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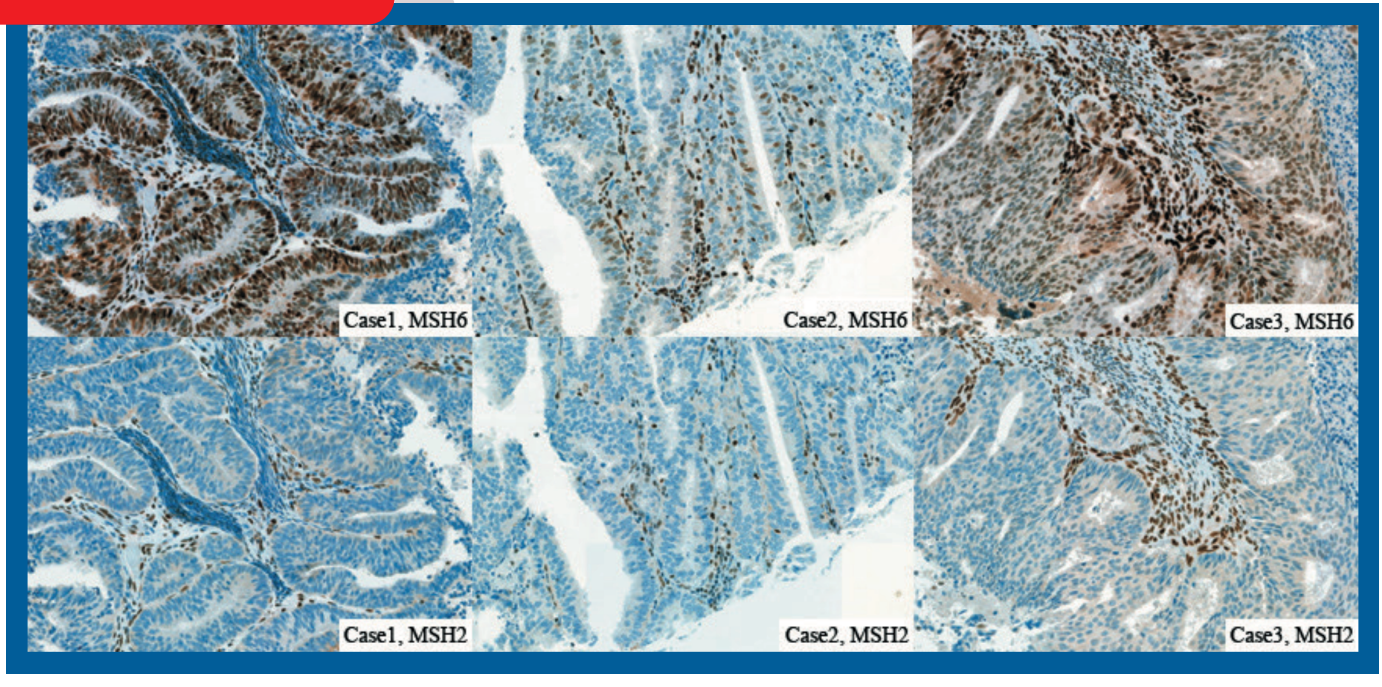

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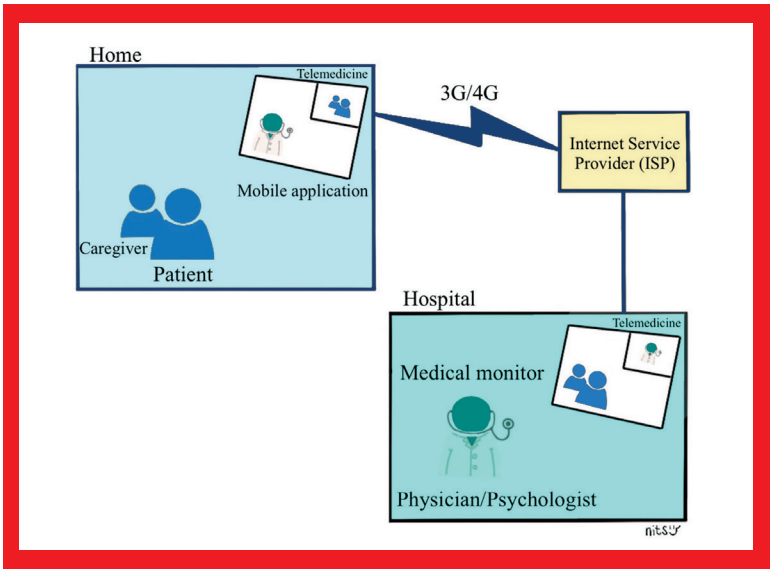

By Hathaichanok Suesat, et al.

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E-mail: sijournal92@gmail.com

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Knowledge of Stroke and Planned Response among Patients Living with Diabetes Mellitus and Hypertension in a Primary Care Unit

Thareerat Ananchaisarp, M.D., Kanyaphim Sa-a, M.D.

Division of Family and Preventive Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.

ABSTRACT

Background: Stroke is an important worldwide public health problem. Lack of knowledge in prevention methods, warning symptoms and planned response of acute stroke are associated with a longer prehospital time, which affect the morbidity and mortality of patients.

Objective: The primary objective was to assess knowledge of stroke prevention methods and warning symptoms among patients living with diabetes and/or hypertension. The secondary objectives were to define planned responses when suspecting acute stroke, and identify associated factors with stroke knowledge scores and planned responses.

Materials and Methods: A cross-sectional study was conducted in patients living with diabetes and/or hypertension, who had continuous follow up at the primary care unit of Songklanagarind Hospital. The outcomes of this study were assessed by a questionnaire, which was developed from a literature review.

Results: This study included 312 participants. Median age was 64.0 years (Q1, Q3 = 58.0, 71.0), and 59.6% were female. Median score of knowledge of stroke prevention methods were 9, from 12 points (Q1, Q3 = 8, 10), and warning symptoms were 7, from 10 points (Q1, Q3 = 6, 8); with 80.1% of them knowing all 3 warning symptoms, according to the acronym FAST. Only 22.8% of participants would go to the hospital immediately, by calling an ambulance when they experienced symptoms of a suspected acute stroke. Participants who had income had statistically significant higher knowledge of stroke prevention methods; while participants under 60 years of age, who had a longer duration of diagnosed diabetes mellitus were associated with appropriate planned responses when suspecting acute stroke.

Conclusion: Patients living with diabetes mellitus and hypertension, who are at a high risk for developing cardiovascular diseases, still do not have enough knowledge about acute stroke and had little concern about developing a stroke; especially the elderly and those with a short duration of having been diagnosed with diabetes mellitus.

Keywords: acute stroke, knowledge, prevention methods, warning symptoms, planned response (Siriraj Med J 2022; 74: 75-84)

INTRODUCTION

Stroke is an important public health problem worldwide; because stroke is life threatening[1] and is the second leading cause of death and disability worldwide.[2] Risk factors of stroke are categorized as non-modifiable risk factors and modifiable risk factors; such as, underlying diabetes mellitus (DM) and hypertension (HT); which are the highest risk factors of stroke (Odds ratio = 3.55 and 2.06, respectively).[3] Although, various studies have shown that reducing risk factors can prevent stroke, only 27.0-37.0% of the population recognized stroke risk factors.[4]

Corresponding author: Thareerat Ananchaisarp
E mail: thareerat.a@psu.ac.th
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ORCID ID: https://orcid.org/https://orcid.org/0000-0002-3386-242X
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There are two types of stroke: ischemic and hemorrhagic stroke. When patients develop acute stroke they should receive appropriate treatment promptly in order to reduce mortality rate and disability.[5,6] Public health systems realize the importance of receiving prompt treatment, and as such many hospitals worldwide have developed a stroke fast tract; including Songklanagarind Hospital. Although, the stroke fast tract can reduce the time for diagnosis of stroke and increases the usage rate of thrombolytic therapy in ischemic stroke[7]; only 14.9% of acute stroke patients in Thailand can arrived at the hospital in time to receive thrombolytic therapy.[4] Lack of knowledge of stroke risk factors, warning symptoms and planned response when suspecting acute stroke were found to be reasons of delayed prehospital time in acute stroke.[8-12] Clinical practice guidelines recommend patients who have warning symptoms of stroke should go to the hospital immediately by calling emergency medical services (EMS)[13]; however, a study in Thailand found that only 5.0-16.0% of stroke patient used EMS as their transportation.[14]

The National Stroke Association, American Heart Association propagate the mnemonic abbreviation "FAST", which stands for facial palsy, arm drip (which means weakness of upper and/or lower extremities), abnormal speech and going to the hospital in time; to make people easy to remember common warning symptoms of acute stroke and emphasize people who have warning symptoms of stroke should go to hospital without delay; or in time, as per the 'T' in FAST.[15] Beyond the 3 most common warning symptoms of stroke, included in the acronym FAST[16], there are also a number of other warning symptoms of stroke.[17] A previous population-based study found that 63.0-75.1% of participants could not recognize any stroke warning symptoms; however, 86.1% of them knew that they should go to the hospital if they had stroke warning symptoms.[4,5]

Factors that related to good knowledge of stroke risk factors, prevention methods, warning symptoms and appropriate planned response when there is a suspicion of acute stroke were younger age, female, married, higher education, living in the city, being employed, sufficient income and underlying DM.[4,7,18-20] While hypertensive patients may have more knowledge of stroke over a normotensive population[19], some studies found that 77.0% of hypertensive patients could not identify any stroke risk factors or warning symptoms.[20]

This study was developed with the primary objective being to assess knowledge of stroke prevention methods and warning symptoms among patients living with diabetes and/or hypertension, who are in the high risk group of stroke, as there are currently few studies concerning this topic; especially in Thailand and in this specific group of patients. The secondary objectives were to define planned response when suspecting acute stroke, and identify associated factors with stroke knowledge scores and appropriateness of planned response when suspecting acute stroke.

MATERIALS AND METHODS

Study design

A cross-sectional study was conducted from; 1st May – 31st August 2019, amongst patients living with diabetes and/or hypertension who had continuous follow up at the Primary Care Unit (PCU) of Songklanagarind Hospital, Hat Yai, Thailand; this being a tertiary care hospital in Southern Thailand.

Study sample and sampling

This study included patients living with diabetes and/or hypertension who had continuous follow up at the Primary Care Unit (PCU) of at least 1 year, came for follow-up during the study period, had good consciousness and consented to participate in our research. We excluded patients who required emergency treatment, and whom were already diagnosed with stroke or transient ischemic attack. The sample size was calculated for the primary objective, by using estimate of the mean in the population formula; with standard deviation (S.D.) being calculated from the pilot study (S.D. =3.3 and 4.5 for knowledge of stroke prevention methods and warning symptoms, respectively); and error (d) = 0.5. According to the maximum calculated value, 312 participants were required. We enrolled participants who were compatible with our eligibility criteria by convenience sampling method.

Variables

The outcomes were assessed by a questionnaire, which was divided into 3 parts: 1) knowledge of stroke, consisting of prevention methods (yes-no questions 12 items) and warning symptoms of stroke (yes-no questions 10 items); 2) planned response when suspecting acute stroke, assessed by an opened-end question: "If you have symptoms suspected to be acute stroke, what is the first thing you will do?"; and 3) factors associated with stroke knowledge scores and planned response, consisting of participant characteristics, general knowledge and attitude of stroke disease. The questions to explore knowledge of stroke and associated factors were applied from previous studies.[19–23] The questionnaire was verified for validity by 3 family physicians, and calculated Item Objective Congruence Index for each question. The results of all

questions were more than 0.5; however, we adjusted some questions according to the specialist's suggestion. Then we conducted a pretest in thirty patients living with diabetes mellitus and/or hypertension in another PCU; which was close to our study setting. Test-retest reliability was used to verify correlation coefficient of stroke knowledge scores in prevention methods and warning symptoms; the results were 0.9 and 0.7, respectively.

Data collection

Those patients who fit into our eligibility criteria were invited to participate in our research, and written informed consent was obtained. The participants completed the questionnaire by themselves; except for participants who could not read the questionnaire, in such cases the researcher helped by interviewing.

Data management and analysis

The data were entered in Epidata (version 3.1, Denmark), with double entry basis, and analyzed using the R program (R Core Team 2021, Vienna, Austria). Descriptive statistical analysis was used to report the sociodemographic characteristics of the participants, score and detail of answer. For variables in age of participants, we used the definition of age ≥ 60 years old to be cut-off point of elderly; according to United Nations and Thailand's Elderly Act. We presented categorical data in terms of frequencies and percentage; while continuous data were checked for normal distribution, and median with interquartile range (IQR) was used when normal distribution assumption was not met. We used multiple linear regression and multiple logistic regression to assess associated factors with stroke knowledge scores and appropriateness of planned response when suspecting acute stroke. Variables were eliminated in a stepwise model, until a final model resulted. Finally, significant factors were identified, based on adjusted coefficient (β) and adjusted Odds ratio, with 95% CI. A p-value < 0.05 was considered as significant.

Ethics statement

The study protocol was approved by the Office of Human Research Ethics Committee (HREC), Prince of Songkla University (REC 63-082-9-4). All participants signed informed consent forms after reading the participant information sheet.

RESULTS

The baseline characteristic of 312 participants are shown in Table 1; two thirds of them were elderly [age range from 30.0 to 93.0 years; median (Q1, Q3) = 64.0 years (58.0, 71.0)] and more than half of them were female. Median duration from time of diagnosis to having diabetes and hypertension was ten years for both diseases.

Table 2 shows general knowledge and attitude about stroke disease in our participants; 20.5% of them had no prior knowledge of "stroke", and most of them thought that a stroke was preventable. Only 5.0% of patients living with diabetes and/or hypertension thought that they had a high risk of developing stroke, and about one third of the participants believed they had no risk of developing stroke. The participants were tested for 2 parts of stroke knowledge, consisting of: prevention methods and warning symptoms of stroke. Table 3 shows the details of the answers in each question concerning knowledge of stroke prevention methods (full score = 12 points). The median of score (Q1, Q3) was 9 points (8, 10), and most of them had the correct knowledge concerning a lifestyle that could prevent stroke. However, almost half of the participants are of the opinion that using herbal medication can prevent stroke. Table 4 shows the answers of each question concerning warning symptoms of stroke (full score = 10 points). The median score (Q1, Q3) was 7 points (6, 8), and more than half of them had correct knowledge in stroke warning symptoms; especially the 3 symptoms in the acronym FAST, by the National Stroke Association, American Heart Association.[15] More than a third of participants did not know that sudden and severe unexplained headaches, sudden confusion as well as sudden trouble in seeing may be presenting symptoms of acute stroke. Details of action that participants undertook when having symptoms of suspected acute stroke are shown in Table 5. More than half of them would go to the hospital immediately by themselves or with family members, only 22.5% of them would go to the hospital immediately by calling EMS, and about 10.0% of them would not go to the hospital immediately; instead they first rested or used alternative medicine.

Multivariate analysis of factors associated with stroke knowledge scores and appropriateness of planned response when suspecting acute stroke are shown in Table 6. Participants who thought that they had a low chance of having a stroke, and who had income were significantly associated with a higher score of stroke prevention methods when compared with participants who thought that they had no chance of having a stroke, and whom did not have income (β = 0.40 and 0.57, p = 0.035 and 0.04; respectively). Additionally, adult patients and those with a longer duration of being diagnosed with DM were significantly associated with an appropriate planned response when suspecting acute stroke (adjusted OR = 2.22 and 1.10; p = 0.01 and 0.02; respectively).

TABLE 1. Sociodemographic characteristics of participants (n=312).

Characteristics	
Age (years)	
< 60	106 (34.0)
≥ 60	206 (66.0)
Gender	
Male	126 (40.4)
Female	186 (59.6)
Occupation	
Unemployed	79 (25.3)
Employed	160 (51.3)
Retirement	73 (23.4)
Income	
No income	46 (14.7)
Having incomes	266 (85.3)
Highest level of education	
Primary education level	150 (48.1)
Secondary education level	76 (24.4)
Tertiary education level	86 (27.5)
Marital status	
Single	25 (8.0)
Married	271 (86.9)
Divorced/widow	16 (5.1)
Smoking status[a]	
Never	231 (74.0)
Ex-smoker	63 (20.2)
Current	18 (5.8)
Subgroup of participants	
DM alone	34 (10.9)
HT alone	170 (54.5)
DM with HT	108 (34.6)
Duration of diagnosed DM (year) [median (Q1, Q3)] (n=142)	10.0 (4.0, 15.0)
Duration of diagnosed HT (year) [median (Q1, Q3)] (n=278)	10.0 (5.0, 15.0)

Data are presented as n (%) unless indicated otherwise.
Abbreviations: DM : diabetes mellitus; HT : hypertension
[a] Smoking status[24];
- never = participant who has never smoked, or smoked less than 100 cigarettes in their lifetime
- ex-smoker = participant who has smoked at least 100 cigarettes in their lifetime, but quit smoking at the time of interview
- current = participant who has smoked at least 100 cigarettes in their lifetime and is currently smoking

TABLE 2. General knowledge and attitude about stroke disease (n=312).

General knowledge and attitude	n (%)
Knowing about stroke before participating in the research	
No	64 (20.5)
Yes	248 (79.5)
Aware that stroke is a preventable disease	
No	11 (3.5)
Yes	301 (96.5)
The chance you could have a stroke	
No	91 (29.2)
Low	158 (50.6)
Moderate	50 (16.0)
High	13 (4.2)

TABLE 3. Knowledge on methods to prevent stroke (n=312).

Methods	Correct answer n (%)
Regular exercise at least 3-5 times/week	304 (97.4)
Well control of blood pressure, plasma glucose and serum lipid	303 (97.1)
Knowing risk factors of stroke and preventing them	300 (96.2)
Smoking cessation	282 (90.4)
Weight reduction in overweight or obese patients	281 (90.1)
Decrease consumption of salty foods	265 (84.9)
Increase consumption of unsweet vegetables and fruits	261 (83.7)
Decrease consumption of sweetened beverages	255 (81.7)
Decrease amount of alcohol drinking in alcoholic consumers	246 (78.8)
Using herbal medication[a]	179 (57.4)
Be careful of head trauma[a]	90 (28.8)
Drinking pure water[a]	58 (18.6)

[a] lifestyles that cannot prevent stroke.

TABLE 4. Knowledge of the warning symptoms of stroke (n=312).

Question	Correct answer n (%)
Sudden numbness of unilateral face, arm or leg[a]	289 (92.6)
Sudden weakness of unilateral face, arm or leg[a]	287 (92.0)
Sudden trouble in walking/dizziness, loss of balance or coordination	280 (89.7)
Sudden trouble in speaking[a]	279 (89.4)
Sudden, severe headache with unknown cause	231 (74.0)
Sudden muscle strain of arm or leg[b]	195 (62.5)
Sudden chest pain[b]	190 (60.9)
Sudden confusion or misunderstanding	189 (60.6)
Sudden trouble in seeing (one or both eyes)	186 (59.6)
Sudden numbness or weakness of bilateral face, arm or leg[b]	53 (17.0)

[a] 3 symptoms in the acronym FAST, by the National Stroke Association, American Heart Association[15]
[b] symptoms that are not warning symptoms of stroke

TABLE 5. Planned response when suspecting acute stroke.

Planned response	n (%)
Go to hospital immediately by themselves or family members	210 (67.3)
Go to hospital immediately by calling emergency medical services[a]	71 (22.8)
Rest at home	20 (6.4)
Using alternative medicine	7 (2.2)
Go to hospital next day	4 (1.3)

[a] appropriated planned response when suspecting acute stroke

TABLE 6. Factors associated with stroke knowledge scores and appropriateness of planned response when suspecting acute stroke.

	Stroke knowledge score				Appropriate planned response when suspecting acute stroke by calling EMS[b]	
	Prevention methods[a]		Warning symptoms[a]			
	β (95%CI)	p-value	β (95%CI)	p-value	adjusted OR (95%CI)	p-value
General knowledge and attitude of stroke disease						
Knowledge of stroke before participating in the research						
No	Ref.		Ref.		Ref.	
Yes	0.36 (-0.04,0.76)	0.076	0.29 (-0.14,0.73)	0.189	1.57 (0.75,3.31)	0.235
Aware that stroke is a preventable disease						
No	Ref.		Ref.		Ref.	
Yes	-0.09 (-0.94,0.77)	0.845	-0.21 (-1.14,0.73)	0.664	1.03 (0.20,5.23)	0.970
The chance you could have a stroke						
No chance	Ref.		Ref.		Ref.	
low chance	0.40 (0.03,0.77)	0.035*	0.19 (-0.21,0.59)	0.359	0.7 (0.36,1.35)	0.289
Medium chance	0.46 (-0.03,0.96)	0.068	-0.17 (-0.71,0.38)	0.543	0.55 0.22,1.38)	0.202
High chance	0.55 (-0.3,1.39)	0.206	0.32 (-0.61,1.25)	0.497	0.84 0.21,3.36)	0.806
Sociodemographic characteristics of participant						
Age (years)						
≥ 60	Ref.		Ref.		Ref.	
< 60	0.13 (-0.24,0.50)	0.493	0.31 (-0.09,0.72)	0.127	2.22 1.18,4.17)	0.014*
Occupation						
Unemployed	Ref.		Ref.		Ref.	
Employed	-0.30 (-0.77,0.17)	0.205	0.10 (-0.41,0.61)	0.691	2.31 0.87,6.18)	0.095
Retirement	-0.06 (-0.58,0.47)	0.831	0.26 (-0.31,0.83)	0.369	2.40 0.82,7.04)	0.111
Income						
No income	Ref.		Ref.		Ref.	
Having incomes	0.57 (0.02,1.12)	0.041*	0.40 (-0.19,1.00)	0.184	0.92 0.29,2.93)	0.888
Marital status						
Single	Ref.		Ref.		Ref.	
Married	0.15 (-0.74,0.45)	0.626	0.15 (-0.50,0.80)	0.647	0.44 0.17,1.12)	0.085
Divorced/widow	-0.06 (-0.97,0.85)	0.897	0.03 (-0.97,1.02)	0.958	0.20 0.03,1.22)	0.081
Subgroup of participants						
DM alone	Ref.		Ref.		Ref.	
HT alone	0.31 (-0.41,1.03)	0.401	0.08 (-0.71,0.87)	0.84	3.32 (0.89,12.41)	0.074
DM with HT	0.02 (-0.61,0.65)	0.956	0.10 (-0.59,0.80)	0.768	0.76 (0.24,2.40)	0.645
Duration of diagnosed DM (years)	0.04 (0,0.08)	0.076	0.02 (-0.02,0.07)	0.324	1.10 (1.01,1.19)	0.021*
Duration of diagnosed HT (years)	-0.02 (-0.05,0.01)	0.138	0.01 (-0.02,0.04)	0.475	0.96 (0.91,1.02)	0.173

Abbreviations: EMS : emergency medical services; DM : diabetes mellitus; HT : hypertension
* statistical significant, [a] multiple linear regression, [b] multiple binary logistic regression

DISCUSSION

Most patients living with diabetes and/or hypertension, which are a high risk group for cardiovascular disease (CVD), could identify more than half of the stroke prevention methods and warning symptoms; especially in the income group. However, they still did not show enough awareness with regards to their attitude of concern in having a stroke. Additionally, they had an inappropriate planned response when suspecting acute stroke; especially the elderly and those with a short duration of being diagnosed with DM.

Our study was conducted in patients living with diabetes and/or hypertension, and most of our participants were elderly. This is in contrast with previous studies[25–27], in which they were population-based survey's; therefore, reporting on younger aged participants. Women outweighed the proportion of men in this study, which is the same as in the previous studies.[18,28] Almost half of the study participants graduated to the level of primary school; this is close to a previous study that reported their participants were of a low education level.[25]

Most of the participants claimed that they knew of the disease, namely "stroke", before participating in our research, similar to a previous study in hypertensive patients[29]; and knew that a stroke is preventable. However, we were surprised that our participants, who were in a high risk group for CVD, had little awareness about developing a stroke; this result was lower than that observed amongst hypertensive patients in Pakistan.[29] It may be due to our participants having a higher proportion of elder patients, and lower education levels.

Most of our study participants had appropriate knowledge with regards to the lifestyle measures to adopt for stroke prevention. This finding corresponds with a previous study.[30] This could be due to Thailand having emphasized the prevention of CVD for many years; such as, the campaign in reduction of CVD risk via various advertising media. However, more than half of our participants misunderstood that herbal medication can help to prevent stroke; it may be due to herbal usage being common in Thailand; including among the elderly who were the majority of participants in our research.[31] Use of herbal medication may make people feel self-reliant[30]; so it is common in patients with chronic diseases.[32,33] With regards to knowledge of stroke warning symptoms; most of the participants had the correct knowledge concerning symptoms that may be a presentation of acute stroke. The results are in accordance with previous studies[12,18,27–29,34,35], and may be due to three out of four of them being an element in the acronym FAST, by the National Stroke Association, American Heart Association.[15] Additionally, this is well known worldwide in medical advertising for mnemonics in acute stroke symptom detection and early management by immediately going to the hospital. "FAST" is one of the more successful public campaigns in promoting knowledge of common stroke warning symptoms; as we found that most of our participants knew all 3 symptoms in "FAST", similar to a previous study.[16] However FAST still has flaws, in that is does not include the less common stroke warning symptoms; as nearly half of our participants did not know that sudden trouble in seeing can be a warning symptom of stroke; this was similar to previous studies.[18,20,30,36] Although, the 'T' in FAST makes mention that when a patient suspects an acute stroke they should go to the hospital immediately, it does not mention this should be done via EMS, according to guideline recommendations.[13] The most common planned response when acute stroke was suspected, was to go to the hospital immediately by themselves, or with family members; while only a quarter of them have an appropriate planned response by calling EMS; this is in line with a previous study.[19] Previous studies have shown that most people believed in the benefit of EMS; however, the low rate of EMS usage may be caused from a concern in the difficulty in its process and longer ambulance waiting times.[37,38] Alternative medicine was still a choice of planned response when suspecting acute stroke, as was the case in previous studies.[12,19,34] This may be due to Thai traditions and faith in alternative medicines.

This study shows that participants who have income had a statistically significant association with increasing of scores of stroke prevention methods; which is the same as a previous study.[28] However, the increasing score of 0.57 points may not have any clinical benefit. Adult participants and those with a prolonged diagnosed of DM have an appropriate planned response when having symptoms suspected of being an acute stroke when compared with elderly patients, and those with a shorter diagnoses of DM; these results correspond with previous studies.[21,39] It may be due to cognitive impairment problems in the elderly, resulting in not retaining knowledge from their physicians or public advertising, or that they cannot call EMS by themselves.[38] For patients with longer diagnoses of DM, it may increase the patient's awareness and chance of receiving knowledge over time.

The strengths of this study consist of: firstly, our research is one of the few studies that evaluated knowledge of stroke in a high risk population. Secondly, we assessed participant's awareness of the chance of developing a stroke, which was a topic of little interest in previous studies. Thirdly, we asked for information

on planned responses when suspecting acute stroke; instead of assessing knowledge in early management. This was because knowledge is only just one factor that affects behavior, and different contexts of each person influences action; such as, ease of accessibility to a nearby hospital or ability to call EMS. In addition, this study is one of the few study's that explored planned responses by an opened-end question, instead of multiple-choice questions[12,19,34]; in order to decrease a chance of bias from choosing the 'good answers' from choices. However, there were some limitations. Firstly, we did not explore the reason of planned response answers, for which the results could be used for developing a method to the solution of problems. For example; if their inappropriate planned response came from a lack of knowledge, the proper intervention would be education on the benefits of EMS usage. Secondly, this study was conducted in a primary care unit of a tertiary care hospital, so the results cannot be generalized to other hospital settings. Lastly, the sample size was calculated for the primary objective, so the number of the sample size may not be large enough to show significance in factors associated with stroke knowledge scores, and appropriateness of planned response. For future research, we suggest extending this to other health care settings, increasing the sample size for increasing statistical power of results, and adding another helpful topic for solving some problems directly; such as, reason of planned response as well as ability and barrier in using EMS.

CONCLUSION

Patients living with diabetes and/or hypertension still do not have enough knowledge of stroke. Additionally, they have a less than acceptable level of awareness in concerns to the risks of developing a stroke. The participants in this study had an inappropriate planned response when suspecting acute stroke. FAST is a successful public campaign for promoting knowledge of the most common warning symptoms of stroke.

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The Analgesic Effect of Cryotherapy on Patients Undergoing Extracorporeal Shock Wave Lithotripsy: A Randomized Controlled Trial

Chaowat Pimratana, M.D.*, Kornkanok Hengsawat, M.D.**

**Department of Surgery, **Department of Anesthesiology, Buri Ram Hospital, Buri Ram 31000, Thailand.*

ABSTRACT

Objective: To compare the degree of pain between cryotherapy and standard preoperative care in the treatment of urolithiasis with extracorporeal shock wave lithotripsy (ESWL).

Materials and Methods: A total of 180 ESWL patients were randomly assigned to experience the standard preoperative method, or additional cryotherapy (ice pack application on the ESWL site) for 10 minutes before ESWL. The primary outcome was the maximum difference of pain intensity score from baseline during ESWL and the secondary outcomes, which were analgesic consumption, pulse rate, adverse events, stone free rate, and complications that were gathered and analyzed.

Results: The maximum change in pain intensity score from baseline during ESWL in the cryotherapy group was significantly lower than in the control group (VAS score 4.0±1.9 vs. 5.2±2.7, p=0.002). The cryotherapy group showed significantly less total fentanyl consumption than the control group (85.3±22.0 mcg vs. 93.6±25.6, p=0.021). We found no significant difference in stone free rate, adverse events or complications in either group.

Conclusion: Preoperative cryotherapy using ice packs for 10 minutes can provide an effective analgesic for ESWL. Adequate pain control with cryotherapy should be an option of pain management during ESWL.

Keywords: Cryotherapy; ESWL; pain; urolithiasis (Siriraj Med J 2022; 74: 85-90)

INTRODUCTION

Extracorporeal shock wave lithotripsy (ESWL) has been a less-invasive option for the treatment of the majority of patients with urolithiasis since 1980.[1] The advancement of the new-generation lithotripter machines has made ESWL more effective with minimal morbidity, making it possible to perform ESWL without the need for general or spinal anesthesia.[2] However, this procedure can be painful because the continuous shock waves act on the cutaneous superficial skin nociceptors and visceral nociceptors, such as the renal capsule, peritoneal, and musculoskeletal pain receptors.[3] Adequate pain control is an important role in achieving successful ESWL treatment. Opioid and sedative drugs are common analgesics for ESWL, but certain amounts of opioids may cause nausea, vomiting, and delayed recovery of patients.[4]

Cryotherapy involves cold applications, which have effects on both the local site around the treatment area and at the level of the spinal cord via neurologic and vascular mechanisms.[5] It is hypothesized that cold applications can control pain by increasing the pain threshold and tolerance by reducing nerve conduction velocity, as described by the gate control theory, whereby pain is transmitted to the dorsal horn of the spinal cord

Corresponding author: Chaowat Pimratana
E-mail: pchaowat@gmail.com
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ORCID ID: http://orcid.org/0000-0003-3754-774X
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via C-fibers and Aß fibers; C-fibers release substance P, which opens the gate and Aß fibers close the gate. Cold applications activate the Aß fibers, thereby stopping the transmission of pain stimuli.[6,7] Many studies have been published in regard to cryotherapy for pain reduction, for example with knee operations, thoracic operations, gynecologic operations, and abdominal operations.[8–11] However, no previous study has reported the analgesic effect of cryotherapy on ESWL.

The aim of this study was to evaluate the effectiveness of cryotherapy regarding pain control during ESWL for urolithiasis treatment.

MATERIALS AND METHODS

This randomized controlled trial was conducted at Buri Ram Provincial Hospital, Buri Ram, Thailand. The protocol of this research was reviewed and approved by the ethical review board of Buri Ram Hospital (BR 0032.102.1/46) and registered in the Thai Clinical Trials Registry (TCTR20201226002). Patients with indication for ESWL treatment were randomly assigned into two groups. Patients in the first group were given ice pack compression at the skin on the ESWL site while patients in the second group were given standard preoperative ESWL. Between October 2020 and March 2021, kidney and ureteric stone patients scheduled for ESWL aged 18 to 80 years with the American Society of Anesthesiologists (ASA) physical status of I, II or III were eligible for the study. This study excluded patients with a history of allergies to the drugs that were used for the ESWL treatment, patients with psychological disorders, neurological disorders, dermatologic disorders (inflammation or eczema within the field of cold therapy), and patients that were unable to comprehend or use the visual analog scale (VAS).

After obtaining the informed written consent, the patients that met the criteria were enrolled and divided into two groups using computer-generated random numbers and opaque sealed envelopes. The patients in group I received cryotherapy; ice pack compression before ESWL. The ice pack was kept at -10°C in a thin cloth bag ready for future use. The ice pack was then placed on the skin at the site of the ESWL for 10 minutes before beginning the procedure. Control group II received standard preoperative care. Both groups received premedication-1000 mg paracetamol and 5 mg diazepam-orally 30 minutes before the ESWL. Initial intravenous fentanyl 1 mcg/kg began five minutes before the beginning of the ESWL for every patient from both groups and a supplementary dose of fentanyl 20 mcg intravenously was given to patients whose pain score was greater than 4 or whose pain tolerance was low. All patients underwent ESWL using a Dornier Delta III Lithotripter machine in a fully integrated operating room, and the procedure was carried out using a similar protocol. An anesthetic nurse, who was not involved in the study, recorded the patients' perioperative anesthetic parameters every five minutes. These parameters included blood pressure, pulse rate, respiratory rate, oxygen saturation, sedative score, pain score, and nausea/vomiting. The pain scores were placed on an 11-point visual analog scale (VAS) and ranged from 0 to 10, with 0 representing no pain and 10 representing the worst pain.

The primary outcome was the maximum of changes in the pain intensity score from baseline during the ESWL, using the VAS score. The secondary outcomes were total fentanyl consumption, pulse rate, perioperative nausea/vomiting, stone free rate at one month after ESWL, and any adverse events or complications.

Statistical analysis

A preliminary study containing 40 patients (20 per group) was conducted. The preliminary study reported the VAS score at 4.9±2.1 in the control group and 3.8±2.1 in the experimental group. The mean scores from the preliminary study were used to calculate an appropriate sample size for the main study. The sample size calculation for the two independent mean tests using a power 90% and a significance level of 0.05 revealed a minimum sample size of 81 participants in each group. We added 10% of the subjects in order to accommodate the projected dropout rate. Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR) and were analyzed between the two groups by using a t-test or the Mann-Whitney U test. The categorical data were expressed as number and percentage and were compared using a chi-squared test or Fisher's exact probability test. Statistical significance was set at p-value <0.05.

RESULTS

One hundred and eighty-two ESWL cases were enrolled in the study and control groups. Two patients in our study were excluded. Each group was composed of 90 cases (Fig 1). There was no difference in the demographic data for either group, including age, sex, body mass index (BMI), ASA physical status, stone location, time of ESWL, lateralization, or stone size (Table 1). Intra-operative parameters, base line pain score, pulse rate, and initial fentanyl doses were not statistically different between the two groups. The maximum of change in the pain intensity score from baseline during the ESWL in the cryotherapy group was significantly lower than in the

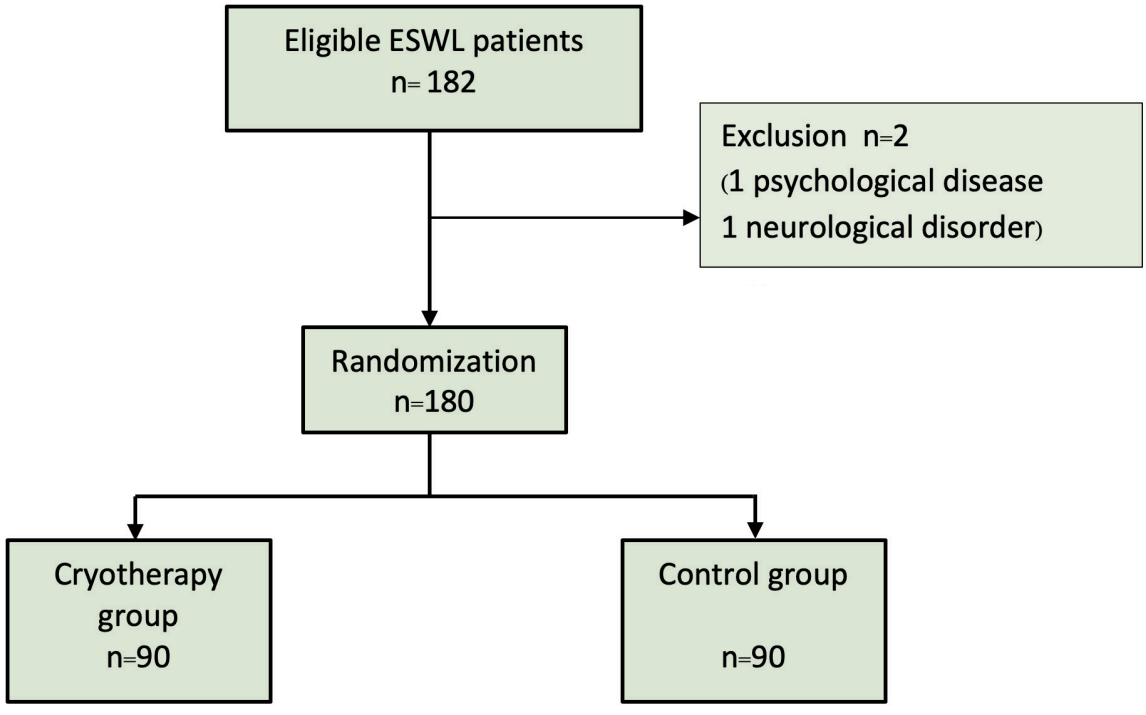

Fig 1. Consort diagram demonstrating the flow of participants through each stage of the randomized trial.

TABLE 1. Knowledge of the warning symptoms of stroke (n=312).

	Cryotherapy group (n=90)	Control group (n=90)	*p*-value
Gender			
Male n (%)	56 (62.2)	56 (62.2)	1.00
Age, year mean±SD	55.5±11.6	55.9±11.0	0.812
BMI, kg/m^2 mean±SD	24.3±3.9	24.4±4.8	0.862
ASA n (%)			0.875
1	29 (32.2)	31 (34.4)	
2	50 (55.6)	50 (55.6)	
3	11 (12.2)	9 (10.0)	
Stone location n (%)			0.414
Renal calculi	61 (67.8)	66 (73.3)	
Ureteric calculi	29 (32.2)	24 (26.7)	
Stone lateralization n (%)			
Right side	49 (54.4)	45 (50.0)	0.551
Time of ESWL n (%)			
First time	32 (35.6)	33 (36.7)	0.877
More than 1 time	58 (64.4)	57 (63.3)	
Stone size, mm median (IQR)	10.0 (10.0,15.0)	10.0 (9.5,15.0)	0.450

control group (VAS score 4.0±1.9 vs. 5.2±2.7, p=0.002). The cryotherapy group showed significantly lower total fentanyl consumption than the control group (85.3±22.0 mcg vs. 93.6±25.6 mcg, p=0.021). The stone free rate at one month after procedure was not difference between both groups (58.8% in the cryotherapy group vs. 62.2% in the control group. We found no significant difference in terms of adverse events regarding nausea/vomiting and bradycardia in either group (Table 2).

DISCUSSION

The introduction of ESWL has been revolutionary for the treatment of urolithiasis since 1980[1], and new-generation lithotripter machines have made ESWL more effective with less morbidity, less pain, shorter recovery time, and shorter hospital stays.[2] Nevertheless, the most common complaint is pain and discomfort during the treatment.[3] The pain experienced during ESWL is due to the continuous shock waves acting on its targets, whether from cutaneous tissue or deeper afferent nerves.[3] An adequate analgesia is mandatory for maintaining patient comfort and improving treatment outcomes[12], but sometimes the patients discharge from the hospital is delayed because of persistent sedation, and nausea and vomiting due to the anesthetic medication administered, so non-pharmacological methods may attract some attention.[13] Cryotherapy for pain relief has been used for many years, based on the gate control theory; cold application can inhibit cutaneous input to the spinal cord and reset the pain threshold in the central nervous system.[6,7,9] By this means, cryotherapy is able to block pain sensation from urinary calculi, whether its origin is from the skin or from the deeper structures. However, the major concern about cryotherapy is the decline in the patient's body core temperature and the local effects on the areas exposed to cryotherapy. The decline in the patient's body temperature has harmful physiological effects, such as Raynaud's phenomenon, while exposure to extreme cold can cause cold urticaria and frostbite of the skin.[14,15] The study by Palmieri et al. showed that

TABLE 2. Outcomes

	Cryotherapy group (n=90)	Control group (n=90)	*p*-value
VAS score			
At baseline median (IQR)	0.0 (0.0,0.0)	0.0 (0.0,0.0)	0.946
Maximum VAS during ESWL mean±SD	4.3±1.7	5.5±2.6	<0.001*
Change of maximum VAS from baseline mean±SD	4.0±1.9	5.2±2.7	0.002*
Pulse rate mean±SD			
At baseline	63.3±9.4	65.7±11.9	0.135
At 15 minutes	63.2±9.4	65.5±11.6	0.144
At 30 minutes	61.2±10.7	63.5±11.4	0.170
Initial fentanyl dose (mcg) mean±SD	69.9±19.4	74.3±20.3	0.140
Total fentanyl consumption (mcg) mean±SD	85.3±22.0	93.6±25.6	0.021*
Nausea and vomiting n (%)	2 (2.2)	2 (2.2)	1.00
Bradycardia n (%)	11 (12.2)	9 (10.0)	0.635
Skin complication (necrosis or frostbite) n (%)	0 (0)	0 (0)	NA**
Stone free rate (%)	58.8	62.2	0.760

* p-value <0.05
** NA = not applicable

a cold application at the skin site for 10-20 minutes did not decline the body core temperature.[16] Thienpont et al. demonstrated that continuous cryotherapy of not more than 20 minutes did not cause frostbite on the skin flap after knee arthroplasty.[15] Further, Natalia et al. demonstrated that a cryotherapy application of not more than 20 minutes was safe and not uncomfortable for any participants.[17] In this study, our protocol applied 10-minute cryotherapy and close monitoring for any adverse event from cryotherapy during the procedure.

Many studies have used cryotherapy to decrease pain in musculoskeletal surgery, gynecologic surgery, cardiothoracic surgery, and abdominal surgery. The overall results revealed beneficial outcomes in terms of pain reduction and pain management.[8–11] The present study is the first to emphasize the effects of cryotherapy or cold application in ESWL treatment. In addition, this study used the change of maximum VAS score from baseline for the primary outcome instead of the patient's stated VAS score after he or she had his/her operation. Traditionally the VAS score is clinically meaningful from the patient's perspective and clinical decisions are made based on such. However, the VAS mean scores cannot capture the complete pain experience because pain is both subjective and multidimensional.[18] Thus this study used the change of maximum VAS score from baseline for the primary outcome, which offers an alternative measurement of the analgesic effect during the actual procedure for the main outcome of our study. We found that the pain intensity score in the cryotherapy group was significantly lower than in the control group (VAS score 4.0±1.9 vs. 5.2±2.7, p=0.002).

Regarding the objective parameter, we used total fentanyl consumption for the secondary outcome. The cryotherapy group showed significantly lower total fentanyl consumption than the control group (85.3±22.0 mcg vs. 93.6±25.6 mcg, p=0.021).

In our study, bradycardia was a common side effect, which may have been caused by both opioid usage and cryotherapy; nevertheless, the pulse rate and bradycardia events did not differ between the two groups (12.2% in the cryotherapy group vs. 10.0% in the control group, p=0.635), as the incidence was likely to be a minor adverse effect of opioids rather than the effect of cryotherapy.[13] The emetic effect of opioids has been documented; in our study, there was no significant difference in nausea or vomiting between both groups (2% in the cryotherapy group vs. 2% in the control group, p=1.000). Local skin complications from cryotherapy were not present in our study. Taking into consideration the significant benefit of pain management from cryotherapy during ESWL, we believe that cryotherapy is a safe, inexpensive, practical, and effective adjuvant pain relief method.

There were some limitations in this study. First, we could not blind the cold application between the cryotherapy group and the control group. Secondly, the VAS score for pain was subjective and multidimensional. Even though this study used the change of maximum VAS score from baseline as an alternative measurement, this measurement is prone to variation regarding the patient's pain tolerance level. In our study the stone free rate was not significant difference between both groups. Thus, future studies should consider using a more objective measurement regarding outcome evaluation such as the success rate of the stone treatment.[3,12,19,20]

CONCLUSIONS

In this study we demonstrated that preoperative cryotherapy using an ice pack for 10 minutes can provide an effective analgesic for ESWL treatment. Adequate pain control with cryotherapy should be an option of pain management during ESWL.

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Stability and Sterility of Extemporaneously Prepared 0.01% Atropine Ophthalmic Solution in Artificial Tears and Balanced Salt Solution

Jureeporn Sri-in, M.Sc.*, Waraphorn Sisan, M.Sc.*, Phonphailin Kingkhangphloo, B.Sc.*, Pinpilai Jutasompakorn, M.D.*, Weerawadee Chandranipapongse, M.D.*, Somruedee Chatsiricharoenkul, M.D.*, Onchira Buranakan, MD.**, Arpha Pornseth, M.D.**, Thammanoon Surachatkumtonekul, M.D.**

*Department of Pharmacology, ** Department of Ophthalmology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Objective: The aim of this study was to investigate the physicochemical and microbiological stability of extemporaneously prepared 0.01% atropine ophthalmic solution in unopened eyedropper and in simulated use condition.

Materials and Methods: Two formulations of 0.01% atropine solutions, atropine in artificial tear and atropine in balanced salt solution (BSS), were prepared using 0.5 mL insulin syringes. In unopened conditions, 0.01% atropine solutions were stored for six months at refrigerated temperature (2-8°C) or room temperature (25±2°C). Visual inspection, atropine quantification, pH measurements, and sterility assay were analyzed at baseline, and every month for six months. In simulated use condition, 0.01% atropine solutions stored at refrigerated and room temperature were analyzed at 0, 15 and 30 days.

Results: In unopened conditions, both of 0.01% atropine formulations stored at refrigerated temperature showed satisfactory stability. Atropine remained within 90% to 110% of the initial concentration up to six months. Under room temperature, both formulations of atropine were less than 90% of their initial value after 4 months storage. In simulated use condition, atropine concentration was within 90% to 110% of initial value after 30 days at refrigerated and room temperature. All atropine solutions prepared in artificial tear and BSS were free from bacterial contamination throughout the study. No alteration of physical appearance (i.e., precipitation, discoloration) was observed, and pH values also remained nearly unchanged.

Conclusion: Both formulations of 0.01% atropine are physicochemically stable for up to 6 months when kept unopened in refrigerator, and for 1 month at refrigerated and room temperatures in simulated use condition.

Keywords: Myopia; atropine; stability; sterility; artificial tear; balanced salt solution (Siriraj Med J 2022; 74: 91-99)

INTRODUCTION

Myopia is an eye disorder and is the principal type of refractive error. Previous population-based studies have reported that the prevalence rates of myopia are highest in East Asian populations.[1,2] Their findings showed that 80% of schoolchildren in Taiwan, Hong Kong, and China, as well as up to 96% of schoolchildren in South Korea, suffered from myopia.[3] It is estimated that myopia will affect nearly 5 billion people by 2050.[2,4,5] Currently, there are many methods for controlling myopia progression, such as spectacles, contact lens, and pharmaceutical strategies. Most of the studies in this field use atropine

Corresponding author: Thammanoon Surachatkumtonekul
E-mail: si95thim@gmail.com
Received 15 September 2021 Revised 28 October 2021 Accepted 17 November 2021
ORCID ID: https://orcid.org/0000-0002-0037-6863
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eye drops to reduce the rate of myopia progression in children.[6]

Atropine is a nonselective muscarinic antagonist, it binds to and inhibit muscarinic acetylcholine receptors, producing a wide range of anticholinergic effects. The precise mechanisms underlying the efficacy of atropine in slowing myopia progression are remains unclear. Various hypotheses have been postulated, including the action via muscarinic receptor pathways in the retina, choroid and sclera. These resulting in the prevention of axial elongation, inhibition of scleral proliferation and matrix synthesis. Moreover, atropine may be exerting its effect via other receptors present in the eye.[7-9]

Most "Atropine for the Treatment of Myopia (ATOM)" studies focus on the efficacy and safety of 1%, 0.5%, 0.1%, and 0.01% atropine in myopic Asian children aged 6-12 years old. Their findings illustrated that 0.01% atropine is effective for retarding myopia with minimal side effects, compared with higher doses of atropine.[10-13] Recently, the studies of Low-concentration Atropine for Myopia Progression (LAMP) have demonstrated the efficacy and safety of atropine concentrations of 0.05%, 0.025%, and 0.01% in China in children aged 4-12 years old with myopia. All these concentrations drastically reduced the rate of myopia progression without any vision-threatening side effects.[14] Generally, 0.01% atropine is the most common strategy for managing childhood myopia and is widely used all over the world, including in Asian countries, such as Singapore, Taiwan, China, and Thailand.[2] The treatment period usually lasts for at least 2 years, and may take longer if myopia progression persists.[15]

Since 0.01% atropine ophthalmic solution is not commercially available in Thailand, eye drops are prepared by ophthalmologists or hospital pharmacists. The 1% commercial atropine is diluted with 0.9% sodium chloride solution, balanced salt solution, or various brands of artificial tears depending on the discretion of the ophthalmologist. Long-term treatment with atropine is required for myopia control, and hence a longer shelf-life is necessary to extend the follow-up intervals for patients. However, there is little data concerning the long-term stability of low-dose atropine eye drops. Only two studies have been published demonstrating that 0.01% atropine in 0.9% sodium chloride solution with or without preservatives is stable for six months in an unopened container, both at room temperature and refrigerated temperature.[16,17] However, there are no studies on the stability of 0.01% atropine eye drops prepared in artificial tears (with preservatives) or balanced salt solution (without preservatives). The lack of long-term stability and sterility data limits the conservation period of these preparations. Consequently, the aim of this study was to determine the long-term chemical, physical, and microbiological stability of extemporaneously prepared atropine in artificial tears containing preservatives and in balanced salt solution at refrigerated and room temperature. The chemical, physical, and microbiological stability of both formulations were also tested in simulated use conditions.

MATERIALS AND METHODS

Reagents and materials

Atropine sulfate monohydrate, the reference standard of atropine, and scopolamine hydrobromide, the internal standard for atropine, were obtained from The United States Pharmacopeial Convention, Inc., USA. 1% Atropine sulfate solution was obtained from Alcon-Couvreur, Belgium. Balanced salt solution (BSS) was obtained from Alcon Research LLC, USA. Hydroxypropyl methylcellulose (HPMC), an artificial tears solution with sodium perborate as a preservative, was obtained from Silom Medical Co., Ltd., Thailand. LC/MS grade acetonitrile and formic acid were obtained from Scharlau, Barcelona, Spain. HPLC-grade methanol was obtained from Fisher Scientific UK, the United Kingdom. Type I water was produced using a Milli-Q water purification system from Millipore Corporation, USA.

0.01% Atropine eye drops preparation

The preparation processes were undertaken by scientists in a clean room of class 1.0×10^5 (air cleanliness level of a maximum of 2.93×10^4 particles (≥0.5 μm) per cubic meter). Two formulations of 0.01% atropine ophthalmic solutions were prepared aseptically using a 0.5 mL insulin syringe:

- Atropine in preserved artificial tears (HPMC), prepared by dissolving 0.1 mL of 1% atropine sulfate solution into 10 mL artificial tears.
- Atropine in balanced salt solution, prepared by dissolving 0.15 mL of 1% atropine sulfate solution into 15 mL balanced salt solution (BSS).

Clear low-density polyethylene commercial eyedroppers of HPMC and BSS were used as the containers in this study.

Study design

The stabilities of the 0.01% atropine ophthalmic solutions were studied at refrigerated temperature (2-8 °C) or room temperature (25±2 °C). The durations of the study for the unopened eyedroppers and in simulated use conditions were 6 months and 1 months, respectively.

In short, there were 4 subgroups in each study (for the unopened eyedroppers and simulated use conditions) as shown below:

(i) 0.01% atropine in HPMC at refrigerated temperature,
(ii) 0.01% atropine in HPMC at room temperature,
(iii) 0.01% atropine in BSS at refrigerated temperature,
(iv) 0.01% atropine in BSS at room temperature.

The eyedroppers stored at room temperature were kept on the shelf, protected from light in their commercial packages at 50%±10% residual humidity (RH).

Physicochemical and microbiological stability of the 0.01% atropine ophthalmic solutions in simulated use conditions

At day 0, 60 eyedroppers with two formulations of 0.01% atropine solutions were prepared, with 15 eyedroppers for each subgroup (i–iv). For illustration purposes, subgroup (i) with a total number of 15 eyedroppers was used as an investigation process example. Each of the 15 eyedroppers was emitted daily (1 drop of the 0.01% atropine solutions), that is, a drop was squeezed out of the eyedropper and collected for analysis instead of being dropped into the eye. Out of the 15 eyedroppers, 10 eyedroppers were obtained for visual inspection and sterility assay. Next, 5 eyedroppers were tested at day 0, 15, and discarded. Another 5 eyedroppers were tested at day 0 and 30. It is important to note the reason why the eyedroppers were discarded after the sterility assay on day 15. Namely, subgroups (i) and (ii) both had an approximate volume of 10 mL, while the sterility assay required at least 4 mL. Hence, after the daily emission and two sterility assays, there would be an insufficient amount of solution remaining for another sterility assay and so these were discarded. The remaining 5 eyedroppers from the 15 totals were used for the atropine quantification and pH measurements at days 0, 15, and 30.

After completing the 1-month study under simulated use conditions, further investigations were planned, with the aim to extend the experimental period of both formulations at refrigerated temperature to 2 months. There were 2 subgroups of eyedroppers here: (i) 6 eyedroppers of 0.01% atropine in HPMC at refrigerated temperature, and (ii) 6 eyedroppers of 0.01% atropine in BSS at refrigerated temperature. These two subgroups were investigated in the exact same manner as in the 1-month study. Out of the 6 eyedroppers in each subgroup, 4 eyedroppers were obtained for visual inspection and sterility assay at day 0, one at another time point (days 15, 30, 45, or 60), and one discarded (n = 1 for each time point/subgroup). The remaining 2 from the 6 eyedroppers were used for atropine quantification and pH measurements at days 0, 15, 30, 45, and 60 (n = 2 for each time point/subgroup).

Physicochemical and microbiological stability of 0.01% atropine ophthalmic solutions in the unopened eyedroppers

In total, 120 eyedroppers of 0.01% atropine solutions were prepared, comprising 30 eyedroppers for each subgroup: atropine in HPMC at refrigerated temperature, atropine in HPMC at room temperature, atropine in BSS at refrigerated temperature, and atropine in BSS at room temperature. In each subgroup, 5 unopened eyedroppers were used for the analysis at days 30, 60, 90, 120, 150, and 180 (n = 5 for each time point/subgroup). Each eyedropper was subjected to the following analyses: visual inspection, atropine quantification, pH measurement, and sterility assay. The baseline values for atropine quantification, pH measurement, and the sterility assay were obtained from the studies of the 0.01% atropine ophthalmic solutions under the simulated use conditions.

Analyses

Quantification of atropine

The liquid chromatography with tandem mass spectrometry (LC-MS/MS) method was applied for quantitative analysis of the extemporaneously prepared atropine solution. LC-MS/MS analysis was performed using an Acquity Ultra Performance LC™ (Waters, Co., Ltd. USA) coupled to a Quattro Premier XE Mass Spectrometer (Micromass Technologies, UK) equipped with an electrospray interface. For data acquisition and processing, a MassLynx 4.1 SCN627 system (Micromass Technologies, UK) was used.

Scopolamine hydrobromide was used as an internal standard (IS). The chromatographic separation of atropine and the internal standard was performed using a Kinetex C18 column (50×2.10 mm, 1.7 µm; Phenomenex Ltd., USA). The mobile phase was an 85:15 (v/v) mixture of 0.1% (v/v) formic acid and acetonitrile in an isocratic elution mode over a 2 min total run time. The flow rate was 0.3 mL/min and the column temperature were set at 30±5 °C. The injection volume was 1 µL. MS analyses were carried out using the multiple reaction monitoring (MRM) mode with positive electrospray ionization (ESI+). The mass transition ion-pair was selected as m/z 290.1 to 124.1 for atropine and m/z 304.1 to 138.1 for the IS.

Validation of this method was performed according to the International Conference on Harmonisation (ICH) guidelines.[12] Linearity was determined by preparing one calibration curve daily using six concentrations of atropine (50, 100, 150, 200, 300, and 400 ng/mL), obtained from atropine standard solution diluted in diluent solutions (methanol and Milli-Q water at a ratio of 1:1, v/v). The influence of different weighting factors ($1/x$ and $1/x^2$) on the sum of the percentage relative error

was evaluated and the results were compared with an unweighted calibration curve. Accuracy was tested by spiking the atropine reference standard with the atropine test sample (at a concentration of 200 ng/mL) to obtain three concentration levels, namely 80%, 100% and 120%, of the test sample concentration. The accuracy was evaluated on the basis of the calculated recovery values, and the results should be found within the range of 95%-105%. The precision of the methods was determined in terms of the intra-day precision (repeatability) and intermediate precision (within-laboratory reproducibility). The intra-day precision was assessed by injecting six replicates of three different concentrations of atropine standard solutions (100, 200, and 300 ng/mL) on the same day. The intermediate precision was determined by injecting the same solutions for three consecutive days. The intra-day and intermediate precisions are expressed as the relative standard deviation (RSD, %). A value of less than 5% was acceptable for both RSDs.

For sample preparation, 0.01% atropine solution from each eyedropper was diluted with the diluent solution to obtain a theoretical concentration of 200 ng/mL. A 100 µL aliquot of diluted atropine was transferred into a 1.5 mL micro tube and mixed with 20 µL internal standard solution at a concentration of 1,000 ng/mL. The micro tubes were thoroughly mixed by vortex mixing for 10 seconds. Then, 1 µL of the mixed solution was collected and transferred into an autosampler vial and submitted to LC-MS/MS analysis.

In the chemical stability assessment, the baseline concentration (day 0) was defined as 100% and the subsequent concentrations of each time point were calculated as percentages of the initial concentration. Acceptance criteria for the stability were defined as 90%-110% of the baseline concentration (including the limit of a 95% confidence interval of the measures).[13,14]

Visual inspection and pH measurements

During the study period, the physical appearance of the solutions was examined when the samples were taken from each eyedropper for the sterility assay. An approximately 4 mL sample was dispensed from each eyedropper into a 5 mL sterilized tube. Before sending the sample for the sterility assay, the atropine solutions were visually inspected under white light. The transparency, color, and presence of visible particles or haziness were noted.

For pH measurement, a 0.5 mL aliquot of 0.01% atropine from each sample was transferred into a 2.0 mL micro tube. Hand-held pH testing was performed on a SevenCompact S220 pH/ion meter with an InLab Micro Pro-ISM electrode (Mettler Toledo, Switzerland), which was calibrated at 25 °C in pH 4.01, 7.00, and 9.21 buffer solutions (Mettler Toledo, Switzerland). The pH change was considered acceptable if it did not vary by more than one pH unit from the initial value.[14]

Sterility assay

The sterility assay was carried out by the Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, in line with the United States Pharmacopeia (USP) for pharmaceutical microbiology testing.[15] First, 4 mL of 0.01% atropine solution from each eyedropper was aseptically taken and sent to the Department of Microbiology in a 5 mL sterilized tube for the sterility assay, using a direct inoculation method. Each sample was transferred directly to a fluid thioglycolate medium and soybean casein digest medium, and then incubated at 30-35 °C and 20-25 °C, respectively, for 14 days. The culture medium was then carefully examined for microbial growth.

RESULTS

Quantification of atropine

The retention times were 1.02 min for atropine and 0.70 min for scopolamine. The method was shown to be selective, as no interferences were observed at the retention times corresponding to 0.01% atropine in the artificial tears or in the BSS (Figs 1A-E). The calibration curve was linear for the concentrations ranging from 50-400 ng/mL and the determination coefficient R^2 was greater than 0.999 (Fig 1F). The weighting factor of 1/x was selected, since it was the one that reproduced the least sum of percentage relative errors (%RE). This method showed acceptable accuracy as the percentage recovery ranged from 99.42%-102.18% in the three different concentrations of atropine standard solutions. The precision was satisfactory, with the RSD of the intra-day and intermediate precision ranging from 1.05%-2.99% and 1.66%-2.94%, respectively.

Chemical stability

In the simulated use study, both formulations demonstrated chemical stability (concentration range between 90%-110% of the initial concentration) for up to 30 days at room temperature and 60 days at refrigerated temperature. The concentrations of the 0.01% atropine solutions stored at room temperature were between 97.60%-99.44% of the initial concentrations in HPMC and 102.26%-106.93% in BSS, and the 95% confidence interval was a maximum of +4.34%. For the 0.01% atropine solutions stored in refrigerator, the concentrations were

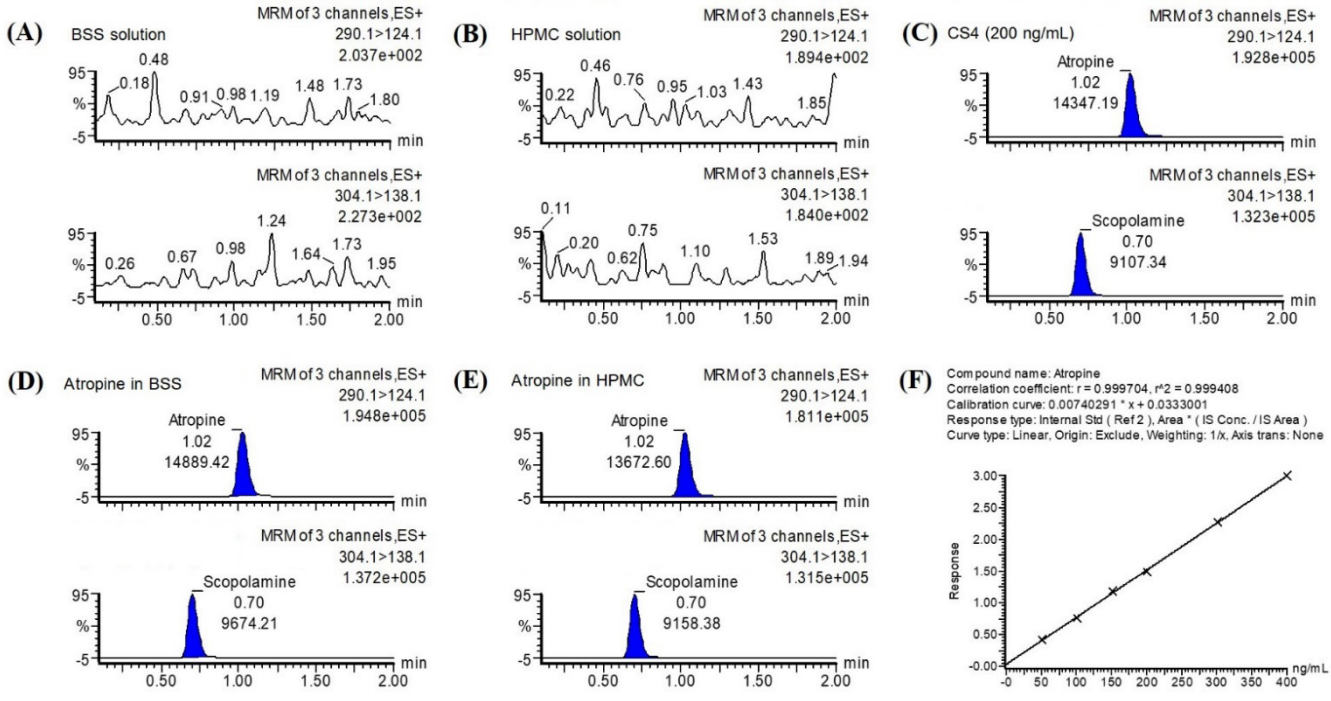

Fig 1. Chromatograms of: (A) BSS solution, (B) HPMC solution, (C) atropine reference standard spiked at 200 ng/mL, (D) atropine test sample in BSS at 200 ng/mL, and (E) atropine test sample in HPMC at 200 ng/mL. (F) Calibration standard of atropine.

between 96.57%-105.80% of the initial concentration in HPMC and 97.40%-104.50% in BSS, and the 95% confidence interval was a maximum of ±3.70%. The chemical stability results for the simulated use conditions are presented in Table 1.

In the unopened study, the 0.01% atropine in HPMC and BSS stored at refrigerated temperature remained stable up until 180 days of storage. The concentrations were between 93.61%-102.99% of the initial concentrations in HPMC and 92.66%-105.11% in BSS, with the maximal and the 95% confidence interval at a maximum of +4.61%. At room temperature, the 0.01% atropine solutions were still within an acceptable range for 60 days in HPMC and for 90 days in BSS. The chemical stability results for the unopened study are presented in Table 2. The chemical stability trend for all the conditions are presented in Fig 2.

TABLE 1. Percentage of atropine concentration remaining (mean ± 95% CI) of 0.01% atropine for each formulation and conservation condition in the simulated use study.

Storage conditions	Solutions	Percentage of atropine concentration remaining (mean ± 95% CI)				
		Day 0	Day 15	Day 30	Day 45	Day 60
At room temperature (25±2 °C)	HPMC (n=5)	100 (n=5)	97.60±4.10 (n=5)	99.44±4.34 (n=5)	ND	ND
	BSS (n=5)	100 (n=5)	106.93±1.15 (n=5)	102.26±2.72 (n=5)	ND	ND
In refrigerator (2–8 °C)	HPMC (n=7)*	100 (n=7)	101.44±2.20 (n=7)	104.86±2.47 (n=7)	96.57±1.53 (n=2)	105.80±3.53 (n=2)
	BSS (n=7)*	100 (n=7)	104.50±2.29 (n=7)	103.73±2.31 (n=7)	102.75±3.70 (n=2)	97.40±1.87 (n=2)

* n = 5 in the 1-month study and n = 2 in the 2-month study.
ND = not determined.

TABLE 2. Percentage of atropine concentration remaining (mean ± 95% CI) of 0.01% atropine for each formulation and conservation condition in the unopened eyedroppers.

Storage conditions	Solutions	Percentage of atropine concentration remaining (mean ± 95% CI)						
		Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
At room temperature (25±2 °C)	HPMC (n=30)	100 (n=5)*	96.51±1.46 (n=5)	94.07±1.36 (n=5)	83.67±2.94 (n=5)	78.71±0.92 (n=5)	73.09±3.21 (n=5)	67.74±3.15 (n=5)
	BSS (n=30)	100 (n=5)*	98.32±2.36 (n=5)	93.32±2.38 (n=5)	92.54±2.92 (n=5)	80.90±1.61 (n=5)	78.53±1.84 (n=5)	74.57±1.25 (n=5)
In refrigerator (2–8 °C)	HPMC (n=30)	100 (n=5)*	100.71±1.69 (n=5)	102.99±3.19 (n=5)	100.30±1.62 (n=5)	93.61±1.53 (n=5)	95.17±1.95 (n=5)	99.62±1.38 (n=5)
	BSS (n=30)	100 (n=5)*	100.57±2.04 (n=5)	105.11±3.93 (n=5)	104.27±3.32 (n=5)	92.66±1.63 (n=5)	99.34±3.78 (n=5)	101.58±4.61 (n=5)

* Baseline values of the atropine concentration were obtained from the studies of 0.01% atropine ophthalmic solutions in 1-month simulated use conditions.

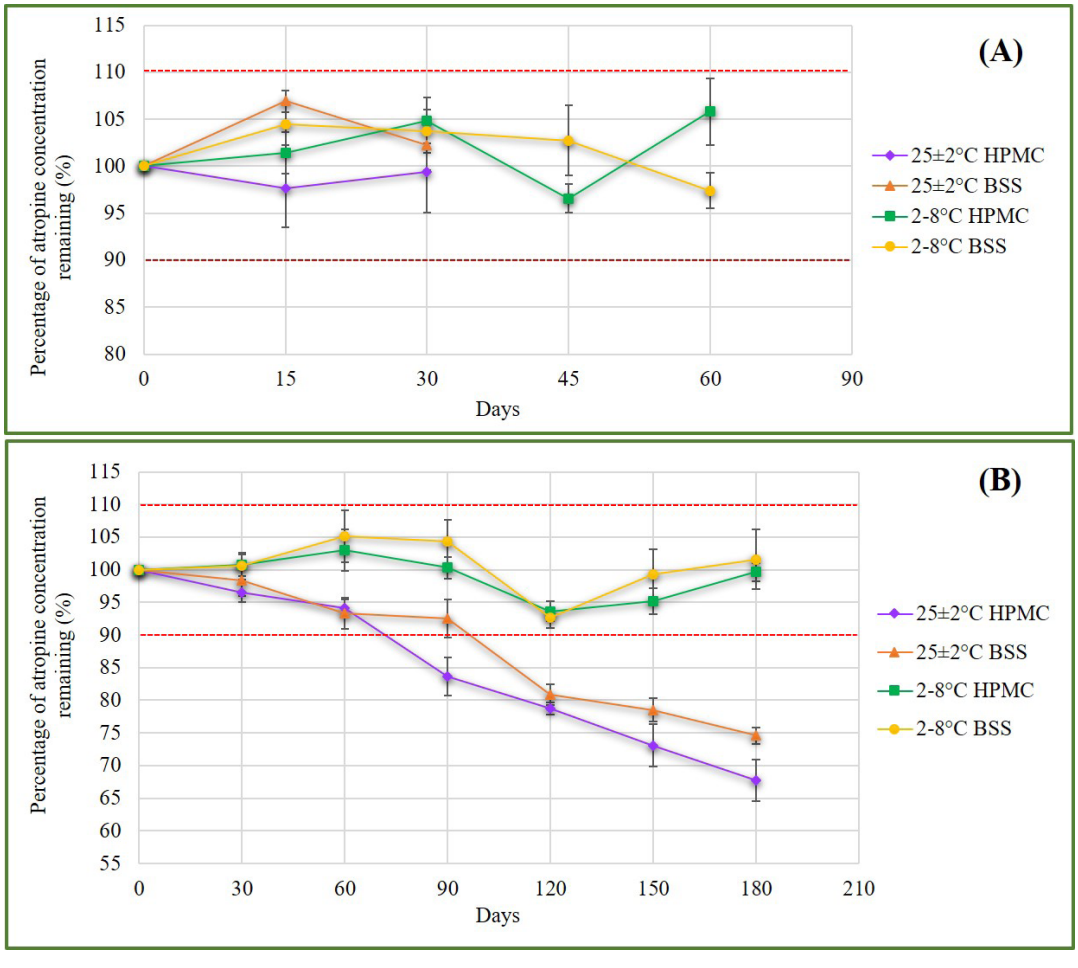

Fig 2. (A) Percentage of atropine concentration remaining (mean ± 95% CI) for each formulation and conservation condition in the simulated use study. (B) Percentage of atropine concentration remaining (mean ± 95% CI) for each formulation and conservation condition in the unopened eyedroppers.

Visual inspection and pH measurements

All the samples that were sent for the sterility assay (from 168 eyedroppers) remained clear and colorless, with no precipitation or visible particles observed during the study period in all the study conditions. The pH of all the samples showed insignificant changes throughout the study. For both formulations, when stored at refrigerated temperature and room temperature, the pH did not vary by more than 0.20 and 0.23 pH units from the initial value for the simulated use conditions and unopened conditions, respectively (Table 3 and 4).

Sterility assay

The results indicated that the sterility was preserved in all the samples, i.e., for every subgroup in the simulated use and unopened conditions. No microbiological growth was observed when incubated for 14 days at 30-35 °C in fluid thioglycolate medium and at 20–25 °C in soybean casein digest medium.

TABLE 3. The pH value of 0.01% atropine sulfate for each formulation and conservation condition in the simulated use study.

Storage conditions	Solutions	pH value (mean ± SD)				
		Day 0	Day 15	Day 30	Day 45	Day 60
At room temperature (25±2 °C)	HPMC (n=5)	6.93±0.02 (n=5)	6.90±0.04 (n=5)	6.92±0.01 (n=5)	ND	ND
	BSS (n=5)	7.02±0.04 (n=5)	6.82±0.08 (n=5)	6.88±0.04 (n=5)	ND	ND
In refrigerator (2–8 °C)	HPMC (n=7)*	6.95±0.05 (n=7)	6.86±0.05 (n=7)	6.93±0.02 (n=7)	6.89±0.02 (n=2)	6.90±0.03 (n=2)
	BSS (n=7)*	6.98±0.08 (n=7)	6.92±0.06 (n=7)	6.90±0.06 (n=7)	6.81±0.05 (n=2)	6.78±0.07 (n=2)

* n = 5 in the 1-month study and n = 2 in the 2-month study.
ND = not determined.

TABLE 4. The pH value of 0.01% atropine sulfate for each formulation and conservation condition in the unopened eyedroppers.

Storage conditions	Solutions	pH value (Mean ± SD)						
		Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
At room temperature (25±2 °C)	HPMC (n=30)	6.93±0.02 (n=5)*	6.91±0.01 (n=5)	6.92±0.01 (n=5)	6.91±0.01 (n=5)	6.83±0.00 (n=5)	6.85±0.01 (n=5)	6.90±0.02 (n=5)
	BSS (n=30)	7.02±0.04 (n=5)*	6.86±0.05 (n=5)	6.89±0.02 (n=5)	6.81±0.07 (n=5)	6.79±0.10 (n=5)	6.81±0.06 (n=5)	6.83±0.05 (n=5)
In refrigerator (2–8 °C)	HPMC (n=30)	6.97±0.03 (n=5)*	6.90±0.01 (n=5)	6.88±0.03 (n=5)	6.92±0.01 (n=5)	6.87±0.01 (n=5)	6.91±0.03 (n=5)	6.92±0.03 (n=5)
	BSS (n=30)	7.02±0.04 (n=5)*	6.88±0.04 (n=5)	6.85±0.06 (n=5)	6.85±0.04 (n=5)	6.85±0.02 (n=5)	6.86±0.05 (n=5)	6.88±0.05 (n=5)

* Baseline values of the pH measurement were obtained from the studies of 0.01% atropine ophthalmic solutions in the 1-month simulated use conditions.

DISCUSSION

To assess the accuracy of the extemporaneous prepared ophthalmic solutions in clinical practice, this study investigated the accuracy of using 0.5 mL and 1 mL insulin syringes compared to an auto pipette for the extemporaneous preparation. The 0.01% atropine solutions were prepared using an auto pipette, and 0.5 mL and 1 mL insulin syringes. Here, 0.1 mL of 1% atropine sulfate was mixed with 9.9 mL of HPMC and 0.15 mL of 1% atropine sulfate was mixed with 14.85 mL of BSS (n =5 for each apparatus in each formulation). The preparation using the 1 mL insulin syringe was the same as for the preparation using the 0.5 mL insulin syringe. The mean concentration of atropine in HPMC compared to the expected concentration ranged from 98.24%-104.37%, 100.25%-102.91%, and 154.29%-157.05% for the auto pipette, and the 0.5 mL and 1 mL insulin syringes, respectively. The mean concentration of atropine in BSS ranged from 98.00%-103.03%, 100.01%-104.79%, and 140.63%-145.89% for the auto pipette, and the 0.5 mL and 1 mL insulin syringes, respectively. In this study, a 0.5 mL insulin syringe was then used in the preparation process, since it was more accurate than the 1 mL insulin syringe.

In the stability assessment of the ophthalmic solutions, the physicochemical and microbiological stability should be evaluated. Previous recent studies have also focused on the long-term stability of ophthalmic atropine solutions. Saito et al.[16] demonstrated that the physical, chemical, and microbiological stability of 0.01%, 0.10%, 0.25%, and 0.5% atropine in 0.9% sodium chloride solution were maintained for at least 6 months when stored unopened in polyethylene bottles at 25 °C or 5 °C. Berton et al.[17] showed that 0.01% atropine in 0.9% sodium chloride solutions with and without antimicrobial preservative were physicochemically stable for 6 months when stored unopened in low-density polyethylene bottles at 25 °C. The aim of our study was to investigate the long-term stability of 0.01% atropine in HPMC and BSS when stored unopened at room temperature (25 °C) or at refrigerated temperature (5 °C), and the stability of the 0.01% atropine solutions in a simulated use condition for up to 2 months.

In the simulated use study, 0.01% atropine in HPMC and BSS demonstrated physicochemical and microbiological stability for up to 30 days at room and refrigerated temperature. For the 2-month extension study at refrigerated temperature, 0.01% atropine in HPMC and BSS also maintained its physicochemical and microbiological stability throughout the study period.

In the unopened conditions, 0.01% atropine in HPMC and BSS stored at refrigerated and room temperature showed both physical and microbiological stability over 6 months. The pH values remained nearly constant, and no visual changes or microbial contamination were observed over the study period. Regarding the chemical stability, the mean atropine concentrations in HPMC and BSS remained well within 90%-110% of the initial concentration for 6 months at refrigerated temperature. However at room temperature, the mean atropine concentrations in HPMC and BSS were considered to be at an acceptable level of stability for only 2 and 3 months, respectively. These results supported the effect of temperature on the chemical stability. The differences in chemical stability at room temperature between our study and previous studies[16-18] are particularly related to the formulation. In previous studies, atropine was mostly prepared in 0.9% sodium chloride solution with a pH value of 5.3-6.2, compared to the pH value ranging from 6.8-7.0 for atropine in HPMC and BSS. The stability of atropine sulfate solution is enhanced in acidic conditions, as it has a lower degree of hydrolysis. Atropine sulfate solution is most stable at a pH between 3–6, and the ideal storage pH ranges between 3-4.[19-20] However, ophthalmic solution should better fall within the ocular comfort range (pH 6.6–7.8) to avoid eye discomfort and irritation.[21]

From the results from the unopened study, the conservation period of 0.01% atropine in HPMC and BSS could be ensured for 6 months when stored at 5 °C and for 2 months when stored at 25 °C.

Hence, the follow-up intervals for patients receiving these formulations could be extended to up to 6 months when a refrigerator is available.

There are some limitations of this study to note. First, the number of samples in the 2-month extension simulated use study was limited. Second, the room temperature in this study was 25±2 °C, which is actually lower than the average indoor temperature in most parts of Thailand. Since the storage temperature significantly affects the chemical stability, the conservation period for 0.01% atropine in HPMC and BSS outside the refrigerator might be, consequently, shorter than in our study.

CONCLUSION

This study demonstrated that 0.01% atropine solution both in HPMC and BSS retained good physicochemical and microbiological stability for 6 months both when left unopened and when stored at 5±3 °C; whereas, the atropine concentration in unopened eyedroppers stored at 25±2 °C generally declined over time. This study also confirmed the physicochemical and microbiological stability of both formulations at 5±3 °C or 25±2 °C for 30

days after opening. In conclusion, the extemporaneously prepared 0.01% atropine ophthalmic solution both in HPMC and BSS could be kept for up to 6 months in the refrigerator at a temperature of 2-8 °C until the bottle is opened.

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Potential conflicts of interest

The authors have no conflicts of interest with the manufacturers or suppliers of any of the products or materials in this study. It is to be noted though that the authors were supported by a Chalermprakiat grant from the Faculty of Medicine Siriraj Hospital, Mahidol University.

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Psychometric Properties of the Thai Mental Health Literacy Scale in Sixth-Year Medical Students

Gobhathai Sittironnarit, M.D., Rungsipohn Sripen, M.Sc., Sucheera Phattharayuttawat, Ph.D.

Department of Psychiatry, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Objective: To assess the psychometric properties of the Thai Mental Health Literacy Scale (TMHLS) in sixth-year medical students.

Materials and Methods: By using the purposive sampling method, we enrolled 202 participants in this study. Descriptive statistics were used to analyze demographic data. The index of item-objective congruence (IOC) was used to verify content validity. Exploratory factor analysis (EFA) was performed to establish the construct validity of the TMHLS. The internal consistency was estimated by computing Cronbach's coefficient alpha.

Results: The TMHLS had good content validity (IOC=.85) and construct validity. The EFA resulted in five factors, which included 32 of the 35 items and accounted for 46.86% of the variance. The factors were the ability to recognize mental disorders; confidentiality of mental health practitioners; skills of mental health information seeking; beliefs about mental illnesses; and attitudes toward patients with mental illness. The reliability coefficient of the TMHLS total test was .851, and reliability coefficient in subdomains were range from .197 to .872. Individuals who had a mental health professional as an intimate contact and individuals who had a history of seeking help from mental health professional(s) in person showed significantly higher mental health literacy than those who did not.

Conclusions: The TMHLS has good psychometric properties. Dynamic knowledge transfer and exchange with a close mental health professional should be applied to promote mental health literacy in medical students.

Keywords: assessment; experience; help-seeking; medical externs; professional; reliability; validity (Siriraj Med J 2022; 74: 100-107)

INTRODUCTION

Mental health problems have been increasing throughout the world[1], with young adults being the most affected group. Thirty percent of them have mental disorders while the remaining are also at risk.[2] Because of poor mental health literacy, high mental health problems and low engagement in help-seeking behaviors were reported in these individuals.[3-7]

Mental health literacy reduces the risk of developing mental disorders along with increasing help-seeking behaviors.[8] People with high mental health literacy will be able to recognize, manage, and prevent mental health problems. Oppositely, people with low mental health literacy may not be able to appropriately manage and often end up with more serious complications.[9] Unfortunately, there is no assessment tool for mental health literacy in Thai at the time.

Sixth-year medical students were targeted in this study because they were young adults at risk of mental disorders[2,10-11] who already gained mental experiences that may affect their mental health literacy.[12-13]

Due to the lack of an instrument to measure mental health literacy among Thai people, this study aimed to assess the psychometric properties of the Thai mental

Corresponding author: Gobhathai Sittironnarit
E-mail: gobhathai.kua@mahidol.edu
Received 1 October 2021 Revised 26 October 2021 Accepted 16 November 2021
ORCID ID: https://orcid.org/0000-0001-8902-4903
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health literacy scale (TMHLS) in sixth-year medical students who may exemplify the young adults at risk of mental disorder.

MATERIALS AND METHODS

Participants

The number of participants in this study was determined by the Cochran formula.[14] We enrolled 250 sixth-year medical students from the Faculty of Medicine Siriraj Hospital in Bangkok who had registered for the first semester in academic year 2017 and voluntarily answered the questionnaires using purposive sampling method.

Tools

A demographic questionnaire was used to collect data from participants including gender, age, sources of mental health experiences, and their mental illness if applicable.

The translation of mental health literacy scale (MHLS)

The MHLS was translated to Thai under the supervision of a language expert. The index of item-objective congruence (IOC) was used to verify content validity by three mental health experts: one psychiatrist and one licensed clinical psychologist from the Department of Psychiatry, Faculty of Medicine Siriraj Hospital; and one licensed clinical psychologist from the Faculty of Psychology, Chulalongkorn University. All mental health experts discussed the translated version until reaching a consensus. The Thai mental health literacy scale (TMHLS) was finally completed following expert opinion.

The TMHLS is a self-reporting questionnaire with 35 items covering six attributes of mental health literacy: the ability to recognize a disorder; knowledge of where to seek information; knowledge of risk factors and causes; knowledge of self-treatment; knowledge of professional help available and attitudes that promote recognition or appropriate help-seeking behavior. The total score is the summation of all items. Therefore, the maximum score is 160 whereas the minimum score is 35. A higher score means greater mental health literacy.

Statistical analyses

All statistical analyses were performed by PASW 18.0.[16] Descriptive statistics were used to analyze demographic data. The IOC was used to verify content validity. The factor solution was determined based on the number of eigenvalues greater than one.[17] We conducted the exploratory factor analysis (EFA) using .30 as a factor loading criterion[18], five to ten participants per item[19], and a minimum sample size of 200.[20-21] The EFA began with an initial analysis run to obtain eigenvalues for each factor in the data. The Kaiser-Meyer-Olkin (KMO) Measure of Sampling Adequacy test and Bartlett's Test of Sphericity were executed to determine construct validity and to confirm those data were appropriate. The KMO test was used to verify the sampling adequacy for the analysis, and Bartlett's Test of Sphericity was used to determine if correlations between items were sufficiently large for EFA. Bartlett's Test of Sphericity should reach a statistical significance of less than .05 in order to conduct an EFA. The reliability of an instrument is concerned with the consistency, stability, and dependability of the scores.[22] For this reason, the internal consistency was tested using Cronbach's alpha for each competency.

RESULTS

The sixth-year medical students

Two-hundred and two of the 250 participants (80.8%) answered the questionnaires. The majority of respondents were female (n=133; 65.8%) aged between 22-24 years (M = 23, SD = 0.46). Psychiatric rotation was the most popular source of their mental health experience (n=190; 94.1%). Thirteen out of 202 medical students had major depressive disorder (6.4%), the most common diagnoses among the samples (Table 1).

The psychometric properties of the Thai mental health literacy scale (TMHLS)

Content validity

The first-round IOC of the TMHLS was .67 with 9 of 35 items (items number 2, 3, 5, 6, 7, 8, 15, 20 and 24) defined as required revision (IOC > .05). After revision of those 9 items, content validity in the second round increased to .85. However, 4 of 9 items (items number 3, 5, 15 and 20) were still defined as required revision (IOC > .05).

Construct Validity

The EFA revealed five meaningful constructs emerged, namely, ability to recognize mental disorders (item 1, 2, 3, 4, 5, 6, 7, 8); confidentiality of mental health practitioners (item 22, 23, 25, 26, 27, 28); skills of mental health information seeking (item 16, 17, 18, 19); beliefs about mental illnesses (item 9, 11, 12, 13, 20, 21, 24); and attitudes toward patient with mental illness (item 29, 30, 31, 32, 33, 34, 35), which accounted for 46.86% of the cumulative variance. Three items (item 10, 14 and 15) did not load on any of the factors (Table 2).

Reliability

Total Cronbach's alpha coefficient of the TMHLS

TABLE 1. Demographic data of the sixth-year medical students (n=202).

Attributes		Frequency (n)	Percent (%)
Response rates		202	80.8
Sex	Female	133	65.8
	Male	69	34.2
Age (years)	22	22	10.9
	23	158	78.2
	24	22	10.9
(M =23, SD = 0.46, Range 22-24 years)			
Sources of mental health experiences (Mutual items and answers reasonable)			
	• Fifth-year rotation (psychiatry)	190	94.1
	• Media (internet/ newspaper/ television)	139	68.8
	• Having family members or friends with mental disorder(s)	110	54.5
	• Self-experience of mental disorder(s)	31	15.3
	• Having a mental health professional as an intimate contact	29	14.4
	• History of seeking help from mental health professional(s) in person	19	9.4
	• History of seeking help from mental health professional(s) for family members or friends	16	7.9
Types of mental illness			
	• Major depressive disorder (MDD)	13	6.4
	• Panic disorder	3	1.5
	• Adjustment disorder	2	1
	• Attention deficit hyperactivity disorder (ADHD)	2	1
	• Bipolar disorder	1	0.5
	• Premenstrual dysphoric disorder (PMDD)	1	0.5
	• Relationship problems	1	0.5
	• Unspecified	8	4

was .851. Still, there were 6 items (items 9, 10, 11, 12, 15 and 20) in the reliability coefficients of all items that do not meet the criterion (CITC < .20). The Cronbach's alpha if item deleted was .872 which was in the same interval before withdrawing the 6 items. The Cronbach's alpha if item deleted for each item was slightly different from the Cronbach's alpha of all items. Therefore, all items that do not meet the criterion still remain (Table 3). The reliability coefficient in subdomains of TMHLS were range from .197 to .872 (Table 4).

The mental health literacy in sixth-year medical students

The medical students' mean score of mental health literacy was 123.09 (S.D. ± 11.55, 95% CI = 121.49–124.69). Multiple comparisons of our participants' mental health experiences showed having intimate contact with a mental

TABLE 2. Factor structure of the Thai Mental Health Literacy Scale (TMHLS).

Item	F1	F2	F3	F4	F5
Q8	.866				
Q5	.831				
Q7	.752				
Q3	.714				
Q6	.696				
Q4	.662				
Q1	.648				
Q2	.540				
Q28		.697			
Q27		.683			
Q26		.612			
Q22		.529			
Q25		.524			
Q23		.397			
Q19			.799		
Q17			.791		
Q16			.753		
Q18			.634		
Q11				.558	
Q20				-.502	
Q21				-.461	
Q24				-.442	
Q13				.422	
Q12				-.351	
Q9				.337	
Q33					.781
Q32					.775
Q30					.758
Q31					.747
Q34					.725
Q35					.725
Q29					.724

Note: F1 =ability to recognize mental disorders, F2 = confidentiality of mental health practitioners, F3 = skills of mental health information seeking, F4 = beliefs about mental illnesses, F5 = attitudes toward patient with mental illness

TABLE 3. Reliability coefficients of all 35 Items from the Thai Mental Health Literacy Scale (TMHLS).

Items	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted
1	120.0050	127.146	.388	.847
2	120.1608	126.206	.440	.845
3	119.7186	127.203	.444	.846
4	120.0101	125.677	.442	.845
5	119.6734	125.160	.509	.844
6	120.0151	127.096	.339	.848
7	119.8442	126.263	.402	.846
8	119.6482	124.320	.544	.843
9	120.1709	132.405	.124**	.853
10	120.4121	136.213	-.122**	.857
11	119.9447	133.113	.089**	.853
12	120.6783	133.586	.028**	.856
13	119.9146	130.887	.223	.850
14	119.6131	128.370	.394	.847
15	120.3568	131.443	.140**	.853
16	119.1005	129.444	.318	.848
17	119.1256	129.878	.295	.849
18	118.9447	129.578	.292	.849
19	118.8543	129.085	.399	.847
20	120.4472	131.945	.077**	.857
21	119.2563	125.616	.367	.847
22	118.9095	126.770	.384	.847
23	119.1859	126.657	.413	.846
24	118.9548	124.649	.492	.844
25	119.3920	129.179	.254	.850
26	118.7286	126.936	.420	.846
27	118.7337	127.762	.414	.846
28	118.6734	128.504	.374	.847
29	120.4573	125.886	.411	.846
30	119.7688	125.360	.461	.845
31	119.3618	123.444	.572	.842
32	119.5879	124.233	.494	.844
33	120.4874	124.776	.414	.846
34	120.0050	124.601	.401	.846
35	119.6784	124.957	.454	.845

**Items that have corrected item-total correlation less than 2 are not pass the criterion.

TABLE 4. Reliability coefficients in subdomain and total of the Thai Mental Health Literacy Scale (TMHLS).

Factors (subdomain)	Number of Items	Cronbach's Alpha coefficient
F1	8	.867
F2	6	.683
F3	4	.782
F4	7	.197
F5	7	.873

Note: F1 =ability to recognize mental disorders, F2 = confidentiality of mental health practitioners, F3 = skills of mental health information seeking, F4 = beliefs about mental illnesses, F5 = attitudes toward patient with mental illness; Total Cronbach's alpha coefficient =.851

health professional and a history of seeking help from a mental health professional(s) in person significantly correlated with the participants' mental health literacy score. The mental health literacy of individuals who had intimate contact with a mental health professional was significantly higher than those who did not (mean±SD was 127.41±13.96 and 122.37±10.99, respectively; t (200) = 2.196, $p < .05$). Likewise, mental health literacy of individuals who had a history of seeking help from mental health professional(s) in person was higher than those who did not (mean±SD was 128.84±10.25 and 122.50±11.55, respectively; t (200) = 2.302, $p < .05$.) (Table 5).

TABLE 5. The comparison of mental health literacy by mental health experiences.

Mental health experiences	n	$\bar{x}$	S.D.	t	p
Media (internet/ newspaper/ television)					
have	139	123.98	11.94	1.622	.106
not have	63	121.14	10.49		
Having family members or friends with a mental illness					
have	110	123.83	11.78	.986	.325
not have	92	122.22	11.28		
Self-experience of mental disorder(s)					
have	31	126.16	10.13	1.612	.108
not have	171	122.54	11.74		
Having a mental health professional as an intimate contact					
have	29	127.41	13.96	2.196*	.029
not have	173	122.37	10.99		
History of seeking help from mental health professional(s) in person					
have	19	128.84	10.25	2.302*	.022
not have	183	122.50	11.55		
History of seeking help from mental health professional(s) for family members or friends					
have	16	124.94	10.85	.664	.507
not have	186	122.94	11.63		

* $p < .05$

DISCUSSION

The sixth-year medical students

Major depressive disorder was the most common diagnosis in this study which was in accordance with previous Thai, Malaysian and Chinese Studies.[23-25]

The Psychometric properties of the Thai mental health literacy scale (TMHLS)

The TMHLS has good validity. The content validity by the IOC in the second-round was .85, and only 4 out of 9 items needed to be revised. According to the original study[15] that stated measurement cannot assess all attributes of mental health literacy when some of the items needed to be removed, all items were used in the scale altogether. Consistent with a previous Persian study[26], the EFA of data resulted in five meaningful factors that were similar to the original ones[15], and accounted for 46.86% of the variance. The trivial differences could have been due to cultural diversities of the participants. Socioeconomic status, cultural and language variances interact with health literacy.[27]

Total Cronbach's alpha coefficient of the TMHLS was .851 which was considered in a good criterion. The reliability coefficient in subdomains were range from .197 to .872. Still, there were 6 items that did not meet the criterion. The Cronbach's alpha if item deleted for each item was slightly different from the Cronbach's alpha of all items. According to the original study[15] that stated the measurement cannot assess all attributes of mental health literacy when some of the items needed to be removed. Therefore, those 6 items that do not meet the criterion were persevered.

The mental health literacy in sixth-year medical students

The mental health literacy of our medical students was aligned but slightly lower than a prior British study.[13] Our score was marginally inferior than an Australian study exploring university students.[15] This may uncover differences in mental health literacy between developing and developed countries. The necessity of mental health literacy acknowledgement in village health workers was mentioned in a previous Thai study.[28] A South African study urged for mental health education in healthcare professionals.[29] Language deviance and questionnaire format may also be responsible for the different results.

Our participants had already gained mental health experiences that may affect their mental health literacy. Previous works also showed higher mental health literacy in individuals who encountered mental health problems than the individuals who did not.[12,30] The more exposure someone has, the more mentally health literate they are.[12]

Consistent with the original study[15], the mental health literacy of individuals who had a history of seeking help from mental health professional(s) in person was higher than those who did not. Dynamic knowledge transfer and exchange with a close mental health professional, like in family businesses[31], could be a reason for higher mental health literacy of individuals who had a mental health professional as an intimate partner than those who did not.

The questionnaire comments

The main concern about the TMHLS was the complexity and clarity of the questions. However, the items that should be allocated were not mentioned. A separate version of TMHLS between medical students and general population was advised. Although some participants described the questionnaire as easy and clear to answer, an equal number expressed the overly theoretical concerns. Some of them requested more attitude questions.

Limitations

Information and recall bias may have been presented in this observational descriptive cross-sectional study. Based on purposive sampling method, the results cannot legitimize any generalizations. We did not perform back-translation process; hence the quality assurance of the TMHLS should be concerned. As the EFA is not a sufficient tool to test the theoretical foundations of the instrument, a confirmatory factor analysis (CFA) should be conducted to further the knowledge in this area. Since we used Cronbach's alpha for reliability testing, the interitem covariance and the measurement assumptions error could be considered as the alpha value cannot be equivalent with the reliability of the test score. Additional studies in other population are recommended to validate this instrument to widen its application.

CONCLUSION

The TMHLS has good validity and reliability. Dynamic knowledge transfer and exchange with a close mental health professional should be applied to promote mental health literacy in medical students.

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Two-Antibody Staining Method, A Cost-Saving Strategy for Universal Lynch Syndrome Screening in Endometrial Cancers

Natthakrit Anansitthikorn, M.D., Suchanan Hanamornroongruang, M.D.

Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

ABSTRACT

Objective: Lynch syndrome is an autosomal dominant disorder that increases the risk of cancers in many sites. In women, endometrial cancer is often a sentinel tumor and thus immunohistochemistry for mismatch repair (MMR) proteins MLH1, MSH2, MSH6 and PMS2 is encouraged as a screening test. To reduce cost, staining for only 2 MMR proteins PMS2 and MSH6 has been proposed. This study aimed to determine whether a 2-antibody staining test is enough to screen for Lynch syndrome in endometrial cancer patients.

Materials and Methods: Cases of endometrial carcinoma with immunohistochemistry for 4 MMR proteins were reviewed. Results of immunohistochemistry screening were compared between all four antibodies and only two (PMS2 and MSH6) antibodies.

Results: Loss of expression of any MMR proteins was detected in 51 out of 203 cases (25.12%). Twenty-three cases (45%) showed loss of MLH1 and PMS2; 13 cases (25%) showed loss of MSH2 and MSH6; five cases (10%) showed loss of MSH6; seven cases (14%) showed loss of PMS2 and three cases (6%) showed loss of MSH2. The 2-antibody method detected 48 cases (94%) with a MMR deficiency but failed to detect three cases (6%) with an isolate loss of MSH2. The screening results from the 2-antibody method are 98.5% (200/203) in accordance with the original 4-antibody method.

Conclusion: The 2-antibody method is a quite effective option to screen for Lynch syndrome in endometrial cancers. However, MSH2 mutations may be missed in a few cases.

Keywords: Endometrial carcinoma; Lynch syndrome; MMR proteins; MSH2 loss (Siriraj Med J 2022; 74: 108-113)

INTRODUCTION

Lynch syndrome (LS) is an autosomal dominant disorder which is caused by a germline mutation in mismatch repaired (MMR) genes (MLH1, MSH2, MSH6 and PMS2) or EpCAM deletion.[1] This syndrome is associated with cancer in many organs such as the lower gastrointestinal tract, endometrium, ovary, stomach, pancreas and brain.[2] However, the two most well-known cancers associated with LS are colorectal and endometrium. Women with LS have a lifetime risk of developing colorectal cancer and endometrial cancer at 50%-85% and 40%-60%, respectively.[3,4] Although the prevalence of LS in the general population remains elusive[1], about 1.7%-5% of endometrial cancers are associated with this syndrome.[1,5-10]

For women with LS, endometrial cancer is often a sentinel tumor.[11] According to a study by Meyer et al, 61% of women with LS linked endometrial cancer had a second primary cancer, mostly colorectal cancer.[3]

Identification of LS patients is the first step in achieving proper cancer surveillance and management. Clinical

Corresponding author: Suchanan Hanamornroongruang
E-mail: suchananice@hotmail.com
Received 12 October 2021 Revised 6 December 2021 Accepted 13 December 2021
ORCID ID: https://orcid.org/ 0000-0003-4392-0811
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screening criteria such as Amsterdam II and revised Bethesda guidelines have failed to detect a significant number of LS patients.[2,10,12] Thus, tumor-based testing - immunohistochemistry (IHC) for MMR proteins (MLH1, MSH2, MSH6 and PMS2) and/or microsatellite instability (MSI) - is recommended.[1-4,9,13,14]

Both IHC and MSI have a high sensitivity and specificity, however, IHC is more practical and cost effective.[3,4] In addition, MSI is less sensitive to the MSH6 germline mutation.[1,2] Many studies claim that IHC for only PMS2 and MSH6 is sufficient for initial screening[15-18] due to the binding properties of MMR heterodimer complexes; MSH2 binds with MSH6 and MLH1 binds with PMS2. With a 2-antibody approach, universal LS screening in endometrial cancers is easier to achieve, especially in places with limited resources. According to an international survey on LS screening in gynecologic cancers by Ryan et al, most pathologists still prefer the 4-antibody method.[19] In our experience and personal communication with pathologists and gynecologists, most were not confident or did not acknowledge in this cost-saving method. Moreover, most studies on a 2-antibody approach were conducted in cases of colorectal cancer. Thus, the purpose of this study was to determine the utility of the 2-antibody method in cases of endometrial cancer compared to the original 4- antibody method.

MATERIALS AND METHODS

The study was conducted at the Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand and was approved by the Siriraj Institutional Review Board (COA no. Si058/2020).

All cases of endometrial carcinoma with an immunohistochemistry conducted for the 4 MMR proteins between January 1st, 2010 and December 31st, 2019 were included in this study. Cases without available H&E and immunostained slides were excluded. Immunohistochemical staining was performed by the Ventana BenchMark ULTRA autostainer. Monoclonal antibodies for MMR proteins were as follows: anti-MLH1 (M1; Ventana), anti-PMS2 (EPR3947; Cell marque; USA), anti-MSH2 (G219-1129; Cell marque; USA) and anti-MSH6 (44; Ventana; USA). Intact expression was defined as positive nuclear staining within tumor cells. Loss of expression was defined as absence of nuclear staining within tumor cells. Stromal cells and nonneoplastic epithelial cells were used as internal control. Cases with absence of staining in internal control cells were excluded from the study. Focal and weak nuclear staining was considered as "cannot be determined".

All H&E and immunostained slides were reviewed. Results of immunohistochemistry screening were recorded and compared between all four antibodies against two (PMS2 and MSH6) antibodies. Clinical information including age at diagnosis, specimen type was retrieved from database records.

RESULTS

A total 203 cases of endometrial carcinoma with an age range of 23-62 were included in this study. Most specimens (97.54%) were from total or subtotal hysterectomy. Endometrioid carcinoma was the most common histologic subtype (89.66%). Specimen characteristics are summarized in Table 1. Loss of expression of any MMR protein was detected in 51 out of 203 cases (25.12%). Of these 51 cases with MMR deficiency, 23 cases (45%) showed loss of MLH1 and PMS2; 13 cases (25%) showed loss of MSH2 and MSH6; five cases (10%) showed loss of MSH6; seven cases (14%) showed loss of PMS2 and three cases (6%) showed loss of MSH2 (Table 2). The 2-antibody method detected 48 cases (94%) with MMR deficiency but failed to detect three cases (6%) with an isolate loss of MSH2. Isolate loss of MLH1 was not observed. One MSH2-absent/ MSH6-intact case was dedifferentiated carcinoma while the others were endometrioid type. All three cases showed convincing MSH6 expression in 20-40% of tumor cells, although the staining intensity in one case (case 2) was slightly less than internal control. (Fig 1) Overall, 98.5% (200/203) of the results from the 2-antibody method were in accordance with the original 4-antibody method.

DISCUSSION

Immunohistochemistry for MMR proteins has been acknowledged as the most practical screening test for LS and is performed routinely in many developed countries. Rates of MMR deficiency in endometrial cancer range from 19.8%- 35%.[4,7,10,12,17,18,20,21] Recently, Puangsricharoen et al reported MMR deficiency in 34.9% of 166 endometrial cancer cases in Thailand.[22] The rate of MMR deficiency in this study is 25.12% which is lower than a previous Thai study. However, population selection for this retrospective study was based on the presence or absence of immunohistochemistry for four MMR proteins and not randomized.

This study supports that IHC testing for only PMS2 and MSH6 is acceptable for initial screening. We found that PMS2 can detect all cases with loss of both MLH1 and PMS2 and PMS2 alone. While MSH6 can detect all cases with loss of both MSH2 and MSH6 and MSH6 alone. In fact, the 2-antibody method failed to identify three cases with an isolate loss of MSH2.

TABLE 1. Specimen Characteristics (n = 203).

Characteristic	Value
Age at diagnosis, average (range), years	45.06 (23-62)
Specimen type	
Total or subtotal hysterectomy	198 (97.54)
Endometrial sampling or curettage	5 (2.46)
Tumor cell type	
Endometrioid carcinoma	182 (89.66)
Endometrioid carcinoma - grade 1	96 (47.29)
Endometrioid carcinoma - grade 2	63 (31.03)
Endometrioid carcinoma - grade 3	21 (10.34)
Endometrioid carcinoma - not graded	2 (0.99)
Serous carcinoma	9 (4.43)
Mixed carcinoma	6 (2.96)
Clear cell carcinoma	3 (1.48)
Undifferentiated carcinoma	1 (0.49)
Dedifferentiated carcinoma	1 (0.49)
Carcinosarcoma	1 (0.49)

TABLE 2. Mismatch repair protein immunohistochemical staining pattern (n=203).

Immunohistochemical pattern	Number (%)
No loss of nuclear expression of MMR proteins	152 (74.88)
Loss of nuclear expression of any MMR proteins	51 (25.12)
Loss of nuclear expression of MLH1 and PMS2	23 (11.33)
Loss of nuclear expression of MSH2 and MSH6	13 (6.40)
Loss of nuclear expression of MSH6 only	5 (2.46)
Loss of nuclear expression of PMS2 only	7 (3.45)
Loss of nuclear expression of MSH2 only	3 (1.48)

Selected studies on patterns of IHC for 4 MMR proteins in endometrial cancers were reviewed (Table 3). Modica et al reported one case of isolate MSH2 loss which showed MSI-H in MSI testing.[20] Meanwhile, a study by Crim el al reported one case of isolate MLH1 loss which is impossible in the 2- antibody method, however, there was no associated germline mutation.[18] Pearlman et al also reviewed 1730 colorectal cancer cases with IHC conducted to screen for LS and reported isolate MSH2 loss in 19 cases; eight had an ambiguous MSH6 expression

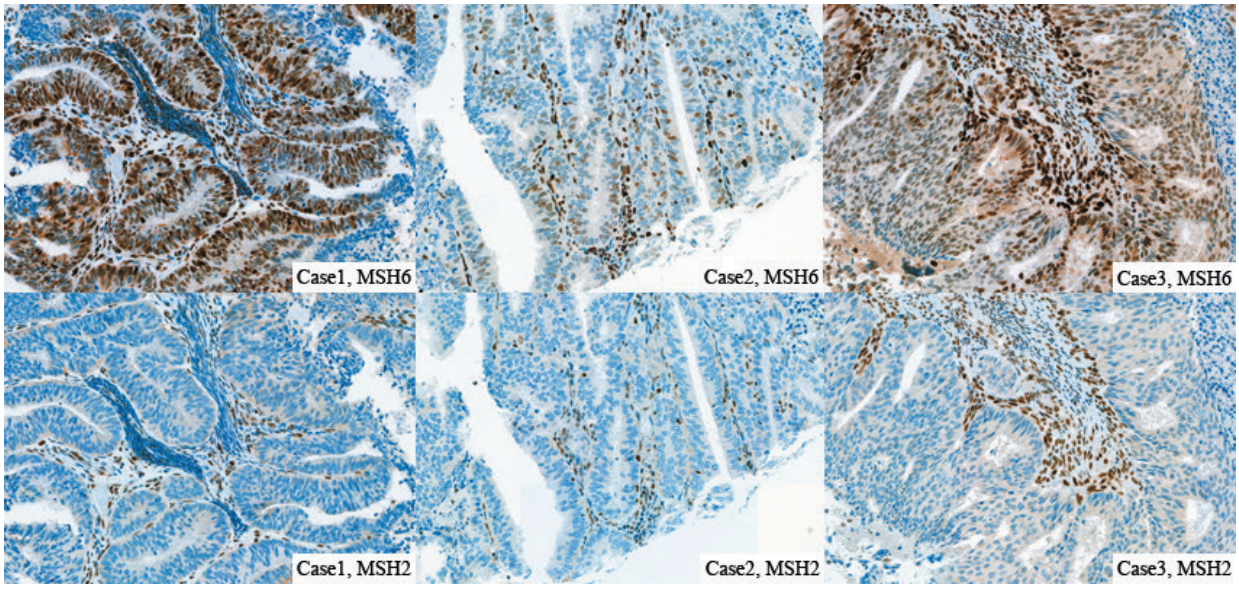

Fig 1. Three cases with an isolate loss of MSH2.

TABLE 3. Literature reports on patterns of immunohistochemical staining for MLH1, MSH2, MSH6 and PMS2 in endometrial carcinomas

Reference	Total	IHC patterns							
			Loss of any nuclear expression						
		Intact	MLH1 and PMS2	MSH2 and MSH6	MSH6 only	PMS2 only	MLH1 only	MSH2 only	Others
Modica 2007	85	37 (43.53%)	23 (27.06%)	6 (7.06%)	9 (10.59%)	6 (7.06%)	0 (0%)	1* (1.18%)	3 (3.53%)
Garg 2009	71	39 (54.93%)	19 (26.76%)	9 (12.68%)	4 (5.63%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Backes 2009	140	110 (78.57%)	24 (17.14%)	4 (2.86%)	2 (1.43%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Mojtahed 2011	40	21 (52.50%)	9 (22.50%)	4 (10%)	4 (10%)	0 (0%)	0 (0%)	0 (0%)	2 (5%)
Egoavil 2013	173	115 (66.47%)	42 (24.28%)	5 (2.89%)	7 (4.05%)	1 (0.58%)	0 (0%)	0 (0%)	3 (1.73%)
Long Q 2014	173	132 (76.30%)	10 (5.78%)	21 (12.14%)	7 (4.05%)	3 (1.73%)	0 (0%)	0 (0%)	0 (0%)
Watkins JC 2017	242	194 (80.17%)	39 (16.12%)	4 (1.65%)	3 (1.24%)	2 (0.83%)	0 (0%)	0 (0%)	0 (0%)
Crim 2017	116	92 (79.31%)	15 (12.93%)	1 (0.86%)	3 (2.59%)	2 (1.72%)	1* (0.86%)	0 (0%)	2 (1.72%)
Puangsricharoen 2020	156	99 (63.46%)	42 (26.92%)	10 (6.41%)	5 (3.21%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Our study 2021	203	152 (74.88%)	23 (11.33%)	13 (6.40%)	5 (2.46%)	7 (3.45%)	0 (0%)	3* (1.48)	0 (0%)

*cases in which the 2-antibody method could not detect defects compared to the 4-antibody method

and 11 had convincing MSH6 expression. Germline testing of these cases revealed MSH2 mutations in 7/8 cases with ambiguous MSH6 expression and 9/11 cases with convincing MSH6 expression.[23] In clinical practice, isolate MSH2 loss is unusual. Genetic consultation and further investigations, such as MSI testing or germline testing should be performed. Failure to identify these rare cases by the 2-antibody method may lead to missed opportunities for cancer surveillance and carrier testing in relatives at risk.

IHC interpretation for MMR proteins can be difficult, especially in cases with focal and weak staining. There is still no official guideline that provides the cut-off proportion and staining intensity in tumor cells. Thus, discordance results and incorrect interpretation are possible pitfalls of IHC testing.

CONCLUSION

The results from the 2-antibody method are in high accordance with the original 4-antibody method. However, the 2-antibody method fails to detect a few cases of isolate MSH2 loss which have a potential to represent those with MSH2 germline mutation.

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Nomogram as a Predictor for Postoperative Acute Kidney Injury in Super-Elderly Patients Undergoing Noncardiac Surgery

Thadakorn Tantisarasart, M.D.*, Sunisa Chartmongkolchart, M.D.*, Rassamee Chotipanvithayakul, M.D.**

**Department of Anesthesiology, **Department of Epidemiology, Faculty of Medicine, Prince of Songkla University, Hatyai Songkhla 90110 Thailand.*

ABSTRACT

Objective: There has been increase the incidence of postoperative acute kidney injury (AKI), especially among super-elderly patients. The study aimed to identify risk factors and develop a nomogram among super-elderly patients undergoing noncardiac surgery.

Material and Methods: A single-center retrospective cohort study of patients aged greater than or equal to 80 years that underwent non-cardiac surgery between January 2018 to December 2020. Acute kidney injury (AKI) was identified by Kidney Disease Improving Global Outcome (KDIGO) during seven days after surgery. Multivariate logistic regression was constructed from preoperative and intraoperative data with variables with P-value<0.2 included in the final model. The performance of model function was conducted by area under the receiver curve (AUC) and calibration curves.

Results: Eight hundred and twenty patients were included; 124 (15%) developed postoperative AKI. A multivariate logistic regression model consisting of COPD, ASA classification, part of surgery, propofol and Succinylated gelatin was displayed as the nomogram. The model showed good discrimination with an AUC 0.746. The cutoff point of 63, which had the highest Youden index, was chosen with sensitivity and specificity of 83% and 45%, respectively. The nomogram showed good performance by the Hosmer-Lameshow goodness-of-fit test (X^2= 6.0697 and P value = 0.6394).

Conclusion: The nomogram predicted model for predicting postoperative AKI among super-elderly patients showed moderate discrimination ability and was instituted. It can help physicians to detect high-risk patients early and promptly prevent episodes of AKI.

Keywords: Postoperative acute kidney injury; super-elderly patient; nomogram (Siriraj Med J 2022; 74: 114-125)

INTRODUCTION

Mortality due to surgery and anesthesia has been reduced from >25% to 19% among elderly aged >80 years.[1] This substantially increased surgery and anesthesia among them. AKI, a preventable condition, has been one of the leading cause of post-operative morbidity and mortality rates, especially among super-elderly patients. The incidence of acute kidney injury is highest in elderly about 16.98%[2] and increase is age-dependent.[3] Therefore, Postoperative acute kidney injury was associated with 30-day unplanned readmission, postoperative renal failure, dialysis, risk of infection and prolonged mechanical ventilation.[4–6] Postoperative AKI is likely related to multifactorial factors including preoperative factor which is intractable, and intraoperative and postoperative factors which can be improved and are preventable.

Corresponding author: Thadakorn tantisarasart
E-mail: thadakorn.t@psu.ac.th
Received 1 November 2021 Revised 7 December 2021 Accepted 26 December 2021
ORCID ID: https://orcid.org/0000-0002-2448-3276
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Previous literature has reported that baseline decrease in eGFR, renal artery involvement, radiocontrast media, intraoperative hypotension, operative time, surgical technique, intraoperative crystalloid use, low preoperative Hemoglobin (Hb), intraoperative blood loss, peripheral arterial disease, perioperative transfusion, amount of blood loss, preoperative hypoalbuminemia, general anesthesia and preoperative use of angiotensin-converting enzyme inhibitor(ACEI), angiotensin II receptor blockers (ARB) and diuretics are associated with acute kidney injury . The most important factors in early detection of high risk patients include enhanced invasive monitoring, maintain fluid balance and avoid nephrotoxic agent.[8-10]

However, there are many predictive tools such as preoperative GFR, preoperative proteinuria and preoperative creatinine, but none of these tools are best validated for prediction of AKI the incidence, risk factor of postsurgical AKI. Studies documenting predictive score use in postoperative acute kidney injury among elderly people are rare. Therefore, this study aims to develop a predicting tool for postoperative acute kidney injury in super-elderly patients.

MATERIALS AND METHODS

Ethical approval and reporting guidelines

The study was approved by the institutional review board (IRB) of Prince of Songkla University Hospital (IRB number: 63-408-8-1). The inform consent was waived due to the study being observational study without medical intervention. The study comply with the transparent reporting of a prediction model for TRIPOD statement.[13]

Study hospital and study designs

This retrospective cohort study was conducted in a tertiary medical center in Thailand from 1 January 2018 to 31 December 2020. A total 820 records of all patients aged ≥80 years who underwent non-cardiac surgery and received either general anesthesia or spinal anesthesia were included.

Exclusion criteria were patients who had preoperative end-stage renal disease requiring renal replacement therapy, diagnosed acute kidney injury since the time of admission to a day prior to the surgery, amputee[12], bed-ridden, patients without baseline serum creatinine or follow-up serum creatinine levels to identify postoperative AKI and no urine output recorded. Eligible patients' data were retrieved from anesthetic records in the hospital information system (HIS) (Fig 1). An anesthesiologist reviewed patients' profiles and clinical data including ASA classification, vital signs, and urine outputs. Laboratory results, details of surgery and anesthesia, and clinical and hospital-related outcomes were also recorded.

Outcome and definition of acute kidney injury (AKI)

Postoperative AKI was assessed within 7 days after surgery. According to Kidney Disease-Improving Global Outcomes (KDIGO) 2012 guideline[14], AKI was defined as either one of the following 3 conditions a) serum

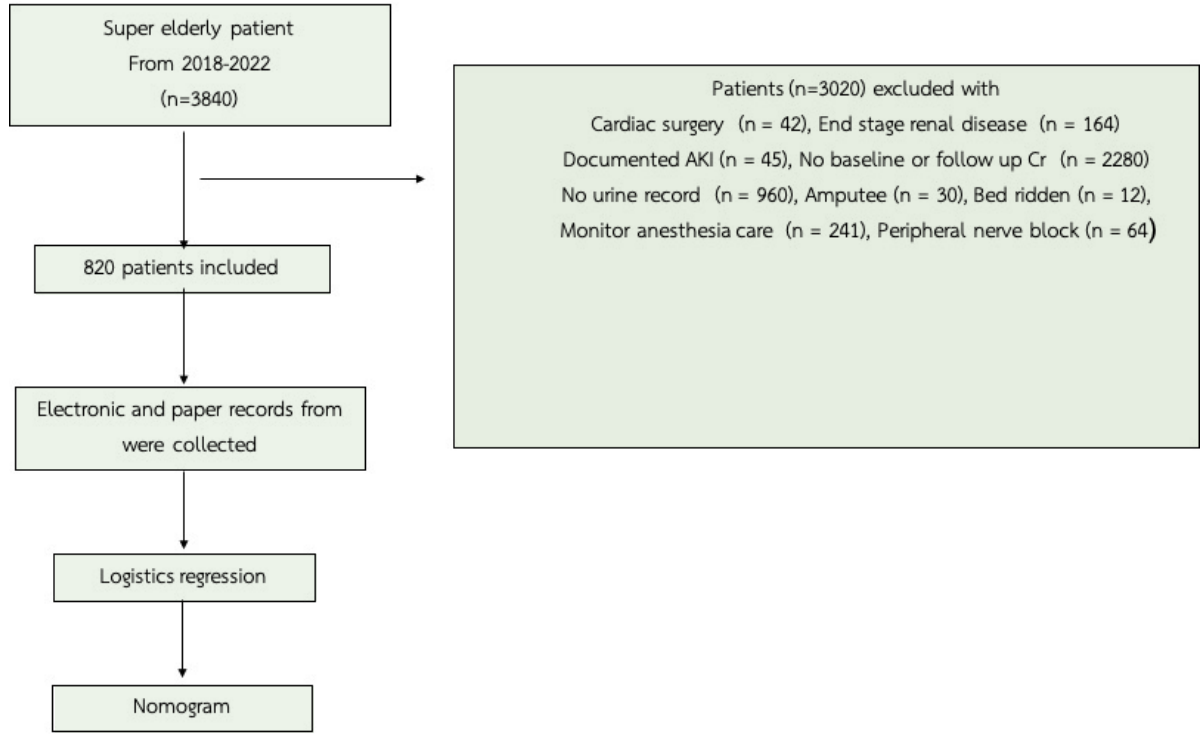

Fig 1. Study flow chart.

creatinine increased by more than 1.5-1.9 times of the baseline level, b) increase in serum creatinine level more than 0.3 mg/dL within 48 hours and c) urine output less than 0.5 mL/kg/h for 6 hours continuously.

The primary outcome was the 7-days AKI rate, according to KDIGO. Secondary outcomes were post-operative 30-day mortality rate, period of hospital stay, period of ICU stay and requirement of renal replacement therapy.

Sample size determination

Estimated sample size estimated base on ability to detect association with exposure and outcome at odds ratio at least 1.5 with 80% power was 820. The statistical significance was set at P value ≤ 0.05.

Statistical analysis

Statistical analysis was performed using R program version 4.0.2.[15] Categorical variables were presented as percentage and proportion such as gender, Body mass index (BMI), ASA classification, type of surgery, choice of anesthesia, part of surgery, and anesthetic drug use. The categorical parameters were compared between AKI group and non-AKI group by Student t-test or Wilcoxon Rank Sum test. In case of small size population, Chi-squared test or Fisher' exact test were used to compare the two groups. Continuous variables were presented as mean ± standard deviation (SD) and median ± interquartile range was used to describe normal and non-normal distribution, respectively. Categorical variables were analyzed using Chi-square test or Fisher' Exact test as appropriate.

Predictors of postoperative acute kidney injury

Predictive factors included preoperative and perioperative data. Pre-operative factors included patient's profile, clinical condition, current medication, and laboratory variables including Hemoglobin and creatinine. Preoperative serum creatinine level was analyzed within 24-48 hours prior to surgery. Baseline creatinine clearance was computed by the standard Cockcroft Gault formula using age, lean body weight (kg), serum creatinine (mg/dL) and sex. Perioperative factors included anesthetic drugs, fluid resuscitation, use of vasopressors, estimated blood loss, duration of surgery, duration of hypotension and intraoperative urine output and intraoperative hemodynamic. (Supplement 1)

Model development

Variables included in multivariate analysis were chosen from univariate analysis results that had p-value < 0.2 (Tables 1 and 2). Multi-collinearity was tested by using variance inflation factor (VIF) > 5 criteria. Stepwise logistic regression method was used to get the final model (Table 3). The Hosmer-Lameshow goodness-of-fit test also displayed a good performance (X^2= 6.0697 and P value = 0.6394). The variable with p-value< 0.05 in multivariate logistic regression model was considered statistically significant.

Score derivation and validation

The prediction score variable was selected from multivariate analysis and weight adjustment was conducted by nomogram. For the final model, each predictor score was summarized in total postoperative acute kidney injury score. Youden's index was used to reveal maximize specificity and sensitivity cutoff values of prediction score.

RESULTS

A total 3840 patients who underwent non cardiac surgery were assessed for eligibility from 1 January 2018 to 31 December 2020. Eight hundred and twenty eligible patients were retrieved in this study. The incidence of postoperative acute kidney injury among super-elderly was 15%.

Table 1 shows clinical characteristics at preoperative period between patients with AKI (acute kidney injury) and non AKI with no statistically significant differences in demographic data such as age, sex, BMI, comorbidities and preoperative blood pressure among groups. The mean age of the super elderly was 87 years old and the average BMI was 21 kg/m^2. Generally, half of the AKI group underwent emergency surgery and had a higher proportion of ASA classification IV and V than non AKI group. No differences in part of surgery, choice of anesthesia and current medication were found between the two groups. Creatinine clearance among AKI group was significant lower than that in the non-AKI group.

Table 2 illustrates clinical characteristics in intraoperative period between AKI group and non-AKI group. According to induction agent, midazolam use was significantly higher among AKI group, while, Propofol use tended to lower incidence of AKI compared to others. Opioid drugs, muscle relaxant drugs and inhalation agents displayed no significant differences in the postoperative AKI. However, AKI group more frequently used vasopressors than non-AKI group. The incidence of postoperative acute kidney injury was higher among patients with duration of hypotension more than 15 minutes. The AKI group tended to use Succinylated gelatin for resuscitation more than non AKI group.

Supplement 1. Univariate analysis of risk factors for acute kidney injury.

Variable	OR	95%CI	P-value
Age*	1.04	(1,1.09)	0.05
Male*	1.24	(0.84,1.82)	0.28
Comorbidities			
HT	1.37	(0.9,2.07)	0.13
DM	1.36	(0.85,2.18)	0.19
COPD	1.84	(0.93,3.73)	0.09
PVD	1.12	(0.13,9.7)	0.09
CHF	1.88	(0.19,18.2)	0.58
Type (ref=elective)			
Emergency	1.92	(1.3,2.82)	<0.05
Part (ref=Neuro)			
Abdomen	1.9	(0.92,3.95)	0.08
Orthopedic	2.13	(1,4.55)	0.05
Urology	3.93	(1.48,10.39)	<0.05
Vascular	1.91	(0.89,4.11)	0.09
Others	1.77	(0.6,5.2)	0.30
ASA (ref=II)			
III	2.19	(1.23,3.9)	<0.05
IV-V	13.13	(6.37,27.08)	<0.05
CrCl	0.99	(0.97,1)	0.04
Hb	0.93	(0.84,1.02)	0.10
Current medication			
ACEI	0.76	(0.38,1.52)	0.43
ARB	0.96	(0.48,1.94)	0.92
Choice (ref=spinal block)			
General anesthesia	1.02	(0.54,1.89)	0.96
Induction			
Propofol*	0.38	(0.25,0.56)	<0.05
Etomidate*	2.24	(1.15,4.39)	0.01
Ketamine*	2.44	(0.62,9.57)	0.02
Midazolam*	1.98	(1.34,2.92)	<0.05
Narcotic			
Morphine*	1.1	(0.31,3.85)	0.88
Fentanyl*	4.96	(0.67,36.83)	0.11

Supplement 1. Univariate analysis of risk factors for acute kidney injury. (Continue)

Variable	OR	95%CI	P-value
Muscle relaxant			
Cisatracurium*	1.35	(0.67,2.7)	0.39
Roccuronium*	0.78	(0.39,1.56)	0.47
Succinylcholine*	0.94	(0.48,1.83)	0.84
Inhalation (ref=no)			0.09
Sevoflurane	0.84	(0.5,1.4)	
Desflurane	0.55	(0.3,1)	
Contrast media	0.78	(0.47,1.29)	0.33
Hypotension	1.52	(0.97,2.39)	0.06
Duration of hypotension (>= 30mins)	2.32	(1.52,3.52)	<0.05
Total urine output (ref>=2)			
<= 0.5	6.87	(3.8,12.42)	<0.05
0.51-2	1.81	(1.05,3.12)	0.03
Diuretic use	1.24	(0.65,2.4)	0.51
Vasopressor use			
Norepinephrine*	1.95	(1.33,2.88)	<0.05
Ephedrine*	0.59	(0.39,0.89)	0.01
Epinephrine*	4.57	(1.67,12.5)	<0.05
Dopamine*	9.58	(3.64,25.23)	<0.05
Fluid			
NSS*	1.74	(1.11,2.71)	0.01
Balanced salt solution*	0.51	(0.34,0.76)	<0.05
Succinylated gelatin use*	2.55	(1.66,3.93)	<0.05
Blood transfusion	1.65	(1.12,2.42)	0.01
RBC*	1.58	(1.07,2.34)	0.02
FFP*	2.04	(1.25,3.34)	<0.05
PC*	2.09	(1.07,4.05)	0.03

Abbreviations: COPD: chronic obstructive lung disease, CHF: chronic heart failure, Hb: hemoglobin, Crcl: creatinine clearance, ACEI: Angiotensin-converting enzyme inhibitors, ARB: Angiotensin receptor blockers, NSS: normal saline solution, PRC: packed red blood cell, FFP: fresh frozen plasma, PC: platelet count *Continuous variables

TABLE 1. Clinical characteristics of preoperative period in super-elderly patients with and without Acute Kidney Injury (AKI) (N=820).

Characteristics	AKI group (N=124) N (%)	Non-AKI group (N=696) N (%)	P-value
Age (years) mean±SD	87.5 ±4.5	86.7±3.9	0.05
Gender			0.32
Female	53 (41.1)	334 (48)	
Male	71 (57.3)	362 (52)	
BMI (kg/m^2)*	21.9 (18.9,24.9)	21.7 (19.1,24.2)	0.54
Comorbidities			
Diabetes	27 (21.8)	118 (17.0)	0.24
Hypertension	87 (70.2)	440 (63.5)	0.16
COPD	11 (8.9)	35 (5)	0.13
Congestive heart failure	1 (0.8)	3 (0.4)	0.48
Type of surgery			<0.05
Scheduled	59 (47.6)	442 (63.5)	
Emergency	65 (52.4)	254 (36.5)	
ASA type			< 0.05
II	15 (12.1)	197 (28.3)	
III	78 (62.9)	468 (67.2)	
IV	28 (22.6)	31 (4.5)	
V	3 (2.4)	0 (0.0)	
Preoperative Hb (g/dL)*	10 (9,11.2)	11 (9,12)	0.04
Preoperative Cr (mg/dL)*	1.1 (0.8,1.4)	0.9 (0.8,1.2)	<0.05
Preoperative CrCl (mL/min)*	34.7 (26.8,44.9)	38.8 (29.6,49.7)	<0.05
Part of surgery			0.14
Abdomen	40 (32.3)	223 (32.0)	
Orthopedic	30 (24.2)	149 (21.4)	
Neurology	10 (8.1)	106 (15.2)	
Urology	10 (8.1)	27 (3.9)	
Vascular	28 (22.6)	155 (22.3)	
Others	6 (4.8)	36 (5.2)	
Current medication			
ACEI	10 (8.1)	72 (10.3)	0.53
ARB	10 (8.1)	58 (8.3)	1
Choice of anesthesia			1
General anesthesia	111 (89.5)	622 (89.4)	
Spinal block	13 (10.5)	74 (10.6)	
Preoperative blood pressure			
SBP (mmHg)*	131 (115.8,146.2)	130 (119,146.2)	0.72
DBP (mmHg)*	69 (60,77)	70 (62,79)	0.18
MAP (mmHg)*	88.5 (81,98.2)	90 (82,99)	0.47

Abbreviations: BMI: body mass index, COPD: chronic obstructive lung disease, CHF: chronic heart failure, Hb: hemoglobin, Cr: creatinine, Crcl: creatinine clearance, ACEI: Angiotensin-converting enzyme inhibitors, ARB: Angiotensin receptor blockers, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure *Continuous data were reported as median and IQR 1-3

TABLE 2. Clinical characteristics of intraoperative period in super-elderly patients with and without Acute Kidney Injury (AKI) (N=820).

Characteristics	AKI (N=124) N (%)	Non-AKI (N=696) N (%)	P-value
Induction agent			
Propofol	73 (58.9)	551 (79.2)	<0.05
Thiopental	0 (0)	12 (1.9)	0.23
Etomidate	13 (11.5)	35 (5.5)	<0.05
Ketamine	3 (2.4)	7 (1.0)	0.18
Midazolam	57 (46)	209 (30)	<0.05
Narcotic			
Morphine	120 (97.6)	658 (97.8)	0.74
Fentanyl	123 (100)	669 (99.4)	1
Muscle relaxant			
Succinylcholine	11 (10.3)	67 (10.9)	0.98
Cisatracurium	97 (90.7)	539 (87.8)	0.49
Rocuronium	10 (9.3)	72 (11.7)	0.58
Inhalation			
Sevoflurane	72 (58.1)	371 (53.3)	0.37
Desflurane	29 (23.4)	226 (32.5)	0.05
No	23 (18.5)	99 (14.2)	0.26
Vasopressor			
Ephedrine	38 (30.6)	298 (42.8)	0.01
Epinephrine	7 (5.6)	9 (1.3)	<0.05
Norepinephrine	71 (57.3)	283 (40.7)	<0.05
Duration of surgery (minutes) mean ± SD	177.5 (135, 256.3)	180 (135, 256.2)	0.88
Intraoperative hypotension	96 (77.4)	482 (69.3)	0.08
Duration of hypotension (minutes)*	15 (5,82.5)	10 (0,20)	<0.05
Contrast media use	21 (16.9)	144 (20.7)	0.39
Diuretic use	12 (9.7)	55 (7.9)	0.63
Fluid intake (mL)*	1150 (500 ,2000)	1100 (700, 1675)	0.63
Balanced salt solution (mL)*	1000 (500, 1450)	1000 (700, 1500)	0.56
NSS (mL)*	500 (100, 1212.5)	500 (0, 1000)	0.07
Succinylated gelatin*	500 (450, 675)	500 (300, 500)	0.02
Blood transfusion			0.01
PRC	54 (43.5)	228 (32.8)	0.02
FFP	26 (21)	80 (11.5)	<0.05
PC	13 (10.5)	37 (5.3)	0.04
Intraoperative blood loss (mL)*	150 (50,412.5)	100 (50,300)	0.06
Intraoperative urine output (mL/kg/hr)			<0.05
≤0.5	46 (37.1)	80 (11.5)	
0.51-2	59 (47.6)	389 (55.9)	
>2	19 (15.3)	227 (32.6)	

Abbreviations: NSS: normal saline solution, PRC: packed red blood cell, FFP: fresh frozen plasma, PC: platelet count *Continuous data were reported as median and IQR 1-3

TABLE 3. Best predictive score revealed by multivariate logistic regression.

Risk factor	Ref	Crude OR (95%CI)	Adj.OR (95%CI)	P (LR-test)	Risk Score
COPD	No	1.84 (0.91, 3..73)	2.02 (0.93, 4.38)	0.07	24
Type	Scheduled				
Emergency		1.92 (1.3, 2.82)	1.43(0.89, 2.31)	0.14	14
ASA					
II				<0.05	
III		2.19 (1.23, 3.9)	2.58 (1.41, 4.73)		37
IV-V		13.13 (6.37,27.08)	13.73 (5.94, 31.76)		100
Part of surgery	Neuro			<0.05	
Abdomen		1.9 (0.92,3.95)	2.85 (1.27,6.38)	<0.05	42
Orthopedic		2.13 (1,4.55)	3.22 (1.4,7.4)	<0.05	48
Urology		3.93 (1.48,10.39)	8.31 (2.86,24.12)	<0.05	90
Vascular		1.91 (0.89,4.11)	1.8 (0.78,4.12)	0.16	25
Others		1.77 (0.6,5.2)	2.35 (0.73,7.57)	0.15	35
Propofol	Yes	2.65 (1.78,3.97)	1.63 (1,2.66)	<0.05	19
Succinylated gelatin	no	2.55 (1.66,3.93)	1.94 (1.2,3.14)	<0.05	26

Nonetheless, Blood transfusion consists of red blood cells, fresh frozen plasma and platelet concentrate that were used in AKI group more than others. Intraoperative urine output less than 5 mL/kg occurred in 75% of AKI group, while an intraoperative output less than 5 mL/kg were found a higher risk in AKI group than non-AKI group (P-value<0.05). Other independent variables were not statistically different.

Table 3 presents multivariate logistic regression model adjusted for patient's status and anesthetic drug. Independent variables significantly associated with postoperative AKI were COPD, ASA classification, part of surgery, Propofol and Succinylated gelatin. There was greater frequency of AKI with higher ASA classification with ASA III and ASA IV with V [OR 2.58 (1.41,4.73), OR 13.73 (5.94,31.76), P-value <0.05], respectively. Part of surgery differed significantly between AKI and non-AKI group (P-value <0.05). The risk of AKI was associated with abdominal surgery, orthopedic surgery and urology surgery [OR 2.85 (1.27,6.38), OR 3.22(1.4,7.4), OR 8.31(2.86,24.12)], respectively, excluding patients that underwent vascular surgery. In contrast, Propofol conserved renal function [OR 1.95 (1.21,3.16), P-value<0.05]. There was a statistically significant risk for AKI with Succinylated gelatin use [(OR 1.94(1.2,3.14), P-value <0.05)].

The nomogram comprised of COPD, ASA classification, Part of surgery, Type of surgery, Propofol and Succinylated gelatin (Fig 2). The score ranged from 20 to 100. The nomogram has a good discriminative ability to identify patients with postoperative AKI with AUC 0.746. The calibrate curve shows that the apparent value is almost the same as bias-corrected value; mean squared error = 0.00043 (Fig 3). Cutoff point at 63 was chosen, which had the highest Youden index. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the prediction score was 83, 45, 33, and 89%, respectively.

The rate of renal replacement was higher in patients with postoperative AKI compared with patients without AKI (P-value<0.05). Period of ICU stay and hospital stay was significantly longer in AKI group than other groups (P-value<0.05). Furthermore, 30-day mortality was significantly greater in AKI group (P-value <0.05).

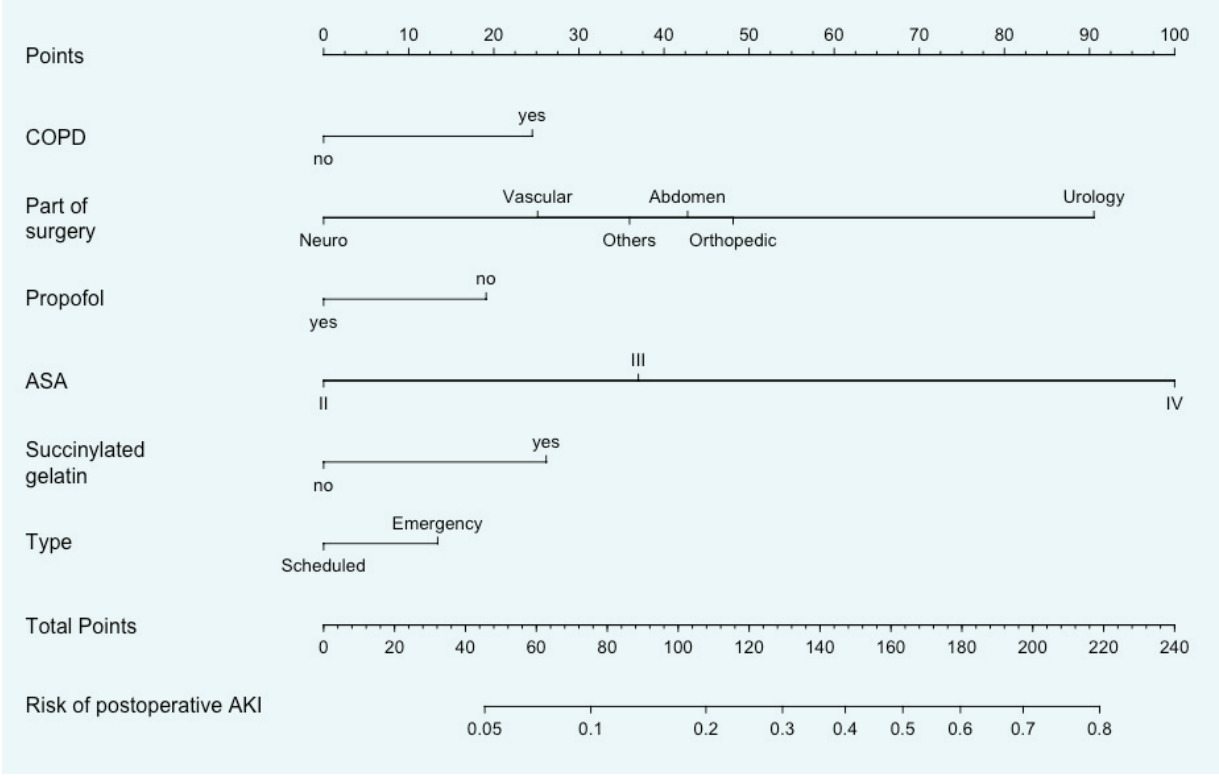

Fig 2. The nomogram explicated by logistic regression. Each predictor variable is presented by a line in the figure. The prediction scores ranged from 0 to 100. The variables were gathered with point and paired with the probability of postoperative AKI. A drawing from was made from each variable to the "Points" axis to indicate the points of the variable. The scores for all variables were summed and placed on the "Total score" line. The predicted risk of postoperative AKI ranged from 5 to 80%.

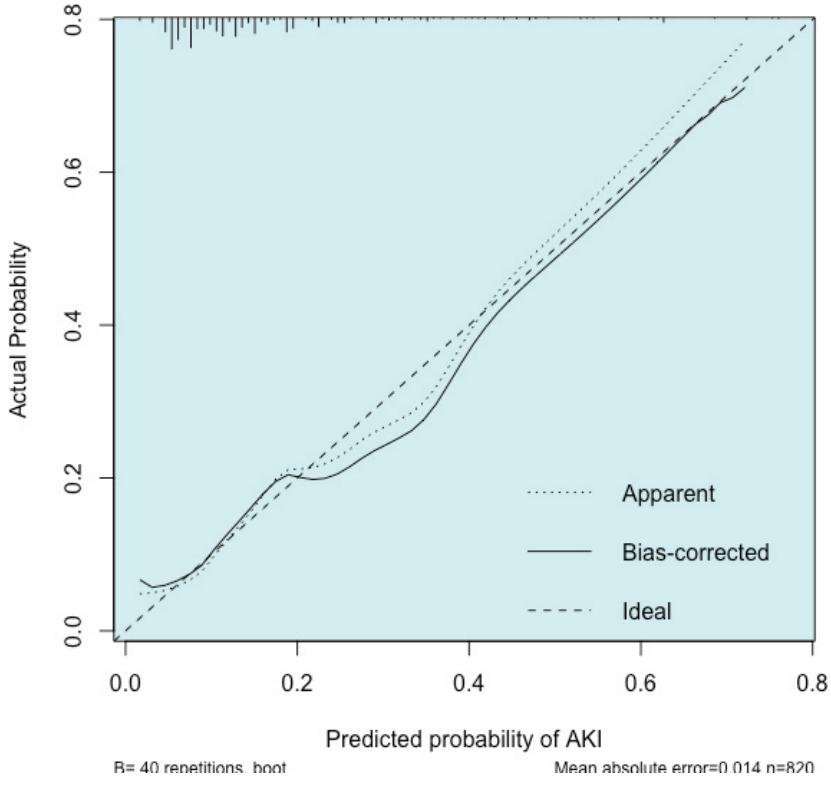

Fig 3. The AKI nomogram and its performance. The Apparent value has a closer fit to Bias-corrected value, which means the nomogram had a good performance. Mean absolute error=0.014 Mean squared error=0.00036 Quantile of absolute error=0.03.

TABLE 4. Postoperative consequence in patients with and without Acute Kidney Injury (AKI).

Characteristics	AKI (N=124)	Non-AKI (N=696)	P-value
Renal replacement n (%)	15 (12.1)	1 (0.1)	<0.05
Period of ICU stay (days)*	2.5 (0,12)	0 (0,1)	<0.05
Period of hospital (days)*	14.5 (8.8,28)	10 (7,18)	
Death 30 n (%)	30 (24.2)	11 (1.6)	<0.05

*Continuous data were reported as median and IQR 1-3

DISCUSSION

Nowadays, there is no effective equipment to identify the probability of postoperative AKI in surgical patients, particularly in super-elderly patients. AKI is a serious consequence after surgery. A prior study demonstrated that approximately 6.8% of patients undergoing abdominal surgery developed an episode of AKI.[16] In the present study, almost 15% of AKI among super-elderly and 24.2% with AKI died in 30 days. The incidence of acute kidney injury is highest in elderly and increases with age.[3,17] There are many reasons for the comparatively high probability of AKI in super elderly patients, for instance, comorbidities that produce AKI, comorbidities that need intervention, medication or surgery that disarrange kidney function, and structure and function that alter with time.[3,9] According to meta-analysis, elderly patients fail to recover from AKI and develop to chronic kidney disease.[18]

The risk of postoperative acute kidney injury is associated with patient factors as age, type of surgery, emergency surgery, part of surgery, ASA classification, preoperative hemoglobin (Hb), preoperative creatinine, preoperative creatinine clearance, and medical comorbidities such as chronic obstructive lung disease (COPD). It is not surprising that emergency surgery was found as a part of the AKI prediction score, as in this study. A US national study revealed that not only cardiac surgery, but also urology, thoracic, orthopedic and malignancy were associated with AKI.[19] In patients following non-cardiac surgery, ASA classification III and IV were shown to comprise a higher proportion of AKI than those with ASA classification I and II.[4]

Other preoperative laboratory data, preoperative anemia[7] high creatinine[20], low creatinine clearance[6] and low eGFR[2] were shown to be similar with previous studies. In cardiac surgery, preoperative anemia increased AKI rate from 1.8% to 3.2%. Meanwhile, patients receiving blood transfusion had relative risk of AKI more than two times compared without transfusion.[21,22] The mechanism of blood transfusion that could cause AKI is still unclear. Although, It was suggested that pathophysiology can aggravate tissue oxygen delivery and stimulate inflammatory process and oxidative stress.[23-25] Previous study showed that higher baseline preoperative creatinine was significant risk factor for AKI.[26] Apart from urine output, Mizota T et al. found perioperative urine output less than 0.3 mL/kg/h increased probability of AKI.[27] Even if perioperative urine output was defined differently, the result remains the same.

Neither the univariate analysis nor the nomogram of the study found a significant difference with diuretic use, which is a known AKI stimulator. It is probably due to the reason that we are not given in lower creatinine clearance patient. Nonetheless, The use of diuretic, particularly furosemide has been demonstrated deleterious in prevention and treatment of AKI.[28,29]

In this study, the use of norepinephrine and epinephrine was essential factor. Norepinephrine, is frequently used to restore MAP from 60 to 75 mmHg and increase renal oxygen delivery (RDO_2), Glomerular filtration rate (GFR) and renal oxygenation.[30] On the other hand, It has been shown that norepinephrine decreases renal blood flow (RBF) and renal oxygen delivery, which provoke renal ischemia.[31] Epinephrine has the same efficacy as norepinephrine, but also causes hyperglycemia, hyperlactatemia and acidosis.[31] Based on current evidence, norepinephrine should be used to restore blood pressure within autoregulatory values

in hypotensive vasodilated patients with acute kidney injury.[31] According to previous knowledge, propofol is down regulated by nitric oxidase synthase to preserve renal function[32] and protect renal tissue via peroxidation of lipid membrane.[33] Lee et al. reported that propofol reduced the new onset of chronic kidney disease after nephrectomy.[34] Therefore, the anti-inflammatory effect of propofol might be significant in the use of TIVA technique, whereas propofol in this study was used as a sole induction agent.

Resuscitation fluid, such as Succinylated gelatin another factor which impacts postoperative acute kidney injury. Recent guidelines suggest avoiding the use of gelatin, as systemic colloids gather in proximal renal tubules and disturb renal function, increasing the risk of anaphylaxis, mortality, renal failure and bleeding.[35]

AKI Predictor in surgical super-elderly patients consist of baseline characteristics and perioperative data. We have found a relationship AKI: COPD, type of surgery, ASA classification, part of surgery and Succinylated gelatin. Propofol was used as a defensive factor in this study. Prior study on AKI prediction score in a different setting, displayed a good discrimination which AUC range above 0.7. For instance, for the eGFRpreSurg as a predictive role among very elderly patients, AUC was at 0.703.[2] This represented eGFRpreSurg at cut off point 70mL/min/1.73 m^2 as a risk factor of postoperative AKI. Another study by Hong et al. reported the relationship between HUGE formula and mortality in elderly patients from hip fracture surgery with highest AUC of 0.78 (95%CI 0.667-0.892).[26] However, both of the predictors were in super-elderly patient settings and the score obtained from only preoperative data.

This nomogram score in the study is easily understood and handly to adopt in preoperative period. Accordingly, AKI is a preventable condition, nomogram may help physicians to early detect AKI and handle it in proper time. According to resource-limited countries such as Thailand, invasive monitoring in every case might be impossible. The most effective prevention is early detection in high risk, special monitoring, optimal fluid administration and nephrotoxic agent avoidance. From the research principle, it is expected that the nomogram will effectively assist in selection high risk AKI patients for prevention and aggressive intervention.

Strength and limitation

The incidence of AKI might be precise because AKI in this study was determined by both urine output and serum creatinine. The result may be in indubitable in the occurrence of AKI.

There are some limitations of this study. Firstly, although all super-elderly patients who underwent surgery in the study period were retrieved to reduce selection bias, patient bias was unavoidable due to retrospective study. Secondly, this study could not analyze the cause of AKI categorized into three groups: prerenal, renal and postrenal due to lack of information in our electronic hospital system. The rate of renal replacement therapy was compared between AKI and non-AKI patients; nonetheless there was a lack of information to identify the reason for renal replacement therapy.

Finally, due to the lack of external validation of the scoring system since this is a single center study and there could have been sampling bias. Future studies performed on the scoring system using data of multiple centers can validate it.

CONCLUSION

This study found an increase incidence of postoperative AKI among super-elderly patients and relationship between AKI and morbidity and mortality. Despite, AKI prediction score not being a definitive tool for observation and monitoring, it can help physicians consider various clinical risk factors in evaluating the chance of AKI. This nomogram can help clinical physicians improve the prognosis among super-elderly patients undergoing surgery.

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Detection of Postoperative Cognitive Dysfunction by Telemedicine Among Octogenarian Patients Who Underwent Minor Elective Surgery; Prospective Cohort Study

Hathaichanok Suesat, M.D.*, Varalak Srinonprasert, M.D., ***, Panita Limpawattana, M.D.****, Salinee Nakyos, M.D.*****, Jiraporn Poontananggul, M.D.*, Chalita Jiraphorncharas, B.S.***, Wiraphon Manatarinat, M.D.******, Thanachai Noomprom, B.S.******, Arunotai Siriussawakul, M.D.*, ***

Department of Anesthesiology, **Division of Geriatric Medicine, Department of Medicine, ***Siriraj Integrated Perioperative Geriatric Excellent Research Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand, ****Division of Geriatric Medicine, Department of Internal Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand, *****Division of Anesthesiology, Buddhachinaraj Hospital, Phitsanulok 65000, Thailand,****Siriraj Center of Telemedicine (SiTEL), Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand*

ABSTRACT

Objective: Postoperative cognitive dysfunction (POCD) is associated with permanent disability, increased mortality, and diminished quality of life. The incidence of acute POCD among geriatric patients who have undergone minor surgery is uncertain because they are typically discharged before acute POCD is detected. Owing to the efficient postoperative care that can be provided, telemedicine is an attractive tool to investigate POCD. The primary objective of our research was to explore the incidence of acute POCD, while its secondary objective was to describe the consequences of POCD on functional recovery and quality of life.

Materials and Methods: This prospective cohort study enrolled patients aged ≥ 80 years and scheduled for minor elective surgery. During pre-anesthetic visits, we installed a telecommunications program on the patients' smartphones. Assessments of cognitive and other functions were performed preoperatively and 1 week postoperatively via telemedicine.

Results: Forty octogenarian patients undergoing minor surgery were included in the final analysis. The acute-POCD incidence was 10% (95% CI 4.79-18.39). Recall memory was the main cognitive domain impaired after the procedures. Nevertheless, there were no significant differences in the functional recovery and quality of life of the POCD and non-POCD patients.

Conclusion: The acute-POCD patients demonstrated minor symptoms that were unrelated to delayed postoperative functional recovery or decreased quality of life.

Keywords: Anesthesia; geriatrics; postoperative cognitive dysfunction; RUDAS-Thai; telemedicine. (Siriraj Med J 2022; 74: 126-133)

Corresponding author: Arunotai Siriussawakul
E-mail: arunotai.sir@mahidol.ac.th
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ORCID ID: https://orcid.org/0000-0003-0848-6546
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INTRODUCTION

Multiple comorbid conditions are typical in the elderly, resulting in an increased possibility of surgical intervention and anesthesia.[1] Postoperative cognitive dysfunction (POCD), defined as an impairment of cognitive function arising after surgery, frequently occurs among elderly patients.[2] The systemic stress response arising during surgical procedures includes changes in the brain function and is involved in a decline in cognitive function.[3] Factors that elevate the risk of POCD include increasing age, pre-existing cerebrovascular and cardiovascular disorders, a history of alcohol abuse, and a low educational level.[4] Perioperative hypoxemia and hypotension, postoperative infection, and respiratory complications are some of the recognized risk factors for POCD.[5] POCD is associated with poorer recovery, an increased risk of permanent disability, and the need to utilize social financial assistance.[4,6]

POCD can be divided into acute, intermediate, and long-term changes. "Acute POCD" is used to describe cognitive declines detected within 1 week of surgery, "intermediate POCD" for changes occurring within 3 months, and "long-term POCD" for declines persisting up to 1-2 years following surgery. However, the exact significance of detecting POCD at these various time points is unclear.[7] POCD was found to be present in 25.8% of patients 1 week after non-cardiac surgery and in 9.9% after 3 months.[5] Other research on patients aged ≥ 60 years who had undergone minor surgery established that their POCD incidence was 6.8% at 1 week and 6.6% at 3 months.[8]

The symptoms of acute POCD may be subtle and might be difficult to detect among geriatric patients who have undergone minor surgery. Patients are often discharged before any symptoms occur. Neuropsychological testing is required to detect POCD by comparing preoperative and postoperative scores.[4] The Rowland Universal Dementia Assessment Scale (RUDAS) is a short, cognitive-screening instrument designed to minimize the effects of cultural learning and language diversity on the assessment of baseline cognitive performance. The Thai version of RUDAS can be utilized for assessments conducted via telemedicine. Telemedicine facilitates the post-discharge monitoring of remotely delivered health care in a cost- and time-saving manner.[9,10] The primary objective of this study was to establish the incidence of acute POCD detected via telemedicine among octogenarian patients who had undergone minor surgery. The secondary objective was to describe the consequences of POCD on their functional recovery and quality of life.

MATERIALS AND METHODS

Study design and participants

The study was approved by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (protocol approval number Si. 168/2018) and was registered in the Thai Clinical Trials Registry (TCTR) under study number TCTR20201216001 date registered on December 16, 2020. Retrospectively registered. Written informed consent was obtained from all study participants. A prospective study was conducted at a large university-based national tertiary referral center during the July 2018 to April 2019 study period. The inclusion criteria were patients aged ≥ 80 years who were scheduled for minor elective surgery. Such surgery had an expected blood loss of < 500 ml, no significant fluid shift, and no need for complex post-operative care typically done on an ambulatory basis (breast surgery without reconstruction; laparoscopic cholecystectomy; hernia repair; most cutaneous, superficial, soft tissue excision; and endoscopic procedures such as ERCP, bladder, and ureteric surgery).[11] Patients or their caregivers needed to use smartphone support provided by way of the "Polycom RealPresence Mobile" application. Patients were excluded if they had factors that might affect the execution of remote cognitive assessments, such as an inability to understand the Thai language, a severe visual or auditory dysfunction, an unstable mental status, or being bedridden. Patients reluctant to complete the preoperative and postoperative RUDAS-Thai test were also excluded. The study protocol followed the guidelines of the Declaration of Helsinki and all of its later amendments.

The day before surgery, a staff member installed the RealPresence Mobile application on the smartphones of the patients or their primary caregivers, who were then trained in its usage. The caregivers helped the patients to establish the connection. However, they did not have any active role during the interview or examination. The mobile application enabled high-quality audio and video communications to be had during preoperative and postoperative assessments. Audio-visual data were shared and transferred via a real-time video stream over a 3G or 4G mobile phone network, with the intermediary Internet Service Provider providing the software interface between the applications held by the hospital-based physicians and the patients. Fig 1 illustrates the broad process of collecting data for telemedicine purposes using a technological network. The tests for each patient were performed in about 30 minutes. They were conducted by a psychologist who was trained to communicate with patients by oral and visual questioning based on a questionnaire.

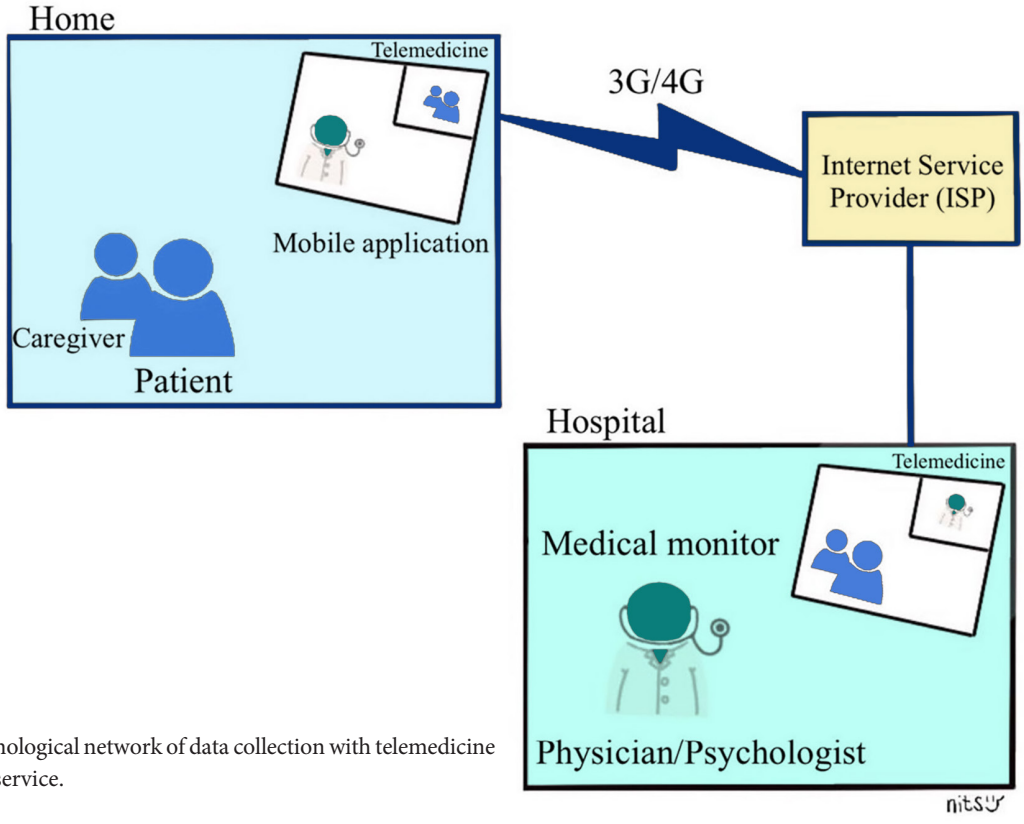

Fig 1. The technological network of data collection with telemedicine in health care service.

Assessments

The RUDAS-Thai version was applied to assess cognitive functions preoperatively. The 6 cognitive domains that RUDAS assesses are memory, praxis, language, judgment, drawing, and body orientation.[12] The maximum total score is 30. In elderly patients with a pre-elementary education level, preoperative cognitive impairment was suspected if the total score was ≤ 23 (AUC = 0.79; sensitivity and specificity of 71.43% and 76.92, respectively), while in the case of elderly patients with a post-elementary education, a score of ≤ 24 (AUC = 0.8, sensitivity and specificity of 77% and 70%, respectively) was considered the threshold. The RUDAS-Thai can be an effective alternative test and can be utilized instead of the Mini-Mental State Examination (MMSE) for dementia screening.[13] The present study therefore used the RUDAS-Thai to detect POCD. Acute POCD, detected within 1 week post-operatively, was diagnosed if a score had decreased by ≥ 3 compared with its pre-operative level.[14,15]

Several other tests were carried out to comprehensively assess potentially affected aspects. The Barthel Activities of Daily Living index was used to measure activity limitations in the domains of personal care and mobility.[16] The 5-level EQ-5D questionnaire was administered to assess quality of life.[17] Montgomery–Asberg Depression Rating Scale testing was conducted to establish the severity of depressive symptoms.[18] Finally, a numeric rating scale was utilized to evaluate postoperative pain levels, while a verbal rating scale was employed to identify the degrees of postoperative nausea and vomiting.

Statistical analysis

The sample size calculation was based on a study by Canet et al., which found a POCD incidence of 6.8%.[8] Therefore, 43 patients were needed for the rare-event analysis in this study. (nQuery Advisor version 7.0; Statistical Solutions Ltd., Cork, Ireland).[19] Once an estimated 10% loss to follow-up was added, the number of participants required was determined to be 48.

The demographic data and clinical variables were summarized using descriptive statistics. The continuous data were reported as means and standard deviations, or as medians with minima and maxima, as appropriate. The categorical data were reported as frequencies and percentages. The statistical analyses were calculated using SPSS Statistics for Windows (version 18; SPSS Inc., Chicago, Ill., USA). A p-value of < 0.05 was considered statistically significant.

RESULTS

Fifty-six octogenarian patients were recruited for the study (Fig 2). Of those, sixteen (28%) were subsequently withdrawn due to surgery postponement, loss to follow-up,

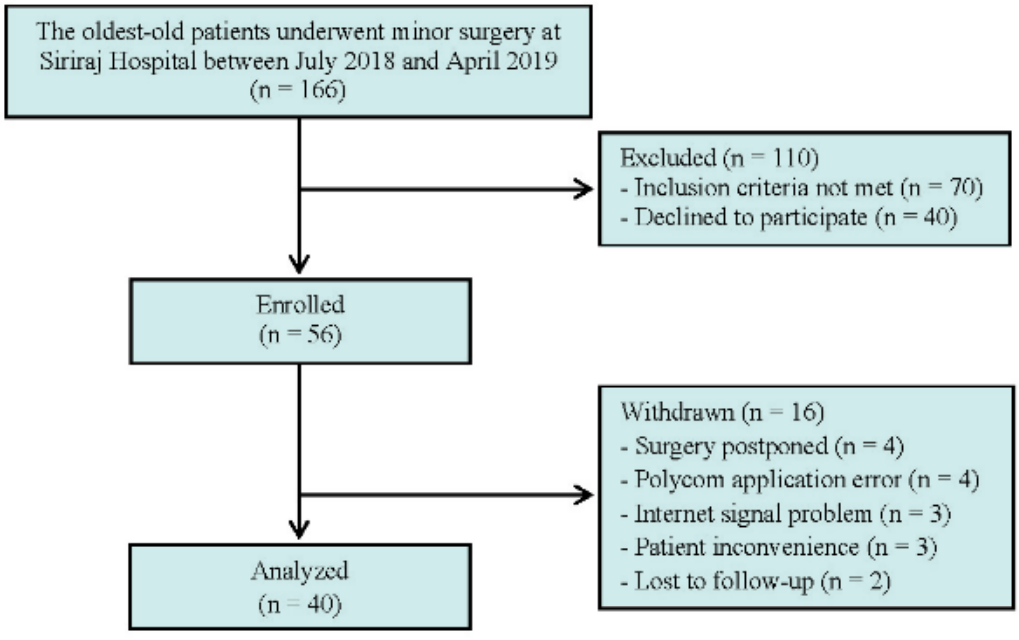

Fig 2. Flowchart of study design.

or incomplete postoperative data collection resulting from patient inconvenience or technical issues (application errors and internet-signal problems). The data relating to 40 patients were therefore included in the final analysis. The number of patients needed for the study was re-calculated, based on the actual probability of event (π) with a 95% confidence interval (CI), to confirm that a sample size of 30 cases was adequate for the achievement of the primary objective.

The characteristics of the octogenarian patients who underwent minor elective surgery and anesthetic management are detailed in Table 1. The mean preoperative RUDAS score, Barthel Index score, EQ-5D-5L score, and MARDS score were 23.40, 17.38, 0.860, and 2.45, respectively. Four octogenarian patients were diagnosed with acute POCD during postoperative Days 5-9, giving an incidence of acute POCD of 10% (95% CI 4.8-18.4). The characteristics of those patients are summarized in Table 2. All four had graduated from primary school, and hypertension was one of their coexisting diseases. Impairment in recall memory was found with each POCD patient. One patient received benzodiazepine to achieve adequate sedation before surgery. Two patients experienced intraoperative adverse events (bradycardia or hypotension) requiring fluid resuscitation and a vasopressor.

There were no differences in the functional declines, decreases in the quality of life, or levels of depression of the POCD and non-POCD patients (Table 3). About 3 days after surgery, the incidence of POCD patients who had experienced mild-to-moderate pain was 7.5% (95% CI 3.45-15.76). The median (range) pain score for the numeric rating scale was 0 (0, 6). Only one of the 40 octogenarian patients had a mild severity of nausea and vomiting, occurring on the first day after anesthesia; consequently, the overall incidence of postoperative nausea and vomiting was 2.5% (95% CI 0.61-8.76).

DISCUSSION

The incidence of acute POCD in this study was 10%. This was higher than the figure reported by a previous study, which revealed that the POCD incidence among patients aged ≥ 60 years and undergoing minor surgery was 6.8% (95% CI 4.3–10.1).[8] Increasing age significantly elevates the incidence of POCD because, relative to younger age groups, individuals with advanced age more frequently have physical and mental frailty as well as a decreased ability to cope with stresses, such as anesthesia and surgery.[2] Yon et al, reported that anesthesia-induced apoptotic neuro-degeneration might also be a potential pathway mediating the development of POCD in the older brain.[20] Glumac et al. showed that preoperative dexamethasone administration may ameliorate the incidence of early POCD after cardiac surgery. This may be because the inflammatory response to surgical procedure is a key factor in the development of POCD.[21]

All 4 POCD patients had impaired recall memory performance. A deterioration of the memory functions is one of the most consistently reported complaints by the elderly.[22] Work by Philp et al. demonstrated that the associations between thalamic structure, integrity, and higher-order cognitive processes-including the

TABLE 1. Patient characteristics, type of surgical procedures, and anesthetic management.

Variables	n = 40
Age (years)	84.20 ± 3.6
Gender	
Female	20
Male	20
Education levels	
Pre-elementary	29 (72.5)
Post-elementary	11 (27.5)
Monthly income (Thai baht)	
≤ 20,000	12 (30.0)
> 20,000	28 (70.0)
Marital Status	
Married	17 (42.5)
Widowed	23 (57.5)
Type of surgery	
Urological endoscopic surgery	20 (50)
Laparoscopic surgery	7 (17.5)
Breast surgery	4 (10.0)
Endoscopic retrograde cholangiopancreatography	4 (10.0)
Wound debridement	3 (7.5)
Anesthetic technique	
General anesthesia	23 (57.5)
Spinal anesthesia	11 (27.5)
Deep sedation	6 (15.0)
Preoperative Scores	
The Rowland Universal Dementia Assessment Scale	23.40 ± 5.00
The Barthel Activities of Daily Living index	17.38 ± 3.41
The 5-level EQ-5D	0.860 ± 0.188
The Montgomery–Asberg Depression Rating Scale	2.45 ± 3.60
Intraoperative benzodiazepine administration	4 (10.0)
Intraoperative adverse events	
Hypotension	19 (47.5)
Bradycardia	2 (5.0)
Hypertension	2 (5.0)

Values expressed as the mean ± SD or n (%).

TABLE 2. Characteristics of the POCD patients.

Case no.	Age	Gender	Education	Coexisting diseases	BDZ	Operation	Anesthetic technique	Intraoperative adverse events	Cognitive domain impairments		
									Recall memory	Drawing	Language
1.	80	Male	Primary school	HT, DLP, DM	No	Urological endoscopy	SA	Bradycardia	√	√	√
2.	82	Male	Primary school	HT, DLP, CVA	No	Urological endoscopy	GA	No	√	√	
3.	83	Female	Primary school	HT, DM	No	Debridement	GA	Hypotension	√		√
4.	85	Female	Primary school	HT, IHD, DLP, CVA	Yes	ERCP	Deep sedation	No	√	√	

Abbreviations: BDZ: Benzodiazepine, CVA: cerebrovascular accident, DLP: dyslipidemia, DM: diabetic mellitus, ERCP: endoscopic retrograde cholangiopancreatography, GA: general anesthesia, HT: hypertension, IHD: ischemic heart disease, SA: spinal anesthesia

TABLE 3. Comparison of the postoperative functional recovery, depression, and quality of life of the POCD and non-POCD patients.

Variables	POCD (n = 4)	Non-POCD (n = 36)	p-value
Functional decline	2 (16.7)	10 (83.3)	0.35
Depression	1 (6.7)	14 (93.3)	0.58
Decreased quality of life	2 (13.3)	13 (86.7)	0.58

Values expressed as the n (%).

component processes of memory and the executive functions of attention and information processing-typically decline with age.[23] Therefore, reductions in the functional connectivity to the thalamus may contribute to age-related cognitive decline.[24] This may explain why most of the cognitive-domain effects in our study were related to recall memory.

POCD is associated with functional dependence and a poor quality of life.[21,25] Previous research has demonstrated that even the early stages of cognitive impairment adversely affect the quality of life.[26] In contrast, our research found that there was no significant development of functional dependence or lowering of the quality of life of the acute-POCD patients. This suggests that minor elective surgery, ambulatory surgery, and anesthesia are quite suitable for octogenarian patients. Depression is also one of the most common illnesses in the elderly population.[27] Steinmetz et al. found that the occurrence of depression was not associated with the incidence of POCD at 1 week.[28] Likewise, we found that there was no significant development of depression among the POCD patients.

Improvements to the population's health literacy has the potential to allow individuals to access health services, to understand basic health-related information,

to communicate their health statuses well enough, and to make appropriate health decisions.[29] In other words, adequate health literacy is key to patients' abilities to maintain their health, achieve behavioral change, and effectively utilize medical services.[30] eHealth requires the use of everyday technology, such as telephones, computers, and services available through the Internet; unfortunately, this can prove to be very challenging for elderly patients.[31] Our study found that many of the octogenarian patients had limited experience with new technological devices, and their eHealth literacy skills were low. The assistance of their caregivers was therefore vital in allowing them to communicate effectively via the application. It follows that the provision of basic training in communications technology and the use of a less complex eHealth application are needed to significantly improve the eHealth literacy of the older population.

There were several limitations of our study. Firstly, the anesthetic techniques and surgical procedures employed were varied. Although all of the procedures were categorized as minor surgery, further research should be considered to assess the impact of technique variations on POCD, such as the use of moderate sedation, deep sedation, and general anesthesia. Secondly, data collection was interrupted on occasion by technological hindrances, such as internet-signal loss and the application not being sufficiently user-friendly for the elderly. Sixteen participants were therefore terminated from our study due to their inability to complete the postoperative cognitive tests. Lastly, the sample size was too small to identify the risk factors for acute POCD in the elderly Thai population. Future studies are recommended to establish those risk factors and to discover means of preventing POCD onset.

In summary, the incidence of early POCD after minor surgery in octogenarian Thai patients was higher than the figure reported by previous research, most probably due to the present study focusing on a much older population. The acute POCD revealed by the current work was not related to a delayed postoperative functional recovery or a poor quality of life. Hence, it can be concluded that minor elective surgery and anesthesia are quite suitable for octogenarian patients.

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Potential conflicts of interest

The authors declare that there are no conflicts of interest.

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The Prevalence and Risk Factors of Storage Urinary Symptoms in Symptomatic COVID-19 Patients Who were Treated in Cohort Ward and Field Hospital

Valeerat Swatesutipun, M.D., Teerayut Tangpaitoon, M.D.

Division of Urology, Thammasat University Hospital, Pathumthani 12120, Thailand.

ABSTRACT

Objective: The primary aim of this study was to focus on the prevalence of storage symptoms in COVID-19 patients and the factors associated with those symptoms.

Material and Methods: We collected the data of COVID-19 patients who were admitted to the cohort ward, ICU and field hospital of Thammasat University Hospital, Thailand, between May and June 2021. Patients answered online survey questions and undertook urinalysis by urine dipstick test. The online survey questions related to symptoms of COVID-19 infection, number of daytime voiding, nocturia, frequency and urgency symptom during COVID-19 infection, OABSS and ICIQ-LUTS in the part of storage symptoms subscale.

Result: There were 136 COVID-19 patients who met with the eligible criteria and were willing to participate in the study. Patients who had storage symptoms totaled 61 (44.85%) and had average daytime frequency, nocturia and proportion of urgency higher than no storage symptom group (5.9 VS 3.8, 2.0 VS 1.0 and 67.21% VS 6.67% (p-value <0.001), respectively). The OABSS and ICIQ storage subscale in the storage symptoms group were higher than normal group, 3.2 VS 0.9 and 4.5 VS 1.7 (p-value < 0.001), respectively.

Conclusion: Our study demonstrated that the SARS-CoV-2 virus infection is associated with abnormal storage symptoms which include frequency, urgency and nocturia. The storage symptoms may be associated with the severity of COVID-19 disease.

Keywords: Storage symptoms; COVID-19; SARS-CoV-2 virus; viral cystitis (Siriraj Med J 2022; 74: 134-141)

INTRODUCTION

Since the pandemic of the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) or Coronavirus Disease 2019 (COVID-19) has spread around the world in 2019, the outbreak is still ongoing with no end in sight. The main infected organ is the respiratory tract[1,2]; however, the SARS-CoV-2 virus can also infect the urinary tract, especially the bladder. Previous studies have reported that SARS-CoV-2 virus could be isolated from a urine sample.[3] Patients who were infected had a high prevalence of abnormal urinary storage symptoms; urinary frequency, urgency and urinary incontinence.[4,5] Moreover, the severity of urinary storage symptoms and the presence of hematuria and proteinuria from urinalysis is related to the severity of COVID-19 disease and mortality rate.[6] In Thailand, the high rate of outbreak occurred during the second, third and fourth wave. The second wave was caused by the SARS-CoV-2 strain GH,

Corresponding author: Teerayut Tangpaitoon
E-mail: jojoteerayut@gmail.com
Received 15 November 2021 Revised 7 January 2022 Accepted 13 January 2022
ORCID ID: https://orcid.org/ 0000-0001-7408-1876
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the third wave by the SARS-CoV-2 strain B1.1.7 (alpha strain) and the fourth wave by the SARS-CoV-2 strain B.1.617 (delta strain). The intensive care requirement and the mortality rate of the third and fourth waves were significantly higher than those of the second wave. The primary aim of this study was to focus on the prevalence of storage symptoms in COVID-19 patients and the factors associated with those symptoms. Another aim of our study was to study the abnormal urinalysis related to the severity of COVID-19 infection.

MATERIAL AND METHODS

This study was cross-sectional. We collected the data of COVID-19 patients, aged between 18 to 60 years old, who were admitted to the cohort ward, ICU and field hospital of Thammasat University Hospital, Pathumthani, Thailand, between May and June 2021. Inclusion was restricted to COVID-19 patients who could use smartphones to respond to our online survey questions. Patients who had clinical presentation of suspicious bacterial cystitis (had dysuria or urine dipstick test positive for nitrite or leukocyte esterase), patients who were foreigners, patients who had unstable medical conditions or indwelling urethral catheter, were excluded.

We reviewed the hospital records relating to collected data about age, gender, body mass index (BMI), underlying diseases, symptoms and complications of the COVID-19 infection. Patients were informed and consented to this study by telephone. Patients who met with the eligible criteria and were willing to participate in the study answered the online survey questions by themselves and undertook urinalysis by urine dipstick test at wards. The online survey questions consisted of symptoms of COVID-19 infection, number of daytime voiding, number of nocturia, whether they voided more frequently during COVID-19 infection, whether they had urgency symptom during COVID-19 infection, whether they had increased number of nocturia during COVID-19 infection, or whether they had urgency incontinence during COVID-19 infection. OABSS (Overactive Bladder Symptoms Scores) questionnaire (total score 15) and ICIQ-LUTS (International Consultation on Incontinence Modular Questionnaires - Lower Urinary Tract Symptoms) in the part of storage symptoms subscale (total score 16).

Patients who had any one of the following, which were cough, runny nose, sore throat and nasal congestion were defined as having upper respiratory tract infection symptoms (URI). Patients who had any one of the following, which were URI symptoms, fever, anosmia, chest discomfort, rash, diarrhea, pneumonia were defined as being in the symptomatic COVID-19 infection group. Patients who did not develop any symptoms were defined as being in the asymptomatic COVID-19 infection group. Pneumonia was diagnosed by chest x-ray that was reported by a radiologist.

The OABSS is the questionnaire that contains questions including all the important content of storage symptoms, which are frequency >7 times, urgency, nocturia and urge incontinence. Therefore, storage symptoms group was defined by using the criteria that patients had to answer that they developed one or more symptoms of frequency, urgency, nocturia, urge incontinence concomitant with having OABSS equal to or greater than 1 score during infection. Patients who did not develop any storage symptoms during infection or had OABSS score 0 were defined as no storage symptoms group.

Patients who had BMI equal to or greater than 25 kg/m^2 were defined as obesity group in this study.

The urine dipstick test that showed any of the following; those which were leukocyte positive, proteinuria or hematuria were classified as a positive result.

The data were analyzed by using STATA statistical software version 15.0. We used the student T-test for parametric data, Mann-Whitney U test for non-parametric data and Fisher-Exact test for categorical data. The binary regression analysis was used for analyzing factors that were associated with storage symptoms in COVID-19 infection patients. The statistically significant were defined as p-value <0.05.

RESULTS

From 2,357 COVID-19 patients admitted, there were 136 (5.77%) COVID-19 patients who met the eligible criteria and were willing to participate in the study; 61 (44.85%) patients had storage symptoms and 75 (55.15%) patients did not have storage symptoms.

For the demographic data as shown in Table 1, patients were divided into two groups by answering the question as to whether they had storage symptoms concomitant with OABSS score ≥1 during the COVID-19 infection, which was defined as having storage symptoms and patients who not develop any storage symptoms or had OABSS score 0 were defined as no storage symptoms. The mean age of COVID-19 patients who had storage symptoms was 36 years old and 33 years old for patients who did not have storage symptoms, which were not statistically difference between the groups. There were 37 (60.66%) patients in the storage symptoms group who had one or more of the underlying diseases, which was obesity, diabetic disease, hypertension, dyslipidemia, asthma/COPD. Meanwhile, 38 (50.67%) patients in the no storage symptoms group had one or more underlying

TABLE 1. Demographic data.

	Storage symptoms[1] (n=69)	No Storage symptoms[2] (n=67)	p-value
Age, year mean (SD)	36 (14)	33 (12)	0.221
Gender			
Male, n (%)	26 (42.62)	28 (37.33)	
Female, n (%)	35 (57.38)	47 (62.67)	0.598
Overall underlying disease			
Yes, n (%)	37 (60.66)	38 (50.67)	
No, n (%)	24 (39.34)	37 (49.33)	0.299
Obesity			
Yes, n (%)	28 (45.90)	39 (52.00)	
No, n (%)	33 (54.10)	36 (48.00)	0.864
Diabetic disease			
Yes, n (%)	5 (8.20)	10 (13.33)	
No, n (%)	56 (91.80)	65 (86.67)	0.416
Hypertension			
Yes, n (%)	5 (8.20)	10 (13.33)	
No, n (%)	56 (91.80)	65 (86.67)	0.416
Dyslipidemia			
Yes, n (%)	3 (4.92)	7 (9.33)	
No, n (%)	58 (95.08)	68 (90.67)	0.511
Asthma/COPD			
Yes, n (%)	1 (1.64)	3 (4.00)	
No, n (%)	60 (98.36)	72 (96.00)	0.628
Admitted ward			
ICU, n (%)	3 (4.92)	3 (4.0)	
Cohort, n (%)	32 (52.46)	21 (28.00)	
Field hospital, n (%)	26 (42.62)	51 (68.00)	0.007
Intravenous fluid therapy			
Yes, n (%)	3 (4.92)	3 (4.00)	
No, n (%)	58 (95.08)	72 (96.00)	1.000
Overall COVID-19 symptoms			
Asymptomatic, n (%)	10 (16.39)	26 (34.67)	
Symptomatic, n (%)	51 (83.61)	49 (65.33)	0.019
URI symptoms[2]			
Yes, n (%)	44 (72.13)	45 (60.00)	
No, n (%)	17 (27.87)	30 (40.00)	0.151
Fever			
Yes, n (%)	35 (57.38)	24 (32.00)	
No, n (%)	26 (42.62)	51 (68.00)	0.003

TABLE 1. Demographic data. (Continue)

	Storage symptoms[1] (n=69)	No Storage symptoms[2] (n=67)	p-value
Anosmia			
Yes, n (%)	11 (18.03)	10 (13.33)	
No, n (%)	50 (81.97)	65 (86.67)	0.482
Chest discomfort			
Yes, n (%)	11 (18.03)	10 (13.33)	
No, n (%)	50 (81.97)	65 (86.67)	0.482
Rash			
Yes, n (%)	2 (3.28)	1 (1.33)	
No, n (%)	59 (96.72)	74 (98.67)	0.587
Diarrhea			
Yes, n (%)	11 (18.03)	5 (6.67)	
No, n (%)	50 (81.97)	70 (93.33)	0.060
Pneumonia			
Absent, n (%)	26 (42.62)	17 (22.67)	
Present, n (%)	35 (57.38)	58 (77.33)	0.016

Abbreviation: SD: standard deviation, URI: respiratory tract infection.

[1] Storage symptom were defined as patients who had one or more symptoms of frequency voiding, urgency, urge incontinence, nocturia concomitant with OABSS score ≥ 1 during COVID-19 infection.

[2] Patients who did not develop any storage symptom during infection or had OABSS score 0 during COVID-19 infection.

[3] URI symptoms were defined as having one or more symptoms of cough, runny nose, sore throat, nasal congestion.

diseases, which was not statistically different between two groups. Most of the patients in the storage symptoms group (52.46%) were admitted to the cohort ward, in contrast to no storage symptoms group that most of the patients (68%) were admitted to the field hospital. Patients in the storage symptoms group were admitted to the cohort ward greater than the no storage symptoms group (52.46% VS 28.00%, p-value 0.007). There were only six patients who received intravenous fluid during admission, 3 patients in the storage symptoms group and 3 patients in the no storage symptoms group, which was not statistically different between the two groups.

Patients who had storage symptoms had statistically significantly COVID-19 symptoms greater than the no storage symptoms group (p-value 0.019). Moreover, fever and pneumonia were significantly related to storage symptoms (p-value <0.05). Although diarrhea was not statistically significant but it had the tendency to be related to storage symptoms (p-value 0.06)

From Table 2, Patients who had storage symptoms had an average daytime frequency higher than that of the no storage symptoms group, at 5.9 and 3.8 (p-value <0.001), respectively. Meanwhile, the number of nocturia were higher in who had storage symptoms, which were 2.0 and 1.0 (p-value <0.001). Patients who had storage symptoms had urgency significantly higher than no storage symptoms group (67.21% VS 6.67%, p-value <0.001). There were only 4 patients who had urge incontinence higher than in storage symptoms group but not statistically significant. Moreover, the OABSS and ICIQ storage subscale in the storage symptoms group were higher than no storage symptoms group, 3.2 VS 0.9 and 4.5 VS 1.7 (p-value < 0.001), respectively.

TABLE 2. Data of storage symptoms between two groups.

	Storage symptoms[1] (n=61)	No Storage symptoms[2] (n=75)	p-value
Daytime frequency, mean (SD)	5.9 (2.9)	3.8 (2.1)	< 0.001
Nocturnal frequency, mean(SD)	2.0 (1.2)	1.0 (1.2)	<0.001
Urgency			
Present, n (%)	41 (67.21)	5 (6.67)	
Absent, n (%)	20 (32.79)	70 (93.33)	<0.001
Urgency urinary incontinence during admission			
Present, n (%)	3 (4.92)	1 (1.33)	
Absent, n (%)	58 (95.08)	74 (98.67)	0.325
OABSS, mean (SD)	3.2 (2.2)	0.9 (1.0)	< 0.001
ICIQ (storage), mean (SD)	4.5 (2.7)	1.7 (2.3)	< 0.001

Abbreviation: SD: standard deviation, URI: respiratory tract infection, OABSS: overactive bladder symptom scores, ICIQ: International Consultation on Incontinence Modular Questionnaires.
[1] Storage symptoms were defined as patients who had one or more symptoms of frequency voiding, urgency, urge incontinence, nocturia concomitant with OABSS score ≥ 1 during COVID-19 infection.
[2] Patients who did not develop any storage symptom during infection or had OABSS score 0 during COVID-19 infection.

The factors associated with storage symptoms

From univariable analysis, the underlying diseases which were obesity, diabetes, hypertension, dyslipidemia, asthma and COPD were not associated with storage symptoms during COVID-19 infection. However, the symptoms and complication of the COVID-19 infection related to storage symptoms, particularly fever and pneumonia are significant related to storage symptoms but the URI symptoms, rash, anosmia and chest discomfort were not related to storage symptoms. After using the multivariable analysis factor associated with storage symptoms in COVID-19 patients by using the factors that would relate to storage symptoms, pneumonia is the only factor that was significantly related to storage symptoms in this study (OR 2.92 (1.04-8.21), p-value 0.042). Fever had a tendency to be related to storage symptoms but was not statistically significant (OR 2.12 (0.93-4.85), p-value 0.072) (Table 3).

TABLE 3. The Multivariable analysis factors associate with storage symptoms in COVID-19 patients.

Factor	Odd ratio (95% CI)[1]	p-value
Age in year	1.01 (0.98 - 1.04)	0.346
Male gender	1.24 (0.57 - 2.71)	0.584
Diabetic mellitus	0.34 (0.09 - 1.31)	0.119
Obesity	0.46 (0.19 - 1.13)	0.091
Fever	2.12 (0.93 - 4.85)	0.072
Pneumonia	2.92 (1.04 - 8.21)	0.042
Diarrhea	2.84 (0.79 - 10.15)	0.108

[1] The binary regression analysis was used for analyzing factors that associated with storage symptoms in COVID-19 infection patients.

The presence of leukocyte, hematuria or proteinuria related to the severity of COVID-19 infection and storage symptoms

From 136 COVID-19 patients, there were 34 patients who undertook the urine dipstick test. Even though they were not statistically significant, the patients who had abnormal urine dipstick tests were more likely to have more storage symptoms, fever, URI and pneumonia (Table 4).

DISCUSSION

The prevalence of the urinary storage symptoms in COVID-19 patients from our study population was 44.85%. Patients in the storage symptoms group had frequent voiding, urgency, nocturia, OABSS and ICIQ storage subscale significantly higher than the no storage symptoms group (p-value <0.001). The prevalence of these symptoms was quite high but there was no information about the abnormal storage symptoms to warn people that this might be a sign of COVID-19 infection. Similar to the previous studies[4,5], our study demonstrated that most COVID-19 patients could have more frequent voiding, nocturia and urgency during the infection. Patients in our study who had COVID-19 pneumonia were more likely to have abnormal storage symptoms than patients who had not. All patients were treated by conservative treatment which were prompt voiding, avoiding caffeine and adjusting fluid intake. None of these patients took medication or underwent any intervention to relieve symptoms.

The SAR-CoV-2 virus could be isolated from the urine sample. Hence, this virus would be contagious by urine.[3] The study by Kashi AH et al and Roshandel et al. demonstrated that the rate of SAR-CoV-2 viral shedding in urine was 1.18% and the detection rate of virus in urine was 4.5-8%. Even though the rate of shedding was lower than in nasopharyngeal and rectum, the viral shedding in urine was higher in patients who had greater severity of the disease and also related to higher mortality.[7,8] From our study, patients who had fever, diarrhea and pneumonia had a higher number of storage symptoms than the asymptomatic or mild symptoms group. Therefore, as well as showing storage symptoms of COVID-19 patients, the SAR-CoV-2 virus might also be transmitted to other people by urine contamination.

Currently, the pathophysiology of storage symptoms is still unclear. There are several mechanisms that could explain the storage symptoms. Firstly, the SAR-CoV-2 can transmit and replicate in the urothelial cells of the bladder via angiotensin converting enzyme 2 (ACE2) receptors in the viremia stage and result in viral cystitis.[9,10] Secondly, the SAR-CoV-2 virus can cause endothelitis which could irritate bladder and result in storage symptoms. Lastly, the SAR-CoV-2 virus can cause inflammation from immunologic response as a previous study found inflammatory cytokine IL-6, IL-8, IP-10 increase in urine

TABLE 4. The presence of leukocyte, hematuria or proteinuria related to the severity of COVID-19 infection and storage symptoms.

Symptoms	Abnormal urine dipstick[1](25)	Normal urine dipstick(9)	p-value
Storage symptoms[2], n (%)	14 (56.00)	3 (33.33)	0.438
Frequency, n (%)	11 (44)	2 (22.22)	0.427
Urgency, n (%)	7 (28)	3 (33.33)	1.000
Daytime frequency, mean (SD)	4.7 (3.1)	4.4 (3.3)	0.823
Nocturia, mean (SD)	1.8 (1.4)	1.2 (0.6)	0.241
Fever, n (%)	7 (28)	2 (22.22)	1.000
URI[3], n (%)	11 (44)	6 (66.67)	0.438
Pneumonia, n (%)	2 (8)	1 (11.11)	1.000

Abbreviation: SD: Standard deviation, URI: upper respiratory tract infection.

[1] abnormal urine dipstick was defined by positive for any of the following, leukocyte, hematuria, or proteinuria.

[2] Storage symptom was defined as patients who voided more frequently or had urgency symptom during COVID-19 infection.

[3] URI symptoms were defined as having one or more symptoms of cough, runny nose, sore throat, nasal congestion

of COVID-19 patients and these patients had urgency, frequency and nocturia.[11]

The limitation of our study was that this study was carried out as a cross-sectional study because it was difficult to communicate with patients and collect the data during this severe pandemic situation. Therefore, we cannot identify the true causal relation of the risk factors associated to abnormal storage symptoms. We could simply imply the probability of the factors that might be related to storage symptoms from the high rate of concomitance with the storage symptoms during the illness as the prediction model of the study. To identify the true effect of the factors, a cohort study could be undertaken in the future. Moreover, we did not collected the data about the frequency of voiding and nocturia before infection because from our pilot study, most of the patients were not willing to answer too many questions and most of them could not remember. In order to avoid recall bias and disturb patients during their illness, therefore, we did not collect the data before the illness. Another limitation of our study was we could not follow up the symptoms of patients after recovery from the illness because of limited access to the contact information of the patients after they went home. However, the study by Welk et al. reported that COVID-19 patients who had storage symptoms during illness did not have increased risk of long term bladder dysfunction.[12]

From our study, underlying disease, which were obesity, diabetes, hypertension, dyslipidemia, and asthma/COPD were not significantly related to storage symptoms. Most of the patients who had storage symptoms were admitted to the cohort ward (52.46% VS 28%, p-value 0.007). This data was coherent with the data regarding COVID-19 symptoms in patients who had fever and pneumonia were statistically significantly related to storage symptoms. These might occur because most of the patients who had pneumonia and more severe disease were admitted to the cohort ward. However, apart from the viral cystitis from the COVID-19 infection that resulted in storage symptoms, there were many factors that might also be related to storage symptoms, for example, resulting from increasing fluid intake during illness, anxiety or cold temperature of the air conditioning room, glucosuria in diabetic patients.[5] Generally, patients who were admitted to ICU might be affected from the intravenous fluid, colder temperature than cohort ward and field hospital that could cause storage symptoms such as frequency urination. Even though our study demonstrated that intravenous fluid was not associated with storage symptoms, there were only 6 patients who were admitted to ICU, therefore the sample size of this group was too small to conclude the association between ICU patients and storage symptoms in this study. None of the patients in this study received a bronchodilator.

From multivariable analysis, only pneumonia was significantly associated with storage symptoms (p-value 0.042). Fever had a tendency to be related to storage symptoms but was not statistically significant. These might be because patients who had fever not only had involvement from viral cystitis but also affected from increased fluid intake and cold temperature of the air conditioning room.

Rui Liu et al. found red blood cell and proteinuria are significantly higher in COVID-19 patients. Patients who had glucosuria or proteinuria were more likely to have more severe COVID-19 disease.[13] From our study, even though there they were not statistically significantly different, the patients who had leukocyte, erythrocyte or proteinuria positive from the dipstick test tended to have more storage symptoms and more fever, URI, and pneumonia.

Regarding the information from our study and previous studies, medical personnel, especially urologists and general physicians, should be aware that the patients who were infected by SARS-CoV-2 virus might present at the hospital with abnormal storage symptoms and abnormal urinalysis that mimic the urinary tract infection. Moreover, in order to increase the awareness of carrying the disease and risk of transmitting the virus to others, people should know that storage symptoms could also be the symptoms of the COVID-19 virus.

CONCLUSION

Our study demonstrated that SARS-CoV-2 virus infection in patients who had pneumonia and fever was associated with abnormal storage symptoms, including frequency, urgency and nocturia. These storage symptoms may be related to the severity of COVID-19 disease. Patients and medical personnel should be aware that storage symptoms might be found together with fever and pneumonia as well as presenting symptoms of COVID-19.

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Conflict of interest

We have no conflict of interest.

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