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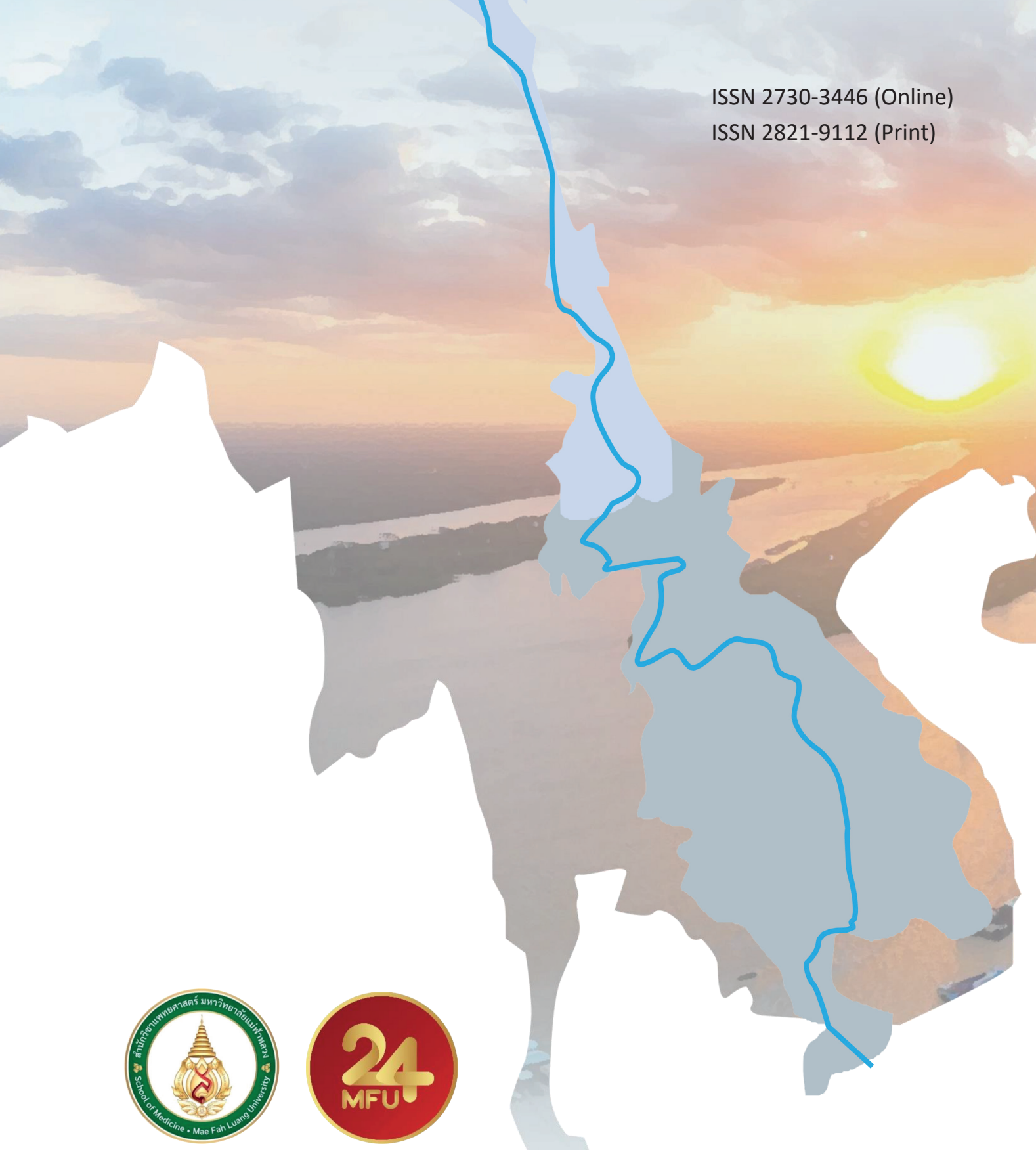

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Aims and Scope	Greater Mekong Subregion Medical Journal is an online and printed, peer reviewed international scientific journal published by Mae Fah Luang University. The journal aims to publish articles in the field of basic and advanced clinical research in medicine and related health sciences, medical education as well as community medicine in Thailand, international and especially in countries of Greater Mekong Subregion. Manuscripts submitted to Greater Mekong Subregion Medical Journal will be accepted on the conditions that the author must not have previously submitted that paper to another journal elsewhere. The journal will not charge for any submission. The reproduction or copy of the articles included the pictures should be under the permission of the publisher.
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School of Medicine • Mae Fah Luang University

Original Article **GMSMJ** **Greater Mekong Subregion Medical Journal**

Manual Small Incision Cataract Surgery (MSICS) in Mature Cataract Using Lerprat Technique: Visual Outcome and Complication

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Abstract:

Background: Mature cataract remains a problem in developing countries included Thailand. The World health organization (WHO) data shows that 60% of blindness in developing countries is caused by cataract. For surgical management, Lerprat Technique has been developed by using conventional extracapsular cataract extraction (ECCE) equipment (Sinskey hook, Iris spatula, Simcoe cannula) without the need for costly or invent new instruments. It can be used in cataract hardness, mature cataract that is common in developing countries. Moreover, it does not interfere with the upper conjunctivae which is used in glaucoma surgical treatments if needed in the future. "Lerprat technique" is a new technique by integrating the "Ruit technique" with the "Modified Blumenthal technique".

Objective: To report visual outcome and complication of manual small incision cataract surgery (MSICS) in mature cataract using Lerprat technique.

Methods: This is a retrospective descriptive study, between January to September 2016. Approval to conduct this study was obtained from the Institutional Review Board Royal Thai Army Medical Department No. IRBRTA 437/2559 and Thai Clinical Trials Registry No. TCTR 20171208003. Data were collected on 60 eyes of 60 patients who performed manual small incision cataract surgery (MSICS) in mature cataract using Lerprat technique. The postoperative visual acuity at first day, 1 week, 6 weeks and 3 months was collected. The intraoperative and postoperative complications were evaluated.

Results: All patients had poor preoperative visual acuity (below 20/200) and achieved improved visual acuity after surgery. On the first postoperative day, the best corrected visual acuity (BCVA) were excellent (20/20-20/30) in 30 cases (50%), good (20/40-20/70) in 28 cases (46.7%) and borderline in 2 cases (3.3%) respectively. On the first postoperative week, visual acuity was excellent (20/20-20/30) in 52 cases (86.7%) and good (20/40-20/70) in 8 cases (3.3%). At 6 weeks and 3 months after operation, visual acuity was excellent (20-20-20/30) in 60 cases (100%). Complications were hyphema in 3 cases (5%) and increased intraocular pressure in one case (1.7%).

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Conclusion: Manual small incision cataract surgery (MSICS) in mature cataract using Lerprat technique is safe and effective procedure.

Keywords: Cataract, Surgery, Lerprat technique, MSICS

Introduction

Mature cataract remains a problem in developing countries included Thailand. The "Thailand Population Data" estimates that in 12 years times there will be 14.4 million elderly people in the country. This group of population will stand the highest chance of having cataracts as the eye lens deteriorates with age. The World Health Organization (WHO) data shows that 60% of blindness in developing countries is caused by cataract. It is estimated that in 2030 there will be 34.865 million people around the world will become blind as a result of untreated cataract.

At present, there are 3 common cataract surgery methods. Firstly, Phacoemulsification, this method uses a small machine to break up the lens into small pieces before sucking the broken pieces out. The advantage of this method is the small incision size of around 3 mm. However, this method is costly. Also, the use of forces to break "mature cataracts" into smaller pieces creates a lot of unwanted heat in the process which can then lead to endothelial cell of cornea loss and decompensation. Second method, Extracapsular Cataract Extraction (ECCE). With this method, the surgical incision size is larger than 10 mm as the whole lens is brought out to remove the cataract. So, the patients will take longer time to recover since the ECCE requires multiple corneal sutures due to the larger incision size. Moreover, the ECCE method interferes with the upper part of the conjunctivae which is an important surgical area used in treating glaucoma if needed in the future. On the other hand, no unwanted heat is created in this method.

The last method, the 'Manual Small Incision Cataract Surgery' (MSICS). There are several techniques in the MSICS method. These include the "Ruit technique"[1], "Modified Blumenthal technique"[2], "Kongsap technique"[3], "Nylon loop technique"[4], "Double nylon loop technique"[5] and etc. Comparing to other methods, the MSICS method causes less bruising on the cornea, requires very little to none suture, less astigmatism and fewer post-operative appointments. Unfortunately, the existing MSICS techniques require the care operators to purchase or invent specific surgical instruments for each technique.

Lt.Col. Lerprat Mangkornkanokpong, M.D., Ph.D., an ophthalmologist with glaucoma expertise has experience in MSICS and understood the existing techniques. He has developed a new technique by integrating the "Ruit technique" with the "Modified Blumenthal technique" to create a new technique, the "Lerprat technique."[6] The advantages of using the "Lerprat technique" in MSICS are 1) Reduced cost as it requires no additional surgical instruments as the standard set of surgical ECCE instruments can be used. 2) Requires only a small amount of subconjunctival anesthesia. 3) Easy surgical procedures. and 4) It does not interfere with the upper conjunctivae which is used in glaucoma surgical treatments if needed in the future.

Materials and methods

This study was performed in accordance with the tenets of the Declaration of Helsinki. Approval to conduct this study was obtained from the Institutional Review Board Royal Thai Army Medical Department No. IRBRTA437/2559 and Thai Clinical Trials Registry No. TCTR20171208003

This retrospective case series was made up of 60 patients with mature cataract who underwent Manual Small Incision Cataract Surgery (MSICS) using Lerprat technique performed by two surgeons (LM and PS) between January to September 2016. All patients with mature cataract who were admitted for surgery were potentially eligible. Patients with ocular infection or ocular pathology such as corneal scar, retinal detachment, optic atrophy, glaucoma were excluded.

Patient data included sex, age, ocular examination, preoperative visual acuity, intraocular pressure by computerized non-contact air puff tonometry, postoperative visual acuity and operative complication. Follow-up patients were examined at 1 day, 1 week, 6 weeks, 3 months and 6 months after surgery. Visual acuities were analyzed of excellent (20/20-20/30), good (20/40-20/70), borderline (20/80-20/200), and poor (less than 20/200) outcomes.

Retrobulbar or subconjunctival anesthesia of 2% xylocaine was administered. There are 5 steps in using the Lerprat technique in MSICS. Step 1: Open anterior capsule of the lens (Capsulorhexis/Capsulotomy) by using a bent needle, together with viscoelastic substance, which makes the operation easier. Step 2: Scleral tunnel incision from temporal side, leaving the upper conjunctivae untouched for glaucoma surgery if needed in the future. The scleral tunnel incision looks like a closed valve. The inside is larger than the outside part so the wound is smaller. Step 3: The lens is removed from the capsule (Nuclear luxation). The lens is tilted and placed on a spatula instrument. Then rotate to remove the entire lens. This looks similar to a car tire changing machine. Step 4: Remove the lens from the eye through scleral tunnel incision (Nuclear delivery). Balance salt solution is used to accelerate the removal by Simcoe cannula via side port incision at 6 or 12 o'clock area. Step 5: Remove the cortex of the lens and intraocular lens (IOL) implantation. Finally remove viscoelastic substance from anterior chamber and check the wound. Generally, this technique does not require any stitches but if so only one suture is sufficient as showed in figure 1-6.

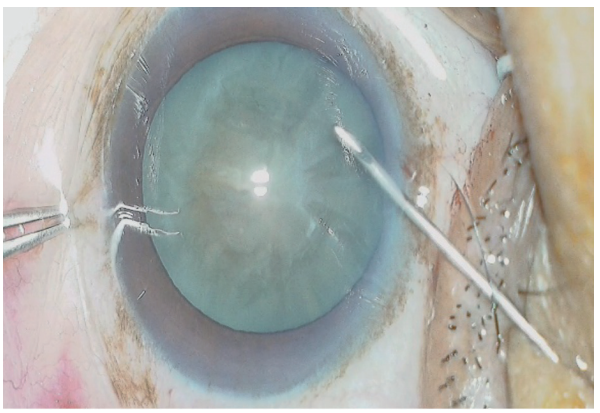

Figure 1 Open anterior capsule of the lens (Capsulorhexis/Capsulotomy) by using a bent needle

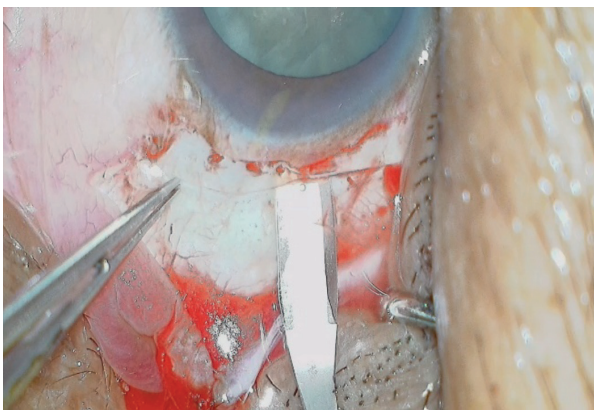

Figure 2 Scleral tunnel incision from temporal side

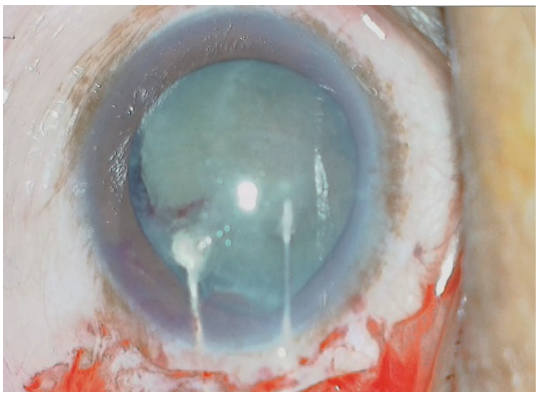

Figure 3 The lens is removed from the capsule (Nuclear luxation)

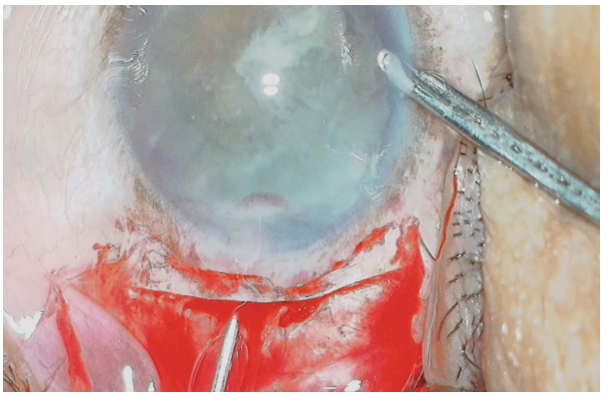

Figure 4 Remove the lens from the eye through scleral tunnel incision (Nuclear delivery)

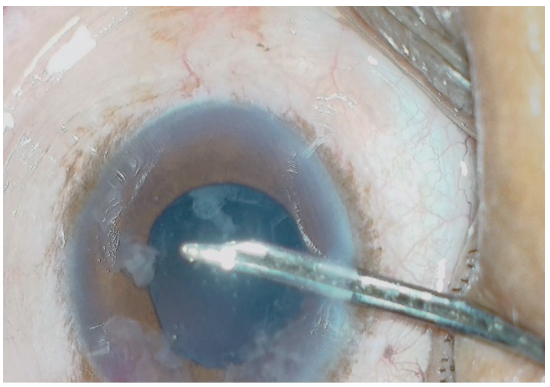

Figure 5 Remove the cortex of the lens

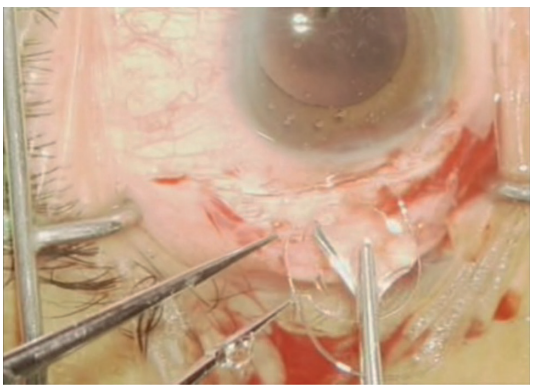

Figure 6 Intraocular lens (IOL) implantation in posterior capsule

Statistical analysis was validated and saved in the form of a file with the STATA/MP 12 program. Then analyzed by statistics: 1) Analyze general data using descriptive statistics such as percentage, mean, standard deviation, minimum and maximum values. 2) Compare preoperative and postoperative visual acuity in 1 day, 1 week, 6 weeks, and 3 months using the McNemar test statistic. Consideration of statistical significance when $p < 0.05$. For exclusion criteria, patients who had any ocular infection, history of allergy to anesthesia, or other underlying ocular conditions (such as corneal scar, retinal detachment, optic atrophy, and glaucoma) were excluded.

Results

Manual small incision cataract surgery (MSICS) using Lerprat technique was successfully performed in 60 eyes of 60 patients (28 men and 32 women) whose age ranging from 48 to 87 years (mean age, 71.07 years). Suture-less wounds were achieved in 22 patients. The rest was only one scleral tunnel wound suture. The preoperative uncorrected and corrected visual acuity was less than 20/200 (poor) in 60 patients (100.0%). All of the patients in this study had mature cataracts and improved visual acuity following surgery. The uncorrected visual acuity (UCVA), shown in Table 1, the most UCVA was good in 40 eyes (66.7%) at first day postoperatively. After follow up the postoperative visual acuity without correction, there were 34 eyes (56.7%), 46 eyes (76.7%) and 47 eyes (76.7%) in excellent group at 1 week, 6 weeks and 3 months respectively.

As shown in table 2, BCVA (best corrected visual acuity), there were 30 eyes

(50%) and 52 eyes (86.7%) were in excellent group at Day 1 and 1 week postoperatively. We found that all eyes 60 eyes (100%) were in excellent group at postoperative 6 weeks and 3 months respectively.

The most common postoperative complication was hyphema (3 eyes, 5 %) that occurred on the first day after surgery. All of the patients were resolved within one week after treated with frequency topical steroid eye drop, bed rest and head elevation 30-45 degree. The other postoperative complication was elevated intraocular pressure (1 eye, 1.67%) which caused by retained viscoelastic substance. The rising of intraocular pressure was controlled by antiglaucomatic drugs. The pressure was back to normal range after discontinuing the medication. No other significant complications, such as endophthalmitis or corneal decompensation, occurred in any of the eyes.

Table 1 Postoperative uncorrected visual acuity n (%)

Visual acuity	Follow-up visit			
	Day 1	1 week	6 weeks	3 months
20/20-20/30 (excellent)	8 (13.3)	34 (56.7)	46 (76.7)	47 (76.7)
20/40-20/70 (good)	40 (66.7)	24 (40.0)	14 (23.3)	13 (23.3)
20/80-20/200 (borderline)	12 (20.0)	1 (1.7)	0	0
< 20/200 (poor)	0	1 (1.7)	0	0

Table 2 Postoperative corrected visual acuity n (%)

Visual acuity	Follow-up visit			
	Day 1	1 week	6 weeks	3 months
20/20-20/30 (excellent)	30 (50.0)	52 (86.7)	60 (100)	60 (100)
20/40-20/70 (good)	28 (46.7)	8 (13.3)	0	0
20/80-20/200 (borderline)	2 (3.3)	0	0	0
< 20/200 (poor)	0	0	0	0

Discussion

The goal of cataract surgery is to improve patient's vision and their quality of life. Lerprat technique used Simcoe cannula and iris spatula, which is already in the conventional extracapsular cataract extraction set, for lens removal. This technique can also improve patients' vision with less complications. For the surgical wound, this technique is suture less or using only one suture for wound closure so the patients will have less wound irritation, less corneal astigmatism, better wound healing and don't have to come to follow up very often. The successful outcome is the rate of visual gain postoperatively. We use Snellen chart to compare between preoperative and postoperative visual acuity

of 60 eyes from 60 patients. All eyes have preoperative visual acuity worse than 20/200. For UCVA, after the surgery 40 eyes (66.7%) were in good visual acuity group at postoperative day 1. There were 34 eyes (56.7%), 46 eyes (76.7%) and 47 eyes (76.7%) in postoperative excellent visual acuity group at one week, 6 weeks and 3 months respectively.

As mention previously, mature cataract extraction with Lerprat technique has a satisfactory outcome. Although this technique has less complications, we found that the common postoperative complication from this technique were hyphema (3 eyes, 5 %) and elevated intraocular pressure (1 eye, 1.67%). There were no other significant complications, such as endophthalmitis or corneal decompensation. Compared to conventional technique such as Phacoemulsification which machine cost about 3-4 million baht and have to use with consumable such as cassette (1400-1800 baht/each), this technique can reduce the cost per case. Compared to MSICS which have to use with special equipment cost about 5000-6000 baht, Lerprat technique needs no new equipment (used only Simcoe cannula, which is already in the conventional extracapsular cataract extraction set). Furthermore, Lerprat technique creates wound at temporal area (preserve superior area) in case that the patients also have glaucoma, they can do other surgery such as Trabeculectomy in the future.

Conclusion

Cataract surgery with Lerprat technique had a satisfactory outcome. Further study with more patients and more factors evaluation such as endothelial cell count and surgical induced astigmatism were recommended.

Acknowledgement

I would like to thank Dr.Pipat Kongsap, Associate professor, Head, Department of Ophthalmology, Prapokklao hospital, for his supervision, advice and guidance from this research.

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Barcode Scanning Technology to Improve Pre-dispensing Errors

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Abstract:

Background: Unsafe medication practices and medication errors are leading causes of avoidable harm in health care systems across the world. Medication errors occur when weak medication systems and/or human factors. World Health Organization (WHO) suggests using computer technology to improve safety at all levels of healthcare. Pre-dispensing errors incident at Mae Fah Luang University Medical Center Hospital was higher than other university hospital that led to dispensing errors.

Objective: To describe handheld computer improving pre-dispensing errors.

Materials and Methods: This research was prospective and quasi-experimental study. This study was divided into 2 phases; the first phase was the development and run-in with handheld computer. The second phase was the effectiveness before and after study using handheld computer for packing.

Results: Handheld computer could reduce pre-dispensing errors caused by handling wrong drug, wrong dosage form and wrong strength of medicines as compared with those using traditional methods significantly (p-value < 0.01). This was a statistically significant reduction of the errors from 41.7% to 26.4% especially in look-alike, sound-alike (LASA) drugs 8.3% to 2.8%. Two errors were found because of the returned medication in the wrong location and pharmacist assistant not using the handheld computer. Median outpatient waiting time was decreased from 11 minute to 9 minute. The satisfaction of users was statistically significant with an increase from moderate to high level of the average scoring (3.2 to 3.8) (p-value = 0.03).

Conclusion: The study showed that barcode scanning technologywith handheld computer could help reduce medication errors, especially LASA drugs. Furthermore, this technology could improve efficacy and patient safety other than the use of barcode scanning system by pharmacist and barcode medication administration (BCMA) by nurse

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Keywords: Pre-dispensing errors, Medication errors, handheld computer, Barcode, Look-Alike Sound-Alike (LASA) drugs

Introduction

Global Patient Safety Challenges identify patient safety burden that poses significant risk to health, then develop frontline interventions and partner with countries to disseminate and implement the interventions. Each Challenge focuses on topic that poses major and significant risk to patient health and safety. WHO provides leadership and guidance in collaboration with Member States, stakeholders and experts, to develop and implement interventions and tools to reduce risk, improve safety and facilitate beneficial change. Unsafe medication practices and medication errors are leading causes of avoidable harm in health care systems across the world. The scale and nature of this harm differs between low-, middle- and high-income countries. Globally, the cost associated with medication errors has been estimated at US$ 42 billion annually. Medication errors occur when weak medication systems and/or human factors such as fatigue, poor environmental conditions or staff shortages affect prescribing, transcribing, dispensing, administration and monitoring practices, which can then result in severe harm, disability and even death.[1]

Implementing system changes and practices are crucial to improve safety at all levels of healthcare. Recognizing the paucity of accessible information on primary care, World Health Organization (WHO) set up a Safer Primary Care Expert Working Group. The Working Group suggests using clinical pharmacists, computer technology and educational programmes, often within multifaceted interventions such as medication reviews and reconciliation.[2]

Several types of information technologies can be used to decrease rates of medication errors. Computerized physician order entry with decision support significantly reduces serious inpatient medication error rates in adults. Other available information technologies that may prove effective for inpatients include computerized medication administration records, robots, automated pharmacy systems, barcoding.[3]

Barcodes are simple, universal and low cost, which makes them the most common form of auto-identification and data capture. Barcodes and scanning technology systems implemented in the medication management process in Mae Fah Luang University Medical Center Hospital for decrease medication errors is limited. The most common initiatives are the use of barcode scanning technologies can be used from medication dispensing. In the packing process, scanning technology is the use of machine-readable codes with standard terminologies, and is also known as, auto-identification and data capture with handheld computer (Zebra® the Wi-Fi/cellular TC26) connect to Hospital information system improving pre-dispensing errors.

Materials and Methods

This research was a prospective and quasi-experimental study. This study was divided into 2 phases; the first phase was the development and run-in with handheld computer. The second phase was the effectiveness before and after study using handheld computer for packing during 1 April to 15 May 2022 and 16 May to 30 June 2022 at department of pharmacy, Mae Fah Luang University Medical Center Hospital

In phase 1 of the research, pharmacist assisants used handheld computer for packing during 4 p.m. to 8 p.m. feedback and interview measured by questionnaire. In phase 2, primary outcomes evaluate the efficacy of interventions to reduce the prevalence caused by handling the wrong

drug, wrong dosage form and wrong strength of medicines, especially look-alike, sound-alike (LASA) medication name errors. Secondary outcomes evaluate the Outpatient waiting time and record the same questionnaire with phase 1.

The total number of medication orders placed in both study periods was obtained from the pharmacy department. Using this information, the rates of errors and items in the pre- and post-implementation periods were compared using rate ratio. Error rates within medication-use phases were then calculated, and rate ratios were calculated to determine if there was a change between pre- and post-implementation. To determine whether the severity of errors had changed, a chi-square test was used to compare the mean values within handheld computer use phases. The satisfaction of users and outpatient waiting time, a paired t-test and Mann-Whitney U Test were used to compare respectively.

Results

Amount items for packing pre- and post-implementation were 77,938 items and 80,963 items. This wasn't statistically significant (p-value = 0.86). Handheld computer could reduce pre-dispensing errors caused by handling wrong drug, wrong dosage form and wrong strength of medicines as compared with those using traditional methods significantly (p-value < 0.01). This was a statistically significant reduction of the errors from 41.7% to 26.4% especially in look-alike, sound-alike (LASA) drugs 8.3% to 2.8% (table 1). Two errors were found because of the returned medication in the wrong location and pharmacist assistant not using the handheld computer (table 2).

Table 1 Work load and Pre-dispensing errors (N = the total number of medication orders 158,901 items)

	pre-implementation	post-implementation	p-value
The total number of medication orders	77,938	80,963	0.86
Pre-dispensing errors (per event)	144	72	<0.01
Wrong drug, wrong dosage form and wrong strength of medicines	60 (41.7%)	19 (26.4%)	<0.01
- LASA drugs	12 (8.3%)	2 (2.8%)	0.01
- Non-LASA drugs	48 (33.4%)	17 (23.6%)	<0.01
Others (such as wrong amount)	84 (58.3%)	53 (73.6%)	<0.01

Table 2 Description of LASA drugs in post-implementation

LASA drugs	Cause
AMITRIPTYLINE TAB 10 MG – BETAHISTINE TAB 12 MG	pharmacist assistant returned medication in the wrong location
LOSARTAN POTASSIUM TAB 50 MG - LORAZEPAM TAB 0.5 MG	pharmacist assistant not using the handheld computer

Table 3 Outpatient waiting time

	pre-implementation	post-implementation	p-value
Overall outpatient waiting time (min)	11	9	<0.01
Rush hour outpatient waiting time (min)	17	13	<0.01
Amount of prescription in rush hour time*	5,647	6,713	0.09
Amount of rush hour time prescription coverage in 30 min (percentage)	1,415 (25)	704 (11)	<0.01

* rush hour time 10.00 a.m. to 1.59 p.m.

Median Outpatient waiting time was decreased from 11 minute to 9 minute. Subgroup analysis in rush hour interval (10.00 a.m. to 1.00 p.m.) was decreased from 17 minute to 13 minute. This was a statistically significant reduction of both percentage of coverage in rush hour over a 30-minute period was a statistically significant reduction of the errors from 25% to 11% (table 3).

The satisfaction of users was statistically significant with an increase from moderate to high level of the average scoring (3.2 to 3.8) (p-value = 0.03) (table 4).

Discussion

Barcode scanning technology has been used in many industries to more accurately reconcile and verify the identity of objects and would have logical application in verifying the identity of medicines, the persons handling them, and the patient to whom they are administered. This technology has been used in both the pharmacy to improve the safety and efficiency of drug storage, preparation, and dispensing, and by nursing to improve the safety and efficiency of drug administration and documentation in the medication administration record.[4] Dispensing and drug preparation errors made in the pharmacy are significant sources of medication errors. A barcode-assisted dispensing system at a large academic hospital was associated with a large and significant reduction in target dispensing errors (i.e. errors that barcode technology is designed to address) by 85%, target potential ADEs by 74%, and all potential ADEs by 63%.[5] The use of machine-readable coding to verify the accuracy of drug dispensing has increased from 5.7% in 2002 to 61.9% in 2017.[6]

Safety design and error proving when the pharmacist assistant used a handheld computer was the screen showing the right drug. These were shown a green screen when scanning with the right location that the doctor ordered in prescription and a red screen in the incorrect location. Data screen showed important data such as name, amount, bin location and picture. Safety design for high alert drugs was a cosign system between the pharmacist assistants (figure 1).

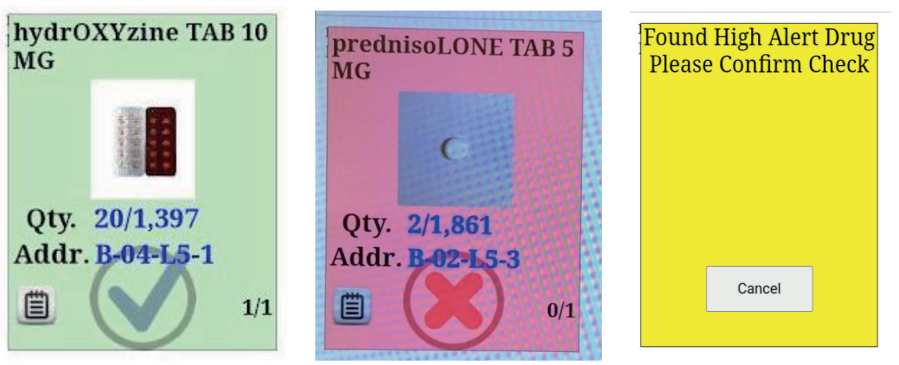
Table 4 The satisfaction of users in pre- and post-implementation periods (N=28)

	pre-implementation	post-implementation	p-value
Infrastructure	3.0	3.6	
1. Handheld was easier to pack the medicine			
2. Handheld detected the QR code correclty	3.0	2.9	
Implementation			
1. User trusted using handheld computer could reduce pre dispensing errors	3.8	4.5	
2. User felt comfortable	3.2	3.9	
3. Overall in post-implementation periods	3.1	3.8	
Average	3.2	3.8	0.03

The effect of the barcode scanning in our study was similar to the effect of the shelf controlled by the barcode system. The LASA safety shelf could reduce pre-dispensing errors caused by handling the wrong drug, wrong dosage form and wrong strength of medicines as compared with those using traditional shelves. There was a statistically significant (p-value <0.01) reduction of the errors from 26.50% to 6.45%.[7]

Although our study suggests that the prevention of many of the pre-dispensing errors could be reduced by using handheld computer, the pharmacist assistants weren't packing the medicine following work instructions that lead to pre-dispensing errors.

There are some limitations to outpatient waiting time because before study was covid-19 situation lead to workload or working in a pharmacy room. The relation between time and technology needs more study. Handheld signaling failure that couldn't connect with the hospital information system led to non-compliance with the use of handheld computer of pharmacist assistants so the data center accepted to fix them.



For right medicine For wrong medicine For High alert drug

Figure 1 Safety design on handheld computer screen

Conclusion

The study showed that barcode scanning technology with handheld computer could help to reduce medication errors, especially LASA drugs. Furthermore, this technology could improve waiting time and patient safety other than the good satisfaction on using of barcode scanning system by pharmacist.

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Perspectives of Health System Sciences Education among Young Physicians in Provincial Settings

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Abstract:

Background: Thai Medical Council determines the internship training program in order to develop clinical practice skills of newly graduated physicians, which will be called young physicians in this study, as well as to give the resolution to insufficient physicians in other regions of the country. As a result, every medical graduate must go through the process of training. However, various studies indicated concerns about preparedness of newly graduated physicians as well as solutions and preparations by medical schools.

Objective: To explore and compare perspectives regarding medical school preparation of young physicians graduated from Phramongkutklao College of Medicine (PCM) for clinical practice.

Materials and Methods: This study was observational mixed model cross-sectional study, conducted in workplaces of intern physicians and focused on first-year and second-year intern physicians graduated from PCM. The questionnaire of this study included two parts; quantitative and qualitative sections. Quantitative part included 5-Likert scale covering 6 perspectives toward preparation for clinical practice (knowledge, health system, medical ethics & laws, continuity of medical education, technology usage and communication skills). Qualitative part was comment section. The questionnaire was made online and distributed via messaging applications and stored in online sheet. One-way ANOVA was used to compare all perspectives with independent t-test for comparing perspectives between groups.

Results: Seventy-four young physicians responded of which 62 were first-year interns. Young physicians perceived knowledge about knowledge of health system significantly lesser than other five aspects of preparations from medical school (F=11.082, p=0.001). Military and civilian physicians had different perspectives on preparation for technology usage (t=2.716, p=0.008). Second-year intern had lower knowledge on medical ethics and laws than first-year intern (t=2.066, p=0.042). Female physicians had lower preparation for communication

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skills than males (t=2.412, *p*=0.018). Qualitative data addressed educational issues about patient management, health system sciences and financial management.

Conclusion: Health system education was to be emphasized in medical schools for young physicians' competencies as well as technology usage, medical laws and ethics, communication skills and financial management.

Keywords: Young physician perspective, Health system sciences, Clinical practice

Introduction

Presently, Thai Medical Council determines the internship training program in order to develop clinical practice skills of newly graduated physicians, which will be called young physicians in this study, as well as to give the resolution to insufficient physicians in other regions of the country. As a result, every medical graduate must go through the process of training. However, various studies indicated concerns about preparedness of newly graduated physicians as well as solutions and preparations by medical schools.[1-5]

All young physicians must be qualified by Thai Medical Council before graduated. The process included three steps of national license examination during their third, fifth and sixth years. The examinations cover 3 steps of basic science examination, clinical science examination and objective structured clinical examination (OSCE) during their respective years. Despite these steps, many young physicians felt uncertain about their upcoming works after their graduation.[1,2]

Several factors render young physicians feel unprepared for clinical practices such as insufficient clinical knowledge and trainings by medical schools[2,6], inadequate or even lacks of practicing some medical procedures since they were medical students[2], lacks of knowledge regarding medical laws[6], unpreparedness for multidisciplinary communication[4], limited guidance from specialists[7] and unconfident in clinical practice.[3,8] In addition, rapid change of status from medical students to young physicians is considered one of the weakest links during their medical career which might causes stresses.[3,8,9]

The objective of this study was to explore and compared perspectives regarding medical school preparation of young physicians graduated from Phramongkutklao College of Medicine (PCM) – a military medical college in Bangkok, Thailand which provide graduated physicians for both civilian public healthcare system and armed forces medical branches – for clinical practices.

Materials and Methods

Study population and setting

This study focused on first-year and second-year intern physicians graduated from PCM. The setting for this study was classified into two settings; civilian hospitals which included public and university hospitals and military hospitals which were army, navy, and air force hospitals. The study was conducted during 2020 to 2021.

Study design

This study was observational mixed model cross-sectional study. This included quantitative and qualitative studies which address perspectives of the preparedness of young physicians in clinical practice.

Questionnaire and data collection

The questionnaire of this study included two parts; quantitative and qualitative sections. The quantitative part included another two parts which were general characteristics data (gender, internship year, average grades and medical branches of service (civilian or armed forces)) and perspectives toward medical school preparation for clinical practice which was 5-Likert scale. Perspectives toward medical school preparation for clinical practice included six aspects regarding physicians' work field; 1) medical knowledge: knowledge in internal medicine, surgery, paediatrics, obstetrics-gynaecology, emergency medicine, preventive medicine, holistic care of patients, history taking and physical examination, proper investigations and treatment, important medical procedures and confidence in patient care 2) knowledge of health system: knowledge regarding Thai health system including health coverage service, hospital accreditation, disease investigation and health economics, etc. 3) medical ethics & laws: knowledge and practice regarding medical ethics and medical laws 4) continuity of medical education: knowledge and practice of evidence-based medicine and use of literatures in patient management 5) technology usage: comprise of both literature search, use of hospitals' computerizing programs and use of medical technology with patient care and 6) communication skills: included communications between patients, patients' relatives and other healthcare providers as well as English and third language communication.

The questionnaire was made online on Google form and distributed via messaging applications to participants. Responses from participants were stored on Google sheet which can be downloaded for statistical analysis.

Qualitative part followed quantitative part. It included filling the comments on overall clinical preparedness and other knowledges important for internship situations.

Statistical analysis

SPSS 26.0 (Armonk, New York) was used for statistical analysis. General characteristics were calculated using descriptive statistics. Comparison between perspectives was conducted using one-way ANOVA with post-hoc tests by Bonferroni test. Comparison between internship years and genders were assessed using independent t-test. Significant differences between comparisons were counted at p-value < 0.05.

Ethical approval

This study was approved by Institutional Review Board Royal Thai Army Medical Department. The approval number was R061q/63_Exp.

Results

General characteristics

Seventy-four young physicians responded the questionnaire including 62 (83.78%) first-year interns. Males accounted for 58.11% of total participants. Most (79.73%) work in armed forces medical branches. Their Grade Point Average (GPA) of <3.50 were 60.81% as data shown in Table 1.

Table 1 General characteristics of young physicians giving perspectives toward preparation for clinical practice by Phramongkutklao College of Medicine (n=74)

Characteristics	Participants	
	First year interns N (%)	Second year interns N (%)
Gender		
Male	35 (81.40%)	8 (18.61%)
Female	27 (87.10%)	4 (12.90%)
Status		
Armed forces physicians	48 (81.36%)	11 (18.64%)
Civilian physicians	14 (93.33%)	1 (6.67%)
GPA		
< 3.50	35 (79.55%)	9 (20.46%)
≥ 3.50	27 (90.00%)	3 (10.00%)

Comparison between perspectives of medical school preparation for clinical practice

One-way ANOVA analysis shown that there were significantly different between perspectives of young physicians (F=11.082, *p*=0.001). Post-hoc analysis showed that young physicians perceived knowledge on health system significantly the least of five aspects of preparations from medical school (*p*=0.001). Other aspects did not show any significant differences in young physicians' perspectives as data shown in Table 2.

Table 2 Comparison between perspectives toward six aspects of medical school preparation for interns in clinical practice (n=74)

Mean score						One-way ANOVA				Pair comparison	p
MK	KHS	MEL	CME	TU	CS	Levene statistics	p	F	p		
4.01± 0.55	3.38± 0.83	4.05± 0.76	4.08± 0.59	3.97± 0.70	4.00± 0.66	3.14	0.009	11.082	<0.001	MK>KHS	<0.001*
										MEL>KHS	<0.001*
										CME>KHS	<0.001*
										TU>KHS	<0.001*
										CS>KHS	<0.001*

MK, medical knowledge; KHS, knowledge of health system; MEL, medical ethics & laws; CME, continuity of medical education; TU, technology usage; CS, communication skills; * Significant at 95% confidential interval

Comparison between young physicians working in civil and military hospitals demonstrated significant difference regarding to preparation for technology usage (t=2.716, *p*=0.008). For internship year, it was found that second-year interns had lower knowledge on medical ethics and laws than the first-year as significantly (t=2.066, *p*=0.042). Female young physicians had lower preparation for communication skills than those of male significantly (t=2.412, *p*=0.018) as data shown in Table 3, 4 and 5, respectively.

Table 3 Comparison between perspectives of military and civilian physicians toward six aspects of medical school preparation for interns in clinical practice (n=74)

Aspects	Mean score ± SD		t	*p*
	Military physicians (n=59)	Civilian physicians (n=15)		
Medical knowledge	4.06 ± 0.54	3.84 ± 0.58	1.386	0.170
Knowledge of health system	3.41 ± 0.83	3.24 ± 0.83	0.697	0.488
Medical ethics & laws	4.05 ± 0.79	4.07 ± 0.70	0.071	0.944
Continuity of medical education	4.08 ± 0.60	4.07 ± 0.55	0.073	0.942
Technology usage	4.08 ± 0.63	3.56 ± 0.81	2.716	0.008*
Communication skills	4.06 ± 0.62	3.77 ± 0.75	1.559	0.123

* Significant at 95% confidential interval

Table 4 Comparison between perspectives of first and second-year interns toward six aspects of medical school preparation for interns in clinical practice (n=74)

Aspects	Mean score ± SD		t	*p*
	First-year interns (n = 62)	Second-year interns (n = 12)		
Medical knowledge	4.03 ± 0.48	3.94 ± 0.84	0.362	0.724
Knowledge of health system	3.40 ± 0.75	3.25 ± 1.18	0.432	0.673
Medical ethics & laws	4.13 ± 0.67	3.65 ± 1.07	2.066	0.042*
Continuity of medical education	4.09 ± 0.57	4.03 ± 0.70	0.312	0.756
Technology usage	4.01 ± 0.65	3.80 ± 0.89	0.738	0.473
Communication skills	4.01 ± 0.61	3.94 ± 0.89	0.359	0.721

* Significant at 95% confidential interval

Table 5 Comparison between perspectives of male and female interns toward six aspects of medical school preparation for interns in clinical practice (n=74)

Aspects	Mean score ± SD		t	*p*
	Males (n=43)	Females (n=31)		
Medical knowledge	4.05 ± 0.62	3.97 ± 0.44	0.602	0.549
Knowledge of health system	3.47 ± 0.88	3.25 ± 0.75	1.156	0.252
Medical ethics & laws	4.16 ± 0.80	3.91 ± 0.69	1.372	0.174
Continuity of medical education	4.16 ± 0.61	3.97 ± 0.55	1.360	0.178
Technology usage	4.02 ± 0.79	3.91 ± 0.55	0.617	0.539
Communication skills	4.15 ± 0.66	3.79 ± 0.61	2.443	0.017*

* Significant at 95% confidential interval

Qualitative results

There were total of 29 comments which can be categorized into three major categories; 1) patient management and procedures by self-individual, 2) health system sciences and 3) financial management.

Patient management and procedures by self-individual

Fourteen physicians commented on not having enough experiences for medical procedure, too few occasions for ordering the management by themselves including sufficient exposure for real patients. One physician commented as follows:

'I think that regarding to knowledge, the medical school had sufficiently provided but some medical procedures and order of treatments were still be supervised by residents. We needed to do some of these procedures by ourselves due to real working situation, these things must be done on our own.'

Another physician comment regarding insufficient skills on medical procedure as follows:

'I want the medical school to emphasize more on medical procedures due to real working situation, these procedures such as intercostal drainage and joint aspiration must be done by ourselves without supervision.'

Regarding to insufficient exposure to real patients, some comments included as follows:

'I want the medical school to rotate us to other affiliated hospitals (provincial general hospitals and community hospitals) more than usual for exposure to more patients.'

'Medical school did not prepare enough to manage patients with non-communicable diseases for diagnosis, investigations, medicine prescription and follow-up patients due to not enough patients for learning.'

Health system science

Eleven physicians commented on health system science practice during their student years. These comments included hospital accreditation, referral system, patients' health scheme, disease reporting system such as influenza and dengue fever and health economics. These comments included:

'I think that medical school did not teach us about patient's scheme because charging cost of treatment can determine hospital's profit.'

Some physicians suggested topics for teaching them on how patient's schemes are related to cost of treatment per each one including hospital accreditation with

complex processes in getting their hospital accredited.

Financial management

Two physicians suggested to teach financial management including tax for physicians and savings. One comment suggested to teach categories of incomes regarding to tax paying type. Other comments suggested to teach about how to save money and manage income.

Discussion

This study addressed perspectives of young physicians toward medical schools' preparations for real world career. Many studies had already addressed the issues focusing on knowledge, clinical skills and interpersonal communications.[2,6,10-12] This study expanded the scope covering real life situations facing with their work, medical ethics and laws, health system sciences, technological skills and continuation of education.

Overall, it was found that young physicians' perspectives toward medical schools' preparation for health system science was significantly less than others. In context of Thailand's health schemes, there were several issues regarding difficulties in managing patients according to their schemes, especially national health policy, patients' schemes budget, medical oversupply and recording of treatments (e.g., Diagnosis Related Group). Many large-scale hospitals had financial loss in recent years with these factors as issues.[13,14] Most Thai medical schools educated their students well enough on clinical management of patients by investigations to treatments; however, public health education was not emphasized well, resulting in limited public health competencies.[15] The current curriculum of PCM does not incorporate health system sciences which should be integrated for young physicians.

This study also found that perspectives toward technology usage was differed between military and civilian physicians with lower mean score from civilian physicians. In our setting, clinical practice between military and civilian hospitals was different regarding to workload and number of patients. This could indirectly affect the use of technology for patient care especially online clinical management data. Patients and workloads were fewer in military hospitals than in civilian hospitals as most of the patients in military hospitals were military officers and their families while in civilian hospitals, patients were general citizens. Previous studies had demonstrated that technology usage, e.g., medical-related mobile applications, were used among physicians for education-learning, assisting in disease diagnosis and drug references.[16,17] This study implied that excessive workloads in civilian hospitals rendered technology using skills such as evidence-based medicine and online literature search, practiced by medical schools were insufficient for young civilian physicians in constraint of times and works. As the trend of healthcare has shifted to technology-based, it is recommended medical schools to prepare their medical students to step forward and handle technology-involved clinical practice such as artificial intelligence and telemedicine, etc.

Comparison between first-year and second-year interns found that there was significant difference in medical ethics and laws. Previous studies addressed ethical decline among young physicians who were attributed to multiple roles (as learners and healthcare providers simultaneously), stressful working environments, prioritization of patients' physical rather than psychological well-being and the attitudes of senior colleagues.[18,19] This might be implied that second-year interns had endured these situations for longer duration than first-year

interns, resulting in decline of medical ethics and knowledge regarding medical laws as factor attributed to limited preparation for coping with these situations for young physicians. One previous study suggested ethical education would halt the ethical decline among young physicians.[18]

Regarding difference between genders, it was found that female physicians scored communication skills lower than male physicians. Previous study addressed the problems regarding communication issues of female physicians had to face such as their negative viewpoints on patients' autonomy and cross-gender encounters (female physicians to male patients) in which patients were more satisfactory for male than female physicians.[20] Female physicians tended to be more attentive and used longer talking time with their patients.[20] This might be affected by the number of patients and time constraint which rendered female physicians to perceive their communication skills lowly as they had fewer time to talk with their patients. Despite the finding was contrast to previous studies which regarded female physicians had higher communication skills than males,[21,22] it was suspected that due to different workplace settings resulted in communication skills were more preferable to males. In our setting, most physicians were working in military hospitals, usually bound to some military bases. In this unique setting, the society was noticeably masculine and militaristic which required communications with not only physicians to patients or other health care providers, but also with military officers. We assumed that male physicians could be more adaptable to this environment better than females. It is suggested that training female military physicians to apply their communication skills to military officers could be beneficial. Also, further explorations into this unique setting are recommended.

Other issues were also reported from qualitative study, but one noticeable topic was financial management by young physicians. Although this issue was unrelated to clinical practice, but some young physicians concerned that they were unprepared for financial management such as savings, income calculation and tax calculation, etc. Previous studies indicated financial illiteracy could be indirectly related to stress, clinical practice, patient caring and burn out.[23,24] It is suggested that financial education course for senior medical students is important to prepare them for financial management in their career. Future studies that explore the financial literacy and perspectives of young physicians as well as medical students are recommended.

One limitation in this study was included young physicians who resigned from either military or civilian health sectors but the number of them were very few. Military and civilian health sectors are bureaucrat branches of Ministry of Defense and Ministry of Public Health, respectively. Physicians who resigned would enter private sectors, becoming casual workers in hospitals registered to non-governmental organizations, continuing their education in other degrees or even starting their career in other occupations. Qualitative studies with this group could revealed how they perceived medical school preparations different from their cohorts which resulted in their resignation.

This study concluded that in order to prepare young physicians well enough for clinical practice, other aspects of medical education than clinical knowledge and procedural skills should be integrated. Health system education was a topic to be emphasized in medical schools for young physicians' competencies in public health. Technology usage, medical laws and ethics, communication skills and financial management were concerns to be focused as well.

Data availability

No data are associated with this article.

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No competing interests were disclosed

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Case Report

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Sequential Conditioning Un-manipulated Haploidentical Hematopoietic Stem Cell Transplantation in A Patient with High Risk and Refractory Acute Myeloid Leukemia by Using A Second-degree Relative Donor

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Abstract:

Following the second course of induction chemotherapy, only 10 to 20 percent of patients with relapsed/refractory acute myeloid leukemia can achieve complete remission. We reported the case of a patient with high-risk, refractory acute myeloid leukemia who underwent sequential conditioning T cell-replete haploidentical hematopoietic stem cell transplantation with post-transplant cyclophosphamide from a second-degree relative donor. The sequential conditioning regimen consisted of thiotepa 5 mg/kg on days -15 and -14, etoposide 100 mg/m^2 on day-13 to -10, cyclophosphamide 400 mg/m^2 on day-13 to -10, and mesna on day-13 to -10, followed by fludarabine 30 mg/m^2 on day-6 to -2, and melphalan 140 mg/m^2 on day-2. The graft-versus-host disease prophylaxis regimen consisted of cyclophosphamide, tacrolimus, and mycophenolate mofetil. Neutrophil and platelet engraftment occurred on day +15 and +32. On day+56, the patient developed CMV reactivation, which was successfully treated with ganciclovir. The last follow-up was on day +300; the patient remained in complete remission. He had mild chronic graft-versus-host disease of the oral mucosa, mild anemia, and mild thrombocytopenia. This patient demonstrated that sequential conditioning T cell- replete haploidentical hematopoietic stem cell transplantation utilizing a second-degree relative donor is feasible and may be a promising therapy option for patients with relapsed/refractory acute myeloid leukemia.

Keywords: Refractory acute myeloid leukemia, Sequential conditioning, Hematopoietic stem cell transplantation

Introduction

Over the past decade, advances in genetic and molecular studies that provide new insights into how acute myeloid leukemia (AML) develops and is regulated by complex molecular pathways have led to the development of more effective treatments. These include chemotherapy, targeted therapy, and allogeneic hematopoietic cell transplantation. Even though these therapeutic techniques have the potential to cure AML, the 5-year survival rate for individuals aged up to 60 years is still approximately 50 percent, while it ranges from 5 percent to 15 percent for those older than 60 years.[1-3] In addition, the prognosis for patients with relapsed/refractory acute

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myeloid leukemia remains dismal. Only 10 to 20 percent of patients can achieve a complete response following the second course of induction chemotherapy.[4-8] After allogeneic stem cell transplantation under normal conditioning regimen, the range of overall survival for relapsed/refractory acute myeloid leukemia patients is 19-33 percent.[9-10]

The Munich group has developed sequential conditioning regimens consisting of adding a brief course of antileukemia chemotherapy prior to reduced-intensity conditioning hematopoietic stem cell transplantation. The combination of fludarabine, amsacrine, and cytarabine (FLAMSA), followed by cyclophosphamide, 4 Gy of total body irradiation, or busulfan (FLAM SA-BuCy), and anti-thymocyte globulin (ATG) is one of the first and most effective strategies reported, with a 2-year leukemia-free survival rate of 37 percent and an overall survival rate of 40 percent.[11] Various groups have further developed the concept of sequential conditioning regimens. Compared to the original FLAMSA, the antileukemia chemotherapy and pre-transplantation phases of the sequential conditioning regimens have been modified, resulting in increased intensity. In relapsed/refractory myeloid malignancies, protocols such as TEC (thiotepa, etoposide, cyclophosphamide) or clofarabine-based strategies have been reported to have a comparable overall survival rate of 30 percent to 45 percent.[12-18]

In the absence of a matched sibling or unrelated donor, allogeneic stem cell transplantation utilizing other donors is widely utilized.[19] Recently, the use of high-dose post-transplant cyclophosphamide has made it possible to evaluate the potential of selecting first-degree relatives (brother, sister, father, mother, son, or daughter) as haploidentical donors for allogeneic stem cell transplantation. Interestingly, treatment of post-transplant cyclophosphamide decreases the incidence of severe acute/chronic graft-versus-host disease due to the early elimination of alloreactive T cells.[20] Unfortunately, some patients may not have a suitable related donor due to factors such as the parents' advanced age and the absence of siblings or children. The statistical probability of having a haploidentical donor among siblings is fifty percent; however, it approaches one hundred percent when both biological parents and offspring are considered. When considering the kid of a matched or haploidentical sibling as a potential donor, the percentages of the donor and receiver being haploidentical stay at 50 percent and 25 percent, respectively. Thus, second-degree relatives (nephew, niece, uncle, aunt) may be considered a source of stem cell grafts.[20,21] Herein, we describe a patient with high risk and refractory acute myeloid leukemia who underwent a T-replete haploidentical allogeneic stem cell transplantation with post-transplant cyclophosphamide from a second-degree relative donor.

Case presentation

A 56-year-old Thai man presented to the dermatology department with multiple erythematous non-blanchable macules on the tip of his second toe, between his fourth and fifth toes, on the lateral malleolus, and on the lateral side of his left foot, as well as an indurated erythematous plaque on his left elbow that had been tender for one week. The dermatologist selected the left elbow for a punch biopsy. According to the pathology report, there was an epidermal ulcer accompanied by lymphocytic vasculitis. Aside from the rash, he was in good health.

A complete blood count revealed anemia with a reverse neutrophil/lymphocyte ratio; hemoglobin 11.8 g/dL; hematocrit 33.9%; white blood cell counts 5.49 x 10^9/L; neutrophil 12.9%; lymphocyte 64%; monocyte 21%; eosinophil 0.9 %; and platelet 211 x 10^9/L. The patient was referred to the

hematology department for further investigation.

Physical examination revealed mildly pale conjunctivae, no icteric sclerae, no hepatosplenomegaly, and no lymph node enlargement. A bone marrow biopsy revealed sufficient hypercellular (80-100 percent) packed bone marrow with diffuse leukemic infiltrate characterized by pleomorphic blasts with medium to large, round to slightly ovoid nuclei, homogeneously dispersed to condense chromatin, inconspicuous nucleoli, and occasional indented nuclei with scanty cytoplasm. Flow cytometric analysis of bone marrow demonstrated CD13 (+), CD15 (-), CD33 (+), CD34 (+), CD64 (+), CD11 (-), HLA-DR (+), MPO (+) compatible with acute myelomonocytic leukemia. Chromosomal analysis from bone marrow showed 46, XY, del (8) (q13q24.1), add (14) (q32), del (13) (q12q32). After complete investigations, this patient was diagnosed with acute myeloid leukemia at high risk due to a complex karyotype.

He underwent standard induction chemotherapy for acute myeloid leukemia consisting of idarubicin for three days and cytosine arabinoside (Ara-c) for seven days. On day 8, he developed a progressive confluent bright erythematous rash on his lower back, both arms, and forearms; multiple erythematous non-blanchable macules on both thighs and both legs; and dry lips but no genuine mucosal involvement as manifestations of early Steven Johnson syndrome; therefore, drug allergy to allopurinol or cytosine arabinoside was the most likely cause. We did not performed genetic testing for HLA-B* 58:01 due to leukopenia, but we decided to switch from allopurinol to febuxostat.

The presence of blast cells on the peripheral blood smear on day 28 prompted us to assess therapy response. A bone marrow biopsy revealed inadequate hypocellular bone marrow with increased residual leukemic blasts (18–28 percent), finely scattered chromatin, inconspicuous nucleoli, and sparse to abundant cytoplasm consistent with acute myeloid leukemia. In addition, bone marrow flow cytometric analysis identified a large population of CD11b (+), CD13 (+), CD15 (+), CD33 (+), CD34 (+), CD64 (+), HLA-DR (+), MPO (+), whereas chromosomal analysis revealed 46, XY. In Thailand, salvage treatment options for acute myeloid leukemia include 1) re-induction with a 7+3 regimen, but the patient might develop severe allergic reaction to Ara-c, 2) hypomethylating agent, but the remission rate for the treatment with hypomethylating agent alone is very low, 3) intense salvage chemotherapy such as the FLAG/idarubicin regimen, but we are concerned about serious complications such as infections due to prolonged neutropenia, and 4) sequential conditioning T cell-replete haploidentical hematopoietic stem cell transplantation that was developed by the Munich group, combining an intensive conventional anti-leukemic regimen with a reduced intensity conditioning regimen, which we hope that this procedure will provide not only a direct cytotoxic effect from chemotherapy but also a strong graft-versus-leukemia effect. After discussing treatment options with the patient, he decided to undergo a sequential conditioning regimen for hematopoietic stem cell transplantation.

We selected his nephew, a 32-year-old male, as the donor for sequential conditioning T cell-replete haploidentical hematopoietic stem cell transplantation because the patient was childless and had just one 60-year-old haploidentical sister. Both the recipient and donor had an O blood group, Rh positive, and a positive CMV serology. Antibodies against donor-specific antigens were not detected in the patient. Because amsacrine is unavailable in Thailand, we adapted the prototype FLAMSA-based sequential conditioning regimen into the Thio-ETO-Cy-Flu-Mel

regimen. This regimen includes of thiotepa 5 mg/kg on day -15 and -14, etoposide 100 mg/m^2 on day-13 to -10, cyclophosphamide 400 mg/m^2 on day-13 to -10, and mesna on day-13 to -10, followed by fludarabine 30 mg/m^2 on day-6 to -2, and melphalan 140 mg/m^2 on day-2. Graft-versus-host disease prophylaxis regimen consists of cyclophosphamide, tacrolimus, and mycophenolate mofetil administered post-transplant. Acyclovir and voriconazole were given at a prophylactic dose. Peripheral blood stem cells were infused on day 0 at a dose of 10 x 10^6/kg of the recipient.

Neutrophil and platelet engraftment occurred as early as day +15 and day +32, respectively, with full-donor whole-blood (WB) chimerism occurring on day +31. (Engraftment is defined as the first of 3 consecutive days with an absolute neutrophil count higher than 0.5 x 10^9 /L, sustained > 20 x 10^9/L platelets, and hemoglobin > 8 g/dL, free of transfusion requirements.[22] A bone marrow biopsy done on day +27 revealed adequate hypocellular bone marrow (20-40 percent) with substantially enhanced developing myeloid precursors and considerably reduced maturing erythroid precursors and megakaryocytes. There was no residual leukemia detected. Flow cytometric analysis of bone marrow also revealed negative results for minimal residual disease, and chromosomal analysis revealed 46, XY. The patient had no signs and symptoms of acute graft-versus-host disease.

On day +32, he was diagnosed with cytomegalovirus reactivation (CMV viral load 3,420 copies/ml, log 3.53); he was treated as an outpatient with valganciclovir. Before administration of valganciclovir, a complete blood count revealed the following: hemoglobin 10.3 g/dL, hematocrit 30.2%, white blood cell counts 5.31 x 10^9/L, neutrophil 56.2%, lymphocyte 18.2%, monocyte 10.0%, eosinophil 2.0%, and platelet 101 x 10^9/L. On day +56, he was admitted due to high fever and watery diarrhea as the cytomegalovirus viral load increased (CMV viral load 18000 copies/ml, log 4.25). The complete blood count revealed the following: hemoglobin 10.2 g/dL, hematocrit 29.7%, white blood cell counts 10.65 x 10^9/L, neutrophil 52%, lymphocyte 24%, eosinophil 24 %, and platelet 79 x 10^9/L. Differential diagnoses included bacterial infection, CMV colitis, and acute graft-versus-host disease. He received an intravenous antibiotic and ganciclovir treatment. Due to the patient's refusal to have a colonoscopy, oral budesonide was administered to treat acute graft-versus-host disease. A few days later, the fever and diarrhea had subsided.

On day +80, there was no detectable CMV viral load, but the patient developed pancytopenia. In order to rule out a relapse of acute myeloid leukemia, a bone marrow biopsy was performed and revealed significantly hypocellular (5-10 percent) bone marrow with diminished maturing trilineage hematopoiesis. There was no residual leukemia detected. Also negative for the minimal residual disease was the flow cytometric examination of bone marrow. Given that pancytopenia might be a consequence of ganciclovir, the patient was administered eltrombopag, and the dosage was reduced when pancytopenia improved.

On day +200, a repeat bone marrow biopsy and flow cytometric analysis revealed mild hypocellularity and the absence of minimal residual disease. Day+300 was the last follow-up. The patient's health was excellent. He had mild oral mucosal chronic graft-versus-host disease, mild anemia, and mild thrombocytopenia.

Discussion

This 56-year-old Thai man, presented with anemia, a reverse neutrophil/lymphocyte ratio, and cutaneous vasculitis. Investigations concluded with a diagnosis of acute myeloid leukemia with a high risk due to complex

karyotypes. He received standard induction chemotherapy (7+3) and developed Steven Johnson syndrome in its early stages, most likely due to Ara-c. Even though chromosomal tests revealed a normal karyotype, he had a significant number of residual leukemic cells in his bone marrow. Following a discussion with the patient, we considered treatment with sequential conditioning T cell-replete haploidentical hematopoietic stem cell transplantation, as prior studies have shown that patients who failed to achieve a complete remission with initial therapy are associated with 10–20 percent complete remission rates following the second course of induction chemotherapy. In addition, prognostic factors include cytogenetics, age, and the failure of a prior salvage treatment.[4,5,22]

The pioneering study developed by the Munich group has sequential conditioning regimens consisting of adding a brief course of antileukemia chemotherapy prior to reduced-intensity conditioning hematopoietic stem cell transplantation. With fludarabine, amsacrine, and cytarabine (FLAMSA), followed by cyclophosphamide, 4 Gy of total body irradiation, or busulfan (FLAM SA-BuCy), and anti-thymocyte globulin (ATG), remission was described in 66 of 75 patients (88%) with a median age of 52.3 years. With a median follow-up period of 35.1 months (range: 13.6 to 47.6 months), the overall and leukemia-free survival rates at 2 years were 42% and 40%, respectively.[11] Various groups have further developed the concept of sequential conditioning regimens.[12,14,15,17,18] Another group developed a novel sequential method that combined chemotherapy with broad anticancer activity (thiotepa 10 mg/kg, etoposide 400 mg/m^2, and cyclophosphamide 1600 mg/m^2 from day-15 to day-10) with a reduced-intensity conditioning regimen (fludarabine 150 mg/m^2, i.v. busulfan 6.4 mg/kg, and thymoglobulin 5 mg/kg from day-6 to day-2. In cases involving haploidentical donors, cyclophosphamide was added post-transplant. This retrospective multicenter analysis comprised 72 patients (median age, 54 years) with refractory hematologic malignancy (44 with acute myeloid leukemia, 7 with acute lymphoblastic leukemia, 15 with myelodysplastic syndrome/myeloproliferative neoplasms, and 6 with lymphomas). With a median follow-up of 21 months, the 2-year overall survival (OS) and event-free survival (EFS) for recipients of haploidentical donors were 54.7% and 49.3%, respectively.[23] In a recent study published by the Acute Leukemia Working Party of the EBMT to evaluate transplantation outcomes following six types of the sequential conditioning regimen for patients with relapsed/refractory acute myeloid leukemia undergoing allogeneic hematopoietic stem cell transplantation, the Flu-Mel-TBI8 group demonstrated the best leukemia-free survival in patients younger than 55 years of age.[13] According to these studies, since amsacrine is unavailable in Thailand, we adjusted the sequential conditioning protocol into the Thio-ETO-Cy-Flu-Mel protocol. Based on the Thio-ETO-Cy-Flu-Bu protocol proposed by Dulery R. et al.[24], we changed the conditioning regimen component from Flu-Bu to Flu-Mel due to higher survival and decreased non-relapse mortality demonstrated in several studies.[25-27] Surprisingly, early engraftment of neutrophils occurred on day+15 in the absence of severe infection and acute graft-versus-host disease.

Regarding donor selection, since the patient was childless and had only a 60-year-old sister, we selected his 30-year-old nephew to be the donor. To our knowledge, there is a study that uses collateral-related donor for haploidentical hematopoietic stem cell transplantation using ATG as a graft-versus-host disease prophylaxis regimen, and another study reported 3 patients who received a T-replete haploidentical allogeneic stem cell transplantation with post-transplant

cyclophosphamide from their second-degree relative nephews.[22,28]

Importantly, our results suggest that using a second-degree relative as a haploidentical donor should not be a constraint from a matching perspective, hence increasing the likelihood of finding a donor for a particular patient. Conversely, some collateral-related haploidentical donors may be less available or eager to donate than first-degree haploidentical donors and hesitate to do so. The recent evidence that the use of a haploidentical donor who is unrelated is possible may aid in resolving this issue. Our findings also indicated that we might be on the verge of eliminating the critical HLA barrier. In addition, combining solid organ transplantation with allogeneic hematopoietic stem cell transplantation from the same haploidentical donor might eliminate immunosuppression drugs in this circumstance because stable tolerance may be developed, as previously reported for kidney transplantation.

Conclusion

The author demonstrates that sequential conditioning T-replete haploidentical hematopoietic stem cell transplantation with high-dose post-transplant cyclophosphamide using a second-degree relative donor is feasible, allowing for complete engraftment and mild acute and chronic graft-versus-host disease in a patient with refractory acute myeloid leukemia and a high blast count. Regarding the number of mismatched HLA antigens on the non-shared haplotype, the number of mismatched HLA antigens on the second-degree relative donor does not appear to negatively influence sequential conditioning T-cell replete haploidentical transplantation with post-transplant cyclophosphamide.

Conflict of interest

The author has declared no conflict of interest

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Review Article GMSMJ Greater Mekong Subregion Medical Journal

Gut Microbiota in The Pathogenesis of Type 2 Diabetes Mellitus

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Abstract:

There are increased evidences of association between gut bacteria and the pathogenesis of type 2 diabetes mellitus. In humans, the gut bacteria comprise of six main phyla, including *Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria* and *Verrucomicrobia*. Gut bacteria maintain in a certain composition between each phylum and subphylum. Disturbance in gut microbiota composition is called dysbiosis. The microbial dysbiosis could result in many diseases, for examples: Celiac disease, obesity and certainly diabetes mellitus. The proposed mechanisms of gut microbiota on the pathogenesis of type 2 diabetes mellitus are resulted from: effects of gut microbiota on energy metabolisms, effects on intestinal integrity, effects on metabolic endotoxemia and low-grade inflammation, effects on intestinal motility, effects on immune system. Clinical information from the treatments to alter the gut flora composition by probiotics, prebiotics and fecal transplantation potentiate the novel alternatives for the future treatment of type 2 diabetes mellitus.

Keywords: Gut Microbiota, Type 2 DM, Pathogenesis

Introduction

Type 2 Diabetes Mellitus is a leading global health problem, with resultant long term social and economic dilemmas. The International Diabetes Federation reported in IDF Atlas 2021 that 537 million people live with diabetes and 783 million people of the world will have developed diabetes by 2045, leading to one person dying of diabetes every 5 seconds.[1] In Thailand, the prevalence of diabetes mellitus in adults is 9.9 percent.[2] The pathogenesis of type 2 diabetes is an interplay between genetic predisposition and environmental factors. Characterized by pathophysiologic abnormality, there are at least 8 organs of the body involved and this reality can be described as follows.[3]

1. Muscle insulin resistance, characterized by reduced muscle glucose uptake and reduced glycogen synthesis.[4,5]

2. Hepatic insulin resistance, leading to excessive hepatic glucose output.

3. Adipocyte insulin resistance, characterized by accelerated lipolysis and abnormal adipocytokine production (for example:increased resistin, decreased adiponectin).[6,7]

4. Progressive beta cell failure and apoptosis.

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5. Increased glucagon secretion by alpha cells and increased liver sensitivity to glucagon.

6. Reduced incretin effects, related to a reduction of Glucagon like peptide -1(GLP-1) levels and beta cell resistance to GLP-1 and Glucagon inhibitory peptide (GIP).[8,9]

7. Increased renal tubular glucose reabsorption, as a result of increased expression of SGLT-2 gene.[10]

8. Inappropriate function of the hypothalamus, via insulin receptor antisense oligodeoxynucleotides, leading to impaired appetite suppression.

The range of pathophysiology as mentioned above, is already known to be responsible for the development of type 2 diabetes mellitus This pathogenesis is evoked by interactions between genetic predisposition and environmental factors. However, there is an unexplored environmental factor that is related to the essential environment inside of the human body, that also contributes to the pathogenesis of type2 diabetes mellitus: the gut environment and the organisms living in this environment – the gut bacteria.

Human gut microbiome and their functions

The human gut contains a microbial community, termed microbiome. The number of gut microbiotas in individuals is diverse, up to 1,500 species having been reported.[11] There are six main phyla of gut bacteria in humans, comprising *Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria* and *Verrucomicrobia*. Generally, a normal balance of gut microbiota in the eubiosis contains a predominance of beneficial microbiota species, mainly comprised of phyla *Bacteroidetes* and *Firmicutes*.[12] However, variation in infants depends on the mode of delivery. Metagenome analysis has demonstrated that the maternal gut is the major source for microbiota in the gastrointestinal tract of healthy infants.[13] Breast-feeding and the mode of delivery affect the early gut composition and plays a role in the development of the immune system.[14,15] The colonization rate of *Bacteroide*s is higher in vaginal delivery-born infants compared to those in the cesarean delivery group. while cesarean delivery infants have a greater abundance of *Clostridium, Lactobacillus, Enterobacter, Streptococcus,* and *Enterococcus*.[14] The colonization of the gut microbiome during neonatal life is thought to affect gut maturation, metabolism, immunity, and brain development.[15] Infants exposed to antibiotics at birth showed an effect on mother-to-infant colonization and an increased the risk of horizontal transfer from the environment.[16] For example, antimicrobial resistance strains from the hospital may be the cause of infection in the perinatal period. The use of intrapartum antimicrobial prophylaxis increased the incidence of some β-lactamase coding genes and related aberrant profiles, with lower relative proportions of *Actinobacteria* and *Bacteroidetes* and increased proportions of *Proteobacteria* and *Firmicutes*.[17] This intestinal microbiota modulation, during the first month of life, can alter the development of individual microbiota-induced host homeostasis.[18,19]

A recent paper reported bacterial dispersal strategies, describing strong correlations among bacterial persistence, family association, and the phylogeography of the human gut flora.[20] The first group are termed *tenacious bacteria*. These bacteria live permanently during childhood through to adult life. They are well-adapted in the human hosts, with different nutritional needs, but tend to be lost permanently by antibiotic intervention. The second group, *herediper-sistant bacteria*, notably *Firmicutes*, have strong family associations and persistence. However, they lack notable phylogeographic signals. The third group, *spatiopersistent bacteria*. show a strong cluster to their own

geographic regions but family associations are not observed, due to a reduced strain persistence in infants.[20] Clearly, the variation of the individual microbiome is related to exposure to environmental factors.

Host and microbes live in association by depending on each other, described as symbiosis.[21] The host serves nutrients and provides an environment for microbes to survive, whilst the gut microbiome confers several advantages to its host, such as fermentation of some undigested food into absorbable compounds, to modify xenobiotics for human health benefit, reduction of toxicity of harmful industrial compounds and pollutants, synthesis of essential nutrients, competing with pathogens, and modulation of mucosal homeostasis by interaction with host immunity.[22-26] The intestinal microbiome can activate the immune system through presentation of microbe-associated molecular patterns (MAMPs), can maintain the intestinal barrier integrity and control mucosal inflammation, particularly during early life, and can therefore educate the immune system to protect the body from harmful microbes later in life.[22,27] Perturbation of optimal host-commensal interactions during this time may result in potentially persistent immune abnormalities.[27]

Studies of 16S rRNA gene amplicon sequencing and metagenomics explores the structure of gut microbiota on human physiology, and also explores links affecting the association between the microbiome and the host's diet, chemistry and health.[28] However, pinpointing specific functions of specific microbiota, requires more sensitive functional omics studies from metatranscriptomic, metaproteomic, and metabolomic analyses.[29-31] Metabolites, mainly short chain fatty acids (SCFAs), are produced by the gut microbiome via the fermentation of dietary fiber, particularly by anaerobic bacteria. Acetic acid (acetate), propionic acid (propionate), and butyric acid (butyrate) are the main bacterial metabolites with immunomodulatory and homeostasis roles.[32] After SCFAs are synthesized in the gut lumen, these metabolites are transported into the intestinal epithelial cells, where butyrate becomes the major energy fuel for metabolism of the intestinal epithelial cells. On the other hand, acetate and propionate are transferred to other organs via the blood circulation.[33,34] Moreover, acetate and propionate are primarily produced by the gut bacteria phylum Bacteroidetes, whilst butyrate is synthesized mostly by the phylum Firmicutes.[35] Interaction between SCFAs and the host cells activates cellular responses, resulting in the proliferation and differentiation of the cells, including inhibition of the zinc-dependent histone deacetylases (HDACs), that act as epigenomic erasers on the chromatin architecture.[33] The inhibition of HDACs provokes the hyperacetylation of histones, leading to anti-inflammatory gene activation.[36] In addition, a recent study has shown that SCFAs can induce neutrophil extracellular traps (NETs) formation, which is mediated, in part, by the free fatty acid 2 receptor expressed in neutrophils.[37] NETs play a role in protection against infection, for example the sequential step of stimulation is mediated by intracellular mediator production, such as reactive oxygen species (ROS), neutrophil elastase (NE) and protein-arginine deiminase type 4 (PAD4).[37] Studies on lymphoid (Epstein-Barr virus-positive) and cancerous epithelial cells, demonstrate that butyrate can stimulate interleukin-6 (IL-6) and interleukin-8 (IL-8) expression, including enhanced NF-kB activity. The consequence of this stimulation results in the removal of the EBV-infected cells and cancerous cells.[38] Microbiota-derived butyrate exhibits increasing antimicrobial activity.[39] Butyrate enhances macrophage differentiation though the inhibition of histone deacetylase 3 (HDAC3), to drive metabolic changes and microbicidal function without inflammation,

and inhibits mTOR kinase activity.[39] SCFAs could also play a role in the activation of adaptive immune response. The promotion of T cell differentiation into effector T cells and regulatory T cells (Treg) is mediated by the inhibition of HDAC and the regulation of mTOR pathway.[40] The mTOR pathway is also found to be involved in the promotion of some regulatory cytokine expressions, such as IL-10, IFN-γ, and IL-17, via STAT3 activation.[41,42]

Gut microflora and dysbiosis

The intestinal microbiome maintains homeostasis of immune reactions, host-pathogen interactions, and pathogen clearance.[42,43] Loss of balance between the gut microbiome composition in the host results in impaired intestinal cell function and increased gut permeability, including altered host immune responses, potentially increasing host susceptibility to infectious pathogens.[44] Some diseases have been found to be linked to dysbiosis of the gut microbiota. However, so far there is no clear evidence whether this is the cause of these diseases or is simply related to the progression towards the dysbiosis.

The microbial dysbiosis and related lower level of butyrate are believed to contribute to inflammatory bowel disease (IBD) immunopathogenesis.[45] Patients and animal models with IBD and colorectal cancer showed lowered levels of butyrate-forming bacterial species, including *Faecalibacterium prausnitzii*, the major bacterium of the *Clostridium leptum* group.[46,47] In addition, it has been found that butyrate production from *F. prausnitzii* could regulate T helper-17 cell/ Treg balance, and exert anti-inflammatory effects in colorectal colitis in murine models.[48] Furthermore, a reduction of butyrate may impair the function of bactericidal activities of macrophages, as described earlier.

Disturbance in microbiota components could also result in susceptibility to *Clostridium difficile*-induced colitis infection. Fachi et al. demonstrated that butyrate could protect intestinal epithelial cells damage by *C. difficile* toxin in infected mice via HIF-1 stabilization, while acetate administration could lessen the disease sequelae via promotion of innate immunity.[49] Several studies reported a decrease in phyla Bacteroidetes and Firmicutes in the human gut in *C. difficile* infection.[50-52] These groups of bacteria play a role in carbohydrate metabolism and SCFAs synthesis. However, a recent study has shown that the composition of these phyla were increased in *C. difficile* infections with community acquired onset. This contradiction may be due to the physical and nutritional components available from the community, which help preserve stable gut community and immune homeostasis, leading to a slower progression of *C. difficile* infection states.[53]

Dysbiosis in the gut microbiome was reported in Celiac disease, which is an autoimmune disorder, affected by genetic predisposition and triggered by gluten ingestion.[54] The impaired immunity and deficiencies in modulation of intestinal permeability result in mucosal inflammation and contribute to the pathogenesis of Celiac disease.[55] A decrease in *Bifidobacteria* and *Lactobacilli*, which play a role in the protective effect against inflammation, was reported in patients with Celiac disease.[56] On the other hand, rod-shaped bacteria, *Bacteroides, Clostridium*, and Prevotella, were more frequently reported in the small bowel of children with Celiac disease.[57-59] Nonetheless, there has been no specific bacterial strain identified as causing the disease or acting as a specific marker for the diagnosis of Celiac disease. Research in intestinal cell culture conditions has demonstrated that *Bifidobacteria* and *Lactabacilli* inhibit the breakdown of gluten and its peptide derivatives that are the cause of this toxicity.[60] So far, the proven therapy for Celiac disease is a gluten-free diet, with supplementation of probiotics, to regulate intestinal integrity and decrease inflammatory responses.[61]

The composition of the gut microbiome in obesity has been studied in both animal and human subjects. It has been proposed that relationships of gut microbiome may act as the pathogenesis of obesity. Studies in obese mice showed an increased ratio of *Firmicutes* and *Bacteroidetes* as compared to the control.[62,63] Findings on overweight pig models also demonstrated a lower abundance of *Bacteroidetes* in the colon compared to lean animals.[64] The transplantation of fecal microbiota from lean twin mice showed modulation of metabolism and prevention of increased adiposity phenotypes, including reconstitution of the number of *Bacteroidetes* in the gut of obese twins.[65] In addition, several studies have reported the major composition of the gut bacteria in obese humans. *Bacteroidetes* are frequently found in a lower abundance in the gut microbiota of obesity versus lean controls.[66-68] Species-specific variations of *Lactobacillus* in obesity were also reported.[66] On the other hand, Patil et al. compared the proportion of dominant bacteria between lean, normal, obese and surgically treated obese individuals of Indian origin and found that there was no correlation in the trends between *Firmicutes* and *Bacteroidetes* among these groups.[69] Observation in obese women showed an increase in levels of inflammatory markers. Calorie restriction improved the inflammatory markers transiently and reduced gut permeability.[70] However, the overall bacterial phylogenetic make up was not changed statistically after the calorie restriction. More specifically, a recent study demonstrated that obese subjects, who lost at least 5 percent of their body weight, had significantly different baseline microbiomes when compared to those that did not lose weight. *Escherichia, Shigella, Klebsiella, Megasphaera,* and *Actinomyces* were substantially enriched in the subjects who did not lose their weight. These bacteria, therefore, have been suggested as the gut microbiota associated with weight response to a calorie-restricted diet.[71]

Proposed mechanisms of gut microbiota on pathogenesis of type 2 Diabetes Mellitus

Effects of gut microbiota on energy metabolism

Dietary polysaccharides and oligosaccharides are digested by gut microbiota into monosaccharides and short chain fatty acids (SCFAs). The main SCFAs are composed of acetate, propionate and butyrate, which contribute to 5-10 percent of energy resource.[72] These SCFAs are the direct energy resource of intestinal epithelial cells (IEC).[73] In adipose tissue, SCFAs and free fatty acids (FFA) interact with the G-protein coupled recepter-41 (GPR-41) and G-protein coupled recepter-43 (GPR-43), located on the adipocyte membrane, to create energy accumulation.[74] At the L-cells of the small intestine SCFAs interact with GPR-41 and GPR-43 to promote GLP-1 production.[75,76] Consequently, SCFAs act as metabolomics, exerting effects on insulin secretion, glucose homeostasis and energy metabolism. Comparison of the individual major SCFAs reveals that acetate diminishes food intake while butyrate and propionate suppress weight gain, as shown in studies on healthy mice.[77,78] In addition, a study of normal laboratory mice, compared with germ free mice (GF), revealed that the level of triglycerides in the normal mice adipose tissue and liver was higher than that of germ-free mice.[79] Hence this study suggested the role of gut microbiota in lipid metabolism and energy storage. In the liver when lipogenesis take places, gut microbiota increases synthesis of hepatic triglycerides via activation of both ChREBP and SREBP-1C.[77,80]

In further interventional studies in mice, introduction of gut bacteria, from normal mice to germ free mice, had the effect of increasing fat mass and insulin resistance.[81] This study proves that gut bacteria have the capacity to enhance energy harvest from food.[82]

Effects of gut microbiota on fatty acid oxidation and synthesis

Different kinds of gut microorganism have different effects on fatty acid oxidation and fatty acid synthesis. *Akkermansia muciniphila* enhances fatty acid oxidation in adipose tissue, via increased levels of 2-oleoyl glycerol, 2-palmito glycerol and 2-acylglycerol. This process leads to increased adipocyte differentiation.[83] *Bacteroides acidificiens* enhances fatty acid oxidation in adipose tissue, via the PPAR-γ pathway.[84] *Lactobacillus gasseri* increases fatty acid oxidation by enhancing fatty acid oxidation genes and reducing fatty acid synthesis related genes, to reduce obesity.[85]

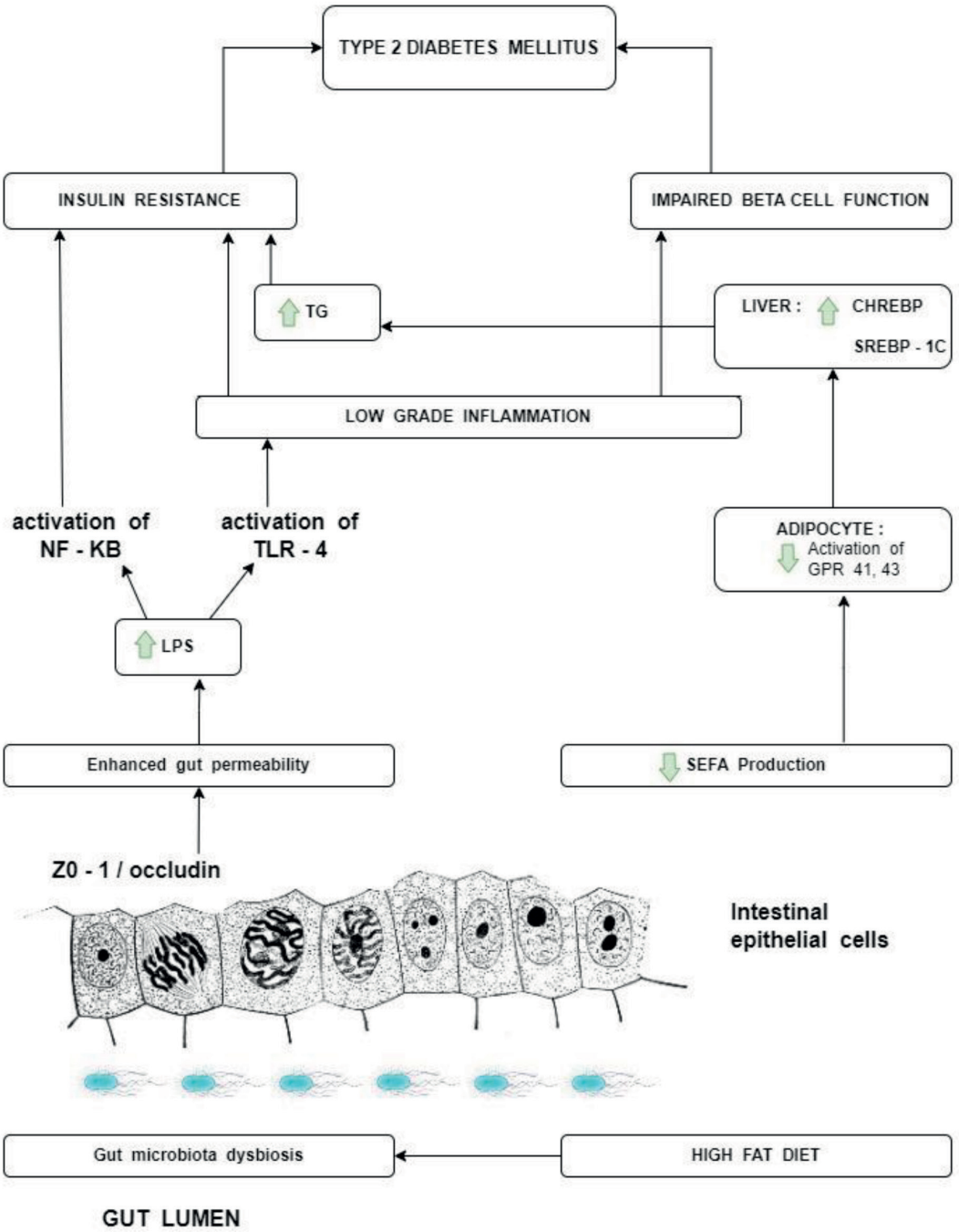

Figure 1 The proposed mechanisms of gut microbiota on contribution to pathogenesis of type 2 diabetes mellitus (modified from He C, Shan Y, Song W. Nutr Res. 2015; 35(5): 361-7.)

Effects of gut microbiota on glucose metabolism

The major organs involved in the development of type 2 diabetes, and creating insulin resistance, are the liver, muscles and adipose tissue. These functions of these organs are affected by gut microbiota and are related to glucose metabolism and homeostasis. There are published studies of certain gut bacteria that have demonstrated these associations. *Bifidobacterium lactis* has good effects on glucose homeostasis, by increasing glycogen synthesis in the liver and decreasing expression of hepatic gluconeogenesis related genes. This bacterium increased translocation of glucose transporter-4 (GLUT-4), in insulin sensitive tissue, to stimulate glucose uptake.[86] Similarly, a bacterium that can increase GLUT-4 expression in muscle is *Lactobacillus gasseri*.[85] Another of the same genus, *Lactobacillus casei*, has a beneficial effect on insulin resistance, by enhancing the mRNA level of phosphatidylinositol 3-kinase (PI3K), AMPK, which increases glycogen synthesis in the liver.[87,88] The Lactobacillus species *L. rhamnosus* increases insulin sensitivity in epididymal fat by increasing adiponectin levels.[89] *Akkermensia muciniphila* potentiates α-glucosidase inhibitor activity, to prevent the breakdown of complex carbohydrate, resulting in reduction of postprandial hyperglycemia.[90]

Interaction of gut microbiota and host genetics in obesity development

Current knowledge on the pathogenesis of obesity is concentrating on the role of gut flora, linking genetic predisposition and development of obesity. The host genome impacts on the individual gut microbiota composition and functions, those influence the breakdown of indigestible dietary polysaccharides, for body energy harvest from food.[73] Experimental studies in mice, by the transfer of gut microbiota from obese mice or humans into germ free mice, caused more weight gain when compared to transfer of gut microbiota from lean mice.[91] Early studies on the gut microbiota composition, comparing results obtained from obese mice and lean mice, gave similar results to those obtained from obese humans. The results showed an increase in Firmicutes and a decrease in Bacteroidetes or an increase in the Firmicutes/Bacteroidetes ratio.[92-95] Furthermore, various studies revealed an association of certain bacterial populations with weight gain, including *Akkermansia muciniphila, Faecalibacterium prausnitzii, Lactobacillus reuteri, Roseburia intestinalis*.[96] Not only were these specific bacterial groups linked to weight gain in human and animal studies, but a decrease in gut flora diversity was associated with obesity and certain metabolic diseases. Reduction of dietary diversity in most countries leads to loss of gut microbial diversity.[97]

Effects of gut microbiota on intestinal integrity (gut permeability)

Increased gut permeability or gut leakage has a deleterious effect on glucose homeostasis. The paracellular permeability of the gut is manipulated by multiple proteins, for example claudin, occludin and the zona occludens.[98] Experimental high fat feeding of mice revealed the expression of the tight junction proteins and zona occluden-1.[99] Hence, gut barrier disruption is responsible for microbes and molecules, derived from bacterial compound production, such as lipopolysaccharides (LPS), pepitidoglycans and flagellin, entering from the gut lumen into the circulation. This process is called metabolic endotoxemia.[82] Consequently, it can be surmised that gut barrier disruption leads to metabolic endotoxemia. Studies on obese mice showed disruption of intestinal barriers, enhanced intestinal mucosal permeability and leakage of LPS. LPS is a molecule that is derived from components of the cell wall of gram-negative bacteria.

Entry of LPS into the circulation was seen to promote inflammatory cytokines activation.[100]

The endocannabinoid system (eCB) is one of the body systems that has an important role in energy homeostasis. In the obese state the endocannabinoid system exerts its effects through gut epithelium permeability, by alteration of the tight junction protein, via activation of cannabinoid receptor1 (CB1) and 2 (CB2). CB1 and CB2 are expressed through the GI tract at various levels and gut microbiota can manipulate these CB1 receptors. The CB1 antagonist could decrease gut permeability and reduce metabolic endotoxemia.[101]

Intestinal alkali phosphatase (IAP) is the enzyme involved in the breaking down of dietary lipid. IAP acts on LPS detoxification and reduction of LPS level by dephosphorylation of the lipid portion of the LPS.[102] Gut microbiota regulated expression of IAP and a study in obesity showed a decrease in IAP activity.[103,104] Nevertheless, an increase in IAP activity also leads to reduction of metabolic endotoxemia.[105]

Glucagon like peptide-2 (GLP-2) is another system involved in gut permeability. Increased endogenous production of GLP-2 is associated with strengthening of the tight junctions of the intestinal epithelium cells.[106] A study in ob/ob mice, incorporating GLP-2 related pharmacological treatment, resulted in improvement of tight junctions and reduction of LPS levels in the plasma.[107] Certain gut bacteria have beneficial effects on tight junctions. *Bacteroidetes valgaris* and *Bacteriodetes dorei* upregulated the expression of the tight junction gene in the colon of mice, inducing reduction of gut permeability and metabolic endotoxemia. *Akkermansia muciniphila* augmented intestinal tight junctions via AMPK activation in intestinal epithelium cells to reduce gut permeability.[108] *Faecalibacterium* and *Roseburia* produce butylate that acts on serotonin transporters and PPAR-γ pathways, to reduce gut permeability and improve intestinal barrier functions.[109]

In human with type 2 diabetes, intestinal permeability substantially increased in comparison to controls.[110] In mice studies, a high fat diet reduced the epithelial integrity of the gut lining, via reduction in tight junction proteins: zonula occluding-1 (ZO-1) and occludin. Dietary fatty acid also activated toll-like receptor 2 (TLR-2) and toll-like receptor 4 (TLR-4). TLR-4 is the component of the complex proteins that mediate metabolic endotoxemia, by intervention of LPS translocation into the intestinal capillaries.[111]

Effects of gut microbiota on metabolic endotoxemia and low-grade inflammation

Low grade inflammation promotes the development of insulin resistance and diabetes.[112] As mentioned earlier, lipopolysaccharides (LPS): a glycol-lipid molecule derived from cell wall of gram-negative bacteria in the gut wall, can induce an innate immune response. LPS stimulates a cascade of responses that leads to release of proinflammatory molecules that contribute to insulin resistance and glucose homeostasis.[113] An increased level of LPS was found in high fat intake mice.[114] A similar study in obese and diabetic humans, with high fat intake, showed increased level of LPS in their blood.[115] LPS mediates inflammatory responses via TLR-2 and TLR-4 pathways.[116] TLR-2 incites inflammatory signaling by activation of nuclear factor kappa-B (NF-kB) and cellular pro-inflammatory cytokines.[117] Similarly, TLR-4 activates the release of pro-inflammatory cytokines which interfere with glucose and insulin metabolism.[118] A study in mice, lacking TLR-2, demonstrated improved insulin sensitivity with faster glucose clearance, according to reduced expression of inflammatory cytokines.[119]

While certain kinds of gut microbes and microbial products aggravate metabolic endotoxemia and low-grade inflammation, some gut microbiota exert beneficial effects by stimulation of anti-inflammatory cytokines. For instances, *Roseburia intestinalis, Bacteroides fragilis, Akkermansia muciniphila, Lactobacillus plantarum* and *Lactobacillus casei* increase IL-10 and IL-22 production. These anti-inflammatory cytokines are known to improve insulin sensitivity and reduce blood glucose excursion in diabetic patients.[120-125] Similarly, exerting beneficial effects on glucose homeostasis, *Lactobacillus, Bacteroides* and *Akkermansia* inhibit pro-inflammatory cytokines: TNF-α.[126-127] Certain species of *Lactobacillus (L. plantarum, L. paracasei, L. casei)* can reduce pro-inflammatory cytokines: IL-1β, IL-8, CD-30, C-reactive protein.[128-129] Some gut microbiota inhibits pro-inflammatory activity, via induction of short chain fatty acids, for example, *Roseburia* and *Faecalibacterium* produce butyrate to repress NF-kB.[130-131]

Effects of gut microbiota on intestinal motility

Microbiota can interact with gut motility through several mechanisms. By stimulation of pro-inflammatory cytokine production, and modulation of immune cell functions in the intestine, the increased inflammation affects Peptidergic Enteric Neurons, resulting in neurodegeneration. This process causes decreased gut motility.[132] In addition, gut microbiota can affect intestinal motility, by interaction with the gut-brain axis, through modification of afferent sensory nerve impulses, enhancing neuronal activity and modulation of pain perception.[133] Moreover, gut microbiota can modify enteric nervous system activity, via production of local gut neurotransmitters, for example GABA, serotonin, melatonin, histamine and acetylcholine, leading to effects on gut motility.[134]

Effects of gut microbiota on immune system

Malfunction of the innate intestinal immune system plays an important role in glucotoxicity and lipotoxicity, resulting in the development of obesity and metabolic syndrome.[135] The chronic inflammatory state, presenting in obesity, stimulates innate and adaptive immunity. The immune response process functions via a Toll like receptor (TLR) to promote production of inflammatory cytokines, such as IL-1β, resulting in beta cells destruction.[136]

TLR-5: one of the components of innate immune system, is expressed in intestinal mucosa.[137] A study on TLR-5 deficient mice showed features of hyperphagia, obesity, hyperlipidemia, high blood pressure and insulin resistance. A further study on transplantation of gut microbiota, from TLR-5 deficient mice to the wild type germ free mice, resulted in increased levels of inflammatory cytokines, development of features of insulin resistance as well as obesity in the recipients.[138] *Akkermansia muciniphila* stimulated FOXP3 regulatory T cells in visceral adipose tissue to enhance glucose tolerance.[139]

Conclusion and perspectives

With the advance of genomic medicine and the advent of novel techniques on genome sequencing, there is a great opportunity to understand the involvement of human gut bacteria on the pathogenesis of many diseases. New generation approaches such as metagenomics, metabolomics and transcriptomics have had tremendous effects on explanations of the molecular basis of interaction between gut microbes and the pathogenesis of type 2 diabetes mellitus and even the association between gut microbiota and diabetic related complications in humans. Then intervention, by alteration of composition of gut microbiota, is a novel therapeutic challenge for treatment of type 2 diabetes.

The use of probiotics, prebiotics and even fecal microbiota transplantation, constitute an explosion of new information, revealed in recent clinical studies in diabetic patients. In the near future, with more studies in human subjects, a better understanding of the molecular interaction of gut microbiota and type 2 diabetes will lead to the application of these measures on type 2 diabetic prevention, alongside treatment with conventional antidiabetic medications.

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Author contribution

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Conflict of interest

The authors declared no conflict of interest.

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Rice Starch Anchor for Osteoporotic Bone Strengthening

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Abstract:

Background: Osteoporosis and osteopenia are important diseases for weakening and fracturing the bones of patients. Both diseases are caused by low bone mass and deterioration of bone structure until severe pain and unable to function normally. Most doctors will treat bone fractures using a metal plate and screw to hold the bone in place. Then fix and pull the bone back to its original position by using screws to fix the bone. But in osteoporosis, the screw is usually to be wobble without tighten bone fixation.

Objective: This research aims to increase bone mass in specific position that enhances bulk structure by implanting the rice starch bone anchor into the porous bone.

Materials and methods: Rice starch anchor (RSa) was carried out in third steps. Characteristics of the final products were then investigated scanning electron microscopy (SEM), XRD, and swelling ratio, PH and, pull out strength. All quantitative data were analyzed with origin 8.0 (Origin Lab Corporation, USA) and presented as the mean ± standard deviation. Statistical comparisons were carried out using analysis of variance (ANOVA, Origin 8.0). A value of $p < 0.05$ was considered to be statistically significant. Materials characteristics were determined by scanning electron microscopy (SEM), X-ray diffraction (XRD) and swelling ratio.

Result: The material was completely biodegradable in the human body. The optimal composition of the material is 50% of rice starch, 50 wt % of additives. It had physical characteristics. In parts that can be used to hold human bones, such as the upper tibia below the knee joint. The rice anchor can be used 3.95 mm of metal screws with a pullout strength of 117.27 ± 1.58 N.

Conclusion: Rice starch anchor had chemical and mechanical properties suitable for used with metal screws to help hold screws tighten in decayed surfaces for human bone fixation. It was suitable material for bone strengthening of osteoporosis. In the future should be tested for compatibility in laboratory animals and further testing for safety and clinical efficacy.

Keywords: Rice starch, Anchor, Cow bone, Metal screw, Osteoporotic

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Introduction

In 2021, Thailand has reached to aging society. Thai population is over 60 years old or 14 million people (70 million of Thai population), while those over 60 years old are at risk of osteoporosis in bone mass. Due to the deficiency of calcium and phosphate minerals is more than growth a new bone and the weakening of organic matter and bone mass. So, the deteriorating elderly organs throughout the body deteriorate when accident or broken bone.[1] It can easily cause fractures. This case is extremely painful. Especially, if a bone is fractured at a load bearing body, such as a hip or leg, it requires bed rest for 2 to 3 months, putting the risk of bedridden and complications. Most doctors recommend surgery and fixing the fractured bone in place with bone plate and screws. For the reduce pain and the patient can move into weight within a few days. But in the condition of osteoporosis, standard screws may not be able to securely tighten. There is a risk of being completely disconnected from the fracture site. Because doctors need to use larger screws and reposition of the screw holes or use another treatment. This case was more serious consequences for the patient.[2]

Rice starch gel was cross-linked by macromolecules to form a hydrophilic polymer and allowing the material to swell in water or retain large amounts of water in the microstructure. From cross linking, the degree of swelling and the amount of water contained depends on two factors 1) the hydrophilic capacity of the polymer chain and 2) the cross-linking density.[3] Bonding can arise from physical crosslink between polymer molecules, such as ionic bonds, hydrogen bonds, van der Waals forces, or hydrophobic reactions. Researchers could apply gel to fabricate biomaterials for a wide application of academic fields, such as medicine, agriculture, and biology.[4] In this research, we developed a novel rice starch anchor which fix to bone weaken with medical screw for bone strengthening and using a composite material of rice starch, polyvinyl alcohol (PVA), gelatin and glycerol. The characteristic of rice starch anchor was then characterized by scanning electron microscopy (SEM), XRD, swelling ratio and, pull out strength.

Materials and Methods

Materials. Pharmaceutical grade rice starch (RS) was purchased from Chiang Mai, Thailand. Polyvinyl alcohol (PVA) with an average molecular weight of 8.5 x 10^4 g/mol and 99+% hydrolyzed was purchased from Sigma-Aldrich, Germany. Gelatin was purchased from Fluka, Switzerland. Cow bone derived from Macro supermarket. Maleic acid used as a cross linker and copper sulfate used as catalyst were purchased from Sigma-Aldrich, Germany. Glycerol was purchased from Fluka, Switzerland.

Sample preparation. Rice starch anchor (RSa) was carried out in third steps. In the first step, 42-50 wt% RS mixed with additives. The additives composed of 7 wt% cow bone, 7 wt% PVA, 7 wt% gelatin, 3.8 wt% maleic acid and 0.2 wt% copper sulfate. The mixture was dissolved in distilled water and 25 wt% glycerol solution. The resulting solution was stirred at 95°C for 1 hour. In the second step, metal screws dipped in the obtained solution then dried in hot air oven at 70°C for 30 min. This step was repeated 4 times. Finally, the part of RSa was removed from metal screw and dried in oven at 70°C for 24 hrs. This procedure followed by flow chart that shown in Figure 1.

Characterization. Characteristics of the final products were then investigated scanning electron microscopy (SEM), XRD, and swelling ratio, pH and, pull out strength. All quantitative data were analyzed with origin 8.0 (Origin Lab Corporation, USA) and presented as the mean ± standard deviation. Statistical comparisons were carried out using analysis of variance (ANOVA, Origin 8.0). A value of $p < 0.05$ was considered to be statistically significant.

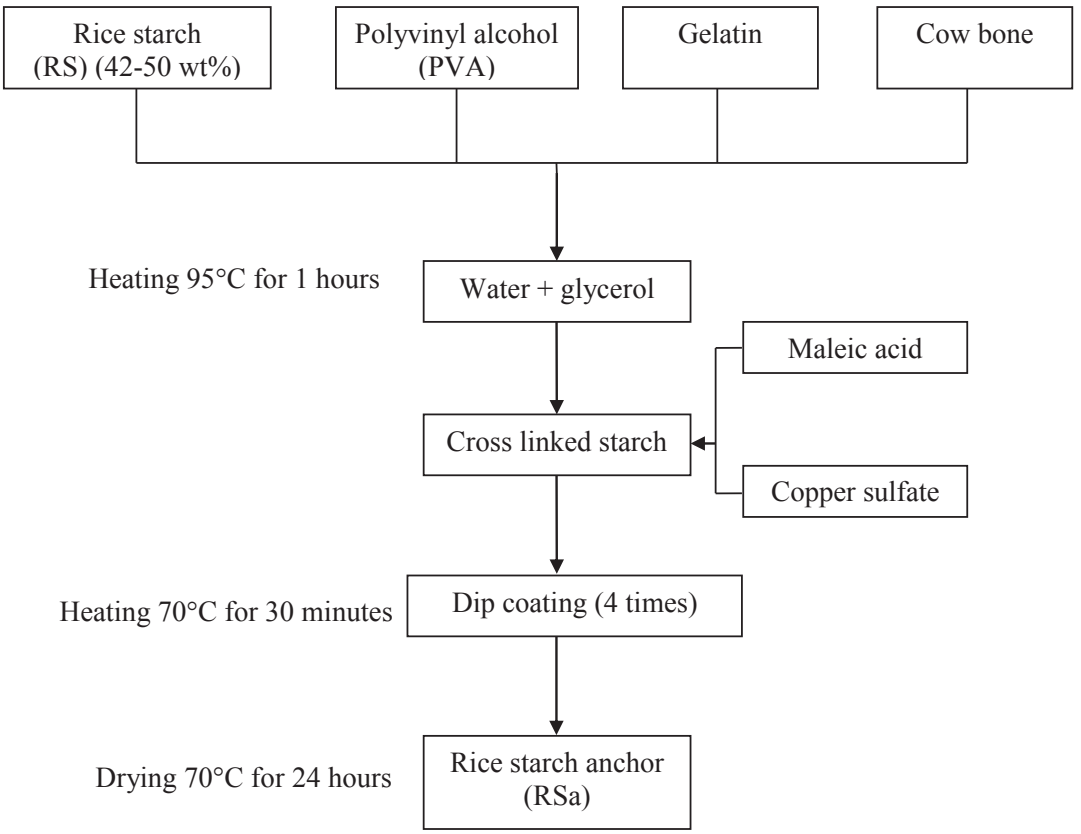

Figure 1 Flow chart of RSa process

Results

Prototype of rice starch anchor for treatment of osteoporosis for bone strengthening shown in Figure 2 and 3. Figure 2 shows that the dimension of rice anchor was inner diameter of 3.95 mm. and length as 14.75 mm. Figure 3 showed metal screw with Rsa.

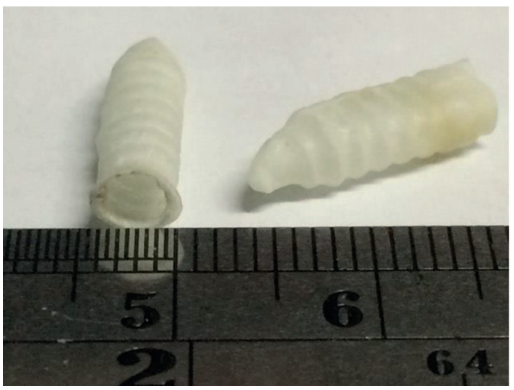

Figure 2 Prototype of rice starch anchor

Figure 3 Samples of rice starch anchor with metal screw

SEM of surface microstructure of RSa is illustrated in Figure 4. The crossection the fracture rice starch anchor indicated that microstructure of cow bone powder embeded under the sufface of RSa in size about 100 nm (Figure 5). The cow bone powder might be assisted for strength and bioactivity of rice starch bone anchor.[5]

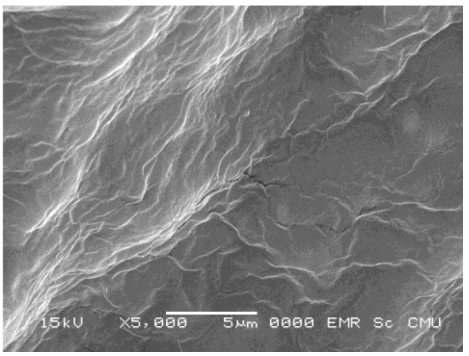

Figure 4 SEM of Rsa surface

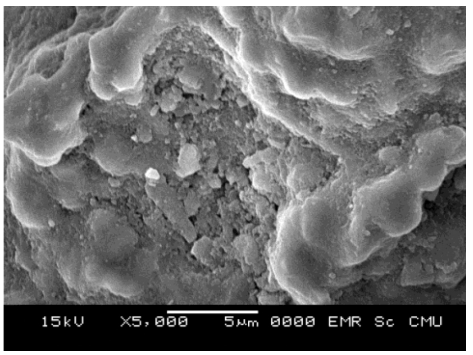

Figure 5 SEM of cross section area of Rsa

Starch hydrogel are amorphous phase which obtained from thermal and chemical cross linked of rice starch with maleic acid, copper sulfate and glycerol. Figure 6 showed the best condition of RSa compared with rice starch gel and cow bone.

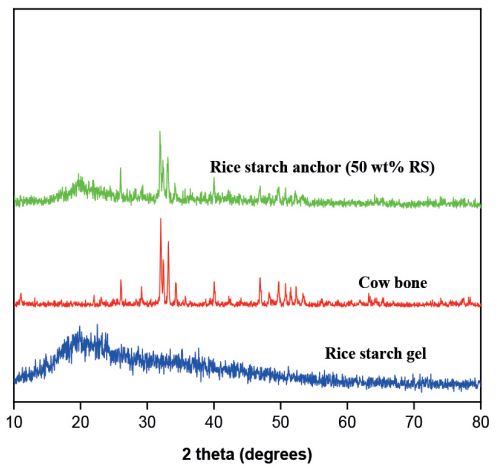

Figure 6 XRD pattern of RS gel, cow bone and RSa

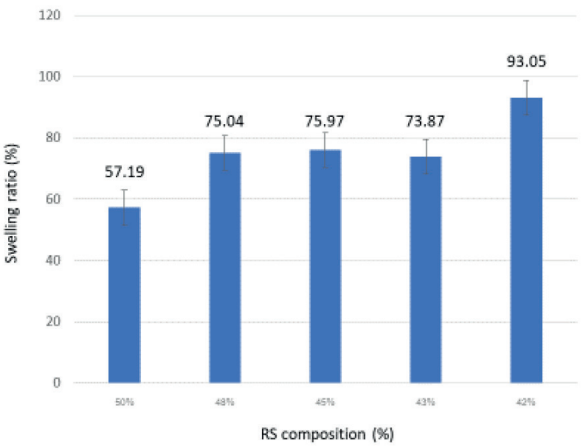

Figure 7 Effect of RS on swelling ratio of RSa

Rice starch gel is amorphous structure and the main XRD patterns of the cow bone were 25.88, 31.78, 32.18, 32.91, 39.82, 46.71 and 49.47°. This result was in agreement with previous work.[5] When rice starch was crosslinked with additive such as cow bone, PVA, gelatin, maleic acid and, $CuSO_4$. Afterthat, the XRD pattern of the rice starch hydrogel blended with the additves at a ratios of RS:additive of 42:58, 43:57, 45:55, 48:52 and 50:50 wt%, respectively. The peak intensity of gel were increased at 25.88°, 31.78°, 32.18° and 32.91°, respectively. This showed that the intensity of the XRD pattern of the hydrogel increased with increasing proportions of the cow bone in the blend. This is due to the impregnation of cow bone powder during the gelatinization process.

The physical properties of the RS hydrogel were evaluated by measuring the degree of swelling in distill water. The effect of the ratio of RS and additive and cow bone on the swelling of the polymer hydrogel in water is shown in Figure 7. It is clear that the swelling ratio of the hydrogel decreased to a minimum with increasing RS content and then increased with further increases in the proportion of RS in the mixture. For instance, the swelling ratio of the hydrogels with ratio of RS:additive of 42:58, 43:57, 45:55, 48:52 and 50:50 wt% were 93.05%, 73.87%, 75.97%, 75.04%, and 57.19%,

respectively. This was due to the hydrophilic behavior of RS and polymer additives.[6] However, these data indicate that at high proportions of cow bone in the hydrogel, there is a loss of rice starch from the hydrogel, especially at ratio of 42:58 due to the phase separation between cow bone and RS.

A suitable ingredient of these ratios of the production of Rsa, it was found that all five ingredients had pH about 6, weak acid, which had no effect on human use. It was found that ratio 50:50 suitable for the production of Rsa. Because the water absorption is 57.19 % and the surface expansion is 32.00%, which is the lowest value compared to other ratios. This indicates that ratio of 50:50 mixtures has slow water absorption causing less area expansion accordingly and there is likely a degree of cross-linking between rice starch and additives.[7] This ratio is suitable for production of bone anchor and flexibility to fasten metal screws without decaying too quickly.

Good mechanical is vital for bone anchor in bone fixation of osteoporotic. The effects of the RS content on the pull out strength of the RS:additive was 117.27 $\pm$ 1.58 Newton which in level of pull out strength ranged (56-1230 N) of metal screw compared with human tibial plateau.[8,9]

Conclusion

Rice starch anchor was material for bone strengthening of osteoporosis. The material was completely biodegradable in the human body. The optimal composition of the material is 50% of rice starch, 50 wt% of additives. It had physical characteristics, chemical and mechanical properties suitable for used with metal screws to help hold screws tighten in decayed surfaces for human bone fixation. In parts that can be used to hold human bones, such as the upper tibia below the knee joint. The rice anchor can be used 3.95 mm of metal screws with a pullout strength of 117.27 $\pm$ 1.58 N. In the future should be tested for compatibility in laboratory animals and further testing for safety and clinical efficacy.

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Prevalence and Factors Associated with Unsuccessful Pulmonary Tuberculosis Treatment in Thai Military Hospitals

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Abstract:

Background: Thailand is one of high TB burden countries. Military hospitals have been providing TB care for both military officers and civilians. However, there has never been studies of TB treatment in these hospitals in large scale.

Objective: This study aimed to report prevalence and associated factors of unsuccessful pulmonary TB treatment outcomes among Thai military hospitals.

Materials and Methods: A cross-sectional study was conducted in nine military hospitals across four regions of Thailand. Data from 2012 to 2021 were collected which included demographic and follow-ups data. Outcomes were successful and unsuccessful treatment. Logistic regression was used for predicting associated factors of unsuccessful pulmonary TB treatment.

Results: Prevalence of unsuccessful TB treatment was 17.72%. Associated factors of unsuccessful treatment included being agriculturist, laborer and household business owner, fever and weight, not investigated sputum culture, abnormal liver function test (LFT) and blood urea nitrogen (BUN) at the start, positive sputum AFB and uninvestigated sputum AFB at second month follow-up, uninvestigated chest radiograph at fifth month and unmonitored weight throughout treatment. Protective factor was having cutaneous adverse reactions during follow-up.

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Conclusion: Several factors associated with unsuccessful pulmonary TB treatment outcomes were system-related or individual factors. Establishing community-based treatment system can be a solution.

Keywords: Pulmonary tuberculosis, Prevalence; Associated factor, Thailand

Introduction

TB has been one of the leading causes of death globally despite advanced and adequate medical care in the past five decades.[1] It was estimated that a quarter of world's population is infected with TB.[2] Although new TB cases are decreasing, but the process was slow.[1]

Regarding Thailand, challenges to TB management strategies in the past years included high mortality, late diagnosis, duplications in monitoring and evaluation systems, under-reported from non-Ministry of Public Health settings, insufficient coverage of multi-drug resistance tuberculosis (MDR-TB) detection and difficulties in accessing TB care for migrant workers.[3]

Military hospitals, as secondary and tertiary care units, have long been providing healthcare services for both military, civilian officers and civilian as well as their families including TB diagnosis, monitoring and treatment. However, military hospitals are not under supervision by Ministry of Public Health and the specific reports of TB treatment in military hospitals were sporadic. In addition, there has never been studies of TB treatment in these hospitals in large scale. To address this problem, this study aimed to report prevalence and associated factors of unsuccessful pulmonary TB treatment outcomes among Thai military hospitals.

Methods

Study design and setting

A cross-sectional study was conducted in nine military hospitals across four regions of Thailand. Data from 2012 to 2021 were collected. Two military hospitals are from each region except for central region which has three hospitals. One hospital in central region located in Bangkok, capital city of Thailand. Eight hospitals are secondary care units and one hospital in Bangkok is tertiary care center as well as being a teaching hospital.

Study population

This study included all patients receiving pulmonary TB treatment. Excluded were patients who has extrapulmonary TB without co-existing pulmonary TB and latent TB.

Data collection

Data stored in TB registration cards, outpatient records and computer-stored information of patients receiving TB treatment at each hospital from 2012 to 2021 were collected with permission from hospitals' directorial boards. Collected data included demographic data (gender, age, occupation, hospital and past medical history), baseline characteristics at start of treatment (registered status, weight, clinical symptoms, chest radiographs, sputum acid-fast bacilli (AFB), sputum gene X-pert, sputum culture, liver function tests, renal function tests and drug regimen) and follow-ups data before 2 months, at second month and at fifth month (sputum AFB, chest radiographs and drug adverse effects). Results of treatment were collected at either sixth month or final month of treatment in case of prolonged treatment regimen. The results included 'cure', 'complete', 'fail', 'transferred out', 'loss to follow-up' and 'death'.

Operational definitions

According to WHO[4] and Thai national tuberculosis control programme guideline[5], pulmonary TB is a case of TB involving the lung parenchyma. Treatment outcomes

included 'cure', 'complete', 'fail', 'transferred out', 'loss to follow-up' and 'death'. Cured is defined as sputum AFB smear or culture is negative at the end of treatment. Completion is considered if the treatment was completed, but without evidence of negative sputum AFB smear or culture results in the last month of treatment, but the latest sputum smear is negative. Failure is defined when the sputum AFB smear or culture is positive at the fifth month or later. Death includes all patients who died from any causes during treatment. Loss to follow-up is characterized by interruption of treatment for two or more consecutive months. Transferred out is considered when a patient was transferred to other treatment facilities without known final treatment result. Cure and complete were categorized into 'successful treatment', while fail, transferred out and loss to follow-up were regarded as 'unsuccessful treatment.

Statistical analysis

Statistical analysis was performed with SPSS 23.0 (Armonk, New York, U.S.). Descriptive statistics was used for describing characteristics of the studied population. Univariate analysis was used for predicting associated factors of unsuccessful pulmonary TB treatment. Factors which had p-value < 0.20 and significant in previous studies were recruited for multivariate analysis using 'Enter' function. Factors with p-value ≤ 0.05 at 95% confidential interval (CI) were considered statistically significant.

Ethical consideration

This study was approved by Institutional Review Board of Royal Thai Army Medical Unit numbering S040h/63_Exp. Data were collected with permission from each hospital's directorial boards.

Results

A total of 2,307 cases were collected from all hospitals. After data cleaning by filtering out incomplete demographics, follow-ups data and final treatment results, 2,003 cases were available for analysis.

Demographic data

Most patients were from hospital in Bangkok Metropolis (74.39%) and male (70.19%). Average age was 48.35 ± 19.44 years with most patients' age range was 21-30 years (22.27%) followed by 51-60 years (20.57%). Approximately 64.35% had HIV co-infection. Around 8.09% had history of TB contact, 4.89% had history of previous TB and 2.08% had co-existing extrapulmonary TB. Most patients presented with chronic cough (59.41%). Around 34.90% had reticulonodular infiltration. Most were smear-negative TB (50.50%). Around 40.84% did not receive DOT (directly observed therapy). Demographic data and baseline characteristics were shown in Table 1.

Table 1 Demographic and baseline characteristics of patients receiving TB treatment in Thai military hospitals

Characteristics	N (%)
Hospital region	
Bangkok Metropolis	1490 (74.39)
Southern region	220 (10.98)
Northern and Northeastern regions	127 (6.34)
Central region	166 (8.29)
Gender	
Male	1406 (70.19)
Female	597 (29.81)
Occupation	
Laborer	602 (30.05)
Military officer	526 (26.26)
Civilian officer	144 (7.19)
Trader/merchant	88 (4.39)
Student	71 (3.54)
Household business	57 (2.85)
Agriculturist	51 (2.55)
Healthcare providers	14 (0.70)
Unemployed	450 (22.47)
Age (years)	
< 20	32 (1.60)
20-29	439 (21.92)
30-39	256 (12.78)
40-49	291 (14.53)
50-59	402 (20.07)
60-69	237 (11.83)
70-79	212 (10.58)
≥ 80	134 (6.69)
Mean (age ± S.D.)	48.35 ± 19.44
Registration status	
New	1905 (95.11)
Relapse	71 (3.54)

Characteristics	N (%)
Treatment after loss to follow-up	23 (1.15)
Treatment after failure	4 (0.20)
TB drug regimen at start	
2 HRZE/4HR	1924 (96.06)
2 HRZES/1HRZE/5HRE	32 (1.60)
MDR regimen	39 (1.95)
Other regimens	8 (0.40)
Medical history and co-morbid illnesses	
HIV infection	
Yes	1289 (64.35)
No	714 (35.65)
Diabetes mellitus	
Yes	906 (45.23)
No	1097 (54.77)
Chronic lung diseases	
Yes	34 (1.70)
No	1969 (98.30)
Kidney diseases	
Yes	21 (1.05)
No	1982 (98.95)
Liver diseases	
Yes	11 (0.55)
No	1992 (99.45)
History of TB contact	
Yes	162 (8.09)
No	1841 (91.91)
Previous history of TB	
Yes	98 (4.89)
No	1905 (95.11)
Presence of extra-pulmonary TB	
Yes	56 (2.80)
No	1947 (97.20)

Characteristics	N (%)
Malnutrition	
Yes	13 (0.65)
No	1990 (99.35)
BCG vaccination	
Yes	1872 (93.46)
No	131 (93.46)
History of imprisonment	
Yes	15 (0.75)
No	1988 (99.25)
Initial clinical presentation	
Chronic cough (> 2 weeks)	
Yes	1190 (59.41)
No	813 (40.59)
Fever	
Yes	577 (28.81)
No	1426 (71.19)
Weight loss	
Yes	575 (28.71)
No	1428 (71.29)
Hemoptysis	
Yes	249 (12.43)
No	1754 (87.57)
Initial chest radiographs	
Reticulonodular infiltration	
Yes	699 (34.90)
No	1304 (65.10)
Miliary shadow	
Yes	57 (2.85)
No	1946 (97.15)
Lung cavity	
Yes	242 (12.08)
No	1761 (87.92)

Characteristics	N (%)
Pleural effusion	
Yes	190 (9.49)
No	1813 (90.51)
Initial sputum AFB	
Positive	985 (42.70)
Negative	1165 (50.50)
Not investigated	157 (6.81)
Initial sputum Gene X-pert	
MTB not detected	221 (11.03)
MTB detected	50 (2.50)
Error	1 (0.05)
Not investigated	1731 (86.42)
Initial sputum Culture	
No growth	463 (23.12)
M.TB detected	549 (27.41)
Contaminated	3 (0.15)
Not investigated	988 (49.33)
Initial AST level	
Normal	824 (35.72)
Abnormal (> 40 U/L)	215 (9.32)
Not investigated	1268 (54.96)
Initial ALT level	
Normal	858 (37.19)
Abnormal (> 40 U/L)	186 (8.06)
Not investigated	1263 (54.75)
Initial BUN level	
Normal	849 (36.80)
Abnormal (> 20 mg/dL)	121 (5.24)
Not investigated	1337 (57.95)
Initial creatinine level	
Normal	909 (39.40)
Abnormal (> 1.2 mg/dL)	93 (4.03)
Not investigated	1305 (56.57)

Characteristics	N (%)
DOT	
By hospital staffs	964 (48.13)
By healthcare volunteers	190 (9.49)
By relatives	8 (0.40)
By unknown personnel	23 (1.15)
Not DOT	818 (40.84)
Vitamin B6 prescription	
Yes	1091 (54.47)
No	912 (45.53)

Follow-ups and results of treatment

Most patients were followed-up before second month (81.13%) with 4.39% had cutaneous adverse drug reactions. At second month follow-up, 30.65% still had cough, 35.45% had reticulonodular infiltration in chest radiographs and 6.59% still had positive sputum AFB. During fifth month follow-up, 14.63% still had reticulonodular infiltration. Most patients had increasing weight compared with weight at the start of treatment (39.59%). At the end, 33.15% completed treatment, 19.87% cured, 9.34% loss to follow-up, 3.99% died, 3.94% transferred out and 0.45% failed the treatment. Follow-up data was displayed in Table 2.

Table 2 Follow-ups of pulmonary TB treatment and results of treatment

Follow-up characteristics	N (%)
Follow-up before 2nd month	
Follow-up	
Yes	1625 (81.13)
No	378 (18.87)
Cutaneous adverse drug reactions	
Yes	88 (4.39)
No	1915 (95.61)
Follow-up 2nd month	
Cough	
Yes	614 (30.65)
No	1389 (69.34)
Chest radiographs	
Reticulonodular infiltration	710 (35.45)

Follow-up characteristics	N (%)
Pleural effusion	95 (4.74)
Lung cavity	45 (2.25)
Miliary shadows	21 (1.05)
Multiple lung lesions	98 (4.89)
Normal	25 (1.25)
Not investigated	1009 (50.37)
Sputum AFB	
Positive	132 (6.59)
Negative	1195 (59.66)
Not investigated	676 (33.75)
Follow-up 5th month	
Chest radiographs	
Reticulonodular infiltration	293 (14.63)
Pleural effusion	48 (2.40)
Lung cavity	24 (1.20)
Miliary shadows	6 (0.30)
Multiple lung lesions	40 (2.00)
Normal	38 (1.90)
Not investigated	1554 (77.58)
Sputum AFB	
Positive	29 (1.45)
Negative	658 (32.85)
Not investigated	1316 (65.70)
Overall weight difference from start	
Increased	793 (39.59)
No change	172 (8.59)
Decreased	597 (29.81)
Not weighed	441 (22.02)
Change of regimen from start	
Yes	253 (12.63)
No	1750 (87.37)

Follow-up characteristics	N (%)
Results of treatment	
Successful	
Completed	984 (49.13)
Cured	664 (33.15)
Unsuccessful	
Loss to follow-up	187 (9.34)
Death	80 (3.99)
Transferred	79 (3.94)
Failed	9 (0.45)

Factors associated with unsuccessful pulmonary TB treatment

Associated factors of unsuccessful treatment included being agriculturist (p = 0.005, 95% CI 1.47-8.56), laborer (p = 0.017, 95% CI 1.13-3.66) and household business (p = 0.041, 95% CI 1.04-6.24), symptoms presented with fever (p = 0.024, 95% CI 1.12-1.81) and weight loss (p = 0.044, 95% CI 1.05-1.91) at the beginning of treatment, sputum culture was not collected for investigation at the start of treatment (p = 0.001, 95% CI 1.28-2.46), having abnormal liver function test (LFT) at the start (p = 0.002, 95% CI 1.25-2.72) and having abnormal blood urea nitrogen (BUN) at the start (p = 0.034, 95% CI 1.11-3.40). During second month follow-up, positive sputum AFB (p = 0.001, 95% CI 1.38-3.79) and sputum AFB not collected for investigated (p < 0.0001, 95% CI 1.32-2.35) were associated with unsuccessful treatment as well as uninvestigated chest radiograph (p = 0.046, 95% CI 1.03-59.31). Patients whose weight was not monitored through the treatment were associated with unsuccessful treatment as well (p < 0.0001, 95% CI 1.56-3.10). On the contrary, protective factors of unsuccessful treatment included having cutaneous adverse reactions during follow-up (p = 0.019, 95% CI 0.15-1.21). The whole results were displayed in Table 3.

Table 3 Univariate and multivariate analysis of associated factors of unsuccessful TB treatment outcomes

Factors	Treatment outcomes		Univariate analysis			Multivariate analysis		
	Successful N (%)	Unsuccessful N (%)	Crude OR	95%CI	*p*-value	Adjusted OR	95%CI	*p*-value
Hospital setting								
Bangkok Metropolis	1204 (80.81)	286 (19.19)	1	-	-	1	-	-
Other provinces	444 (86.55)	69 (13.558)	0.65	0.49-0.87	0.003	0.65	0.40-1.06	0.082
Gender								
Male	1143 (81.29)	263 (18.71)	1	-	-	1	-	-
Female	505 (84.59)	92 (15.41)	0.792	0.61-1.03	0.078	0.87	0.64-1.17	0.353
Occupation								
Civilian officer	127 (88.19)	17 (11.81)	1	-	-	1	-	-
Agriculturist	38 (74.51)	13 (25.49)	2.56	1.14-5.73	0.023	3.54	1.47-8.56	0.005*
Laborer	487 (80.90)	115 (19.10)	1.76	1.02-3.04	0.041	2.04	1.13-3.66	0.017*
Military officer	426 (80.99)	100 (19.01)	1.75	1.01-3.04	0.046	1.81	1.00-3.28	0.051
Traders/merchant	74 (84.09)	14 (15.91)	1.41	0.66-3.03	0.374	1.67	0.73-3.80	0.222
Household business	46 (80.70)	11 (19.30)	1.79	0.78-4.10	0.171	2.55	1.04-6.24	0.041*
Healthcare provider	12 (85.71)	2 (14.29)	1.25	0.26-6.05	0.786	2.23	0.42-11.97	0.348
Students	58 (81.69)	13 (18.31)	1.67	0.76-3.68	0.199	1.87	0.80-4.38	0.150
Unemployed	380 (84.44)	70 (15.56)	1.38	0.78-2.43	0.269	1.51	0.82-2.78	0.189
Age								
< 20	26 (81.25)	6 (18.75)	1	-	-			
20-29	344 (78.36)	95 (21.64)	1.12	0.48-2.99	0.701			
30-39	215 (83.98)	41 (16.02)	0.83	0.32-2.13	0.693			

Factors	Treatment outcomes		Univariate analysis			Multivariate analysis		
	Successful N (%)	Unsuccessful N (%)	Crude OR	95%CI	*p*-value	Adjusted OR	95%CI	*p*-value
40-49	232 (79.73)	59 (20.27)	1.10	0.43-2.80	0.838			
50-59	348 (86.57)	54 (13.43)	0.67	0.27-1.71	0.404			
60-69	204 (86.08)	33 (13.92)	0.70	0.27-1.83	0.469			
70-79	175 (82.55)	37 (17.45)	0.92	0.35-2.38	0.858			
≥ 80	104 (77.61)	30 (22.39)	1.25	0.47-3.32	0.654			
Medical history and co-morbid illnesses								
HIV infection								
No	605 (84.73)	109 (15.27)	1	-	-	1	-	-
Yes	1043 (80.92)	246 (19.08)	1.31	1.02-1.68	0.032	1.06	0.73-1.54	0.763
Diabetes mellitus								
No	902 (82.22)	195 (17.78)	1	-	-			
Yes	746 (82.34)	160 (17.66)	0.992	0.79-1.25	0.946			
Chronic lung diseases								
No	1620 (82.28)	349 (17.72)	1	-	-			
Yes	28 (82.35)	6 (17.65)	0.995	0.41-2.42	0.991			
Liver diseases								
No	1640 (82.23)	352 (17.67)	1	-	-			
Yes	8 (72.73)	3 (27.27)	1.75	0.46-6.62	0.412			
Chronic kidney disease								
No	1629 (82.19)	353 (17.81)	1	-	-			
Yes	19 (90.48)	2 (9.52)	0.49	0.11-2.10	0.333			

Factors	Treatment outcomes		Univariate analysis			Multivariate analysis		
	Successful N (%)	Unsuccessful N (%)	Crude OR	95%CI	*p*-value	Adjusted OR	95%CI	*p*-value
Malnutrition								
No	1636 (82.21)	354 (17.79)	1	-	-			
Yes	12 (92.31)	1 (7.69)	0.39	0.05-2.97	0.360			
History of TB contact	1517 (82.40)	324 (17.60)	1	-	-			
No	131 (80.86)	31 (19.14)	1.11	0.74-1.67	0.624			
Yes								
Previous history of TB								
No	1564 (82.10)	341 (17.90)	1	-	-			
Yes	84 (85.71)	14 (14.29)	0.76	0.43-1.36	0.362			
Presence of extra-pulmonary TB	1601 (82.23)	346 (17.77)	1	-	-			
No	47 (83.93)	9 (16.07)	0.89	0.43-1.83	0.743			
Yes								
History of imprisonment								
No	1647 (82.19)	357 (17.81)	1	-	-			
Yes	12 (80.00)	3 (20.00)	1.15	0.32-4.11	0.826			
BCG vaccination								
Yes	1557 (82.47)	331 (17.53)	1	-	-	1	-	-
No	102 (77.86)	29 (22.14)	1.34	0.87-2.05	0.184	1.18	0.72-1.94	0.500
Initial clinical presentation								
Chronic cough (> 2 weeks)								
No	660 (80.49)	160 (19.51)	1	-	-	1	-	-
Yes	999 (83.32)	200 (16.68)	0.83	0.66-1.04	0.103	0.78	0.58-1.04	0.092

Factors	Treatment outcomes		Univariate analysis			Multivariate analysis		
	Successful N (%)	Unsuccessful N (%)	Crude OR	95%CI	*p*-value	Adjusted OR	95%CI	*p*-value
Hemoptysis								
No	1453 (82.84)	301 (17.16)	1	-	-	1	-	-
Yes	195 (78.31)	54 (21.69)	1.32	0.96-1.83	0.089	1.35	0.93-1.96	0.113
Fever								
No	1187 (83.24)	239 (16.76)	1	-	-	1	-	-
Yes	461 (79.90)	116 (20.10)	1.27	1.00-1.62	0.054	1.35	1.12-1.81	0.024*
Weight loss								
No	1188 (83.19)	240 (16.81)	1	-	-	1	-	-
Yes	460 (80.00)	115 (20.00)	1.26	0.99-1.61	0.061	1.41	1.05-1.91	0.044*
Initial chest radiographs								
Normal	25 (80.65)	6 (19.35)	1	-	-			
Reticulonodular infiltration	927 (82.25)	200 (17.75)	0.90	0.36-2.22	0.817			
Miliary shadows	42 (89.36)	5 (10.64)	0.50	0.14-1.80	0.285			
Lung cavity	86 (85.15)	15 (14.85)	0.73	0.26-2.07	0.550			
Pleural effusion	122 (80.26)	30 (19.74)	1.03	0.39-2.72	0.961			
Multiple lung lesions	155 (85.64)	26 (14.36)	0.70	0.26-1.87	0.475			
Not investigated	291 (79.95)	73 (20.05)	1.05	0.41-2.64	0.925			
Initial sputum AFB								
Negative	831 (82.11)	181 (17.89)	1	-	-			
Positive	718 (82.43)	153 (17.57)	0.98	0.77-1.24	0.856			
Not investigated	99 (82.50)	21 (17.50)	0.97	0.59-1.60	0.917			

Factors	Treatment outcomes		Univariate analysis			Multivariate analysis		
	Successful N (%)	Unsuccessful N (%)	Crude OR	95%CI	*p*-value	Adjusted OR	95%CI	*p*-value
Initial sputum gene X-pert								
M.TB not detected	181 (81.90)	40 (18.10)	1	-	-			
M.TB detected	37 (74.00)	13 (26.00)	1.59	0.78-3.26	0.206			
Error	1 (100.00)	0 (0.00)	-	-	-			
Not investigated	1429 (82.55)	302 (17.45)	0.96	0.66-1.38	0.810			
Initial sputum culture								
No growth	399 (86.18)	64 (13.82)	1	-	-	1	-	-
M.TB detected	481 (87.61)	68 (12.39)	0.88	0.61-1.27	0.499	0.80	0.54-1.20	0.280
Contaminated	3 (100.00)	0 (0.00)	-	-	-	-	-	-
Not investigated	765 (77.43)	223 (22.57)	1.82	1.34-2.46	< 0.0001	1.78	1.28-2.46	0.001*
Initial LFT								
Normal	581 (82.88)	120 (17.12)	1	-	-	1	-	-
Abnormal (AST or ALT > 40 U/L)	169 (72.53)	64 (27.47)	1.83	1.29-2.60	0.001	1.84	1.25-2.72	0.002*
Not investigated	171 (16.00)	0.92	0.71-1.19	0.533	0.75	0.51-1.11	0.148	
Initial BUN level								
Normal	621 (82.14)	135 (17.86)	1	-	-	1	-	-
Abnormal (> 20 mg/dL)	75 (71.43)	30 (28.57)	1.84	1.16-2.92	0.010	1.90	1.04-2.96	0.034*
Not investigated	952 (83.36)	190 (16.64)	0.92	0.72-1.17	0.490	1.29	0.82-1.75	0.350
Initial creatinine level								
Normal	655 (81.77)	146 (18.23)	1	-	-			
Abnormal (> 1.2 mg/dL)	67 (77.91)	19 (22.09)	1.27	0.74-2.18	0.382			
Not investigated	926 (82.97)	190 (17.03)	0.92	0.73-1.17	0.495			

Factors	Treatment outcomes		Univariate analysis			Multivariate analysis		
	Successful N (%)	Unsuccessful N (%)	Crude OR	95%CI	*p*-value	Adjusted OR	95%CI	*p*-value
DOT (directly observed therapy)								
Yes	995 (83.97)	190 (16.03)	1	-	-	1	-	-
No	653 (79.83)	165 (20.17)	1.32	1.05-1.67	0.018	1.15	0.89-1.49	0.299
Vitamin B6 prescription								
Yes	912 (83.59)	179 (16.41)	1	-	-	1	-	-
No	736 (80.70)	176 (19.30)	1.22	0.97-1.53	0.092	1.29	0.91-1.68	0.055
Cutaneous adverse drug reactions after treatment								
No	1566 (81.78)	349 (18.22)	1	-	-	1	-	-
Yes	82 (93.18)	6 (6.82)	0.33	0.14-0.76	0.009	0.35	0.15-0.85	0.019*
Cough at the end of 2nd month								
No	1113 (80.13)	276 (19.87)	1	-	-	1	-	-
Yes	535 (87.13)	79 (12.87)	0.595	0.45-0.78	< 0.0001	0.88	0.64-1.21	0.445
Chest radiographs at the end of 2nd month								
Normal	24 (96.00)	1 (4.00)	1	-	-	1	-	-
Reticulonodular infiltration	619 (87.18)	91 (12.82)	3.53	0.47-26.40	0.219	3.66	0.48-28.19	0.200
Miliary shadows	18 (85.71)	3 (14.29)	4.00	0.38-41.70	0.246	5.54	0.51-60.58	0.161
Lung cavity	40 (88.89)	5 (11.11)	3.00	0.33-27.23	0.329	3.52	0.37-33.53	0.273
Pleural effusion	85 (89.47)	10 (10.53)	2.82	0.34-23.17	0.334	2.14	0.25-18.40	0.488
Multiple lung lesions	80 (81.63)	18 (18.37)	5.40	0.69-42.57	0.109	6.38	0.78-52.47	0.085
Not investigated	782 (77.50)	227 (22.50)	6.97	0.94-51.78	0.058	4.31	0.56-32.92	0.159

Factors	Treatment outcomes		Univariate analysis			Multivariate analysis		
	Successful N (%)	Unsuccessful N (%)	Crude OR	95%CI	*p*-value	Adjusted OR	95%CI	*p*-value
Sputum AFB at the end of 2nd month								
Negative	1047 (87.62)	148 (12.38)	1	-	-	1	-	-
Positive	105 (79.55)	27 (20.45)	1.82	1.15-2.87	0.010	2.29	1.38-3.79	0.001*
Not investigated	496 (73.37)	180 (26.63)	2.57	2.02-3.27	< 0.0001	1.76	1.32-2.35	< 0.0001*
Chest radiographs at the end of 5th month								
Normal	37 (97.37)	1 (2.63)	1	-	-	1	-	-
Reticulonodular infiltration	274 (93.52)	19 (6.48)	2.57	0.33-19.73	0.365	2.23	0.28-17.86	0.450
Miliary shadows	6 (100.00)	0 (0.00)	-	-	-	-	-	-
Lung cavity	23 (95.83)	1 (4.17)	1.61	0.10-27.00	0.741	1.55	0.09-27.72	0.765
Pleural effusion	45 (93.75)	3 (6.25)	2.47	0.25-24.72	0.443	2.00	0.19-21.33	0.566
Multiple lung lesions	38 (95.00)	2 (5.00)	1.95	0.17-22.40	0.593	1.18	0.10-14.56	0.900
Not investigated	1225 (78.83)	329 (21.17)	9.94	1.36-72.70	0.024	7.83	1.03-59.31	0.046*
Overall weight difference from start								
Increased	694 (87.52)	99 (12.48)	1	-	-	1	-	-
No change	150 (87.21)	22 (12.79)	1.03	0.63-1.69	0.912	1.07	0.62-1.86	0.801
Decreased	509 (85.26)	88 (14.74)	1.21	0.89-1.65	0.223	1.30	0.93-1.81	0.119
Not weighed	295 (66.89)	146 (33.11)	3.47	2.60-4.63	< 0.0001	2.20	1.56-3.10	< 0.0001*
Change of regimen from start								
No	1447 (82.69)	303 (17.31)	1	-	-			
Yes	201 (79.45)	52 (20.55)	1.24	0.89-1.72	0.208			

* Significant at 95% CI

Discussion

This study addressed prevalence and associated factors of unfavorable TB treatment outcomes. At 17.72%, the prevalence of unsuccessful pulmonary TB treatment in military settings was comparable to previous studies conducted in other secondary or tertiary care units.[6-8] This number might reflect the universal rate of TB treatment outcomes among all settings, disregarding supervision by Ministry of Public Health. However, most of the unsuccessful treatment cases were attributed to loss to follow-up which contrasted to previous studies in Thailand of which death was the most common cause of unsuccessful treatment.[6-9]

In this study, being agriculturists, laborers and business owner were associated with unsuccessful treatment. Agriculturists and laborers likely to be linked to low socio-economic status, low educational level and rural living.[10,11] Household business, in this context, usually referred to small household business which usually associated with low- and middle-income socioeconomic levels. These factors were addressed to be associated with unsuccessful TB treatment in previous studies.[9,12,13] To cope with unsuccessful treatment, management in community level and to be more exact, individual level, is essential.

Initial clinical presentation also correlated with clinical outcomes. In this study, fever and weight loss were associated with unsuccessful treatment. Fever was reported to be one of the most common clinical features of pulmonary TB, along with chronic cough and weight loss.[14,15] In a previous study, people who were underweight were significantly presented with fever and weight loss.[16] This might be implied that people who were underweight (BMI<18.5) at the diagnosis, which might be initially presented with fever and weight loss, usually had higher risk of treatment failure and death.[16,17] As a result, fever and weight loss were associated with unsuccessful treatment outcome. However, this study did not indicate that underlying malnourishment was associated with unfavorable treatment outcomes as well as decreased weight in the overall treatment course.

This study found that people who was not weighed or monitored throughout the treatment were significantly associated with treatment failure. Patients who were not weighed at the beginning of treatment might be missed for low BMI status, which associated with unsuccessful treatment. Also, patients whose weight were not monitored in the subsequent follow-ups might include those who loss to follow-up, died or transferred out. A study in Vietnam found that weight loss during first two months of treatment might associated with poor treatment response due to drug resistance, malnourishment and HIV co-infection.[18] Weight reduction during TB treatment was also linked to drug-induced hepatotoxicity[18,19] of which the patient usually had lower favorable treatment outcome.[20] As a result, in patients who remain at the treatment facilities, weight monitoring should be done in follow-ups.

People whose sputum culture was not investigated at the beginning of treatment were more likely to have unfavorable treatment outcome. Diagnostic methods of MDR-TB included drug susceptibility test and culture.[21] Sputum culture also plays a major role in monitoring response to treatment in MDR-TB patients.[21,22] In some settings where laboratory resources are limited for sputum culture or gene X-pert, logistic processing is required for sputum transportation to more advanced laboratory of which it usually add additional duration to obtain the result.[21,23] As a result, some facilities decide not to send the sputum for culture or gene X-pert at all. This might lead to the treatment regimen does not match TB strains.

The trend of unsuccessful treatment among patients whose sputum AFB was positive or not performed at the end of intensive phase (2nd month) was observed. According to Thai National Guideline, in new pulmonary TB patients, sputum AFB should be performed at the end of intensive phase and at the fifth month.[5] Sputum AFB follow ups can determine treatment outcomes, especially at the end of intensive phase.[24,25] Previous studies indicated that positive sputum AFB at the end of intensive phase might be due to patients' poor compliance and drug-resistance TB[8,24,26] , while positive sputum AFB at the 5th month was an indicator of treatment failure.[4,5] In a previous study, uninvestigated sputum AFB can be caused by poor treatment compliance, receiving out-of-track management or missing cases follow-up.[8]

This study found that the proportion of unsuccessful treatment among patients whose sputum AFB positive or not investigated at the end of intensive phase were towering. Thai National Guideline had imposed the regulations for patients whose sputum AFB were positive at the end of intensive to have sputum gene X-pert and culture investigated.[5] However, these indicators took several days to weeks to accomplish the result.[21,23] Other rapid diagnostic tests for treatment resistance should be developed to alleviate this problem.

Uninvestigated chest radiograph at the fifth month was associated with unsuccessful treatment. Chest radiograph was usually taken for all patients at the end of intensive phase and at the end of treatment, according to Thai National Guideline.[21] There was no recommendation on chest radiography at the fifth month of treatment. However, unimproved chest radiograph at the fifth month can predict treatment failure.[25] In a previous study, no radiographic improvement on CXR at the fifth month.[25] Thus, monitoring this parameter at fifth month might enhance treatment success.

This study found that abnormal LFT (either elevated AST or ALT or both) was significantly related to unsuccessful TB treatment. Hepatitis was noted to be associated with unsuccessful TB treatment due to various factors such as change in regimen, treatment interruption, liver failure and death.[20,27,28] Thus, it is suggested to examine liver function of every TB patient before initiate treatment.

The unsuccessful treatment was also found in patients with elevated BUN. It cannot be concluded that elevated BUN solely is defined as chronic kidney disease. Elevated BUN can be caused by several factors, not only chronic kidney disease, which is associated with unsuccessful TB treatment.[29,30] However, elevated BUN in the absence of renal disease or high creatinine was previously reported to be associated with death in miliary TB patients due to dehydration tendencies or hypercatabolism.[31]

Cutaneous adverse drug reaction was reported to be a protective factor of unsuccessful treatment. There are limited data on suggestion on patients with severe cutaneous adverse drug reaction are required to interrupt the treatment with most interruptions are based upon the knowledge that treatment should be interrupted if any adverse drug reaction occurred.[32] There is still controversy regarding cutaneous adverse drug reaction and treatment outcomes as well as patients' adherence to therapy following adverse drug reaction.[32] Although treatment interruption might be associated with treatment failure and death[33], it was usually due to patients' own incompliance to treatment than interruption due to adverse drug reaction by physicians.[32] However, non-severe cutaneous adverse drug reaction might not require treatment interruption, but usually require only anti-histamine medication.[34]

This study hypothesized that in this setting, most patients were not affected by serious cutaneous adverse drug reaction and having good adherence to the treatment. Also, having cutaneous drug reaction might provoke concerns of both patients to be adhere to treatment and healthcare providers to be more specifically monitor the patient.

This study discovered several factors associated with unsuccessful pulmonary TB treatment outcomes. Many of these factors were system-related such as patient tracking, patient follow-up visits and monitoring, proper facilities and recording of patient treatment history. Others included individual factors such as individual's compliance and adherence to the treatment system. To enhance treatment success, establishing community-based treatment system can be a solution. Community-based treatment system can deliver fast and efficient TB diagnosis, treatment, monitoring and follow ups better than hospital-based system which patients require several resources to access the treatment and healthcare workers have multiple workloads to deal with than specifically monitoring and exploring each patient's problems. In addition, attaching TB treatment system in military hospitals to the national TB information program established by Ministry of Public Health would make each patient's data regarding TB treatment history be systematically collected, standard and easier to monitor.

There were some limitations in this study. First, some patients' treatment data from some of the studied hospitals, especially the older data, were registered in paper form and was not scanned to the computer system. Thus, some data in the older years were loss. Second, some hospitals in this study did not register to national TB information program. This resulted in difficulty to retrieve patients' data, laboratory results and date of treatment. Third, the database did not include CD4 level of HIV patients.

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