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Aims and scope

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

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

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

Objectives

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Influence of short-term iodinated radiographic contrast media exposure on reactive oxygen species levels in K562 cancer cells

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ABSTRACT

Background: Iodinated radiographic contrast media (IRCM) are commonly used for evaluating cancer diseases in diagnostic radiology. There are several studies that have showed the effects of IRCMs on various biological endpoints in normal cells. However, the effects of IRCMs on cancer cells is still a bit of a mystery.

Objectives: To investigate the effects of short-term iodinated radiographic contrast media exposure on reactive oxygen species levels in K562 cancer cells.

Materials and methods: Five commercially available IRCMs used were iohexol, iopamidol, iobitridol, ioxaglate, and iodixanol. A trypan blue exclusion assay was performed to evaluate the cytotoxicity of each IRCMs on K562 cancer cells. The effect of IRCMs on cell proliferation was further determined by counting the number of cells in metaphase. The reactive oxygen species (ROS) levels was determined at short-term by the use of a spectrofluorometric method.

Results: All IRCMs decreased in percentage of cell viability, number of metaphase cells, and levels of ROS in a concentration-dependent manner.

Conclusion: This study suggested that all IRCMs showed a short-term effect on K562 cancer cells by decreasing ROS levels in a concentration-dependent manner. In addition, IRCMs exhibited effect on cell viability and cell proliferation as well.

Introduction

Iodinated radiographic contrast media (IRCM) is a tri-iodinated derivative of benzoic acid.[1] IRCMs are the most commonly used methods in clinical practice for both diagnostic and therapeutic examinations. It can be involved in plain radiography, fluoroscopy, angiography, percutaneous cardiac and arterial interventions, and computed tomography (CT).[1, 2] The most common justifications for using contrast media is for evaluating cancer diseases. Injection of IRCMs are generally safe, however, there are notable adverse effects that are more likely to occur such as hypersensitivity reactions, contrast-induced nephropathy, and thyrotoxicosis.[3] In addition, results from many studies using a variety of biological endpoints have shown the effects due to exposure to IRCMs in cells and animal models.[4-8] However, information on such effects is mainly limited to only normal cells or animal models. Therefore, information on the potential risks from exposure to IRCM for cancer cells is lacking. As an initial step to fill this knowledge gap, we focused on erythromyelogenous leukemia cells line (K562) following short-term exposure to the IRCMs. Three biological endpoints (i.e.; cytotoxicity, a cell in metaphase, and reactive oxygen species)were determined in these studies. We used these biological endpoints due to the cytotoxicity and number of cells in metaphase which was referred to as toxicity and cell proliferation, respectively.Reactive oxygen species (ROS)are known to cause oxidative stress in several cellular molecules (i.e.; DNA, lipids, and proteins) and subcellular

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organelles (i.e.; mitochondria and plasma membranes).[9-12] Oxidative stress is one of the risk factors that play an important role in contrast-induced renal diseases.[13, 14]

Materials and methods

Chemicals

Five commercially available iodinated radiographic contrast media (IRCM) used were iohexol (omnipaque; GE Healthcare, China), iopamidol (iopamiro; Bracco, Italy), iobitridol (xenetix; Guerbet, France), ioxaglate (hexabrix; Guerbet, France), and iodixanol (visipaque; GE Healthcare, Ireland). These IRCMs are commonly used in diagnostic radiology.

Cancer cell and culture

Cancerous cell lines were erythromyelogenous leukemia cells line (K562). Cells were cultured in a tissue culture flask containing RPMI 1640 medium, supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin at 37 °C in a humidified 5% CO_2 atmosphere. The cell line cultures initiated a total of $1x10^5$ cells/mL before exponentially proliferating to a total of 8-10 x 10^5 cells/mL over 3 days. For the experiment, cultures were initiated at 5 x 10^5 cells/mL to obtain cells in the exponential growth phase to reach a total of about 8-10 x 10^5 cells/mL over 24 hours. Total number of viable cells was determined by a trypan blue exclusion assay. Total number of cells was determined by haemocytometer.

Cell viability

K562 cancer cells ($3x10^5$ cells/mL) were treated with IRCMs (10, 50, 100 mgI/mL) in 24-well plates at 37 °C for 72 hours. Total number of viable cells was determined by a trypan blue exclusion assay. Total number of cells was determined by haemocytometer. The percentage (%) of cell viability was calculated as followed;

% Cell viability = (Number of cells treated with IRCM/ Number of cells un-treated with IRCM)x100

Number of cells in metaphase

Number of cells in metaphase can be referred to as cell proliferation. K562 cancer cells ($3x10^5$ cells/mL) were treated with IRCMs (10, 50, 100 mgI/mL) in 24-well plates at 37 °C, 5% CO_2 in a humidified incubator for 72 hours. Next, 30 µL of 10 µg/mL colcemid was added to each well of the 24-well plates. After a 45 minutes incubation with colcemid, cells were washed with a phosphate buffer saline (PBS) and a total of 500 µL of 0.075 M KCl was added, followed by 45 minutes of additional incubation at 37°C, 5% CO_2 in a humidified incubator. Next, cells were washed with PBS and 5 mL of fixatives (Carnoy's solution, 3:1 v/v methanol: acetic acid) were added. Microscope slides were routinely at 4 °C until used for cell fixing. Fixed cells were dropped gently on clean microscope slides, were air-dried, and stained with a Wright Giemsa solution for 3 minutes. The number of metaphase cells was scored and recorded (Figure 1). For consistency, the microscopic analysis was performed by a single individual. Slides were coded so that the analyst was not aware of the treatment until after the slides were scored and the code was broken.

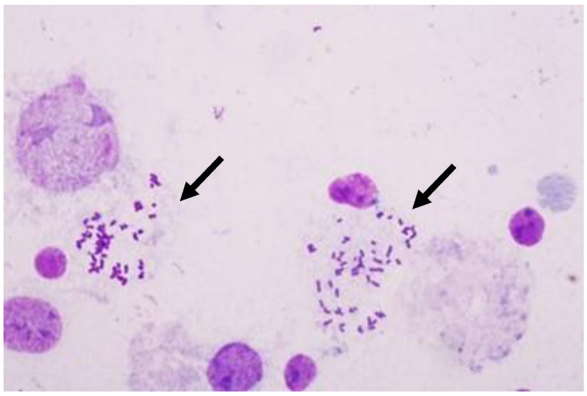

Figure 1. *Metaphase cells (Arrow). Magnification 100X*

Determination of reactive oxygen species (ROS) levels

Determination of reactive oxygen species levels was performed based on the work of Loetchutinat *et al*.[15] with some modifications. Briefly, a $1x10^5$ cells/mL suspended in HEPES-Na^+ buffer (pH 7.25) at 37 °C were treated with IRCMs (1, 10, 50 mgI/mL) for 5 minutes. That treated time is considered as short-term. After 100 seconds, 100 nM 2',7'-dichlorofluorescein diacetate (DCHF-DA) was then added into the system. Dichlorofluorescein (DCF) fluorescence intensity at 523 nm (excitation at 502 nm) was recorded as a function of time. Slope (dF/dt) of the tangent of the curve (experimental spectrofluorometric data) after time at the presence of DCHF-DA to 200 seconds was measured (Figure 2). The dF/dt was related to level of ROS. Thus, when dF/dt increases, it means that ROS levels are high. Conversely, when dF/dt has decreased, it means that ROS levels are low.

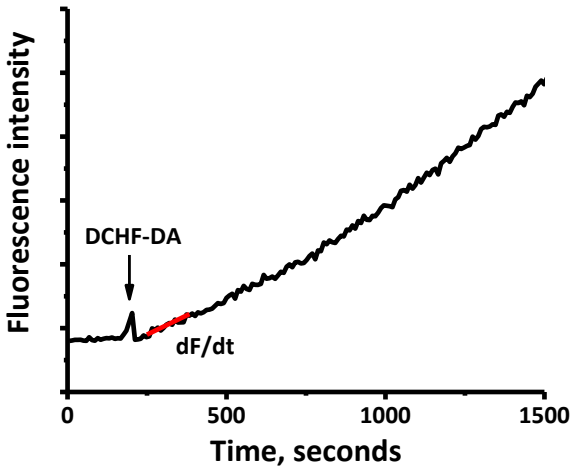

Figure 2. *Dichlorofluorescein (DCF) fluorescence intensity at 523 nm (excitation at 502 nm) as a function of time. Slope (dF/dt) of curve after time at the presence of DCHF-DA to 200 seconds.*

Statistical analysis

We presented the results as a mean±standard error of the mean (SE). Student's t-test was used independently to evaluate any statistical differences in the mean values between each test group and the corresponding control. A *p*-value of less than 0.05 was considered as statistically significant.

Results

Cell viability

Figure 3. shows the effects of IRCMs on K562 cancer cell viability. IRCMs decreased percentage of cell viability in a concentration-dependent manner. This result suggests that all IRCMs exhibited cytotoxicity on K562 cancer cells. However, four IRCMs (iodixanol, ioxaglate, iohexol, and iopamidol) significantly exhibited inhibition of cell viability at 50 and 100 mgI/mL when compared to a corresponding control. Iobitridol significantly exhibited inhibition of cell viability at 100 mgI/mL.

Metaphase cells

Figure 4. shows the number of cells in metaphase of K562 cancer cell after exposure to IRCMs. IRCMs reduced the number of metaphase cells in a concentration-dependent manner. However, all IRCMs except iopamidol significantly decreased the number of metaphase cells at 100 mgI/mL only when compared to a corresponding control. The result suggests that all IRCMs exhibited inhibition of K562 cancer cell proliferation.

Reactive oxygen species (ROS) levels

Figure 5. shows dF/dt of curve of K562 cancer cells after exposure to IRCMs. IRCMs reduced dF/dt of the curve in a concentration-dependent manner. However, all IRCMs except iodixanol significantly decreased dF/dt at 50 mgI/mL only when compared to a corresponding control. The result suggests that IRCMs could decrease ROS levels in K562 cancer cells.

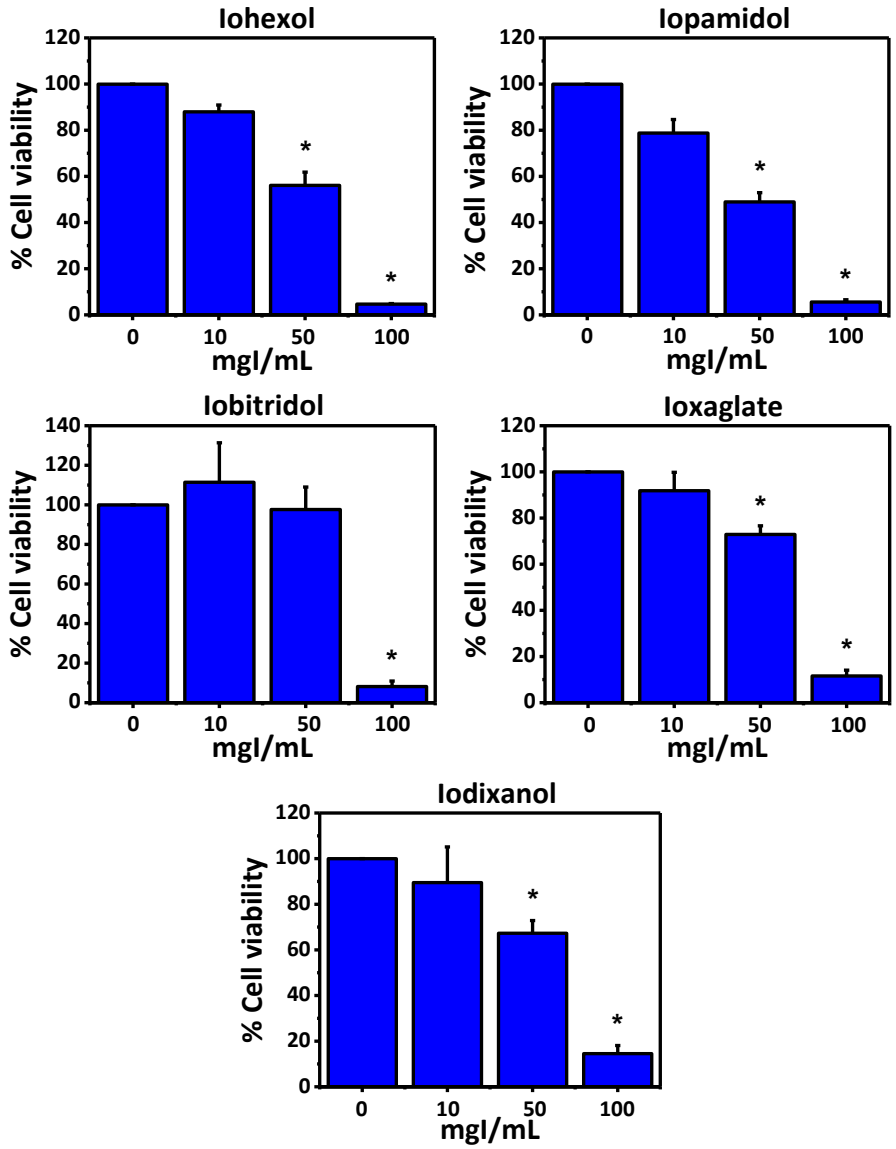

Figure 3. *Effects of IRCMs on K562 cancer cell viability. * p<0.05.*

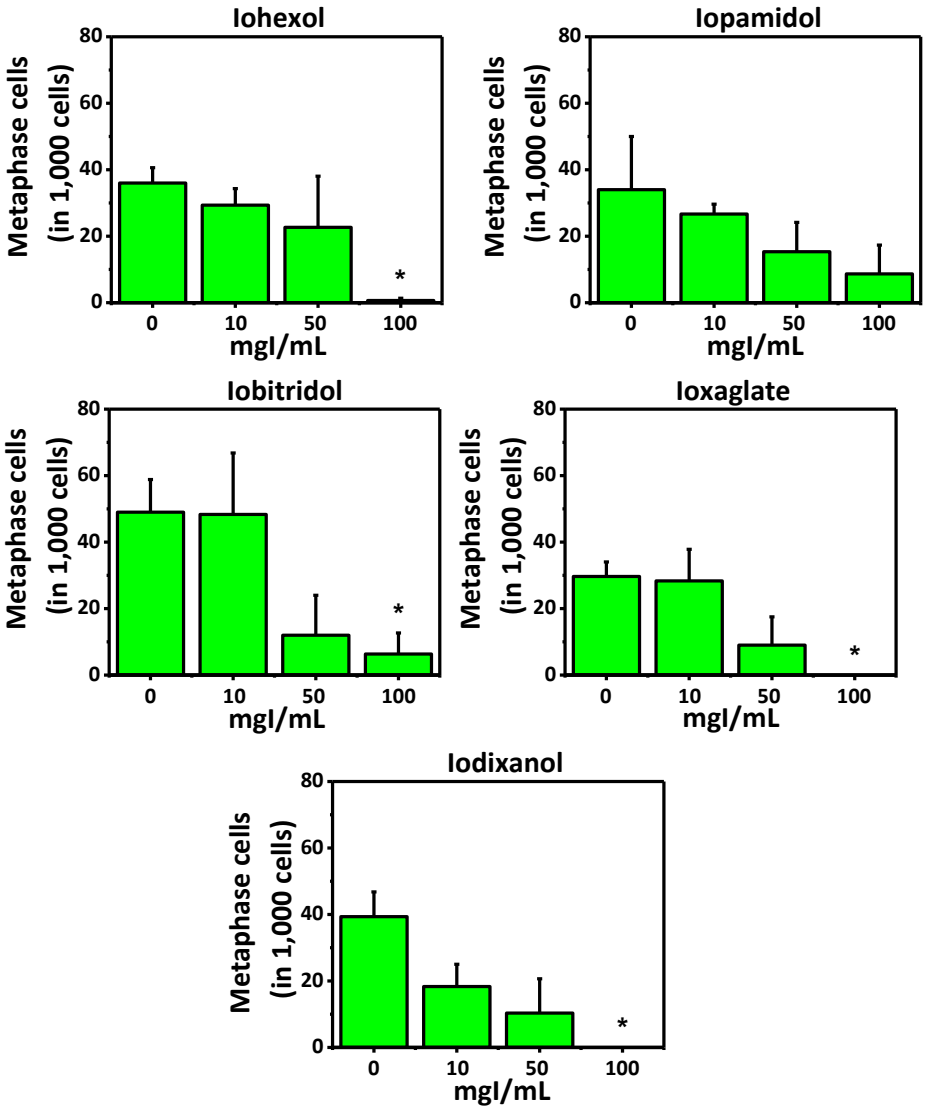

Figure 4. *Number of cells in metaphase in 1,000 cells of K562 cancer cells after exposure to IRCMs. * p<0.05.*

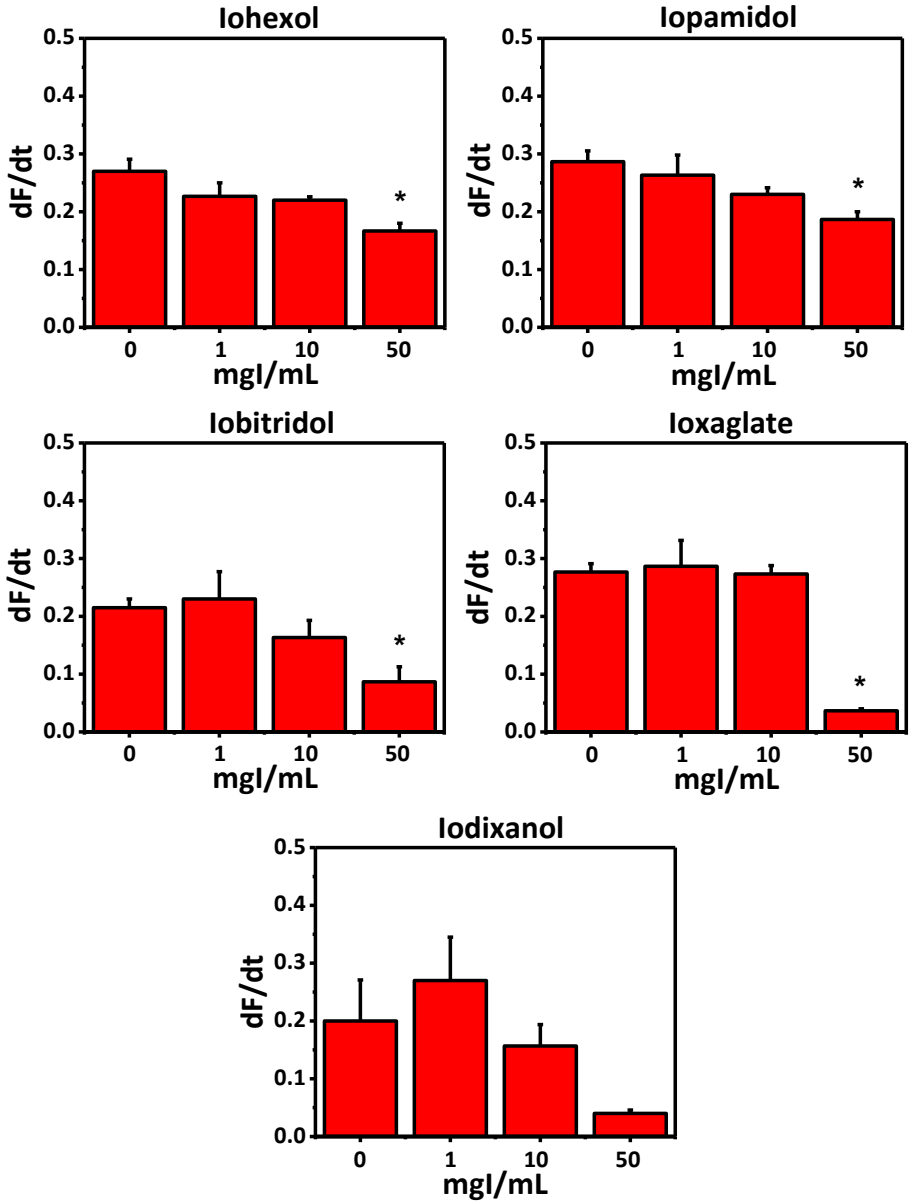

Figure 5. *The dF/dt of curve of K562 cancer cells after exposure to IRCMs. * p<0.05.*

Discussion

Oxidative stress resulting from an imbalance between free radicals and antioxidant agents, is one of the risk factors that plays an important role in IRCM-induced renal disease.[16] Our previously studies evaluated the potential properties of IRCMs (iohexol, iopamidol, iobitridol, ioxaglate, and iodixanol) *in vitro* free radical generating reactions. The results showed IRCMs exhibited weak *in vitro* antioxidant properties. This finding suggested that antioxidant ability depended on type of free radical production and concentration of IRCMs.[17] Our previous studies corresponded to the studies conducted by Berg *et al*.[18] These authors concluded that IRCMs (iodixanol, iohexol, ioxaglate, and diatrizoate) showed *in vitro* antioxidant properties in concentrations relevant for their clinical applications.[18] Furthermore, Xiong *et al*. observed increased intracellular ROS formation in renal tubular cells after exposure to IRCMs (ioversol). These studies suggested that ioversol induced renal tubular cell death in a concentration-dependent manner via an increase in oxidative stress.[19] In contrast, Zager *et al*. showed IRCM toxicity could be dissociated from tubular cell oxidant stress.[20] Current studies showed that IRCMs decreased ROS levels in K562 cancer cells in a concentration-dependent manner. Of note, Xiong *et al*. observed increased ROS levels in cells after exposure to IRCM for 1 hour[19] whereas the current studies observed decreased ROS levels occurring in cells after exposure to IRCMs for 5 minutes. It might be suggested that the effects of IRCMs was not only dependent on concentration but dependent on exposure time and cell type, as well.

Furthermore, our findings demonstrate that all IRCMs (iohexol, iopamidol, iobitridol, ioxaglate, and iodixanol) in present studies showed cytotoxicity and anti-proliferation effects on K562 cancer cells as being concentration-dependent

manner at a concentration of 10, 50, and 100 mgI/mL in ways similar to the studies conducted by Kim *et al*.[21] The authors investigated the effects of IRCMs (iodixanol, iopromide, ioxaglate, and ioxithalamate) on human disc cells. They showed human disc cells death had occurred in a concentration-dependent manner after exposure to IRCMs at a concentration of 0.1, 10, and 100 mg/mL.[21] It should be noted that there was a difference in the experimental design between the current studies and the studies conducted by Kim *et al*[21] in terms of the cell types used to investigate the effects of IRCMs. In the present studies, cancer cells were used instead of human disc cells in the studies conducted by Kim *et al*.[21] In addition, there are studies that have determined the effects of IRCMs on various cell types such as renal epithelial cells,[22, 23] endothelial cells,[23, 24] smooth muscle cells,[23, 25] human fibroblasts,[23, 26] and human neutrophils.[23, 27] Of note, toxic effects caused by IRCMs are considered multifactorial in that they can involve osmolarity and ionic strength.[23]

Conclusion

Taken together, we concluded that IRCMs such as iohexol, iopamidol, iobitridol, ioxaglate, and iodixanol showed short-term effects on K562 cancer cells by decreasing ROS levels in a concentration-dependent manner. In addition, IRCMs exhibited effect on cell viability and cell proliferation as well.

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Conflicts of Interest

The authors declare none of conflict of interest.

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Inhibition of nitric oxide production and COX-2 protein expression in LPS-stimulated RAW 264.7 cells by the hexane fraction of *Murdannia loriformis*

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ABSTRACT

Background: *Murdannia loriformis* (ML) is a medicinal plant traditionally used for chronic bronchitis, cancers in the initial stage, colds, throat infections, pneumonia, the flu, and wound healing. The crude ethanolic extract of ML has been reported anti-inflammatory, analgesic, antipyretic and gastroprotective activities in various *in vivo* experiments.

Objectives: This study aimed to isolate the active fractions of ML and assess the effect on nitric oxide (NO) inhibition and cyclooxygenase 2 (COX-2) expression.

Materials and methods: The dry powder of ML was extracted with 80% ethanol. The crude ethanolic extract afterward was brought to partition with the various solvents base on polarity difference. Finally, accepted hexane, chloroform, ethyl acetate, and water fractions, respectively. The ML extract and its fractions were screened the cytotoxicity on RAW264.7 cells by sulforhodamine B assay. The non-toxic doses were selected for the next NO inhibition experiment. RAW264.7 cells were treated with the various non-toxic doses of the ML. Lipopolysaccharide (LPS) was added to induce cells inflammation and stimulate NO production. Additionally, the culture supernatants were collected and measured NO levels by Griess reagent. The fraction that revealed potent anti-inflammatory activity by reduced NO accumulation was selected to study COX-2 protein suppression by western blot analysis.

Results: The results showed that crude ethanolic extract and all fractions except for the water fraction significantly inhibited NO production of RAW264.7 cells. The hexane fraction demonstrated a superior on nitric oxide reduction as same as the standard drugs L-NAME and indomethacin. This fraction also reduced COX-2 protein expression in LPS-stimulated RAW264.7 cells.

Conclusion: The hexane fraction possesses an anti-inflammatory activity by reducing nitrite level and COX-2 protein expression. Suggesting that, the active ingredient of ML is the non-polar compound. Further studies should be carried out to isolate the pure compound from the hexane fraction and structure identification.

Introduction

Inflammation is a response process that triggering by a variety of factors including invading pathogens, toxic compounds, and endogenous signals like damaged cells.[1] Tissue macrophages and dendritic cells represent a key role in danger signals detection. These cells can release pro-inflammatory mediators to promote leukocyte migration and eliminating the cause of infection and contributing to tissue repair.[2] Activated macrophages and other immune system cells inhibit pathogen replication by releasing many kinds of effector molecules, including nitric oxide (NO).[3] Nitric

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oxide is an important mediator of inflammation. It causes vasodilation and tissue injuries in the inflammation process.[4] It has two principal divergent functions in cells: homeostasis and cytotoxicity. For regulatory functions, NO is produced in small amounts under physiological conditions and mediates vasodilation, controls the adhesion and aggregation of platelets and neutrophils, and is involved in neurotransmission.[5] It is considered as a pro-inflammatory mediator that induces inflammation due to overproduction in abnormal situations and involved in the pathogenesis of inflammatory disorders.[4] Therefore, NO inhibitors represent an important therapeutic advance in inflammatory diseases management.[4] The cyclooxygenase (COX) is a key enzyme involved in the inflammation process.[6] The role of NO on the constitutive and induced forms of COX activity were studied in the mouse macrophages cell line RAW264.7. The results showed that NO directly interacts with COX to cause an increase in the enzymatic activity and prostaglandins (PGs) production.[5]

NSAIDs are frequently prescribed drugs for relieving inflammation. In addition to their anti-inflammatory effect, they have antipyretic and analgesic properties.[7, 8] Anti-inflammatory mechanisms of NSAIDs based on cyclooxygenase enzymes (COXs) inhibition. COXs are the key enzymes required in PGs synthesis.[9] COX-1 involves in PGs production that supports platelets and protects the stomach. COX-2 is induced by various inflammatory stimuli, contributed to PGs production that involves pain and swelling.[9, 10] The traditional NSAIDs reduced inflammation, pain, and fever because of both COX-1 and COX-2 inhibition. However, inhibition of COX-1 cause reduction of the PGs that protect the stomach and support platelets and blood clotting then it leads to the gastric ulcer and bleeding.[11] For this point, the NSAIDs drug that selective inhibition on COX-2 was developed. This group increased the selectivity for COX-2 inhibition but also enhanced the risk of cardiovascular diseases.[12] The results from several trials showed that coxib drugs increased the risk of atherothrombotic vascular events.[12, 13] Although, NSAIDs produced an excellent anti-inflammatory activity their adverse effects caused the limitation of NSAIDs using. The critical side effect of NSAIDs including gastrointestinal, renal, and cardiovascular toxicity caused the limitation for NSAIDs user. For these reasons, there is a continuous effort in NSAIDs development with decreasing side effect profiles from the alternative sources.[14]

Murdannia loriformis (ML) is a medicinal plant traditionally used for treating diseases like colds, throat infections, pneumonia, diabetes mellitus, flu, and inflamed wound.[15] In Thailand, the cancer patients used this plant to relieve side effects of chemotherapy and radiotherapy.[16] The crude ethanolic extract of this plant showed anti-inflammatory, analgesic, and antipyretic activities in the various *in vivo* models.[17] In the cotton pellet-induced granuloma formation in the rats, it showed anti-inflammatory activity without gastric ulceration. Suggested that, the anti-inflammatory activity of the extract is the non-steroidal like action since it did not affect the thymus weight of the rats.[17] The ethanolic extract also decreased gastric ulcer in the rats induced by ethanol-hydrochloric acid, indomethacin, and stress.[18] The crude ethanolic extract of this plant revealed both anti-inflammatory and gastro-protection properties. Therefore, it might be the alternative source of the anti-inflammatory agent. This study aimed to isolate active fractions of ML and examine the effect of its fraction on nitric oxide production and COX-2 expression in RAW264.7 cells

Materials and methods

Preparation of plant crude extract

Dry powders of *M. loriformis* was purchased from Abhaibhubejhr's hospital, Prachinburi Province, Voucher specimen with number 25135 was deposited at the herbarium section of Queen Sirikit Botanical Garden. For preparing of crude ethanol extract, the *M. loriformis* powders (300 gm) was macerated with 80% ethanol at room temperature for 24 hr, evaporated by a rotary evaporator, and lyophilized. The yield of crude EtOH extracted was 14.45%.

Liquid-Liquid partition

Crude ethanolic extract (10 gm) was suspended in double distilled water (100 mL) and partitioned with n-hexane, chloroform ($CHCl_3$), and ethyl acetate (EtOAc), respectively. The partition process was repeated three times. Eluent of each fraction was pooled, evaporated and lyophilized.

Cell lines and cell culture

The RAW264.7 cells were provided by Assoc. Prof. Dr. Siriwan Ongchai, Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Thailand. Cells were cultured in Dulbecco's Modified Eagle Medium (GIBCO™, Thermo Fisher Scientific, MA, USA) supplemented with 10% fetal bovine serum (GIBCO™, Thermo Fisher Scientific, MA, USA), and 1% antibiotics (penicillin 100 units/mL and streptomycin 100 µg/mL; GIBCO™, Thermo Fisher Scientific, MA, USA). Cells were incubated in a CO_2 incubator at 37 °C, 5% CO_2, and humidified atmosphere and sub-cultured every 2-3 days or when the confluence reaching 70–80%.

Cytotoxicity assay

RAW264.7 cells were seeded into 96 well plates ($5x10^4$ cells/well) and were incubated in the CO_2 incubator for 24 hr. Crude ethanolic extract and its fractions, in various concentrations (0.5-800 µg/mL), was added to the cells. At 24 hr after the incubation time, cell viability was measured by sulforhodamine B assay (SRB assay).[19] Briefly, 100 µL of 10% cold TCA was added to the cells and incubated at 4 °C. One hour later, the plate was washed with tap water and let air dry. Cells were stained with 0.057% SRB (Sigma-Aldrich) and incubated for 30 min, then added 1 % (v/v) acetic acid to remove the excess dye. Two hundreds microliter of 10 mM Tris base solution (Sigma-Aldrich) were added to dissolve protein-bound dye and measured the OD at 510 nm using a microplate reader.[20]

Nitrite assay

To study the effect of ML fractions on nitric oxide reduction, we stimulated RAW264.7 cells with LPS and measured the nitrite, the stable product of NO, in the culture supernatant. Cells were seeded in 96-well plate ($5x10^4$ cells/well) and

incubated for 24 hr. Various concentrations of ML fractions were added before cell stimulating with LPS (Sigma- Aldrich). Twenty-four hours after the incubation time, nitrite level in cell culture supernatant was measured by Griess reaction. Briefly, 100 µL of culture medium was mixed with 100 µL of Griess reagent (Sigma-Aldrich). The reaction was performed at room temperature for 15 min and the absorbance was measured at 540 nm. Nitrite level in the sample was determined by comparing with the sodium nitrite standard curve.[21] Non-selective inhibitor of nitric oxide synthase, nitroarginine methyl ester (L-NAME) and non-selective COX inhibitor, indomethacin was used as positive control.[22, 23] The positive controls were kindly provided by Assist. Prof. Dr. Nuttakarn Jiruntanat, Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Thailand.

Western Blot analysis

We chose the potent ML fraction from the earlier nitrite inhibition experiment for COX-2 expression study. RAW264.7 cells ($1x10^6$ cells/well) were seeded in the 6-well plate for 24 hr. ML fraction was added for 1 hr before stimulating with LPS and cells were incubated for another 24 hr. RIPA buffer was added to lyse cells. The lysate was centrifuged at 12,000 g 4 °C for 10 min and supernatant was collected for protein determination using a protein assay kit. Total 20 µg of proteins were subjected to SDS-PAGE and electrotransferred to a nitrocellulose membrane. The membranes were blocked with 5% skim milk overnight and incubated with anti- COX-2 antibody (Merck). After incubation, membranes were washed three times with PBS-Tween 20 and incubated with horseradish peroxidase (HRP) conjugated goat anti-mouse IgG (Merck). Beta actin antibody (Merck) was used as a loading control. The enhanced chemiluminescence system was applied to detect the signals. Protein bands intensity from western blot films was quantified by ImageJ software.

Statistical analysis

Data from three independent experiments were presented as the mean±standard error of the mean (S.E.M). Statistical comparisons between the mean of each group were analyzed using the One-way analysis of variance (One-way ANOVA). The statistically significant was considered at $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

Results

Liquid-Liquid extraction yield

Dry weight of crude ethanolic extract and its fractions obtained from experiment was shown in the extraction process flowchart (Figure 1). Yields of crude ethanolic extract, hexane, $CHCl_3$, EtOAc, and water fractions were 14.45%, 11.2%, 1.49%, 2.18%, and 87% respectively (Table 1).

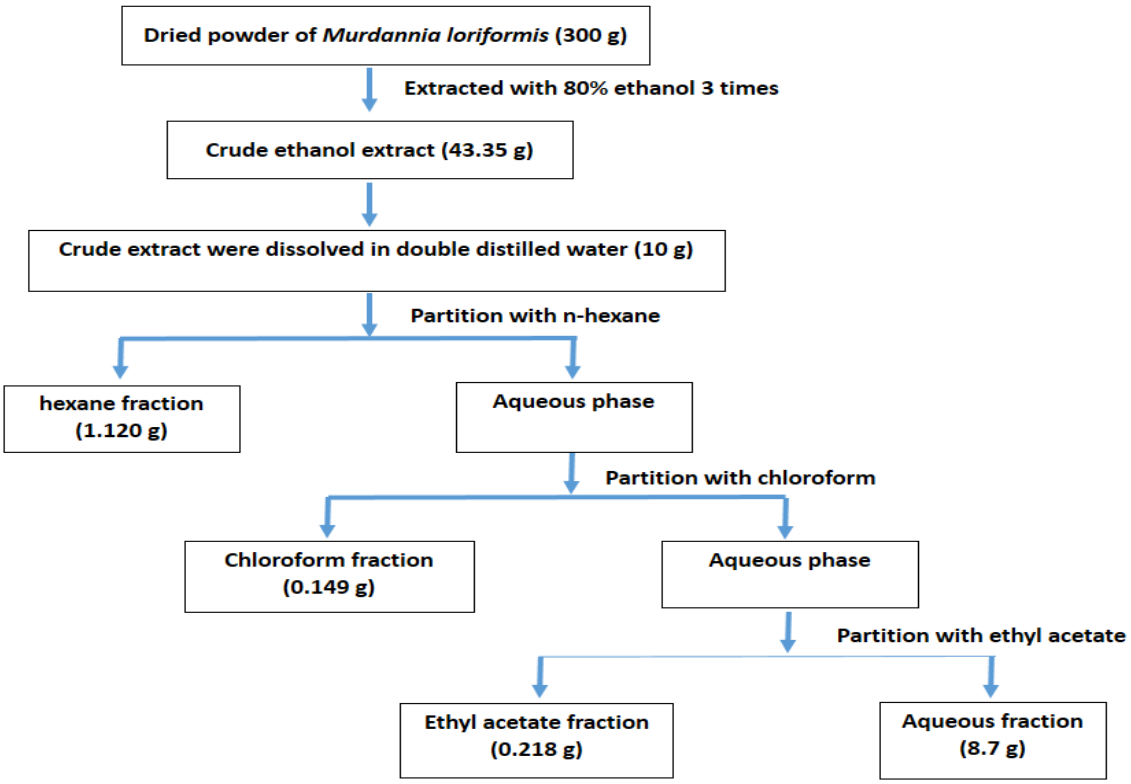

Figure 1. *Flow chart of Extraction process.*

Table 1 *Extraction yield*.

Extracts	Yield (%)	Weight (g)
1. Crude ethanolic extract	14.45	43.35
2. Hexane fraction	11.2	1.120
3. $CHCl_3$ fraction	1.49	0.149
4. EtOAc fraction	2.18	0.218
5. Water fraction	87	8.7

Cytotoxicity assay

Effect of crude ethanolic extract and its fractions on RAW264.7 cells availability were determined using SRB assay. As shown in Figure 2, no significant difference in cell viability was observed for crude EtOH extract, EtOAc, and water fractions up to concentrations of 100 µg/mL. The difference in cell death was shown in hexane and $CHCl_3$ treated-cell at a concentration of 100 µg/mL. From these results, non-toxic concentrations of crude EtOH extract, EtOAc, and water fractions (up to 100 µg/mL) and hexane and chloroform fractions (up to 50 µg/mL) were selected for subsequent NO inhibition assay.

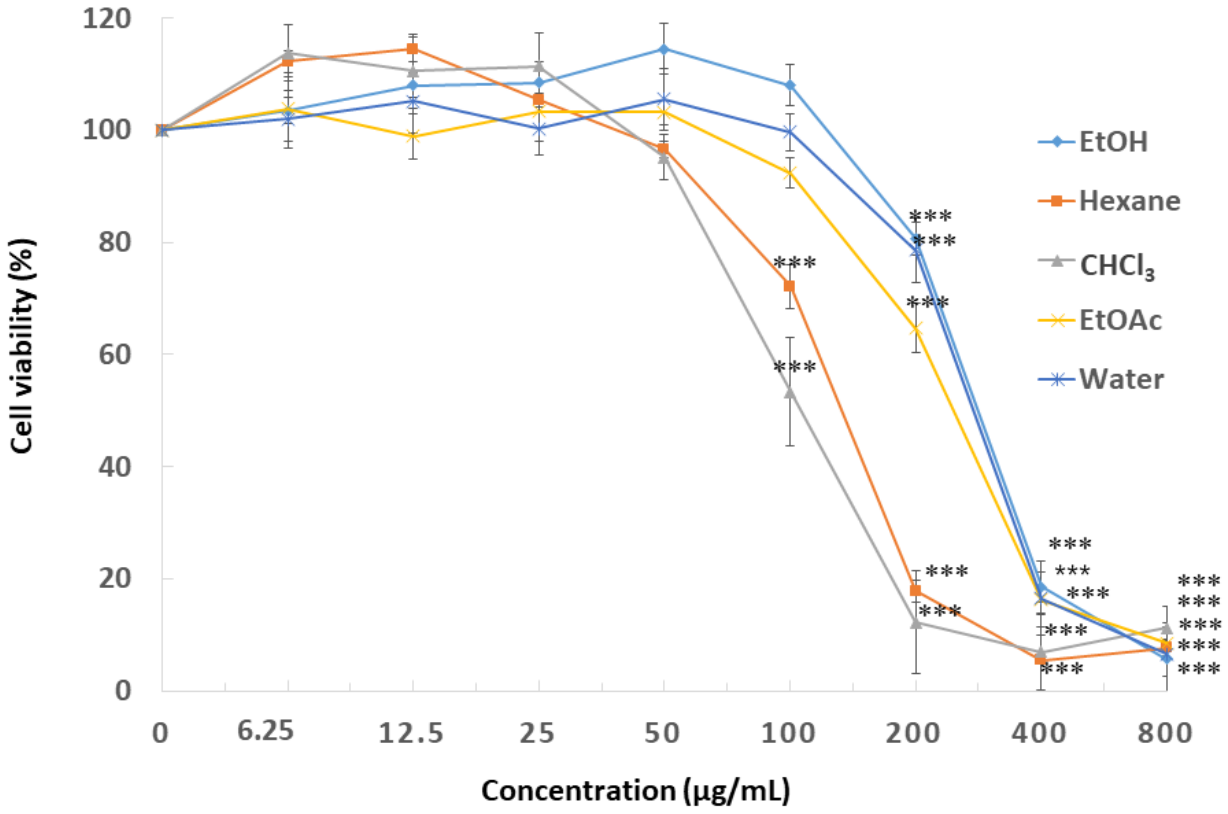

Figure 2. *Effect of crude ethanolic extract of ML and its fractions on RAW264.7 cells viability. Each line represents the mean of three replicates with error bar representing the S.E.M. ***: significant difference compared with media control (concentration 0 µg/mL).*

Inhibition of NO production in LPS-stimulated RAW264.7 cells

Nitrite levels of cells stimulated with LPS were 19.1±5.9 µM. Cells treated with the positive control, L-NAME and indomethacin, caused nitrite reduction to 2.2±0.32 and 2.8±1.19 µM, respectively. Crude ethanolic extract and EtOAc treatment caused nitrite levels reduction comparable with positive control. The potent NO reduction effect was seen in hexane and $CHCl_3$ fractions at a high dose of 50 µg/mL. Nitrite level in the culture supernatant of hexane and $CHCl_3$ –treated cells were 0.83±0.63 µM and 1.1±0.54 µM, respectively. Water fraction tended to reduce nitrite level; however, no significant difference from LPS-induced group (Figure 3). Ethanolic extract and almost ML fractions reduced NO accumulation in culture supernatants without significant reduction of RAW264.7 cells viability as shown in Figure 4.

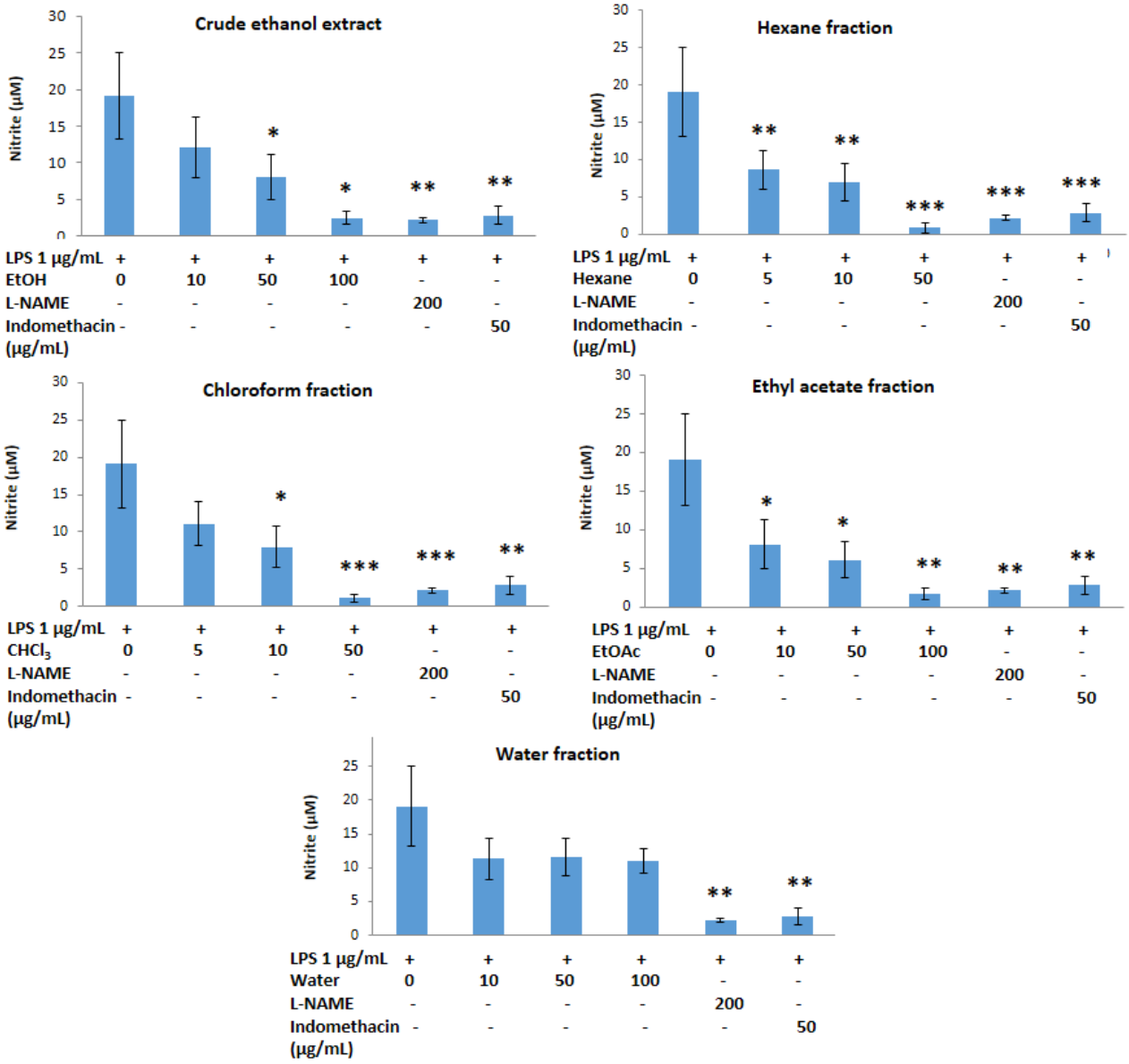

Figure 3. *Effect of crude ethanolic extract and its fractions on the nitrite production in LPS-stimulated RAW264.7 cells. RAW264.7 cells were treated with various concentrations of ethanol extract and its fractions for 1 hr. LPS (1 µg/mL) was added to the cells and incubated for 24 hr. Nitrite level was determined by using the Griess reagent. *, **, *** : significantly different (p<0.05), (p<0.01), and (p<0.001), respectively, from that of LPS-treated cells.*

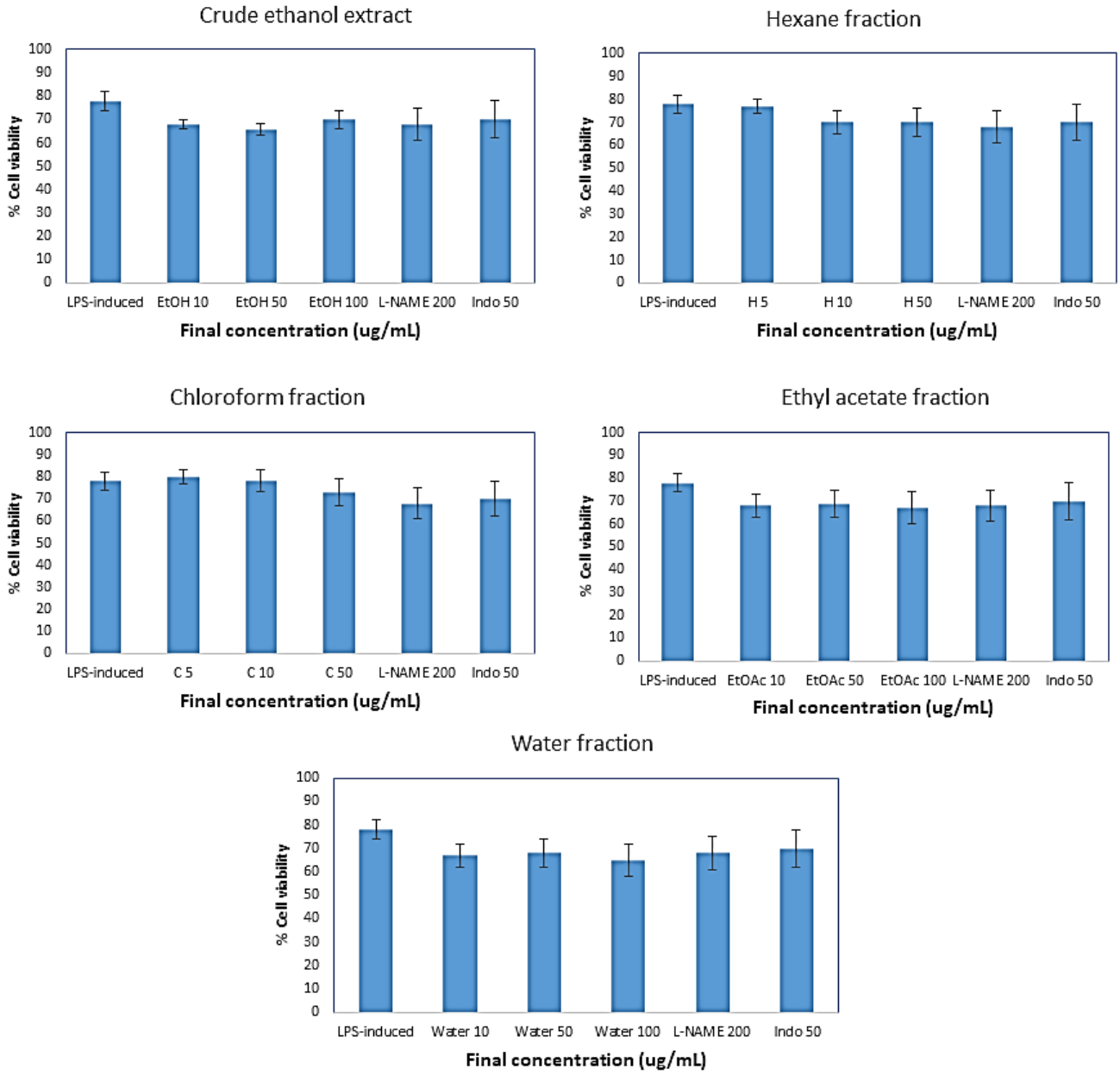

Figure 4. *Cells viability assay after NO experiment. Each bar shows the mean of three replicates experiments with the standard error of the mean (S.E.M.).*

Effect of hexane fraction on COX-2 protein expression

Hexane fraction showed the most potent anti-inflammatory property against LPS-induced inflammation. For this reason, it was selected to study the effect on COX-2 expression by western blot analysis. As showed in Figure 5A, result from western blot analysis demonstrated that COX-2 protein expression was not observed in unstimulated RAW264.7 cells. COX-2 protein expression was presented in LPS treatment cells. Density ratio of COX-2/β-actin was shown in Figure 5B. Indomethacin at a concentration of 50 µg/mL significantly reduced COX-2 expression of LPS-stimulated RAW264.7 cells (p<0.01). RAW264.7 cells-treated with hexane (10 and 50 µg/mL) showed a dose-dependent inhibition of COX-2 protein expression when compared with the LPS-treated cells (p<0.05 and p<0.001, respectively).

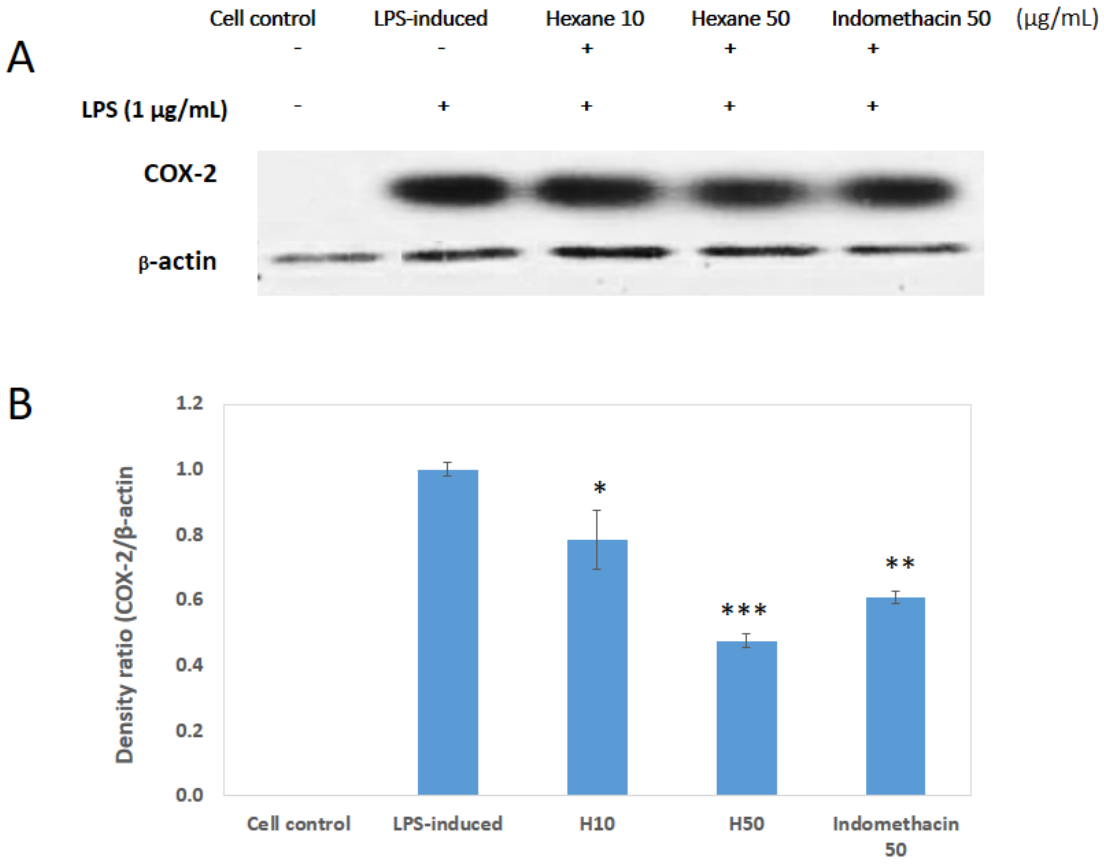

Figure 5. *Effect of hexane fraction of M. loriformis on COX-2 protein expression in LPS-stimulated RAW264.7 cells. A: Expression of the COX-2 protein was assessed by Western blot analysis; β-actin was used as the loading control, B: Density ratio of COX-2/β-actin. Densitometry data represents mean±S.E.M from three independent experiment, *, **, ***: significantly different (p<0.05), (p<0.01), and (p<0.001), respectively, from that of LPS-treated cells.*

Discussion and Conclusion

Macrophages play an essential role in the steps of inflammation including the initiation, maintenance, and resolution.[24] These cells are activated by various activation signals such as cytokines, bacterial lipopolysaccharide, extracellular matrix proteins, and other chemical mediators.[24] RAW264.7 cells are macrophage-like, derived from BALB/c mice. RAW264.7 cells stimulation by LPS causes increasing of NO production and enhance phagocytosis of cells.[25] NO produces from a family of enzymes called nitric oxide synthases (NOSs). The three distinct isoforms of NOS are endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS).[26] Inducible NOS normally is not present in resting cells it can be induced by cytokines, bacterial products or infection in various cells, including smooth muscle cells, endothelium, hepatocytes, monocytes, mast cells, and macrophages.[27] Overproduction of NO from iNOS can result in either protective or damaging effects. Protective effect involves in produces NO against microbial and viral pathogens infection.[28] However, at excessive concentrations, NO can display pathogenic properties. Production of ONOO-, NO•, and other reactive oxidizing compounds in the presence of superoxide radicals or peroxidases finally causing the oxidative damage of tissues.[26, 29]

We investigated effects of ML fractions on NO inhibition in LPS-stimulated RAW264.7 cells. Results showed all fractions except water fraction significantly decreased nitrite accumulation in culture supernatant. The high dose of ML fractions caused reduction of nitrite level similar to the positive controls. Among these, hexane fraction exhibited a potent effect on nitric oxide inhibition compared with other fractions. In NO inhibition assay, we used L-NAME (200 µg/mL) as a positive control. The results indicated its efficacy in reduced nitrite accumulation nearly ninety percent. Mechanism of L-NAME in decreasing of NO level involved iNOS inhibition.[30] We also used indomethacin as the second positive control for nitrite assay. Our results revealed that indomethacin (50 µg/mL) decreased nitrite level close to that of L-NAME. Inducible NOS has been reported correlation with COX-2 pathway.[31, 32] COX-2 an inducible enzyme is stimulated by LPS and several cytokines and involved in pathology of inflammation. COX-2 expression results in inflammatory mediators production including prostaglandins, prostacyclin, and thromboxane.[6] The results

from previous studies, demonstrated that NO activates COX to produce large amounts of prostaglandins and may result in an exacerbated inflammatory response.[32, 33] These studies led to the concept that COX enzymes as an important endogenous "receptor" target for amplifying or modulating the varied roles of NO in physiology and pathology.[31, 33]

Macrophages stimulation by LPS for iNOS expression represents a secondary effect requiring autocrine signaling of endogenously produced PGE_2. It seems to be mediated by up-regulation of COX-2 and followed by produced PGs that stimulate iNOS expression in an autocrine manner.[34] Our results found that indomethacin reduced NO accumulation better than L-NAME. In this study, COX-2 inhibition by indomethacin may be diminished PGE_2 production then decreased iNOS stimulation and afterward reduced NO production.

Since hexane fraction exhibited the most potent effect on NO inhibition, we further evaluated effect of this fraction on COX-2 expression. The results demonstrated that hexane fraction significantly decreased COX-2 expression in LPS-stimulated RAW264.7 cells. Anti-inflammatory mechanisms of the fraction may be via reducing of COX-2 expression and NO inhibition. From this present study, active anti-inflammatory substances of ML fraction are considered non-polar compound.[35] There were many reports about anti-inflammatory activity of non-polar solvent extracts from various plants species. Othman and colleagues have been reported anti-inflammatory activity of hexane partition from root *Jatropha curcas* towards RAW 264.7 cells.[36] In an *in vivo* study, hexane extract of Alchornea and a non-polar extract from *Rhododendron L*. exhibited anti-inflammatory activity over indomethacin.[37, 38] In 2013, Da and colleagues reported that hexane fraction of *Urtica dioica* exhibited anti-inflammatory activity by reduced rat paw edema.[39] These results along well with our findings of present study where hexane fraction demonstrated the most potent anti-inflammatory activity, indicating non-polar compounds were involved.

In conclusion, ML fractions reduced NO production in RAW264.7 cells. Nitric oxide inhibition remains an essential goal in inflammatory management. Therefore, ML fractions may be useful in inflammatory-related conditions. Hexane fraction demonstrated the most effectiveness in reducing NO accumulation and COX-2 expression. These results suggested that hexane fraction of ML might be a potent anti-inflammatory agent for inflammatory-related disease treatment. The active components of hexane fraction should be identified in future study.

Conflict of interests

The authors declare no conflict of interest.

Acknowledgements

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A study of self-esteem and academic achievement of undergraduate students with physical or locomotion disability in Chiang Mai University

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ABSTRACT

Background: Self-esteem reflects an individual's judgment concerning worth and accepting or rejecting of self that shows in one's attitude. Low self-esteem is a weakening condition that shields an individual from understanding their full potential and limits their engaging in an occupation.Academic achievement is viewed as a key criterion to judge one's total capabilities and potentialities. There are several studies about the self-esteem and academic achievement of undergraduate students; however, limited studies exist for undergraduate students with physical or locomotion disability.

Objectives: To determine the level of self-esteem in undergraduate students in Chiang Mai University with a physical or locomotion disability and explore the associations between self-esteem level and academic achievement.

Materials and methods: Data collection was performed during the academic year 2017, in a sample of 27 undergraduate students in Chiang Mai University with physical or locomotion disability using Rogers Rubin's Self-Esteem Scale, in order to identify the self-esteem level of the participants. Fisher's exact tests were used to explore the associations between the self-esteem level and the background characteristics of the participants.

Results: Unexpectedly, the undergraduate students with physical or locomotion disability had average or high self-esteem level, although there was no statistically significant association between academic achievement and self-esteem. Gender variable was significantly associated to the participants' self-esteem.

Conclusion: To promote self-esteem among undergraduate students with a physical or locomotion disability, occupational therapists should encourage them to engage in positive occupations that the person has the capability to accomplish and chooses to accomplish to regulate their rhythm of personal and community life.

Introduction

Number of people with a disability has been increasing with population growth and medical advances. In 2018, Thailand reported that 3.08% of the population were people with some disability.[1] There are seven categories of people with disability: hearing or communication disability, physical or locomotion disability, mental or behavioral disability, intellectual disability, learning disability, autism, and multiple disabilities. The majority, approximately 1,000,000 people, has physical impairments and locomotion limitations.[1]

The college years are a developmentally critical period in light of the fact that college students are making the transition from late adolescence to emerging adulthood.[2] During this period, college students are faced with numerous

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challenges and have an extensive variety of necessities.[3] On one side, the progress from secondary school to college is a noteworthy life change for some students. For students who move far from home, the transition to college decreases contact and, likely help, from family and companions. They have to adapt to changes autonomously and deal with their college life to adjust to the new condition.[4] On another side, they are faced with academic adjustment, or how well they manage educational demands, including inspiration to finish academic work, achievement in meeting academic requirements, academic exertion, and fulfillment with the academic condition.[5] Compas *et al*. reported that university life has more stress than students anticipate.[6] Troubles taking care of these stresses may prompt diminished academic performance and increased psychological distress.[7]

Self-esteem is one of the important resources for students undergoing the transition to university. Self-esteem is a personal judgment of his or her self-worth.[8] It is also designated as self-worth, self-respect, self-acceptance, self-regard, self-feeling, and self-evaluation.[9] Self-esteem ranges from extremely positive to extremely negative. An environment of success raises it while an environment of failure lowers it. A few indications of positive self-esteem will be certainty, an awareness of personal strengths, optimism, self-direction, an independent and cooperative attitude, a capacity to confide in others and to feel good with an extensive variety of feelings. Whereas a person who has low self-esteem feels incapable, dependent, unworthy, and incompetent, has a fear of being derided. Low self-esteem is a weakening condition that shields an individual from understanding their full potential.[10]

Academic achievement viewed as a key criterion to judge one's total capability and potential as students with high self-esteem appear to be more successful than those with low self-esteem. There are a few investigations that do not distinguish a significant relationship between self-esteem and academic performance. A few authors contend that low self-esteem does not really demonstrate poor academic performance.[11-14] Nevertheless, a number of other studies have shown a significant positive correlation between high-self-esteem and good academic performance,[15-16] thus, reinforcing the view that self-esteem is associated with academic achievement.

According to the Education for Individuals with Disabilities Act B.E. 2551 (2008), the Thai government sets out a few intentions to amplify instructive open doors for persons with disabilities through the improvement of special education and the promotion of integrated education in regular schools.[17] To respond to the government policy, Chiang Mai University (CMU) started enrolling disabled students from 2012. Most of them are students with physical or locomotion disability. Regardless of the increased number of disabled students attending CMU compared to a few decades ago, little is known about the self-esteem of undergraduate students with physical or locomotion disability. [18]

The World Federation of Occupational Therapists defines the profession as concerned with promoting health and well-being through occupation and identifies the profession's primary goal as enabling people to participate in activities of daily life.[19] A previous study reported that low self-esteem is like a loss of inner certainly and power and has an impact on occupational performance.[20] Therefore, this study aimed to investigate the self-esteem level and explore the associations between self-esteem level and academic achievement of undergraduate students in CMU with physical or locomotion disability. Our results may help occupational therapists supervise disabled students who experience difficulties related to low self-esteem.

Materials and methods

Participants

A cross-sectional study was conducted among the total population of undergraduate students in CMU with physical or locomotion disability, Thailand, in the academic year 2017 (N=27).

Ethical approval for the study was obtained from the ethical review committee for research in humans, Faculty of Associated Medical Sciences, Chiang Mai University (approval number: AMSEC-60EX-052). All participants signed a written informed consent before the commencement of the study.

Measurements

All participants completed a general questionnaire designed to collect demographic data, characteristics of gender, age, faculty of studies, and grade point average (GPA).

Rogers Rubin's Self Esteem Scale is a widely used self-report instrument for evaluating individual self-esteem. Rogers Rubin's Self Esteem Scale was developed by Rosenberg and adapted by Paganan and Noisuwan to Thai. The scale was shown to have good reliability and validity.[21-22] In the current study, the Cronbach alpha coefficient of Rogers Rubin's Self Esteem Scale was 0.77. This scale contains 62 items with 3 components; 30 of the items measure the self-concept, 10 of the items measure the self-esteem scale, and 22 of the items measure self-concept rating. The positive and negative items were presented in random order to reduce the effect of respondent set. Each component had a different rating. Participants rated each self-concept item using a dichotomous scale which is a two-point scale (yes/no). Items 1, 2, 3, 4, 8, 10, 13, 16, 17, 18, 19, 20, 22, 23, 25, 27, 29 were reversed scored. Scale format ranging was categorized as follows: "yes"- 2 point, "no"- 1 point. Participants rated each self-esteem item using a 4-point Likert scale format ranging from strongly agree to strongly disagree. Items 33, 35, 38, 39, 40 were reversed scored. Scale format ranging was categorized as follows: "strongly agree"/ "agree"- 2 point, "disagree"/ "strongly disagree" - 1 point. Participants rated each self-concept rating item using a 5-point Likert scale format ranging from never to most of the time. Items 50, 56, 59 were reversed scored. Scale format ranging was categorized as follows: "never" - 1 point, "rarely"- 2 point, "sometimes"- 3 point, "often" - 4 point, "all of the Time"- 5 point. All of the scoring of items was performed reversely for negative questions and directly for positive questions. Scores ranged from 62 to 190, scores between 159-190, 95-158 and 62-94 were

considered as high, average, and low self-esteem, respectively.

Statistical analysis

Data analysis was performed using the statistical package SPSS version 17. Descriptive statistics such as frequencies, mean, and standard deviations were carried out for the variables of the study. Fisher's exact tests were used to explore the associations between the self-esteem level and the background characteristics of participants. The level of significance was set at $p<0.05$.

Results

A total of 27 students with physical or locomotion disability, from undergraduate study programs participated in the study. Their ages ranged from 19 to 25 years (mean=20.74, SD=1.63), with more female than male participants. The years of study ranged from 1 to 7 years (mean=2.78, SD=1.65). The faculty with the highest number of registered students was the faculty of Education with 25.93% of the research participants. The GPA, which was used as an index of academic achievement, ranged from 0.82 to 3.2 (mean=2.28, SD=0.63). Among students with physical or locomotion disability, 70.37% had a GPA of more than 2.00. Demographic characteristics of participants are presented in Table 1.

The results revealed that most of the students with physical or locomotion disability had self-esteem in an average level (59.26%) and high level (40.74%). However, none of the students had a low self-esteem level (Table 2).

Bivariate analyses revealed statistically significant differences ($p<0.05$) in levels of self-esteem with regard to gender (Table 3). However, the GPA was not significantly associated with self-esteem level ($p>0.05$). The results are presented in Table 4.

Table 1 Demographic characteristics of the participants (N=27).

Characteristics	Number	Percentage
Gender		
Female	19	70.37
Male	8	29.63
Age (years)		
19	6	22.22
20	8	29.63
21	7	25.93
22	3	11.11
23	1	3.70
24	0	0
25	2	7.41
Year of study		
Year 1	6	22.22
Year 2	8	29.63
Year 3	6	22.22
Year 4	4	14.81
Year 5	1	3.70
Year 6	0	0
Year 7	2	7.41
Faculty of studies		
Faculty of Humanities	4	14.81
Faculty of Fine Arts	3	11.11
Faculty of Science	4	14.81
Faculty of Engineering	1	3.70
Faculty of Education	7	25.93
Faculty of Business Administration	4	14.81
Faculty of Law	2	7.41
Faculty of Political Science and Public Administration	2	7.41
Grade point average (GPA)		
<2.00	8	29.63
2.00-4.00	19	70.37

Data are presented as number and percentage.

Table 2 Distribution of the undergraduate students with physical or locomotion disability on self-esteem.

Levels	Physical or locomotion disabled students	
	Number	Percentage
High self-esteem	11	40.74
Average self-esteem	16	59.26
Low self-esteem	0	0
Total	27	100

Table 3 Association between gender and self-esteem in students with physical or locomotion disability.

Group	Total numbers of students	Self-esteem level		*p*-value
		High	Average	
Female	19	5 (26.3%)	14 (73.7%)	0.033*
Male	8	6 (75.0%)	2 (25.0%)	
Total	27	11	16	

**Significant values p<0.05-Fisher's exact tests.*

Table 4 Association between academic achievement and self-esteem in students with physical or locomotion disability.

Group	Total numbers of students	Self-esteem level		*p*-value
		High	Average	
GPA <2.00	8	2 (25.0%)	6 (75.0%)	0.405
GPA 2.00-4.00	16	9 (47.4%)	10 (52.6%)	
Total	27	11	16	

GPA: Grade point average

Discussion

The study demonstrated that undergraduate students with physical or locomotion disability had an average and a high self-esteem level. Surprisingly, none of the students had a low self-esteem level (Table 2). Previous studies reported that people with disabilities often suffer stigmatization. They often receive negative remarks related to their body, which contributes to the development of the low self-esteem.[23-25] Possible mechanisms through which stigma may decrease self-esteem have been proposed. Self-esteem may be decreased if disabled people incorporate others' stigmatizing reactions into their self-representations.[26-27] Self-esteem is a form of personal appreciation, self-acceptance, and subjective respect of one's own.[28] Self-esteem is to assess the contrast between their ideal self and image of self. We comprehend the self-esteem level of the person by looking at the disparity between how the individual sees oneself and the self they might want to be.[28] The results from this study revealed that 77.8% and 74.07% of disabled students answer "no" in statement "I have a bad shape" and "Now, I want to be different from myself", respectively (data not shown). It is implied that the individual's feelings such as personal appreciation, self-acceptance, and overall acceptance of personality was found in students with physical or locomotion disability. Therefore, self-esteem ascends in the consequence of self-evaluation of the individual.

Moreover, the results of the study indicated that none of the students with physical or locomotion disability had a low self-esteem level. It might be due to these students having a sense of competence. Previous studies reported that the advancement of competence in the tasks and activities of one's appreciated roles promotes a sense of self-esteem and self-efficacy. Effective interaction with the physical and social environments refers to competence. To be competent means to have the skills that are adequate or sufficient to meet the demands of a task or situation.[29] It does not compare to magnificence, ordinariness, or the capacity to do everything, and it perceives that there are degrees of adequacy and sufficiency in individuals. The education of people with disabilities has been a part of policy development in Thailand for the past few decades. However, the Department of Empowerment of Person with Disabilities reported that only 0.35% of people with disabilities are studying in college. CMU is currently perceived as a complete standout amongst the best colleges in the northern part of Thailand. The Office for National Education Standards and Quality Assessment of Thailand presently ranks it as one of the Top Three Universities in the country with their mission comprised to provide academic service to society and cultural preservation, produce research, and produce graduates. By getting a placement

to study in CMU, the students with a handicap had a higher perceived self-esteem. In addition, providing educational opportunity for students with physical or locomotion disability removed society's barriers that confine life decisions for students with disabilities. At the point when obstructions are removed, incapacitated individuals can be autonomous and break even within the public arena, with decision making and command over their own lives. This independence and equality of disabled students increase their self-esteem.

The current study was conducted for the purpose of investigating if there is any relationship between students' self-esteem and their academic performance. Although several studies showed associations between self-esteem and academic achievement,[15-16] the results of the study showed that there was no statistically significant association between academic achievement and self-esteem (Table 4). According to The Model of Human Occupation (MOHO), volition includes a deep human drive for action, combined with thoughts and feeling about a thing, shaped by previous experience and linked to the future. Volitional thoughts and feelings pertain to (1) how effective one is in acting on the world, (2) what one holds as important and meaningful in doing, and (3) what one finds enjoyable and satisfying to do.[30] These three sets of thoughts and feelings are referred to as personal causation, values, and interests. From these statements, we can explain the reason why there was no association between self-esteem and academic achievement in disabled students. It may be due to the participants have other activities than studying that are valuable to them. For example, a student who is committed to attending a competitive college may anticipate anxiety, put forth substantial effort studying and then react negatively to getting an average grade, whereas another student who plans to enter a skilled trade may be much less concerned with preparing for an examination and be quite pleased with receiving an average grade.

Another finding of the research indicated an association between self-esteem and gender (Table 3). In other studies, the same findings were stated. Termini and Roya Valibeygi (2011) studied the impact of gender, age and academic branch on self-esteem of normal students from Sistan and Baluchestan University using self-esteem scale and revealed that male students showed significantly higher total scores of self-esteem than female students.[31] Studies conducted by McMullin and Cairney (2004) in normal students also emphasize that female students had lower self-esteem than male students.[32] Although there were several studies on the correlation between gender and self-esteem which found that male students had more self-esteem than female students, these studied were conducted with normal students. Therefore, it is recommended to explore the association between self-esteem and gender with disabled students in future studies.

Occupational therapists define occupation in various ways, but all definitions include the idea of activities of everyday life that are meaningful to individuals.[33] "Occupation is everything people do to occupy themselves, including looking after themselves, enjoying life and contributing to the social and economic fabric of their communities".[34] Personal identity emerges from a harmonious balance of the many meaningful occupations in which a person engages over time.[35] Individuals occupied with occupation characterize their way of life as well as accomplish a feeling of capability and report a feeling of fulfillment and satisfaction.[33] Occupational engagement contributes to the experience of a life worth living[36] even though undergraduate students with physical or locomotion disability had an average and high self-esteem level. Occupational therapists should be encouraging them to engage in positive occupations that the person has the capability to achieve and chooses to achieve in order to manage the rhythm of individual and community life. Such occupations bring out imagination and absorb the attention, promote feelings of fulfillment with achievement, and contribute to a sense of self-efficacy and self-esteem.

There are some limitations of this study. This study included one research university. Further study may include other universities and may compare differences by region, education types, or disability types. Moreover, further studies should look more closely at factors affecting the self-esteem level.

Conclusion

The undergraduate students with physical or locomotion disability had an average and a high self-esteem level. However, no association was found between self-esteem and academic achievement. Students should be encouraged to engage in an activity meaningful to them, in order to improve self-esteem.

Conflicts of Interest

The researchers claimed no conflicts of interest.

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Validity and reliability of the pediatric voice handicap index: Thai version

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ABSTRACT

Background: Pediatric Voice Handicap Index (pVHI) is one of the most widely used self-assessment tools for pediatric voice disorders which is completed by the children's parents. pVHI has been translated into several languages, but not in Thai.

Objectives: This study was conducted in order to translate pVHI into Thai and to assess the validity and reliability of the Thai version of pVHI.

Materials and methods: The original pVHI has been translated into Thai using the World Health Organization's (WHO) backward standard translation approach. Thai-pVHI was administered to 30 parents whose children had voice disorders. Two qualified speech-language pathologists and two translation experts verified the content validity. Internal consistency was determined using Cronbach's alpha coefficient, and the test-retest reliability of Thai-pVHI was calculated using intraclass correlation coefficient.

Results: The result showed that Thai-pVHI had excellent internal consistency on both total subscales (α=0.855) and functional subscale (α=0.851), strong internal consistency on the physical subscale (α =0.747) and the emotional subscale (α=0.716). Moreover, Thai-pVHI also had excellent test-retest reliability on its subscales: total (ICC=0.917), functional (ICC=0.973), physical (ICC=0.896), and emotional (ICC=0.914).

Conclusion: Thai-pVHI met the acceptance criteria for psychometric evidence relative to internal consistency and test-retest reliability. This suggests that this instrument might be used as a Thai parental proxy for measuring the severity of children's voice disorders, impact on a child's quality of daily life, and efficacy of treatments in pre- and post-interventions.

Introduction

Human beings use their voice to communicate and sing as well as to express their thoughts, emotions, and feelings. Consequently, voice is vital to individuals of all ages. Children express what they feel and think by speaking. If there is an abnormality in their voice, it will have negative effects on their communication and quality of life. Voice handicaps in children refer to a change in their sound, pitch, loudness, and voice quality, generally impeding their communication capability.[1-3] Incidence rate of voice handicaps in children is between 6% and 23%.[4-6] Children with voice handicaps encounter negative effects regarding communication, social relationships, self-esteem, participation in school activities, as well as negative feedback from adults and their peers.[2, 7-12] There are now available number of instruments used to evaluate voice handicaps in children. For example, abnormalities of vocal structure can be assessed using traditional endoscopic imaging. However, this technique can not provide information about the effects of voice handicaps on the quality of the children's lives.

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Moreover, some instruments for assessing the effects of voice handicaps in children are available, including Pediatric Voice Related Quality of Life (PVRQOL),[13] Pediatric Voice Outcome Survey (PVOS),[14] Pediatric Voice Symptom Questionnaire (PVSQ),[15] Children's Voice Handicap Index-10 for Parents (CVHI-10-P),[16] and Pediatric Voice Handicap Index (pVHI).[17]

Parents use Pediatric Voice Handicap Index (pVHI)[17] to assess voice handicap indices of their children and to evaluate the effects of their voice handicaps. This index was developed and validated in the United States by Zur et al.[17] and was adapted from an index used for adults.[18] The index is composed of 23 question items scoring from 0 to 4 on a Likert-type rating scale. The question item responses assess the severity levels of the effects of voice handicaps in the following three aspects: seven items for functional aspect (F), nine items for physical aspect (P) and seven items for emotional aspects (E).[17] This index is widely utilized and is accepted in pediatrics because it is used friendly with fewer items for evaluation and full coverage of the three aspects. Moreover, its validity and reliability are at high levels. The index has been translated into many languages such as Italian,[19] Korean,[7] Arabic,[20] Malayalam[21] and Turkish.[22] However, in the Thai context, pVHI has not yet been translated or validated, and reliable evaluation indices for assessing voice handicaps in Thai children had thus far not been available in Thai. As a consequence, the objectives of this research were to translate the Pediatric Voice Handicap Index (pVHI) from English into Thai and to investigate the validity and reliability of the translated index by using internal consistency and test-retest reliability.

Materials and methods

1. Translation of Thai-pVHI

Initially, permission to translate the three aspects of pVHI into Thai was granted by the copyright owners, the copyright office of the American Speech, Language and Hearing Association (ASLHA), and the International Journal of Pediatric Otorhinolaryngology. Afterwards, the index was forward-translated and back-translated based on an adaptation of WHO articles.[23] The steps of forward-translation and back-translation were as follows: (I) Translation of English index into Thai was conducted by a speech therapist who was proficient in both Thai and English. Thus, the first Thai version of the index was provided; (II) Back-translation of Thai version into English was done by a proficient translator who did not have any knowledge regarding the index. Accordingly, a verified back-translated version of the index was provided; (III) determination of content validity and reliability of forward-translated and back-translated Thai versions of the index were conducted by the researcher, her supervisor, and two translators who were not the same individuals as in step II. Purpose of this step was to analyze and improve the accuracy of the index items. Thus, a second translated Thai version of the index was available; (IV) The second Thai version was pilot tested for its content validity with five participants who were parents of 3-to 12-year old children in December 2017. They completed the assessment index and were interviewed for their understanding and responses on the items of each topic. After that, improvements were made and the final Thai version of the index with adequate content validity and reliability was completed. It contained 23 question items with scores ranging from 0 (never) to 4 (always). This index assessed the severity of the effects of voice handicaps of children relative to three aspects: seven items for functional aspect, nine items for physical aspect, and seven items for emotional aspect (Appendix A).

2. Participants

The participants were parents of 30 children, aged 3-12 years, who had been diagnosed by otorhinolaryngologists. They suffered from voice handicaps without a hearing impairment. They received medical treatment at the Department of Otolaryngology, Faculty of Medicine, Chiang Mai University, during December 2017 to March 2018. Parental participants volunteered and were willing to participate in this research study by completing a written consent form.

3. Evaluation of psychometric properties

3.1 Content validity

Question items of the index were validated after completion of forward- and back-translation process. The consensus was reached among researcher, her major thesis advisor, and two translation specialists in order to improve the accuracy of index and determine its content validity.

3.2 Reliability

Internal consistency of the index was computed using Cronbach's alpha coefficient and test-retest reliability of the final Thai version was determined using intraclass correlation coefficients (ICC). Moreover, ICC model (3, 1) was used again with the same sample participants two weeks after the first administration.

3.3 Statistical analysis

SPSS Version 25 was used to analyze the index data relative to frequencies, percentages, and means. Descriptive statistics were used to analyze the general information of the participants. Cronbach's alpha coefficient, with a 95% confidence interval, was used to determine the internal consistency. Intraclass correlation coefficient, based on the ICC model (3, 1), with a 95% confidence interval, was used to determine test-retest reliability of the index.

Results

Demographic characteristics

Table 1 contains general information of the 30 participants involved in this study. They were parents of children who were diagnosed as having a voice handicap without hearing impairment. These children included 17 males (56.67%) and 13 females (43.33%) with ages ranging from 3 to 12 years. Most of the participants, 11 children or

36.67%, were diagnosed as having subglottic stenosis and 28 of them, 93.33%, had undergone a tracheostomy. However, one child diagnosed as having a vocal nodule was an exception.

Table 1 Demographic characteristics and diagnoses of voice disorders of the participating children.

Characteristics	Children (n = 30)
Gender	**n (%)**
Male	17 (56.67)
Female	13 (43.33)
Age	
3-3.11 years	4 (13.32)
4-4.11 years	6 (20.00)
5-5.11 years	8 (26.67)
6-6.11 years	2 (6.67)
7-7.11 years	2 (6.67)
8-8.11 years	2 (6.67)
9-9.11 years	0 (0.00)
10-10.11 years	3 (10.00)
11-12 years	3 (10.00)
Types of diseases	
Subglottic stenosis	11 (36.67)
Tracheal stenosis	5 (16.67)
Tracheomalacia	2 (6.67)
Bilateral true vocal cords paralysis	2 (6.67)
Bilateral true vocal cords impaired mobile	1 (3.33)
Vocal nodules	1 (3.33)
Subglottic web	1 (3.33)
Tracheal papilloma	1 (3.33)
Laryngeal papilloma	1 (3.33)
Frontoethmoidal encephalomeningocele	1 (3.33)
Lymphatic malformations	1 (3.33)
Micrognathia	1 (3.33)
Supraglottic swelling	1 (3.33)
Posterior glottic stenosis	1 (3.33)

Internal consistency

Total internal consistency of Thai version of the index had an excellent Cronbach's alpha coefficient (α=0.855). The functional aspect also had an excellent level (α=0.851) while the physical aspect had a high level (α=0.747) similar to the emotional aspect which also had a high level (α=0.716). These results are in Table 2.

Test-retest reliability

Total test-retest reliability was at an excellent level (ICC=0.917). The functional, physical and emotional aspects were all at the excellent level; ICC=0.973, ICC=0.896 and ICC=0.914, respectively, as shown in Table 2.

Table 2 Distribution of the undergraduate students with physical or locomotion disability on self-esteem.

Domain	No. of items	Internal consistency (Cronbach's alpha)	Test-retest reliability (ICC)
Total	23	0.855	0.917
Functional	7	0.851	0.973
Physical	9	0.747	0.896
Emotional	7	0.716	0.914

Discussion

The study findings showed that total internal consistency and functional aspect of the Thai version of pVHI were at the highest levels. The internal consistencies of physical and emotional aspects were at a high level (Table 2) and are within the acceptable criteria. These findings may be due to quality of the original English index which had undergone reliability assessments and internal consistency assessments. These results indicated that its reliability was at a high level and were considered acceptable.[17] Moreover, specialists in the effects of voice handicaps of children directed the development of the original index, which was adapted from the Voice Handicap Index (VHI) for adults.[18] The VHI, upon which the pVHI is based, has been standardized and its validity and reliability determined using various sample groups with different cultural backgrounds.[24-26] These results reported that the original pVHI was standardized and that pVHI is useful in thoroughly assessing the effects of voice handicaps in children. When Thai language version pVHI underwent the systematic forward-translation and back-translation processes of the WHO,[23] the results indicated that the original concepts and contents were well matched. The assessment methods for Thai version were consistent with those of the original pVHI. Additionally, translators in each translation step were highly qualified and proficient in both Thai and English, resulting in Thai-version index having an internal consistency at the highest level. These findings indicate that Thai index is consistent with the original pVHI and evaluates the same aspects: functional, physical, and emotional, respectively.

The analysis results of test-retest reliability showed that ICC of the three aspects were between 0.896 and 0.973, and total ICC was 0.917. From consideration of the reliability coefficient criteria,[27] these findings were between 0.75 and 1.00, which means the highest levels of reliability of Thai-pVHI. This finding may be due to the fact that two-week interval between assessments was suitable[28] and that interval did not change the effects of voice handicaps of children. Furthermore, the assessments were behavioral observations and the scoring was given by the same parents, which were more consistent than if the assessments had been administered and analyzed by different evaluators. This finding further confirmed test-retest reliability of the original pVHI as reported by Zur et al.[17] It was found that the reliability was at a high level, indicating that Thai-pVHI demonstrated an evaluation quality consistent with the original. Simply stated, no matter how many times the test-retest assessment was conducted, results remained the same. These consistent results may have also been due to the consistency of evaluators and the short interval between the first and second evaluations, resulting in the evaluation results being consistent and virtually unchanged.

One limitation of this research was that before administering this index, the evaluators needed to ensure that the procedures of index were clearly understood. This extra step was required because some items may have been difficult to understand since the original item words were matched during translation process. Another limitation of the present study was its small sample size.

Conclusion

Based upon the results of this study, the validity and reliability of Thai-pVHI were acceptable. Therefore, Thai-pVHI can be used to evaluate the severity and effects of voice handicaps of children reported by their parents. These assessments can also be used to evaluate pre- and post-training effectiveness of therapy programs designed for each child with a voice handicap.

Conflict of interest statement

The authors declare no conflict of interest to report.

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Appendix A. The Thai version of the pVHI

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Effects of multi-faceted cognitive training program for elders with cognitive impairment living in social welfare home for older persons

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ABSTRACT

Background: Elders living in social welfare home are at risk of developing dementia due to lacking of opportunities to perform problem solving activities under non-familiar situations. Dementia declines cognitive functions leading to a limitation of performing individual activities of daily living. Therefore, the elders need a cognitive training program which may be enhancing their cognitive abilities. That program's effectiveness would be beneficial in slowing down the rise in numbers of demented elders who live with cognitive impairment at the social welfare home.

Objectives: To study the effects of a multi-faceted cognitive training program on cognitive abilities in elders with cognitive impairment living in social welfare home.

Materials and methods: Participants were the elders living at Thammapakorn Social Welfare Development Center for Older Persons, Chiang Mai Province. Twenty-four elders with cognitive impairment were selected through purposive sampling method combined a set of screening tests, including the Mini-Mental State Examination (MMSE), the Montreal Cognitive Assessment (MoCA), and the Depression Assessment 9 Questions (9Qs). Participants were divided into a control and an experimental group (n=12 each). The experimental group underwent multi-faceted cognitive training program 3 times per week, for 6 consecutive weeks. The outcome measurements were the Digit Span Test, the Thai Cognitive-Perceptual Test (Thai-CPT), and the Dynamic Loewenstein Occupational Therapy Cognitive Assessment (DLOTCA). Data was analyzed using descriptive, Mann-Whitney U Test, and Wilcoxon Signed Rank Test.

Results: After completing the multi-faceted cognitive training program, both experimental and control groups had demonstrated statistically significant differences in post-test in attention, memory, and executive functions. Within the experimental group, there was no significant difference in those cognitive areas, whereas the reduction of cognitive scores was significantly found within the control group.

Conclusion: The multi-faceted cognitive training program could prevent cognitive deterioration for elders with cognitive impairment living in social welfare home.

Introduction

Currently, dementia is more prevalent worldwide. It was found that 91-98% of people with dementia are older than 65 years old.[1] In Thailand, prevalence of dementia in elderly people is 12.4% of the total elderly population.[2] Elderly people with dementia has cognitive impairments in 3 main areas including attention, memory, and executive function.[3] The impairments cause a reduction of independent daily living affecting quality of life of the elders themselves, their caregivers and families, society and nation.[4] There is no cure for dementia and the symptoms tend to worsen over

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time.

Current research focused on reducing the risk factors which cause dementia and in early rehabilitation of cognitive skill in elders with mild cognitive impairment (MCI) to prevent or slow down dementia in high risk elders. Elderly people at high risk are those with mild cognitive impairment (MCI),[5] whose cognitive ability is regarded as less compared to same-aged individuals, but their daily activities are not yet affected.[6] These people can be identified by preliminary dementia screening. In Thailand, 95.6% of elderly people in community are not diagnosed in early stages of dementia. This shows limited access to healthcare for diagnosis and rehabilitation.[7]

Treatment guidelines for dementia are divided into 2 main types. There are pharmacological treatment, which provides good results in the first year for people with mild to moderate dementia,[8] and non-pharmacological treatment. Cognitive training is a commonly used treatment in the latter. Studies of the effectiveness of different cognitive trainings found that they could prevent and slow down the impairment of cognitive skill in elderly people with MCI.[9-12] Characteristics of cognitive training programs can include specific skill training using tabletop activities, drills, computer-based activities, strategy training, and multi-faceted cognitive training e.g. providing information on nutrition, exercise, leisure, relaxation techniques, group activities, and lifestyle modification.[13,14]

Through literature reviews of related studies in Thailand, it was found that there were some studies on developing cognitive training programs in samples who were elderly people with cognitive impairment or dementia suspects. The programs included specific skill training or multi-faceted training. The trainings were conducted in clinical settings by healthcare providers[15,16] or community public health volunteers[17] and by the samples themselves at home.[18] This shows that the studies gave priority to the home and community contexts in order to decrease elderly people's barriers of accessing healthcare services. However, there is an increasing number of elders who needed to live in social welfare homes for older persons due to the increase of the elderly population in Thailand and the change in society, culture, and economy. For example, there is an increase in the number of elderly people living alone or with income below the poverty line. The elders in need of care could not obtain it and there are limited healthcare services. From a survey in 2010, there were 2,610 elderly living in social welfare homes for older persons[19]. Elderly living in social welfare homes for older persons are at 41.6% risk of having dementia.[1] From reviewing the past studies regarding the contexts of social welfare homes for older persons, there were nominal reports about cognitive training. Elders lived in those contexts may lack the opportunity for health prevention of dementia designed by providing in-house cognitive training.

Therefore, this study aimed to develop a cognitive training program for the elders living in social welfare homes for older persons and examined its effects on cognitive abilities in 3 cognitive skills; attention, memory, and executive functions. This cognitive training program was multi-faceted created based on occupational therapy frameworks and models, relevant research, and the researcher's clinical experiences. The researcher expected that this cognitive training program might help to promote, or at least, maintain cognitive ability for elders living in the social welfare home and delay the possible occurrence of dementia.

Materials and methods

Research Design and Participants

The study employed a quasi-experimental, assessor-blinded research design, using before and after experiment tests with a control group. Participants were elders with cognitive impairment living in Thammapakorn Social Welfare Development Center for Older Persons, Muang District, Chiang Mai Province. Calculation of sample size was done by G*power 3.1.[20] The study by Pratumtarn, Rattakorn, and Munkhetwit[18] found 1.29 effect size with 0.05 deviation score, and 0.80 test power. Calculation revealed sample size of 8 people in each group. Thus, researchers added 50% on top, which was 4 people, in case of sample drop out from research. Therefore, total sample size for this study was 24 people; 12 in each group. Inclusion criteria for the participants were: 1) either male or female aged 60-80 years old 2) able to read and communicate in Thai language, 3) scored more than 18 on the MMSE for those who graduated from primary school, more than 23 for those who graduated from higher educational levels, 4) scored between 11-25 with cognitive impairment for the Montreal Cognitive Assessment (MoCA) Thai version, 5) no dementia, 6) no severe depression with scored equal to or less than 18 for the Depression Assessment 9 Questions (9Q) and not receiving anti-depressive drugs, 7) no physical impairment which could not perform the multi-faceted cognitive training program, 8) no diagnosis of any other mental health conditions, and 9) was willing to participate with volunteer basis. The participants were excluded if they had no intention to finish the research project or participated in the experimental group less than 80% of the program.

Outcome Measure

Outcome measurement for assessing the effects of the multi-faceted cognitive training program on cognitive skills were:

1. Digit span test, subtests forward attention for assessing attention
2. Thai Cognitive-Perceptual Test: Thai-CPT, subtest memory for assessing memory
3. Dynamic Lowenstein Occupational Therapy Cognitive Assessment (DLOTCA, subtests colored block design, clock drawing, and pictorial sequence A & B for assessing executive functions

Intervention Program

The multi-faceted cognitive training program was aimed to promote attention, memory, and executive function skills which were commonly found in elders with mild cognitive impairment. The program was developed with the concept based on occupational therapy models called

'Person Environment Occupation Performance Model (PEOP)' by Christiansen and Baum[21] and the 'Dynamic Interactional (Multi-Context) Approach' by Toglia.[22] These two models were employed as a conceptual framework for determining factors, appropriate conditions or situations to stimulate the cognitive ability for elders living in the social welfare home. Cognitive performance is a complex phenomenon that is interconnected among many variables. These include person, unique environment in which one functions, and occupations.[21] Thus, the program is focused on changing of person's strategies and self-awareness, modifying external factors such as activity demands, and environment to facilitate cognitive performance.[23] In addition, activity features, duration and frequency of the training were designed based on related researches.[15-18] This multi-faceted program composed of providing knowledge about dementia and its effects, specific cognitive skill training using games and drills, group activities, role play activities, field trip activities, and teaching relaxation technique. Training time composed of 18 sessions at the frequency of 3 sessions per week for 6 consecutive weeks. Each session was approximately 60 minutes.

Statistical Analysis

General information of the participants was analyzed using descriptive statistics and the Chi-Square test. The Mann-Whitney U test was employed for comparing cognitive scores before and after the experiment between the control and experimental group. Comparison of cognitive scores before and after experiment within groups was analyzed using the Wilcoxon Signed Rank test.

Results

Results of the analysis and distribution of general information including gender, age, education level, and scores from the Mini-Mental State Examination (MMSE), the Montreal Cognitive Assessment (MoCA), and the Depression Assessment 9 Questions (9Q) of the participants are shown in Table 1.

Table 1 Participant characteristics (control group n=12, experimental group n=12).

Characteristics	Control group n (%)	Experimental group n (%)	Chi-square
Gender			0.68
Male	6 (50%)	7 (58.33%)	
Female	6 (50%)	5 (41.67%)	
Age			0.26
60-65 yr.	1 (8.34%)	2 (16.67%)	
66-70 yr.	4 (33.33%)	2 (16.67%)	
71-75 yr.	3 (25%)	4 (33.33%)	
76-80 yr.	4 (33.33%)	4 (33.33%)	
Mean±SD	72.25±5.01	72.75±5.98	
Education			0.36
Primary	6 (50%)	5 (41.67%)	
Secondary	3 (25%)	1 (8.33%)	
High school	3 (25%)	4 (33.33%)	
Bachelor degree	0	2 (16.67%)	
Screening score (mean±SD)			
MMSE	25±3.39	26±2.73	0.41
MoCA	17.75±4.37	18.17±3.59	0.48
9Q	3.25±5.01	4.17±2.89	0.47

From Table 1, distribution of gender between control group and experimental group was fairly equal. The average age of the control and the experimental group was 72.25±5.01 years and 72.75±5.98 years, respectively. Education levels of the control and the experimental group were primary education level at 50% and 41.67%, respectively. Results from cognitive screening using the MMSE revealed the average scores for the control group was 25±3.39 and 26±2.73 for the experimental group. On the MoCA assessment, the control group's average score was 17.75±4.37 and 18.17±3.59 for the experimental group. On the 9Q, the control group's average score was 3.25±5.01 and the experimental group's score was 4.17±2.89. On the Chi Square test used to test the differences of participant characteristics before experiment between control and experimental groups, correlation was found on general information on gender, age, and education level at 0.68 0.26 and 0.36. Correlation of scores from the MMSE, the MoCA and the 9Q were 0.41, 0.48, and 0.47, respectively. This showed that before experiment, characteristics of the control and

the experimental group were not statistically different.

Table 2 Mean rank and p value before and after experiment between control and experimental groups.

Cognitive skills	Pre-test		*p* value	Post-test		*p* value
	Mean rank			Mean rank		
	Control group (n=12)	Experimental group (n=12)		Control group (n=12)	Experimental group (n=12)	
Attention	13.54	11.46	0.45	9.08	15.92	0.02*
Memory						
Recall	12.08	12.92	0.77	9.29	15.71	0.02*
Recognition	12.46	12.54	0.95	11.50	13.50	0.15
Total	12.08	12.92	0.77	8.96	16.04	0.01*
Executive functions	11.54	13.46	0.50	9.42	15.58	0.03*

*NB: *statistically significant difference in means.*

Table 2 demonstrated the statistical analysis of the cognitive scores between the control and the experimental group before and after the experiment. At base line, the cognitive scores of all cognitive skills; attention, memory, and executive functions, between the control and the experimental group were not significantly different (p=0.45, 0.77, and 0.50 respectively). This indicated that the two groups did not differ in cognitive ability before the experiment. After the experiment, it was found that there were significant differences of the cognitive scores between the control and the experimental group (p=0.02, 0.01, and 0.03 respectively), which the experimental group's average scores were higher than the f control group (17.33>7.67, 16.04>8.96, and 15.58>9.42, respectively). However, in memory skill, it was found that recognition showed no significant difference.

Table 3 Mean rank, z-score, p value before and after within control group.

Cognitive skills	Negative ranks (post<pre)		Positive ranks (post>pre)		Ties (post=pre)	z	*p* value
	n=12	Mean rank	n=12	Mean rank			
Attention	10	5.50	0	0.00	2	-2.81[a]	0.001*
Memory							
Recall	12	6.50	0	0.00	0	-3.09[a]	0.001*
Recognition	1	1.50	1	1.50	10	0.00[b]	1.00
Total	11	6.00	0	0.00	1	-2.96[a]	0.001*
Executive functions	9	5.44	1	6.00	2	2.23[a]	0.03*

*NB: *statistically significant difference in means, a: based on positive ranks, b: the sum of negative ranks equals the sum of positive ranks*

Comparison of cognitive scores before and after the experiment within the control group is shown in Table 3. Significant differences of the scores was found between baseline and post experiment (p=0.001, 0.001, and 0.03). Most of the participants had negative ranks on attention (10 persons), memory (11 persons), and executive functions (9 persons) after the experiment. This indicated lower cognitive ability of the control group after the experiment. In memory skill, there was no significant difference in recognition score (p=1.00) in this group.

Table 4 Mean rank, z-score, p-value before and after within the experimental group.

Cognitive skills	Negative ranks (post<pre)		Positive ranks (post>pre)		Ties (post=pre)	z	*p* value
	n=12	Mean rank	n=12	Mean rank			
Attention	5	5.60	5	5.40	2	-0.05[a]	0.96
Memory							
Recall	4	5.50	7	6.29	1	-1.00[b]	0.32
Recognition	0	0.00	1	1.00	11	-1.00[b]	0.32
Total	4	5.00	7	6.57	1	-1.18[b]	0.24
Executive functions	5	3.90	4	6.38	3	-0.36[b]	0.72

*NB: *statistically significant difference in means, a: based on positive ranks, b: Sum of negative ranks equals sum of positive ranks.*

Comparison of cognitive score before and after the experiment within the experimental group is shown in Table 4. The scores before and after the experiment showed no statistical difference in attention, memory, and executive functions (p=0.96, 0.24, and 0.72, respectively). In addition, It was found that some participants scored in the positive ranks in attention, memory, and executive functions (5 persons, 7 persons, and 4 persons respectively) and some were able to maintain the scores (ties) after the experiment. The results indicated that the experimental group maintained or increased their cognitive ability after receiving the training program.

Discussion and Conclusion

The present study aimed to investigate the effects of multi-faceted cognitive training program for elders with cognitive impairment living in social welfare home for older persons. The results showed that both sample groups were similar (Table 1). Before the experiment, the cognitive scores of the control and the experimental group were not significantly different as shown in Table 2. This demonstrated similarity of cognitive skills between the two groups at baseline. After the experiment, the control group who did not participate in the cognitive training program and spent time on basic daily activities for 6 weeks showed lower cognitive scores while the experimental group who received the cognitive training program for 6 weeks could maintain or increase their cognitive ability in all areas (Table 2, 3, and 4). These results implied that the cognitive training program might be effective to maintain attention, memory, and executive functions in the experimental group. It confirmed the findings from previous studies regarding the deteriorating prevention for the elderly persons with cognitive impairment who had engaged in the training program.[9-12] It is acknowledged that cognitive training program is a good early intervention for elders to change or maintain their cognitive ability.[5] The participants in this study were cognitively impaired elders and could progressively develop into the late stage of dementia. In addition, the multi-faceted cognitive training program developed in this study was based on occupational therapy models and related researches regarding features the activities, duration and frequency of the program.[15-18] The program combined rehabilitation and adaptation methods doing specific activities for promoting cognitive skills with strategic integration of various activities including the use of specific techniques, knowledge provision, and strategy training, for example, the "Position Puzzles" (group activity) used the method of loci technique, the "Which one is the odd one out?" game used chunking and grouping technique and relaxation activities used deep breathing, meditation and progressive muscle relaxation technique.

The control group's scores in attention, memory, and executive functions significantly reduced after 6 weeks. Most participants of the control group (75.00-91.67%) had reduced cognitive scores due to the aging process of physiological deteriorations. In addition, personal and contextual factors might affect cognitive function in this group.[24] When considered the elders' lifestyle in the social welfare home for older persons, which focused only on their performance of basic daily activities, there was a lack of opportunities to be trained by using some complex activities under supportive environment. The study of Sasat and co-researchers[25] found that the elders living with passive routines in the social welfare home had high risk for developing a spectrum of dementia around 41.6% of Thai elderly population. However, it was found that, for memory skill, recognition score did not changed after the experiment. This conformed the theory about memory impairment in dementia that recall memory was more likely to be affected than recognition.[26-28]

At baseline and after receiving the cognitive training program for 6 consecutive weeks, there was no statistical difference of cognitive scores in the experimental group. This result could be implied that the participants in this group might be able to maintain their cognitive abilities in this period of time. The cognitive training program enables a provision of knowledge about dementia and its changeable impacts on basic daily activities in combination with cognitive challenge and leisure activities such as games and drills, memory strategies, social dynamic group activities, role play activities, field trip activities, yoga for relaxation.[9-14] Those occupations-based practice would provide friendly opportunities to perform solving problem activities during facing on any non-familiar living situations with supportive environments in order to utilize their assistive and creative cognition for a consecutive 6 week.[16-18] Diversional activities designed in this study could be applied using gradation and simplification in relation with the elders' educational levels. However, this study had some limitations that could not be generalized to the other population. The content of the training program was designed specifically to the Thammapakorn Social Welfare Development Center and might not be able to cover the other social welfare homes. The sample size was small, and long term effect was not investigated. Randomized control trial study with larger sample size and investigation for the long term effect are suggested for further studies. As preliminary study, the results from this research might be used as guideline for developing or applying the multi-faceted cognitive training program for elders who live in limited environment context like social welfare home.

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Relationship between cognition, disease severity and balance performance in individuals with Chronic Obstructive Pulmonary Disease

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ABSTRACT

Background: Accumulating evidence reveals that balance and cognitive impairment often coexist in individuals with Chronic Obstructive Pulmonary Disease (COPD), particularly in severe stage of COPD. However, the correlations among the disease manifestations remain uncertain. Understanding the contribution of disease severity and cognition to balance may lead to an optimal clinical assessment and intervention in this population.

Objectives: To examine the correlations between disease severity, cognitive function and balance performance in individuals with COPD.

Materials and methods: Fifty individuals with COPD aged 40 years and over participated in this cross-sectional study. Disease severity was evaluated using spirometry and classified according to the Global Initiative for Obstructive Lung Disease (GOLD). Cognitive performance was measured using the Montreal Cognitive Assessment-Basic (MoCA-B) Thai version. Balance performance was assessed using the Timed Up and Go test (TUG) under single- and dual-task conditions.Pearson's correlation coefficient was used to examine the relationship between the parameters evaluated. Independent sample student's t-test was conducted to compare balance performance between participants with and without cognitive impairment. Significance level was set at 0.05 for all analyses.

Results: The time taken to complete TUG for both single- and dual-task conditions was negatively correlated with the MoCA-B score (TUG-single r = -0.47; TUG-dual r = -0.48; $p \leq 0.001$). There were no correlations between the time to complete TUG both conditions and %predictedFEV1. Subgroup analyses demonstrated that the cognitively-impaired group took significantly longer time to complete TUG for both conditions than the cognitively-intact group ($p<0.01$).

Conclusion: Balance performance significantly correlated with cognitive performance but not COPD severity. Cognitive impairment adversely affected balance ability and posed individuals with COPD at risk of falls. Balance and cognitive performances should be assessed in individuals with COPD regardless of the disease severity.

Introduction

Chronic Obstructive Pulmonary disease (COPD), a respiratory disease that results in progressive airflow limitation, is a leading cause of morbidity, mortality, and health care expenditure worldwide. The prevalence of COPD has continued to increase and estimated to be the fourth leading cause of mortality by the year 2030.[1] Although the primary underlying pathophysiology is lung function, other systemic functions such as musculoskeletal

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and neuromuscular functions are affected in individuals with COPD. Thus, a wide range of impairments including muscle weakness, balance and cognitive impairments are presented in individuals with COPD.

Balance impairment is highly prevalent in individuals with COPD. Previous studies have demonstrated balance impairment across all stages of COPD severity.[2, 3] Lower limb muscle weakness, fatigue, and physical inactivity have often been reported to be associated with impaired balance in individuals with COPD.[4-6] Although central processing is essential for balance control[7], few studies have investigated the contribution of cognitive function to balance control in this population. Among several clinical tests, Timed Up and Go (TUG) test has been widely used to identify functional mobility, balance deficit, and risk of falls in healthy older adults and those with different medical diagnoses including COPD.[4, 8] Recently, the TUG dual task, adding either a cognitive or motor task, has often been used as previous research found that it increased the sensitivity to detect balance problem, consequently enhancing the ability to predict falls.[8, 9]

Cognitive impairment has been increasingly recognized in individuals with COPD. A large longitudinal study found that individuals with COPD had 4.2-fold increased odds of having mild cognitive impairment and 15.5-fold increased odds of having dementia compared to non-COPD.[10] Although a previous study suggested that the cognitive impairment in COPD is primarily relevant with hypoxia [11], non-hypoxemic COPD individuals also demonstrate cognitive impairment.[12] As both cognitive and balance impairments are independent risk factors for falls, balance and cognitive function are recommended as routine assessment in the elderly.[13, 14] These tests, however, are not yet routinely included in COPD population.

Taken together, accumulating evidence demonstrated that balance and cognitive impairment often coexist in individuals with COPD.[3, 10] However, the correlation of these disease manifestations remains unclear. Further, the severity of COPD has also been documented to influence balance impairment. Previous work found that COPD patients with severe to very severe stage (%predictedFEV_1< 50%) demonstrate greater balance impairment than those with mild stage (%predictedFEV_1 ≥80%).[2] Therefore, the aim of this study was to determine the correlations between cognition, disease severity and balance performance in individuals with COPD. In addition, balance performance in COPD participants with and without cognitive impairment was examined. Understanding the contributions of disease severity and cognition to balance may lead to an optimal clinical assessment and intervention in this population.

Materials and methods

Participants

Fifty participants with COPD were recruited from a local hospital in Chiang Mai. This sample size was based on the recommendation for the sufficient sample required for correlation analysis.[15] All participants were diagnosed by a physician according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria.[16] The GOLD criteria are considered by two aspects; a ratio of forced expiratory volume in one second and forced vital capacity (FEV_1/FVC<70%, and a classification of severity using the percentage predicted level of forced expiratory volume in one second (%predictedFEV_1) following a standard spirometer recommended by American Thoracic Society/ European Respiratory Society (ATS/ERS) 2005.[17] Other inclusion criteria included age 40 years and older, and walking independently. Participants were excluded if they had a previous history of neurological conditions, musculoskeletal conditions that could interfere with standing and walking, and impaired visual ability. Ethical approval was gained from the ethical review committee for research in humans according to the Declaration of Helsinki (AMSEC-61EX-009), and all eligible participants signed a written informed consent prior to participating in this study.

Baseline characteristic assessments

Baseline characteristic assessments including age, gender, Body Mass Index (BMI), smoking history, COPD onset, modified Medical Research Council (mMRC) dyspnea, and six-minute walk test (6MWT) were recorded. The mMRC dyspnea questionnaire consists of five-grade scales (0-4) describing the severity of participants' breathlessness associated with physical activity from low breathlessness (0) to almost complete incapacity (4). The 6MWT was performed following the methodology specified by the American Thoracic Society.[18] The participants were instructed to walk as far as possible along the 30-meter corridor during the 6-minute duration. During the test, they were allowed to rest at any time as needed and walk again when ready. Dyspnea, heart rate, and oxygen saturation were continuously monitored throughout the test and blood pressure was assessed before and after the test. The 6MWT was measured for two trials with 30-min rest between trials. The longest distance was recorded.

Balance assessment

The TUG (single task) was used to measure balance performance as it has been widely used to identify balance deficit and risk of falls in individuals with COPD.[3, 4] In addition, TUG dual-task was also included to increase test's sensitivity for identifying balance problem.[8, 9] The secondary cognitive task was chosen over the motor task as previous study demonstrated that it was more challenging than the motor task.[19] The TUG has been shown to be a simple and reliable test for assessing balance and risk of falls.[20-22] Participants were asked to perform the TUG under single- and dual-task conditions in a random order. For TUG with dual-task, participants were asked to do a naming task while performing TUG test. Specifically, they were instructed to say as many names from a given category as possible. Category such as animals, fruits, flowers, provinces was given to the participants prior to each testing trial. A practice trial was allowed prior to each test condition, followed by two testing trials. Average time used to complete each condition was recorded.[23] A rest period of 3 minutes was given between each test. Tests were administered by an independent assessor who was blinded to the participant's disease severity

and cognitive condition.

Cognitive assessment

Montreal Cognitive Assessment-Basic (MoCA-B) Thai version was administered to all participants by a trained assessor according to standard instruction. The assessor was blinded to the participants' disease severity and balance performance. MoCA-B is a 30-point test that evaluates six cognitive domains including visual perception, executive functioning, language, attention, memory, and orientation. A score of 1 point is added to participants who had less than 4 years of education. MoCA-B score lower than 25 points indicates cognitive impairment and the score of 25 points and greater indicates normal cognitive function.[25] MoCA-B (Copyright Z. Nasreddine, MD) is freely available for clinical use in Thai, English, Chinese, and French (www.mocatest.org, visit Basic section). MoCA-B Thai version has excellent sensitivity and specificity and high test-retest reliability and internal consistency.[25]

Measure of COPD severity

FEV_1, FVC, and %predictedFEV_1 values with post-bronchodilator were assessed using spirometer (HI-101, CHEST M.I., Inc, Japan). The testing protocol was conducted by an experienced, certified investigator following the instruction specified by the European Respiratory Society recommendations.[17] The classification of COPD severity using the %predictedFEV_1 value was defined as follows; mild stage (GOLD I) %predictedFEV_1 ≥80%, moderate stage (GOLD II) %predictedFEV_1≥50% and <80%, severe stage (GOLD III) %predictedFEV_1 ≥30% and <50%, and very severe stage (GOLD IV) %predictedFEV_1<30%.[16]

Statistical analyses

The statistical analyses were performed using SPSS software. Descriptive statistics were used to describe demographic data and outcome measures. Kolmogorov-Smirnov test was used to determine the assumption of normality. Pearson's correlation coefficient was used to determine the association between the time to complete TUG and %predictedFEV_1; and between the time to complete TUG and MoCA-B score. The correlation coefficient between 0.9 to 1.0 indicates very high correlation, 0.7 to 0.9 indicates high correlation, 0.5 to 0.7 indicates moderate correlation, 0.3 to 0.5 indicates low correlation, and 0.0 to 0.3 indicates negligible correlation.[26] Participants were further classified into either cognitively-intact (MoCA-B score ≥25 points) or cognitively-impaired (MoCA-B score <25 points) subgroups. Independent sample student's t-test was used to determine the differences of balance performance between the two cognitive subgroups. A significance level was set at 0.05 for all analyses.

Results

Demographics of participants

Study participants included 50 individuals with COPD (37 men, 13 women) aged between 54 and 85 years. Of the 50 participants, 11 (22%) had mild, 24 (48%) had moderate, and 15 (30%) had severe stage of COPD. The average MoCA-B score was 22.9±4.5 points with the range between 9 and 29 points. Demographic characteristics of the participants are presented in Table 1.

Table 1 Demographic characteristics of participants (n=50).

Variables	Values (mean±SD)
Age (years)	69.8±7.8
Gender (male, %)	37 (74)
Body Mass Index; BMI (kg/m^2)	19.9±3.8
Smoking history (pack-years)	13.0±18.6
mMRC dyspnea (median, IQR)	1(1)
FEV_1 (L)	1.3±0.5
FVC (L)	2.3±0.7
FEV_1/FVC (%)	55.9±9.7
Disease severity (n)	
GOLD stage I	11
GOLD stage II	24
GOLD stage III	15
PredictedFEV_1 (%)	64.3±22.5
COPD onset (years)	3.9±3.9
MoCA-B score (points)	22.9±4.5
6MWD (m)	347.1±96.3

The data are expressed as mean±standard deviation, otherwise as indicated.
Notes: *SD: standard deviation, %: percentage, kg: kilogram, m: meter, mMRC: modified Medical Research Council, IQR: Interquartile range, FEV_1: forced expiratory volume in 1 second, FVC: forced vital capacity, L: liter, GOLD: Global Initiative for Chronic Obstructive Lung Disease, n: number, MoCA-B: the Montreal Cognitive Assessment-Basic, 6MWD: six-minute walk distance*

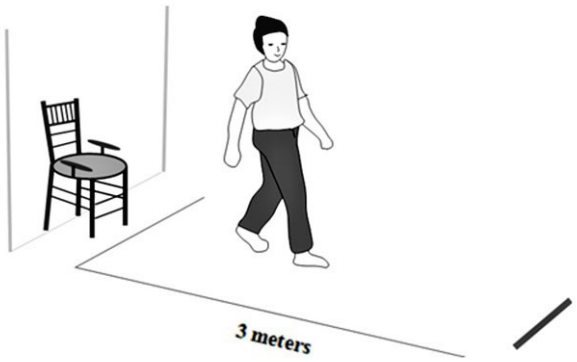

Figure 1 *The Timed Up and Go test*[24]

Balance performance and cognitive function, disease severity

Average time taken to accomplish TUG was 11.9±3.1 seconds for the single-task condition and 15.2±4.6 seconds for the dual-task condition. Pearson's correlation analysis revealed that the time taken to complete TUG for both single- and dual-task conditions was negatively correlated with MoCA-B score (p≤0.001) (Figure 2, 3). The correlation coefficients were comparable between both TUG conditions (TUG-single r =-0.47; TUG-dual r =-0.48), and indicated low relationship. There were no correlations between time to complete TUG in both conditions and %predictedFEV_1 (p>0.05).

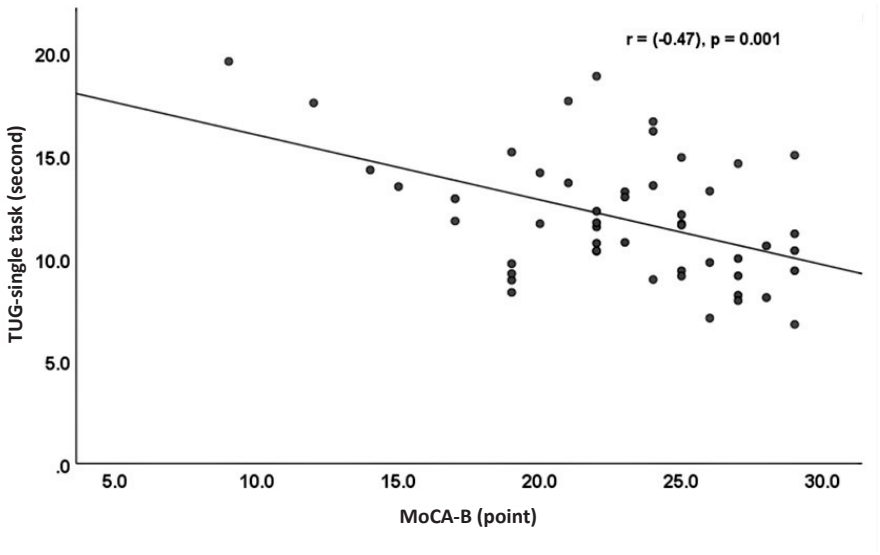

Figure 2 *The correlation of TUG-single task time and MoCA-B score*

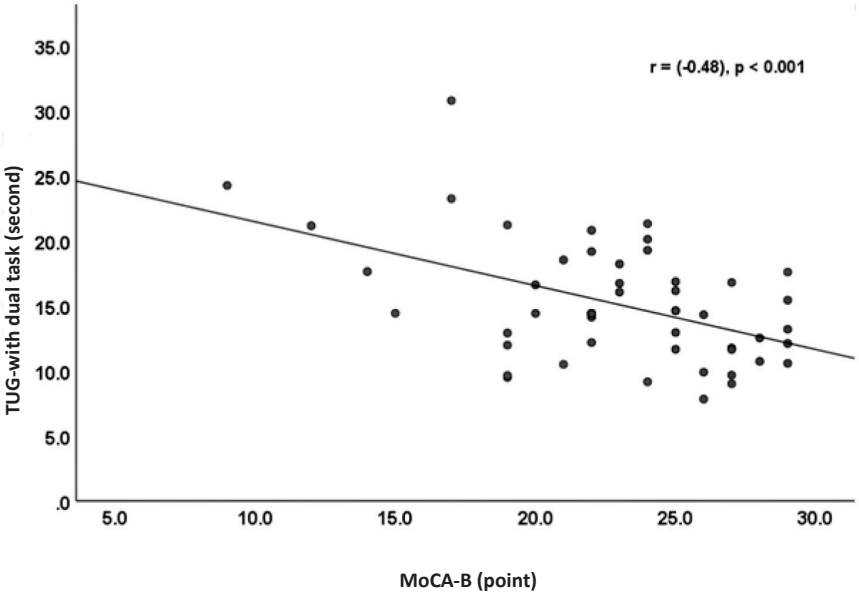

Figure 3 *The correlation of TUG-dual task time and MoCA-B score*

Comparison between cognitively-intact and cognitively-impaired COPD subgroups

Of the 50 participants, 29 participants were classified as having cognitive impairment based on MoCA-B cut-off score at 25 points.[25] Characteristics of the cognitively-intact and cognitively-impaired subgroups are shown in Table 2. There were no significant differences between groups for age, gender, BMI, dyspnea score, disease onset and exercise capacity (p>0.05).The cognitively-impaired group had significant lower of FEV_1, FEV_1/FVC and higher mean pack-years of smoking than the cognitively-intact group. The time to complete TUG-single and TUG-dual task was significantly longer for the cognitively-impaired group as compared to the cognitively-intact group (Table 3).

Table 2 Demographic characteristics of the cognitively-intact (n=21) and cognitively-impaired (n=29) subgroups.

Variables	Cognitively-intact COPD	Cognitively-impaired COPD	*p* value
Age (year)	67.5±7.5	71.4±7.8	0.08
Gender (male, %)	17 (81)	20 (69)	0.53
Body Mass Index; BMI (kg/m^2)	20.8±3.8	19.3±3.7	0.15
Smoking history (pack-years)	7.5±9.1	20.5±25.1	0.03
mMRC dyspnea (median, IQR)	1(1)	1(1)	1.00
FEV_1 (L)	1.4±0.5	1.1±0.4	0.03
FVC (L)	2.4±0.8	2.1±0.7	0.12
FEV_1/FVC (%)	58.9±9.1	53.7±9.8	0.06
PredictedFEV_1 (%)	71.8±24.0	58.9±20.1	0.05
COPD onset (year)	3.8±2.9	4.0±4.6	0.88
6MWD (m)	354.2±110.0	342.0±86.7	0.66

Notes: *%: percentage, kg: kilogram, m: meter, mMRC: modified Medical Research Council, IQR: Interquartile range, FEV_1: forced expiratory volume in 1 second, FVC: forced vital capacity, 6MWD: six-minute walk distance*

Table 3 TUG performance between the cognitively-intact (n=21) and cognitively-impaired (n=29) subgroups.

Variables	Cognitively-intact COPD	Cognitively-impaired COPD	Mean difference	95% CI	*p* value
TUG time (s)	10.5±2.5	13.0±3.1	2.5	-4.1,-0.9	0.003
TUG with dual task time (s)	12.9±2.8	16.8±5.0	3.9	-6.2,-1.7	0.001

Notes: *s: second, CI: confidence interval*

Discussion

The main findings of this study demonstrated that TUG performance, both single- and dual-tasks, of individuals with COPD were significantly correlated with MoCA-B score but not %predictedFEV_1. In addition, TUG performance was poorer in COPD individuals with cognitive impairment compared to those without cognitive impairment.

Balance control requires complex integration of multiple sensorimotor and cognitive processes.[27] The pertinent role of cognition in balance control has been revealed by several studies that demonstrated balance impairment in individuals with cognitive impairment such as mild cognitive impairment and Alzheimer's disease.[28, 29] The present study demonstrated negative correlation between MoCA-B score and time to complete TUG test. Lower MoCA-B score was correlated with longer time to complete TUG. This finding is in agreement with a previous study that reported a significantly negative correlation between Mini-Mental State Examination (MMSE) score and static posturography path length in elderly people (r=-0.25).[30] The correlation coefficient in both previous studies[29,30] and this study was, however, relatively low which might be explained partly by the use of global cognitive function tests (i.e. MoCA-B, MMSE). It has been suggested that balance control was related to some more than other cognitive domains.[29] Tangen *et al* [29] found that Balance Evaluation System Test (BESTest) score was significantly correlated with Trail Making test-part B (TMT-B) score but not with TMT-part A, clock drawing, word-list learning, verbal fluency, and MMSE scores. The authors concluded that balance control relies more on executive function than other cognitive domains. However, it remains unclear which cognitive domain is mostly correlated with TUG performance. Future study should examine the influence of specific cognitive functions on TUG performance.

The findings of comparable correlation coefficient between the single (r=-0.47) and dual-task (r=-0.48) conditions suggested that adding a cognitive task did not strengthen the relationship between balance and cognitive performance. However, the dual-task condition appeared to make the TUG test more challenging as reflected by an increase in the time to complete TUG under this condition in both cognitively-intact COPD (TUG single=10.5 seconds, TUG dual=12.9 seconds) and cognitively-impaired COPD (TUG single=13.0 seconds, TUG dual=16.8 seconds). This finding was supported by several studies that demonstrated significant increase in time taken to complete TUG under dual-task compared to single task conditions.[8, 23]

In the present study, there was no correlation between TUG performance and disease severity which was in accordance with findings from previous studies.[2, 31] de Castro *et al* [31] found no correlation between balance and pulmonary function. In addition, results from subgroup analyses based on the COPD severity demonstrated that performance on both static and functional balance, as assessed by one-legged

stance and TUG, were comparable across these subgroups.[31] Possible explanation for this finding is that TUG performance may be related to factors such as exercise capacity and lower limb muscle strength rather than disease severity.[2, 31] Jacome *et al*[2] also found that body mass index, number of medications, restriction in recreational activities, and depression were the multivariate predictors of balance impairment as measured by TUG.

The results derived from subgroup analysis demonstrated that the time taken to perform TUG in both conditions was significantly longer for the COPD with cognitive impairment than non-cognitive impairment subgroups. This result was in line with a recent systematic review and meta-analysis which demonstrated adverse effect of MCI on gait and balance.[28] Using TUG cut-off times for COPD at 11.2 seconds as recommended by Mesquita *et al*[32], the present finding indicated that the COPD with cognitive impairment subgroup were at risk of falls.[32] These findings suggested that cognitive impairment has negative impact on balance and consequently place individuals with COPD at risk of falls. A higher disease severity observed in the cognitive impairment subgroup was in accordance with previous systematic review which indicated significant link between disease severity and cognitive impairment in this population.[33]

Certain limitations must be considered when interpreting the results of this study. Given that all stages of disease severity were not included and the sample size was small, the generalizability of our results to the COPD population is limited. In addition, as this study was a cross-sectional design, the causal relationship of the study outcomes could not be inferred. In future studies, a longitudinal design with large sample size that includes all COPD severity is warranted. Furthermore, the role of specific cognitive domains on TUG performance should be investigated.

Conclusion

The present study demonstrated that balance performance was significantly correlated with cognitive performance but not COPD severity. Cognitive impairment adversely affected balance ability and put individuals with COPD at risk of falls. These findings confirmed the role of cognition in balance control. Thus, cognitive function should be included as part of a routine assessment in individuals with COPD and fall risk assessment should particularly be administered to COPD individuals who have cognitive impairment.

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Test-retest Reliability of the Five Times Sit-to-Stand Test measured using the kinect in older adults

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ABSTRACT

Background: The Kinect camera system is a low-cost portable device that can be used to time the Five Times Sit-to-Stand Test (FTSST). However, there is insufficient information and limited studies concerning the reliability of the Kinect measurements when compared with standard clinical stopwatch measurements.

Objectives: The aims of this study were to investigate the test-retest reliability of the FTSST as measured using a Kinect camera and to determine its agreement with standard clinical stopwatch measurements.

Materials and methods: Thirty older adults were timed while performing four repeated FTSSTs under two conditions: with and without their arms crossed. There were 10-minute breaks between the four tests. The times were simultaneously recorded by a Kinect camera system and by a research assistant using a stopwatch.

Results: The intraclass correlation coefficient (ICC) values for the test-retest reliability were 0.664 with the arms crossed and 0.843 with the arms free. In addition, the determination of the agreement between the Kinect and the stopwatch measurements in this study produced ICC values of 0.944 with the arms crossed and 0.954 with the arms free.

Conclusion: The test-retest reliability of the FTSST as measured using the Kinect system was good to excellent, and its agreement with the standard clinical stopwatch was excellent.

Introduction

Fall prevention is a very important strategy used to protect older adults from injuries, disabilities, and premature deaths. Falls can devastate the quality of life of older adults by compromising their activities of daily living. In addition, they can affect an individual's socioeconomic status, and they can have implications on government resource allocations. There are several methods that physical therapists use in clinics to assess the functional mobility of older adults in order to predict their fall risks, such as the Berg Balance Scale, the Timed Up and Go (TUG) test, and the Short Physical Performance Battery test. However, previous research has suggested that due to their good psychometric properties and clinical utility, the Five Times Sit-to-Stand Test (FTSST) and other clinical tests, such as the Alternate Step Test and the Six-meter Walk Test, are the best tests of general mobility.[1]

Moving from sitting to standing is a critical movement task performed in daily life. It involves the functional ability to control the center of gravity while moving the base of support from the hips to the feet in order to attain an upright standing posture.[2] Even individuals with functional deficits

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commonly perform sit-to-stand tasks more than 45 times daily.[3] Older adults who have reported multiple falls have been found to perform poorly in tests of functional ability, such as the FTSST.[4] Moreover, the FTSST also reflects the functional strength of the lower extremities, and thus, it is commonly used in the clinic for functional assessments of various populations.[5,6] Due to the ease of its application in the clinical setting, there are several studies that have reported the value of the FTSST in predicting the potential for future falls in older adults.[7,8] However, comparing clinical studies is difficult because there are many variables that can affect the results, including the seat height of the chair, the arm positions, the movement speed, the patient's balancing ability, and the patient's psychological status.[9,10]

The original FTSST used in clinical settings involves the use of manual timing devices, such as a stopwatch, but these cannot be used for isolated assessments, and they do not report the kinematic movements of the lower extremities. However, new technology has been developed to enhance the test by obtaining additional information about the kinematic movements themselves and the timing of the movement phases. For example, excellent measurement consistency has been reported when using a smartphone application for the laboratory assessment of an FTSST.[11] In addition, a sensor-based sit-to-stand movement test was also found to have excellent validity in older adults.[12] Kinect-based systems have also become increasingly popular when used for functional clinical assessments. They are commonly used for gait assessments in various populations, but they can also be used for other clinical tests, such as the TUG test and the FTSST.[13,14] A Kinect-based system is a valuable clinical tool due to its ability to discriminate between fallers and non-fallers in the FTSST, as well as its correlation with the Physiological Profile Assessment, which indicated that the Kinect was feasible to use in clinical practice.[14]

The Kinect is a low cost device that can easily be used in the clinic and by older adults to monitor their fall risks by themselves at home. However, it requires specialized software to control the tests, collect and analyze the data, and report the psychometric property outcomes required for clinical decision-making.[15] Reliability is important when determining the consistency of an assessment tool. Even though the psychometric properties of the Kinect system used for the FTSST have been reported in a previous study[16], there is insufficient information and limited literature concerning the reliability of the Kinect measurements when compared with standard stopwatch measurements. Thus, the primary purpose of this study was to determine the test-retest reliability of the FTSST as measured using a Kinect system. The second purpose was to examine the agreement between the FTSST measured using the Kinect system and the FTSST measured using a standard clinical stopwatch in older adults. This would provide valuable reference data to inform clinical decision-making with regard to the selection of the most reliable measurement tools in the clinic.

Materials and methods

Study design

An observational design with repeated measures was used in this study.

Participants

This study was conducted at the Mae Khao Tom subdistrict hospital in Chiang Rai, Thailand. Thirty adults from the Mae Khao Tom community who were older than 60 years of age and who could stand independently participated in this study. The subjects were excluded if they had a cognitive impairment (Thai Mental State Examination score of less than 23), a balance impairment (Berg Balance Scale score of less than 45), a past medical history that could have affected their postural control (such as a stroke or Parkinson's disease), a problem with their vestibular system (as tested via the Dix-Hallpike test), or a pain score of more than 5 out of 10 on a Visual Analog Scale.

Ethical approval

A research assistant explained the study protocol to all of the participants. Each participant signed an informed consent form before participating in this study, and they understood that they could withdraw from the study at any time. The study protocol was approved by the Mae Fah Luang University Ethics Committee according to the tenets of the Declaration of Helsinki (ethical record number: REH-60093).

Equipment

The Kinect camera (Microsoft, Redmond, WA, USA) has been shown to provide valid measurements for movement analyses, such as sit-to-stand tests.[17] Therefore, a Kinect camera was used in this study in conjunction with "MFU fall risk detection" customized software. One Kinect camera was placed 2 meters lateral to the participant, on his or her right side, and the whole body movement was captured using an infrared sensor attached over the participant's humeral head. The customized software captured the motion of the shoulder throughout each movement sequence of the sit-to-stand-to-sit motion. The sequence timing was stopped automatically when the participant completed the 5 required movement sequences.

Rater quality

A research assistant conducted the FTSST using a standard stopwatch. He had previously practiced administering the test with young adults in the university laboratory before taking part in this research. For the standard stopwatch measurements, the time was recorded from the start, when the hip left the chair. It ended when, after 5 completed sit-to-sand-to-sit movement sequences, the participant was again seated in the chair with his or her trunk vertical. The intrarater reliability for the FTSST showed an intraclass correlation coefficient (ICC) of 0.960. The same research assistant conducted the stopwatch measurements for all of the participants as part of the research protocol.

Procedure

After being screened for eligibility, each participant performed the FTSST in a quiet room with suitable lighting.

The participant sat on a standard chair with a seat height of 0.46 meters. For the starting position, the participant placed his or her feet 0.1 meters behind the knees, as shown in Figure 1.[18] The participant was asked to stand up and sit down five times as quickly as possible, while ensuring that his or her hips and knees were fully extended when standing. The test was performed 4 times sequentially, twice under each of two conditions: with the participant's arms folded across the chest and with the arms unrestricted and free to move about as required. The participants were allowed one practice FTSST in order to familiarize themselves with the instructions and test requirements. The FTSST was then performed first with the arms folded across the chest, and then with the arms free, then again with the arms folded, and again with the arms free. They were given 10-minute breaks between the tests. The durations of the FTSSTs were simultaneously recorded by both the Kinect system and by a standard stopwatch. Both the software and the stopwatch were controlled by the same research assistant. The data were then used for further statistical analyses.

Software and system overview

In order to measure the FTSST times, the Kinect was placed approximately 0.5 meters above the floor and 2 meters lateral to the participant, on his or her right side (Figure 1). The "MFU fall risk detection" software was used to detect the subject's joints by obtaining a video feed from the Kinect at 30 frames per second with a 640 by 480 image resolution. The shoulder joint was used as a reference landmark based on our findings that it was the most stable position when compared to the other landmarks, such as the head or hip joint.

The main algorithm of our software used to measure the FTSST times is briefly explained in following steps:

1. Detect the shoulder joint.
2. Check whether the subject sits still for a while and keep this shoulder position as a sitting reference. Set the current state to sitting.
3. Detect whether the subject is standing by comparing the current shoulder position with the sitting reference. If the difference is greater than the defined thresholds and the previous state was sitting, set the state to standing and start the counting time.
4. If the current state is standing and then the subject's shoulder changes to less than the sitting reference, set the state to sitting and count a complete sit-stand-sit loop.
5. Repeat steps 3 and 4. When the count of the sit-stand-sit loop reaches five, stop and record the time.
6. Compute the elapsed time for the FTSST. Reset the software and wait for the next test.

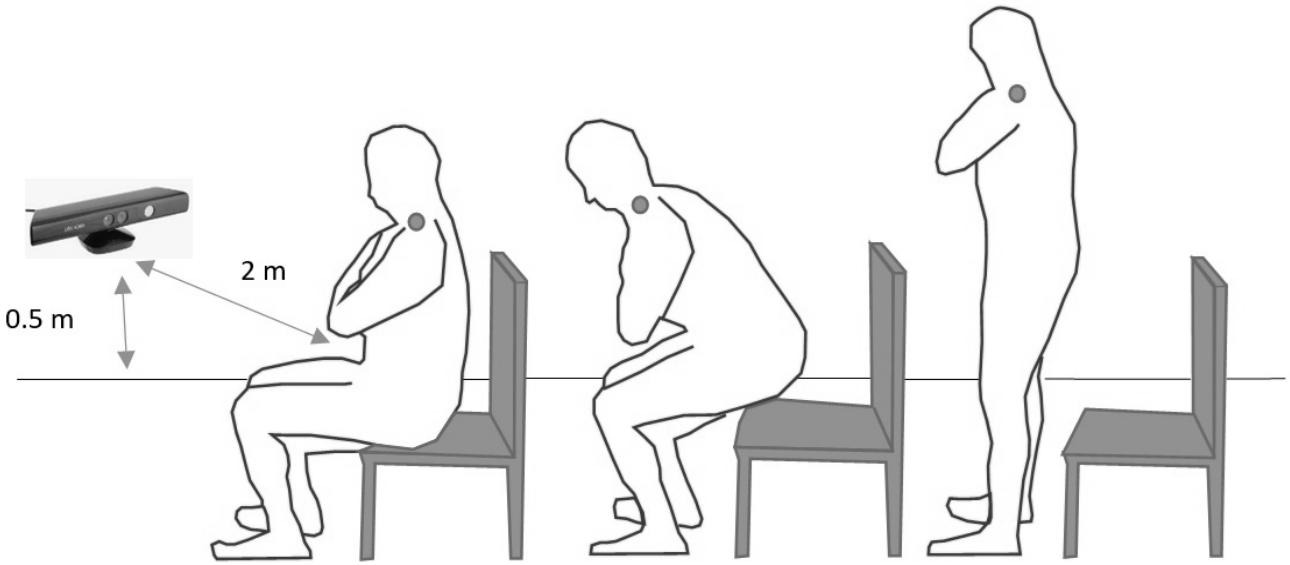

Figure 1 *Testing environment and sit-to-stand detection phases of Kinect. Right shoulder joint was a referece point.*

Statistical analysis

The baseline characteristics were presented using descriptive statistics. ICCs (two-way mixed model, type consistency, single measurement) were used to measure the consistency of each condition. One sample t-test was used to determine the difference between the means of trial 1 and trial 2 for each condition. The absolute reliability was measured using the standard error of the measurement (SEM) and the minimal detectable change at the 95% confidence interval (MDC95). Bland-Altman plots were used to determine the systematic bias between the test and retest. The values were computed using the following formulas:

$$SEM = SD \times \sqrt{(1-ICC)}$$

$$MDC95 = 1.96 \times \sqrt{2} \times SEM$$

Additionally, ICCs (two-way random model, type absolute agreement, average measurement) and Bland-Altman plots were used to assess the agreement between the two measurement methods. Between the repeated measures and between the methods, ICCs between 0.75 and 1.00

could be interpreted as excellent, ICCs between 0.60 and 0.74 could be interpreted as having good reliability, ICCs between 0.40 and 0.59 could be interpreted as having fair reliability, and ICCs of less than 0.40 could be interpreted as having poor reliability.[19] IBM SPSS Statistics for Windows (version 20.0; IBM Corp., Armonk, NY, USA) was used to analyze the data, with a significance level set at 0.05.

Results

Test-retest reliability of the FTSST measured using the Kinect

The baseline characteristics are presented in Table 1. As shown in Table 2, there were no differences between the means of trial 1 and trial 2 for both the FTSST with arms crossed (p=0.696) and with arms free (p=0.778). The test-retest reliability results showed good reliability [ICC=0.664, 95% confidence interval (CI)=0.404–0.824, SEM=0.808] to excellent reliability [ICC=0.843, 95%CI=0.697–0.843, SEM=0.389) with arms crossed and arms free, respectively. In addition, the test-retest reliability for the arms free condition was higher than that for the arms crossed condition. The Bland-Altman plot showed a positive bias of 0.170 seconds for the arms crossed condition (95% limits of agreement, from -2.561 seconds to 2.907 seconds; Figure 2) and a negative bias of -0.130 seconds for the arms free condition (95% limits of agreement, from -2.057 seconds to 1.799 seconds; Figure 2), indicating an overestimation of the repeated measurement for the arms crossed condition and an underestimation of the repeated measurement for the arms free condition. The MDC95 of the FTSST as measured using the Kinect system for the arms crossed condition was 2.239 seconds; for the arms free condition it was 1.078 seconds.

Table 1 Baseline characteristics.

Characteristics	Older adults (n=30)
Gender, n (%)	
Males	8 (26.67)
Females	22 (73.33)
Age in years, mean (SD)	65.80 (5.72)
Weight in kg, mean (SD)	58.48 (10.12)
Height in m, mean (SD)	1.56 (0.07)
Number of education years, mean (SD)	3.60 (1.22)
Thai Mental State Examination, mean (SD)	25.58 (1.61)
Berg Balance Score, mean (SD)	55.57 (0.67)
Dix-Hallpike test, n (%)	
Negative	30 (100)
Positive	0 (0)

SD: standard deviation

Table 2 Reliability and minimum detectable change for the FTSST using the Kinect.

	Trial 1	Trial 2	Different (SD_{diff})	$ICC_{3,1}$	95% confidence interval	SEM	MDC_{95}
	Mean (SD)						
Arms crossed (seconds)	9.536 (1.658)	9.363 (1.744)	0.173 (1.395)	0.664	0.404 to 0.824	0.808	2.239
Arms free (seconds)	9.184 (1.663)	9.313 (1.845)	-0.129 (0.984)	0.843	0.697 to 0.922	0.389	1.078

FTSST: Five Times Sit-to-Stand Test, SD: standard deviation, ICC: intraclass correlation coefficient, SEM: standard error of the measurement, MDC95: minimal detectable change at the 95% confidence interval

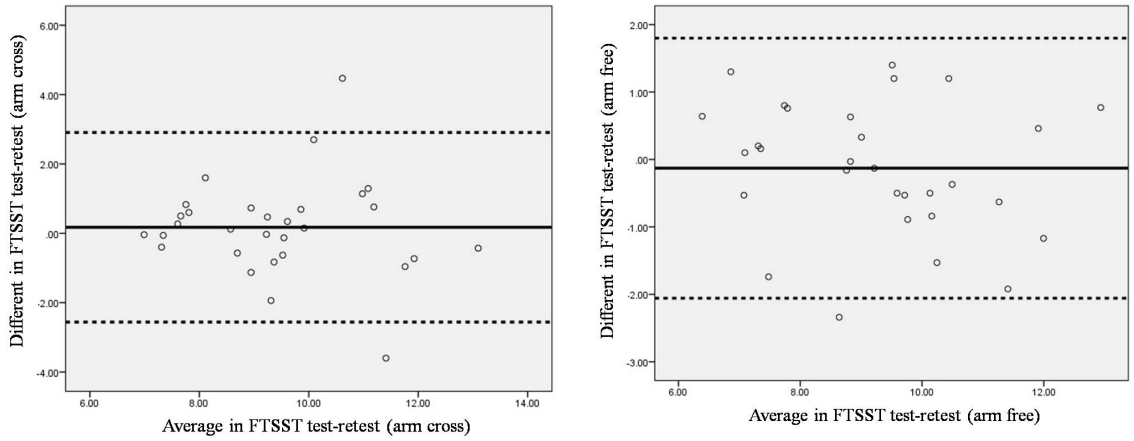

Figure 2 *Bland-Altman plot representing the average of two trials for test-retest for FTSST for the arms crossed and arms free conditions.*

Agreement of the FTSST measured using the Kinect system and a standard stopwatch

The measurement consistency between the Kinect system measurements and the standard stopwatch measurements was excellent, which was indicated by the ICCs for the Kinect system and standard stopwatch measurements for both the arms crossed condition and the arms free condition, which were 0.944 (95%CI=0.224–0.986) and 0.954 (95%CI=0.222–0.989), respectively (Table 3). The Bland-Altman plots represented negative biases of -0.464 seconds (95% limits of agreement, from -1.056 seconds to 0.128 seconds; Figure 3) for the arms crossed condition and -0.462 seconds (95% limits of agreement, from -1.015 seconds to 0.090 seconds; Figure 3) for the arms free condition, indicating an underestimation of the time by the Kinect system.

Table 3 The FTSST agreement analysis between the Kinect and the standard stopwatch measurements.

	FTSST using Kinect (seconds)*	FTSST using standard stopwatch (seconds)*	$ICC_{2,k}$	95% confidence interval	SEM
Arms crossed	9.452 (1.552)	9.915 (1.658)	0.944	0.224 to 0.986	0.071
Arms free	9.251 (1.687)	9.713 (1.797)	0.954	0.222 to 0.989	0.062

**Values are expressed as mean (SD)*
FTSST: Five Times Sit-to-Stand Test, SD: standard deviation, ICC: intraclass correlation coefficient, SEM: standard error of the measure

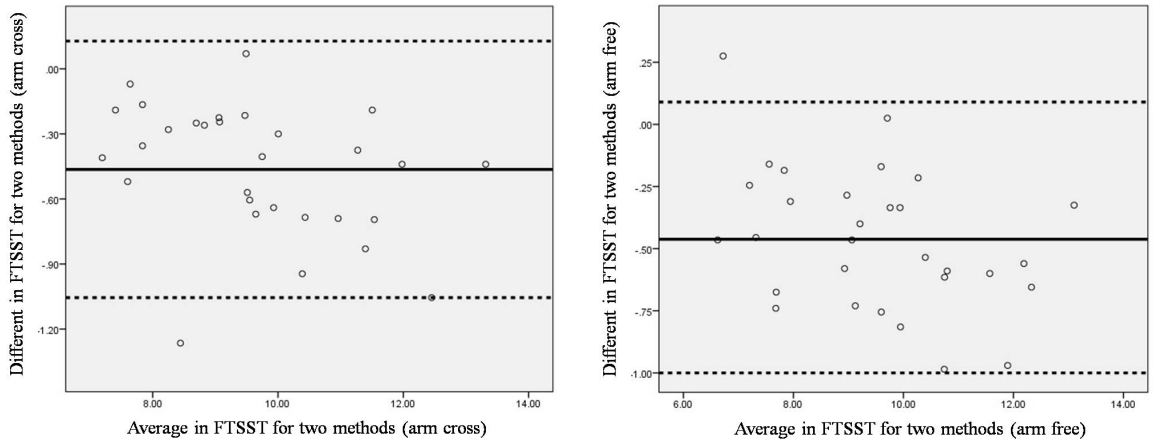

Figure 3 *Bland-Altman plot representing the agreement between two methods for the FTSST.*

Discussion

The aim of this study was to investigate the reliability of the Kinect system for the measurement of the FTSST in older adults and its agreement with standard clinical stopwatch-measured tests. This research represents the development of customized software using a low-cost device. The results showed good to excellent test-retest reliability for the FTSST by the Kinect system for both conditions. In addition, excellent agreement was found between the Kinect system and the standard clinical stopwatch measurements.

Reliability of the Kinect system for the FTSST

The results of this study showed that there was good to excellent test-retest reliability, indicating that the FTSST timed by the Kinect system is the optimal choice for clinical use. One previous study suggested that an ICC of more than 0.6 was a useful consideration in clinical outcome measurements.[20] Our results were not found to be different from those of other studies, and this is supported by a previous systematic review that reported a test-retest reliability for the FTSST in which the ICC values ranged between 0.64 and 0.96.[21] However, a higher test-retest reliability for the arms free condition than the arms crossed condition indicated that the arm position affected the outcome measurements. This is in contrast to the results from the study by Ng et al., who found that there was no difference between the times obtained by older adults while performing a FTSST with different arm positions.[22] This may be due to the "MFU fall risk detection software" used with the Kinect system, which timed the movement pathway of the shoulder. Thus, the motion capture by the Kinect system may have been affected when the arm position was out of neutral alignment.

In this study, the smallest change that could be considered to be a true change was found to be different for each arm condition: 2.239 seconds and 1.078 seconds for the arms crossed and arms free conditions, respectively. This is in contrast with the results from the study by Goldberg et al. in 2012, who found a minimal detectable change that exceeded 2.5 seconds in older adults[7]; however, the measurement protocol in our study was different.

FTSST agreement between the Kinect system and the standard stopwatch measurements

A strong correlation between the Kinect system and clinical standard measurements, such as those obtained using a Vicon motion analysis system, for timed sit-to-stand tests can be found in Parkinson's disease studies (r=0.999).[17] Therefore, the measurements of the time taken in the sit-to-stand studies using the Kinect camera system are considered to be valid. The FTSST test is easily performed, and tests using a standard stopwatch to measure the time have been commonly used in clinical practice.

This study found agreement between the Kinect system and the standard stopwatch measurements for the FTSST, indicating that the Kinect system is an optimal tool for this clinical test, even though there was a difference in the measurement methods: the standard stopwatch measurements were obtained by focusing on the hip movement, while the Kinect system focused on the shoulder movement. This is supported by a previous study that found a high correlation for the FTSST using the Kinect system and standard stopwatch measurements (r=0.704 to 0.931).[14] The Kinect camera is superior to the standard stopwatch measures because it can provide a self-assessment method to determine the risk of falling. Older adults can monitor their own risk of falling at home by themselves. Moreover, Kinect cameras can also provide kinematic joint data during movement; therefore, they are useful tools for clinical analyses conducted by both physical therapists and researchers.

Study limitations

The older adults who participated in this study were healthy older adults. Therefore, the reliability of these tests in various populations, such as older adults with balance impairments and health problems as well as patients suffering from strokes or Parkinson's disease, is unknown. The other psychometric properties of the Kinect system used for the FTSST, such as the test-retest reliability between days and a cut-off score for the prediction of falls in older adults, are also unknown. Further studies should aim to fill these gaps in the knowledge.

Conclusion

A good-to-excellent within-day test-retest reliability was found for the FTSST measured using the Kinect system. The excellent agreement of the measurements between the Kinect system and a standard stopwatch could inform the decision-making of the management of clinical practices, such as the promotion of FTSST self-assessments using the Kinect at home.

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Conflict of interest

The authors declare no conflicts of interest.

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