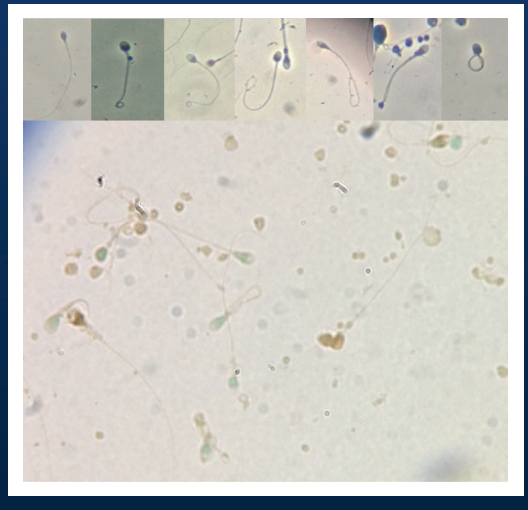
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Effect of Kao-Ta (9-Square Step Exercise) and Kao-Ten (9-Square Dance Exercise) on Balance Rehabilitation in Patients with Balance Disorders

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ABSTRACT

Objective: To study the effect of Kao-ta (9-square step exercise) and Kao-ten (9-square dance exercise) on balance improvement in patients with balance disorders.

Methods: This prospective pilot study in patients with balance disorders was conducted at the outpatient clinic, Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand from December 2015 to December 2016. Patients diagnosed by clinical symptoms and at least one abnormal condition on posturography were taught how to perform Kao-ta and Kao-ten exercise. Participants were provided with the equipment necessary to create a nine square grid at home. They were instructed to perform 3 minutes of Kao-ta followed by 2 minutes of Kao-ten twice per day for at least 45 days in an 8-week period. Posturography and visual analogue scale (VAS) of balance symptom severity were compared between before and after exercise program.

Results: Eleven patients with balance disorders were included. The mean age was 57.2±12.9 years (range: 33-70), and all patients were women. The average composite equilibrium score at baseline was 64.4±8.1. After 8 weeks of Kao-ta and Kao-ten, the average composite equilibrium score increased to 73.8±10.2 (p<0.01). The median (P25, P75) of the abnormal equilibrium score condition decreased from 2 (1, 3) at baseline to 1 (0, 2) after 8 weeks (p=0.016). The median VAS of balance symptom severity decreased from 4 (3, 6) at baseline to 2 (0.2, 5.5) after 8 weeks (p=0.028).

Conclusion: Kao-ta and Kao-ten exercise can improve symptoms in patients with balance disorders after 8 weeks of exercise

Keywords: Kao-ta; Kao-ten; balance rehabilitation; patients; balance disorders; Thai traditional medicine (Siriraj Med J 2019;71: 1-7)

INTRODUCTION

Balance disorders can be found in any age group, but they are more commonly observed in older adults. The prevalence of balance disorder complaints is 5-10% of patients among general practitioners, and 10-20% of patients among otorhinolaryngologists.[1] During 2014-2016, about 12% of the patients (7,250 patients) that visited the outpatient clinic of the Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital had balance disorder, and 48.12% of those were aged greater than 60 years.

Balance requires coordination among the visual,

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proprioceptive, and inner ear vestibular systems. The central nervous system receives inputs from these systems, and then sends back information to effectuate muscle control of the eyes, neck, torso, and extremities to maintain balance. Any impairment along any of these pathways can cause balance disorders and/or vertigo.

Treatments of balance disorders and vertigo include:

1. Specific treatment, such as canalith repositioning for benign paroxysmal positional vertigo. It should be noted that many diseases have no specific treatment, and some diseases are incurable.
2. Symptomatic treatment, which consists mostly of medication therapy
3. Balance rehabilitation

When balance disorders occur, the vestibular system will initiate compensatory adaptations in an effort to correct the aberration. These adaptations and recovery of defective functions can take days to weeks. However, balance may not fully recover in all patients, and rehabilitation plays a key role in the recovery of balance in this group.

The goal of rehabilitation is to improve overall body balance. Cawthorne-Cooksey exercise, which was introduced in 1940, is one of the most well-known imbalance rehabilitation methods.[2] This exercise is indicated in patients with prolonged symptoms or partial recovery. Cawthorne-Cooksey exercise can improve balance in up to 50-80% of patients[3-5], and it has demonstrated benefit in patients with acute vertigo. Other traditional exercises, such as Tai chi[5-7] and Wii Fit[8], have shown rehabilitation benefit in patients with balance disorders. A 2008 study in aquatic physiotherapy using whirlpool as part of an exercise protocol revealed positive effects on unilateral vestibular hypofunction.[9]

Kao-ta (9-square step exercise) or Ouay's Test is a form of exercise invented in 1970 by Professor Dr. Ouay Ketusinh, a renowned Professor of Physiology from the Faculty of Medicine Siriraj Hospital, Mahidol University. Professor Dr. Ouay Ketusinh also founded a Thai traditional medical school that was later named the Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. In 1972, he presented his invention Nine Square Test or Ouay's Test to the International Committee on the Standardization of Physical Fitness Test (ICSPFT) in Cologne, Germany. He later presented his Nine Square Health Twist exercise (original name of the 9-square dance exercise) at the Olympic Conference in the same year. He published his article describing Kao-ta and Kao-ten in Thai language in 1984.[10]

Kao-ta was originally employed as a speed test. Kao-ten (9-square dance exercise), which was developed from Kao-ta, requires more coordination than Kao-ta because it includes many body turns.[11] A user of either exercise maneuvers his/her body within a square somehow drawn or represented on the floor or ground. The box, which can have overall dimensions of 90 x 90 cm, 120 x 120 cm, or 150 x 150 cm, is divided into 9 equal sized boxes – 3 at the bottom, 3 at the middle, and 3 at the top (Fig 1 and 2). Both exercises are safe and easy to perform without elaborate equipment, and both are health promotion techniques that are taught in Thai traditional medicine. Although another original goal of these exercises was to strengthen the cardiovascular system among older adults, the fact that both involve head turning and body balance indicates that they are rooted in the same principles as other balance improvement exercises.

The aim of this first ever pilot study was to investigate the efficacy of Kao-ta and Kao-ten Thai exercises for improving symptoms in patients with balance disorders. Subjective evaluation using VAS symptom score, and objective evaluation using posturography score were compared between before and after the prescribed 8-week Thai exercise program.

MATERIALS AND METHODS

This prospective pilot study in patients with balance disorders was conducted at the outpatient clinic of the Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand during the December 2015 to December 2016 study period. Siriraj Hospital is Thailand's largest national tertiary referral center. Patients aged 18-70 years with persistent and prolonged (more than 4 weeks) balance disorder were enrolled. Balance disorders included vestibular neuritis, dizziness in the elderly or vestibulopathy, and inability to maintain balance in at least one (out of six) condition on posturography. Patients having one or more of the following were excluded: inability to properly or adequately perform Kao-ta and/or Kao-ten (e.g., neuromuscular disorder, vision defect); having central cause of balance disorders; having psychiatric problems; and/or, having disease with specific treatment (e.g., benign paroxysmal positional vertigo). The protocol for this study as approved by the Siriraj Institutional Review Board (COA no. SI 704/2015), and all included patients provided written informed consent.

The objective evaluation of balance was performed using SMART Equitest® Computerized Dynamic Posturography (NeuroCom International, Inc., Clackamas, OR, USA). Briefly, patients stand on a support surface that can be

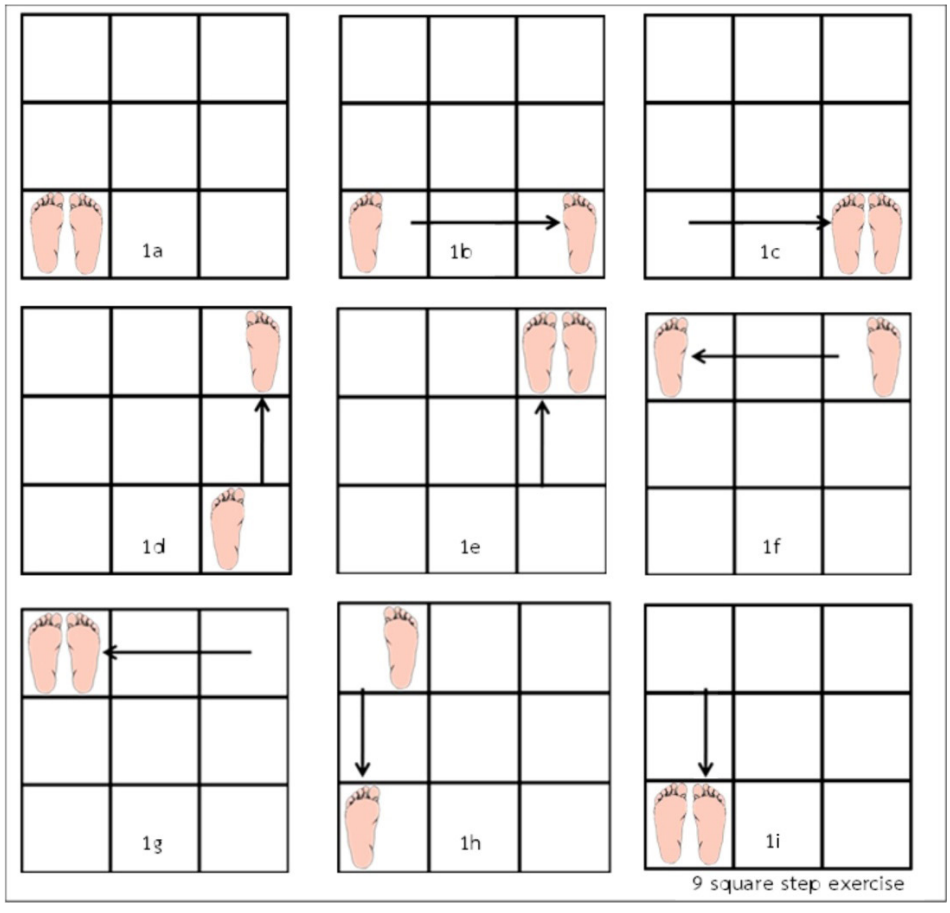


Fig 1. Kao-ta (9-square step exercise)
Preparatory position: Stand with both feet within the bottom left square (1a).
Exercise steps: Moving in a counterclockwise direction, move your right foot directly to the right into the bottom right square (1b), followed then by your left foot. Now both of your feet are once again in the same square (1c). Now move your right foot straight ahead to the top right square (1d), followed then by your left foot (1e). Now move your left foot directly to the left into the top left square (1f), followed then by your right foot (1g). Now, move your left foot directly backwards to the bottom left square, which is where you first started (1h), followed then by your right foot (1i). Now that you have completed one cycle of the exercise, move to the bottom right square to begin, except now you will repeat these movements going in the opposite (clockwise) direction.

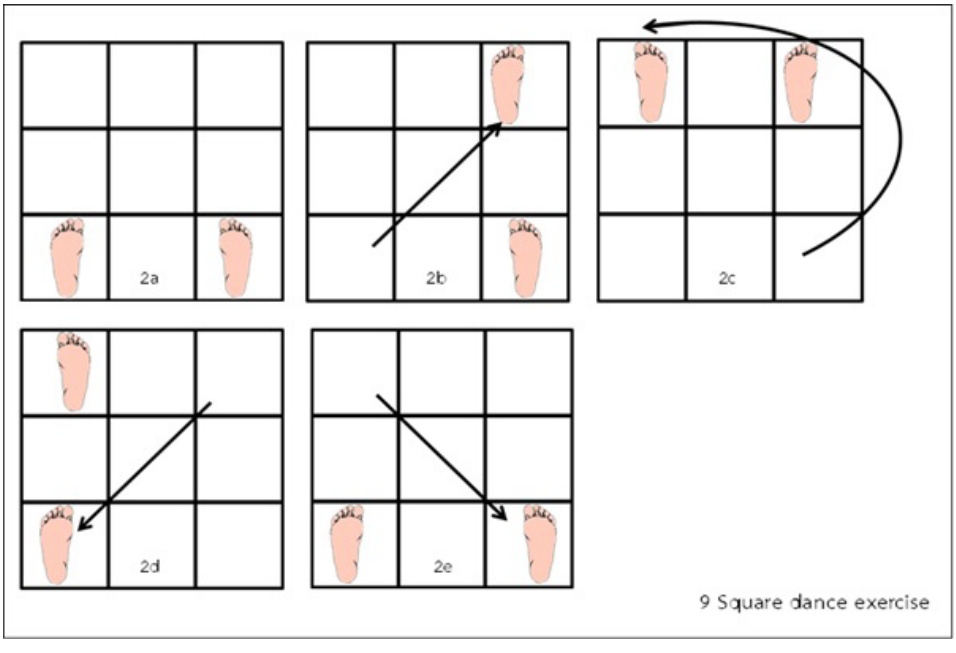


Fig 2. Kao-ten (9-square dance exercise)
Preparatory position: Stand with your feet apart, with your left foot in the bottom left square, and your right foot in the bottom right square (2a).
Exercise steps: Move your left foot diagonally to the top right square (2b). Now bring your right leg around the front of your left leg, and position your right foot in the top left square (2c). Now move your left foot back to the bottom left square where you started with your left foot (2d), followed by movement of your right foot to the bottom right square where you started with your right foot (2e). Then you will repeat these movements going in the opposite direction.

controlled to be level or tilted. Located in front of the patient is a visual field that can be stable or moved in and out. The result of this test is the "equilibrium score", which is an average of body balance in 6 different conditions. Persons with normal balance have normal results in all conditions. The result is also reported as how many of the equilibrium conditions are abnormal out of the six conditions. The program then uses the equilibrium score and the degrees of sway to calculate a "composite equilibrium score", which is a weighted average of all 6 tested conditions. An improvement in the composite equilibrium score of greater than 2 standard deviations or 8 points compared to age-matched normative data within the system is considered a meaningful improvement in balance.[12,13]

The subjective evaluation of the severity of patient imbalance was performed using a visual analogue scale (VAS). Patients rated the intensity of their imbalance using a 10 cm VAS, with a 0 indicating no symptoms of imbalance, and a 10 indicating the worst possible level of imbalance.[14]

Patients were taught how to correctly perform the Kao-ta and Kao-ten exercises by instructors from the Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University. Each participant was also given an instruction manual (Fig 1 and 2), a music CD (with a rhythm and beat matched to the steps of the exercises), and corrugated plastic sheets with a roll of colored adhesive tape that were together used to make a nine square grid. A video clip of the Kao-ta and Kao-ten exercises is available at http://www.si.mahidol.ac.th/ent/knowledge/videos/kao-ta_kao-ten_exercise.php.

Patients had to perform the Kao-ta and Kao-ten exercises to music twice a day for a total of 5 minutes per session. Each session consisted of Kao-ta for 3 minutes, followed by Kao-ten for 2 minutes. Each patient had to perform at least 45 days of exercise within the 8-week study period (80% of days). Since balance disorder patients are at higher risk for falling, all study participants were asked to perform their exercises in their bare feet. No socks or slippers were allowed. Moreover, it was recommended that a caretaker participate as an observer during each exercise session in case of a fall or some other unexpected event. Patients were advised to take a dimenhydrinate tablet as rescue medication if severe symptoms developed. Patients were instructed to record the time and date of their exercises, and any adverse events in a logbook that was provided to each study participant.

The results of posturography testing and VAS scores of all patients were recorded at baseline and after the 8-week exercise program. Patients were asked to return to the clinic for a follow-up visit at 4 weeks so that we could check their logbooks, inquire about adverse events, evaluate patient satisfaction, and ensure that the exercises were being performed correctly.

Statistical analysis

PASW Statistics for Windows version 18.0 (SPSS, Inc., Chicago, IL, USA) was used to perform all statistical analyses. Descriptive statistics are reported as mean ± standard deviation, number and percentage, or median (P25, P75). Paired t-test was used to compare composite equilibrium scores, and Wilcoxon signed-rank test was used to compare equilibrium scores and VAS symptom scores between baseline and after 8 weeks of exercise. A p-value less than 0.05 was regarded as being statistically significant.

RESULTS

Of the 12 patients that initially enrolled, one patient was not able to complete the study due to imbalance symptoms that were too severe to perform Kao-ta and Kao-ten. The remaining 11 patients completed the study and were included in the final analysis. The mean age of patients was 57.2±12.9 years (range: 33-70), and all of them were women. The diagnoses of study participants were, as follows: nonspecific dizziness (7 patients, 63.6%), vestibulopathy (3 patients, 27.3%), and probably Meniere's disease (1 patient, 9.1%) (Table 1).

Posturography

The average composite equilibrium score at baseline was 64.4±8.1. After 8 weeks of Kao-ta and Kao-ten, the mean±standard deviation composite equilibrium score increased to 73.8±10.2 (p<0.01). The median (P25, P75) of abnormal equilibrium score condition decreased from 2 (1, 3) at baseline to 1 (0, 2) after 8 weeks (p=0.016). After 8 weeks of exercise, 10 patients (90.9%) had improvement in their composite equilibrium score, and 6 of them (54.5%) had scores that increased by at least 8 points. Seven of 11 patients (63.6%) had at least one condition that returned to normal after completion of the 8-week exercise program (Tables 2 and 3).

Visual analogue scale (VAS)

The median (p25, P75) VAS of balance symptom severity decreased from 4 (3, 6) at baseline to 2 (0.2, 5.5) after 8 weeks (p=0.028). Nine patients (81.8%) rated their severity of imbalance as improved (Tables 2 and 3). Four patients (36.6%) were unable to complete a full 5 minutes of exercise during the first one or two days; however,

TABLE 1. Demographic characteristics and clinical diagnosis of the 11 study participants.

Age (years), mean±SD (range)	57.2 ± 12.9 (range: 33-70)
Gender, n (%)	
Male	0 (0.0%)
Female	11 (100%)
Diagnosis, n (%)	
Nonspecific dizziness	7 (63.6%)
Vestibulopathy	3 (27.3%)
Probable Meniere's disease	1 (9.1%)

Abbreviation: SD=standard deviation

TABLE 2. Clinical diagnosis and result of objective test and subjective VAS symptom score.

Patient	Age (yr)	Diagnosis	Composite equilibrium score		Equilibrium score (number of abnormal conditions)		VAS	
			Pre	Post	Pre	Post	Pre	Post
1	51	Probable Meniere's disease	43	50	5	5	6.8	6
2	69	Vestibulopathy	66	72	2	1†	4.5	5.5
3	70	Vestibulopathy	65	73*	1	1	3	1
4	33	Vestibulopathy	67	85*	3	0†	3	2
5	65	Nonspecific dizziness	69	76	2	1†	7.5	5.5
6	67	Nonspecific dizziness	70	70	2	2	4	0.2
7	68	Nonspecific dizziness	69	78*	1	0†	5	3
8	42	Nonspecific dizziness	71	84*	1	0†	4	1
9	58	Nonspecific dizziness	68	81*	2	0†	6	0
10	43	Nonspecific dizziness	63	80*	2	0†	0	0
11	63	Nonspecific dizziness	57	63	3	3	3	4

*Composite equilibrium score increased ≥8 points

†Abnormal equilibrium score that returned to normal in at least 1 condition

Abbreviations: VAS=visual analog scale; Pre=before exercise; Post=after 8 weeks of exercise

TABLE 3. Comparison of composite equilibrium score, abnormal equilibrium score condition, visual analogue score (VAS) before and after 8 weeks of exercise.

	Before exercise	After exercise	*P*-value
Composite equilibrium score	64.4 ± 8.1	73.8 ± 10.2	*<0.01*
Abnormal equilibrium score condition*	2 (1, 3)	1 (0, 2)	*0.016*
VAS	4 (3, 6)	2 (0.2, 5.5)	*0.028*

Data are presented as mean ± standard deviation or median (P25, P75)

A *p*-value<0.05 indicates statistical significance

*Abnormal equilibrium score condition indicates the median (range) number of the total of 6 conditions that had an abnormal equilibrium score at baseline and after 8 weeks of exercise

those patients developed the stamina to exercise for a full 5 minutes in all subsequent exercise sessions. No patients had to take rescue medication, and no adverse events were reported or observed in this study.

DISCUSSION

Balance rehabilitation is one of the most effective methods for treating balance disorders. Kao-ta and Kao-ten Thai exercises, which were invented by Professor Dr. Ouay Ketusinh, were designed to improve coordination among the eyes, head, torso, and extremities. The design features of Kao-ta and Kao-ten inspired this research team to investigate these exercises for balance benefit in patients with balance disorders.

After 8 weeks of Kao-ta and Kao-ten exercise in this study, 90.9% of patients had a better composite equilibrium score, and 54.5% of those had an increase at least 8 points, which indicates that they had better balance.[12,13] Moreover, the equilibrium score of at least one condition returned to normal in 63.6% of patients. Nine patients (81.8%) reported improved balance. These results are comparable to those observed after Cawthorne-Cooksey exercise in Thai elderly population with imbalance disorders.[4] Cawthorne-Cooksey exercise was reported to effectuate 50-80% improvement in patient balance.[3-5]

Compensation usually occurs 2-3 days after symptoms of balance disorders develop, but 30% of patients do not compensate well enough.[15] Therefore, we only recruited patients with symptoms for 4 weeks or more in order to exclude patients that might develop spontaneous compensation.

All patients in our study were female, so there were no gender or physical strength biases. We also endeavored to reduce the probability of incorrect exercise technique by inviting instructors from the Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University to teach correct Kao-ta and Kao-ten technique to our study patients. A logbook was given to each patient to record daily exercise times, and to note any accidents or adverse events that occurred during the study period. In addition, all patients attended our outpatient clinic at 4 weeks for a follow-up to assess patient satisfaction and to inquire about adverse events. During that visit, patient logbooks were checked, and questions were asked to elicit information specific to the correctness and regularity of the 2 prescribed Thai exercises.

The results of this pilot study revealed that Kao-ta and Kao-ten exercise can significantly improve patient balance. However, a controlled study in a larger study population is needed confirm our findings, and to further elucidate the scope of the benefit conferred by these Thai exercises. Comparatives studies that compare Kao-ta and Kao-ten with other balance rehabilitation methods are also recommended.

Limitations

This pilot study has a mentionable limitation. Patients were required to perform at least 45 days of exercise (80%) during the 8-week study period. It is, therefore, possible that patients that performed more than 45 days of exercise may have realized better outcomes than patients that performed only the minimum 45 days of exercise. No provision was made in this study to evaluate performance based on the number of days of exercise beyond the 45-day minimum.

CONCLUSION

The results of the first ever pilot study revealed that Kao-ta and Kao-ten exercise can improve symptoms in patients with balance disorders after 8 weeks of exercise when evaluated by visual analogue scale and computerized dynamic posturography. No exercise-related adverse events were observed or reported.

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Conflict of interest declaration

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

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Correlation between the Hypoosmotic SwellingT and DNA Fragmentation Assessed by the TUNEL Assay in Asthenozoospermia

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ABSTRACT

Objective: To study the correlation between the hypoosmotic swelling test (HOST) and DNA fragmentation in asthenozoospermia assessed by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end-labeling (TUNEL) assay.

Methods: This cross-sectional study was conducted in 27 semen samples obtained from infertile men with asthenozoospermia. Both HOST and TUNEL assay were performed for each sample. The sperm swelling pattern and positive apoptosis staining of individual spermatozoa were evaluated. HOST and TUNEL scores, and the proportion of positive staining in each grade were calculated in each sample.

Results: The results showed a negative correlation between HOST and TUNEL scores ($r = -0.428$, $P = 0.026$). Sperm swelling grade A had a higher incidence of positive apoptosis staining when compared with other grades ($P < 0.01$). There was no statistically significant difference in positive apoptotic staining between other grades; nevertheless, sperm swelling grade D tended to have a lower incidence of positive apoptosis staining.

Conclusion: Based on the results of this study, HOST may be used as an optional test to identify DNA-intact spermatozoa whereby sperm with a grade D swelling pattern should be selected preferentially for intracytoplasmic sperm injection (ICSI), whereas sperm with a grade A swelling pattern should be avoided for ICSI.

Keywords: Hypoosmotic swelling test; TUNEL; asthenozoospermia; male infertility; intracytoplasmic sperm injection (Siriraj Med J 2019;71: 8-13)

INTRODUCTION

Male factor infertility constitutes 30% of infertility causes. The World Health Organization (WHO) defines the lower limit of normal sperm motility as 32% of progressively motile sperm (5th centile, 95% confidence interval [CI]: 31-34) and 40% of total motile sperm (5th centile, 95% CI: 38-42).[1] The prevalence of asthenozoospermia has been reported to be 18.71-24.19%.[2,3] In Thailand, 10.78% of infertile couples are affected by asthenozoospermia.[4] During natural human fertilization, sperm velocity is essential for its transition through the vagina and fallopian tubes, and for the penetration of the cumulus oophorus and zona pellucida. Therefore, sperm motility impacts fertilization rates; i.e., low-velocity sperm are associated with a reduced chance of zona pellucida penetration and thereby fertilization potential.[5-7] Intracytoplasmic sperm

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injection (ICSI) was developed to overcome impaired fertilization as a result of reduced semen parameters, including asthenozoospermia. However, higher DNA fragmentation rates are reported in asthenozoospermia and the introduction of ICSI for asthenozoospermia has promoted the transmission of disintegrated sperm DNA to the resulting offspring, which consequently affects fetal or postnatal development.[8] Many studies have reported a negative correlation between sperm DNA damage and fertilization, implantation and miscarriage rates, in addition to embryo quality and rates of childhood diseases and cancer.[9-14] Thus, it is important to discriminate DNA-intact spermatozoa in asthenozoospermia to improve pregnancy rates and reproductive health outcomes.

DNA integrity can be analyzed by several diagnostic tests, such as terminal deoxynucleotidyl transferase mediated deoxyuridine triphosphate nick end-labeling (TUNEL) assay.[15,16] However, drawbacks associated with the TUNEL assay are that it is a time-consuming technique, which requires specific skills and, most importantly, causes sperm toxicity. Therefore, the adoption of a nontoxic sperm selection test is essential for ICSI. In cases of severe asthenospermia, it is difficult to distinguish viable and nonviable sperm. Since some viable sperm are known to contain DNA-intact components, it is important to be able to select viable sperm for ICSI to optimize fertilization rates.[17]

The hypoosmotic swelling test (HOST) is the most commonly used method for assessing sperm vitality.[1] The basis of HOST relies on the semipermeable nature of the sperm cell membrane, which allows the influx of water when placed in a hypoosmotic solution, and results in the expansion and coiling of the tail.[18] HOST presumes that only cells with intact membranes (live cells) will swell when within a hypotonic solution, which allows easy identification of viable sperm.[1] It is a simple, quick, safe, and cost-effective test. HOST was recently introduced as a test for sperm function because membrane integrity is important for sperm capacitation, the acrosome reaction, and sperm–oocyte binding and penetration. Moreover, HOST has been suggested as an alternative test for DNA integrity. Previous studies have demonstrated a favorable association between HOST and many reproductive outcomes, including fertilization and pregnancy rates.[19-21] Some studies have shown benefits from using HOST for the selection of viable nonmotile spermatozoa.[22,23] Casper et al. found increased fertilization and cleavage rates (43% and 39%, respectively) when HOST was used to select viable sperm compared with when sperm was randomly selected in cases of complete asthenozoospermia (26% and 23%, respectively).[24] Ortega *et al.* also reported improved fertilization rates using HOST in complete asthenozoospermia.[23] In contrast, recent studies have failed to report a benefit of HOST utilization to select sperm on the basis of lower DNA fragmentation levels.[20,21] However, these studies were conducted on semen samples with parameters within the normal range; therefore, they may not be applicable in asthenozoospermia cases.[20,21] There is insufficient data in relation to asthenozoospermia; therefore, we conducted this study to clarify the correlation between HOST outcomes and DNA fragmentation levels in asthenozoospermia as assessed by the TUNEL assay. The secondary objective was to identify the positive apoptosis staining in each sperm-swelling grade.

MATERIALS AND METHODS

This cross-sectional study was approved by the Institutional Review Board of the university hospital and it was conducted in males aged 18 years or older who were attending a university-based infertility unit. All patients who requested a semen analysis and provided written informed consent were screened. Semen samples were collected by masturbation. Twenty-seven patients with asthenozoospermia classified according to WHO 2010 were eligible for this study.[1] Two aliquots of each sample were collected within 90 minutes after semen sample collection and liquefaction. Both aliquots were first prepared for HOST and then fixed for TUNEL assay. The fixed and stained semen sample slides were examined using a phase contrast microscope (BX40; Olympus, Tokyo, Japan). A single interpreter assessed a total of 200 spermatozoa per semen sample twice. The semen parameters, i.e., average HOST score, average TUNEL score, individual spermatozoa HOST grading, individual apoptotic staining, and proportion of positive apoptosis staining in each grade of sperm swelling, were calculated and recorded.

HOST

According to WHO 2010,[1] the hypoosmotic solution was prepared by dissolving 0.375 g of sodium citrate dehydrate and 1.351 g of D-fructose in 100 mL of purified water. An aliquot of 100 mL of semen was mixed with 1 mL of the swelling solution before being incubated at 37°C for 30 minutes. Ten microliters of the mixed sample was then placed on a clean slide and covered with a coverslip. In live sperm with normal membrane function, the hypoosmotic buffer diffused through the membrane into the sperm tail. Under microscopy, live spermatozoa showed various degrees of tail swelling and curling, whereas dead cells exhibited no membrane

changes. All membrane-change patterns were categorized from grades A to G (Fig 1), where grade A corresponds to no tail swelling. The HOST score was obtained from the average percentage of total swollen spermatozoa (excluding the natural swelling before treatment with hypoosmotic buffer). The lower reference limit for normal vitality was 58% (5th centile, 95% CI: 55-63).[1]

TUNEL assay

Both semen slides were fixed after HOST. The TUNEL assay was then performed according to the manufacturer's instructions for ApopTag® Plus Peroxidase *In Situ* Apoptosis Detection Kit (Merck, Kenilworth, NJ, USA). In the assay, spermatozoa with DNA fragmentation stained brown, while sperm containing intact DNA stained green (Fig 2).

The TUNEL score was calculated using the formula:

$$\% \text{ Average positive staining} = \frac{\text{Average of stained apoptotic spermatozoa}}{200} \times 100$$

The proportion of positive apoptosis staining in each grade of sperm swelling was calculated as follows:

$$\text{Positive apoptosis staining (\%)} = \frac{\text{number of positive apoptosis staining in that grade}}{\text{total number of spermatozoa in that grade}} \times 100$$

Statistical analysis

The semen parameters were presented as mean, median, and standard deviation. The intraclass correlation coefficient was used to evaluate consistency in two time measurements for the HOST and TUNEL scores in each sample. The correlations between HOST and TUNEL scores were analyzed using Pearson correlation and Wilcoxon signed-rank tests. $P < 0.05$ was accepted as statistically significant. Data were analyzed using PASW Statistics (v. 18.0; SPSS Inc., Chicago, IL, USA).

RESULTS

The characteristics of 27 semen samples are shown in Table 1. A fairly negative correlation between HOST and TUNEL scores ($r = -0.428$, $P = 0.026$) was revealed using Pearson correlation (Fig 3). There was high agreement in

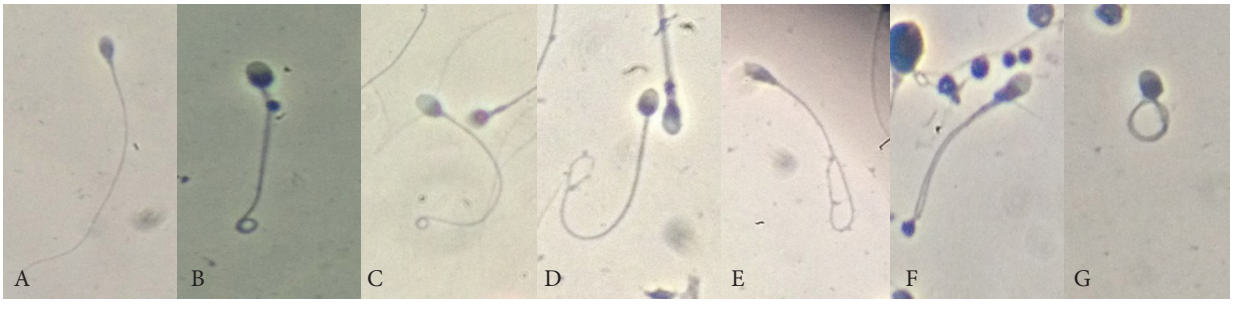


Fig 1. After incubation in hypo-osmotic solution for 30 minutes, every sperm was assessed under phase-contrast microscopy and categorized in 7 groups, from grade A to G as demonstrated in the figure. Grade A showed the sperm with no membrane swelling which indicated a non-viable sperm. Grade B to G showed different grades of tail swelling in viable sperms.

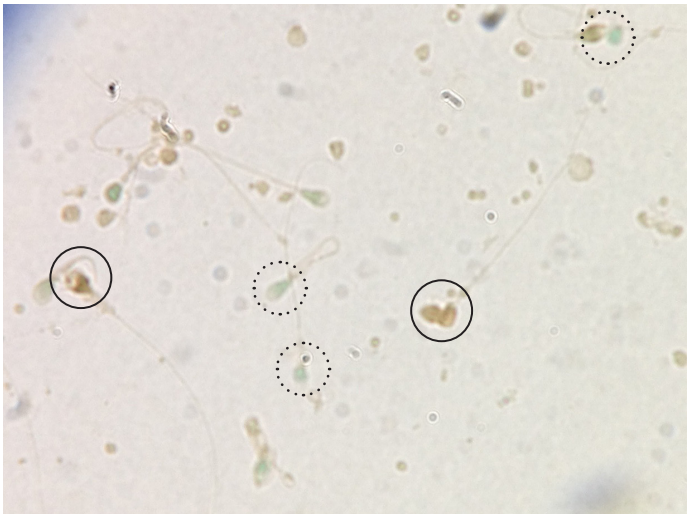


Fig 2. Apoptosis staining under normal light microscopy was demonstrated in the figure.

The positive apoptosis staining was demonstrated in the solid circle.

The negative apoptosis staining was displayed in the circle of dotted line.

TABLE 1. Characteristics of semen parameters (N = 27) were displayed.

Semen parameters	Mean ± SD
pH	7.7 ± 0.3
Volume (mL)	2.5 ± 1.8
Sperm concentration (10^6/mL)	21.4 ± 15.1
Total sperm count (10^6/mL)	51.9 ± 43.7
Progressive motility (%)	24.1 ± 5.1
Vitality (%)	58.9 ± 11.3
Normal morphology (%)	8.9 ± 5.9
Natural sperm swelling (%)	7.2 ± 3.2

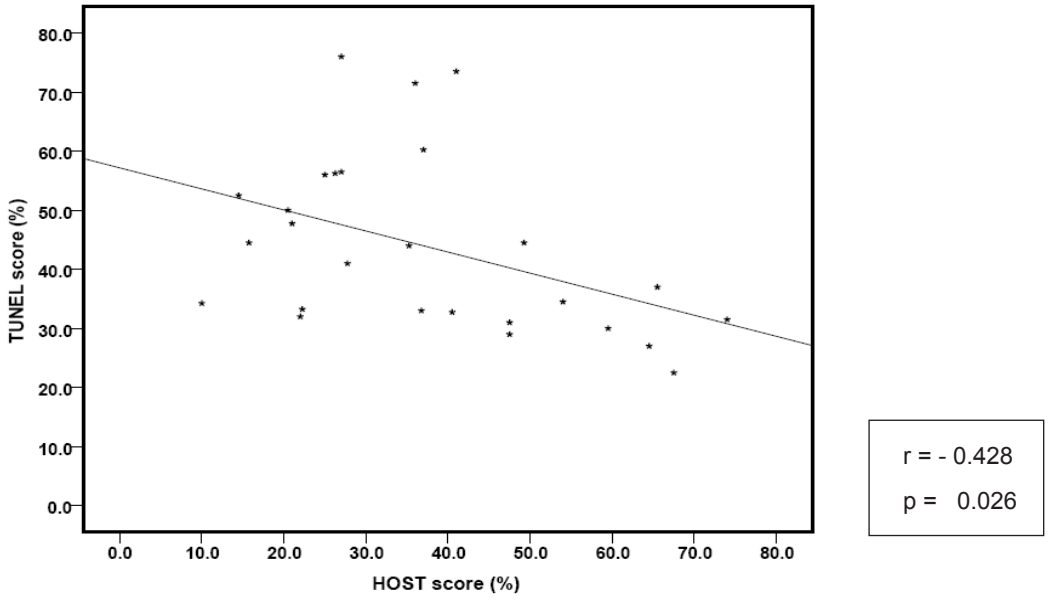


Fig 3. Correlation between HOS TEST score and TUNEL score.

the duplicate test evaluations by the same investigator. The intraclass correlations were 0.97 (95% CI = 0.935-0.986) and 0.952 (95% CI = 0.898-0.978) for the HOST and TUNEL score assessments, respectively.

When the positive staining of TUNEL was compared among the groups with various tail swelling patterns, grade A sperm demonstrated the highest proportion of apoptosis staining ($P < 0.01$). There was no significant difference in the DNA integrity between other grades. Nevertheless, the sperm swelling grade D tended to have a lower proportion of positive apoptosis staining (Fig 4).

DISCUSSION

The quality of sperm is highly associated with the outcome of assisted reproductive technology. The selection of sperm with high DNA integrity is an important step prior to ICSI to optimize the outcome because DNA

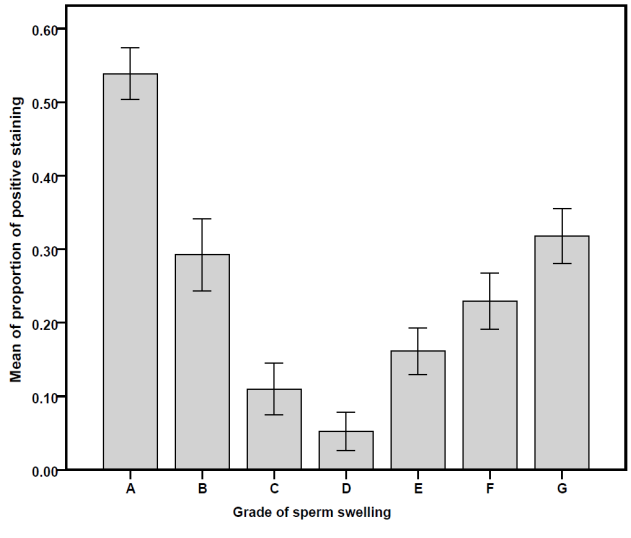


Fig 4. Proportion of positive apoptosis staining in each grade of sperm swelling.

fragmentation is known to affect fertilization rates and embryo development.[25] Assessment based solely on morphology or vitality alone may not be enough because many sperm with normal morphology and motility still contain damaged genetic material, especially in asthenospermia.[8]

Membrane function contributes a key role in various sperm function competencies, i.e., capacitation, the acrosome reaction, and fertilization. Membrane disintegration is suggested to be associated with implantation failure and miscarriage.[26] HOST is a vitality test used to reveal the sperm membrane integrity and it is not only useful in increasing fertilization rates in cases of complete asthenospermia, but can also be used to assess sperm DNA fragmentation.[19,20]

This study demonstrates the relationship between HOST and TUNEL assay in cases of asthenozoospermia and highlights the benefits of using HOST to select sperm with lower levels of DNA fragmentation. The results are consistent with the study by Stanger,[20] which also showed a negative correlation (r = -0.81) between HOST and TUNEL assay outcomes. However, this correlation was stronger than in this study. This discrepancy is likely to be the result of the difference in study populations, with this study enrolling only asthenozoospermic males whose samples are prone to DNA fragmentation.[8]

Stranger reported significantly higher grade A and lower grade D swelling patterns in abnormal semen samples compared with normal samples.[20] However, that study did not directly identify the DNA status in each grade. In this study, the DNA integrity and tail swelling pattern was assessed individually for each sperm; therefore, the DNA integrity pattern for each grade can be calculated. In the DNA fragmentation analysis in each grade of sperm swelling, grade A was found to contain the highest portion of DNA fragmentation, while grade D obtained the lowest percentage. In the remaining viable sperm, healthy DNA was identified mostly in grade C followed by grades E, F, B, and G (Fig 4), which concurs with previous studies that suggested a correlation between tail swelling pattern and DNA damage.[20,21] These results are also consistent with a study conducted in males with normal semen parameters.[27] The HOST or hypotonic resistance test distinguishes the sperm with different capacities in the function of Na^+/K^+ and Na^+/H^+ exchange of the membrane. Sperm that exhibited the minimal swelling patterns, as in grade D, are supposed to contain higher membrane competency along the flagellum.[28] Nevertheless, a high level of swelling signifies impaired Na^+/K^+ ATPase function.[20] The normal HOST-sensitive membrane feature is suggested to be linked with less or no DNA damage.[20] Therefore, this finding suggests that HOST is clinically applicable for selecting better-quality sperm with a grade D swelling pattern for ICSI.

CONCLUSION

There is a fairly negative correlation between HOST outcomes and DNA fragmentation levels in asthenozoospermia as assessed by the TUNEL assay. The study results support the use of HOST as a tool to identify viable spermatozoa in terms of intact DNA in asthenozoospermia to improve reproductive health outcomes. As sperm swelling grade A has the highest incidence of positive apoptosis staining when compared with other grades, they should not be selected for ICSI, whereas grade D tend to have a lower incidence of positive apoptosis staining and therefore should be prioritized for selection during ICSI.

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Lipid-Poor Adrenal Lesion: Differentiation of Benign from Malignant Disease by Using Imaging Features on Routine Contrast-Enhanced CT

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ABSTRACT

Objective: To assess the effectiveness of CT imaging features of lipid-poor adrenal lesions on routine contrast-enhanced CTs in differentiating benign from malignant masses.

Methods: A retrospective study was performed on 84 lipid-poor adrenal lesions (HU >10 on unenhanced CT scans), which were sized 1-4 cm, had a proven final diagnosis, and were detected during routine contrast-enhanced MDCT studies. Of those, 58 were found in patients with an underlying extra-adrenal malignancy. Two authors determined the morphological features according to their shape, margin, density on unenhanced images, and enhancement pattern. The sensitivity, specificity, and positive and negative predictive values were also calculated for each feature which suggested benignancy, plus a combination of those features.

Results: There were 46 (55%) benign and 38 (45%) malignant adrenal masses. The low-density feature (10-20 HU on unenhanced CT images) indicated as benign with a high specificity of 92%, even in patients with known malignancy. The other features (round/oval shape, smooth margin, and homogenous enhancement) showed a high sensitivity (75%-85%) but a low specificity (39%-56%) in predicting benignity. The combined features for presumed benignancy could predict a benign mass with the highest specificity of 95%.

Conclusion: The small, lipid-poor, adrenal masses detected by routine contrast-enhanced CTs are likely to be benign when their internal density on unenhanced images is not higher than 20 HU and/or, especially, when a combination of all morphological features for presumed benignancy presents.

Keywords: Adrenal adenoma; adrenal gland; CT (Siriraj Med J 2019;71: 14-20)

INTRODUCTION

The prevalence of an incidental adrenal mass discovered on chest or abdominal contrast-enhanced CT scans is approximately 4% which is mainly a benign adenoma.[1] Using internal attenuation of ≤ 10 Hounsfield units (HU) on unenhanced CT images, sensitivity and specificity for the diagnosis of adrenal adenoma were 71% and 98%, respectively.[2,3] Previous study reported that approximately 30% of non-fat-containing adrenal masses (HU > 10) were lipid-poor adrenal adenomas.[2] Currently, an adrenal gland CT with a 10- or 15-minute delayed protocol is usually performed to evaluate lipid-poor adrenal lesions. A diagnosis of lipid-poor adenoma is established by calculating the absolute contrast washout (ACW) or relative contrast washout (RCW) values. The thresholds for ACW and RCW for 10-minute delayed

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protocol were 37% and 53%, respectively - with the sensitivity and specificity of 100% and 98%, respectively.[4,5,6]

Despite the high sensitivity and specificity of specific CT adrenal protocol, this protocol requires another appointment, more expense and exposure to radiation and contrast media.[7] There have been efforts to differentiate benign from malignant adrenal masses by using the morphological features apparent on the first routine CT scan.[7-10] One study found that benign adrenal masses were associated with homogeneous low attenuation, an enlarged gland with the adrenal configuration maintained, a round or oval shape, and a thin or absent rim enhancement, whereas malignant masses were associated with a size exceeding 4 cm, a thick or nodular enhancing rim, and adjacent organ invasion.[9] Another study showed that an irregular margin and a thick rim enhancement were highly associated with malignancy but had low sensitivity.[7] Therefore, the aim of this study was to evaluate the CT imaging features of lipid-poor adrenal lesions on routine contrast-enhanced CTs to differentiate benign from malignant masses.

MATERIALS AND METHODS

Subjects

This retrospective study was approved by the Siriraj Institutional Review Board (Si 588/2015). We identified 175 patients with lipid-poor adrenal lesions detected on routine contrast-enhanced CTs of the chest or abdomen at our hospital between January 2013 and March 2014. A lipid-poor adrenal lesion was defined as any adrenal lesion with an HU level > 10 on an unenhanced CT scan. We excluded those patients who had adrenal masses sized smaller than 1 cm or larger than 4 cm, had no accessible pathological reports, or had had no follow-up studies for more than 1 year. Per exclusion criteria, 78 patients with 84 proven lipid-poor adrenal masses (1-4 cm) left for the analysis.

Adrenal masses

The final diagnosis of adrenal masses was confirmed by histology or imaging studies, as follows:

- A benign mass was considered if it was proven by pathology or imaging studies (in detail, if there was a lesion with calculated ACW and RCW values of > 53% and/or > 37%, respectively, on a 10-minute delayed CT adrenal protocol; or a lesion with calculated ACW and RCW values of > 60% and/or > 40%, respectively, on a 15-minute delayed CT adrenal protocol; or a lesion with a signal drop on opposed phase compared with in-phase chemical shift MR images (CS-MRI); or a lesion with 1-year stability during follow-up.)[4,7]
- A malignant mass was considered if it was proven by pathology or a change of nodule size, or a new lesion developed during chemotherapeutic sessions or imaging follow-up in patients with known malignancy; the changes should be in keeping with primary tumors or the patient's conditions.

MDCT technique

Routine contrast-enhanced chest and/or abdominal CT examinations were performed by 64-slice scanners. The intravenous low-osmolar contrast medium was administered at the rate of 3 ml/sec, with a dose of 100 ml for standard abdominal CTs or 80 ml for standard chest CTs. The post-contrast images were performed 80 seconds after contrast administration for the abdomen, and 35 seconds after contrast administration for chest studies. The images were reconstructed to 1.25 mm thickness.

Image interpretation

All lipid-poor adrenal masses on the CT images obtained via the Picture Archiving and Communication System (PACS) system were evaluated by 2 radiologists who had a subspecialty in abdominal imaging and were blinded to the final diagnoses. The imaging features were evaluated according to their shape (round/oval, irregular), margins (smooth/microlobulated, irregular), densities on the unenhanced images (10–20 HU or > 20 HU), and patterns of enhancement (homogeneous, rim/heterogeneous). The location, laterality, and lesion size were also recorded. An adrenal mass was presumed to be benign if a mass had a round or oval shape, a smooth or microlobulated margin, a low density (10–20 HU) on an unenhanced CT image, and a homogeneous enhancement, whereas a malignant mass was presumed to have an irregular shape, an irregular margin, a higher density (> 20 HU) on unenhanced CT images, and a heterogeneous or rim enhancement.

Statistical methods

The features presented in the benign and malignant lesions were compared using a chi-square test, and a P-value of < 0.05 was considered statistically significant. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each morphological feature for presumed benignancy and the combined features were calculated. The corresponding 95% CIs were also reported. The statistical analyses were performed by using SPSS Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patients

There were 50 men and 28 women - with mean age of 60 years (range 18–89). Of them, 56 (66.7%) had a history of malignancy, while 19 (24.4%) had bilateral adrenal masses.

Overall adrenal lesions

Of the 84 proven, lipid-poor, adrenal masses detected on routine contrast-enhanced CT studies, 46 (55%) were benign, and 38 (45%) were malignant masses. The final diagnosis of benign adrenal masses was pathologically proven for 4 lesions (two adrenal adenomas, 1 bland adrenal cortical tissue, and 1 granulation tissue), and by clinical and/or imaging follow-up for another 42 lesions. Of those 42 lesions, 17 were considered benign adenomas according to the imaging criteria for the 10-minute delayed CT adrenal protocol and/or CS-MRI; a further 4 lesions were considered benign pheochromocytomas by MIBG scans and clinical follow-up; while the remainder (23 masses) were considered benign due to lesion stability during the 1-year follow-up.

Thirty-seven malignant masses were diagnosed as metastases by either tissue diagnosis (n=1) or the imaging criteria (n=36; nodule size changes or new nodules developed after chemotherapeutic session or during imaging follow-up in patients with known malignancy). The other was a pathologically-proven adrenocortical carcinoma.

The mean ages of patients with benign and malignant masses were 59.3 years and 60.2 years, respectively. The average size of all adrenal masses was 2 cm (range 1-4 cm in maximal width). The mean sizes of the benign and malignant masses were 18.2 and 22.0 mm, with an SD of 7.4 and 8.2 mm, respectively (P = 0.027). Of the adrenal masses, 59 (70%) were unilateral and 25 (30%) bilateral. Among the 59 unilateral masses, 20 (36%) were benign, and 36 (64%) were malignant. As for the bilateral location, 10 out of the 25 (40%) were benign, and the remaining 15 (60%) were malignant. There were no significant differences between the benign and malignant masses in terms of age, sex, or laterality (P > 0.05).

A total of 56 out of the 84 (67%) lesions were found in patients with an underlying extra-adrenal malignancy, while the other were found in patients without one. In the case of the 56 adrenal lesions, the masses were significantly malignant etiologies (n=36, 64%) rather than benign (n=20, 36%; P < 0.001).

CT morphological features of benign and malignant adrenal lesions

A statistically significant difference between benign and malignant lesions is shown in Table 1. Most benign adrenal masses had a round/oval shape (91%), a smooth/microlobulated margin (93%), and a homogenous enhancement (82%); those three features showed a very high sensitivity but rather low specificity to differentiate from malignant lesions. In addition, the density of 10–20 HU on unenhanced CT images showed the highest level of specificity (92%) among other features for presumed benignancy. However, the detection of this feature may be limited due to its low sensitivity (40%; Table 2).

TABLE 1. CT features of 84 lipid-poor adrenal masses.

Variable		Malignant N=38 (%)	Benign N=46 (%)	P-value
Shape	Round/oval	22 (58)	42 (91)	< 0.001
	Irregular	16 (42)	4 (9)	
Margin	Smooth/microlobulated	22 (58)	43 (93)	< 0.001
	Irregular	16 (42)	3 (7)	
Density (HU) on unenhanced CT images	10–20	3 (8)	20 (43)	< 0.001
	> 20	35 (92)	26 (57)	
Pattern of enhancement	Homogeneous	16 (42)	38 (82)	< 0.001
	Rim/heterogeneous	22 (58)	8 (18)	

TABLE 2. The sensitivity, specificity, PPV, and NPV of CT features for presumed benignancy in 84 adrenal masses.

Morphology	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV %	NPV %	P-value
Round shape	91.3 (79-98)	42.1 (26-59)	65.6	80	< 0.001
Smooth margin	93.5 (82-99)	42.1 (26-59)	66.1	84.2	< 0.001
Low density (10-20 HU)	43.5 (29-59)	92.1 (79-98)	86.9	57.3	< 0.001
Homogenous enhancement	82.6 (69-92)	57.8 (41-74)	70.4	73.3	< 0.001

Abbreviations: PPV, positive predictive value; NPV, negative predictive value

We also specifically analyzed patients with a history of malignancy (n=56), finding that the low density (10-20 HU) showed the highest specificity (91%) in the determination of a benign etiology, and with statistical significance. This result was similar to that for the patients-without-malignancy group. However, the other features for presumed benignancy, including a smooth margin and homogenous enhancement, showed a high sensitivity (75%–85%) but low specificity (42%-56%). A round/oval shape was the only feature that showed no statistical significance to differentiate between benign and malignant diseases in this patient population (P = 0.06; Table 3).

Combined CT morphological features of benign and malignant adrenal lesions

We combined three morphologies for presumed benignancy (round shape, smooth margin, and homogenous enhancement), for which the sensitivities, specificities, PPVs, and NPVs are presented at Table 4. We also combined all morphological features for presumed benignancy, including the low density feature; although this increased the specificity to 95%, it decreased the sensitivity to 34.8%.

In predicting malignant adrenal lesions, the combination of the high density (HU > 20) feature and a history of malignancy showed a specificity, sensitivity, PPV, and NPV of 86.8%, 73.9%, 73.3%, and 87.2%, respectively ($p < 0.001$).

DISCUSSION

In 2017, the ACR Incidental Findings Committee released an updated version of its White Paper on the management of adrenal incidentalomas. Those lesions are being detected more often than in the past due to the increased use of, as well as improvements to, the spatial resolution of cross-sectional imaging modalities.[11] The guidelines in the White Paper help radiologists and clinicians decide whether an incidentaloma should be further investigated, followed up, or left alone. The guidelines focus mainly on nodules of more than 1 cm

TABLE 3. The sensitivity, specificity, PPV, and NPV of CT features for presumed benignancy in 56 adrenal masses in patients with known extra-adrenal malignancy.

Morphology	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV %	NPV %	P-value
Round shape	85.0 (62-97)	38.9 (23-57)	43.6	82.4	0.06
Smooth margin	85.0 (62-97)	41.7 (26-59)	44.8	83.3	0.04
Low density (10-20 HU)	40.0 (19-64)	91.7 (78-98)	72.7	73.3	0.004
Homogenous enhancement	75.0 (51-91)	55.6 (38-72)	48.4	80.0	0.03

Abbreviations: PPV, positive predictive value; NPV, negative predictive value

TABLE 4. The sensitivity, specificity, PPV, and NPV of combined CT features for presumed benignancy in 84 lipid-poor adrenal masses

Combination	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV %	NPV %	P-value
Benign A	73.9 (59-86)	65.8 (49-80)	72.3	67.6	< 0.001
Benign B	34.8 (21-50)	94.7 (82-99)	88.9	54.6	0.001

Benign A: round shape, smooth margin, and a homogenous enhancement.
Benign B: round shape, smooth margin, a homogenous enhancement, and a low density (10–20 HU).
Abbreviations: PPV, positive predictive value; NPV, negative predictive value

in size and on lipid-poor nodules (nodules with an internal density > 10 HU on unenhanced CT scans), both of which are usually troublesome.[11]

A nodule should be evaluated whether its size is more or less than 4 cm because size is an important indicator for malignancy.[10,11] In the evaluation of benign or malignant adrenal nodules, the specific adrenal CT, CS-MRI, PET/CT, or tissue diagnosis can provide the solution.[4,6,11] However, those do not serve as initial tools, which consequently necessitates another appointment, causing additional costs as well as patient worry and inconvenience. In this study, we aimed to evaluate the CT imaging features of lipid-poor adrenal lesions discovered on routine contrast-enhanced CTs as those may help to initially differentiate benign from malignant masses.

Our study showed that there were significant differences in shape, margin, density on unenhanced images and the pattern of enhancement, to determine the nature of lipid-poor adrenal masses. However, three morphological features for presumed benignancy, which were a round/oval shape, a smooth margin, and a homogeneous enhancement, had high sensitivity but relatively low specificity to suggest benignity. That poor specificity implied that recognizing those imaging features on routine CT scans cannot be helpful in differentiating benign from malignant adrenal diseases. The findings were also in concordance with the results in other studies.[7-10,12] For example, a study by Berland et al. reported two features for benign lesions showing 100% PPV but no statistical significance, namely, homogenous attenuation with little punctuate enhancement, and a gland enlargement with the normal configuration maintained.[9] Another interesting finding of a homogenous attenuation lower than that of muscle was reported with a high PPV, but it was not investigated in our study.[9] Homogenous enhancement is considered a feature suggesting benignancy, and it has been reported to be seen in adrenal adenomas rather than adrenal carcinomas, metastases, and pheochromocytomas by many investigators.[7,10] Nevertheless, a heterogenous enhancement may not be considered an absolute finding for suggesting malignancy since this usually presents in pheochromocytomas.[13]

According to the widely used guidelines for adrenal incidentalomas, a nodule can be considered as benign by measuring the CT density with a threshold less than 10 HU.[11] Despite that, using the higher threshold value (> 10 HU), which has been evaluated in many studies, also demonstrates an impressive performance for lesion characterization.[2,10,12,14] In a meta-analysis study, the reported sensitivity and specificity for adenomas at the cut-off values of 20 HU versus 10 HU were 88% and 84% versus 71% and 98%, respectively.[2] Park et al. also supported the application of a threshold value of 20 HU for characterizing an adrenal adenoma as they reported a sensitivity, specificity, PPV, and NPV of 60%, 100%, 100%, and 67%, respectively.[14] In our study, we showed a specificity and sensitivity of a low-density feature (10-20 HU) on unenhanced CT images of 92% and 44%, respectively. This was the only feature with a high specificity among the other features for presumed benignancy, and we also observed a similar trend in patients either with or without known malignancy. Despite the low sensitivity of this feature, we believe that using a threshold of 20 HU for distinguishing benign and malignant adrenal lesions can be acceptable, subject to the careful consideration of some false negative rates. In the case of patients with a history of cancer, an adrenal nodule may be found on a routine chest or abdominal CT during their metastatic workup. It is therefore important to know whether an adrenal nodule is of a metastatic or benign incidental nature. In our study, a benign-appearing margin, shape, and enhancement pattern were not helpful in predicting benignity except for the low-density feature on unenhanced images, as discussed

earlier. In the study by Song et al., the findings of rim enhancement and an irregular margin were nearly 100% specific to malignant lesions.[7] Our results also revealed that adrenal masses were significantly malignant rather than benign in patients with previously known malignancy. As a history of malignancy is considered significant, diagnosis of a benign or malignant mass using the features on a contrast-enhanced CT alone is usually not reliable in this patient population. Further evaluation such as CT with an adrenal protocol is recommended.[11]

Due to the high sensitivity but low specificity of the individual morphological features for presumed benignancy to indicate a benign mass, we further combined all those features; they included a round/oval shape, a smooth margin, a density at 10–20 HU on unenhanced images, and a homogeneous enhancement. This combination was able to achieve greater percentages for specificity (95%) and PPV (89%). This finding encourages more confidence to diagnose a lipid-poor adrenal mass as being benign if it presents all those features for presumed benignancy within the lesion.

Our study had several limitations. First, it was a retrospective study with a small sample size. The interpretation of the morphological features was also subjective as it depended on the readers' experiences. Still, as this review process was close to daily practice, the results can be applied to real-life situations. In addition, we did not calculate the inter-observer agreement, but instead used a consensus approach to resolve discordant readings. Finally, there were a variety of phases of contrast-enhanced CTs in our study. However, this did not affect our results because different enhancements between phases was not within the scope of our study.

CONCLUSION

Lipid-poor adrenal masses with a density lower than 20 HU can be considered as benign even in patients with known malignancy. However, the rest of the individual features for presumed benignancy have a poor performance in differentiating benign from malignant adrenal masses.

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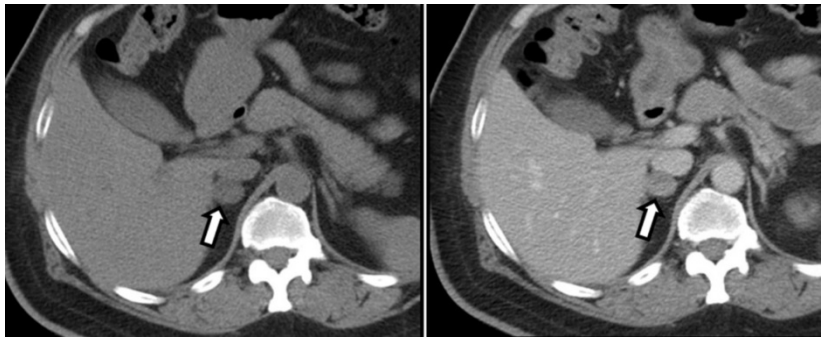


Fig 1. A 58-year-old man with known rectal cancer was found to have a 19-mm right adrenal mass (arrow) during a metastatic workup. The mass had a density of 14 HU on unenhanced CT images, and had smooth margins, an oval shape, and a homogeneous enhancement. As the mass showed no significant change at the 20-month CT follow-up, it was considered to be benign.



Fig 2. A 74-year-old woman with no history of malignancy presented with significant weight loss; bilateral adrenal masses were found on abdominal CT images. The masses showed an oval shape, irregular margins, and an internal density > 20 HU on unenhanced CT images. They were subsequently proven to be malignant by CT-guided core needle biopsy.

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Normative Values of Second-Trimester Maternal Serum Markers Using an Automated Assay Platform for Down Syndrome Screening

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ABSTRACT

Objective: Automated chemiluminescent immunoassay has several advantages over manual ELISA with comparable test performance. Few studies have reported the reference values of the second-trimester serum markers maternal serum alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), unconjugated estriol (uE3), and inhibin A (Inh A) by automated immunoassay in Asian population. Accordingly, this study aimed to determine the median values of second trimester serum markers as a function of gestational age (GA) in Thai population using an automated immunoassay.

Methods: This prospective cross-sectional study of serum markers in healthy singleton second trimester (14-22 weeks) pregnant women was conducted at Siriraj Hospital from September 2012 to April 2015. Maternal serum AFP, hCG, uE3, and Inh A were analyzed by automated immunoassay. Predicted median values as a function of GA were calculated from best-fit regression equations.

Results: A total of 1,526 women were included. Median values serum markers were constructed from the following optimal models: AFP (ng/mL) = 99.082 - 14.195 GA + 0.662 GA^2, r^2=0.995; hCG (mIU/mL) = 390168.106 - 35 968.397GA + 876.708GA^2, r^2=0.972; uE3 (ng/mL) = -3.388 + 0.274 GA, r^2=0.997; and, Inh-A (pg/mL) = 1206.875 - 114.171 GA + 3.174 GA^2, r^2=0.882. Using the same platform analysis and maternal weight adjustment, the reference values in Thai population were shown to be different from those of other ethnicities.

Conclusion: Median values of second-trimester serum markers for Thai population were determined. Maternal weight and the use of population-specific normal values have to be taken into account for Down syndrome screening in the second trimester.

Keywords: Automated immunoassay; Down syndrome; maternal serum screening; quadruple test; reference values; second trimester (Siriraj Med J 2019;71: 21-24)

INTRODUCTION

Alterations in the serum levels of alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), unconjugated estriol (uE3), and inhibin A (Inh A) during the mid-trimester (quadruple test), offer a detection rate of 81% for a 5% false positive rate for prenatal detection of Down syndrome.[1,2] The quality of the results from risk calculation is affected by the analytical performance of the assay used for serum marker determination, the accurate dating of pregnancy, population-specific median values of serum analytes, and the reliable relationship between serum markers and gestational age.[3-6] Well-

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established assays often provide automated quantitation of serum AFP, hCG, and uE3, but not Inh A. A new totally automated quantitative assay for these four serum biomarkers has recently been developed. This automated immunoassay is different from the manual enzyme-linked immunosorbent assay (ELISA) in terms of sample treatment, incubations, washes and detection systems, attributed to the advantage of less labor and fast analysis. Up to now, only few studies have been reported regarding the reference values of these four second-trimester serum markers analyzed by an automated immunoassay among Asian population.[7,8] Moreover and importantly, no data derived from an automated immunoassay is available for Thai population. Accordingly, the aim of this study was to determine the median values of second trimester serum markers as a function of gestational age (GA) in Thai population using an automated immunoassay.

MATERIALS AND METHODS

Study population

This prospective validation study was carried out in pregnant women at 14 to 22 weeks' gestation who attended antenatal care at the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital from September 2012 to April 2015. The study protocol was approved by Siriraj Institutional Review Board (Si 413/2012). In order to generate median values of serum levels for each analyte, at a significance of 5%, standard deviation of 32, margin of error in estimating mean of 5%, and reservation for data loss of 10%, 160 subjects were required for each gestational week (total of 1,600 subjects). Inclusion criteria were women with singleton pregnancy, Thai racial origin, and ≥ 18 years of age. Gestational age was estimated by either a reliable menstrual history and/or by ultrasound examination before 13 +6 weeks of gestation. Exclusion criteria were multiple pregnancies or prior invasive prenatal diagnostic procedures before the time of enrollment. The peripheral blood samples were shipped for analysis to the laboratory no later than 2 hours after blood drawing. Serum sample was transferred at least 500 µL aliquot.

Sample analysis

A new paramagnetic particle chemiluminescent immunoassay on the Access 2 Immunoassay Systems (Beckman Coulter, CA, USA) using Beckman Coulter Access Reagents (cat. No. 33210, 33500, 33570, and A36097 for AFP, hCG, uE3, and Inh-A, respectively) was used. Serum levels of these quadruple markers were then calculated from a stored, multi-point calibration curve. The lowest detection thresholds of AFP, hCG, uE3, and Inh A with 95% confidence were 0.5 ng/mL, 0.5 mIU/mL, 0.017 ng/mL, and < 1 pg/mL, respectively. Results of invasive prenatal genetic testing were obtained from voluntary self-reporting, with an absence of reported abnormalities until the time of birth adjudicated to be euploid.

Statistical analysis

Demographic characteristics are presented as numbers and percentages for categorical data and as mean ± standard deviation or median and interquartile range (IQR) for continuous data, depending on the distribution. Median values of AFP, hCG, uE3, and Inh A were calculated for each completed gestational week. Regression analysis was used to estimate the relationship of serum markers and gestational age and the optimal model was then selected to predict median values of each marker. Patient results were then stratified into six groups according to maternal weight (< 45, 45-54.9, 55-64.9, 65-74.9, 75-84.9, and ≥ 85 kg). To adjust for maternal weight, predicted multiples of the median (MoMs) values of each marker were calculated from the best fit equation using regression analysis. All data were analysed statistically by using SPSS (IBM SPSS Statistics for Windows version 18, Microsoft Corporation; Chicago, IL, USA) and GraphPad Prism (GraphPad software for Windows version 7.00, San Diego, California, USA).

RESULTS

Of the 1,600 women enrolled in this study, 1,526 (95.38%) women with complete data set were selected for further analysis. Median maternal age, weight, and body mass index (BMI) at the time of study enrollment were 27 years (IQR, 23 - 31), 52 kg (IQR, 47 - 59.25), and 20.82 kg/m^2 (IQR, 18.90 - 23.51), respectively. Only 3% (46/1526) of this study population were obese (BMI ≥ 30 kg/m^2). Most patients (1,417/1,526, 92.9%) were aged less than 35 years and 843 patients (55.24%) were nulliparous. Median gestational age at delivery and birthweight were 39 weeks (IQR, 38 - 39) and 3,110 grams (IQR, 2,890 - 3,330), respectively. All patients included in this study had a naturally conceived pregnancy, no one reported being a current smoker.

The regression equations for serum markers as a function of gestational age (GA) in weeks from 14 to 22 weeks of gestation are described as follows:

AFP (ng/mL) = $99.082 - 14.195\ GA + 0.662\ GA^2$, $r^2 = 0.995$, $P < 0.001$

hCG (mIU/mL) = $390168.106 - 35968.397GA + 876.708GA^2$, $r^2 = 0.972$, $P < 0.001$

uE3 (ng/mL) = -3.388 + 0.274 GA, $r^2 = 0.997$, $P < 0.001$
Inh-A (pg/mL) = 1206.875 – 114.171 GA + 3.174 GA^2, $r^2 = 0.882$, $P = 0.002$.

Median values of all serum markers at each gestational week calculated from the regression equations are shown in Fig 1. Serum levels of AFP and uE3 significantly increased, and serum levels of hCG significantly decreased from 14 to 22 gestational weeks. Serum levels of Inh A continuously dropped until reaching the nadir at 18 weeks of gestation, then continuously elevated.

Significant inverse relationships between multiples of the median (MoMs) serum levels of AFP, hCG, uE3, and Inh A and maternal weight (Wt) (in kilograms) in each category were observed with the best-fit equations described, as follows:

AFP (MoMs) = 1.972 – 0.024 Wt + 0.00011 Wt^2, $r^2 = 0.994$, $P < 0.001$
hCG (MoMs) = 1.484 – 0.008 Wt, $r^2 = 0.921$, $P = 0.002$
uE3 (MoMs) = 1.322 - 0.006 Wt, $r^2 = 0.934$, $P = 0.002$
Inh-A (MoMs) = 1.302 – 0.006 Wt, $r^2 = 0.998$, $P < 0.001$

Predicted MoM values of all serum markers adjusted for maternal weight are shown in Table 1.

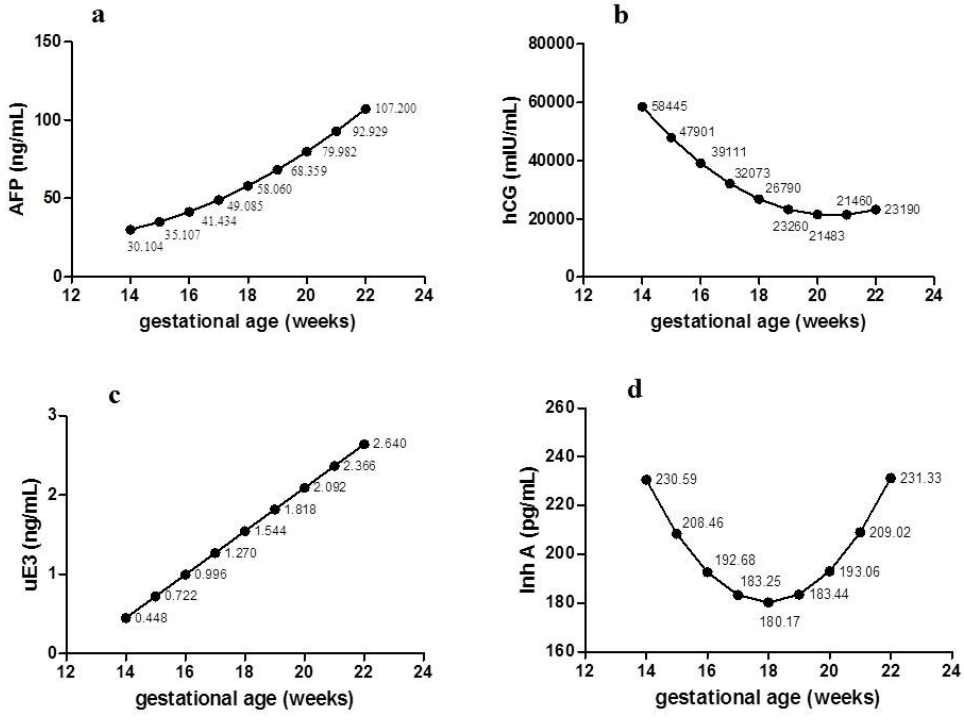


Fig 1. Median values of serum markers in Thai women by gestational age. (a) alpha-fetoprotein (AFP) levels expressed in ng/ml, (b) human chorionic gonadotropin (hCG) levels expressed in mIU/ml, (c) unconjugated estriol (uE3) levels expressed in ng/ml, (d) inhibin A (Inh A) levels expressed in pg/ml.

TABLE 1. Predicted median MoM values of serum markers by maternal weight category.

Maternal weight category		Number of cases	AFP (MoM)	hCG (MoM)	uE3 (MoM)	Inh A (MoM)
I	< 45 kg	199	1.158	1.148	1.070	1.050
II	45 – 54.9 kg	718	1.060	1.092	1.028	1.008
III	55 – 64.9 kg	376	0.950	1.020	0.974	0.954
IV	65 – 74.9 kg	178	0.849	0.940	0.914	0.894
V	75 – 84.9 kg	39	0.769	0.860	0.854	0.834
VI	≥ 85 kg	16	0.710	0.776	0.791	0.771

Abbreviations: AFP: alpha-fetoprotein; hCG: human chorionic gonadotropin; uE3: unconjugated estriol; Inh A: inhibin A; MoM: multiples of the median; kg: kilogram.

DISCUSSION

In this study, median values of serum levels of AFP, hCG, uE3, and Inh A in Thai women carrying singleton pregnancy from 14 to 22 weeks of gestation were generated using a new Access 2 automated chemiluminescence immunoassay system. Previous studies supported the highly correlated results of dimeric Inh A between this new immunoassay platform and manual ELISA, with a comparable performance of prenatal detection of Down syndrome.[9,10] Several advantages of an automated chemiluminescent immunoassay over a manual ELISA with a comparable test performance explain its preference for serum Inh A analysis.[9,10] The effects of gestational age and maternal weight were consistent with previously published data from other platforms.[11,12]

Maternal weight is another influential determinant of these serum marker levels, displaying negative affiliations. The pathophysiology behind these affiliations is at present indistinct. In order to compare the median values of maternal serum markers in our population to those previously reported from other populations using the same automated immunoassay, it is essential to adjust the median values by weight-correction models. Each of four serum markers had a similar pattern of change during the second trimester of pregnancy among various ethnic groups.[7,9,10,13] Nevertheless, unique normal values were reported among different ethnic groups. Higher levels of serum AFP, hCG, and Inh A and a lower level of serum uE3 in our population were observed in comparison of those in the Caucasian groups.[9,10,13] Despite the fact that Thai and Korean people are both racially classified as Asian and have similar serum AFP and hCG levels in the second trimester, there are some more subtle differences in uE3 and Inh A levels between these two ethnic groups.[7] Our population seemed to have a lower level of serum uE3 and a slower progression of serum Inh A after reaching its nadir level at 18 weeks of gestation.[7] These would seem to signify the necessity of population-specific normal values of these serum markers.

The potential impact of this study is reinforced by a number of key strengths. This is the first prospective, well-designed study of normal values of second-trimester serum markers using an automated immunoassay in a large cohort of Thai population with a high rate of available outcomes. Limitations of this study include a highly selected, low-risk study population, no accuracy assessment of these reference values, and no data comparison between the normal values derived from the automated immunoassay and those derived from the manual ELISA method in the studied Thai population. The impact of maternal age, smoking status, and the method of conception on these reference values could not be determined. Further studies on test performance are needed to support the application of these normative values to second-trimester screening for Down syndrome.

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Economic Evaluation of Ready-to-use Injectable Medications by Pharmacy Department Compared with the Traditional System of Individual Preparation by Nurse

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ABSTRACT

Objective: To dispense medication in a form of ready to use (RTU) medication was recommended by the standards of Joint Commission International (JCI) and Standard Guidelines of Hospital Pharmacy for preventing the medication error. However, the cost and benefit were questionable. The costs may increase while benefits were unclear. Before making the implementation decision, the cost of investment and benefit between traditional (injectable medication is prepared by nurse) and RTU systems (injectable medication is prepared by pharmacy department) should be evaluated.

Methods: This study compared the cost and benefit of injectable medication administration between the traditional system and the RTU system within a large academic hospital. The decision tree was designed to produce comparable data on the hospital's perspective. The time horizon was 10 years thus all costs were discounted at 3% annually. Sensitivity analysis was performed to test the stability of the results.

Results: The cost of investment at 10-year intervals of the RTU system was lower than the traditional system by about 18,710,160 baht. The benefit was decreased 19.32 full time equivalents (FTEs) of nurse when compared with the traditional system. The result showed that the five most sensitive variables were number of doses, mixing time per dose (prepared by nurse), space for production, salary and fringe benefits of pharmacists, and mixing time per dose (prepared by pharmacist).

Conclusion: The RTU system saved 1,871,016 baht per year and 19.32 FTEs of nurse. Moreover, the RTU system enhanced the opportunity of nurses and pharmacists to play more professional role and promoted the efficient health care system.

Keywords: Ready-to-use medication; premixed medication; intravenous admixture; medication administration; economics (Siriraj Med J 2019;71: 25-30)

INTRODUCTION

The growing nursing workforce shortage has increased nurse workload and reduced the amount of nursing time available for patient care activities.[1] The prior study found that nurses time spend on specific activities such as documentation, medication administration,

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care coordination, and patient care activities.[2] In the hospital view, some parts of nurses' activity especially admixing in step of medication administration would be considered to decrease workload of nurse. Some injection medications such as high alert drugs were prepared by pharmacy department and dispensed in ready to use dosage form. Thus medication would be administered to the patient without the admixing step by nurse. Totally, nurses could decrease their workload and have more times for patient care. From previous study, 73.3% of nurses agreed that ready to use (RTU) medication preparation reduced nurses' workload.[3]

For the pharmacist's role, dispensing medications in form of ready-to-use medications or premixed medications in order to decrease medication errors was recommended by the standard of Joint Commission International (JCI) and Standard Guidelines of Hospital Pharmacy.[4] Having RTU medications may help reduce admixing errors or administration errors.[5] Thus, to prevent the medication error, enhance pharmacist role, and decrease nurse workload, RTU medication was considered. Previous study found that advantages of RTU medications are as follows:

1. RTU medications assured that patients received accurate dosages, and reduced medication errors.[6-10]

2. RTU medications could be administered more quickly especially in a busy time in hospital. Thus they could reduce overloading in emergency rooms and other treatment areas.[10]

3. The cost of RTU medications preparation was less than cost of individual preparation by nurse.[10,11]

4. RTU medications reduced risk of microbial contamination and cross contamination.[10]

5. RTU medications could reduce needle-prick injury which was a major occupational health and safety issue facing healthcare professionals especially nurses.[10]

Previous studies [6-9] found that injectable medication preparation by pharmacist decreased medication errors whereas the system may increase cost of investment. However, the overall cost was expected to be decreased from prevented medication errors.

For this setting, the cost of investment for the RTU system and the evidence of benefit were controversial issues that provoke differing views from the relevant health care personnel. The costs may increase from the capital cost for the standard practice of sterile preparation and the involved personnel in the production process while benefits were unclear. Before RTU medication implementation, cost of investment and benefit of the system needs to be evaluated. Thus the objective of the study was comparison on the cost of investment and benefit of injectable medication preparation system between traditional and RTU systems.

MATERIALS AND METHODS

The study evaluated two intravenous admixture systems; the traditional system (injectable medications were prepared by nurse) and the RTU system (injectable medications were prepared by pharmacy department. Nine high volume antibiotics after reconstitution is stable under refrigeration (2-8 °C) for at least seven days were chosen for RTU medication. The recommended dose of preparation was the usual adult dose for treatment in the hospital. Cost of investment was analyzed in hospital perspective. Direct cost including capital cost, labor cost, material cost, and other costs incurred in ten years were accumulated and discounted to present value with 3% discounting rate.[12] To analyze cost and sensitivity analysis, TreeAge Pro Healthcare was used. The benefit in terms of full-time equivalents (FTE), of the number of staff required for work process, was analyzed for comparison. In medication preparation process, the traditional system required nurse, whereas pharmacist and pharmacy technician were involved in the RTU system. The FTE of the study was calculated based on 6 hours per day and 230 days per year. Data included in the study was extracted from the hospital data and directly recorded at ward and pharmacy department. (This study was approved by the Institutional Review Board Ethics Committee IRB. No. 558/2558(EC2).)

Traditional system

Injectable medication was prepared for administration with aseptic technique at ward environment by nurse. Then medication would be immediately administered to patient. Cost of investment comprised of labor cost and material cost. Labor cost including salary and fringe benefits of nurse was calculated from time spent in preparation process. Time spent of work process was collected at ward and calculated as full-time equivalent (FTE). Data of salary and fringe benefits were obtained from human resource unit and estimated that in every year salaries increased 5% (salary increase between 3-7% per year). Sterile syringe and needle were used and accumulated for cost per dose in preparation process.

RTU system

Injection medication was prepared by pharmacist (R.Ph) and pharmacy technician (Ph.Tech) in cleanroom as the standard practice of pharmaceutical compounding - sterile preparations. RTU medication was stored at controlled temperature (2-8 °C) and dispensed with cold

chain system. Cost of investment consisted of capital cost, labor cost, material cost, and other costs. Capital costs were fixed cost incurred in the production of RTU medication such as laminar airflow hood, vial roller mixer, sealer, repeater, pharmaceutical refrigerator, autoclave, hot air oven. For this study, a lifetime of 10 years has been chosen for all production equipment by an expert panel. Computer and printer would be changed every 3 - 5 years. Cleanroom and laminar airflow hood needed maintenance since the first year of production. For other equipment, for the first two years of implementation the support and maintenance were free. In this system, opportunity cost of space for production was included. Labor cost was defined as labor cost of pharmacist and pharmacy technician. Time spent in preparation and packaging process was collected at pharmacy department. Data of salary and fringe benefits were obtained from human resource unit and estimated that in every year salaries increased 5%. RTU medication was contained within the sterile packaging labeled medication name, concentration, lot number, and expiration date. Material including sterile syringe, sterile needle, packaging, and labeling was used for RTU medication.

Sensitivity analysis

Sensitivity analysis was used to evaluate how uncertainty in the model inputs affected the outputs of the model. There was a wide range of input data for sensitivity analysis as Table 1. The use of sensitivity analysis results were classified to four categories: making decision or development of recommendations for decision makers, communication, increased understanding or quantification of the system, and model development. While all these uses were potentially important, this study focused on decisions making or recommendations. When the optimal option was insensitive to parameter changes, policy maker considered higher confidence of implementing the optimal option. On the other hand, if the option was sensitive, sensitivity analysis could be used to specify the level of importance of changes and recommend solutions. Even if the levels of variables in the optimal solution were changed dramatically by a higher or lower parameter value, the stability of the outcomes should examine the difference in profit (or another relevant objective) between these solutions and the base-case solution.[13] One way sensitivity analysis was performed on all variables in order to test the stability of the outcomes and presented as the tornado diagram. Tornado diagram showed changes in the net present value under the feasible range of each variable.

RESULTS

The estimated number of medications per year was 300,827 doses. 10-year cost of investment for traditional and RTU system were analyzed and presented as present value. Direct cost was accumulated from capital cost, labor cost, material cost, and other costs. The total 10-year cost of the traditional system included labor and material costs which was 98.24 and 9.25 million baht, respectively. For the RTU system, total 10-year cost of labor and material were 39.52 and 5.07 million baht, respectively. Capital and opportunity costs were also included in the RTU system. Total 10-year capital and opportunity costs were 4.65 and 39.54 million baht, respectively. The cost of investment of the traditional system and the RTU system were 107,492,820 baht and 88,782,660 baht, respectively as shown in Table 2. The RTU system reduced overall cost about 18,710,160 baht in 10 years when compared with the traditional system.

For workload, the RTU system could shorten some preparation processes so lower human resource requirements per dose were needed. As Table 1, medication preparation time in the traditional system took 319 seconds per dose compared with 240 seconds per dose in the RTU system. The result showed that the traditional system required 19.32 FTEs of nurses while the RTU system required 3.63 FTEs of pharmacists and 10.90 FTEs of pharmacy technicians. Thus using the RTU system could replace 19.32 FTEs of nurses.

Sensitivity analysis

From the Fig 1, the most sensitive variable was number of doses per year of RTU medication. The RTU system saved cost when the minimum number of RTU medications was 211,346 doses per year. The following sensitive variables were nursing time for medication preparation, space of production, salary and fringe benefits of pharmacist, and pharmacy technician, respectively. Decreasing the nursing time for medication preparation from 319 sec/dose to 258 sec/dose, the lowest expected value changed from the RTU system to the traditional system. When the space of production was more than 552.46 square meters, the lowest expected value changed from the RTU system to the traditional system. Increasing the salary and fringe benefits per month of pharmacist and pharmacy technician to 77,018.29 and 28,191.26 baht, respectively, the lowest expected value changed from the RTU system to the traditional system. For the salary and fringe benefits of nurse with less than 31,418.62 baht/month, the lowest expected value changed from the RTU system to the traditional system. With the increase

TABLE 1. All variables and intervals used in the model and sensitivity analysis.

Variable	Value	Low	High
Number of medications (doses/year)	300,827	220,934	391,075
Working time per year (sec/FTE)	4,968,000	4,968,000	5,796,000
Increasing rate of salary per year (%)	5	3	7
Discount rate (%)	3	0	7
Traditional system			
Medication preparation time: nurse (sec/dose)	319	30	2,730
Salary and fringe benefits of nurse (baht/month)	38,809.96	23,250.00	124,257.10
Material cost (baht/dose)	3.50	2.50	5.00
Ready to use (RTU) system			
Laminar Airflow Hood cost (baht/piece)	400,000	300,000	500,000
Vial Roller Mixer cost (baht/piece)	25,000	25,000	30,000
Sealer cost (baht/piece)	25,000	25,000	30,000
Repeater	300,000	300,000	350,000
Pharmaceutical Refrigerator cost (baht/piece)	38,000	26,000	38,000
Autoclave cost (baht/piece)	350,000	350,000	400,000
Hot Air Oven cost (baht/piece)	130,000	130,000	150,000
Computer and Software cost (baht/piece)	25,000	20,000	30,000
Printer cost (baht/piece)	20,000	20,000	25,000
Refrigerator for storing RTU medications	128,000	104,000	152,000
Maintenance cost of Laminar Airflow Hood (baht/machine/year) *	7,000	4,900	9,100
Maintenance cost of cleanroom (baht/year) *	60,000	60,000	100,000
Maintenance cost of product equipment (baht/year) †	50,000	35,000	65,000
Space of production (square metre : Sq m)	375	255	555
Opportunity cost (baht/m^2/year)	12,000	12,000	18,000
Medication preparation time: R.Ph (sec/dose)	60	36	120
Medication preparation time: Ph.Tech (sec/dose)	180	120	180
Salary and fringe benefits of R.Ph (baht/month)	37,720.96	21,290.00	124,236.90
Salary and fringe benefits of Ph.Tech (baht/month)	15,092.14	11,422.00	54,291.30
Material cost (baht/dose)	1.92	1.51	9.00

Abbreviations: R.Ph = Pharmacist, Ph.Tech = Pharmacy technician

* Cleanroom and laminar airflow hood required maintenance every 6 months since the first year of implementation.

† Maintenance cost occurred in the third year after implementation.

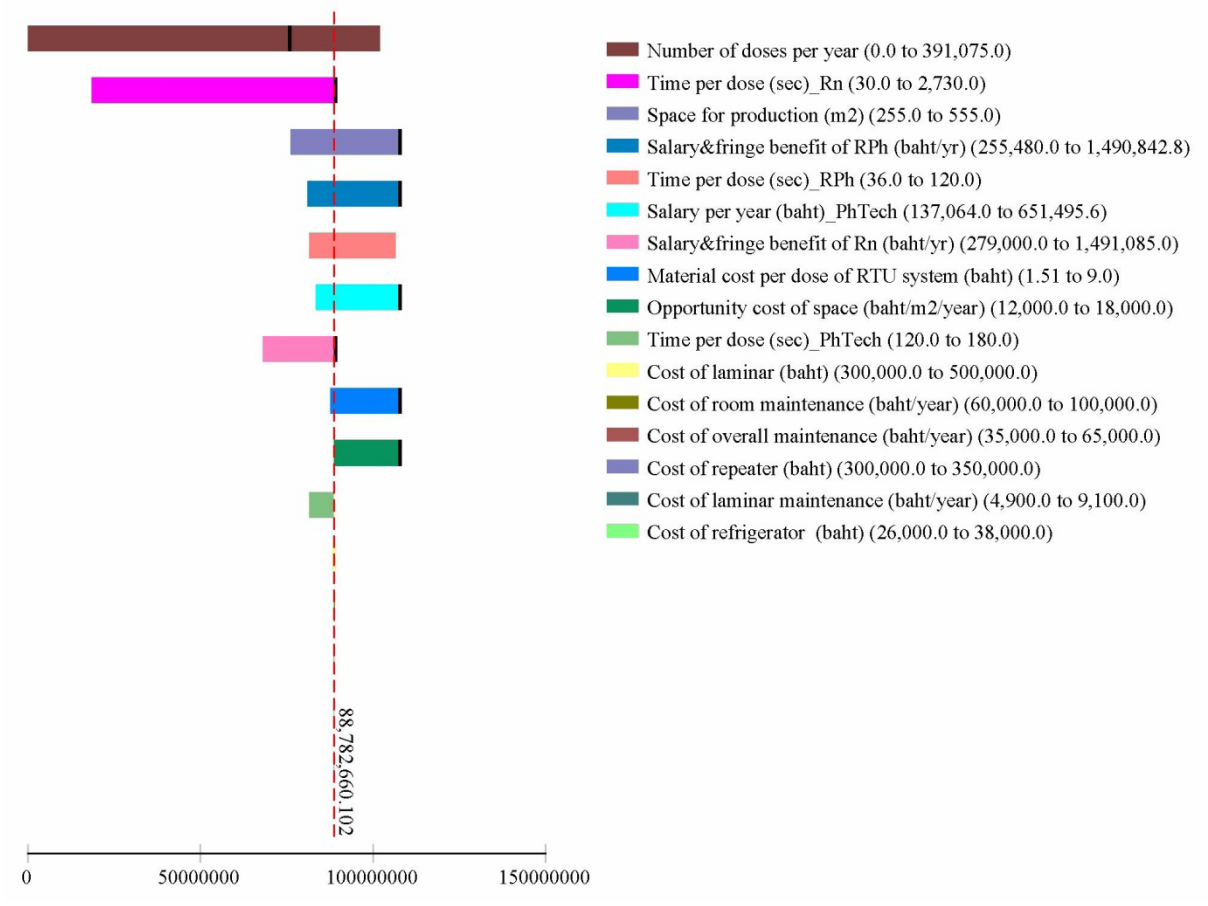


Fig 1. Tornado diagram

in material cost for RTU preparation to 9 baht/dose, the traditional system would be cost saving. Not only space of production, but opportunity cost of space also affected the alternative. Increasing the opportunity cost from 1,000 baht/m^2/month to 1,473 baht/m^2/month, the lowest expected value changed from the RTU system to the traditional system.

DISCUSSION

This study focused on medication in standard dose with extended shelf life at least seven days. The estimated numbers of medications per year were 300,827 doses. In the RTU system, capital, maintenance, and opportunity costs increased. However, the RTU system saved labor and material costs. Material cost per dose in the RTU system lowered from fewer needle and syringe volumes per dose preparation. As a result, cost saving of labor and material costs from the RTU system were 58,725,439 baht and 4,176,096 baht in 10 years, respectively. Overall in a 10-year period, the RTU system saved 18,710,160 baht. As a result, the RTU system could save nursing time 19.32 FTEs from reducing administration time while workload of pharmacist and pharmacy technician increased 3.63 FTEs and 10.90 FTEs, respectively. The benefits of the RTU system implementation were the opportunity of nurse to provide a patient care and pharmacists to play the role as the standard of JCI and good pharmacy practice. Moreover, the RTU system is able to enhance the safety of medication administration and promote the efficient healthcare system.

From sensitivity analysis, the number of medications was the most sensitive variable. To increase items covered by the RTU system could save more cost of the system. The result would be influenced by the change of number of medications, admixing time per dose by nurse, space for production and opportunity cost, salary and fringe benefits of pharmacist, pharmacy technician, and nurse, and material cost of the RTU system. To decrease labor cost in the RTU system, offering overtime compensation could decrease labor cost compared with full-time staff employment. When nurse took admixing time less than 258 seconds, the traditional system would be the lower cost alternative. In this observation, 44.43% of admixing doses took less than 258 seconds. However, nursed can be disturbed by calls and other notifications during administer or mixing injectable drug. The previous study

showed that the interruption event occurred one in 1.7 times of admixing. Thus nurse required more time in real practice during admixing from the interrupted event.

The study did not take into account the cost of medication and diluent which were equal in both systems. RTU medication was the dose of standard usually used with long stability, so cost from expired RTU medications was not accumulated. However, the efficient system would be planned for the RTU system implementation to prevent waste. Cost saving from unused medication in vial as prior study was not included because dose of RTU medication was similar to the company packaging.[3] Initially, the RTU system was implemented in nine antibiotics and cost saving from reducing of administrative errors was not accumulated as study from Colombia which explored in high alert medication which cause patient harm.[14]

CONCLUSION

The RTU system was the lowest cost alternative, saves time and workload of nurse by eliminating reconstitution at the point of care. As well as the economic interest, the RTU system could enhance the opportunity of nurses and pharmacists to play more professional role and contribute to patient safety improvement and hospital quality following the standard of JCI.

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Administration of Renin-Angiotensin System Inhibitor Affects Tumor Recurrence and Progression in Non-Muscle Invasive Bladder Cancer Patients

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ABSTRACT

Objective: To evaluate the effects of renin-angiotensin system inhibitors (RASIs) on tumor-recurrence and disease-progression in non-muscle invasive bladder cancer (NMIBC) patients.

Methods: From 2006-2015, 348 NMIBC patients at Siriraj Hospital were recruited for this study. Tumor-recurrence was identified after the transurethral resection of bladder cancer (TUR-BT) and pathological confirmation of NMIBC, while stage-progression was defined as muscularis-propria invasion after pathological review or metastases. Cox proportional hazards models were used to assess the recurrence-free survival (RFS) and progression-free survival (PFS) rates.

Results: Of the 348 patients, 86 (24.7%) received RASIs at the first TUR-BT. The median age was 68 years, and it was significantly older for the RASI cohort. No differences in the tumor characteristics of the groups were found. The median follow-up periods for tumor-recurrence and stage-progression were 2.3 and 3.7 years, respectively. Forty percent of the patients experienced tumor-recurrence, with the no-RASI cohort experiencing a significantly higher tumor-recurrence rate (46% versus 22%, $p<0.001$). The 5-year RFS rates were 54% and 78% for the no-RASI and RASI cohorts, respectively ($p=0.001$). Stage-progression was observed in 6% of the patients. The 5-year PFS rates were 87% and 97% for the no-RASI and RASI cohorts, respectively. On univariate and multivariate analyses, a tumor size ≥3 cm and tumor multifocality were associated with recurrent bladder cancer ($p<0.02$). On the other hand, the administration of RASIs was associated with a reduced recurrence ($p\leq0.002$).

Conclusion: Our study suggests that RASI administration might be a potential factor to prevent bladder cancer recurrence. Further study is needed to evaluate the effects of RASIs.

Keywords: Non-muscle invasive bladder cancer; renin-angiotensin system inhibitors; tumor recurrence; stage progression (Siriraj Med J 2019;71: 31-37)

Abbreviations

AJCC	= American Joint Committee on Cancer
Ang II	= Angiotensin II
AT1R	= Angiotensin type 1 receptor
BCG	= Bacillus Calmette–Guerin
CI	= Confidence interval

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EORTC = European Organization for Research and Treatment of Cancer
HR = Hazard ratio
IQR = Interquartile range
MIBC = Muscle invasive bladder cancer
MMC = Mitomycin C
MP = Muscularis propria
NMIBC = Non-muscle invasive bladder cancer
OR = Odd ratio
PFS = Progression-free survival
RASI = Renin-angiotensin system inhibitor
RFS = Recurrence-free survival
RR = Relative risk
TUR-BT = Transurethral resection of bladder tumor

INTRODUCTION

Bladder cancer is the second most common urologic malignancy and the eighth most common overall malignancy in Thailand.[1] Most patients present with gross hematuria, and 75% of patients have non-muscle invasive bladder cancer (NMIBC) stages Ta, T1, and Tis.[2-4] Transurethral resection of bladder tumor (TUR-BT) is the standard treatment and the diagnostic procedure.[2-4] Up to 50% of NMIBC patients experience tumor recurrence, and 6% -17% progress to muscle invasive bladder cancer (MIBC).[4] The use of adjuvant agents after a TUR-BT has been introduced to reduce the risk of tumor recurrence and progression.[2-4]

Adjuvant intravesical therapy, such as Bacillus Calmette–Guerin (BCG) and mitomycin C (MMC), has been utilized in current practice to decrease the incidences of recurrent NMIBCs and disease progression.[2-6] A recent meta-analysis demonstrated that adjuvant intravesical BCG as immunotherapy was associated with reduced recurrent NMIBC (RR 0.56, 95% CI 0.43-0.71).[5] Additionally, intravesical chemotherapy such as MMC, doxorubicin, and epirubicin have also been associated with a decreased risk of bladder cancer recurrence (RR 0.68, 95% CI 0.55 - 0.83; RR 0.80, 95% CI 0.72 - 0.88; and RR 0.63, 95% CI 0.53 - 0.75, respectively). As to tumor progression, only adjuvant intravesical BCG has been associated with a reduced risk of progression (RR 0.39, 95% CI 0.24 - 0.64). Given its potential role in both reduced tumor recurrence and progression, immunotherapy such as checkpoint blockade has also been studied as an option for the treatment of NMIBC patients.[7] However, all agents are adjuvant treatments after the TUR-BT.

Angiotensin II (Ang II) is a key biological peptide in renin-angiotensin systems. Ang II is involved in the regulation of blood pressure, water, and sodium homeostasis, and in the control of other neurohumoral systems. It also leads to the excessive production of reactive oxygen species, and to the hypertrophy, proliferation, migration, and apoptosis of vascular cells.[8] Angiotensin type 1 receptors (AT1Rs) are expressed in various malignancies, including bladder cancer, and are significantly involved in tumor growth, metastasis, and angiogenesis.[9] Ang II-AT1R signaling leads to the potent induction of vascular endothelial growth factors.[10] Recent publications have outlined that renin-angiotensin system inhibitors (RASIs) have an antiangiogenic effect on bladder cancer.[11–12]

Our primary objective was to determine the effects of RASIs on tumor recurrence and disease progression in NMIBC patients.

MATERIALS AND METHODS

After receiving Siriraj Institutional Review Board approval (Si 708/2015), patients diagnosed with NMIBC at Siriraj Hospital between 2006 and 2015 were recruited for the study. Excluded were those patients who were followed up for less than 1 year, underwent a cystectomy due to unresectable lesions, took RASIs after the initial TUR-BT, or had concurrent upper urinary tract tumors. A total of 348 patients were ultimately available for the study.

The patient and tumor characteristics were collected retrospectively. The RASIs had been prescribed by physicians as anti-hypertensive drugs or for other indications, such as cardiac or renal disorders. The pathological reports were in accord with the guidelines of the American Joint Committee on Cancer (AJCC) current during that period. NMIBCs were defined as Ta, TI or Tis. Regarding the past records, some tumors were reported pathologically as NMIBC by non-muscularis propria invasion (Tu). MIBCs had been confirmed by histological muscularis propria invasion (T2-T4). Tumor-recurrence had been identified after the TUR-BT and pathological confirmation of NMIBC. Stage-progression was identified as MIBC or

lymph nodes or distant metastases. Tumor multifocality was defined as the presence of 2 or more tumors. The surveillance schedules had followed the standard guidelines for each NMIBC risk group. The TUR-BT technique and the administration of intravesical therapy depended upon the preferences of the attending staff.

Continuous variables were shown as medians and interquartile ranges (IQRs), and they were compared with the Mann–Whitney U test. Categorical variables were presented as numbers (percent) and compared with the Chi-square or Fisher's exact tests. Cox proportional hazards models were employed to assess the recurrence-free survival (RFS) and progression-free survival (PFS) rates. The RFS curve was generated using the Kaplan-Meier method and compared with the log rank test. Variables with $p<0.05$ were considered significant. The analyses were performed using SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Overall, 348 patients were analyzed. Their median age was 68 years. The patient and tumor characteristics are listed in Table 1. Of those patients, 86 (25%) were taking RASIs at the time of the first TUR-BT. The types of RASI are listed in Table 2. Male gender was predominant in both cohorts. The smoking histories, tumor sizes, grades, and multifocality of the cohorts were similar. One hundred and eighty-one patients (29%) had never smoked, 101 (52%) used to smoke, and 66 (19%) were current smokers. The tumor size was less than 3 cm in 233 patients (67%), while it was equal to, or greater than, 3 cm in 115 patients (33%). Ta was found in 95 patients (27.3%), T1 and Tis in 43 patients (12.4%), and Tu in 210 patients (60.3%). One hundred and sixty-eights patients (48%) had high grade tumors, with 119 and 49 patients in the no-RASI and RASI cohorts, respectively. Multifocal tumors were found in 170 patients (49%), with 130 and 40 patients in the no-RASI and RASI cohorts, respectively. MMC was administrated after the TUR-BT in 50 patients (14.4%), while 113 patients (32.5%) received BCG therapy, and a further 25 (7.2%) received both agents.

The median follow-up times for tumor recurrence and stage progression were 2.3 years (IQR 1.1-4.2) and 3.7 years (IQR 2.0-5.8), respectively. One hundred and forty patients (40%) experienced tumor recurrence, with patients in the RASI cohort having a significantly lower rate of tumor-recurrence (22% versus 46%, $p<0.001$), as shown in Table 3. The 5-year recurrence-free survival (RFS) rates were 54% and 78% for the no-RASI and RASI cohorts, respectively ($p=0.001$). The RFS between the 2 cohorts was demonstrated with Kaplan–Meier curves (Fig 1). Stage progression was observed in 19 patients (6%), with 3 and 16 patients in the RASI and no-RASI cohorts, respectively ($p=0.5$). The 5-year PFS rates were 87% and 97% for the no-RASI and RASI cohorts, respectively.

On univariate and multivariate analyses (Table 4), a tumor size equal to or greater than 3 cm, tumor multifocality, and patients without RASIs were associated with recurrent bladder cancer (all $p<0.02$). However, there was no significant association between RASI administration and decreased disease progression in both the univariate and multivariate analyses.

DISCUSSION

In Thailand, bladder cancer is the second most common urologic malignancy and the eighth most common overall malignancy, with a prevalence of 4.5/100,000 in males and of 1.2/100,000 in females.[1] NMIBC is the most common presentation, and a variety of adjuvant treatments have been investigated to prevent tumor recurrence and disease progression.[2-7] In a recent meta-analysis, only intravesical BCG demonstrated an association with reduced bladder cancer recurrence and progression in NMIBC patients.[5] In contrast, adjuvant chemotherapy has been solely associated with decreased tumor recurrence, not progression.[5] However, the adverse effects of intravesical BCG are still a concern. Targeted therapies such as checkpoint inhibitors, which function as immunotherapy, have been explored for their substantial effects.[7] In addition, the AT1R has been found in various cancer cells and been shown to be involved in tumor growth and angiogenesis.[9-10] Shirotake and colleagues, who studied the AT1R of bladder cancer patients, reported that it was an independent predictor of the RFS rate on multivariate analysis.[9] As such, RASIs, which are prescribed as anti-hypertensive drugs, may have a potential role in diminishing the risks of tumor recurrence and progression.

The AT1R has been found in bladder cancer specimens and has been significantly associated with intramural neovascularization.[9] AT1R could therefore be a potential factor to identify patients with a high risk of tumor recurrence. Ang II-AT1R also has an impact on tumor microenvironments and thus promotes tumor growth, survival, invasive behavior, and tumor cell migration.[10] This suggests that RASIs, such as angiotensin-converting enzyme inhibitors and angiotensin receptor blockages, which are prescribed as anti-hypertensives, might have substantial roles. Blocking the AT1R might reduce tumor growth and angiogenesis and, in turn, inhibit tumor proliferation.[9,10] Previous studies from Yuge et al. and Blute et al. showed that the administration of RASIs was

TABLE 1. Patient and tumor characteristics.

	All patients (n=348)	No-RASIs (n = 262)	RASIs (n = 86)	*P-value*
Age , years, median (IQR)	68.2 (30.9-93.2)			0.03
< 65 years, *n* (%)	127 (36.5)	104 (39.7)	23 (26.7)	
≥ 65 years, *n* (%)	221 (63.5)	158 (60.3)	63 (73.3)	
Gender, *n* (%)				0.89
Male	261 (75.0)	196 (74.8)	65 (75.6)	
Female	87 (25.0)	66 (25.2)	21 (24.4)	
Smoking, *n* (%)				0.10
Never	181 (29.0)	135 (51.5)	46 (53.5)	
Former	101 (52.0)	71 (27.1)	30 (34.9)	
Current	66 (19.0)	56 (21.4)	10 (11.6)	
Tumor size, *n* (%)				0.37
< 3 cm	233 (67.0)	172 (65.6)	61 (70.9)	
≥ 3 cm	115 (33.0)	90 (34.4)	25 (29.1)	
Tumor stage, *n* (%)				0.04
Ta	95 (27.3)	68 (26.0)	27 (31.4)	
T1+Tis	43 (12.4)	27 (10.3)	16 (18.6)	
Tu	210 (60.3)	167 (63.7)	43 (50.0)	
Tumor grade, *n* (%)				0.06
Low	180 (51.7)	143 (54.6)	37 (43.0)	
High	168 (48.3)	119 (45.4)	49 (57.0)	
Tumor multifocality, *n* (%)				0.62
No	178 (51.1)	132 (50.4)	46 (53.5)	
Yes	170 (48.9)	130 (49.6)	40 (46.5)	
Presence of MP in specimen, *n* (%)				0.22
No	208 (59.8)	141 (53.8)	67 (77.9)	
Yes	140 (40.2)	121 (46.2)	19 (22.1)	
Intravesical agents, *n* (%)				0.50
None	160 (46.0)	124 (47.3)	36 (41.9)	
MMC	50 (14.4)	40 (15.3)	10 (11.6)	
BCG	113 (32.5)	80 (30.5)	33 (38.4)	

Abbreviations: BCG = Bacillus Calmette-Guerin; IQR = interquartile range; MMC = mitomycin C; MP = muscularis propria; RASI = renin-angiotensin system inhibitor

TABLE 2. Types of renin-angiotensin system inhibitors.

Renin-angiotensin system inhibitors	n = 86
Angiotensin-converting enzyme inhibitor, n (%)	38 (44.2)
Enalapril	32 (37.2)
Imidapril	1 (1.2)
Nootropril	1 (1.2)
Peridopril	1 (1.2)
Quinapril	3 (3.5)
Angiotensin II receptor blockers, n (%)	48 (55.8)
Irbesartan	5 (5.8)
Losartan	28 (32.6)
Olmesartan	1 (1.2)
Telmisartan	2 (2.3)
Valsartan	12 (14)

TABLE 3. Tumor recurrence and stage progression.

	All patients (n=348)	No-RASIs (n = 262)	RASIs (n = 86)	*P-value*
Tumor recurrence	140 (40.2)	121 (46.2)	19 (22.1)	<0.001
Stage progression	19 (5.5)	16 (5.67)	3 (3.5)	0.46

Abbreviations: RASI = renin-angiotensin system inhibitor

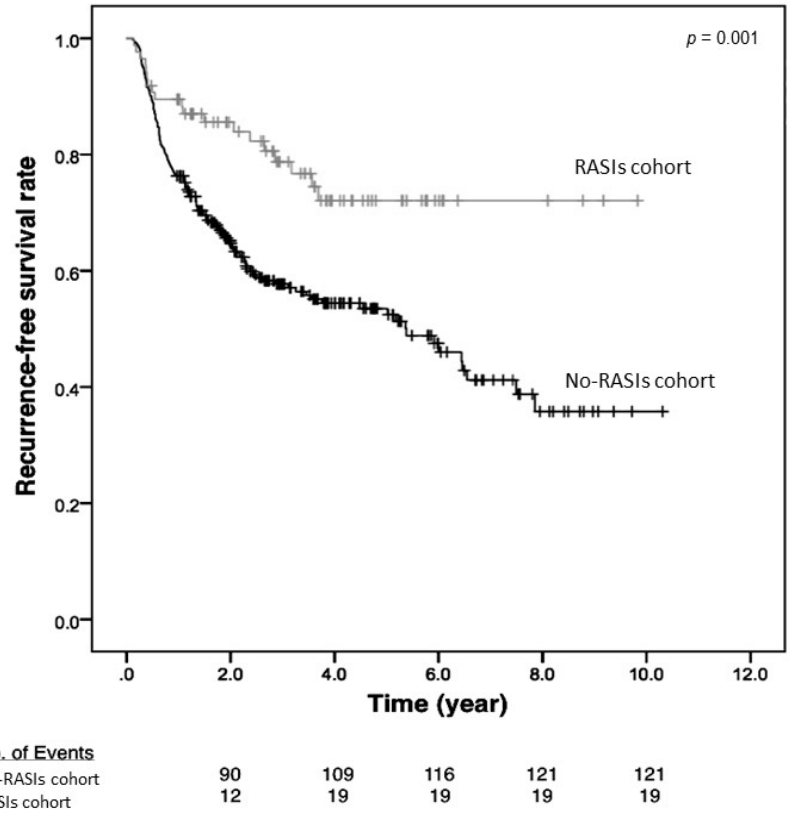


Fig 1. Kaplan-Meier curves demonstrates recurrence-free survival between 2 cohorts of patients: RASIs (gray) and no-RASIs (black).

TABLE 4. Univariate and multivariate analyses for factors associated with tumor recurrence.

	Univariate analysis		Multivariate analysis	
	HR (95 % CI)	*P-value*	HR (95 % CI)	*P-value*
Age ≥ 65 years	1.25 (0.88-1.78)	0.22		
Female	0.92 (0.63-1.36)	0.69		
Smoking		0.31		
Former	0.92 (0.63-1.34)			
Current	0.69 (0.43-1.12)			
Intravesical		0.26		
MMC	1.12 (0.70-1.81)			
BCG	0.83 (0.57-1.21)			
MMC+BCG	0.55 (0.25-1.20)			
High grade	1.16 (0.83-1.61)	0.39		
Tumor size ≥ 3 cm	1.56 (1.12-2.19)	0.009	1.50 (1.07-2.10)	0.018
Tumor multifocality	1.53 (1.10-2.14)	0.012	1.49 (1.07-2.09)	0.019
Administration of RASIs	0.45 (0.28-0.73)	0.001	0.46 (0.29-0.75)	0.002

Abbreviations: BCG = Bacillus Calmette-Guerin, CI = confidence interval, HR = hazard ratio, IQR = interquartile range, MMC = mitomycin C, RASI = renin-angiotensin system inhibitor

associated with reduced risks of tumor recurrence and disease progression in NMIBC patients.[11,12]

Of the 348 patients in the present study, 140 (40%) and 19 (6%) patients experienced tumor recurrence and progression, respectively. The incidences in our study were similar to those in research by Yuge et al., which demonstrated that 39% of patients had tumor recurrence and 5% had stage progression.[11] On multivariate analysis, RASI administration was significantly associated with a reduced risk of tumor recurrence in our study as well as in the work of Yuge et al. and Blute et al.[11,12] Nevertheless, none of the studies showed that RASIs were associated with disease progression. In our study, the RASI cohort had a significantly greater 5-year RFS rate than that of the no-RASI cohort (78% versus 54%, p=0.001). This was comparable to the 5-year RFS rates of the study by Yuge et al., which were 78% and 53% for the RASI and no-RASI cohorts, respectively, (p=0.01).[11]

Patients with NMIBC after a TUR-BT are classified based on the risk stratification of recurrence and progression.[2-4] Different further management strategies are employed for each group of NMIBC patients. As to the European Organization for Research and Treatment of Cancer (EORTC) risk table, patients with multiple or large tumors are at high risk for bladder cancer recurrence and stage progression.[2–4,13–15] Our study revealed that tumor multifocality and a tumor size greater than 3 cm were associated with an increased risk of tumor recurrence. Millán-Rodríguez et al. studied a cohort of 1,529 patients with NMIBC; their Kaplan–Meier analysis demonstrated that tumor recurrence was statistically significant for multiple tumors and a large tumor size (p<0.001). On multivariate analysis, multifocality and a large tumor were also associated with an increased risk of tumor recurrence (OR 2.0, 95% CI 1.6–2.4; and OR 1.7, 95% CI 1.3–2.0, respectively).[13] It has been suggested that high-risk NMIBC patients should receive intravesical BCG to prevent recurrence and progression.[2-4,13-15]

Previous meta-analyses have shown that intravesical therapies significantly decrease the risk of recurrence in NMIBC patients.[5] The type of intravesical therapy used depended on the risk profile of each patient. BCG therapy showed better outcomes in terms of a reduction in tumor recurrence than a TUR-BT alone or intravesical chemotherapy.[5] Nonetheless, some patients could not tolerate its adverse effects, and some tumors still refracted or relapsed after treatment.[6,16] In our study, intravesical therapy, including BCG and MMC, was not associated with tumor recurrence and disease progression. The number of patients recruited for the study could be the

explanation, given that the role of the RASIs was our primary outcome.

The major limitation of our study was its retrospective design with intermediate follow-up. As to the historic data, the tumor stages and grades differed slightly from the current classifications. While patients given intravesical BCG in other studies showed a reduced risk of tumor recurrence and progression, our study had too limited a number of patients to show similar outcomes. In addition, supplies of intravesical BCG were unavailable in Thailand during several periods, thereby precluding the utilization of BCG based upon patient risk classifications. Nevertheless, there was a sufficient number of patients in our study to demonstrate the impact of RASIs on bladder cancer recurrence. To our knowledge, this is the first study to reveal the impact of RASIs on NMIBCs in Thailand. A prospective study will be needed to further evaluate the effectiveness of RASIs in decreasing NMIBC recurrence and stage progression.

CONCLUSION

Our study suggests that RASI administration in patients with NMIBC might be a potential factor to prevent bladder cancer recurrence. To the best of our knowledge, this is the first study in Thailand to address the benefits of RASIs for NMIBC patients. Further study is needed to evaluate the effects of RASIs on NMIBC patients.

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Cancer Pain Management: Is It Still Problematic?

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ABSTRACT

Objective: To evaluate the efficacy of pain management during 3-month follow-ups for outpatients with cancer pain.

Methods: A retrospective chart review was conducted and included all newly diagnosed cancer pain patients visiting the Siriraj Pain Clinic, Mahidol University, between January 2013 and June 2014. Demographic data, pain intensity at first visit, 1-, 2- and 3-month follow-ups, pharmacological therapy and co-treatments were collected. Good treatment response was defined as more than 30% pain reduction from baseline. Predictive factors associated with pain treatment response were also assessed.

Results: Out of 432 new patients, 118 cancer pain patients were included in the study with a mean age of 59.8±13.7 years (range 18-91 years). About half of patients had at least one comorbidity. Over 90% of all cancer patients presented with distance metastasis. Mixed neuropathic/nociceptive pain (53.4%) and nociceptive pain alone (43.2%) were common pain features in cancer pain patients. The mean initial pain intensity described by verbal numerical scales was 7.7±2.1 (range 3-10). The majority of patients (60.2%) received co-treatment. The main pharmacological therapies in all patients were opioids (99.2%) and anticonvulsants (90.7%). At 3-month follow-up, nearly half of patients achieved a good treatment response. However, 44.6% of good responders still had moderate and severe pain. No predictive factors associated with the pain treatment response was found.

Conclusion: Approximately half of patients with cancer pain in the pain clinic achieved a good treatment response whereas one-fifth of the patients had an increase in pain severity at their 3-month follow-up.

Keywords: Cancer pain; clinical response; pain management; predictive factor (Siriraj Med J 2019;71: 38-43)

INTRODUCTION

Pain is one of the most common symptoms in cancer patients (70-90%),[1] negatively affecting their functionality, emotion and quality of life.[2,3] Multiple factors are involved in the pain mechanism, including the disease itself, treatments, and comorbidities.[4] Although cancer pain treatment guidelines were developed by the World Health Organization (WHO) in the year 1986, and subsequently modified in 1996,[5] treatment outcomes still remain unsatisfactory.[6] For example, Deandrea reported 43% of cancer patients suffered from moderate to severe pain.[7] In addition, Yennurajalingam reported that only 45% of cancer patients in the United States receiving treatment experienced 30% pain improvement on a numerical rating scale.[8]

Regarding the difference of culture among the nations

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which may affect to pain acceptance and factor-related to pain treatment response, the treatment outcome data of Thai patients is required to explore. However, there has been no study reporting the effectiveness of cancer pain management services in Thailand. Therefore, this study aimed to evaluate the efficacy of cancer pain management and identify factors associated with the pain treatment response during 3-month follow-ups at the Siriraj Pain Clinic.

MATERIALS AND METHODS

The study was approved by the Siriraj Institutional Review Board (Si 703/2014). The researchers retrospectively and manually reviewed outpatient records of all newly diagnosed cancer pain patients who visited Siriraj Pain Clinic from January 1st, 2013 to June 30th, 2014. Cancer patients with non-cancer pain conditions and patients who were lost before the 3-month follow-up were excluded. Demographic data including age, gender, living region, comorbidities (diabetes mellitus, hypertension, heart disease and chronic kidney disease), primary cancer site (gastrointestinal tract, respiratory tract, urogenital tract, breast, etc.), pharmacological therapy and co-treatments were recorded. Pain intensity using verbal numerical scales (VNS) at first visit, 1-, 2- and 3-month follow-ups were collected as the primary outcomes.

Pain severity was classified into three levels which were mild pain (VNS 0-3), moderate pain (VNS 4-6) and severe pain (VNS 7-10).

Responses to treatments varied from time to time, even for individual patients. The timing of pain evaluation was crucial. If pain was evaluated after a few weeks following treatment, the number subjects in the non-responding group may be appear elevated because it could be too early to see a response. Conversely, it is possible evaluating pain at distant time periods could allow the subjects' cancer to progress and cause more pain, reducing the number in subjects the responsive group. Therefore, the optimum time for evaluating the efficacy of pain treatment in this study was set at three months from the initial treatment. Factors associated with pain treatment responses were also analyzed.

Statistical analysis

The sample size was calculated based on the study of Yennurajalingam et al[8] and required 40% proportion of patients to achieve good treatment response at 3-month follow-up. We selected the confidence level 95% and allowable error 10% to calculate by Query program. Calculated sample size was 93 patients with 10% dropout. Therefore, at least 102 patients were included in this study.

The qualitative data, including gender and main site of cancer, was described as numbers and percentages. The quantitative data, consisting of the initial pain scores, numbers of visits, and age, was described using mean and standard deviation.

According to Farrar's study, the researchers defined good treatment response as the pain reduction of at least 30% using a verbal numerical scale.[9] To compare the good treatment response group and the poor treatment response group (VNS pain responses less than 30%), unpaired t-test (normality) or Mann-Whitney U-test (non-normality) were used to analyze quantitative data and Chi-square test or Fisher's exact test were used for qualitative data. Logistic regression analysis was applied when there were one or more statistically significant factors ($p < 0.05$). All parameters were analyzed by SPSS program version 15.0.

RESULTS

After screening outpatient charts, 432 cancer pain patients were reviewed; of these 314 were excluded due to attrition or incomplete follow-ups during the 3 months. A total of 118 cancer patients were included in this study (Table 1), 50.8% of which were males with the median age of 60 years old (range 18-91 years old). About half of the patients had at least one comorbidity. Most of the patients presented with advanced stage of cancer, accounting for 91.5% of the total number. The majority of patients suffered from nociceptive and mixed pain. The most common adjuvant treatment (apart from pain medication) was radiation (28.8%) while only two patients underwent the pain intervention. Regarding pharmacological treatment, almost all of the patients (117 patients) were prescribed opioids (Table 2). The most common opioid used was morphine (54.7%), while the most common adjuvant drugs were anticonvulsants (90.7%). Dosages for opioid therapy in each visit have been presented in Table 3.

The overall outcome after cancer pain treatment found that the mean VNS decreased from 7.7±2.1 to 5.7±2.6 at the 1-month follow-up and maintained at this level until 3-month (Table 3). Moreover, there were 47.5% of the patients achieving good treatment response while 44.6% of which still suffered from moderate to severe pain at three months after treatment (Fig 1). However, 19.5% of the patients got worse even after 3 months of pain treatment.

Many potential factors associated with treatment outcome were analyzed, including age, gender, comorbidity, primary organ tumour, stage of cancer, initial pain intensity, types of pain, adjuvant therapy, type and dose

TABLE 1. Demographic data.

Patient characteristics	N=118
Age (yrs)	60 (18-91)
Gender M:F	60 (50.8):58 (49.2)
Comorbidity	
0	62 (52.5)
1	34 (28.8)
≥ 2	22 (18.6)
Primary tumor	
Gastrointestinal system	26 (22.0)
Respiratory system	20 (16.9)
Others	72 (61.0)
Stage of cancer	
Local invasion	10 (8.5)
Distant metastasis	108 (91.5)
Initial pain intensity (verbal numerical scale)	8 (3-10)
Types of pain	
Nociceptive	51 (43.2)
Neuropathic	4 (3.4)
Mixed pain	63 (53.4)
Co-treatment	
Radiation therapy	34 (28.8)
Chemotherapy	24 (20.3)
Surgery	10 (8.5)
Pain intervention	2 (1.6)

Data are presented as median (range), n (%)

TABLE 2. Medications used in pain treatment on 3-month follow-up visit.

Drugs	N=118
Opioids	117 (99.2)
Strong opioid	78 (66.1)
morphine	64 (54.7)
fentanyl	12 (10.3)
methadone	2 (1.7)
Weak opioid	39 (33.1)
codeine	4 (3.4)
tramadol	35 (29.9)
Acetaminophen	49 (41.5)
NSAIDs	37 (31.4)
Antidepressant	70 (59.3)
Anticonvulsant	107 (90.7)

Data are presented as n (%), NSAIDs = Non-steroidal anti-inflammatory drugs

TABLE 3. Opioid dosage and average pain intensity in each visit for 3 months.

Opioid	1st visit	1-month	2-month	3-month
Morphine (mg/day)	20 (10-180)	20 (10-200)	20 (10-180)	35 (10-180)
Fentanyl (mcg/hr)	12 (12-25)	25 (12-100)	25 (12-50)	25 (12-37)
Methadone (mg/day)	10 (10-10)	10 (10-15)	20 (10-30)	10 (10-10)
Codeine (mg/day)	60 (45-90)	60 (45-90)	45 (45-60)	45 (30-60)
Tramadol (mg/day)	150 (50-400)	150 (50-300)	150 (100-300)	150 (100-200)
VNS	7.7±2.1	5.7±2.6	5.8±2.9	5.7±3.1

Data are presented as median (range), mean±SD
Abbreviation: VNS = verbal numerical scales

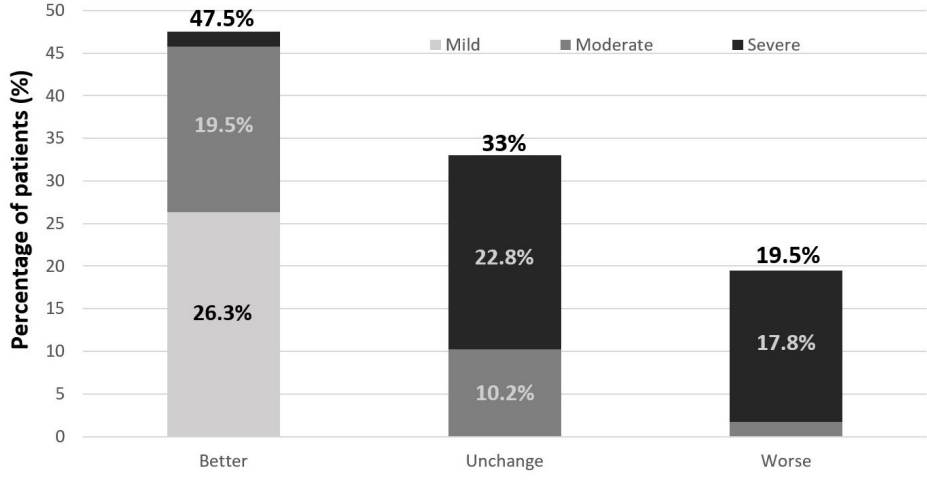


Fig 1. Pain treatment response at 3-month follow-up.

of opioids, etc. However, there were no predictive factors significantly associated with the pain treatment response (Table 4).

DISCUSSION

This study evaluated outcomes of cancer pain treatment at the Siriraj Pain Clinic at three months after treatment. Even though after following regimens provided by guidelines for cancer pain management[5], average pain score was reduced from 7.7 to 5.7 which was about 26% pain reduction. Also, 42.3% of patients were still in severe pain and less than half achieved good treatment response. However, this data was comparable to Yennurajalingam's study, in which the responsive group was accounted for 45% of subjects.[8] Regarding the treatment outcome in this study revealed that cancer pain is still problematic even in the tertiary pain center.

Considering about one-fifth of patients in this study and one-third of patients in the Yennurajalingam's study developed worse pain over 3 months,[8] this could be attributed to either rapid cancer progression or the duration of the visits per month was perhaps too long, particularly in advanced stage cancer patients. Therefore, more frequent follow-up visits should be set in the case of uncontrolled pain. To address some of the difficulties that were encountered, for example, remoteness from the hospital or physical limitations, a telephone interviewing program is advisable. In addition, some patients might not well tolerated to higher dose of opioids or doubted about using opioid therapy which could be another hindrance to increase opioid dosage. Interestingly, mean opioid dosage appeared to be stable from the first visit to 3-month visit, which should have been increased in the following visits if the pain could not be controlled

TABLE 4. Factors associated with pain treatment outcomes.

Factors	Good treatment response n=56	Poor treatment response n=62	P-value
Age	58.7±13.5	60.8±14.0	0.418
Female	28 (50)	30 (48.4)	0.861
Living outside Bangkok and metropolitan area	27 (48.2)	35 (56.5)	0.371
Comorbidities	25 (44.6)	31 (50)	0.561
Primary organ tumor			
Gastrointestinal system	12 (21.4)	14 (22.6)	
Respiratory system	9 (16.1)	11 (17.7)	0.949
Others	35 (62.5)	37 (59.7)	
Advanced cancer stage	50 (89.3)	58 (93.5)	0.515
Initial pain intensity	8.0±2.1	7.4±2.1	0.976
Types of pain			
Nociceptive	28 (50)	23 (37.1)	
Neuropathic	2 (3.6)	2 (3.2)	0.348
Mixed	26 (46.4)	37 (59.7)	
Co-treatment			
Radiation therapy	13 (23.2)	21 (33.9)	0.202
Chemotherapy	13 (23.2)	11 (17.7)	0.461
Surgery	4 (7.1)	6 (9.7)	0.746
Pain intervention	0 (0)	2 (3.2)	0.497
Opioid dosage			
Morphine	45.0±36.5	46.8±35.3	0.803
Fentanyl	24.8±8.8	28.3±9.4	0.492
Tramadol	145±35.9	153±35.2	0.793

Data are presented as mean±SD, n (%)

(Table 3). Nevertheless, the poor treatment response group seemed to consume only a little more opioids than the good response group (Table 4), presenting that opioids might not help in the poor response group or they experienced some problems to increase opioid consumption.

The previous study found that high initial pain intensity was a factor associated with good clinical response in outpatients.[8] In contrast, another retrospective study in inpatients demonstrated that high pain intensity on the first day of consultation was related to poor treatment response.[10] However, there was no association between initial pain intensity and the pain treatment response in this study. In addition, no significant association of any potential factors to the treatment response was observed.

Although this study was a retrospective study, all data were obtained by pain specialists and well-trained nurses in pain management, which made the data more reliable. However, there were some limitations in this study. Firstly, some data were not documented in some included charts, for instance, the side effects, the frequency of incidental pain or effects of pain on emotion and function, which may affect the treatment outcomes. Therefore, we decide not to present and analyze those data in this study. Secondly, the number of patients may not be enough to detect significant factors associated with the

treatment response. Although there were a large number of cancer patients consulted the pain clinic, about 73% of reviewed charts were excluded from this study mainly due to incomplete follow-up in three months, which may cause by patients' death within three months after the first visit or some difficulty of traveling to-and-from the hospital. Despite the limitations, the results of this study serve as baseline data for cancer pain treatment and further development of guidelines for cancer pain management.

Future prospective studies should be conducted to compare the effectiveness of treatments between different cancer service centers. However, future prospective research with cancer pain management protocol should be developed to improve the effectiveness of cancer pain treatment. Also, the psychological aspects played an important role in cancer patients and could not be solved only by pain medications. Multidisciplinary teams, including psychiatrists and behavioral therapists, could offer better care and help improve the treatment's efficacy.

CONCLUSION

Approximately half of patients with cancer pain in the pain clinic achieved a good treatment response whereas one-fifth of the patients had an increase in pain severity at 3-month follow-up. There is no significant factor that associated with treatment response.

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Patient Interviews Improve Empathy Levels of Preclinical Medical Students

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ABSTRACT

Objective: In order to cultivate and maintain empathy during medical school, an experiential learning program, "A Patient as a Human Being", was designed to promote empathy in second-year medical students through interviews with patients focusing on their suffering and the difficulties arising from their illnesses and hospital stays.

Methods: The second-year medical students were divided into groups of three and four. Each group was assigned a patient to interview under close supervision. The selected patients were informed beforehand about the interview and voluntarily agreed to participate. The Thai version of the Jefferson Scale of Physician Empathy–Student Version (JSPE-SV) was used to assess the students' empathy levels.

Results: The baseline JSPE-SV score (n = 310) was 114.10±10.20. After the interview, the scores significantly increased (1.19 [0.21-2.18], P = 0.009). Students in the lower-half group of baseline scores showed a higher improvement (2.64 [1.14–4.15], P < 0.001) than those in the upper-half group. The difference coefficient by multivariate analysis of the improved JSPE–SV scores between the two groups was 3.03 [1.08-4.98] (p = 0.002), accompanied by a correlation between the pre-activity empathy score and the improved score (p = - 0.21, P-value < 0.01).

Conclusion: The patient interviews improved the empathy levels of the preclinical medical students, especially those with lower baseline empathy levels.

Keywords: Empathy; preclinical medical student; experiential learning; patient interview (Siriraj Med J 2019;71: 44-51)

INTRODUCTION

Empathy has been described as the ability to understand and accept the feelings of other people.[1-3] In medicine, empathy may be defined as the capability of physicians to accurately comprehend the mental status of their patients.[1] Importantly, empathy has been described as one of the important components of the doctor-patient relationship in the provision of holistic patient care.[4–8] Nevertheless, the empathy level of medical students tends to decline during medical school.[8-14] Therefore, empathy maintenance and improvement during a course of medical training has been emphasized.[5,7, 15-18] Various activities have been created to enhance empathy among medical students, including communication-skill training in various formats, such as didactic lectures, experiential learning, role-playing, and reflective writing after a learning session with standardized patients. However, students' empathy levels were not measured as part of those activities.[19, 20]

To study empathy in medicine, various questionnaires have been developed as a measurement tool, such as the Jefferson Scale of Physician Empathy (JSPE)[21], the Empathy Test (ET)[22], the Balanced Emotional Empathy

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Scale (BEES)[23], and the Consultation and Relational Empathy (CARE).[24] Among those tools, the JSPE is the most widely used instrument for assessing the empathy of healthcare professionals, including medical students. The JSPE–Student Version (SV) has been developed and validated into many languages, including Thai.

To cultivate empathy in preclinical medical student, the development program entitled "A Patient as a Human Being" was designed as a mandatory activity in the Humanistic Medicine course for second-year medical students. Through a group interview of a patient admitted to Siriraj Hospital, students focused on the patient's suffering and the difficulties arising from the illness and hospital stay. After the interview, each student submitted a piece of reflective writing exploring the interview experience. To evaluate the effects of this experiential learning on students' empathy, the Thai version of the JSPE–SV was utilized.

MATERIALS AND METHODS

Participants

The Thai medical school system has implemented a 6-year curriculum for the bachelor's degree in Doctor of Medicine (M.D.). The first year, referred to as the pre-medicine year, focuses on general education and/or the liberal arts. The next two years are the preclinical years, and the remainder of the course is the clinical years. The present study focused on preclinical medical students at the Faculty of Medicine Siriraj Hospital, in particular, the second-year medical students (n = 325) participating in the activity "A Patient as a Human Being", which is a part of the compulsory Humanistic Medicine course. In all, 310 students (n = 310) voluntarily participated in the study. However, 14 of those students were subsequently excluded because their questionnaires were incomplete.

Instrument

The Thai version of the Jefferson Scale of Physician Empathy–Student Version (JSPE–SV)[25] was used to evaluate the empathy level of each medical student before and after participating in the activity "A Patient as a Human Being". The JSPE–SV comprises twenty self-reported statements. Students rated each statement from one to seven, which represented the spectrum from "strongly disagree" to "strongly agree", respectively. The total score could range from 20 to 140 points. Participants with a higher score were regarded as having a higher level of empathy than those with a lower score. The development of the Thai version of the JSPE–SV by the back-translation procedure had been previously examined, and it had demonstrated acceptable validity and reliability.[25]

Intervention

"A Patient as a Human Being" is one of the learning activities in the Humanistic Medicine course for second-year medical students at the Faculty of Medicine, Siriraj Hospital. It provides them with first-hand experience in interviewing a patient regarding the suffering arising from having a disease and the difficulties associated with a hospital stay. In other words, this activity provides the opportunity for the preclinical medical students to explore and to feel suffering and hardship from a patient's perspective. Before the activity, the professional manners to be employed during the interview were introduced. Each group of three or four medical students subsequently met with an assigned faculty member and a fifth-year medical student to prepare for the patient interview. The recruitment of each selected inpatient was based on voluntary permission to participate in the activity. A thirty-minute interview was conducted by a group of preclinical students under the supervision of a senior medical student or faculty member. After the interview, the second-year students reflected on their experiences through conversation with an assigned faculty member and reflective writing.

To investigate the effects of the activity on the empathy levels of second-year medical students, a study protocol was submitted to, and approved by the Siriraj Institutional Review Board (Si 759/2016). Details of the research were subsequently explained to second-year medical students. Each student who voluntarily decided to participate later anonymously completed the Thai version of the JSPE-SV. This was done before and after their patient interview, using a web-based questionnaire created by the Undergraduate Medical Education Unit of the Faculty of Medicine, Siriraj Hospital. The empathy scores before and after the interview and reflection tasks were designated as the pre-activity and post-activity scores, respectively.

Data analysis

Data acquired from the electronic database consisted of the pre- and post-activity scores, and demographic information such as gender and grade point average (GPA). These underwent a quantitative analysis using the R Statistical System, version 3.2.5, with the built-in library and significance threshold set to 0.05.

To determine the effects of the interview-reflection activity on the empathy scores, the pair-wised difference between the pre-activity and post-activity scores (which represents improvement) was examined with the one-tailed dependent *t*-test. To determine the factors associated with an improvement in the empathy scores, further

analyses were performed on subgroups of students in terms of their gender, GPA, participation in university activities, experience in taking care of patients with chronic illnesses, and their having heard the word "empathy" before admission to the medical school.

To transform the GPA and the pre-activity score from numerical data to categorical data, the median cut-point was applied to classify these samples into two subgroups. For instance, with the "Higher GPA" vs. the "Lower GPA" subgroups, the cut point was the median of the GPAs of the participants. As a further example, in the case of the "Higher pre-activity score" vs. the "Lower pre-activity score" subgroups, the cut point was the median of the pre-activity scores of the participants. Since the data were categorized into at most two subgroups for each variable, the difference between the subgroups for each variable was evaluated by the two-tailed independent *t*-test. The Pearson correlation coefficient was applied to evaluate any association between the numerical factor and the pre-activity empathy score.

To study the factors determining the pre-activity empathy scores, a comparison was made of the score for each subgroup within the same variable (e.g., the "Higher GPA" vs. the "Lower GPA" subgroups). A univariate analysis was performed on each variable using a two-tailed independent t-test for categorical factors. Multivariate analyses were performed on the between-subgroup difference using a multivariate linear regression model to adjust for confounding effects.

RESULTS

Of the 325 second-year medical students, 310 students (95.38%) undertook the web-based questionnaire, with 296 (91.08%) of the questionnaires being fully completed. The 14 students with incomplete data were excluded from this study. The demography of the categorical and numerical variables is presented in Tables 1 and 2, respectively.

Association factors determining pre-activity scores

Unadjusted associations between the pre-activity empathy scores and the candidate variables demonstrated no statistically significant differences. The detailed results of the association factors that determined the pre-activity scores are in Table 3.

Improvements in empathy scores

Overall, the statistical analyses revealed a significant increase in the empathy scores from the pre- to the post-activity scores (1.19 [0.21–2.18], P-value = 0.009). The unadjusted comparisons within the subgroups showed significant improvements in the empathy scores of the following subgroups: male students (1.31 [0.01–2.62], P-value = 0.025); students with a higher GPA (1.60 [0.35–2.85], P-value = 0.007); students with no participation in the extracurricular activities of the Mahidol University (2.06 [0.25–3.87], P-value = 0.014); students who had experience of taking care of a patient with chronic illness (1.84 [0.37–1.31], P-value = 0.008); students with no experience of hearing the word "empathy" before admission to the medical school (1.81 [0.11–3.51], P-value = 0.020); and students with a lower pre-activity empathy score (2.64 [1.14–4.15], P-value < 0.001). When the improved empathy scores were compared between the subgroups, the only statistically significant factor was the pre-activity score. From the multivariate analysis, the students in the "Lower pre-activity" group had a significantly higher improvement in empathy score than those in the "Higher pre-activity" group (P-value = 0.002). When the GPA and pre-activity empathy score were considered as a scale variable, there were significant degrees of correlation between the improvement and GPA (p = 0.13, P-value = 0.03) and between the improvement and pre-activity empathy score (p = - 0.21, P-value < 0.01). These correlations supported the findings of the categorical groups' comparison. The details of the statistical analyses are given in Tables 4 and 5.

DISCUSSION

"A Patient as a Human Being", a truly experiential learning activity that improves the empathy levels of preclinical medical students, involves authentic patients. This distinguishes it from previously reported activities using simulated medical consultations with standardized patients[26] or clinical interview training with simulated situations[27] Through direct communication with authentic patients, "A Patient as a Human Being" offers a unique learning experience designed to cultivate empathy among preclinical students participating in an otherwise traditional medical curriculum. Well-developed communication skills are essential for a doctor to express empathy and to give high quality care.[28-30] Conversations with real patients have previously been reported to increase and maintain the empathy levels of medical students.[31,32]

The JSPE is an instrument that has been used by many previous studies to measure levels of empathy as well as to evaluate the effects of learning activities designed to improve and maintain empathy[13,15,26,33-35] Importantly, the JSPE has been translated into Thai and validated on Thai medical students.[25] Therefore, the Thai version of the JSPE was the best available tool to study empathy levels in Thailand.

TABLE 1. Demographic information of the numerical data.

Numerical variables	Min. - Max.	Mean	S.D.
Age (years)	18 - 21	19.58	0.59
Grade point average	2.13 - 4.00	3.48	0.33
Pre-activity empathy score	76 - 140	114.10	10.20
Post-activity empathy score	72 - 140	115.30	11.88

Abbreviations: min. = the minimum value, max. = the maximum value, S.D. = standard deviation of the mean

TABLE 2. Demographic information of the categorical data.

Categorical variables	N (% of total)
Gender	
Male	181 (61.15)
Female	115 (38.85)
Grade point average	
High (Mean = 3.75, S.D. = 0.13)	149 (50.34)
Low (Mean = 3.20, S.D. = 0.24)	147 (49.66)
Participation of university activities	
Yes	183 (61.82)
No	113 (38.18)
Experience in caring for a patient with chronic illnesses	
Yes	135 (45.61)
No	161 (54.39)
Having heard the word 'empathy' before admission to the medical school	
Yes	186 (62.84)
No	110 (37.16)

Abbreviations: N = numbers of subject, S.D. = standard deviation of the mean

In this study, the baseline empathy score of the second-year medical students was 114.10±10.20, which was similar to the mean score of the second year medical students at another medical school in Thailand.[25] The Faculty of Medicine, Siriraj Hospital, introduces the word "empathy" to first-year medical students through the mandatory course entitled Medical Profession. Therefore, the term "empathy" was not considered a new word for the second-year medical students.

Previous studies have demonstrated associations between empathy levels and factors such as gender and education level. For example, female students have been shown to have a higher mean empathy score than male students.[14,15,25,35,36] In contrast, in the current study, the relatively limited number of female medical students might have been responsible for there being no significant difference in the empathy levels of the genders. In addition, it has been demonstrated that higher education levels result in higher empathy scores.[37] However, a high GPA has also been associated with lower empathy scores.[38] Furthermore, it has been demonstrated that medical students attending any service activity (such as the free clinic for patients without insurance) have a higher empathy score than those who have never attended.[35]

In contrast to the findings of the other studies, the present study did not find any association between

TABLE 3. Associations of the pre-activity empathy score with corresponding subgroup analysis for each candidate variable.

Variables	N	Mean	S.D.	Between Subgroup Difference	Lower 95% CI	Upper 95% CI	P-value
Gender				0.29	-2.07	2.65	0.808
Male	181	114.20	10.55				
Female	115	113.91	9.73				
Grade point average				0.21	-2.13	2.55	0.861
High	149	114.19	10.89				
Low	147	113.99	9.53				
Participation of university activities				- 0.17	- 2.54	2.20	0.889
Yes	183	114.03	10.53				
No	113	114.19	9.75				
Experience in taking care of a patient with chronic illnesses				- 0.79	- 3.12	1.53	0.501
Yes	135	113.66	9.47				
No	161	114.45	10.83				
Having heard the word 'empathy' before admission to the medical school				0.71	- 1.73	3.15	0.567
Yes	186	114.35	10.14				
No	110	113.65	10.39				

Abbreviations: N = numbers of subject, CI = Confidence Interval

the empathy scores and gender, GPA, participation in university activities, experience of taking care of a patient (e.g., a family member) with chronic illnesses, or having heard the word "empathy" before admission to the medical school. According to the current study, for each subgroup comparison (Table 3), male gender, having a higher GPA, the non-participation in university activities, no experience of taking care of a patient with chronic illnesses, and having heard the word "empathy" before medical school admission showed a positive trend to having a higher baseline empathy score. However, the differences in the empathy scores of all factors were without statistical significance.

"A Patient as a Human Being" was designed to improve and maintain the empathy levels of preclinical medical students. From the empathy scores measured by the JSPE–SV, Thai version, the student participants in the present study demonstrated an improvement of 1.19 [0.21–2.18] in the effect size, and with statistical significance (P = 0.009). Many activities[19] have been reported by other studies to improve empathy levels, for example, simulated medical consultations using standardized patients with subsequent reflection by students on the patients' feelings about their diseases and the doctors' feelings towards the patients.[26] In addition, participation in communication skills workshops[27] has also been demonstrated to improve empathy levels. Even though there was not much improvement in the scores, the current study strengthened the finding of many previous studies that experiential learning gained from authentic situations, simulations, or workshops is effective in improving empathy.

"A Patient as a Human Being" had a statistically significantly greater impact on the students who had a lower baseline empathy level than those with a higher level. The students with the lower baseline empathy level had a statistically significant improvement in effect size of 2.90 by univariate analysis and 3.03 by multivariate analysis. This study demonstrated that a medical school could improve the empathy levels of preclinical medical students through a patient interview and subsequent reflection on the patient's suffering. Moreover, a previous study found that medical students with a higher empathy level demonstrated a lower rate of empathy decrease than those with a lower baseline empathy level[14] Therefore, medical schools could sustain the empathy levels among students by providing an interview-reflection activity throughout their curriculum.

TABLE 4. Univariate analysis of within subgroup improvement of the empathy score after participation in 'A patient as human being' activity. Statistical analyses of empathy score within subgroup improvements and corresponding univariate subgroups were demonstrated.

Categorization	N	Within subgroup improvement	Lower 95% CI	Upper 95% CI	P-value
Overall	296	1.19	0.21	2.18	0.009
By Gender					
Male	181	1.31	0.01	2.62	0.025
Female	115	1.01	- 0.46	2.48	0.091
By GPA					
Higher	149	1.60	0.35	2.85	0.007
Lower	147	0.78	- 0.73	2.30	0.157
By experience of participation in university activities					
Yes	183	0.66	- 0.47	1.80	0.127
No	113	2.06	0.25	3.87	0.014
By experience of taking care of a patient with a chronic illness					
Yes	135	1.84	0.37	3.31	0.008
No	161	0.65	- 0.66	1.97	0.166
By experience of hearing the word 'empathy' before admission					
Yes	186	0.83	- 0.36	2.02	0.088
No	110	1.81	0.11	3.51	0.020
By pre-activity empathy score					
High score	148	- 0.26	- 1.47	0.96	0.660
Low score	148	2.64	1.14	4.15	< 0.001

Abbreviations: N = numbers of subject, CI = Confidence Interval

In the subgroup division of an unadjusted comparison (Table 5), differences between each subgroup were demonstrated using both univariate and multivariate analyses. In the case of the male medical students, a lower baseline empathy level, a high GPA, and no participation in university activities showed an improvement in empathy scores after participating in "A Patient as a Human Being". These observations imply that the benefits of this activity were not homogenous among medical students from different subgroups. In agreement with previous studies, there would appear to be no single activity capable of improving the empathy levels of students from diverse backgrounds, but the personal and professional development activities (such as communication skills workshops, interpersonal skills workshops, and literature and medicine programs) would help students realize the importance of empathy in the medical profession.[36]

One of limiting factors of this study was the differences among the patients participating in the interviews. To illustrate, some patients shared their stories with humor, whereas others were highly emotional, expressing their feelings tearfully through a large part of the interview. Furthermore, through the self-reflective writing, it was apparent that students from the same interview session demonstrated different degrees of perception regarding patients' sufferings and difficulties. Another possible limitation could be that "A Patient as a Human Being" is an intra-curricular activity. Therefore, there was no control

TABLE 5. Univariate and multivariate analysis of between subgroup difference of the empathy score after participation in 'A patient as human being' activity. Statistical analyses of empathy score between subgroup and corresponding univariate and multivariate subgroups were demonstrated.

Categorization	Univariate Analysis				Multivariate Analysis			
	Difference*	Lower 95% CI	Upper 95% CI	P-value	Difference* coefficient	Lower 95% CI	Upper 95% CI	P-value
By Gender								
Male > Female	0.30	- 1.68	2.28	0.765	0.41	- 1.60	2.42	0.691
By GPA								
Higher > Lower	0.82	- 1.16	2.79	0.417	1.24	- 0.73	3.20	0.217
By experience of participation in university activities								
No>Yes	1.41	- 0.74	3.55	0.197	1.44	- 0.59	3.47	0.164
By experience of taking care of a patient with a chronic illness								
Yes>No	1.19	- 0.80	3.16	0.240	1.64	- 0.34	3.61	0.104
By experience of hearing the word 'empathy' before admission								
No>Yes	0.98	- 1.12	3.07	0.356	0.97	- 1.06	2.99	0.348
By pre-activity empathy score								
Low score > High score	2.90	0.95	4.84	0.004	3.03	1.08	4.98	0.002

Note: Difference* refers to between subgroup difference
Abbreviation: CI = Confidence Interval

group to emphasize the true effect of this experiential learning. Last but not least, this study demonstrated only the short-term effect of the patient interviews and subsequent reflection; no long-term assessment was made.

CONCLUSION

"A Patient as a Human Being", a mandatory learning activity in a traditional six-year medical curriculum, provided first-hand experience for the preclinical medical students to feel patients' suffering from their current illnesses and hospital stays. After the interviews with the patients, through an experiential learning cycle, students reflected on what they had learned during the interviews and how they could help the patients as preclinical medical students. Participation in "A Patient as a Human Being" increased the second-year medical students' empathy levels, especially in the case of students with lower baseline empathy levels.

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Effect of Intraoperative Hypothermia on Surgical Outcomes after Colorectal Surgery within an Enhanced Recovery after Surgery Pathway

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ABSTRACT

Objective: The adverse effects of intraoperative hypothermia from the published literature were mainly based on non-enhanced recovery after surgery (ERAS) settings. This study aimed to determine association between intraoperative hypothermia and outcomes following colorectal surgery under ERAS pathway.

Methods: A prospectively collected database of patients undergoing elective colorectal surgery under ERAS pathway from 2011 to 2015 was reviewed. Patients were divided into 2 groups: hypothermic group (core temperature <36°C continuously exceeding 30 minutes during an operation) and normothermic group. Short-term outcomes were compared.

Results: This study included 195 patients: 150 (77%) in hypothermic group and 45 (23%) in normothermic group. Rectal surgery (OR=5.15), operative time exceeding 3 hours (OR=3.80), multi-organ resection (OR=3.12) and male gender (OR=2.62) were significant predictors for intraoperative hypothermia. Rates of postoperative complication and wound infection were comparable between hypothermic patients and normothermic patients (23% vs 13%; p=0.17 and 6.0 vs 6.7%; p=0.87, respectively). Hypothermic patients had a longer time to tolerate normal diet (2.0 days vs 1.3 days; p=0.023) but a comparable time to first bowel movement (2.6 days vs 2.6 days; p=0.84). Hypothermic patients had a significant longer hospitalization (5.7 days vs 4.4 days; p=0.048). A multivariate analysis showed that intraoperative hypothermia was an independent predictor for delayed food intake (OR=2.9, 95%CI=1.2-6.9; p=0.014) but not for prolonged hospitalization (OR=1.7, 95%CI=0.7-3.9; p=0.207).

Conclusion: Intraoperative hypothermia prolonged time to tolerate food intake after colorectal surgery within an ERAS setting but it did not adversely affect the return of bowel function, wound infection, complication and length of hospitalization.

Keywords: Hypothermia; enhanced recovery after surgery; colon; rectum; surgery; outcomes (Siriraj Med J 2019;71: 52-58)

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INTRODUCTION

During an intraabdominal operation, patient's body temperature decreases as a result of impaired thermoregulatory mechanisms secondary to anesthesia and heat loss through a surgical wound or to the environment.[1] According to the World Health Organization (WHO)[2] and the U.S. Agency for Healthcare Research and Quality,[3] intraoperative hypothermia is defined as a

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core temperature less than 36°C (96.8°F). Intraoperative hypothermia was associated with poor surgical outcomes after major abdominal operations including colorectal surgery.[4-6] It led to increased surgical bleeding and requirement of blood transfusion,[7] higher incidence of cardiac arrhythmia and ischemia,[4] higher rates of surgical site infection and prolonged hospitalization.[5] Maintaining perioperative normothermia in surgical patients is therefore an essential part of several surgical guidelines such as the latest WHO recommendations for surgical site infection prevention[2] and the Enhanced Recovery After Surgery (ERAS) society recommendations for perioperative care in elective colorectal surgery.[8,9]

Since the adverse effects of intraoperative hypothermia in colorectal surgery from the published literature were mainly based on non-ERAS settings,[5,10] strong evidence supporting this association in patients undergoing colorectal operations within an ERAS pathway is lacking. The current study aimed to determine the association between intraoperative hypothermia and surgical outcomes following colorectal surgery within an ERAS pathway.

MATERIALS AND METHODS

Patients

A prospective, observational study of adult patients undergoing elective segmental resection (colectomy and/or proctectomy) within an ERAS pathway from March 2011 to October 2015 in the Faculty of Medicine Siriraj Hospital, Thailand was conducted. Patients with clinical peritonitis or acute colonic obstruction were excluded. The study was approved by the Institutional Ethics Committee and written informed consent was obtained from each patient (Si 014/2013).

Perioperative and operative care

All of the studied patients were operated on and treated by a board-certified colorectal surgeon (the first author) who has applied an ERAS pathway into colorectal surgery since 2010. ERAS strategies in our institute were adopted from the ERAS society recommendations for perioperative care in elective colorectal surgery.[8,9] Some details of our ERAS program have been described previously.[11-13] Briefly, a practice of mechanical bowel preparation, prophylactic antibiotic regimen, prophylaxis of postoperative nausea and vomiting, anastomosis creation with or without stoma formation, analgesic regimen, early enteral feeding and immediate mobilization was standardized. However, there was no standardized protocol of active warming in an operating theater *except* blood warmer was used for intraoperative blood transfusion (if any). Notably, active warming was not standardized in our ERAS protocol due to the cost and availability of related equipment. Patients would be discharged from the hospital if they had no fever, good appetite, satisfactory gastrointestinal recovery and a good level of ambulation. All of the patients were scheduled for follow-up at 7-10 days and 30 days after an operation.

Diagnosis of intraoperative hypothermia

Intraoperative core temperature of the patients was continuously measured after induction of anesthesia using a single esophageal probe which was inserted by a staff anesthesiologist to the distal half of the esophagus. In this study patients were classified into a hypothermic group if their intraoperative core temperature was continuously below 36°C more than 30 minutes. A cut-off period of half an hour in a hypothermic state (< 36°C) was decided based on a previous study of >50,000 surgical patients which showed a trend of hypothermia-associated adverse outcomes from this time point.[14]

Data collection

Data including patient characteristics, operative details, and postoperative outcomes were prospectively collected. Patient characteristics included age, gender, body mass index (BMI), American Society of Anesthesiologists (ASA) grade, and ColoRectal Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity (CR-POSSUM) score.[15] Operative details included type of operation, operative time, and estimated blood loss. Overall ERAS protocol compliance of each case was determined based on the ERAS society recommendations for perioperative care in elective colorectal surgery.[8,9] Postoperative outcomes included postoperative complications (graded I-V according to the Clavien-Dindo classification system),[16] surgical site infection (based on the criteria of the U.S. Centers for Disease Control and Prevention),[17] time to first bowel movement, time to tolerate normal diet, length of postoperative stay, death and readmission within 30 days after the operation.

Statistical analysis

All of the data were prepared and compiled using the Statistical Package for the Social Sciences program version 18.0 for Windows (SPSS Inc, Chicago, IL). Continuous variables were expressed as mean ± standard deviation or median (interquartile; IQR), and were compared using the Student t-test or Mann-Whitney U test. Categorical data were expressed as number (percentage) and were compared using the Pearson Chi-square test or Fisher exact probability test. Factors influencing poor surgical

outcomes were analyzed using a univariate analysis. Only significant variables from the univariate analysis were included in a multivariate model of logistic regression, and the odds ratio with 95% confidence intervals (95%CI) for each variable was determined. A *p*-value of <0.05 was considered statistically significant.

RESULTS

This study included 195 patients: 150 (77%) in hypothermic group and 45 (23%) in normothermic group. Maximum, minimum and average intraoperative core temperature was significantly lower in the hypothermic group (Table 1). Patients in the hypothermic group tended to had a greater volume of intraoperative IV fluid (2.4 L vs 1.7 L; p=0.141) and more median blood loss (200 mL vs 100 mL; p=0.074). Patient's characteristics of each group are shown in table 1. Factors strongly associated with intraoperative hypothermia were rectal surgery (OR=5.15, 95%CI=2.25-11.79; p<0.001), operative time exceeding 3 hours (OR=3.80, 95%CI=1.82-7.93; p<0.001), multi-organ resection (OR=3.12, 95%CI=1.04-9.32; p=0.034) and male gender (OR=2.62, 95%CI=1.30-5.26; p=0.006).

TABLE 1. Patient's characteristics and intraoperative parameters.

	Hypothermic group (n=150)	Normothermic group (n=45)	P-value
Age, year	64.3 ± 13.0	61.6 ± 13.8	0.227
Male	85 (56.7)	15 (33.3)	0.006*
BMI, kg/m^2	23.3 ± 4.5	23.3 ± 4.7	0.968
ASA class ≥ 3	29 (19.3)	8 (17.8)	0.815
CR-POSSUM predictive mortality, %	1.85 (0.98-3.38)	1.37 (0.95-2.58)	0.382
Hematocrit, %	36.7 ± 5.2	35.9 ± 5.7	0.355
Serum albumin, g/dL	3.8 ± 0.6	3.8 ± 0.6	0.368
Cancer surgery	138 (92.0)	39 (86.7)	0.278
Tumor staging ≥ 3	91 (60.7)	26 (57.8)	0.729
Rectal surgery	79 (52.7)	8 (17.8)	<0.001*
Multi-organ resection	35 (23.3)	4 (8.9)	0.034*
Laparoscopic surgery	23 (15.3)	11 (24.4)	0.158
Epidural analgesia	48 (32.0)	10 (22.2)	0.208
Total IV morphine consumption, mg/kg	0.11 (0-0.57)	0.10 (0-0.50)	0.976
Core temperature, °C			
Maximum	36.0 (35.7-36.3)	36.6 (36.4-36.9)	<0.001*
Minimum	35.4 (35.0-35.7)	36.1 (36.0-36.4)	<0.001*
Average	35.8 (35.4-36.0)	36.5 (36.2-36.6)	<0.001*
Duration of surgery, hour	3.7 ± 1.5	3.1 ± 1.4	0.010*
Intravenous fluid, L	2.4 ± 1.1	1.7 ± 1.0	0.141
Blood loss, mL	200 (100-400)	100 (50-300)	0.074
Intraoperative blood transfusion, yes	20 (13.3)	4 (8.9)	0.426
Overall ERAS protocol compliance#, %	84.4 ± 6.2	85.8 ± 6.5	0.195

* P-value < 0.05

Values are expressed as mean ± standard deviation, median (interquartile range) or number (percentage).

Abbreviations: ASA = American society of Anesthesiologists, BMI = body mass index, CR-POSSUM = ColoRectal Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity, ERAS = enhanced recovery after surgery, IV = intravenous

#Overall compliance of each patient was determined based on the ERAS® society recommendations for perioperative care in elective colorectal surgery.

The incidences of postoperative complication and wound infection were comparable between hypothermic patients and normothermic patients (23% vs 13%; p=0.17 and 6.0 vs 6.7%; p=0.87, respectively). One patient in the normothermic group had a 30-day mortality while the other did not (p=0.12). Hypothermic patients had a longer time to tolerate normal diet (2.0 days vs 1.3 days; p=0.023) but a comparable time to first bowel movement (2.6 days vs 2.6 days; p=0.84). Hypothermic patients had a significant longer hospitalization (5.7 days vs 4.4 days; p=0.048) (Table 2).

A multivariate analysis adjusted for risk factors associated with delayed time to tolerate normal food (> 2 days) showed that intraoperative hypothermia was an independent predictor (OR=2.9, 95%CI=1.2-6.9; p=0.014) (Table 3). For predicting prolonged hospitalization (>5 days), a multivariate analysis showed that postoperative complication (OR=5.2, 95%CI=2.3-11.9; p<0.001) and operative time exceeding 3 hours (OR=3.4, 95%CI=1.8-6.4; p<0.001) were two significant risk factors. Intraoperative hypothermia was not associated with prolonged hospitalization (OR=1.7, 95%CI=0.7-3.9; p=0.207).

TABLE 2. Postoperative outcomes.

	Hypothermic group (n=150)	Normothermic group (n=45)	P-value
Overall complication	34 (22.7)	6 (13.3)	0.174
Complication excluding grade I#	19 (12.7)	3 (6.7)	0.265
Wound infection	9 (6.0)	3 (6.7)	0.870
30-day death	0	1 (2.2)	0.231
30-day readmission	5 (3.3)	3 (6.7)	0.389
Time to tolerate normal diet, days	2.0 ± 2.0	1.3 ± 1.3	0.023*
Time to first bowel movement, days	2.6 ± 1.1	2.6 ± 1.1	0.838
Length of hospitalization, days	5.7 ± 4.2	4.4 ± 2.6	0.048*

*P-value < 0.05
Values are expressed as mean ± standard deviation or number (percentage).
#According to the Clavien-Dindo classification of surgical complications

TABLE 3. Multivariate analysis of factors potentially associated with delayed time to tolerate normal diet (>2 days).

	Odds ratio	95% confidential interval	P-value
Intraoperative hypothermia	2.88	1.20-6.90	0.014*
Postoperative complication	1.99	0.97-4.09	0.059
Operative time exceeding 3 hours	1.81	0.97-3.38	0.059
Rectal surgery	1.58	0.86-2.92	0.142
Open surgery	1.32	0.61-2.89	0.482
Hypoalbuminemia	1.04	0.50-2.19	0.912
No epidural analgesia	0.84	0.44-1.64	0.621
Multi-organ resection	0.75	0.34-1.66	0.483

*P-value < 0.05

DISCUSSION

This study of 195 patients showed that intraoperative hypothermia was an independent risk factor for prolonged time to tolerate normal food in patients undergoing elective colorectal surgery. However, there was no association between intraoperative and time to first bowel movement, overall complication, surgical site infection or length of hospitalization. Rectal surgery, operative time exceeding 3 hours, multi-organ resection and male patients perceived greater risks of intraoperative hypothermia.

This study showed that intraoperative hypothermia was associated with delayed time to tolerate normal diet. Hypothermia-associated prolonged GI recovery could be explained by several possible mechanisms. First, the sympathetic nervous system is stimulated during the period of hypothermia to generate heat production and prevent further heat loss[18]. Neurotransmitters of the sympathetic nervous system such as adrenaline and noradrenaline are known to decrease GI motility and reduce luminal secretion.[19,20] Sympathetic stimulation also led to an inhibition of the vagus nerve-mediated gastric contractions[21] and decreased food appetite.[22] Second, the abnormal activities of the sympathetic nervous system in the GI tract may cause gut inflammation and motility disorders.[23] Third, in animal studies cold temperature diminished spontaneous movements of small bowel and depressed acetylcholine-induced contraction thus indicating that the tonic and phasic component of small bowel contraction are sensitive to cold temperature.[24] Although intraoperative hypothermia was an independent predictor for delayed time to tolerate solid food, it did not affect time to first bowel movement or time to discharge patients.

Within an ERAS setting this study failed to demonstrate a correlation between intraoperative hypothermia and postoperative complications including wound infection. These findings are similar to those reported in several large and recent studies examining an association between perioperative hypothermia and surgical site infection following colorectal surgery.[10,25,26] For example, Baucom and her colleagues showed that, regardless of how hypothermia was defined, intraoperative temperature did not predict infectious complications after laparoscopic and open colorectal operations.[10] Linking to the American College of Surgeons National Surgical Quality Improvement Program, Melton et al also did not find any correlation between intraoperative hypothermia and 30-day surgical site infection in 1008 colorectal procedures.[26]

Our findings were in contrast to the 1996 landmark study by Kurz et al which was a randomized prospective trial of routine care versus additional intraoperative warming in 200 patients undergoing open colorectal resection in non-ERAS setting.[5] Kurz et al reported that patients with hypothermia had three times higher rates of wound infection (19% vs 6%) and 2.6-days longer hospitalization compared with normothermic patients. Although hypothermic patients in our study had 1.3-days longer hospitalization than the other group, in a multivariate analysis the prolonged hospitalization was a result of postoperative complications – not intraoperative hypothermia. Notably, in our study the rates of wound infection in both studied groups were comparable (6% in hypothermic patients and 6.7% in normothermic patients) and almost identical to those with active warming in the study of Kurz et al. It is conceivable that the detrimental effects of 'mild' intraoperative hypothermia on surgical site infection may be negligible in an ERAS setting. Within an ERAS pathway, the implementation of bundled interventions including appropriate administration of prophylactic antibiotics and better glycemic control significantly decreased the rates of surgical site infection after colorectal operation to 4-7%.[27]

The incidence of intraoperative hypothermia in our study was high (77%). This may be explained by the fact that active warming protocol and standardized maneuvers for preventing hypothermia are lacking in our institute even an ERAS pathway has been applied for several years.[11-13] In some institutes, where the routine use of body-warming devices and other efforts to prevent and manage perioperative hypothermia, the incidence of intraoperative hypothermia may be as low as 7%.[28] Forced air warming system appeared to be the most efficient in maintaining perioperative normothermia compared with reflective blanket and warmed cotton blanket.[29] Warming of large amounts of intravenous fluid, blood and inspired air is also commonly used in the theater to preventing hypothermia in developed countries.[26,30] However, the rate of active warming of patients during an operation is low in resource-poor countries including Thailand and other Asian countries.[31-33]

Our data indicated that rectal surgery, operative time exceeding 3 hours, multi-organ resection and male gender were significant predictors for intraoperative hypothermia. Several risk factors for intraoperative hypothermia have been identified in the literature including high ASA physical status, major surgery, operative time exceeding 2-3 hours, use of combined epidural and general anesthesia and intravenous administration of un-warmed fluid or blood components.[33] Meanwhile, active warming, overweight, high baseline core temperature before anesthesia and high ambient temperature were significant protective factors for intraoperative hypothermia.[32]

Our study benefits from the use of a single-center database of ERAS pathway in colorectal operations. Notably, the patients in this registry were taken care of by single surgeon's team with good adherence to the ERAS protocol. However, there are several limitations of this observational study. First, the sample size was relatively small. Potential negative impact of hypothermia on surgical outcomes reported in non-ERAS setting, such as prolonged hospitalization,[5] may be not clearly evident in this study due to the low sample size. Second, the effect of intraoperative hypothermia was evaluated only in patients undergoing colorectal surgery – mainly open surgery for colorectal cancer, making it difficult to extrapolate our results to patients undergoing other operations. Third, it could be argued that active warming is currently the accepted standard of care and laparoscopic surgery has become a common approach with a less incidence or less degree of intraoperative hypothermia. We acknowledge that it is true in developed countries but maybe not in developing and underdeveloped regions[31,33] – thus making this study a great opportunity to re-evaluate the effect of intraoperative hypothermia in the current surgical practice. Furthermore, it would be interesting to examine in the future whether cost savings from the omission of active warming is off-set by additional costs to provide care in the postoperative period. Finally, our findings were analyzed based on a definition of 'mild' intraoperative hypothermia. Whether moderate or severe hypothermia will adversely affect surgical outcomes under an ERAS pathway needs to be examined.

In conclusion, despite these limitations, our data indicated that intraoperative hypothermia prolonged time to resume normal food after colorectal surgery within an ERAS setting but it did not adversely affect the return of bowel function, surgical site infection, postoperative complications and length of hospitalization. These findings suggest that the detrimental effects of 'mild' intraoperative hypothermia on surgical outcomes may be minimal in an ERAS setting.

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A Randomized Controlled Trial of the Correlation between Iodine Supplementation in Pregnancy and Maternal Urine Iodine and Neonatal Thyroid Stimulating Hormone Levels

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ABSTRACT

Objective: To establish the correlation of maternal urine iodine and neonatal thyroid stimulating hormone (TSH) in iodine supplemented and non-iodine supplemented pregnant women.

Materials and Methods: The study was a prospective, randomized, controlled trial, which was conducted at the antenatal care unit, labor ward, and neonatal unit of Siriraj Hospital, Mahidol University, Bangkok, Thailand. In all, 224 pregnant women were recruited during 1 October 2015 and 31 July 2017. They were randomized into 2 groups: an iodine and a non-iodine supplemented group. One woman in the control group left the study as she had delivery at another hospital.

Results: There were no statistically significant differences in the demographic data, original area of domicile, and adverse neonatal outcomes (including preterm labor and low birth weight) of the patients in the two groups. The maternal urinary iodine levels were 84.14 ± 61.85 and 58.41 ± 41.36 microgram/L, and the median values of the neonatal TSH levels were 3.7 ± 1.87 and 4.4 ± 1.99 mIU/ml, in the iodine and non-iodine supplemented groups, respectively. The differences in both values were statistically significant (p-value < 0.05).

Conclusion: This study determined that there were statistically significant differences in the maternal urinary iodine levels and the median values of the neonatal TSH levels of the iodine and non-iodine replacement groups of pregnant women. Even though there were no clinically significant differences and none of the newborns was diagnosed with hypothyroidism, iodine supplementation in all pregnant women should be considered. A larger prospective, RCT trial would confirm the benefits of a strategy of routinely administering iodine to pregnant women at Siriraj Hospital.

Keywords: Iodine supplementation; maternal urine iodine; neonatal TSH; hypothyroidism (Siriraj Med J 2019;71: 59-65)

INTRODUCTION

Iodine is an essential substance for fetal brain development; cell metabolism; cell growth; and the myocardial, hepatic, and muscle functions. Thyroid hormone production requires iodine as the major substance. Iodine deficiency results in impaired thyroid hormone synthesis and/or thyroid enlargement (goiter), hypothyroidism, cretinism, a decreased fertility rate, increased infant mortality, and mental retardation[1], as well as miscarriage and preterm labor in pregnant women. [2] As the development of the fetal thyroid gland and hormone production are delayed during gestation, the fetus is

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totally dependent during early pregnancy on maternal thyroxine for normal brain development.[3] Maternal dietary supplementation of iodine during pregnancy is also beneficial. Irreversible fetal brain damage can result from inadequate iodine supplementation during pregnancy, which may lead to inadequate production of thyroid hormones and hypothyroidism in pregnant women.[3] Iodine supplementation before and during pregnancy can prevent cretinism and improve the cognitive function of the general population.[1]

The growing requirement for iodine in pregnancy arises from the progressive increase in maternal thyroxine (T4) production needed to maintain maternal euthyroidism and to transfer thyroid hormone to the fetus during the first trimester, before the fetal thyroid begins to function. Other reasons are an iodine transfer to the fetus, particularly in later gestation, and an increase in renal iodine clearance.[4] The US Institute of Medicine[5] and the World Health Organization (WHO)[6] recommendations for iodine intake during pregnancy are 220 and 250 micrograms/day, respectively. From WHO global estimates of iodine status, more than half of the children with iodine deficiency came from Southeast Asia and Africa.[7]

Thailand has an iodine deficiency, even though the south and southeast parts of the country have substantial coastal areas. In 2013, a national survey found that iodine deficiency was a major problem among pregnant Thai women. Nearly half of all the women suffering from iodine deficiency were reported to be located in the north and northeast of Thailand, neither of which are near the coast.[8] Thailand's capital city, Bangkok, has plenty of sea food available for consumption. Almost all Thai food in that city is cooked with the ingredients of fish sauce and iodized salt, which may result in adequate iodine intake by pregnant Thai women; hence, iodine supplementation may be unnecessary for pregnant women in Bangkok.

Prior to the present study, no research had been conducted of the degree of correlation between iodine supplementation in pregnancy and the maternal urine iodine and neonatal TSH levels of patients at Siriraj Hospital. This research was carried out to determine the need for iodine-containing medicated supplementation by pregnant Thai women. We hypothesized that iodine supplementation would still be necessary despite pregnant Thai women having access to adequate seafood nutrition.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of the Siriraj Institutional Review Board (Si 524/2014). This prospective, randomized, controlled trial was conducted at the antenatal clinic and labor ward, Faculty of Medicine Siriraj Hospital, Mahidol University, from October 2015 to July 2017. Using nQuery Advisor (Statistical Solutions Ltd., Cork, Ireland), the calculation of the sample size was based on the findings of a study from Denmark[9], which reported that the median values of the neonatal TSH levels in pregnant women with and without iodine supplementation were 9 mU/l and 7.07 mU/l, respectively. However, the standard deviation (SD) employed in the present study's calculations was 4 times greater than that used in the Danish study to account for the variations in the populations of many parts of Thailand. A 10% follow-up loss was also factored in. The final sample size was determined to be 112 cases for each group.

Included in the study were all pregnant women who were older than 18 years and who had a singleton fetus at a gestational age of less than 18 weeks on the day the women commenced participation. The exclusion criteria were patients who had any of the following: a contraindication to the use of iodine, a previous administration of other iodine-containing drugs, a multifetal pregnancy, a pregnancy with a fetal anomaly, or a pregnancy with an abnormal thyroid function (hyperthyroidism or hypothyroidism).

The total of 224 pregnant women were recruited and divided into 2 groups of 112 by block randomization (block sizes of ten). One group received an iodine-containing ferrous tablet, and the other a no iodine-containing ferrous tablet. The patient's demographic data were obtained; the data items comprised age, pre-pregnant body weight, height, occupation, income, area of domicile, socioeconomic status, parity, antenatal care history, and medications received during pregnancy.

All of the pregnant women were given the standard care afforded to other patients, such as gestational diabetes mellitus and thalassemia screening, ultrasonography, and other indicated fetal surveillances. In the case of anemic patients (defined as a hematocrit level under 33%), an iron supplement (FeSO4 tablets) was prescribed.

The primary objective was to find any correlations between the neonatal TSH levels at 48-hours of life in the iodine supplemented and non-iodine supplemented groups. The reference cut-off value of an abnormally high TSH level used by the study was 12 mIU/L. This value was based on the laboratory reference range provided by the Genetics Division, Pediatrics Department, Siriraj Hospital.

All descriptive data were analyzed by descriptive statistics, and an unpaired t-test or Mann–Whitney U test was used to analyze the correlation of the data and the neonatal TSH levels. The data was deemed to have statistical significance at a *p*-value of less than 0.05.

RESULTS

Of the total of 224 pregnant women initially recruited, 1 woman in the control group left the study as she had delivery at another hospital.

The demographic data and neonatal outcomes are presented in Tables 1 and 2. The patients' data included the mean age, parity, body weight, height, body mass index (BMI), gestational age at the first antenatal care unit visit and later at the start of medication, occupation, education level, and monthly income. There were no statistically significant differences between the data for the two groups (p-value < 0.05).

As to the neonatal outcomes, the preterm birth rates of the patients in the iodine and non-iodine supplemented groups were 8.2% and 7.1%, and the rates of low birth weights were 6.1% and 10.1%, respectively. No statistically significant differences were detected in those figures. The mean gestational ages at delivery were 38.1 and 38.3 weeks, and the mean neonatal birth weights were 3,061.8 + 474.7 and 3,075.9 + 407.4 grams, respectively, again with no statistically significant differences (Table 3).

The mean urinary iodine level of the patients in the iodine supplement group was 84.14 ± 61.85 microgram/L, which was higher than that of the patients in the non-iodine supplemented group (58.41 ± 41.36 micrograms/L; Table 5). However, the median neonatal TSH level of the patients in the iodine supplemented group was 3.7 ± 1.87 mIU/L, which was lower than the figure of 34.4 ± 1.99 mIU/L for the patients in the non-iodine supplemented group. There were statistically significant differences in the results for the two groups (p-values 0.001 and 0.01, respectively; Table 6).

DISCUSSION

The most authoritative guidelines on how to assess iodine nutrition in a population were published in 2007 by the WHO, the United Nations Children's Fund (UNICEF), and the International Council for Control of Iodine Deficiency Disorders (ICCIDD, currently named the Iodine Global Network).[10] Iodine deficiency is the most common micronutrient deficiency in the world, especially in Asia.[7] Thailand also has an iodine deficiency even though plenty of seafood is available in most areas of the country. The WHO and the ICCIDD have promoted the usage of iodized table salt to alleviate endemic cretinism in many parts of the world.[10] Despite iodine deficiency affecting both the mother and the child, iodine supplementation during pregnancy tends to be of low concern among physicians in Thailand. Pregnant women require a 50% increase in their iodine intake to ensure there is sufficient available for thyroid hormone production by fetuses.[11] However, inadequate iodine supplementation may result in an iodine deficiency for both mothers and fetuses; alternatively, the mother may achieve euthyroidism, yet the fetus becomes hypothyroid.

In 2013, the annual statistical report of the Pediatric Genetic Division of the Pediatrics Department, Faculty of Medicine Siriraj Hospital, reported a case of a neonate that had an abnormally high TSH level of 0.17%, yet only 0.02% of the neonates in the group of neonates with abnormally high TSH levels had been diagnosed with hypothyroidism.[12] This proportion seems to be small and may not be representative of the extent of hypothyroidism among Thai children generally. However, the current study found that the urine iodine and neonatal TSH levels for

TABLE 1. Demographic data of the study groups.

Demographic data	Iodine supplemented group (N = 112) Mean ± SD	Non-iodine supplemented group (N = 111) Mean ± SD	P-value+
Age	29.9 ± 5.8	29.5 ± 5.8	0.64
Body weight	54.8 ± 10.5	54.9 ± 11.1	0.92
Height	157.9 ± 6.4	158.7 ± 5.5	0.39
BMI	21.9 ± 3.9	21.8 ± 4.0	0.76
GA at 1st ANC unit visit	10.3 ± 3.2	11.0 ± 3.7	0.17
GA at start of medication	14.9 ± 2.1	14.9 ± 2.5	0.15

BMI, body mass index; +, t-test; GA, gestational age; ANC, antenatal care

TABLE 2. Demographic data of the study groups.

Demographic data	Iodine supplemented group (N = 112) N (%)	Non-iodine supplemented group (N = 111) N (%)	P-value+
Parity			0.33
1	39 (34.8)	52 (46.8)	
2	47 (42.0)	34 (30.6)	
3	21 (18.8)	16 (14.4)	
4	5 (4.5)	7 (6.3)	
5	0 (0)	2 (1.8)	
Occupation			0.39
Housewife	16 (14.3)	22 (19.8)	
Farmer	0 (0)	1 (0.9)	
Government officer	4 (3.6)	8 (7.2)	
State enterprise officer	3 (2.7)	1 (0.9)	
Laborer	68 (60.7)	62 (55.8)	
Merchant	16 (14.3)	16 (14.4)	
Other (student, business owner, unemployed)	5 (4.5)	1 (0.9)	
Income (Baht/mo)			0.57
< 10,000	13 (11.6)	9 (8.1)	
10,000–29,999	66 (58.9)	79 (71.1)	
30,000–49,999	21 (18.8)	15 (13.5)	
> 50,000	12 (10.7)	8 (7.2)	
Education			0.64
Primary school	1 (0.9)	0 (0)	
Secondary school	43 (38.4)	39 (38.6)	
Bachelor and higher degree	68 (60.7)	72 (64.9)	

+, Chi-square test

TABLE 3. Neonatal outcomes.

Neonatal outcome	Iodine supplemented group (N = 112) Mean (SD)	Non-iodine supplemented group (N = 111) Mean (SD)	P-value+
GA at delivery	38.1 ± 1.7	38.3 ± 1.9	0.97
Neonatal birth weight	3,061.8 ± 474.7	3,075.9 ± 407.4	0.58

GA, gestational age; +, Chi-square test

TABLE 4. Incidences of preterm births and low birth weights.

	Iodine supplemented group (N = 112) N (%)	Non-iodine supplemented group (N = 111) N (%)	P-value+
Preterm birth (GA < 37 wk)	11 (9.8)	10 (9.0)	0.9
Extremely preterm(< 28 wk)	0 (0)	0 (0)	
Very preterm (28– < 32 wk)	2 (1.7)	1 (0.1)	
Moderate to late preterm (32– < 37 wk)	7 (6.2)	6 (5.4)	
LBW (birth weight < 2,500 g)	8 (7.1)	10 (9.0)	0.36
Extremely LBW (< 1,000 g)	0 (0)	0 (0)	
Very low birth weight (1,000– < 1,500 g)	2 (1.8)	0 (0)	
Low birth weight (1,500– < 2,500 g)	6 (5.3)	10 (9.0)	

GA, gestational age; +, Chi-square test

TABLE 5. Urinary iodine levels of the two groups.

Groups	N	Mean of urinary iodine (range) microgram/L	P-value+
Iodine supplemented group	112	84.14 ± 61.85 (9.40 – 437.00)	0.001
Non-iodine supplemented group	111	58.41 ± 41.36 (3.01–215.60)	

+, Chi-square test

TABLE 6. Median of neonatal TSH levels of the two groups.

Groups	N	Median of neonatal TSH (range) mIU/ml	P-value+
Iodine supplemented group	112	3.7 ± 1.87 (0.66–11.30)	0.01
Non-iodine supplemented group	111	4.4 ± 1.99 (1.31–10.60)	

+, Chi-square test

those mothers with iodine supplementation were higher and lower, respectively, than the corresponding figures for the mothers without iodine supplementation. It has been reported that children born from mothers with an iodine deficiency may lose up to 13.5 IQ points.[13] The International Child Development Steering Group has identified that iodine deficiency is one of the four, key, global health factors for impaired child development that have the most urgent need for intervention.[14]

As about 90% of absorbed iodine is excreted in urine, the median urinary iodine concentration (MUIC) is the best indicator of iodine intake.[15] The MUIC in the general population should be between 100 and 199 mcg/L, while in pregnant women, it should be in the range of

150 to 249 mcg/L. In our study, the MUIC in the group of pregnant women with iodine supplementation was only 84.14 ± 61.85 (9.4–437) mcg/L, which was lower than the standard requirement.

The neonatal TSH level is a biological indicator in national congenital hypothyroid screening programs. The guidelines of WHO/ICCIDD/UNICEF state that a < 3% frequency of TSH values > 5 mIU/L (in whole blood spots) indicates iodine sufficiency in a population. This numerical value was measured from 72 hour–old neonates who were born in iodine sufficient areas. Our study found that the median value of the TSH from the neonates of the mothers receiving iodine supplementation was 3.7 ± 1.87 (0.66–11.3) mIU/ml, which was lower than those without iodine supplementation. However, Smyth[16] and Li[17] suggested that using neonatal TSH levels may not be a reliable method for indicating an iodine deficiency in newborns because of the discrepancy between the MUIC and neonatal TSH levels.

The results of our study indicated that even in pregnant women receiving iodine supplementation, the MUIC and neonatal TSH levels were still abnormal. The main consensus in the WHO/UNICEF/ICCIDD guidance on achieving an adequate iodine intake in the general population as well as in pregnant women is that salt iodization is the key strategy.[10] Iodine supplementation during pregnancy is important, but encouraging the usage of iodized salt should be the primary strategy for all pregnant women. The consensus reached by WHO/UNICEF/ICCIDD was that pregnant women should not be advised to take iodine-containing supplements if the general population they come from is iodine sufficient, indicated by that population having a median UIC ≥ 100 µg/l for at least 2 years.[18] In Thailand's case, the country generally has an iodine deficiency; therefore, household iodized-salt usage and supplementation with iodine-containing iron during pregnancy should prevent iodine deficiency in newborns.

Encouraging the long-term use of household iodized salt is the most effective strategy for eradicating iodine deficiency.[10] Even though the Thai National Iodine Deficiency Disorder Control Project has promoted the regular use of household iodized salt since 1989, an iodine deficiency among pregnant Thai women was still reported in national surveys conducted during the years 2000–2006.[19] The dual promotion of household iodized salt usage and of the prescribing of iodine-containing iron supplementation during pregnancy has been in force since October 2010. A previous study from Thailand reported an improvement in the iodine nutrition of pregnant women after the implementation of those health policies.[20] However, our study showed that although the iodine-containing iron supplementation affected the MUIC and neonatal TSH levels, the MUIC level was still lower than that recommended by WHO/UNICEF/ICCIDD. Extensive repromotion of household iodized salt should be considered in order to improve the iodine nutrition of pregnant women.

The limitation of this study was that the baseline iodine status before the iodine supplementation was not measured. Therefore, some of the pregnant women may have had a severe iodine deficiency status prior to attending the study, which would have affected the MUIC and neonatal TSH levels. Moreover, it was not possible to control the daily iodine level of the participants' diets, which could have affected the iodine status of some of the pregnant women.

Many studies have supported the view that iodine supplementation during pregnancy can improve iodine status and neonatal TSH levels.[20–22] Providing iodine supplementation to all pregnant women is still beneficial to neonatal brain development.

The strength of our study was that it was a randomized controlled trial. The sequence generation and allocation were well designed, which was evidenced by there being no significant differences in the demographic data of the 2 groups. Nevertheless, the study had some limitations. These were some foreign babies missed the screening of the TSH levels due to their parents being unable to afford the related costs, an incompleteness of iodine supplementation, a lack of assessment of the medication adherence by both groups, and the absence of basis data relating to iodine status before participation in the study.

In order to obtain more precise data on the benefits of iodine supplementation during antenatal care, a larger prospective RCT should be performed. Moreover, the baseline iodine status should be determined before commencing the iodine supplementation.

CONCLUSION

There were statistically significant differences in the mean levels of the urinary iodine and the median values of the neonatal TSH levels of the iodine and non-iodine supplement groups of pregnant women. Even in areas with plentiful supplies of iodized food, pregnant women still had an iodine deficiency. Thus, iodine supplementation for all pregnant women should be encouraged, even if only to ensure proper fetal brain development.

What is already known on this topic

From previous studies, iodine has been established as being essential for fetal brain and thyroid development,

and it is recommended for all pregnant women. However, the administration of iodine supplementation for pregnant women is not routinely applied at all centers in Thailand, especially at Siriraj Hospital, which is a tertiary center. The general belief among health professionals that there is sufficient iodine intake during pregnancy has led to a lack of concern about the need for iodine supplementation during pregnancy at Siriraj Hospital. The objective of this study was to ascertain the TSH levels of neonates of iodine- and non-iodine-supplemented mothers during pregnancy.

What this study adds

The study found a statistically significant difference in the median values of the neonatal TSH levels of the iodine and non-iodine supplemented groups. Even though there were no clinical signs of hypothyroidism in the neonates, iodine is still beneficial for brain development. This study indicated that iodine supplementation affected the TSH levels of neonates and should therefore be adopted for all pregnant women in Thailand, especially at Siriraj Hospital. A larger study is needed to confirm the benefits of widespread iodine supplementation.

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Potential conflicts of interest

We have no potential conflicts of interest.

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The Implementation of a Red Blood Cell Transfusion Guideline in Critically Ill Surgical Patients at Siriraj Hospital

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ABSTRACT

Objective: To compare the RBC transfusion rate and clinical outcomes before and after the implementation of a transfusion guideline and to determine the adherence rate to the guideline.

Methods: This is a prospective experimental study in adult patients (≥18 years) admitted to surgical intensive care units. After developing and implementing a transfusion guideline, the data including patients' characteristics, transfusion and outcomes were collected prospectively (post-educational group). Data in pre-educational group was retrieved retrospectively from medical records.

Results: There were 197 patients in pre-educational group and 188 patients in post-educational group. The incidence of RBC transfusion significantly decreased significantly in post-educational group (37.2% vs. 48.7%, p = 0.02). The hemoglobin threshold for RBC transfusion was significantly lower in post-education group (8.16±1.43 vs. 8.97±1.57, p=0.001). The 28-day mortality rate was significantly decreased after the implementation of the guideline. (3.2 % vs. 11.2 %, p = 0.007). The adherence rate to transfusion guideline was reported as 47.1%.

Conclusion: The incidence of RBC transfusion was lower after the implementation of a transfusion guideline.

Keywords: Red Blood cell; transfusion; surgical patients; intensive care; guidelines (Siriraj Med J 2019;71: 66-73)

BACKGROUND

Anemia has been associated with poor clinical outcomes including higher mortality in patients who underwent non-cardiac surgery.[1] Red blood cell transfusions (RBCs) is one of the available methods that is frequently used to treat anemia or hemorrhage in order to improve oxygen delivery to tissues. Nevertheless, it is well established that RBC transfusion is an independent predictor of death, nosocomial infection, multi-organ dysfunction syndrome (MODS) and acute respiratory distress syndrome (ARDS) in critically ill patients.[2] Transfusion related immunomodulation (TRIM) is accounted to be the underlying cause.[3]

However, many studies from diverse locations in North America and Western Europe have demonstrated that 30-40% of the patients admitted to intensive care unit (ICU) still received RBC transfusion.[4-6] In addition, it was increasing up to 73% in patients with an ICU-stay longer than 7 days.[4] The pre-transfusion hemoglobin (Hb) was reported around 8.5 g/dl. Surgical patients have shown to receive more blood transfusions than medical patients, particularly in those with emergency surgery.[4] A retrospective study from Siriraj Hospital in critically ill surgical patients who had been on mechanical ventilation for greater than 24 hours reported an incidence of RBC transfusion was as high as 83 % and the mean RBC

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transfusion threshold was at Hb level of 8.7±1.2 g/dL.[7] The morbidity and mortality were higher in transfused patients.[7] Although recent data suggested that restrictive transfusion strategy with the a Hb threshold of 7.0 g/dl was well tolerated in general critically ill patients[8,9], trauma patients[10], sepsis patients[11,12] and in cardiac surgery patients[13,14] concerns have been raised regarding the actual practices and the remaining high incidence of RBC transfusion.

In addition, there has been lack of high quality data regarding the optimal Hb threshold in certain critically ill populations such as those with preexisting coronary disease, cerebrovascular disease and renal failure. The implementation of a guideline with specifies indication for transfusion might help to reduce the unnecessary transfusion. Although a number of previous guidelines[12,15,25] have been published, the adherence to the guideline might be problematic. Educational program including training course and posters related to the specific guideline for transfusion probably improves the adherence rate to the guideline. As a result, lower RBCs transfusion rate and better clinical outcomes are anticipated. This study aims to compare the RBC transfusion rate before and after the implementation of a guideline and the adherence rate to the guideline in critically ill surgical patients.

MATERIALS AND METHODS

The study was approved by Siriraj Institutional Review Board (Si 602/2013) with the waiver of informed consent. It was a prospective experimental study. All surgical patients admitted to SICU with age of equal to or more than 18 years during the study period were included. Patients with brain death or imminent death required withholding or withdrawing the treatment or had demonstrated an active blood loss (defined as blood loss of more than 30% of blood volume) were excluded.

The study consisted of 5 periods

- 1 month for developing a transfusion guideline and distributing a guideline to ICU physicians and surgeons by setting the meeting and official letters to the head of department
- After an approval from SIRB, 4 months pre-guideline data collection (April to August 2011) from the previous medical records were obtained (pre-educational group)
- 1 month (February 2014) for providing educational programs to the anesthesia attending staff who were the same group in both pre- and post-educational period and anesthesia resident trainees who were working in ICU during the study period. Programs included formal lecture at the beginning, poster presentation, reminder card and reminder about transfusion guideline once a month
- 4 months prospective observational period with data collection after providing educational programs between March and July 2014 (post-educational group)
- 2 months of follow up period

Data collection were 1) patient profiles including age, gender, weight, height, primary diagnosis, co-morbid disease (diabetes mellitus, hypertension, cardiovascular disease, chronic lung disease, chronic kidney disease, hematologic disease), smoking, alcohol drinking, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, type and duration of surgery, type and duration of anesthesia and the presence of sepsis on ICU admission; 2) transfusion data (post-educational group) including Hb/hematocrit (Hct) level before RBC transfusion. In case, the patients were transfused more than one time, we collected the transfusion information at the first transfusion), indication for RBC transfusion and the number, type and storage time of the transfused RBC; 3) clinical outcomes including nosocomial infection, cardiovascular events, acute lung injury (ALI)/ARDS, and MODS, ventilator days, ICU and hospital length of stay and 28-day mortality rate

Guideline for transfusion of RBC in critically ill surgical patients, Siriraj Hospital[15, 16]

Preface

1. World Health Organization (WHO) defines anemia as hemoglobin concentration (cHb) < 13.0 g/dl in men and < 12.0 g/dl in women.

2. Except dramatic emergency events with exsanguinating patients, transfusion of RBC should be generally performed as single-unit transfusion, which means one unit a time; the next unit, if necessary is given after actual Hb recording.

3. Any varyiation from the standards described below should be explained by the decision maker and documented in the patients file.

Indications for red cell transfusion **(Fig 1)**

Patients with prolonged hemorrhagic shock or acute hemorrhage (> 30% blood volume) that is difficult to estimate and not manageable by colloid/crystalloid infusion, and signs of oxygen deficiency, such as drop in central venous oxygen saturation ($ScvO_2$), increase in arterio-venous differences of oxygen ($AVDO_2$), elevated plasma lactate level and increase in base excess (BE).

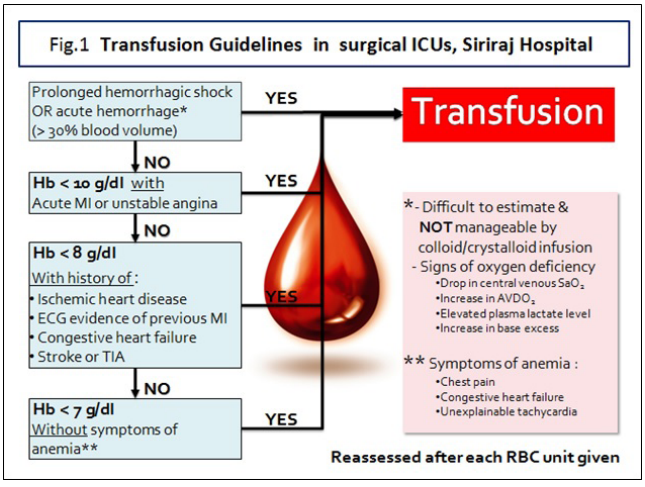


Fig 1. Transfusion Guidelines in surgical ICUs, Siriraj Hospital.

Additional indications

A. Hemoglobin concentration (cHb) < 7 g/dl in normovolemic anemia without pathologic symptoms related to anemia.

A. 1 Symptoms of anemia

I. Chest pain (deemed to be cardiac in origin)

II. Congestive heart failure

III. Otherwise unexplainable tachycardia

Situation has to be reassessed after each RBC unit given.

B. Hemoglobin concentration (cHb) < 8 g/dl in normovolemic anemia with history of:

I. Ischemic heart disease

II. Electrocardiographic evidence of previous myocardial infarction

III. Presence of congestive heart failure

IV. Stroke or transient ischemic attack

C. Hemoglobin concentration (cHb) < 10 g/dl in patients with acute myocardial infarction or unstable angina.

Statistical analysis

The primary outcome was the incidence of RBC transfusion after the guideline implementation and the secondary outcomes included the adherence to the guidelines (Hb threshold of RBC transfusion), ICU and hospital length of stay (LOS), in-hospital and 28-day mortality. The sample size was calculated by predicting the transfusion rate before the implementation using a guideline about 50% and reduced to 35% during the post-implementation period, at test significant level 0.05 and power of 80%. After adding up 20% for possible missing data, the sample size of 200 patients in each group was required.

Continuous data were presented as median with interquartile range (IQR) or mean with standard deviation (SD) with 95% confidential interval (CI). Categorical data were presented as numbers and percentages. Non-parametric Mann-Whitney U test or unpaired t-test for continuous data and Chi-Square or Fisher's exact test for numbers of events were used for comparison between pre- and post- educational program. P-value < 0.05 was considered as statistically significant. Data analysis was performed using SPSS 15.0 (SPSS Inc., Chicago, IL).

RESULTS

The overall population was 400 patients, 200 in a pre-educational group and 200 in a post-educational group. Three patients from pre-educational group were excluded due to massive hemorrhage and twelve patients in post-educational group were excluded due to massive hemorrhage (10) and incomplete data (2). These left 197 and 188 patients in pre- and post-educational groups for analysis. (Fig 2)

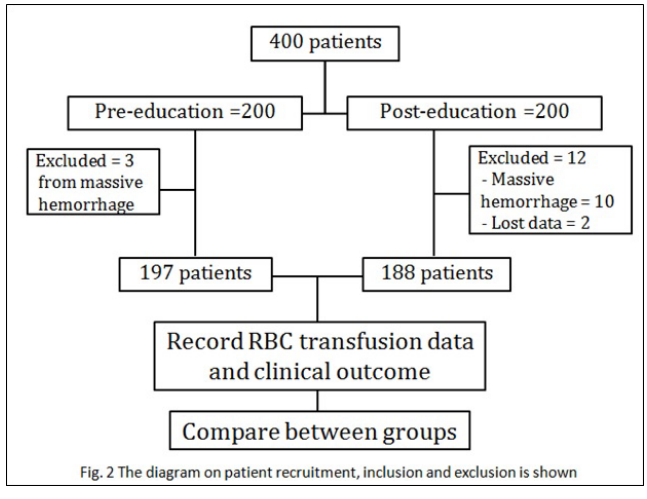


Fig 2. The diagram on patient recruitment, inclusion and exclusion is shown.

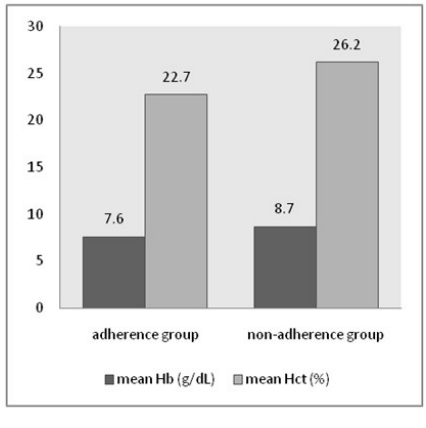


Fig 3. The mean Hb and Hct in adherence and non-adherence group in post-educational patients.

Patient characteristics and intra-operative data were shown in Table 1. There was no significant difference in patient characteristics, underlying medical diseases,

TABLE 1. Characteristic of patients on admission to surgical intensive care unit.

	Pre-education N=197	Post-education N=188	P-value
Age (year)	64.49 ± 17.86	61.59 ± 19.49	0.13
Gender (male) N (%)	103 (52.3)	105 (55.85)	0.54
Weight (kg)	60.66 ± 15.12	60.28 ± 16.08	0.82
Height (cm)	159.78 ± 8.95	160.55 ± 9.06	0.41
Surgical patients N (%)	180 (91.37)	176 (93.62)	0.44
Medical patients N (%)	17 (8.63)	12 (6.83)	
Postoperative day, Median (IQR)	0 (0-6)	0 (0-8)	0.53
Underlying medical diseases N (%)			
Coronary artery disease	31 (15.74)	23 (12.34)	0.4
Vascular disease	34 (17.26)	44 (23.4)	0.16
Respiratory disease	61 (30.96)	62 (32.98)	0.53
End stage renal disease	8 (4.06)	6 (3.19)	0.54
Endocrine disease	57 (28.93)	65 (35.57)	0.27
Stroke	26 (13.2%)	26 (13.8)	0.88
Anemia N (%)	163 (82.74)	146 (77.66)	0.25
Coagulopathy N (%)	40 (20.3)	43 (22.87)	0.62
Immunocompromised N (%)	7 (3.55)	15 (7.98)	0.08
Malignancy N (%)	70 (35.53)	61 (32.45)	0.59
Surgical data N (%)			
Elective surgery	124 (62.94)	114 (60.64)	0.28
Emergency surgery	52 (26.4)	63 (33.51)	
General surgery	77 (39.1)	73 (38.83)	0.81
Vascular surgery	38 (19.29)	41 (21.8)	
Orthopedic surgery	28 (14.21)	22 (11.7)	
Obstetric & gynecological surgery	11 (5.58)	13 (6.91)	
Type of anesthesia N (%)	135 (68.53)	134 (71.28)	0.05
- General anesthesia	11 (5.58)	3 (1.6)	
- Regional anesthesia			
ASA class > 2 N (%)	105 (58.33)	119 (63.3)	0.14
Volume of RBC transfusion in operating room (ml), Median (IQR)	565.5 (324.8, 924.8)	706.5 (350.8,1217.8)	0.11
APACHE II score	9.67 ± 6.62	8.66 ± 5.5	0.1
SOFA score ,Median (IQR)	2 (0,5)	2 (0,3)	0.07
Indication for SICU admission			
Hemodynamic monitoring	196 (99.5)	188 (100)	1.0
Respiratory support	100 (50.76)	120 (63.83)	0.01
Shock			
Septic shock	25 (12.7)	18 (9.57)	0.35
Cardiogenic shock	6 (3.05)	2 (1.06)	
Hypovolemic shock	11 (5.58)	14 (7.45)	
Laboratory on admission			
Hb (g/dl)	10.53 ± 2.05	11.57 ± 7.64	0.71
Hct (%)	31.73 ± 6.28	33.24 ± 6.42	0.21
Creatinine (mg/dl)	1.45 ± 1.6	1.32 ± 1.32	0.39
Albumin (g/dl)	2.84 ± 0.69	2.93 ± 2.61	0.66
PaO_2 (mmHg)	177.25 ± 69.06	164.79 ± 79.80	0.2
PaO_2/FIO_2 ratio	345.05 ± 196.24	359.16 ± 178.33	0.56

Abbreviations: APACHE II = Acute Physiology and Chronic Health Evaluation II score; ASA = American Society of Anesthesiology Physical Status; CI = confidence interval; FiO2 = fraction of inspired oxygen; Hb = hemoglobin; Hct = hematocrit; ;IQR = interquartile range; PaO_2 = partial pressure of arterial oxygenation; RBC = red blood cell; SOFA = Sequential Organ Failure Assessment; SICU = surgical intensive care unit

surgical data and patient conditions on ICU admission between the pre and post- education groups except for the indication for SICU admission. Respiratory support as an indication for SICU admission was higher in post-educational group, than in pre-educational group (63.83% vs. 50.76%, p = 0.01).

Patients in post-educational group had significantly lower RBC transfusion rate (37% vs. 48%, p = 0.02) and the adherence rate to the guideline was 47% .The mean Hb level before RBC transfusion was significantly lower in post-educational group (8.16 ± 1.43 vs. 8.97 ± 1.57g/dL, p = 0.001). The 28-day mortality and ICU mortality were also significantly lower in post-educational group. Patients in pre-educational group had significantly higher incidence of acute respiratory distress syndrome (ARDS) (2.5% vs. 0%, p = 0.05) and acute kidney injury (AKI) (8.1% vs. 2.1%, p = 0.01) than those in post-educational group (Table 2).

TABLE 2. Transfusion data and outcomes.

	Pre-education N=197	Post-education N=188	P-value
Transfusion in SICU, N (%)	96 (48.7)	70 (37.2)	0.02
Threshold			
Hb threshold	8.97 ± 1.57	8.16 ± 1.43	0.001
Hct threshold	27.14 ± 4.76	24.54 ± 4.12	< 0.001
RBC volume transfused in SICU, Median (IQR)	627.5 (316.25, 1030.25)	650 (342.5, 904.0)	0.95
Outcomes			
Ventilator days, Median (IQR)	1 (0,2)	1 (0,2)	0.18
28-day mortality N (%)	22 (11.12)	6 (3.19)	0.007
SICU mortality N (%)	15 (7.61)	3 (1.56)	0.009
SICU LOS, Median (IQR)	2 (1,3)	2 (1,3)	0.73
Hospital LOS, Median (IQR)	15 (9,28)	13 (9,27)	0.37
Complication in SICU,N (%)			
Delirium	5 (2.54)	6 (3.2)	0.55
Stroke	0 (0)	4 (2.1)	0.07
Acute myocardial infarction	2 (1.0)	4 (2.1)	0.4
Pulmonary edema	3 (1.52)	2 (1.1)	0.55
ARDS	5 (2.5)	0 (0)	0.05
Acute kidney injury	16 (8.1)	4 (2.1)	0.01
Pneumonia	4 (2.0)	5 (2.7)	0.54
CRBSI	0 (0)	1 (0.5)	0.35
Severe sepsis	6 (3.1)	8 (4.3)	0.48
Septic shock	10 (5.1)	5 (2.7)	0.28

Abbreviations: ARDS = acute respiratory distress syndrome; CRBSI = catheter-related bloodstream infection; Hb = hemoglobin; Hct = hematocrit; LOS = length of stay; RBC = red blood cell; SICU = surgical intensive care unit; IQR = interquartile range

TABLE 3. Indications for red blood cell transfusion in post-educational group (N = 70).

Indication	N (%)
Low Hb	33 (47.1%)
Suspected anemic symptoms	14 (20%)
Keep Hct > 30 %	10 (14.3%)
History of coronary artery disease	8 (11.43%)
MI/Unstable angina	4 (5.7%)
Undefined indication	1 (1.4%)

Abbreviations: Hb = hemoglobin; Hct = hematocrit; MI = myocardial infarction

TABLE 4. Laboratory result before red blood cell transfusion in transfused patients of post-educational group (N = 70).

Laboratory	Mean ± SD
Serum lactate (mg/dL)	3.22 ± 2.58
ScvO2 (%)	67 ± 4.24
Base excess (mEq/L)	-5.12 ± 5.53

Abbreviation: $ScvO_2$ = central venous oxygen saturation

DISCUSSION

In this study, the implementation of a RBC transfusion guideline using the educational program can reduce the RBC transfusion rate and decrease the hemoglobin threshold for RBC transfusion. In addition the clinical outcomes regarding the complication (ARDS, AKI) and the mortality rate were improved after the educational program. Previous study had reported the high incidence of RBC transfusion up to 83 % in general surgical ICU, Siriraj hospital.[7] The rate of transfusion is considerably high in critically ill surgical patients who have been on mechanical ventilation longer than 48 hours.[7] Therefore, the implementation of a strategy that can reduce the number of transfusions should be considered. As a result, this study regarding the implementation of the RBC transfusion guideline was performed with the aim to decrease the rate of RBC transfusion. The educational program was a tool selected to implement the guideline in this study. Previous study demonstrated that an educational program coupled with the institution policy for RBC transfusion could reduce the total RBC units transfused.[17] In this study, we found that after the implementation of the RBC transfusion guideline, the rate of RBC transfusion was significantly decreased from nearly 50 % to 37 % which was comparable to other regions.[15] In addition, the Hb threshold for RBC transfusion was significantly decreased from 9 to 8 g/dL.

Interestingly, the primary and secondary outcomes were improved, and even the adherence rate to the transfusion guideline was less than 50%. Several issues were concerned including 1) physician might accept the transfusion trigger of Hb around 8-9 g/dL even though evidences from previous randomized controlled trials[8,11,14] have addressed the safety of restrictive transfusion. A survey of physician's attitudes to transfusion practice in critically ill patients in the United Kingdom[18] reported that there was significant variation in transfusion threshold among physicians. It depended on the scenario and the severity of patients. The majority of respondents selected a baseline Hb threshold between 9 and 10 g/dL. In addition, previous studies from different locations reported the average pre-transfusion Hb at 8.5 g/dL.[15] 2) the educational program regarding the transfusion guideline might not be frequently provided or reminded. Previous literatures showed that the implementation of education program has significantly decreased RBC transfusion, increased transfusion safety and helped to prevent the occurrence of transfusion-related adverse effects.[17,19,20] However, only the educational program might not be adequate to improve the outcomes. The combination of the educational program and the support from medical board in terms of integration of the guideline recommendation into RBC transfusion order including the guideline recommendation can result in the better adherence rate and outcomes[17] 3) the plastic surgeon required maintaining the hematocrit level at least 30 % in the operations which involved flap procedures, and 5 out of 37 patients (14 %) of non-adherence were in this category. The researcher did not argue against this concept, although there were studies which reported that transfusion trigger of hematocrit < 25 % can decrease blood transfusion rates without increasing rates of flap-related complications.[21]; and 4) half of the patients in non-adherence group had sepsis, and the recent sepsis guideline implemented by Siriraj hospital recommended the Hb threshold for RBC transfusion at 10 g/dL.[22] However, this recommendation was based on the protocol of "early goal-directed therapy (EGDT)"[23] in sepsis patients that targeted an increase in mixed venous oxygen saturation to ≥ 70%. This was achieved by the administration of fluid resuscitation, then RBC transfusion to keep a hematocrit ≥ 30 % and then administering inotropes (dobutamine). The EGDT group received more fluid and RBC transfusion in the first 6 hours. The EGDT group demonstrated the improvement in survival, although it was not possible to separate the impact of only RBC transfusion strategy on outcome. Moreover, the recent multicenter RCT[11], which compared restrictive and liberal RBC transfusion in patients with

septic shock, did not demonstrate significant difference in terms of mortality between the two groups. Finally, the recent edition of Sepsis ad septic shock guidelines was published in 2016. It recommended to reserve RBC transfusion for patients with a Hb level less than 7 g/dl.[24] Moreover, the updated clinical practice guidelines from the American Association of Blood Banks (AABB) recommended that RBC transfusion is not indicated until the Hb level is 7 g/dL for hospitalized adult patients who are hemodynamically stable, including critically ill patients rather than when the Hb is 10 g/dL.[12]

Although the adherence rate was not high, the RBC transfusion rate significantly decreased after the implementation of the guideline. It might not be the guidelines itself that improved the outcomes, but the advancement in knowledge regarding the adverse effects of blood transfusion or the new recommendation from clinical practice guidelines might be accountable. Nevertheless, some limitation should be addressed. Twelve patients (6%) were excluded from the post-educational group which might alter the adherence rate and outcomes. The different in duration of data collection between pre and post-educational program should be concerned. The data of pre-educational group was collected in 2011 whereas the post-educational information was prospectively collected during 2014. The difference in time frame might result in the variety of treatment modalities especially the RBC transfusion. The lower incidence of RBC transfusion and the improvement in mortality rate may result from the better knowledge from recent publications rather than the implementation of the guideline. The frequency and efficacy of educational program as mentioned earlier might be insufficient to change attitude of transfusion. Other limitation should be addressed including there was no information regarding detail of transfusion in pre-educational group especially the indication of RBC transfusion which might help to interpret the high transfusion rate. In addition the information regarding the RBC transfusion in the operating theaters was not available in both groups, although the hemoglobin at SICUs admission was not significantly different between pre and post educational groups.

In conclusion, the implementation of educational program regarding RBC transfusion guideline can decrease the RBC transfusion rate.

What is already known on this topic?

Although RBC transfusion is an independent predictor of death, nosocomial infection, multi-organ dysfunction syndrome (MODS) and acute respiratory distress syndrome (ARDS) in critically ill patients, the majority of the patients admitted to intensive care unit (ICU) still received RBC transfusion. A pre-transfusion hemoglobin (Hb) was reported around 8.5 g/dl.

What does this study adds?

The implementation of educational program regarding RBC transfusion guideline can reduce RBC transfusion rate, complications and the mortality rates. The Hb threshold for RBC transfusion was significantly decreased from 9 g/dL in the pre-educational group to 8 g/dL in the post-educational group.

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Prevalence of and Factors Associated with Inappropriate Indications for Transthoracic Echocardiography in Adult Outpatients at Siriraj Hospital

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ABSTRACT

Objective: Ordering transthoracic echocardiography (TTE) for inappropriate indications could prolong patient waiting time, increase echocardiography laboratory workload, and compromise the quality of TTE studies. This study aimed to investigate the prevalence of and factors associated with inappropriate indications for TTE in adult outpatients at Siriraj Hospital.

Methods: Two cardiologists prospectively and independently evaluated indications for adult TTE scheduled during regular office hours at Her Majesty Cardiac Center, Siriraj Hospital. Cases were classified as appropriate, inappropriate, or uncertain according to the 2011 Appropriate Use Criteria for Echocardiography proposed by a group of American cardiovascular organizations. Agreement between the two cardiologists was measured using weighted kappa statistic, and disagreement was resolved by consensus. Factors were evaluated for association with inappropriate indications for TTE.

Results: Four hundred and eighty-two patients were included. Weighted kappa statistic was 0.46 [95% confidence interval (CI) 0.34 to 0.59] for agreement between the two cardiologists. Four hundred and thirty-two TTE were appropriate (89.6%, 95% CI 86.6% to 92.1%), 27 were inappropriate (5.6%, 95% CI 3.9% to 8.0%), and 23 were uncertain (4.8%, 95% CI 3.2% to 7.0%). Neither status of ordering physician (cardiologist, cardiology fellow, or cardiothoracic surgeon) nor payment type was found to be significantly associated with the appropriateness of indications for TTE.

Conclusion: The prevalence of inappropriate indications among adult outpatients undergoing TTE during regular office hours at Siriraj Hospital was low. No significant association was observed between the appropriateness of TTE indications and either status of ordering physician or payment type.

Keywords: Transthoracic echocardiography; evaluation of echocardiography; appropriate use criteria; appropriateness (Siriraj Med J 2019;71: 74-79)

INTRODUCTION

Transthoracic echocardiography (TTE) is widely used in clinical practice due to its safe and non-invasive nature, its wide availability, and its relatively low cost compared to other cardiac tests. The demand for TTE is increasing, and this has resulted in longer waiting

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periods for patients. The number of adult TTE procedures performed during regular office hours at Her Majesty Cardiac Center, Siriraj Hospital, increased from 2,683 per year in 2009 to 3,326 per year in 2012. The average waiting time for a patient scheduled for outpatient TTE was 48.9, 74.5, and 58.4 days in 2009, 2010, and 2011, respectively. This increase in demand for TTE increases the workload of the echocardiography laboratory, and this workload increase could compromise the quality of echocardiographic examinations.

At our echocardiography laboratory, TTE examinations were previously performed only by cardiologists, or by cardiology fellows under the supervision of cardiologists. However, as the number of TTE requests increased over the years, non-cardiologist cardiac sonographers were recruited to cope with the increased demand. Even with the increased number of sonographers, long waiting time is still a frequent complaint from patients and requesting physicians. In routine clinical practice, we observed that some patients were scheduled for TTE with doubtful or inappropriate indications. Reducing the number of TTE procedures that are unnecessarily performed due to inappropriate indications may help to shorten the waiting period for TTE, reduce echocardiography laboratory workload, improve the quality of TTE examinations, and reduce healthcare costs. Experts in echocardiography have proposed clinical situations in which echocardiography should be considered indicated as a guideline for the appropriate and effective use of echocardiography in clinical practice.[1,2] Reports from Western countries showed that 56% to 92% of echocardiographic examinations were considered appropriate,[3-10] and that non-cardiac specialists were more likely to inappropriately request echocardiography.[4,6,8] In Thailand, a study published in 2011 from a university hospital found that 89% of echocardiographic requests among inpatients and outpatients were appropriate[11] when evaluated against the 2007 Appropriateness Criteria for Transthoracic and Transesophageal echocardiography.[1] The aim of this study was to investigate the prevalence of and factors associated with inappropriate indications for TTE in adult outpatients at Siriraj Hospital according to the updated Appropriate Use Criteria for Echocardiography published in 2011.[2]

MATERIALS AND METHODS

Methods

We conducted this prospective study to assess the appropriateness of TTE requests among adult outpatients scheduled for TTE at the echocardiography laboratory of Her Majesty Cardiac Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. The Siriraj Institutional Review Board (SIRB) approved the protocol for this study (Si 067/2013), and all patients provided written informed consent before enrollment into the study.

Study population

The principal inclusion criterion was adult outpatients electively scheduled for TTE during regular office hours. Patients were prospectively and consecutively enrolled. Patients scheduled for stress echocardiography, transesophageal echocardiography, echocardiography performed for other research purposes, and TTE performed after office hours were excluded.

Study procedures

Two cardiologists (KU and VC) independently reviewed the medical record of each patient to determine the indication for TTE. Indications were classified as appropriate, inappropriate, or uncertain according to the 2011 Appropriate Use Criteria for Echocardiography proposed by the American College of Cardiology Foundation (ACCF) in collaboration with various other American cardiovascular organizations.[2] There are 98 criteria listed in the appropriate use criteria for TTE, 57 of which are considered appropriate, 29 inappropriate, and 12 uncertain. If the indication for TTE in a particular patient did not match any of the 98 criteria, each of the two reviewing cardiologists used his own judgment to determine the appropriateness of the indication. Disagreement between the two cardiologists was resolved by discussion and consensus. Data relating to the status of the physician that ordered the TTE, and the type of payment to cover the cost of the TTE were also recorded. Regarding physician status, only cardiology fellows, cardiologists, and cardiothoracic surgeons are authorized to order TTE at our center. Payment type was categorized into any one of 3 Thailand health insurance schemes or self-pay.

Statistical Analysis

The primary objective of this study was to estimate the proportion of TTE that were ordered with inappropriate indications among adult outpatients electively scheduled for TTE during regular office hours. We estimated that a minimum of 480 subjects would be required to ensure that the limits of the 2-sided 95% confidence interval (CI) of this proportion would not exceed ±0.045 from the observed proportion. We chose to use an expected proportion of inappropriate TTE of 0.5 in the sample size

calculation, as this value would yield the largest variance of the estimate of proportion, thereby maximizing the required sample size.

Demographic data, status of ordering physician, and payment type were summarized using descriptive statistics. Continuous variables are presented as median and interquartile range (IQR), and Kruskal-Wallis test was used to compare continuous variables between groups. Categorical variables are reported as number and percentage, and Fisher's exact test was used to compare categorical variables between groups. Agreement between the two cardiologists was measured using weighted kappa statistic with linear weights. All statistical tests were carried out at the 2-sided significance level of 0.05.

RESULTS

A total of 482 patients were consecutively enrolled in this study between March 1 and June 11, 2013. Demographic data, status of ordering physician, and payment type of study participants are shown in Table 1. Almost half (47.1%) of subjects were male. Patient age ranged from 16 to 95 years. Cardiologists or cardiology fellows ordered TTE in about 95% of cases. The Civil Servant Medical Benefits system and the National Health Insurance system covered the cost of TTE in 40.7% and 33.8% of patients, respectively.

The two cardiologists concordantly determined the indications for TTE as appropriate in 409 subjects, as inappropriate in 12 subjects, and as uncertain in 11 subjects, representing the observed agreement of 89.6%. When chance agreement was taken into account, there was moderate agreement between the two cardiologists regarding the level of appropriateness of TTE indications (weighted kappa 0.46, 95% CI 0.34 to 0.59). Consensus was achieved in all 50 cases where there was initial disagreement between reviewers.

After consensus was reached, the indications for TTE were considered appropriate in 432 patients (89.6%, 95% CI 86.6% to 92.1%), inappropriate in 27 patients (5.6%, 95% CI 3.9% to 8.0%), and uncertain in 23 patients (4.8%, 95% CI 3.2% to 7.0%). There were six patients whose indications for TTE were not specifically listed in the 2011 Appropriate Use Criteria for Echocardiography, so determination of the level of appropriateness was based on judgement of the reviewing cardiologists. Among those 6 indications, 1 was judged appropriate, 2 inappropriate, and 3 uncertain.

There was a statistically significant difference in age among the appropriate, inappropriate, and uncertain TTE indication groups. Subjects in the uncertain group were significantly younger than those in the appropriate and inappropriate groups ($p = 0.003$; Table 2). Females were significantly more likely than males to have TTE with appropriate indications (92.9% vs. 85.9%, $p = 0.043$).

Although cardiology fellows had a higher proportion of appropriate indications for TTE request (92.7%) than cardiologists (86.0%) and cardiothoracic surgeons (85.7%), there was no statistically significant association between the status of the ordering physician and the level of TTE indication appropriateness ($p = 0.062$). Similarly, patient payment type was not found to be significantly associated with the level of indication appropriateness ($p = 0.071$).

TABLE 1. Characteristics of study participants.

Variables	Value (n = 482)
Age (years), median (IQR)	61 (50, 71)
Male, n (%)	227 (47.1%)
Ordering physician, n (%)	
Cardiologist	200 (41.5%)
Cardiology fellow	261 (54.1%)
Cardiothoracic surgeon	21 (4.4%)
Payment type, n (%)	
Civil Servant Medical Benefits	196 (40.7%)
National Health Insurance	163 (33.8%)
Social Security	48 (10.0%)
Self-pay	75 (15.5%)

Abbreviation: IQR = interquartile range

TABLE 2. Comparison of various factors among different levels of appropriateness relative to indications for adult outpatient transthoracic echocardiography during regular office hours at Siriraj Hospital.

Variables[a]	Appropriate	Inappropriate	Uncertain	P-value[b]
Age (years), median (IQR)	62 (50, 72)	61 (54, 72)	43 (33, 63)	0.003[c]
Gender, n (%)				0.043
Male	195 (85.9%)	17 (7.5%)	15 (6.6%)	
Female	237 (92.9%)	10 (3.9%)	8 (3.1%)	
Ordering physician, n (%)				0.062
Cardiologist	172 (86.0%)	17 (8.5%)	11 (5.5%)	
Cardiology fellow	242 (92.7%)	8 (3.1%)	11 (4.2%)	
Cardiothoracic surgeon	18 (85.7%)	2 (9.5%)	1 (4.8%)	
Payment type, n (%)				0.071
Civil Servant Medical Benefits	176 (89.8%)	14 (7.1%)	6 (3.1%)	
National Health Insurance	150 (92.0%)	6 (3.7%)	7 (4.3%)	
Social Security	38 (79.2%)	3 (6.3%)	7 (14.6%)	
Self-pay	68 (90.7%)	4 (5.3%)	3 (4.0%)	

[a] Percentage data are shown as row percentage. [b] Fisher's exact test, except where indicated otherwise
[c] Kruskal-Wallis test
Abbreviation: IQR = Interquartile range

The most frequent indication for TTE, according to the 2011 Appropriate Use Criteria for Echocardiography, was presentation with symptoms or conditions potentially related to suspected cardiac etiology, such as chest pain, shortness of breath, and palpitations (94 patients, 19.5%; Table 3), followed by initial evaluation for suspected valvular or structural heart disease (48 patients, 10.0%; Table 3). Among the 27 patients with inappropriate indications, the most frequently observed indication was routine surveillance of ventricular function in clinically stable subjects with known coronary artery disease (5 patients, 18.5%; Table 4).

TABLE 3. The 10 most common indications for adult outpatient transthoracic echocardiography during regular office hours at Siriraj Hospital.

	Indication	n (%) (Total n = 482)
1.	Symptoms or conditions potentially related to suspected cardiac etiology including but not limited to chest pain, shortness of breath, palpitations, transient ischemic attack, stroke, or peripheral embolic event	94 (19.5%)
2.	Initial evaluation when there is a reasonable suspicion of valvular or structural heart disease	48 (10.0%)
3.	Re-evaluation of known valvular heart disease with a change in clinical status or cardiac exam or to guide therapy	36 (7.5%)
4.	Prior testing that is concerning for heart disease or structural abnormality including but not limited to chest X-ray, baseline scout images for stress echocardiogram, ECG, or cardiac biomarkers	35 (7.3%)
5.	Routine surveillance (≥ 1 year) of moderate or severe valvular regurgitation without change in clinical status or cardiac exam	31 (6.4%)
6.	Sustained or nonsustained atrial fibrillation, supraventricular tachycardia, or ventricular tachycardia	30 (6.2%)
7.	Initial evaluation of known or suspected heart failure (systolic or diastolic) based on symptoms, signs, or abnormal test results	29 (6.0%)
8.	Initial evaluation of ventricular function following acute coronary syndrome	26 (5.4%)
9.	Routine surveillance (≥ 1 year) of moderate or severe valvular stenosis without a change in clinical status or cardiac exam	18 (3.7%)
10.	Evaluation of suspected pulmonary hypertension including evaluation of right ventricular function and estimated pulmonary artery pressure	15 (3.2%)

TABLE 4. Common inappropriate indications for adult outpatient transthoracic echocardiography during regular office hours at Siriraj Hospital.

	Indication	n (%) (Total n = 27)
1.	Routine surveillance of ventricular function with known coronary artery disease and no change in clinical status or cardiac exam	5 (18.5%)
2.	Initial evaluation of ventricular function (e.g., screening) with no symptoms or signs of cardiovascular disease	2 (7.4%)
3.	Routine perioperative evaluation of ventricular function with no symptoms or signs of cardiovascular disease	2 (7.4%)
4.	Routine surveillance (< 1 year) of moderate or severe valvular stenosis without a change in clinical status or cardiac exam	2 (7.4%)
5.	Routine surveillance (< 3 year after valve implantation) of prosthetic valve if no known or suspected valve dysfunction	2 (7.4%)
6.	Routine evaluation of systemic hypertension without symptoms or signs of hypertensive heart disease	2 (7.4%)

DISCUSSION

We evaluated the indications for TTE among adult outpatients undergoing TTE during regular office hours, and determined their appropriateness according to the 2011 Appropriate Use Criteria for Echocardiography. Our findings revealed that most of outpatient TTE examinations performed at our center during the study period were appropriate, regardless of the status of the ordering physician or the payment type used to cover the cost of TTE.

Our result is very comparable to that reported from another university hospital in Thailand (89% appropriate TTE indications) that enrolled both inpatients and outpatients, and that based the appropriateness of TTE indications on 2007 Appropriateness Criteria for Transthoracic and Transesophageal Echocardiography.[11] In that study, the level of appropriateness of TTE requests was similar between inpatients and outpatients. The most common inappropriate indications for TTE were preoperative evaluation (50% of inappropriate TTE), as determined by expert opinion since this indication is not listed as an indication in the 2007 Appropriateness Criteria for Transthoracic and Transesophageal Echocardiography, and evaluation of endocarditis without evidence of bacteremia or new murmur (19% of inappropriate TTE). In our study, the most common inappropriate indications for TTE were evaluation of left ventricular function in clinically stable coronary artery disease patients, and evaluation of asymptomatic individuals without suggestive evidence of cardiovascular disease (26% of inappropriate TTE for both indications combined). TTE performed for perioperative evaluation was found in only 7% of inappropriate TTE in our study, probably because this indication is clearly listed as inappropriate in the 2011 Appropriate Use Criteria for Echocardiography. We did not have any TTE evaluation for endocarditis in our study, because we included only elective outpatients.

The rate of appropriate TTE requests at our hospital, and probably at most university hospitals in Thailand, is higher than the rates reported from the United States of America (56% to 92%).[3-10] This may be explained in part by the policy imposed at our center, and at other university hospitals in Thailand, to limit the privilege of ordering TTE to only cardiovascular specialists. At many centers in the US, family physicians and general practitioners can order TTE, and the rates of inappropriate requests made by these physicians are usually higher than those made by cardiovascular specialists.[4,6,8] Another possible explanation for the observed high rate of appropriate TTE indications is that ordering physicians may occasionally have to perform echocardiographic examination themselves without being paid extra to do so. This may have the effect of influencing requests for TTE that are more accurately based on guideline recommendations.

The observed tendency of higher rate of appropriate requests among cardiology fellows compared to cardiologists and cardiothoracic surgeons might reflect the nature of practice during training at an academic institution. The management decisions of cardiology fellows are usually monitored by certified cardiologists, and this may inspire them to be more cautious and more likely to consult their mentor before ordering a test.

This study was inspired by the idea that decreasing or eliminating inappropriate TTE requests would lead to

improvement in patient waiting time for, and perhaps the quality of TTE. However, the magnitude of inappropriate requests found in this study was only 5.6%, which indicates that the practice at our center regarding the use of TTE is reasonable, and that only modest improvement would be possible. As such, alternative strategies need to be explored in order to improve outpatient TTE service.

Some limitations of this study need to be mentioned. First, included patients were enrolled over a relatively short 14-week study period, so the indications that were identified may not reflect all of the TTE indications that are used in clinical practice. Second, even though our reviewers are both board-certified cardiologists, which gave our study more credibility than if we had enlisted non-cardiologist reviewers, their agreement was only moderate, which suggests the complexity associated with reviewing medical records to identify TTE indications. The differences between reviewers are likely due to incomplete documentation on echocardiography request forms and/or medical records. Third and last, the results of this study may not be generalizable to other centers with different TTE request system practices, such as non-teaching hospitals or private hospitals.

CONCLUSION

The prevalence of inappropriate indications among adult outpatients undergoing TTE during regular office hours at Siriraj Hospital was low. No significant association was observed between the appropriateness of TTE indications and either status of ordering physician or payment type.

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Esophageal Replacement in Children: A 10-Year, Single-Center Experience

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ABSTRACT

Objective: Various esophageal replacement grafts have been used in children, although none can equal the native esophagus. The purpose of this study was to review the complications and outcomes associated with using different techniques in a single institute.

Methods: A retrospective medical record review was conducted from 2006 to 2016. Patient demographics, perioperative clinical courses, complications and long-term outcomes were reported as percentages and categorized according to the surgical procedure performed.

Results: A total of 15 children underwent esophageal replacement procedures, comprising 7 (47%) isoperistaltic gastric tubes, 3 (20%) colonic interpositions, 3 (20%) gastric transpositions and 2 (13%) reversed gastric tubes. Indications for esophageal replacement included long-gap esophageal atresia (5; 33%), esophageal atresia with severe postoperative complications (6; 40%), and caustic injury (4; 27%).The mean age of patients was 2.9 years (range: 0.2–15 years). The average follow-up duration was 3.6 years (range: 0.4–8 years). There was no perioperative mortality and no graft loss in any group. The long-term outcomes were acceptable, with no late stricture. Eighty-six percent of the patients in the isoperistaltic gastric tube group and all patients in the other procedural groups achieved full oral feeding. Nevertheless, the patients had various degrees of malnutrition.

Conclusion: Esophageal replacement remains a major challenge in children. Our experience indicates that children can be safely operated on using any of these methods, with acceptable outcomes and no deaths. Nevertheless, the long-term consequences and complications should be monitored throughout adulthood.

Keywords: Esophageal replacement; isoperistaltic gastric tube; reversed gastric tube; gastric transposition; colonic interposition (Siriraj Med J 2019;71: 80-88)

INTRODUCTION

Over the last several decades, many different techniques and various visceral substitute grafts have been used for esophageal replacement in children.[1-4] Common indications for the esophageal replacement procedure in children include long-gap esophageal atresia, severe peptic or caustic injuries, and anastomotic strictures.[5-7] The ideal esophageal substitute should closely imitate the native esophagus both in size and function; nevertheless, none can match a normal esophagus.[1,5] Several studies have reported comparable outcomes for each technique, with no significant differences in terms of their early and late complications.[1-7] The procedure selection and graft choice in those studies were based on the anatomy and

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availability of the visceral substitute and, in particular, the experience and preferences of the surgeon. Factors influencing the outcomes were related to the infrequency with which the procedures were performed, the variable expertise among the surgeons, and the lack of an ideal conduit.[5]

The aim of the present study was to review our 10-year-experience using various esophageal replacement techniques and to compare the complications and outcomes of those procedures in children at the Division of Pediatric Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Ethical approval for this study as a retrospective evaluation of practice was obtained from the Siriraj Institutional Review Board (Si 628/2016).

MATERIALS AND METHODS

The medical records of all patients who underwent an esophageal replacement procedure between 2006 and 2016 were retrospectively reviewed. The clinical data extracted included patient demographics, indications for surgery, perioperative courses, complications and outcomes. Each parameter was reported as a percentage and categorized according to the surgical procedure performed. The patient demographics included the associated congenital anomalies and pre-replacement surgical procedures. The early postoperative outcomes were evaluated according to the duration of the overall admissions, the degree of intensive care and intubation needed, and the number of readmissions after the replacement operation. The perioperative adverse events comprised respiratory complications, esophageal leakages, esophagocutaneous fistulas, gut obstructions, delayed gastric function, dumping syndrome, wound infections and death; they were reported as percentages for each surgical procedure. The operative outcomes were categorized into gastrointestinal and respiratory outcomes and listed in the same fashion. The long-term anthropometric assessments were expressed in degrees of malnutrition, using the weight at the final follow-up compared to the standard growth chart.

RESULTS

Between January 2006 and December 2016, 15 patients underwent an esophageal replacement at the hospital. The patients had mostly been referred from other hospitals. The mean age of the patients was 2.9 years (range: 0.2-15 years). There were 11 (73%) males and 4 (27%) females. The average follow-up duration was 3.6 years (range: 0.4-8 years). The operative procedures of the 15 children studied consisted of 7 (47%) isoperistaltic gastric tubes, 3 (20%) colonic interpositions, 3 (20%) gastric transpositions and 2 (13%) reversed gastric tubes. The indications for esophageal replacement included 5 (33%) long-gap esophageal atresias, 6 (40%) esophageal atresias with postoperative complications, and 4 (27%) caustic injuries. The indications for surgery, categorized by procedure, are demonstrated in Table 1.

The majority of patients (73%) had esophageal atresia, either with long-gap or severe postoperative complications after esophagoesophagostomy (anastomotic leakages, strictures unresponsive to dilatation, empyema thoracis and diverticulum with bleeding), whereas 4 patients (27%) had caustic injuries.

The patient characteristics and associated anomalies are summarized in Table 2. The median age at surgery was 12 months for those patients who underwent the gastric tube and gastric transposition procedures, but much older (108 months) for the colonic interposition group. Accordingly, the median body weights at surgery were 9.6 and 8 kg for the isoperistaltic gastric tube and gastric transposition groups, respectively. The smallest baby in each group weighed 4.7 and 6.2 kg, respectively. There were only 2 cases in the reversed gastric tube group weighing 12 and 21 kg at the time of surgery. As the colonic interposition procedure was performed in older children, their median weight was 20 kg. VACTERL association (vertebral, anorectal, cardiac, renal and limbs anomalies) was the most common associated anomaly, due to esophageal atresia (types A, B and C) being the main indication for surgery.

TABLE 1. Indications for esophageal replacement.

Diagnosis	Isoperistaltic gastric tube	Reversed gastric tube	Gastric transposition	Colonic interposition
Long-gap EA	4	-	-	1
EA with complications	2	1	3	-
Caustic injury	1	1	-	2
Total	7 (47%)	2 (13%)	3 (20%)	3 (20%)

Abbreviation: EA= esophageal atresia

TABLE 2. Patient characteristics.

	Isoperistaltic gastric tube (n = 7)	Reversed gastric tube (n = 2)	Gastric transposition (n = 3)	Colonic interposition (n = 3)
Median age at surgery (months)	12 (3–120)	12, 120	12 (6–24)	108 (36–180)
BW at surgery (kg)	9.6 (4.7–27)	12, 21	8 (6.2–10)	20 (17–23)
Caustic injury	1 (14%)	1 (50%)	-	2 (67%)
Type atresia	6 (86%)	1 (50%)	3 (100%)	1 (33%)
No fistula (type A)	2 (33%)	-	-	1 (100%)
Proximal fistula (type B)	2 (33%)	-	-	-
Distal fistula (type C)	2 (33%)	1 (100%)	3 (100%)	-
Congenital anomaly				
Vertebral	-	-	2 (67%)	1 (33%)
Cardiac	2 (28%)	-	3 (100%)	-
Anorectal	-	-	1 (33%)	-
Renal	-	-	1 (33%)	-
Limbs	-	-	1 (33%)	-
Duodenal atresia	1 (14%)	-	1 (33%)	-
Down syndrome	1 (14%)	-	-	-
Pre-replacement surgery				
Esophagoesophagostomy	2 (28%)	1 (50%)	2 (67%)	-
Gastrostomy	7 (100%)	2 (100%)	3 (100%)	2 (67%)
Cervical esophagostomy	6 (86%)	1 (50%)	3 (100%)	-
Endoscope with dilatation	2 (28%)	1 (50%)	-	2 (67%)
Gastrojejunostomy	-	-	-	1 (33%)
Duodenoduodenostomy	1 (1–4%)	-	-	-

All patients with long-gap esophageal atresia unsuitable for elongation techniques underwent cervical esophagostomy and feeding gastrostomy prior to their replacement surgery. Other types of esophageal atresia patients initially underwent esophagoesophagostomy and subsequently required cervical esophagostomy and gastrostomy due to severe complications, as mentioned above. In the case of children with caustic strictures, 4 (27%) underwent esophageal replacement when their endoscopic dilatations failed. Almost all patients (93%) were fed by gastrostomy until the appropriate time and weight for the replacement procedure.

The early postoperative outcomes are presented in Table 3. The gastric transposition group had the longest median admission period of 68 days owing to previous operations and complications. The intensive care periods after the replacement operation for all groups were typically 2 to 3 weeks. The median intubation period for these techniques was about 2 to 6 days. Postoperative readmissions occurred approximately 3 to 5 times for esophagoscopy and anastomotic dilatation, although some were due to respiratory problems.

The perioperative complications are summarized in Table 4. There was no perioperative mortality, and none of the grafts were lost. Respiratory complications were the most common adverse event in all groups. There was 1 esophageal leakage (in the gastric transposition group) and 2 esophageal fistulas (one each in the gastric transposition and isoperistaltic gastric tube groups). The complications of delayed gastric function and dumping syndrome arose after gastric transposition, with an incidence of 33% each. Wound infections developed most frequently in the colonic interposition group, affecting all of its cases. All esophageal leakage, esophagocutaneous fistulas and gut obstructions were successfully treated conservatively. The gastrointestinal and respiratory outcomes are listed in Table 5. Full oral feeding was achieved by 86% of the isoperistaltic gastric tube group and 100% of the other

TABLE 3. Early postoperative outcomes.

	Isoperistaltic gastric tube (n = 7)	Reversed gastric tube (n = 2)	Gastric transposition (n = 3)	Colonic interposition (n = 3)
Admission period	37 (17–106)	18, 27	68 (24–84)	22 (20–55)
Intensive care period	17 (8–44)	8, 15	23 (9–35)	13 (4–14)
Intubation period	2 (1–9)	1, 2	6 (1–10)	2 (1–12)
Postoperative readmissions (number of readmissions)	5 (1–14)	2, 12	5 (2–20)	3 (1–3)

Values expressed as days: median (range).

TABLE 4. Perioperative complications.

	Isoperistaltic gastric tube (n = 7)	Reversed gastric tube (n = 2)	Gastric transposition (n = 3)	Colonic interposition (n = 3)
Respiratory complications	1 (14%)	2 (100%)	3 (100%)	1 (33%)
Pneumothorax	-	1 (50%)	-	-
Pneumonia or aspiration	1 (14%)	1 (50%)	3 (100%)	1 (33%)
Esophageal leakage	-	-	1 (33%)	-
Esophagocutaneous fistula	1 (14%)	-	1 (33%)	-
Gut obstruction	2 (28%)	-	-	1 (33%)
Delayed gastric function	-	-	1 (33%)	-
Dumping syndrome	-	-	1 (33%)	-
Wound infection	1 (14%)	-	-	3 (100%)
Death	- (0%)	- (0%)	- (0%)	- (0%)

TABLE 5. Gastrointestinal and respiratory outcomes of esophageal replacement.

	Isoperistaltic gastric tube (n = 7)	Reversed gastric tube (n = 2)	Gastric transposition (n = 3)	Colonic interposition (n = 3)
Gastrointestinal				
Full oral feeding	6 (86%)	2 (100%)	3 (100%)	3 (100%)
Anastomotic stenosis	6 (86%)	1 (50%)	3 (100%)	3 (100%)
Number of endoscopic dilatations per patient	2 (2–11)	6	4 (2–10)	2 (1–31)
Dysphagia	4 (57%)	1 (50%)	1 (33%)	2 (67%)
Oromotor dysfunction	-	1 (50%)	3 (100%)	-
Reflux	1 (14%)	-	1 (33%)	1 (33%)
Tortuous/redundant	2 (28%)*	1 (50%)*	-	3 (100%)
Respiratory				
Chronic lung disease	1 (14%)	-	-	-
Recurrent pneumonia	1 (14%)	1 (50%)	3 (100%)	-
Restrictive lung disease	-	-	-	1 (33%)

*The tortuosity of the grafts was surgically corrected by manubrium excision

groups. Anastomotic stenosis was found in 50%-100% of the cases in the groups, and all resolved after endoscopic dilatation. The median number of endoscopic dilatations required ranged from 2-6 times per patient. Dysphagia and reflux manifested sporadically in 14%-67% of the cases in the groups. Oromotor dysfunction developed in all patients who underwent gastric transposition. Nevertheless, the majority of patients achieved full oral feeding. Tortuosity of the cervical anastomosis arose in 28%-50% of cases, and mainly in the isoperistaltic and reversed gastric tube groups; all cases were surgically corrected by manubrium resection. Redundancy of the graft was present in the colonic interposition group with minimal symptoms; therefore, no intervention was required. The respiratory outcomes comprised chronic lungs, restrictive lung disease and, the most common, recurrent pneumonia (which was found in all patients in the gastric transposition group).

The long-term anthropometric outcomes at the final follow-up are illustrated in Table 6 and Fig 1. The patients had various degrees of malnutrition, particularly in the gastric tube and gastric transposition groups, with 57%-100% of the aforementioned groups weighing less than the third centile on the standard growth chart. An average weight gain was achieved within the study period by 67% of the patients in the colonic interposition group and 20% of the study cohort.

DISCUSSION

Esophageal replacement remains a major challenge in children. Since there is presently no replacement technique that can replace the features of a normal esophagus, many pediatric surgeons believe patients are best served by their own esophagus. The common indications in children are long-gap esophageal atresia; severe peptic ulcers; caustic and anastomotic strictures; and some rare esophageal disorders such as tumor, prolonged impaction of radiolucent foreign bodies and intractable achalasia.[5,6,8,9] The common indications for replacement procedure in our study were long-gap esophageal atresia (types A and B) and complicated esophageal atresia with distal tracheoesophageal fistula. Caustic injuries following failed dilatations are another common indication for esophageal replacement in children. As in other developing countries[10], caustic injuries in Thailand continue to be one of the most common health hazards because caustic agents, such as household cleaning materials, are frequently stored in unsuitable or poorly labelled secondary containers.

Various esophageal replacement grafts and techniques have comparable outcomes, none of which emulate the normal esophagus.[11] The ideal esophageal conduit should maintain the entire esophageal length, which would allow normal swallowing while technically being simple and adaptable for small children. Accordingly, it should not compress the mediastinum or suppress respiration, should not become tortuous or redundant, should have minimal gastro-esophageal reflux, should not increase the malignancy risk, and should function normally for the lifetime of the patient.[5,10]

Many esophageal replacement techniques are being practiced and recommended without clear consensus. Furthermore, no randomized, controlled trials have yet demonstrated significant, superior, clinical outcomes of any one of the different types of esophageal replacement. The four most commonly used esophageal replacement techniques are gastric transposition, gastric tube interposition (isoperistaltic or reversed), colonic interposition and

TABLE 6. Long term anthropometric outcomes at final follow up.

	Isoperistaltic gastric tube (n = 7)	Reversed gastric tube (n = 2)	Gastric transposition (n = 3)	Colonic interposition (n = 3)
Malnutrition	4 (57%)	2 (100%)	2 (67%)	1 (33%)
Percentile body weight				
< 3 percentile	4 (57%)	2 (100%)	2 (67%)	1 (33%)
3-25 percentile	2 (28%)	-	1 (33%)	-
25-75 percentile	1 (14%)	-	-	2 (67%)
75-97 percentile	-	-	-	-

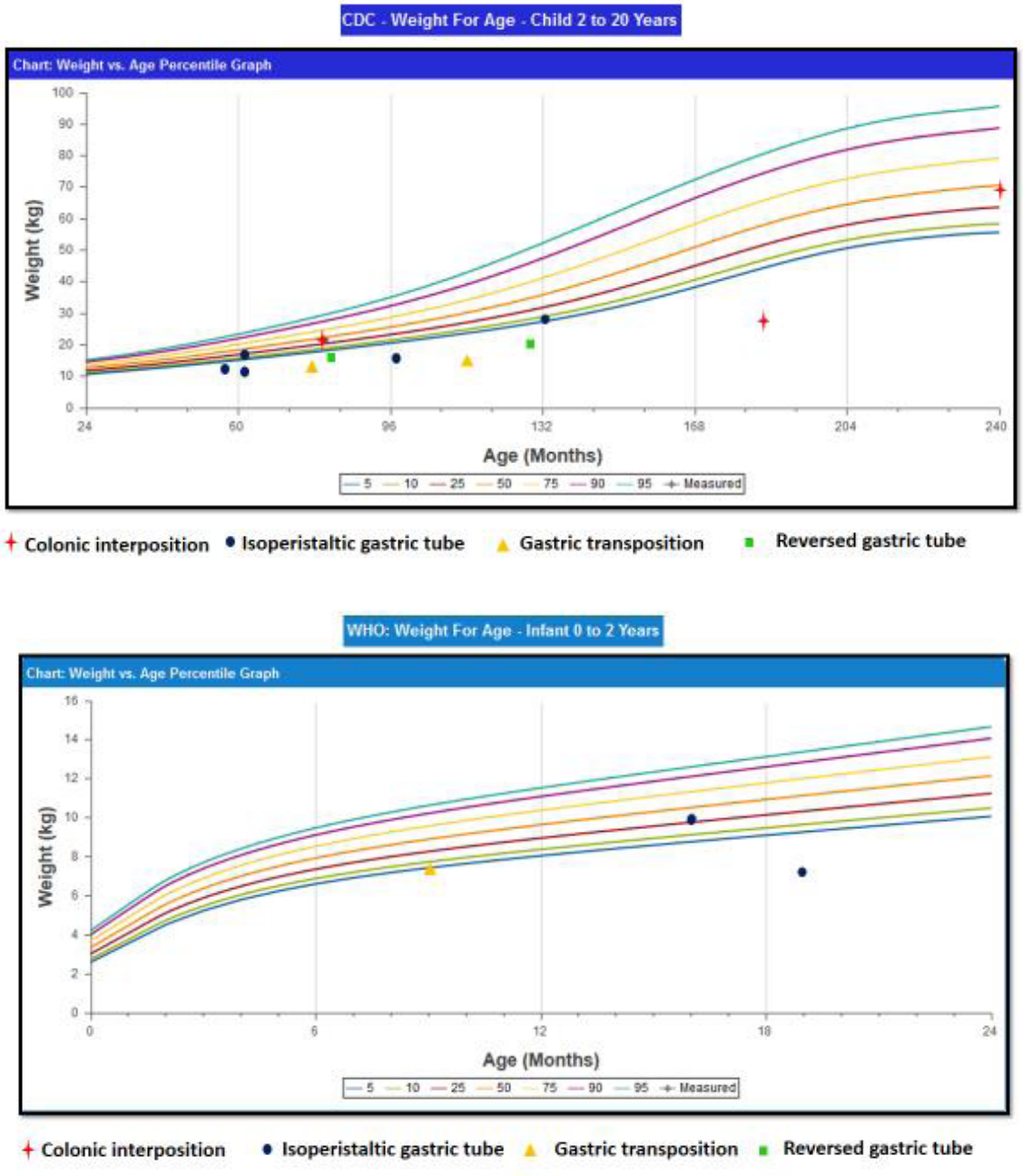


Fig 1. Growth at final follow-up.

jejunal interposition.[8,11–15] At our institute, the more technically demanding jejunal interposition has been the least preferred; therefore, the esophageal replacement procedures practiced have comprised gastric transposition, isoperistaltic or reversed gastric tube, and colonic interposition. Almost half (47%) of the patients studied at our institute underwent isoperistaltic gastric tube, given that the stomach has better vascularity and fewer ischemic complications.[1,11] The graft choices were based on anatomical considerations and the preferred technique of the treating surgeons rather than on any discernible objective data. The factors influencing the outcomes were related to the relatively infrequent need for esophageal replacement, the surgeons' expertise and the absence of ideal grafts.[5]

Various esophageal substitutes have different advantages, technical difficulties and specific complications. The overall morbidity is high, the most common being anastomotic leakage and stricture, with both ranging from 10% to 20%.[8]

The overall replacement-related mortality rate has been assessed to be approximately 2%.[5] In our study, there was no perioperative mortality, and none of the grafts were lost. The early post-operative outcomes of the

methods showed no differences in their intubation periods, intensive care periods or post-operative readmission rates. The intubation period was about 1-2 days, whereas the intensive care period was typically about two weeks. Patients in the gastric transposition group had the longest median intubation and intensive care periods (6 and 23 days, respectively), being complicated by type-C esophageal atresia from previous esophagoesophagostomy operations with associated cardiac anomalies. The post-operative readmissions of all groups were due to pneumonia or esophageal dilatation, and they averaged around 3-5 times per group.

Gastric tube interposition grafts are constructed from the greater curvature in a reversed (antiperistaltic) or isoperistaltic fashion.[9] Tube graft necrosis is rare because the gastric tube has an excellent and reliable blood supply from the submucosal plexus and gastroepiploic vessels.[1] The gastric tube can bridge relatively long gaps and remain as a passive conduit with a tubular shape and without dilatation or tortuosity. Other series using a gastric tube substitute had cervical anastomotic leakage despite being sealed spontaneously with dilatable strictures.[1,16] In our study, the most common perioperative adverse event was respiratory complications, appearing in both isoperistaltic and reversed gastric tube interpositions. Esophagocutaneous fistulas (14%), gut obstructions (28%) and wound infections (14%) were not common after isoperistaltic gastric tube interposition, and all were resolved by conservative treatment.

The evaluation of the long-term gastrointestinal outcomes found that 86% of the patients in the isoperistaltic gastric tube interposition group and all patients in the other groups achieved full oral feeding. Anastomotic stenosis was common, albeit dilatable in all groups. Borgnon et al.[1] reported that of their series of esophageal replacements with isoperistaltic gastric tube, 80% achieved a normal diet, 15% had mild dysphagia, 15% had major dysphagia, and 10% had redundant grafts with dumping syndrome and cervical Barrett's esophagus. Our study demonstrated that 57% of the patients had dysphagia, 14% had reflux and 28% had tortuosity of the cervical anastomosis. In consideration of the proximal esophageal graft anastomosis, most could be achieved through the neck incision. When the anastomosis is high within the neck, the thoracic inlet can act as a constriction to the anastomosis. In that event, the thoracic inlet can be widened by resecting the upper part of the manubrium or the sternal head of the left clavicle. Consequently, the tortuous grafts in 3 patients who underwent gastric tube interposition procedures were surgically corrected by manubrium excision. Although the incidences of long-term respiratory problems, including recurrent pneumonia and chronic lung diseases, ranged from 14%-50%, they did not differ between the two gastric tube groups.

The disadvantage of the gastric tube is an extensive suture line that produces a higher incidence of leakages and strictures. The reduced gastric capacity and the production of acid within the tube graft results in acid reflux into the cervical esophagus, precipitating Barrett's esophagitis.[1,5,16,17] Esophagitis and metaplasia have been described in children following gastric tube replacement. There should be regular monitoring of the esophageal conduit since chronic exposure to acid reflux may predispose to metaplasia and adenocarcinoma.[1]

Gastric transposition or the gastric pull-up procedure has several advantages. It is relatively simple with a single anastomosis at the neck, creating a sufficiently long and very well-vascularized graft.[10,12] It has been shown to have a reduced risk of anastomotic leakage, stricture and necrosis.[5,8] The perioperative complications in our study were esophageal leakage, esophagocutaneous fistula, delayed gastric function and dumping syndrome (each arising in 33% of patients) and pneumonia (in all patients). Vagotomy during gastric mobilization may lead to delayed gastric emptying and dumping syndrome. In addition, pyloromyotomy and pyloroplasty may be performed to prevent delayed gastric emptying. Spitz et al.[8,18] reported the outcomes of gastric transposition in 236 patients, which were a 2.5% mortality rate, a 12% leakage rate and a 20% stricture rate. In our study, the long-term outcomes of the gastric transposition group revealed all patients (100%) achieved full oral feeding, with all having anastomotic stenosis, oromotor dysfunction and recurrent pneumonia. It is speculated that the recurrent pneumonia was a consequence of underlying complicated, type C, esophageal atresia. However, previous studies have acknowledged that gastric transposition is associated with a higher respiratory morbidity.[11] Occupying the mediastinum and chest, the stomach may not empty effectively, causing compression (mass effect) of the intrathoracic organs and a long-term reduction of the lung capacity. The long-term gastrointestinal outcomes also included 33% gastroesophageal reflux and dysphagia rates. The reflux may lead to recurrent heart burn, regurgitation, bad breath and pneumonia. Dysphagia has been found to be common, despite successful and adequate replacements[5,6], which could be due to discoordinated peristalsis, antiperistaltic layout, a tortuous esophageal conduit or significant acid reflux.

Colonic interposition is the most frequently used esophageal replacement procedure and provides superior length.[1,5] The perioperative complications in our study showed 33% respiratory complication and gut obstruction rates. Although there was a 100% wound infection rate, there were no leakages or fistulas. In the long-term follow-up, all patients (100%) achieved full oral feeding, and they developed dilatable anastomotic stenosis and redundancy of the colonic graft; however, the patients did not require intervention. The long-term gastrointestinal outcomes comprised dysphagia (67%) and reflux (33%), while the respiratory problem was restrictive lung disease (33%). In 2015, Lobeck et al.[14] reported that the most common postoperative complication among 10 patients after colonic interposition was esophageal stricture (54%). The disadvantage of colonic interposition is the redundancy of grafts with stasis and dysphagia due to a negative pressure in the thoracic cavity and emptying by gravity.[4,5,19, 20] Complications such as anastomotic leakage and strictures have also been reported to be related to a precarious blood supply.[4] Other renowned, large-scale series[8,15,18] with long-term outcomes have demonstrated no significant differences in the early or late complications of the gastric transposition and colonic interposition techniques.

Jejunal interposition can be used as a pedicle or free graft.[11] The main advantage of this graft type is the most suitable caliber of the grafts with peristaltic activity.[3] Conversely, high failure, morbidity and mortality rates[13] have been reported for this procedure, presumably due to technical difficulties and a tenuous blood supply. Our center has no experience with this technique.

The average follow-up duration in the current study was 3.6 years (range: 0.4-8 years). The measured growth in patients after esophageal replacement at the final follow-up demonstrated growth retardation and malnutrition in all procedural groups, with 57% in the isoperistaltic gastric tube, 100% in the reversed gastric tube, 67% in the gastric transposition and 33% in the colonic interposition groups. Malnutrition was deemed to be present when a child's weight-for-age fell below the third centile of the standard growth chart. Growth retardation is prevalent in patients with esophageal atresia. Oral aversion is common among infants with long-gap esophageal atresia due to delayed oral feeding, caused by either a lack of sham feeding in oral feeds or delays in the replacement.[5] It is important to monitor nutritional status, growth and development in children after an esophageal replacement as they are known to fall below centiles for both height and weight.[1,3,4]

The limitations of a retrospective design, the small number of patients and technical variations led to difficulty in making comparisons to discover any statistical significance in this study. Close monitoring and long-term follow-up through more substantial group studies may elicit the clinically important factors relating to the care of these patients.

CONCLUSION

Esophageal substitution remains a major challenge in children. Our experience indicates that children can be safely operated on using any of the various methods currently available, with acceptable perioperative morbidities and no mortality. Nevertheless, the long-term consequences and complications should be monitored throughout adulthood.

Conflict of interest: The authors have no conflicts of interest to declare.

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Extracellular Vesicles in Malaria Infection

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ABSTRACT

Malaria is one of the tropical diseases which cause high rate of morbidity and mortality. The disease is caused by the infection of protozoan parasites in the genus *Plasmodium*. The severe syndromes of malaria infection arise from the complex sequences of parasite-host interactions. It starts with parasite invasion and followed by the rupture of infected red blood cells causing the release of parasite products that activate the host immune response. During the past decade, research on the functions of extracellular vesicles (EVs) in many diseases including malaria has increased dramatically. This article reviews the role of EVs in malaria immunopathogenesis. Investigations into modulators in immune response, ubiquitous mechanism for intercellular communication between parasite-parasite and parasite-host, as well as its usefulness as the diagnostic biomarkers are highlighted.

Keywords: Extracellular vesicles; exosomes; Malaria; *Plasmodium* spp. (Siriraj Med J 2019;71: 89-94)

INTRODUCTION

Extracellular vesicles (EVs) are heterogeneous types of small membrane-enclosed particles which originated from many cell types and can be found in body fluids such as serum, plasma and cerebrospinal fluids (CSF). EVs released from the cells in physiological conditions have significant role in homeostasis. Its level tends to increase under the pathological conditions. EVs transport cellular components such as proteins, lipids and genetic materials from the originating cells to the recipient cells. These EVs can be directly fused at plasma membrane or internalized by endocytosis resulting in the EVs cargo transfer and function in the recipient cells. Nowadays, the roles of EVs have been widely demonstrated in many diseases such as cardiovascular diseases, cancer and autoimmune diseases.[1,2] The contribution of EVs is also well-established in infectious diseases. However, study of EVs in the context of a parasitic infection is complicated because both the parasites and the host cells release the EVs into the extracellular space which play a role in disease pathogenesis. The EVs in niche environment provide the intercellular communication between parasites and other parasites or host cells. It leads to either activation or modulation in the host immune response to parasites. This review provides an overview of the research studies on EVs in malaria infection.

Characteristics of EVs in malaria infection

Although the definite terminology for different types of EVs has not yet finalized. Three types of commonly known EVs, apoptotic bodies, ectosomes and exosomes, are classified based on their biogenesis and characteristics. Because of their different biogenesis and originating cells, each EV type contains distinct active biological components and has specific biological functions.[3,4]

The apoptosis bodies are the largest particles with 1-5 micron in size. They result from outward blebbing of plasma membrane of cells at the end stage of apoptosis.

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They carry various molecules including the nuclear fractions, cell organelles and genomic DNA which involve in facilitating apoptotic process. The term of ectosomes, also referred as microvesicles (MVs) or microparticles (MPs), are used for defining the membrane-enclosed particles ranging in size from 0.1 to 1 micron. MPs are released by outward budding from plasma membrane of activated cells or early apoptotic cells. The plasma membrane is composed of a bilayer of lipids, including phospholipid and oily substances. Phospholipids normally enriched at the outer leaflet of plasma membrane are cationic and neutral phospholipids such as sphingomyelin and phosphatidylcholine (PC) while anionic phospholipids located at the inner leaflet are phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylethanolamine (PE). Once cells are in the activated stage or undergo apoptosis, there is the alteration of plasma membrane asymmetry, externalization of anionic phospholipids and vesiculation from the plasma membrane. Therefore, the types of ectosomes can be identified by specific surface markers such as phosphatidylserine, integrins, selectins and other compositions expressed on the membrane of originating cells. The exosomes are the smallest particles with the diameter 40-100 nm. They are generated from the endocytosis of plasma membrane into endosomes and later are accumulated as vesicles in multivesicular bodies (MVB). The fusion of MVB with the plasma membrane allows the release of exosomes from the originating cells to the extracellular space. The common proteins used for identifying exosomes include tetraspanins (CD81, CD63, CD9), flotillin, constitutive heat shock protein 70 (Hsc70) and vesicle trafficking-related proteins (TSG101, ALIX, and RAB proteins, syntenin-1).[5] Both ectosomes and exosomes deliver numerous proteins (cytoplasmic proteins, transmembrane proteins and membrane associated proteins), lipid and nucleic acids (mRNA, miRNA and other non-coding RNAs), similar to those expressed in the parental cells, to the membrane or cytosol of the target cells and function in the same way as parental cells do.

The characteristics of EVs derived from plasma of malaria-infected patients and malaria culture supernatant have been determined. The biophysical analysis of EVs derived from *P. falciparum*-infected red blood cells (iRBCs) showed that the majority of EVs were 50-300 nm diameter with single bilayer membrane.[6] The proteomic analysis of EVs revealed that they contained both host and parasite proteins. The MPs isolated from plasma of malaria-infected patients contained host proteins including complement-associated proteins, coagulation-associated proteins and cytoskeletal proteins. Meanwhile, major parasitic protein components were involved in parasite invasion and parasite growth.[7] The EVs from *P. falciparum* culture supernatant are enriched with red blood cell lipid rafts proteins and membrane-associated parasite antigens, especially proteins associated with red blood cell membranes and proteins involved in parasite invasion.[8,9] These abundant parasitic proteins included the parasite proteins found in cytosol of iRBCs and exported to red cell membrane such as Maurer's clefts; the merozoite secretory proteins such as RhopH protein complex (RhopH1, RhopH2 and RhopH3), the RAP complex (RAP2 and RAP3), RALP1 and RON3; the microneme resident proteins such as EBA-175 and EBA-181; and the dense granule proteins such as HSP101, PTEX150, EXP2, SBP1, RESA, and MAHRP1.[9] Not only proteins, but other genetic materials including genomic DNA(gDNA), functional mRNA, miRNA and other small non-coding RNA have also been detected in EVs.[6,8,10] The parasitic gDNA, human and parasitic small RNA between 4-150 nucleotides were present in EVs derived from *P. falciparum*-iRBCs, especially those from ring-stage iRBCs.[6] The miRNA profile of plasma EVs and peripheral blood cells under normal healthy conditions showed that red blood cell contributed the highest cellular miRNA to the blood and different cell lineage expressing different patterns of miRNA. The miR-451 and miR-150 established crucial function in erythroid- and lymphoid differentiation, respectively. The level of miR-223 is abundant in granulocytes and platelets.[11] The highest miRNAs expressed in plasma EVs were miR-223, -484, -191, -146a, -16, -26a, -222, -24, -126, and-32.[12] Since *Plasmodium* spp., do not have a mechanism to produce miRNA, all miRNAs isolated from malaria-infected red blood cells or from the plasma of malaria-infected patients have been confirmed to be of human origin.[13-15] The majority of miRNA found in iRBCs-derived EVs were miR-451, let-7b and miR-106b.[10] However, the expression levels of miRNAs, miR-19b, -4732, let-7a, -16, -183, -18a and 148b in iRBCs-derived EVs were lower than those in uninfected RBCs-derived EVs.[6]

EVs as an amplifier in the pathology of malaria infection

Human malaria is caused by the infection of intracellular protozoan genus *Plasmodium* transmitted by Anopheles mosquitoes. There are five species of *Plasmodium* which are *P. falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi.* Among these, the *P. falciparum* and *P. vivax* are highly prevalent in Thailand and the Southeast Asian region.[16]

Malaria infection begins with the injection of

sporozoites from infected mosquitoes during a blood meal. Sporozoites invade hepatocytes and proliferate into merozoites. It leads to rupture of infected hepatocytes. The hepatic merozoites enter the blood circulation, invade erythrocytes and begin the erythrocytic schizogony. The clinical symptoms are associated with the invasion of asexual erythrocytic-stage parasite to erythrocyte, and the inappropriate immune response to iRBCs and diverse parasite-derived products. The severe *P. falciparum* infection results from the sequestration of iRBCs, leukocytes and platelets to endothelial cells (ECs) within microvessels, the excessive proinflammatory cytokine production and the severe red blood cell hemolysis. The parasite sequestration leads to accumulate host cells within vasculature, injure ECs and disrupt blood flow, causing tissue hypoxia and lactic acidosis. The sequestration mechanisms contribute to organ-specific syndromes such as cerebral malaria and placental malaria.[17] Recent studies in both animal models and malaria-infected patients suggest the significant contributions of EVs to severe malaria.

In rodent malaria model, the association of EVs and malaria pathogenesis was initially studied in ATP-binding cassette transporter (ABC) knockout mice. The ABC is a cholesterol transporter involved in controlling the outward translocation of PS at the plasma membrane.[18] The deletion of this gene results in reducing the externalized expression of PS on the cell surface and inhibit the MPs production. The *Plasmodium berghei* strain ANKA (PbA) infection in the ABCA1 knockout mice ($ABCA^{-/-}$) showed the decreasing of plasma tumor necrosis factor (TNF) level and resistance to cerebral malaria. The MPs from these mice had lower procoagulant activity than those from wide type mice.[19] Treatment with pantethine, a provitamin regulated lipid metabolism, to PbA-infected mice also reduced the MPs production, decreased platelet reactivity and impaired endothelial cell activation by MPs resulting in prevention of cerebral malaria development.[20] By contrast, the adoptive transfer of MPs from PbA-infected mice with the neurological symptoms led to localize MPs in cerebral microvessels of PbA-infected recipient mice. The transfer of endothelial cell-derived MPs (EMPs) also induces the signs of pathologies in the brain and lung of the recipient mice.[21] These findings indicate that host cell-derived EVs have pathogenic roles such as procoagulant activity and proinflammatory potentials in the pathogenesis of cerebral syndrome.

Accumulating evidence of host cell-derived MPs in plasma of malaria-infected patients has also supported the contention that the elevated levels and origin of MPs are associated with the disease severity. The levels of red blood cell-derived MPs (RMPs), platelet-derived MPs (PMPs) and EMPs were markedly increased in severe falciparum malaria patients. The level of RMPs were significantly elevated in patients with severe anemia while the levels of EMPs and PMPs correlated to coma depth and thrombocytopenia.[22-24] Similarly, the levels of MPs derived from leukocytes, platelets, and erythrocytes were significantly increased and the level of PMPs correlated with the presence of fever in acute *P. vivax*-infected patients.[25] These elevated levels of MPs were reduced to normal level at the convalescent phase and the clearance of parasitemia.[24,26]

Although the precise mechanisms underlying the induction of MPs production during the course of infection are not completely understood, many factors have been described. The CD40L-induced platelet apoptosis and thrombocytopenia were associated with increasing plasma PMPs in PbA-infected mice with severe syndrome.[27] The exposure to febrile temperature led to the significant increases of PS expression on the surface of iRBCs, particularly at the late schizont stage before the red blood cell egress which was corresponding to releasing EVs from iRBCs.[28] Moreover, the level of proinflammatory cytokine TNF was positively correlated with the level of circulating plasma MPs in malaria-infected patients, thus TNF might be another factor that induces the releasing of MPs in malaria infection.[26]

The major cell sources of EVs in malaria infected patients were from platelets and red blood cells. The *in vitro* study showed that infected red blood cells produced more EVs per cell than uninfected cells.[8,24] The components that were carried by EVs were associated in driven malaria pathogenesis. The EVs derived from plasma of malaria-infected patients and *P. falciparum* culture supernatant contain the proteins which are involved in parasite invasion and parasite growth. Therefore, these EVs might play a role in facilitating red blood cell invasion by merozoite during intraerythrocytic life cycle. The plasma MPs from cerebral malaria infected mice, but not from non-infected mice, carried proteins that were implicated in molecular mechanisms relevant to cerebral malaria pathogenesis, including endothelial activation.[29] In addition, the reticulocyte-derived exosomes of non-lethal *P. yoelii* 17X-infected mice contained the parasite antigens. The immunization of these purified exosomes induced the specific immune response to *P. yoelii* infected red blood cells.[30] The EVs also transferred the functional miRNAs, especially miR-451, from red blood cells to endothelial cells and targeted on the genes of proteins required for barrier function leading to vascular alteration.[10]

In addition to these biomolecules, hexanal compound was also encompassed with EVs of iRBC, because the

volatile organic compound presented in EV of iRBC is diacetin which is the insect attractant like hexanal. However, both compounds have been proven to play a role in malaria transmission.[31]

EVs as a messenger in parasite communication

Within the past decade, EVs have emerged as important mediators of communication between EVs-secreting cells and recipient cells. Two crucial experimental studies demonstrated the crosstalk between intraerythrocytic stage -*P. falciparum* by EVs. Regev-Rudzki *et al* exhibited the role of EVs cargo in parasite crosstalk by using the transgenic parasites. The EVs derived from drug resistance *P. falciparum*-iRBCs can transfer DNA encoding for a drug resistance marker between individual parasites leading to spreading of drug resistance in the parasite population. Moreover, this study showed that EVs transferring between *P. falciparum* parasites under drug pressure-induced stress conditions allows increased differentiation of gametocytes.[32]

In parallel, Mantel et al also revealed that iRBCs are able to internalize EVs isolated from malarial culture supernatant and transfer them into the parasite cytosol leading to increased gametogenesis.[8] However, the exact underlying mechanism of this phenomenon was not obviously proven in this work. However, another study showed that the endogenous translocation of human miRNA-451 from red blood cells into the parasites leads to chimeric fusion RNAs with regulatory subunit of cAMP-dependent protein kinase (PKA-R) transcripts of *P. falciparum* resulting in reduction of the translation of the regulatory PKA subunit. The suppression of PKA-R is associated with reduced parasite growth and increased numbers of gametocytes.[33] All these findings suggested that the EVs transfer genetic materials of parasite and host cells to parasites of other infected red blood cells resulting in alteration of the parasite cycle.

EVs as a modulator on the host immune response

Internalization of EVs by immune cells leads to either activation or suppression of the immune cell function. Because monocytes are the key immune cells that play a role in phagocytic eradication of iRBCs and free merozoites in blood circulation during intraerythrocytic life cycle, there are several interesting studies in monocyte/macrophage immunomodulation by EVs during malaria infection. The iRBCs-derived MPs from the plasma of PbA-infected mice induce macrophage activation by up-regulation of CD40 expression and proinflammatory cytokine TNF production. The activation is via TLR-4 and MyD88 dependent pathways resulting in systemic inflammation which impacts on both progression of disease complications and generation of adaptive immune responses.[34] In addition, the internalization of iRBCs-derived MVs isolated from malarial culture supernatant by macrophages triggers the strong pro- and anti-inflammatory cytokine responses of TNF and IL-10 production, respectively. The pre-incubation of these MVs with neutrophils also reduces neutrophil function.[8] Not only in malaria infection, the malaria EVs also attenuate neutrophil function in response to bacterial infection by inhibiting ability to produce reactive oxygen species and suppression of cytokine secretion.[35] These data demonstrate that EVs from iRBCs, but not from uninfected red blood cells, strongly modulate the cells of the innate immune system.

Immunization with reticulocyte-derived EVs from *P. yoelii* 17X-infected mice can induce the *P. yoelii* infected red blood cells-specific IgG antibody production.[30] Study showed that the immunization with the combination of these reticulocyte-derived EVs and CpG-ODN to BALB/c mice leads to significant increase in the percentage of effector T cells of both CD4 and CD8 T cells, especially effector memory CD4 subset when compared to mice immunized with reticulocyte-derived EVs isolated from uninfected mice. In addition, *in vitro* experiment showed that the exosome isolated from *P. vivax*-infected patients are captured by splenocytes leading to significant increase of the number of CD3 T cells and CD8 T cells, but there is no change on B or NK cell population. These data suggested that EVs also activate the immune cells of the adaptive immunity.[36] In addition to the immunization of iRBCs-derived EVs in malaria infection, the subcutaneous immunization with the exosomes from excretory/secretory products of *Echinostoma caproni*, an experimental intestinal helminth, in mice can reduce symptom severity during infection.[37] The intraperitoneal immunization of mice with EVs isolated from *Heligmosomoides polygyrus*, a gastrointestinal nematode, in alum adjuvant Is resulted in induction of specific antibody response against larval challenge and reduction of intestinal worm burdens.[38] Collectively, the parasitic EVs can also modulate diverse aspects of the immune system, suggesting that these EVs might be the candidate strategy used for vaccine development.

EVs as a source of diagnostic biomarkers

Today, the circulating miRNAs have been the research subject of interest as they can be used as biomarkers in many diseases. Alteration of miRNA expression reflects the pathological status. Upon malaria infection, infected

hepatocytes or other tissues at pathological sites, such as brain, placenta and bone marrow, may produce and release the EVs containing tissue-specific miRNAs to blood circulation. Detection of such miRNAs may allow discrimination between infected individuals with uncomplicated symptoms and those with specific organ complications or to be used as the surrogate markers for detecting the hypnozoites that remain dormant in the liver.

The differential miRNA expression study in the brain tissues from PbA-infected mice showed that the levels of miR-27a, miR-150, and let-7i, miRNAs in regulation of cellular proliferation and the innate immune response, are upregulated in infected mice with cerebral malaria when compared to those without cerebral malaria symptoms.[39] The development of protective immunity against malarial blood stages of *P. chabaudi* or the lethal outcome of *P. chabaudi* infection in mice are also associated with alteration of miRNA expressions in the liver during the infections.[40,41] As human miRNAs, mir-451 and miR-16, are highly expressed in red blood cells, it is not surprising that these miRNAs are also detected in plasma of both normal healthy and malaria-infected patients.[14,15] However, their levels were significantly downregulated in *P. vivax* infection and negatively correlated with the severity of parasitemia.[42] This might be due to the consumption of miRNAs by parasites during parasite growth inside the red blood cells and the clearance of circulating miRNAs by spleen during infection. A recent study of the EVs-bound miRNAs from plasma of mothers with placental malaria showed overexpression of placenta miRNA, a miRNA in regulatory process during gestation, compared with non-infected group.[43]

CONCLUSION

The involvement of EVs in the pathophysiology of malaria infection have been extensively shown in both malaria-infected patients and in experimental animal studies. EVs are considered as a vehicle necessary for transferring of proteins and nucleic acids which trigger intercellular communication between parasites and host immune cells which lead to change in the parasite biology and regulation of host immune responses. Due to their association with the disease severity, they are now being researched as potential biomarkers and for their use in future vaccine development.

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