6] Another factor that impacts patients' concerns is disease onset.[6] Cognitive decline, anxiety and depression were the common symptoms of PD patients,[7,8] which can deteriorate their quality of life. Additionally, nonmotor symptoms were related to disease progression in PD.[9] The evidence for the impact of these non-motor symptoms on functional disabilities in PD patients is still limited. In addition to disease-related factors, personal factors such as age and sex were investigated to determine whether they influenced the daily living of PD patients.[10] However, a small numbers of evidences demonstrate an association between personal and diseased-personal factors, and functional disabilities of Thai PD patients.

Although individuals with PD usually receive pharmacological and surgical treatments, their functional disabilities are still present.[3] Non-pharmacological approaches have been effective for minimizing both motor and nonmotor symptoms in all stages of disease, including early PD.[11-14] A multidisciplinary approach or allied health care (AHC) team consists of physical therapists, occupational therapists, and speech and language therapists.[4,11,13-15] Physical therapy and occupational therapy are delivered in the community setting and can improve performance in daily activities.[16] Moreover, the use of alternative complementary therapies (ACTs) is common among PD patients, such as tai chi, qigong, massage, acupuncture and nutritional supplements.[15] The utilization of AHC and ACT in PD varies in different countries. For example, approximately 60% of patients were treated by physical therapists in the Netherlands,[17] 7.4-76.6% of patients in South Korea had used ACT,[18] and in the US and United Kingdom, 4-57% were treated by physical therapy, occupational therapy, speech therapy, massage, vitamins, herbs and acupuncture.[19-21]

Patients in need of multidisciplinary health care may reflect their attitude toward health professions. A focus group interviewing revealed a negative image of psychosocial therapy and low level of the need of therapy.[22] In addition, patient satisfaction can reflect the success of treatment. PD patients rated their satisfaction as higher when their problems improved after a physical therapy program than did those whose problems persisted.[17] Thus, patient's need and satisfaction may be consistent with the amount of AHC and ACT utilizing. However, the needs and, understanding role of, utilization and satisfaction with AHC and ACT are still ambiguity.

The present study aimed to survey Thai individuals with PD in terms of self-perceived functional disability in each stage of the disease and determined the correlation to related personal factors. An additional purpose was to determine these patients' needs for, utilization of and satisfaction with AHC and ACT for improving physical functioning. This study will reveal the daily activities restriction in Parkinson's patients and the factors contributing disabilities. The study outcome can be role for multidisciplinary professionals to design a paradigm of health service and patient education. Furthermore, it may emphasis patients for better understanding roles ACH and ACT and making an appropriate choice. On the same hand, these results would be beneficial to improve heath strategy proactively and effective future health policy.

Materials and methods

Study design

This study had a cross-sectional observational design with a questionnaire survey. The participants were enrolled consecutively from March to June 2020. The Central Institutional Review Board in Mahidol University (MU-CIRB, COA No. Si 124/2017) approved the study.

Participants

All participants were recruited from Siriraj Hospital, Bangkok, Thailand. Eligible criteria included a diagnosis of idiopathic Parkinson's disease with modified Hoehn and Yahr stage (H&Y) I-IV by a neurologist.[23] Participants needed to be able to communicate and were "ON" state of regular medications during participate in the study. Following medical records and cognitive assessment using Mini-Mental State Examination Thai version (MMSE) score and Thai Hospital Anxiety and Depression (Thai-HADS) Scale, patients were excluded if they had dementia, psychological and other neurological problems, drug and alcohol abuse, brain surgery or deep brain stimulation (DBS) treatment, or pain from musculoskeletal problems (visual analog scale result at least 6 to 10) 1 week before the interview. This process spent approximately 30 minutes.

Procedures

Outpatients who received information about the study and were interested in participating in the study could contact the researchers by phone call. After the researcher informed them about the details of the study, those who agreed to participate made an appointment to be individually screened and to provide information. Then, eligible participants who passed the screening process were allowed to sign informed consent and participate in the interview.

After informed consent was obtained, all participants were interviewed in 3 parts. Part 1 concerned personal and disease-related information. Part 2 asked about the level of difficulty in their functional disabilities, while Part 3 pertained to the need for and their experience and satisfaction with AHC and ACT. All these steps were performed individually by a trained assessor, who is a lecturer in the Faculty of Physical Therapy and had the experience of more than 12 years in physical therapy rehabilitation in PD patients.

Part 1: Personal and disease-related information interview

Demographic data consisted of sex, age, disease's duration, and living area were interviewed. Modified H&Y scale, MMSE scores and Thai-HADS score in anxiety including depression domains obtained from the initial screening were also collected. The interviewing in this part spent approximately 10 minutes.

Part 2: Functional disabilities interview

We surveyed individuals on their functional disabilities in daily life by using self-evaluation questionnaires adapted from Raggi A. *et al*., 2011.[3] The checklist items were selected from the World Health Organization's International Classification of Functioning, Disability, and Health (ICF) categories in the part about PD problems in activities and participation.[24] The participants were asked to rate the severity of difficulties that disrupted their daily activity over the last 30 days with qualifiers on a 0-4 scale, where 0 refers to no difficulty and scores of 1-4 indicate problems. A score of 1 indicated mild difficulty (presenting problems 1-25% of the time or rarely disrupting their daily activity), a score of 2 indicated moderate difficulty (presenting problems approximately 26–50% of the time), a score of 3 indicated severe difficulty (presenting problems approximately 51-75% of the time), and a score of 4 indicated extreme difficulty or complete inability (presenting problems >75% of the time or totally interfering with their daily activity). The interviewing time for this part was approximately 10 minutes.

Part 3: Allied health care experience interview

To survey participants about their experience and satisfaction with AHC and ACT, information on experiences with physical therapists, occupational therapists, speech therapists, acupuncturists, massage therapists, and nutritional supplement specialists was collected. The questionnaire collected information on experiences with each occupation, treatment, and satisfaction level. Satisfaction was rated as "not at all satisfied", "partly satisfied", "satisfied", and "more than satisfied" or "very satisfied".

Moreover, we asked about their attitudes and the likelihood of receiving AHC and ACT treatment for functional disabilities in the future. The rating scale assessed positive attitude as follows: "much more than helpful", "more than helpful", "as expected", "less helpful" or "not helpful." The interviewing in this part took about 5-15 minutes depending on the individual patient experience.

Sample size calculation

The primary objective of this study was to investigate the percentage of patients with each daily activity limitation. Therefore, we employed formula for cross-sectional study which is $n=[(Z_{\alpha/2})^2*p*(1-p)]/d^2$. Where n is the sample size, Z is the statistic corresponding to level of confidence, p is expected prevalence (obtained from same studies or a pilot study), and d is a precision (corresponding to effect size). The study of Nijkrake and colleagues in 2009[4] reported percentage of patients who have limitation in the performance of daily activities as about 19.9% (eating) to 54.6 % (arm/hand activities). Then, *p* were 0.199 to 0.546. The precision (d) level was set at 0.10. Therefore, the sample size was ranged between 61 and 119.

Outcome measures

Functional disability levels and the percentage of individuals with PD who had experience with AHC and ACT treatment at different H&Y severity stages were reported. Moreover, the correlation coefficient value between each personal- (age and gender) and disease-related factor (disease duration, Hoehn and Yahr staging, cognitive level assessed by the Mini-Mental State Examination Thai version, anxiety and depression assessed by the Thai Hospital Anxiety and Depression Scale) for the participants and the level of each functional disability was also analyzed.

Data analysis

Statistical analyses were performed using IBM SPSS statistics version 24 (IBM Corporation, Armonk, NY). The demographic data consisted of sex, age, disease duration, modified H&Y scale, MMSE score, Thai-HADS score, and living area. Regarding functional disabilities, rating scores from participants were divided into 3 subcategories based on the severity of PD: mild (H&Y 1-1.5), moderate (H&Y 2-3) and severe (H&Y 4). Descriptive statistics were used for demographic and functional disability data. All variables are measured as the mean and standard deviation (SD) or percentage. Percentage from participants who perceived functional limitations and participation problems at levels of difficulty ranging from 1 to 4 are demonstrated for each severity stage. The Kolmogorov–Smirnov goodness-of-fit test was used to evaluate the distribution of the data. Pearson's or Spearman's correlation was used to assess the correlation coefficient between the level of difficulty of each functional disability and the characteristics of participants, including age, sex, diagnosis duration, modified H&Y score, MMSE score and Thai-HADS score. The AHC and ACT data experience and number of patients with moderate to high levels of satisfaction with treatment of AHC and ACT were presented as percentages. The percentage of treatment satisfaction was calculated from a proportion between amount PD patients with moderate to high levels of satisfaction and the number of subjects who received treatments from AHC and ACT. The significance level was set to $p<0.05$.

Results

Characteristics of Participants.

A total of 135 PD patients were enrolled in this study (Figure 1). Sixty-two patients were excluded from reviewing medical records because they were diagnosed with other forms of parkinsonism rather than idiopathic PD, such as multiple system atrophy and progressive supranuclear palsy. Ten patients had a history of surgical DBS treatments. Nine patients had cognitive problems, major depression, or other neurological comorbid diseases. Fifty-four patients who had a diagnosis of idiopathic PD were recruited. The demographic data of the participants are shown in Table 1. Their age range was 39 to 87 years, and the onset duration ranged from 0.5 to 24 years from first diagnosis. None of the participants had a history of dementia or depression. In addition, 13% of patients had H&Y stage 1-1.5, 81.5% had H&Y stage 2-3, and 5.6% had H&Y stage 4. Regarding living areas, 75.9% of patients lived in the Bangkok Metropolitan Region, while 24.1% of patients lived in a rural area.

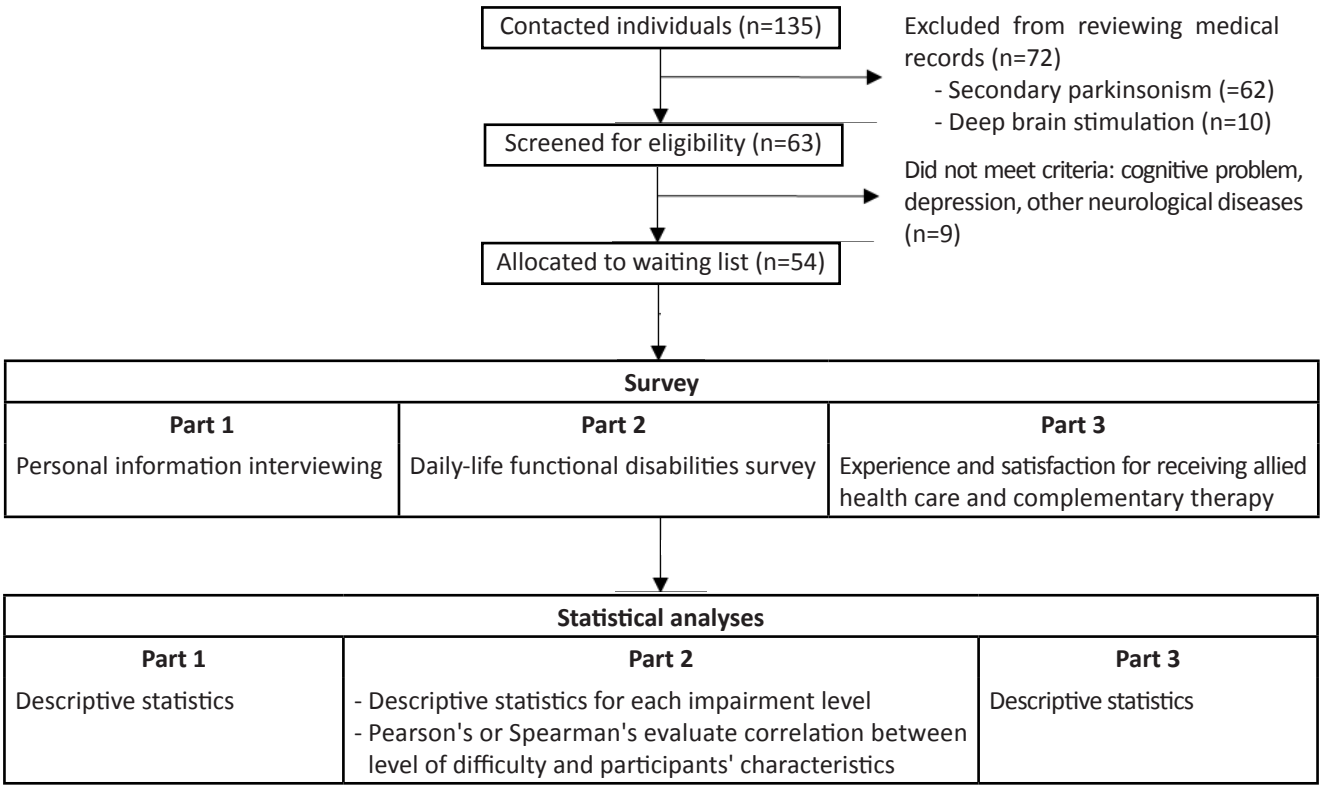

Figure 1. *Flowchart of the experimental design, recruitment process, and survey process.*

Table 1 Participant characteristics.

Characteristics	Value (n=54)	
	Mean (SD)	Range
1. Age (years)	63.96 (9.65)	39-87
2. Gender (male: female)	30 (55.6): 24 (44.4)	
3. Disease duration (years)	6.69 (5.07)	0.5-24
4. MMSE (scores)	25.91 (2.85)	14-30
5. Anxiety score (Thai-HADS)	4.76 (3.30)	0-11
6. Depression score (Thai-HADS)	5.19 (3.34)	1-13
7. Hoehn and Yahr stage		
7.1 Mild stage (1-1.5)	7 (12.96)	
7.2 Moderate stage (2-3)	44 (81.48)	
7.3 Severe stage (4-5)	3 (5.56)	
8. Location (Bangkok Metropolitan Region: rural area)	41 (75.9): 13 (24.1)	

Note*; Gender, Hoehn and Yahr stage and Location are represented in number (percentage). MMSE, Mini-Mental State Examination, Thai version HADS, Hospital Anxiety and Depression Scale.*

Functional disability among different severity stages

The findings reveal approximately 40% of patients in mild H&Y stage had problems in transfer activity and social participation (Figure 2). More than a half of participants those who classified as moderate H&Y stage had additional problems, including, walking, doing housework and balancing. Meanwhile, a severe group of participants was similar problems to mild and moderate stages, but this group faced higher in activities restriction. Moreover, there were other difficulties, such as dressing, fine hands using and washing, whereas public transportation use and writing which had not been performed during a month before.

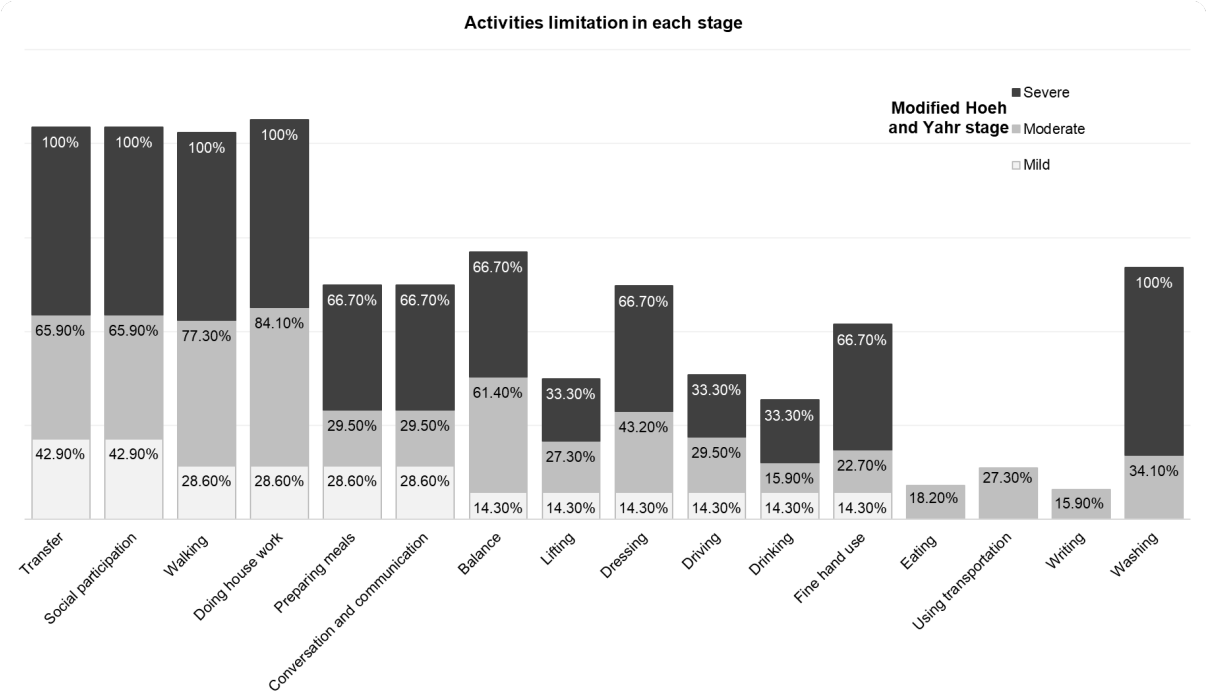

Figure 2. *Percentage of participants who perceived functional limitations and participation problems at levels of difficulty ranging from 1 to 4. Dark gray, gray and light gray bars represent the percentage of participants with Hoehn and Yahr stages 1–1.5, 2–3 and 4, respectively.*

Related factors for each functional disability

The data demonstrated a non-normal distribution. Therefore, nonparametric statistics were used to analyze the correlations and to make comparisons. Further analysis aimed to investigate the relationship between personal factors of patients and the level of their functional limitations. The correlation coefficient is shown in **Table 2**. The findings indicate that the modified H&Y stage has a small but significant positive correlation (r=0.308-0.350, *p*=0.009-0.024) with level of difficulty while walking, doing housework, preparing meals, and dressing, whereas it has a significant and moderate positive correlation with difficulty level (r=0.434-0.458, *p*=0.000-0.001) during balance, lifting and transferring activities. The disease onset duration had a significantly small but positive correlation with the level of difficulty while performing 9 activities (r=0.279-0.360, *p*=0.008-0.038): transfer, social participation, lifting, dressing, eating, drinking, fine hand use, driving and conversation. The depression score based on the Thai-HADS also had a significant small to moderate and positive correlation with the level of difficulty (r=0.269-0.482, *p*=0.003-0.022) in the 9 following activities: social participation, walking, doing housework, preparing meals, engaging in communication, lifting, drinking, eating, and using transportation. The last factor is cognitive performance, which is measured by the MMSE. It has a significant but small negative correlation with the level of difficulty during social participation (r= -0.293, *p*=0.031) and transferring activities (r= -0.321, *p*=0.018).

Perception, utilization, and satisfaction regarding treatment via allied health care and complementary therapy

The results regarding familiarity with AHC and ACT observation showed that most patients knew about the role of these kinds of treatments (Table 3). The largest proportion (98.1%) of patients were aware of the role of massage therapists, and 92.6% knew about physical therapists and acupuncturists. Approximately 50% of patients knew about supplement specialists, while 33.3% and 5.6% of patients knew about the roles of occupational therapy and speech therapy, respectively. For the interview question about attitudes, all patients were asked whether AHC or ACT could improve their daily life function and whether they needed these treatments or not. The results showed that approximately 13% of patients did not feel that they needed AHC or ACT, and 29.6% of participants were unable to decide; however, 57.4% of patients thought that AHC or ACT could enhance their functional performance and that they needed these alternative treatments.

Regarding experience with AHC or ACT, 50% of patients had experience with massage, approximately 40% had experience with physical therapy or acupuncture, and approximately 26% of them had tried to take supplements under the advice of supplement specialists. The satisfaction level revealed that approximately 80% of patients rated their satisfaction with massage and physical therapy at moderate to highest levels, while 42.9% and 23.8% were satisfied with supplement products and acupuncture, respectively.

Table 2 Correlation coefficient between level of difficulty in each activity of daily living (ADL) and other personal patient factors.

	Age	Gender	Diagnosis duration (year)	Hoehn & Yahr	MMSE	HAD (Anxiety)	HAD (Depression)
Transfer	0.081	0.025	0.288*	0.458**	-0.321*	0.165	0.239†
Social participation	0.050	0.041	0.284*	0.257†	-0.293*	0.201	0.357**
Walking	0.186	0.029	0.219	0.329*	-0.212	0.085	0.269*
Doing housework	-0.013	-0.081	0.254†	0.308*	-0.080	0.188	0.369**
Preparing meals	0.102	0.145	0.101	0.350**	-0.257†	0.260	0.398**
Conversation and communication	0.051	0.115	0.360**	0.205	0.013	0.151	0.482**
Balance	0.023	-0.077	0.209	0.447**	-0.118	0.119	0.243†
Lifting	0.153	0.171	0.285*	0.434**	-0.064	0.142	0.394**
Dressing	-0.009	-0.105	0.308*	0.332*	-0.129	0.004	0.237†
Driving	-0.116	-0.165	0.279*	0.265†	-0.232†	0.050	0.078
Drinking	-0.047	-0.108	0.358**	0.079	0.116	0.078	0.312*
Fine motor use of hands	-0.065	-0.094	0.292*	0.130	-0.001	-0.054	0.121
Eating	-0.147	-0.084	0.295*	0.201	0.128	0.187	0.319*
Using transportation	0.021	0.085	0.235†	0.197	-0.232†	0.020	0.366**
Writing	-0.137	-0.202	0.261†	0.238†	0.019	0.100	0.229†
Washing	-0.121	-0.153	0.233†	0.126	0.068	0.011	0.227†

†: *trend approaching a significant level (p value = 0.05–0.10), *:significant level (p value < 0.05), **: significant level (p value<0.01), MMSE, Mini-Mental State Examination, Thai version HAD, Hospital Anxiety and Depression Scale.*

Table 3 Number of patients who knew about the role of allied health or complementary therapies for individuals with Parkinson's disease and who have experience and are satisfied with treatment from allied health or complementary therapies.

Allied health or complementary therapies	Number of patients who knew about the role (n=54)	Number of patients who received the therapy (n=54)	Number of patients who had moderate to high levels of satisfaction
	Number (Percent)	Number (Percent)	Number (Percent)
Physical therapy	50 (92.6)	20 (37.0)	15 (75.0)
Occupational therapy	18 (33.3)	1 (1.9)	0 (0.0)
Speech therapy	3 (5.6)	0 (0.0)	0 (0.0)
Massage	53 (98.1)	27 (50.0)	22 (81.5)
Acupuncture	50 (92.6)	21 (38.9)	5 (23.8)
Supplement specialist	30 (55.6)	14 (25.9)	6 (42.9)

Discussion

This study aimed to investigate 1) the functional disability of Thai individuals with Parkinson's disease (PD) and 2) the role of AHC and ACT for Thai PD patients. The findings were subdivided into 4 parts as follows:

Regressive functional ability when the disease progressed and longer duration of the disease

The top 4 difficult daily activities that were demonstrated in all stages from mild to severe were transferring, social participation, walking and doing housework. Though only patients in the mild stage did not report difficulty in using transport, eating, and writing, they did report other difficulties. We found that among these daily activities, the degree of difficulty increased with disease severity. This result aligns with previous evidence that severity was related to a decline in functional performance and balance.[25] Early-stage patients have less disability, which might be explained by unrepresented axial problem involvement and pharmacological treatment limitations. The occurring progression of axial problems in PD is considered the result of the worsening of nondopaminergic neurons downstream from the basal ganglia after deterioration by dopamine deficiency.[26] It has been found that axial impairment is strongly related to the level of

functional disability.[27] Axial problems such as postural instability, trunk posture alterations, and gait disorder may lead to inconvenience in performing activities that require locomotion or postural adjustment movement.[28] Thus, patients with moderate to severe disease should experience more disability. Unexpectedly, the level of difficulty of conversation, drinking, fine motor use with hands, eating, using transportation, and washing oneself were not related to the stage of disease. Some participants stated that they could not estimate how much difficulty they had because they did not perform these activities at all.

According to disease's onset, higher level of difficulty increased alongside with a longer duration of disease. These findings are contrast to the previous study which found that duration of disease did not significantly influence in living with Parkinson's disease.[10] However, regressive functional abilities have been suggested to be involved in motor fluctuations following long-term dopamine treatment.[29] Using dopamine over the optimum dose brings about uncontrolled involuntary movement (dyskinesia), such as chorea and dystonia, due to excessive glutamatergic transmission within the basal ganglia.[26, 30] Fifty percent of PD patients develop dyskinesia 2 years after starting conventional levodopa,[31] and approximately 90% of those who receive more than 9 years of treatment experience dyskinesia.[32] These symptoms significantly impact their daily activities and quality of life.[31-33]

Personal factors related to the level of functional disability

Apart from the severity and duration of disease, 2 other factors that were related to the level of functional disability were depression score and cognitive performance. A previous study demonstrated that nonmotor symptoms are the main prognostic factors for disease progression in PD.[9] These nonmotor symptoms are mood impairment, anxiety, and cognitive decline. Similarly, a review study also indicated that depression is a prognostic factor for the progression of PD.[34] Moreover, mood and cognitive performance are important factors for deteriorating quality of life and decreased functional capabilities in PD.[35] There are other factors related to functional performance, such as comorbidities, medication state, others non-motor symptoms, and complications.[25, 27]

Perception of the roles of allied health and complementary therapy in Parkinson's disease

The researcher interviewed Thai individuals with PD who had consulted with their doctor and ascertained their perception of the roles and responsibilities of using AHC and ACT in PD. Almost all patients knew about the roles of physical therapy, massage therapy, and acupuncture in PD. However, only a small number of patients were aware of the role of occupational therapy in PD.

Previous evidence was also consistent with this study, finding that the prevalence of PD patients who receive alternative medicine is approximately 20–75%; however, 11-0% of these patients were referred by health care professionals.[2, 4, 36] Obtaining AHC from a professional in Thailand, such as occupational and speech therapy, requires screening and referral from a physician. Unfortunately, evidence regarding the referral rate in Thailand is still lacking. Robert and colleagues in 2021 reported that AHC referrals were given to patients who had experience a fall in the 6 months prior, patients with advanced- and moderate-stage disease, and older patients.[37] Almost all participants in our study were also elderly with moderate-stage disease; however, they were not referred to AHC. It might be occurred because they had a good response to pharmacologic treatment and were able to independently perform routine activities, although they may not understand roles of AHC and ACT. A higher frequency of consultation with allied health might increase utilization. Another possible reason might be the specialized allied health services available for PD. In various countries, such as European countries, specialized allied health care includes physical therapy, occupational therapy and speech therapy.[36] The level of expertise of these health care services could increase the number of patients by increasing confidence and clarifying the role of allied health.

Utilization of and satisfaction with treatment via allied health and complementary therapy

A small number of patients were treated by physical therapists and acupuncturists. However, the most popular alternative treatment for Thai individuals with PDs in the present study was massage therapy. Among patients who received treatment from these 3 types of professionals, they tended to be satisfied with treatment from physical therapists and massage therapists. A smaller number of patients were satisfied with the efficacy of acupuncture.

Physical therapy treatments in PD aims to ameliorate PD-related impairments, minimize or prevent secondary complications alongside, balance and functional improvement in order to reach patients independency.[38] In addition, physical therapy rehabilitation emphasizes functional mobility, physical activity, and improve nonmotor while suppress motor symptoms. According to movement strategy training for individual PD, modalities of treatment are concerned, such as using cueing, cognitive rehabilitation, adapted environments, assistive devices, including caregiver training.[38-41]

Massage is one of common choice of ACT in Asian countries, especially in Thailand.[42] The massage can temporarily release pain or stiffness and promote mood and muscle relaxation.[28] A prior systematic review demonstrated that massage can improve the quality of life of PD patients, such as their sleep quality, pain and depressive symptoms, and muscle stiffness, and also seems to improve motor symptoms.[43]

Approximately 60% of patients need alternative treatment, including allied health and complementary therapy, because they perceived that these treatments could improve their functional ability. Although priority health care for Thai PD patients in recent research is massage therapy, a multidisciplinary team approach of allied health care professionals or others complementary services specifically for PD patients can probably help them obtain more benefits to alleviate motor and nonmotor symptoms, improve level of activities or social participation, and enhance quality of life.[13, 15, 38, 44-47] Consequently, health policy implementation, which supports consulting in cooperation with each health care professional, may help patients be more informed about the roles and benefit more from public health care services.

Limitations

This study did not assess sensory function, pain, functioning of cardiovascular and respiratory systems or motor fluctuation symptoms, all of which may affect functional ability and the need for further treatment. Additionally, the researcher surveyed only one center. Therefore, the findings may not be representative of all Thai individuals with PD.

The sample size was small when compared to number in sample size calculation, particularly individuals with PD in the mild and severe stage. Most of the PD participants were moderate severity level when determined by H&Y stage 2-3. In the present study, the researcher conducted an interview as the same day of physician's appointment. Therefore, less severity patients may not obviously present PD's symptoms during medical consultation. On the other hand, patients with severe symptom may inconvenience to come to the hospital. Apart from these reasons, the COVID-19 pandemic is a potential factor influencing a reduction on number of patients making appointments. Therefore, the findings may not refer to individuals with mild and severe stage of PD. Further research should recruit a larger sample size and both mild and severe groups are required.

Nevertheless, the number of patients who had experience with AHC and ACT in this study was too small, and the correlation of functional disability level and experience with AHC and ACT was not covered in this study because of the sample size and the limitation in using only one observation center. Thus, further research may be necessary to determine why Thai PD patients choose not to receive treatment from AHC and ACT professions.

Conclusion

Progressive deterioration of functional activity was present in relation to disease duration and aging status. Higher functional disability levels corresponded to more severe stages, longer disease durations, and worse cognitive and depression scores in Thai PD patients. More than half of the patients were satisfied and require physical therapy treatment and massage. These findings may reflect the benefits of understanding AHC and ACT treatments.

Conflict of interest

The authors have no conflicts of interest to report

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