E-ISSN 2228-8082

Volume 73, Number 5, May 2021

SMJ

Siriraj Medical Journal

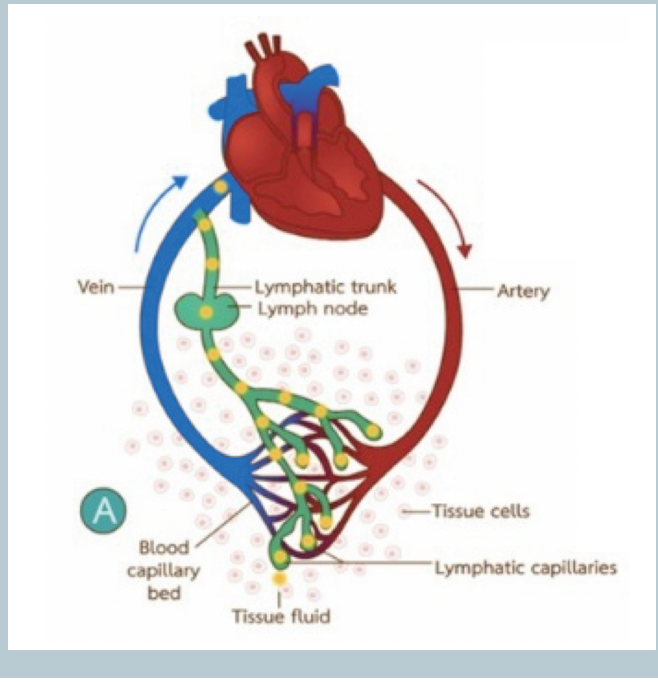
MONTHLY

By Atthawit Mongkronwong, et al.

REVIEW ARTICLE

ORIGINAL ARTICLE

LETTER TO EDITOR



Indexed by

Scopus®

Thai Association for Gastrointestinal Endoscopy

International Association of Surgeons Gastroenterologists & Oncologists Thailand Chapter

https://he02.tci-thaijo.org/index.php/sirirajmedj/index

E-mail: sijournal92@gmail.com

Siriraj Medical Journal SMJ

Volume 73, Number 5, May 2021

REVIEW ARTICLE

ORIGINAL ARTICLE

LETTER TO EDITOR

SIRIRAJ MEDICAL JOURNAL

www.smj.si.mahidol.ac.th

SIRIRAJ MEDICAL JOURNAL is published bimonthly, 6 issues a year (Jan-Feb, Mar-Apr, May-Jun, Jul-Aug, Sep-Oct and Nov-Dec) and distributed by the end of the last month of that issue.
SIRIRAJ MEDICAL JOURNAL is listed as a journal following the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (URM) by the International Committee of Medical Journal Editors (ICMJE) since 9 July 2010 [http://www.icmje.org/journals.html].

Endoscopic Sleeve Gastroplasty in the Armamentarium of Bariatric Treatment in Thailand: A TAGE-TSMBS Joint Addendum Statement to TSMBS Consensus Guideline

Pradermchai Kongkam, M.D.*, Parit Mekaroonkamol, M.D.*, Nonthalee Pausawasdi, M.D.*, Thawee Ratanachu-ek, M.D.*, Rungsun Rerknimitr MD, FRCP*, Voraboot Taweerutchana, M.D., Ajjana Techagumpuch, M.D.**, Suthep Udomsawaengsup, MD, FRCS**, Panot Yimcharoen, M.D.**, Sutdhachit Linananda, M.D.****

**Thai Association of Gastrointestinal Endoscopy, **Thai Bariatric surgery Society of Metabolic and Bariatric Surgery, Thailand*

ABSTRACT

The Thai bariatric surgery Society of Metabolic and Bariatric Surgery (TSMBS) has recently published a consensus guideline for the treatment of obesity, which has become an emerging health crisis in Thailand. As endoscopic sleeve gastroplasty (ESG) lately became available in the country, the TSMBS and the Thai Association for Gastrointestinal Endoscopy (TAGE) then agreed to propose this addendum statement that aims to standardize the patient selection protocol, physician credentialing, and procedural data monitoring in order to safely and effectively incorporate ESG into the armamentarium of bariatric treatment of Thailand.

Keywords: Obesity; endoscopy; bariatric surgery; sleeve gastroplasty; recommendations (Siriraj Med J 2021; 73: 289-292)

INTRODUCTION

Over the past decade, the prevalence of obesity has been rising at an alarming rate and has now become an emerging health crisis in Thailand.[1-3] Obesity is recognized as an epidemic of a chronic, relapsing, and debilitating disease that poses significant health consequences in addition to an enormous economic burden to our healthcare system.[1,4,5] Recently, the Thai bariatric surgery Society of Metabolic and Bariatric Surgery (TSMBS) has published a consensus guideline for the treatment of obese patients.[6] The guideline has standardized bariatric surgery protocol in Thailand, stating that the indication for bariatric surgery includes patients with BMI between 32.5-37.5 kg/m^2 with co-morbidities and those whose BMI of more than 37.5 kg/m^2 without co-morbidities.[6]

The efficacy of laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy for weight reduction is well-established, and they currently remain the standard of care in the Thai population.[6,7] However, the current guideline did not include patients whose BMI are less than 37.5 kg/m^2 without co-morbidities as candidates for primary surgical intervention,[4,6] underscoring a therapeutic gap for which an alternate treatment modality can be offered.[3]

Endoscopic sleeve gastroplasty (ESG) is one of the bariatric endoscopy techniques that offers a less invasive approach that is safe, effective, repeatable, and reversible.[8-11] In addition, ESG has been shown to improve

Corresponding author: Sutdhachit Linananda
E-mail: sutdhacit.li@thaindc.org
Received 29 January 2021 Revised 2 April 2021 Accepted 2 April 2021
ORCID ID: http://orcid.org/0000-0003-4267-0996
http://dx.doi.org/10.33192/Smj.2021.38

obesity-related comorbidities, including diabetes and hypertension.[12-14] The procedure entails an incisionless longitudinal full-thickness plication along the greater curvature of stomach using an overstitch device. ESG has been adopted and widely accepted as an effective endoscopic bariatric therapy according to the American Society for Gastrointestinal Endoscopy (ASGE)/ the American Society for Metabolic and Bariatric Surgery (ASMBS) thresholds of >25% excessive weight loss and <5% adverse events.[9-11,15,16]

As the endoscopic suturing device has recently become available in Thailand, ESG is expected to soon emerge as one of the bariatric therapies being offered to patients with morbid obesity. However, the current TSMBS guideline was developed when ESG was not an available option and focused only on bariatric surgical therapies, pre-operative and post-operative management, which were in a different context compared to the endoscopic counterpart in its early phase. For example, there is no specialized formal training program on ESG in the country yet, together with limited hands-on proctorship from international colleagues due to the COVID-19 pandemic, all expert endoscopists were trained virtually. In addition, although the role of ESG is established in the international communities but there is no currently available local data on ESG to formulate a national high-level evidence guidelines yet. Thai Association of Gastrointestinal Endoscopy (TAGE) and TSMBS recognized the importance of standardizing the patient selection protocol, training process, competency evaluation, and safety data monitoring to incorporate ESG into the armamentarium of bariatric treatment of Thailand. This joint addendum statement therefore aims to address the aforementioned issues and provide a pathway to safely and effectively bring ESG into clinical practice and improve care for our patients in an early era of ESG procedure in Thailand.

MATERIALS AND METHODS

This addendum statement was conceptualized and created using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) and the Strength of Recommendation Taxonomy (SORT) framework[17,18] as described in Table 1.

Nominated experts from both societies convened in January 2021 in Bangkok, Thailand to discuss 4 aspects of bariatric schematic management; 1) Pre-procedural evaluation, 2) Procedural indications and patient selection, 3) Physician training and credentialing, 4) Post-procedure monitoring based on a literature review utilizing Medline, Cochrane library, and Embase databases for existing evidence. The recommendations were approved when the consensus of all experts was reached for each aspect. The summary of

TABLE 1. Classification of the quality of evidence and strength of recommendations.

Quality of evidence	
A	(strong) Strongly confident in the effect of estimate.
B	(moderate) Moderately confident in the effect of estimate.
C	(weak) Confidence in the effect of estimate is limited.
D	(very weak) Almost no confidence in the effect of estimate.
Strength of the recommendation	
Level 1	Recommendation based on consistent and good-quality patient-oriented evidence.
Level 2	Recommendation based on inconsistent or limited-quality patient-oriented evidence.
Level 3	Recommendation based on consensus, usual practice, opinion, disease-oriented evidence, or case series for studies of diagnosis, treatment, prevention, or screening studies

TAGE and TSMBS Obesity Task Force recommends the following:

Pre-procedural evaluation

1. The patient should be evaluated in a multi-disciplinary team approach focusing on behavioral eating habit, endocrinopathies, and other obesity-related co-morbidities. (Quality of evidence A; Recommendation level 1)
2. Patients with any uncontrolled maladaptive eating disorder should be excluded. (Quality of evidence C; Recommendation level 3)
3. For the patient to be considered for any bariatric therapies, he/she should have undergone a maximum effort to his/her capacity with adequate assistance from the multi-disciplinary team for weight reduction. (Quality of evidence D; Recommendation level 3)
4. Although weight loss is always encouraged, the rapid 5-10% weight reduction typically required for bariatric surgery is not necessary for ESG procedure. (Quality of evidence C; Recommendation level 2)
5. All bariatric treatment options should be thoroughly explained to the patients. The physician's credential should also be declared that ESG will be performed by a highly experienced physician in advanced endoscopy and/or minimally invasive surgery after a formal training for ESG. (Quality of evidence D; Recommendation level 3)

Procedural indication and Patient selection

6. The committee agrees that a lower BMI cut-off than the international standard for Caucasians should be used due to the higher risk of obesity-related morbidities at a lower BMI value.[19-21] (Quality of evidence B; Recommendation level 2)
7. For patients with a BMI of more than 37.5 kg/m^2 regardless of co-morbidities, surgery should be the primary treatment. (Quality of evidence C; Recommendation level 3)
8. For patients whose BMI is between 32.5-37.5 kg/m^2 with co-morbidities, surgery should be the primary treatment with ESG as an alternate option. (Quality of evidence C; Recommendation level 3)
9. ESG should be considered a primary treatment for obesity in patients whose BMI is between 32.5-37.5 kg/m^2 without co-morbidities. (Quality of evidence C; Recommendation level 3)
10. The role of ESG in patients whose BMI is between 27.5-32.5 kg/m^2 is yet to be clearly defined and should be reevaluated once the local data becomes more available. (Quality of evidence D; Recommendation level 3)

Physician training and credentialing

11. ESG should be performed in tertiary care hospital where a collaborative multi-disciplinary team comprising of endocrinologists, nutritionists, bariatric surgeons, and gastroenterologists is available. (Quality of evidence C; Recommendation level 3)
12. ESG should initially be performed by expert endoscopists, defined as physicians who have been formally trained in advanced endoscopy or minimally invasive surgery, have performed ESG procedure under a formal proctorship by an expert bariatric endoscopist, and have been approved by the TAGE-TSMBS committee. (Quality of evidence D; Recommendation level 3)
13. Safety and efficacy data should commence periodically post-procedure for credentialing purposes. (Quality of evidence D; Recommendation level 3)

Post-procedure monitoring

14. All procedures should be registered in a National Registry on Safety and Efficacy of Bariatric Endoscopy. (Quality of evidence D; Recommendation level 3)
15. Patients who have undergone ESG should have a regular follow-up with the multi-disciplinary team to assess nutrition status, continued lifestyle modification, and weight reduction efficacy. (Quality of evidence B; Recommendation level 1)

CONCLUSION

The obesity crisis in Thailand continues to rise and threaten the well-being of our population. TAGE and TSMBS recognize an urgent need to optimize and standardize available therapeutic modalities for patients with this chronic, relapsing, debilitating disease. This addendum statement aims to clarify the indication for ESG to be primarily for patients with BMI between 32.5-37.5 kg/m^2 without co-morbidities and emphasizes the importance of post-procedural care with a multi-disciplinary team approach. It is intended to serve as a general recommendation to safely and effectively incorporate ESG into the bariatric treatment armamentarium. As the field is still evolving, TAGE and TSMBS are committed to periodic updates on these recommendations when more local data becomes available.

Conflict of interest: All authors have no conflict of interest to declare.

Disclosures: All authors have no conflicts of interest or financial ties to disclose.

REFERENCES

1. Aekplakorn W, Inthawong R, Kessomboon P, Sangthong R, Chariyalertsak S, Putwatana P, et al. Prevalence and trends of obesity and association with socioeconomic status in Thai adults: National Health Examination Surveys, 1991-2009. J Obes 2014;2014:410259.
2. Jitnarin N, Kosulwat V, Rojroongwasinkul N, Boonpraderm A, Haddock C, Poston W. Prevalence of overweight and obesity in Thai population: results of the National Thai Food Consumption Survey. Eat Weight Disord 2011;16(4):e242-9.
3. All countries significantly off track to meet 2025 WHO targets on Obesity, 2020.
4. Pitayatienanan P, Butchon R, Yothasamut J, Aekplakorn W, Teerawattananon Y, Suksomboon N, et al. Economic costs of obesity in Thailand: a retrospective cost-of-illness study. BMC Health Serv Res 2014;14:146.
5. Viratanapanu I, Romyen C, Chaivanijchaya K, Sornphiphatphong S, Kattipatanapong W, Techagumpuch A, et al. Cost-effectiveness evaluation of bariatric surgery for morbidly obese with diabetes patients in Thailand. J Obes 2019;2019:5383478.
6. Techagumpuch A, Pantanakul S, Chansaenroj P, Boonyagard N, Wittayapairoch J, Poonthananiwatkul T, et al. Thai Society for Metabolic and Bariatric Surgery Consensus Guideline on Bariatric Surgery for the Treatment of Obese Patient in Thailand. J Med Assoc Thai 2020;103:300-7.
7. Benaiges Foix D, Más-Lorenzo A, Goday Arno A, Ramón Moros JM, Chillarón Jordan JJ, Pedro-Botet JC, et al. Laparoscopic sleeve gastrectomy: More than a restrictive bariatric surgery procedure? 2015.
8. Glass J, Chaudhry A, Zeeshan MS, Ramzan Z. New Era: Endoscopic treatment options in obesity–a paradigm shift. World J Gastroenterol 2019;25(32):4567-79.
9. Sartoretto A, Sui Z, Hill C, Dunlap M, Rivera AR, Khashab MA, et al. Endoscopic sleeve gastroplasty (ESG) is a reproducible and effective endoscopic bariatric therapy suitable for widespread clinical adoption: a large, international multicenter study. Obes Surg 2018;28:1812-21.
10. Alqahtani A, Al-Darwish A, Mahmoud AE, Alqahtani YA, Elahmedi M. Short-term outcomes of endoscopic sleeve gastroplasty in 1000 consecutive patients. Gastrointest Endosc 2019;89:1132-8.
11. Barrichello S, de Moura DTH, de Moura EGH, Jirapinyo P, Hoff AC, Fittipaldi-Fernandez RJ, et al. Endoscopic sleeve gastroplasty in the management of overweight and obesity: an international multicenter study. Gastrointest Endosc 2019;90:770-80.
12. de Moura DTH, de Moura EGH, Thompson CC. Endoscopic sleeve gastroplasty: from whence we came and where we are going. World J Gastroenterol Endosc 2019;11:322.
13. Fiorillo C, Quero G, Vix M, Guerriero L, Pizzicannella M, Lapergola A, et al. 6-month gastrointestinal quality of life (QoL) results after endoscopic sleeve gastroplasty and laparoscopic sleeve gastrectomy: a propensity score analysis. Obes Surg 2020;30:1944-51.
14. Lopez-Nava G, Negi A, Bautista-Castaño I, Rubio MA, Asokkumar R. Gut and metabolic hormones changes after endoscopic sleeve gastroplasty (ESG) vs. laparoscopic sleeve gastrectomy (LSG). Obes Surg 2020;30:2642-51.
15. Sullivan S, Kumar N, Edmundowicz SA, Dayyeh BKA, Jonnalagadda SS, Larsen M, et al. ASGE position statement on endoscopic bariatric therapies in clinical practice. Gastrointest Endosc 2015;82:767-72.
16. Ginsberg GG, Chand B, Cote GA, Dallal RM, Edmundowicz SA, Nguyen NT, et al. A pathway to endoscopic bariatric therapies. Gastrointest Endosc 2011;74:943-53.
17. Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, et al. GRADE guidelines: 1. Introduction—GRADE evidence profiles and summary of findings tables. J Clin Epidemiol 2011;64:383-94.
18. Ebell MH, Siwek J, Weiss BD, Woolf SH, Susman J, Ewigman B, et al. Strength of recommendation taxonomy (SORT): a patient-centered approach to grading evidence in the medical literature. J Am Board Fam Pract 2004;17:59-67.
19. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004;363(9403):157-63.
20. Misra A, Chowbey P, Makkar B, Vikram N, Wasir J, Chadha D, et al. Consensus statement for diagnosis of obesity, abdominal obesity and the metabolic syndrome for Asian Indians and recommendations for physical activity, medical and surgical management. J Assoc Physicians India 2009;57:163-70.
21. Pan W-H, Yeh W-T. How to define obesity? Evidence-based multiple action points for public awareness, screening, and treatment: an extension of Asian-Pacific recommendations. Asia Pac J Clin Nutr 2008;17(3):370-4.

What do we know about lymphedema? Review Article

Atthawit Mongkronwong, M.D.*, Chanatip Nilkarn, M.D., Nutthawut Akaranuchat, M.D.*****

**Division of Plastic and Reconstructive Surgery, Department of Surgery, Faculty of Medicine, Prince of Songkla University, Songkhla 90110, Thailand, **Department of Surgery, Faculty of Medicine, Prince of Songkla University, Songkhla 90110, Thailand, ***Division of Plastic Surgery, Department of Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.*

ABSTRACT

Lymphedema can cause by a congenital anomaly, an infectious disease, chronic inflammation, connective tissue disease, and cancer. The most common presenting symptoms are swelling of the affected limb, difficulty wearing clothes, and disturbances to daily life activities. Most of the time, patients have a high chance of developing a soft tissue infection that will jeopardize the quality of their life and socioeconomic status. As to management of the disease, it necessary to make a precise diagnosis and clinico-pathological staging in order to guide the treatment plan and yield optimum results. Currently, surgical management for lymphedema is based on the use of 1) physiological treatment, and 2) reductive or ablative procedures. Conservative treatment (especially for complete decongestive therapy) is still the mainstay for the management of lymphedema.

Keywords: Lymphedema; lymphatic obstruction; lymphatic reconstruction; lymphatic surgery; lymphaticovenous anastomosis; lymph node transfer (Siriraj Med J 2021; 73: 293-304)

INTRODUCTION

The lymphatic system performs three main functions: 1) draining excess fluids from body tissues, 2) fat-absorption, and 3) the production of immune cells. Intercellular fluid is the fluid that leaks from the circulatory system, and 90% of it is reabsorbed by the venous circulatory system. The remaining 10% of this protein-rich fluid accumulates between cells and flows into the lymphatic system (Fig 1); once this fluid enters the lymphatic system, it is termed "lymph". It is transported through the collecting lymphatic vessels and filtered through the lymph nodes, through which approximately 2-3 liters of circulating lymph passes daily. The difference between the accumulated lymphatic and capillary lymphatic pathways is the presence of smooth muscle on the lymph vessel walls that results in compression. Intermittent valve blocking also forces lymphatic flow in one direction. Consequently, a loss of drainage ability (whether caused by a blockage of the lymphatic tract or by the lymphatic system not growing) causes lymph to accumulate between cells, with a subsequent swelling of soft tissue, inflammation, and fibrosis. This adverse condition is called "lymphedema".[1,38,39]

Anatomy and pathophysiology of lymphatic system

The lymphatic system is divided into lymph capillaries, which drain much of the intercellular fluid from the dermal layer. This fluid is subsequently passed to pre-collector and collecting vessels located in the subcutaneous fat layer before moving into the lymph nodes. The lymphatic flow is one direction because there is a valve blocking period (Fig 2).

Corresponding author: Nutthawut Akaranuchat
E-mail: nutthawut.joe@gmail.com
Received 23 November 2020 Revised 18 March 2021 Accepted 2 April 2021
ORCID ID: http://orcid.org/0000-0003-0651-0581
http://dx.doi.org/10.33192/Smj.2021.39

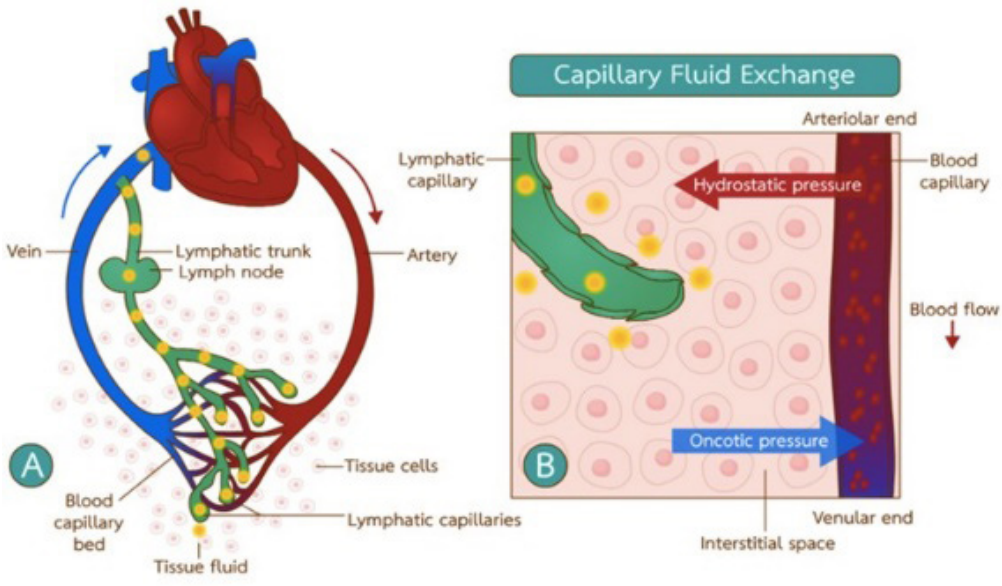


Fig 1. Lymphatic system

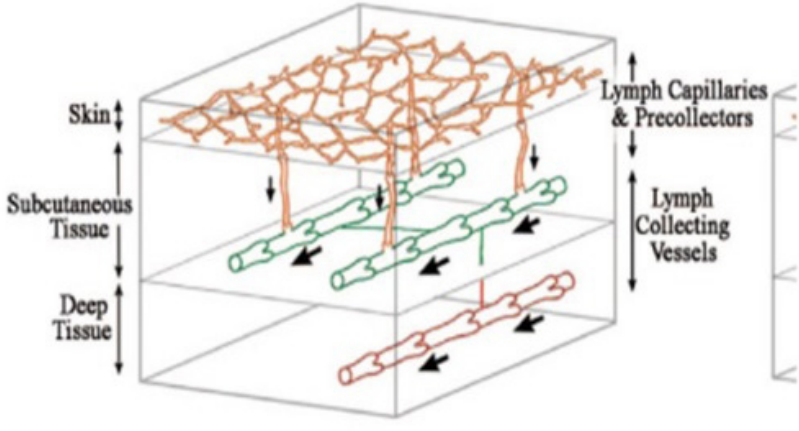


Fig 2. Anatomy of lymphatic system[2]

The etiology of lymphedema

Primary lymphedema

Primary lymphedema is the type of lymphedema that occurs from an inherited abnormality (not a consequence of other conditions). The cause is an abnormal growth of the lymphatic tract (aplasia, dysplasia, or malformation). Primary lymphedema can be subdivided into the following 3 groups, based on their etiology and the onset of the disease.

1. *Congenital lymphedema (Milroy's disease)*
   - The second most common type of primary lymphedema (10%-25%)
   - Occurs within first 2 years of age
   - Usually presents as bilateral extremity edema
   - Autosomal dominant, inherited disorder caused by an inactive mutation of VEGFR-3 tyrosine kinase (VEGFR, vascular endothelial growth factor receptor)
2. *Familial lymphedema praecox (Meige's disease)*
   - The most common form of primary lymphedema (65%–80%)
   - Incidence is about 1:100,000 in the population, with a 4:1 female/male ratio
   - Typically presents during puberty (adolescence), and usually as a unilateral edema (especially of the foot and calf)
   - Transfers through autosomal dominant inheritance, and can associate with multiple anomalies, such as a double row of eyelashes (distichiasis), vertebral and cerebrovascular malformations, and hearing loss
3. *Lymphedema tarda*
   - The rarest form of primary lymphedema (< 10%)
   - Usually occurs after 35 year of age (adulthood)
   - Histological findings of this lymphedema type are usually tortuous and hyperplastic, with an absence of competent valves

Secondary lymphedema

Secondary lymphedema is the type of lymphedema that occurs from adverse events related to other conditions. These include cancer, infectious diseases, inflammation, obesity, and postoperative tumor extirpation with lymph node surgery and/or radiotherapy (Table 1).

Epidemiology and risk factors

Worldwide, the most common cause of lymphedema is filariasis, which results from an infection by the parasitic worm, Wuchereria bancrofti.[4] However, in developed countries, its major cause is cancer and post-cancer treatment (postoperative tumor extirpation with lymph node surgery and/or radiotherapy).[5]

The etiology of lymphedema induced by cancer and post-cancer-treatment can be explained by many mechanisms. For instance, there may be a blockage of the lymphatic ducts from which the cancer is directly pressed, or a cancer may have spread directly into the lymphatic system (lymphangitic carcinomatosis). In addition, surgery for lymph node removal and radiotherapy may have injured the lymphatic partway.

Studies have shown that the incidence of upper extremity lymphedema in breast cancer patients is about 17%. Most patients who undergo axillary lymph node dissection develop symptoms of lymphedema within 3 years of the surgery.[6] Other forms of cancer have been reported to have an overall lymphedema incidence of 15.5%.[7]

Adjuvant radiotherapy after surgery significantly increases the risk of lymphedema. A systematic study in 2001 found that patients who had a mastectomy and received radiotherapy at the axilla subsequently developed lymphedema more frequently than patients who only underwent surgery (41% versus 17%, respectively). Other risk factors for lymphedema include older age and obesity; these populations are at risk of developing a greater level of lymphatic fluid formation and have a higher chance of presenting with symptoms than the general population.[9]

In 2010, Helyer et al. studied the relationship between obesity and the occurrence of lymphedema in breast cancer patients. They found that patients with a body mass index > 30 kg/m^2 had a higher chance of developing lymphedema than those with an index < 25 kg/m^2 (odds ratio, 2.93; 95% CI, 1.03–8.31).[10]

Diagnosis

History and physical examination

Patients with lymphedema often present with unilateral arm or leg edema, and they usually describe a feeling of heaviness around the affected limb. Some patients may present with abnormal skin changes, such as thicker, stiffer, and/or orange-peel-like skin (Paul d'orange).

A physical examination can establish the difference in circumference of the limbs and size will gradually grow equally entire the affected limb. In the early stage of lymphedema, the affected limb can still be depressed

TABLE 1. Causes of lymphedema.[3]

Primary lymphedema
Congenital
Praecox (adolescence)
Tarda (adulthood)
Secondary lymphedema
Cancer
Recurrent cellulitis
Connective tissue disease
Infectious disease (filariasis)
Contact dermatitis
Lymphatic drainage (surgery, radiation therapy, burn, etc.)

when pressure is applied to the skin; however, depression is no longer possible once nonpitting edema forms during the late stage of the disease. The specific physical examination for lymphedema draws upon the "Kaposi-Stemmer sign". This is looked for by trying to pinch the skin on the dorsum of the second toe into a fold (alternatively, the procedure can be performed on the index finger of the hand). If a fold cannot be made, the patient is considered positive for lymphedema (Fig 3). As to the circumference measurement (Fig 4), the patient should be in a standard position; the circumference size is used to calculate the volume of the limb by using the truncated-cone formula (Fig 5). The volume measurement obtained by using this anatomical-landmark circumference method is more accurate than the volume determined by water displacement.[11]

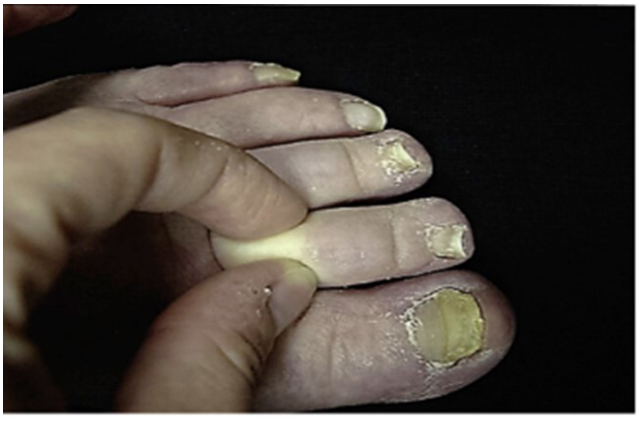


Fig 3. Kaposi–Stemmer sign[1]

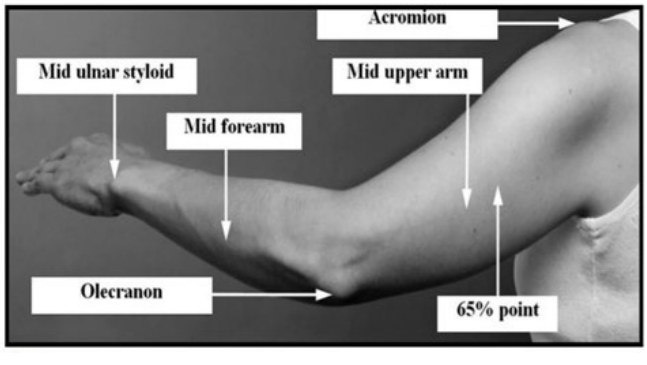


Fig 4. The truncated-cone formula[11]

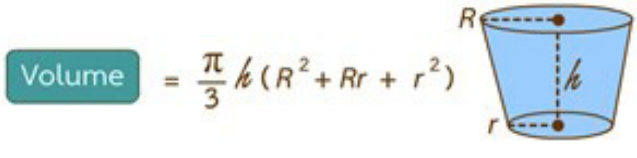


Fig 5. Anatomical landmark[11]

Differential diagnosis

1. *Venous insufficiency*

Lymphedema has some clinical features that mimic chronic venous degeneration. To differentiate them, the limb that has venous insufficiency usually has accompanying symptoms, such as varicose veins, a reddish-brown skin color (from hemosiderin deposition), and possibly ulcerative lesions above the medial malleolus area. The limb swelling that has caused the venous insufficiency often presents as pitting edema, which can be reduced in size by elevation.

2. *Deep vein thromboembolism (DVT)*

Unilateral limb swelling is also the presenting sign and symptom of DVT. However, DVT usually presents during the acute phase with rapid disease progression. Some patients with DVT may present with severe and unexplained pain, or a throbbing and cramping pain (especially at the calf), redness of the skin, and warmth in the affected limb.

3. *Lipedema*

This condition is caused by an accumulation of adipose tissue around the extremities, and it frequently occurs in young women. Normally, lipedema usually presents on both side of the extremities. The affected area is often painful when pressed, which can be used to distinguish it from a lymphatic obstruction (Table 2).

Investigation

1. *Lymphoscintigraphy*

This investigation is used to assess the lymphatic drainage of both the superficial and the deep lymphatic systems, which drain from distal to proximal to the regional lymph node basin. The test involves injecting a radioactive substance (Technetium-99m sulfur colloid) intradermally at the web space of the affected limb; serial radiographic measurements are subsequently used to detect the pattern of lymphatic flow over a period from 15 to 240 minutes.[12,13] This noninvasive investigative technique is relatively safe due to the very low radiation exposure; it also has very good accuracy (sensitivity, 96%; specificity, 100%).[14] The common findings of lymphedema from lymphoscintigraphy are:

- Not found, or a delayed lymphatic drainage
- An absence of, or a decrease in the number of, lymph nodes at the regional lymphatic basin
- A reverse flow of radioactive substance in the dermis (dermal backflow)

TABLE 2. Key clinical differences between lipedema and lymphedema of the lower extremities.[1]

Lipedema	Lymphedema
Almost always female patients	Both males and females
Spares the foot	Foot involved
Usually bilateral	Usually unilateral
Negative Kaposi–Stemmer sign	Positive Kaposi–Stemmer sign
No pitting	No pitting when it becomes chronic
Tender	Usually not tender
Soft	Firm/tense

2. *Computed tomography (CT) scan/magnetic resonance imaging (MRI)*

Both investigative techniques facilitate the evaluation of the fluid accumulation in tissues. The findings of lymphedema from a CT-scan or MRI are:

- Thickening of the skin layer (skin thickening)
- Swelling of the subcutaneous layer (subcutaneous edema)
- A honey-combed appearance

3. *Indocyanine green (ICG) fluorescence lymphography*

ICG is a near-infrared dye that is injected intradermally to facilitate the identification of the superficial lymphatic tract beneath the skin. After injection, the substance binds to albumin before being drained proximally via the lymphatic channel through the regional node basin. This method can be used to assess lymphatic leakage, pumping capacity, and lymph reflux into the dermis.[15] Moreover, ICG lymphography can be utilized to grade the severity of the disease and guide the selection of an appropriate choice of treatment.

4. *Duplex ultrasound*

While not used specifically as a diagnostic tool, it provides benefits in terms of excluding deep venous thromboembolism and venous insufficiency, and it is able to investigate a space-occupying lesion that may be compressing the lymphatic channel. Many surgeons also use it to locate and map the superficial lymphatic vessels and superficial veins before performing a lymphaticovenous anastomosis procedure.

5. *Ankle-brachial pressure index (ABI)*

As with duplex ultrasound, ABI is not used for the diagnosis of lymphedema. However, it is recommended for patients with a history of, or with suspected, peripheral arterial disease. Because of the lymphedema, patients almost always need to do compression therapy, which might affect their peripheral blood circulation. If the ABI value is < 0.5, compression therapy is contraindicated for patients.

Staging of lymphedema

There are currently many clinical staging systems for the grading of lymphedema severity. However, the most commonly used worldwide are the staging system of the International Society of Lymphology (ISL) and the Campisi staging system. The details of each are listed in Tables 3 and 4.[16]

TABLE 3. International Society of Lymphedema (ISL) stagings.

ISL stage	Description
0	Subclinical state; swelling is not evident despite impaired lymph transport
I	Accumulation of tissue fluid (higher protein content), which subsides with limb elevation
IIa	Limb elevation alone rarely reduces swelling, and pitting is manifest
IIb (late stage)	Limb may or may not be pitted as fat and tissue fibrosis is more evident
III	Lymphostatic elephantiasis; the tissue is hard (fibrotic), and pitting is absent. Skin (changes) thickening, hyperpigmentation, increased skin folds, fat deposits, and warty overgrowths develop

TABLE 4. Campisi stagings.

Campisi stage	Description
Ia	No overt swelling despite impaired lymph drainage
Ib	Reversible swelling with limb elevation
II	Mild, persistent swelling with elevation
III	Persistent swelling, with recurrent lymphangitis
IV	Fibrotic change with column-like limb
V	Elephantiasis with limb deformation, including widespread lymphostatic warts

Lymphedema management

Chronic lymphedema is an irreversible process if left untreated. The mainstay of treatment is a conservative protocol. If the clinical status does not improve within 6 months of the commencement of therapy, surgical management is recommended.

The conservative treatment protocol consists of general measures of self-care, compression therapy, and physiotherapy.

1. *Self-care*

This aims to reduce swelling and slow the progression of disease. The measures are comprised of the following.

1.1 Self-monitoring. The size of the swollen arm or leg should be monitored, with observations made of the feeling of the skin, the skin color, and changes in skin appearance.

1.2 Limb elevation. This helps to reduce swelling of the affected limb in the early stages of the disease. The patient should be advised to avoid postures that cause the limbs to fall with gravity, such as standing for long periods of time or sitting cross-legged.

1.3 Diet and exercise. Being obese is not only a risk factor for lymphedema, but it also greatly hampers compression therapy. The patient should therefore be advised to exercise regularly, control food intake, and wear a pressure garment. A study on breast cancer patients with upper extremity lymphedema, divided the patients into two groups: first, those who are advised to control food and lose weight. Second, a group that provides general dietary recommendations. At 12 weeks of follow-up, the first group showed a significant loss weight of arm (P-value = 0.02) and a significant decrease in swelling and arm volume (P-value = 0.03).[9] For exercise by lifting weights in patients with undergoing axillary lymph node dissection without lymphedema. The study did not find that lifting weights made the lymphedema more severe.[17] Additionally, in the lymphedema group, the lifting of weights reduced the pain and swelling in the affected arm, with the incidence of swelling declining from 29% to 14%.[18]

1.4 The avoidance of skin infection. Regular skin and nail care can help to prevent the cracking that leads to skin infections. If there are piercings or abrasions on the skin, an antibiotic cream or ointment should be applied. The use of a sunscreen should also be recommended if the affected limb is likely to be exposed to the sun. In addition, as exposure to extremely hot environments (such as saunas and hot springs) can cause swelling, the patient should be advised to avoid such situations.

1.5 The avoidance of local limb concussion. Wearing tight-fitting clothing or performing local constriction procedures (such as the measurement of blood pressure) exacerbates lymphedema by stimulating the production of lymph while causing a narrowing of lymphatic ducts. This differs from the use of a compression garment (discussed below): it provides a firm and even pressure from the distal to proximal limb, thereby improving the flow of lymph.

2. *Compression therapy*

2.1 Stockings and sleeves. Wearing arm or leg restraints, such as a compression sleeve, is an appropriate treatment for the early stage (ISL I) and should be employed in conjunction with skin care, exercise, and elevation. As the compression apparatus typically has a pressure range of 20-50 mmHg, it is able to stimulate lymphatic return by exerting greater force on the distal than the proximal limb. The firmness of the particular material to be selected depends on the condition of the patient's arteries and, more importantly, the patient's ability to tolerate the proposed compression garment. Evaluation

of the patient's treatment should therefore be undertaken early, after 4-6 weeks' usage of the compression sleeve. Further reviews should be conducted every 3-6 months if the condition has stabilized. In addition, the compression device should be replaced every 3-6 months, or even earlier if it appears to be starting to slacken.

2.2 Multilayer lymphedema bandaging. An elastic bandage is used to treat intermediate-stage lymphedema (ISL II), which is when the limb is so large that stockings or compression sleeves cannot be worn. The bandage is used in conjunction with skin care, limb elevation, and short-stretch bandages. Force is applied to the limbs by the elastic bandage only when the affected muscles are contracted; the pressure that is generated compresses the lymphatic vessels and increases lymph flow, resulting in decreased swelling. The appropriate ankle sub-bandage pressure is 45 mmHg. During the first week of treatment, the bandage should be changed every day; after that, changing it 2-3 times a week is acceptable.

The contraindications to the use of multilayer lymphedema bandaging are:
- ABI < 0.5
- Uncontrolled heart failure
- Severe peripheral neuropathy

2.3 Intermittent pneumatic compression device. The efficacy of intermittent pneumatic compression is still controversial. It involves the placement of cuffs around an affected limb; they are then filled with air. In turn, the cuffs squeeze the limb, compressing it from the distal to the proximal region while applying a pressure of 30-60 mmHg. It is recommended that an intermittent pneumatic compression device be used for 30-120 minutes per day, with a newer generation, multichambered device being preferable.

The contraindications to the use of intermittent pneumatic compression are:
- Untreated, nonpitting, chronic lymphedema
- Deep vein thrombosis
- Pulmonary embolism
- Acute cellulitis
- Uncontrolled heart failure
- ABI < 0.5
- Active tumor at the affected region

3. Physiotherapy

3.1 Manual lymphatic drainage. This massage technique aims to stimulate fluid flow in the lymphatic system. The force produced by the massage causes the lymph to flow from the distal to the proximal. After the massage, the patient still needs to wear a pressure garment during daily activities. A massage of at least one hour per day is recommended. Although the efficacy of manual lymphatic drainage has not yet been clearly identified, this technique is still recommended as a component of complete decongestive therapy (discussed below) during the treatment phase.

The contraindications to manual lymphatic drainage are:
- Acute cellulitis in the position to be massaged.
- Unstable hypertension
- Uncontrolled heart failure
- Tumor at the affected part

3.2 Complete decongestive therapy. This multi-modality treatment aims to reduce the swelling of the limbs in two phases:

3.2.1 ***Treatment phase.*** This comprises 1) skin and nail care, 2) exercise, 3) manual lymphatic drainage or massage, and 4) compression (bandage therapy). Patients must perform these procedures five days a week for 2-4 weeks. During the treatment phase, it is recommended that the patients take circumference and volume measurements themselves once weekly to facilitate the assessment of the treatment outcomes.

3.2.2. ***Maintenance phase.*** The treatment in this period consists of wearing a compression garment (during the day), bandaging (at night), maintaining skin care, and exercise. Lymphatic squeezing can be performed on its own after the patient has received appropriate training. During the maintenance phase, patients should measure the circumference and volume every 6 months to monitor the treatment progress.[19]

The absolute contraindications to complete decongestive therapy are:
- Infection
- Active cancer
- Heart attack
- Acute deep vein thrombosis

The relative contraindications to complete decongestive therapy are:
- High blood pressure that has not been controlled
- Diabetes
- Asthma
- Paralysis

4. Pharmacotherapy

The use of medications to treat edema is not recommended. Some studies investigated the use of diuretics to reduce limb swelling, but the results were not as good as expected. Apart from diuretics, coumarin (a benzopyrone) has been reported to reduce the incidence of cellulitis or lymphangitis.[20] However, recent studies have found that the use of this drug resulted in a reduction in the volume of swollen limbs compared with a placebo, with an incidence of hepatic toxicity of approximately 6%.[21] So, The use of medications to treat edema is still not recommended.

5. Experimental therapy

Low-level laser therapy (or cold laser therapy) has been investigated in patients with lymphedema after mastectomy. The therapy is believed to reduce fibrosis, stimulate macrophages in the immune system, and result in the formation of new lymphatic vessels (lymphangiogenesis). The results of one study showed that 1 in 3 patients who underwent laser therapy demonstrated a reduction in the volume of their swollen arm at their 3-month follow-up visit.[22] Moreover, low-level laser therapy produced a better limb-swelling reduction than pneumatic compression therapy after 12 months of treatment.[23]

6. Surgical management

Surgery is an ideal treatment option for patients with localized disease, a failed conservative treatment, recurrent cellulitis or lymphangitis, leakage of lymph to externally, or a significantly diminished quality of life.

Preoperative assessment

1. Assess degree of lymphedema

Evaluation of the degree of lymphedema is based on the difference in the circumference and volume of the affected limb relative to the contralateral, normal limb.

2. Lymphedema staging

Assessment of the stage of the disease is undertaken in order to select a suitable treatment option. The most commonly used staging system is the ISL system.[24]

3. Venous duplex ultrasound

In patients who plan to undergo surgery, venous duplex ultrasound might be required to rule out venous thrombosis, venous insufficiency, and valvular incompetency.

Surgical treatment

Surgical treatment can be divided into two broad approaches: reductive or ablative procedures, and physiological treatment.

1. Reductive or ablative procedures

1.1 Direct excision. In 1912, Dr. Charles wrote a book entitled "Elephantiasis Scroti", which describes how to treat lymphedema around the scrotum. The principle of the surgery is to remove the swollen tissue of the scrotum and then cover the wound with a skin graft from the thigh.

In 1940, Dr. Macey from the Mayo Clinic applied the Charles procedure to surgery on extremities lymphedema.[25] The benefit of this surgical technique is that it totally removes the swelling and fibrotic tissues. Nevertheless, there are still many postoperative complications, such as surgical site infection, hematoma, graft loss, and scars. Therefore, the procedure is usually recommended only for late-stage lymphedema patients, or for patients with wounds on the affected limb that are significantly disturbing their quality of life.

1.2 Liposuction. This treatment involves the insertion of a steel canula into the subcutaneous layer; the cannula is connected to a vacuum that suctions the fibrofatty tissue via negative pressure. O'Brien et al.[26] reported that this treatment reduced the volume of the swelling by 20% - 23%. The advantages of this treatment are, firstly, it only leaves a very small incisional scar and, secondly, patients recover rapidly (approximately 48 hours). The disadvantage, however, is that there may be injury to the lymphatic tract during the liposuction procedure; consequently, it is recommended that the canula is kept parallel to the limbs to reduce injuries. Another consideration is that, after liposuction, patients need to permanently wear compression devices.

2. Physiological treatment

This treatment principle aims to creates a new lymphatic channel in order to increase the ability of the lymph to flow. These surgical procedures are only suitable for the early stages of lymphedema (ISL stages I or II)[27], being ineffective in late-stage patients. The physiological treatment procedures are comprised of 4 main types, as described below.

2.1 Flap interposition. The procedure uses a flap with good blood vessels and lymphatic vasculature obtained from another site of the body. The flap is placed in the

area where the lymphatic flow is blocked or disrupted, and then anastomosis of the blood vessels and lymphatic vessels is performed.

In 1935, Gills and Fraser[28] were the first doctors to use this procedure to treat a patient with leg edema. Flaps taken from the arms were pasted to the thighs and body of the patient; the flaps became bypasses that allowed lymphatic fluid to cross the obstructed areas.

In 1974, Goldsmith et al.[29] reported the use of a greater omental flap to treat patients with lymphedema. The flap was inset in a subcutaneous layer, thereby allowing lymph in an arm or leg to drain into the lymphatic system. Twenty-two patients from their study reported satisfactory results. Nonetheless, this surgery is not popular because it has a relatively high number of complications, such as intestinal obstruction, blood clots, atherosclerosis, and hernia.

2.2 Lymphatic bypass procedure. The principle of this surgery is to create a link between distal lymphatic vessels and lymphatic vessels above the point of an obstruction. Alternatively, a bypass from the obstructed lymphatic channels into the venous system is created.

Before surgery, the functional lymphatic vessels should be identified by specific dyes transported through the lymphatic channels[27], for example, isosulfan blue or indocyanin green.

The indications for this surgery are:
- No response to conservative treatment
- Recurrence of skin infection
- Disease significantly affecting the quality of life and daily activities

The limitations of lymphatic bypass surgery are:
- Tissue fibrosis
- Late stage lymphedema
- Inferior surgical outcomes for venous hypertension or venous insufficiency patients
- Recurrence of cancer
- Patients unable to take care of themselves after surgery
- Uncooperative patient

2.2.1 ***Lymphatic-lymphatic bypass.*** An attempt was made to bypass the problematic lymphatic tract by connecting the distal lymphatic vessels to the proximal lymphatic vessels above the area of obstruction.

In 1990, Baumeistier and Suida[30,31] performed a series of lymphatic reconstructions to manage arm lymphedemas by using lymphatic vessels from thighs. Lymphatic vessels were harvested from the patients' thighs and then implanted in the subcutaneous fat layer between the upper arm and shoulder area, above the clavicular bone. In the case of lower extremity lymphedema, the surgery was performed by harvesting contralateral, normal lymphatic vessels and moving them to the groin of the affected leg. The procedure was performed on 55 patients. The treatment outcome was reductions of up to 80% in the volume of the affected areas during the 3-year follow-up period.

2.2.2 ***Lymphovenous bypass and lymphaticovenous anastomosis.*** In 1962, Jacobson et al. attempted to connect lymphatic vessels to veins by conducting an experimental study in an animal model. After that, Yamada et al. applied the technique to treat lymphedema patients by using a saphenous vein connection to an obstructed lymphatic tract. However, some concern had been expressed about using a high-pressure gradient large vein, which might cause problems if there is a poor lymphatic flow.

Consequently, Yamada et al. modified the protocol so that venules with a diameter of 0.8 mm or less were used.[32] The outcomes of lymphaticovenous anastomosis were studied in 100 patients with lymphedema of the arms or legs. The results showed clinical improvements for 96% of the patients, and a volume reduction for 74% of the cases. The overall lower-limb-volume reduction at 12 months was 42%; the decline was greater for early-stage patients (61%) than late-stage patients (17%).[33]

The effect of lymphaticovenous bypass surgery has been studied at the cell level by skin biopsy. The findings revealed a decline in the white-blood-cell and CD4 + values of the affected limb, and tissue fibrosis.[34]

Fluorescence lymphangiography is currently been used to identify the location of the lymphatic tract in real time.[15] It is also used to stage the disease. With the early stage, the lymphatic tract is seen to have a clear linear pattern. In contrast, the late stage shows a diffuse stain of substance due to obstruction and a reversed lymphatic flow. If good lymphatic vessels are identified, lymphaticovenous anastomosis is performed. The superficial lymphatic vessels are localized again using isosulfan blue dye during surgery conducted under a surgical microscope.

2.3 Vascularized lymph node transfer (VLNT). The procedure involves harvesting a free lymph node flap from outside the affected region, implanting it into the affected limb, and anastomosing it to recipient vessels via a microsurgical technique (without connecting the

lymphatic tract). Two mechanisms explain the effect of VLNT:

- Bridging mechanism. The transplanted lymph node flap secretes vascular endothelial growth factor C (VEGF-C), which stimulates the formation of a new lymphatic tract.
- Pumping mechanism. The difference in the pressure gradient between arterial anastomosis and venous anastomosis acts like a pump suctioning excess lymph back to the systemic circulation system (like lymphatic-venous drainage).

In 1982, Clodius et al.[35] reported the results of 2 cases of VLNT to manage lower extremity lymphedema, with the lymph node flap being obtained from the groin. Although the swelling rapidly subsided during the early post-operation period, the edema had returned by the 6-month follow-up session.

In 2006, Becker et al.[36] reported the long-term effects of free lymph node flap transfers using inguinal lymph nodes to manage 24, post-mastectomy, upper extremity lymphedema patients, sixteen of who had postoperative lymphoscintigraphic evaluations. The results showed that 5 out of the 16 patients (who had lymphoscintigraphy) had functional lymph nodes and lymphatic tract regeneration.

In 2012, Saaristo et al.[37] performed vascularized lymph node transfer in conjunction with autologous breast reconstruction in 9 patients with post-mastectomy lymphedema. There was an improved lymphatic circulation in 5 out of 6 patients, and 3 out of the 9 patients no longer needed to use a pressure garment after the surgery. Additionally, an increase in endogenous lymphatic vessel growth factors was demonstrated, suggesting that new lymphatic regeneration in the axilla had been stimulated.

The indications for vascularized lymph node transfer are:

- Segmental dermal backflow or a non-functioning lymphatic vessel detected by lymphoscintigraphy
- ISL stage II with repeated cellulitis
- No acute cellulitis
- Follow up > 12 months

There are two principles for lymph node implantation: 1) orthotopic (anatomical) placement, and 2) heterotopic (nonanatomical) placement.

2.3.1 ***Anatomical placement.*** The lymph nodes are implanted in the area where the obstruction occurs, such as the axilla or groin.

The advantages of anatomical placement are:

- Removal of the fibrosis that caused the lymphatic tract obstruction
- Nearby tissue can be sewn or closed without any skin grafting
- Less postoperative scarring

2.3.2 ***Nonanatomical placement.*** The lymph nodes are implanted in the distal limb, with the transplanted lymph node acting like a lymphatic pump.

The advantages of nonanatomical placement are:

- Avoidance of surgery in the area of fibrosis

The disadvantages of nonanatomical placement are:

- Bulkiness of lymph node flap in the distal limb
- Often need to do skin grafting

If a scar cannot be properly removed, it is recommended that the lymph nodes be implanted in an area that will produce less scarring. Many studies have demonstrated that VLNT can relieve symptoms in 100% of cases, reduce the volume in 91% of cases, and allow 78% of patients to stop using pressure garments.

As to complications, lymphatic leakage develops in 15% of cases, postoperative infection arises in about 8%, and a need to reoperate occurs with 3% of patients.

2.4 Lymphatic microsurgical preventive healing approach (LYMPHA). This procedure aims to connect lymphatic vessels to a branch of the axillary vein simultaneously with axillary lymph node dissection. One study found that, after 4 years of follow-up, only 3 (4%) out of 74 patients continued to have lymphedema in the arm that had been operated on.[20] However, this new technique needs further long-term studies to prove its efficacy and to determine any adverse outcomes, such as cancer recurrence.

CONCLUSION

Lymphedema is a condition caused by an obstruction in the lymphatic system arising from a congenital anomaly, infectious disease, chronic inflammation, connective tissue disease, or cancer. Patients who are faced with this disease usually have a poor quality of life due to infection and limits to their ability to perform daily activities. Consequently, it is critical to provide early diagnosis and treatment as these are key to managing and conquering the lymphedema. A multidisciplinary team approach yields the best solutions and long-term outcomes for patients.

What is already known on this topic?

Lymphedema is a congenital and acquired disease with can cause swelling of the limbs. The most common symptoms are limb swelling and recurrent cellulitis of the limbs.

What this study adds?

It reviews the related clinical presentations, physical examinations, radiological studies, and new treatment options.

Potential conflicts of interest

The authors declare that there are no conflicts of interest related to this study.

REFERENCES

1. Grada AA, Phillips TJ. Lymphedema: Pathophysiology and clinical manifestations. J Am Acad Dermatol 2017;77:1009-20.
2. Suami H, Pan WR, Taylor GI. Changes in the lymph structure of the upper limb after axillary dissection: radiographic and anatomical study in a human cadaver. Plast Reconstr Surg 2007;120:982-91.
3. Barak Mehrara M. Clinical staging and conservative management of peripheral lymphedema Wolters Kluwer: Uptodate; 2019 [Available from: http://www.uptodate.com.
4. Ramaiah KD, Ottesen EA. Progress and impact of 13 years of the global programme to eliminate lymphatic filariasis on reducing the burden of filarial disease. PLoS Negl Trop Dis 2014;8:e3319.
5. Brayton KM, Hirsch AT, PJ OB, Cheville A, Karaca-Mandic P, Rockson SG. Lymphedema prevalence and treatment benefits in cancer: impact of a therapeutic intervention on health outcomes and costs. PloS One. 2014;9:e114597.
6. DiSipio T, Rye S, Newman B, Hayes S. Incidence of unilateral arm lymphoedema after breast cancer: a systematic review and meta-analysis. Lancet Oncol 2013;14:500-15.
7. Cormier JN, Askew RL, Mungovan KS, Xing Y, Ross MI, Armer JM. Lymphedema beyond breast cancer: a systematic review and meta-analysis of cancer-related secondary lymphedema. Cancer 2010;116:5138-49.
8. Erickson VS, Pearson ML, Ganz PA, Adams J, Kahn KL. Arm edema in breast cancer patients. J Natl Cancer Inst 2001;93:96-111.
9. Shaw C, Mortimer P, Judd PA. A randomized controlled trial of weight reduction as a treatment for breast cancer-related lymphedema. Cancer 2007;110:1868-74.
10. Helyer LK, Varnic M, Le LW, Leong W, McCready D. Obesity is a risk factor for developing postoperative lymphedema in breast cancer patients. Breast J 2010;16:48-54.
11. Taylor R, Jayasinghe UW, Koelmeyer L, Ung O, Boyages J. Reliability and Validity of Arm Volume Measurements for Assessment of Lymphedema. Phys Ther 2006;86:205-14.
12. Maclellan RA, Zurakowski D, Voss S, Greene AK. Correlation Between Lymphedema Disease Severity and Lymphoscintigraphic Findings: A Clinical-Radiologic Study. J Am Coll Surg 2017;225:366-70.
13. Szuba A, Shin WS, Strauss HW, Rockson S. The third circulation: radionuclide lymphoscintigraphy in the evaluation of lymphedema. J Nucl Med 2003;44:43-57.
14. Hassanein AH, Maclellan RA, Grant FD, Greene AK. Diagnostic Accuracy of Lymphoscintigraphy for Lymphedema and Analysis of False-Negative Tests. Plast Reconstr Surg Glob Open 2017;5:e1396.
15. Narushima M, Yamamoto T, Ogata F, Yoshimatsu H, Mihara M, Koshima I. Indocyanine Green Lymphography Findings in Limb Lymphedema. J Reconstr Microsurg 2016;32:72-9.
16. Executive Committee. The Diagnosis and Treatment of Peripheral Lymphedema: 2016 Consensus Document of the International Society of Lymphology. Lymphology 2016;49:170-84.
17. Schmitz KH, Ahmed RL, Troxel AB, Cheville A, Lewis-Grant L, Smith R, et al. Weight Lifting for Women at Risk for Breast Cancer–Related Lymphedema: A Randomized Trial. JAMA 2010;304:2699-705.
18. Schmitz KH, Ahmed RL, Troxel A, Cheville A, Smith R, Lewis-Grant L, et al. Weight Lifting in Women with Breast-Cancer–Related Lymphedema. N Engl J Med 2009;361:664-73.
19. Lawenda BD, Mondry TE, Johnstone PA. Lymphedema: a primer on the identification and management of a chronic condition in oncologic treatment. CA Cancer J Clin 2009;59:8-24.
20. Casley-Smith JR, Morgan RG, Piller NB. Treatment of Lymphedema of the Arms and Legs with 5,6-Benzo-[alpha]-pyrone. N Engl J Med 1993;329:1158-63.
21. Loprinzi CL, Kugler JW, Sloan JA, Rooke TW, Quella SK, Novotny P, et al. Lack of Effect of Coumarin in Women with Lymphedema after Treatment for Breast Cancer. N Engl J Med 1999;340:346-50.
22. Carati CJ, Anderson SN, Gannon BJ, Piller NB. Treatment of postmastectomy lymphedema with low-level laser therapy: a double blind, placebo-controlled trial. Cancer 2003;98:1114-22.
23. Kozanoglu E, Basaran S, Paydas S, Sarpel T. Efficacy of pneumatic compression and low-level laser therapy in the treatment of postmastectomy lymphoedema: a randomized controlled trial. Clin Rehabil 2009;23:117-24.
24. The Diagnosis and Treatment of Peripheral Lymphedema: 2016 Consensus Document of the International Society of Lymphology. Lymphology 2016;49:170-84.
25. Karri V, Yang MC, Lee IJ, Chen SH, Hong JP, Xu ES, et al. Optimizing outcome of charles procedure for chronic lower extremity lymphoedema. Ann Plast Surg 2011;66:393-402.
26. Brorson H. Liposuction in arm lymphedema treatment. Scand J Surg 2003;92:287-95.
27. Chang DW. Lymphaticovenular bypass for lymphedema management in breast cancer patients: a prospective study. Plast Reconstr Surg 2010;126:752-8.
28. Gillies H, Fraser FR. Treatment of Lymphoedema by plastic operation: (a Preliminary report). Br Med J 1935;1:96-8.
29. Goldsmith HS. Long term evaluation of omental transposition for chronic lymphedema. Ann Surg 1974;180:847-9.
30. Baumeister RG, Siuda S. Treatment of lymphedemas by microsurgical lymphatic grafting: what is proved? Plast Reconstr Surg 1990;85:64-74.
31. Campisi C. Use of autologous interposition vein graft in management of lymphedema: preliminary experimental and clinical observations. Lymphology 1991;24:71-6.
32. Koshima I, Inagawa K, Urushibara K, Moriguchi T. Supermicrosurgical lymphaticovenular anastomosis for the treatment of lymphedema in the upper extremities. J Reconstr

Microsurg 2000;16:437-42.
33. Chang DW, Suami H, Skoracki R. A prospective analysis of 100 consecutive lymphovenous bypass cases for treatment of extremity lymphedema. Plast Reconstr Surg 2013;132:1305-14.
34. Torrisi JS, Joseph WJ, Ghanta S, Cuzzone DA, Albano NJ, Savetsky IL, et al. Lymphaticovenous bypass decreases pathologic skin changes in upper extremity breast cancer-related lymphedema. Lymphat Res Biol 2015;13:46-53.
35. Thompson N. Surgical treatment of chronic lymphoedema of the lower limb. With preliminary report of new operation. Br Med J 1962;2:1566-73.
36. Becker C, Assouad J, Riquet M, Hidden G. Postmastectomy lymphedema: long-term results following microsurgical lymph node transplantation. Ann Surg 2006;243:313-5.
37. Saaristo AM, Niemi TS, Viitanen TP, Tervala TV, Hartiala P, Suominen EA. Microvascular Breast Reconstruction and Lymph Node Transfer for Postmastectomy Lymphedema Patients. Ann Surg 2012;255:468-73.
38. Borman P. Lymphedema diagnosis, treatment, and follow-up from the view point of physical medicine and rehabilitation specialists. Turk J Phys Med Rehabil 2018;64:179-197.
39. Kayıran O, De La Cruz C, Tane K, Soran A. Lymphedema: From diagnosis to treatment. Turk J Surg 2017;33:51-57.

Comparison of the Sensitivity and Specificity of Tzanck Smear and Immunofluorescence Assay for the Diagnosis of Cutaneous Herpes Simplex Virus and Varicella Zoster Virus Infections in a Real-life Clinical Setting

Chayada Chaiyabutr, M.D., Nuttagarn Jantanapornchai, M.D., Chalermkwan Apinuntham, M.D., Charussri Leeyaphan, M.D., Sukhum Jiamton, M.D., Ph.D.

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

ABSTRACT

Objective: This research aims to compare (1) the sensitivity and specificity of Tzanck smear and indirect immunofluorescence assay (IFA) which detect viral antigen for the diagnosis of cutaneous herpes simplex virus (HSV) and varicella zoster virus (VZV) infections; and (2) the detection rates of the tests among various patient groups and lesion morphologies.

Materials and Methods: This retrospective study reviewed 440 and 172 samples from patients with clinically suspicious cutaneous HSV and VZV infections, who underwent both Tzanck smear and IFA, respectively. The gold standard of the study was defined by showing agreement of diagnostic codes between initial and subsequent visits.

Results: For HSV infections, the respective sensitivity and specificity of Tzanck smear were 32.8% and 96.6% whereas those of IFA were 60.7% and 100%. As to VZV infections, the sensitivity and specificity of Tzanck smear were 54.3% and 97.8%, respectively, while the corresponding values of IFA were 71.7% and 100%. According to disease characteristics and lesion morphologies, IFA provided substantially higher ability to detect HSV than the Tzanck smear, especially in patients with immunosuppressed conditions. Tzanck smear and IFA demonstrated no statistically significant difference for early-onset VZV infections (≤ 3 days).

Conclusion: The Tzanck smear and IFA had higher sensitivities for detecting VZV than HSV infections. IFA testing is recommended in patients with immunosuppressed conditions who present with suspected cutaneous HSV infection. Despite the overall sensitivity and specificity of IFA being greater than those of Tzanck smear especially in HSV infections, the latter test is comparable option for early-onset VZV infections.

Keywords: Herpes simplex virus; varicella zoster virus; Tzanck smear; immunofluorescence (Siriraj Med J 2021; 73: 305-311)

INTRODUCTION

Herpes simplex virus (HSV) and varicella zoster virus (VZV) are large, enveloped DNA viruses belonging to the Herpesviridae family.[1] Although cutaneous infections of HSV and VZV are mainly diagnosed by history-taking and clinical characteristics, laboratory examinations are sometimes needed for a definite diagnosis.[2]

Corresponding author: Sukhum Jiamton
E-mail: sukhum.jiamton@gmail.com
Received 25 September 2020 Revised 28 December 2020 Accepted 30 December 2020
ORCID ID: http://orcid.org/0000-0003-1068-1586
http://dx.doi.org/10.33192/Smj.2021.40

Multiple laboratory options currently available to diagnose HSV and VZV infections can be categorized into four groups: (1) morphological tests, such as the Tzanck smear and tissue histopathology; (2) immunomorphological tests, like immunofluorescence and immunoperoxidase staining; (3) serological methods, for instance, enzyme-linked immunosorbent assay and immunoglobulin M/G titer; and (4) virological testing, for example, viral culture and viral polymerase chain reaction. A viral culture was long considered the gold-standard diagnostic test before the advent of polymerase chain reaction testing.[1,3]

HSV and VZV are more commonly observed as cutaneous infections rather than as infections of internal organs.[1,4] Moreover, their cutaneous symptoms are usually not severe and can be self-limited. Ideally, the chosen diagnostic test for these infections should be easy to perform, give a rapid result, and be inexpensive. In outpatient dermatological settings, the Tzanck smear and immunofluorescence staining are therefore the most frequently ordered tests at our clinic.

Previous research has found that the sensitivity of Tzanck smear ranges from 34% to 78% in detecting HSV, and from 26% to 64% in detecting VZV.[5] However, with a proficient technician, the sensitivity of the test may rise to 80% and its specificity to 90%.[6-8] Even though Tzanck smear is currently considered obsolete in many countries[1], it still has an important role in developing countries. There, the newer testing methodologies are not only often deemed to be too expensive, but also have the drawbacks of slower turnaround times and, sometimes, a lack of availability.

In terms of immunofluorescence testing, previous studies revealed that the sensitivity and specificity of immunofluorescence staining was greater than those of Tzanck smear, particularly in the case of VZV infections. The sensitivity of immunofluorescence staining in detecting cutaneous HSV infections was found to be around 50% - 100% compared with the viral culture technique, but its sensitivity in detecting VZV infections exceeded that of the viral culture. Moreover, the specificity of immunofluorescence staining was nearly 100%, and it was able to discriminate between the HSV1/2 and VZV pathogens.[2,8]

Earlier studies of the sensitivity and specificity of the Tzanck smear and immunofluorescence testing were usually performed in a small number of patients, and compared with those of the viral culture technique as the gold standard diagnostic modality.[9] It is also noteworthy that few details of the infected patients or the clinical morphologies of their lesions were reported.[2,5,9] Thus, the objective of the current research was twofold. The first aim was to compare the sensitivity and specificity of the Tzanck smear and immunofluorescence assay for the diagnosis of cutaneous HSV and VZV infections in a larger population and in a real-life setting. The secondary aim of this study was to compare the detection rates of two tests among various subgroups of patients, durations, and clinical morphologies of lesions.

MATERIALS AND METHODS

This retrospective research was approved by the Siriraj Institutional Review Board. (Si 333/2020) The study reviewed the samples taken from patients with clinically suspicious cutaneous HSV (ICD-10 B00) and VZV (ICD-10 B01-B02) infections. During 2012-2019, the samples had initially been collected from the Infection Control Clinic of the Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University.

All eligible patients had to undergo both a Tzanck smear and immunofluorescence testing for HSV or VZV. For each patient, demographic data, onset of lesions, morphology of the lesions, suspicious diagnosis, and comorbidities had been collected at their first visit. Tzanck-smear and IFA specimens that were reported as being inadequate for diagnosis were excluded from the study. The sensitivity and specificity of the tests were subsequently analyzed only in clinically confirmed cases. For the purposes of this study, the reference standard for clinically confirmed diagnosis was an agreement of diagnostic codes between the first and subsequent follow-up visits (determined by a dermatologist).

The Tzanck smear was performed by scraping the base of lesions with a blunt scalpel blade and spreading the sample as a thin layer on microscope slides. The slides were then fixed with 100% methyl alcohol for 10 minutes and stained with eosin solution for 20 seconds. After being rinsed with distilled water, the slides were stained with 3% methylene blue for 60 seconds, rinsed with distilled water, and allowed to dry. The slides were subsequently examined under a light microscope. A positive Tzanck smear was defined as the presence of herpetic cytopathic effects, such as the presence of multinucleated giant cells. Throughout the 7-year study period, the Tzanck tests were performed by the same, proficient technician, which obviated inter-rater variability. The typical test turnaround time was 15 minutes.

As to the immunofluorescence staining, our hospital used the technique of an indirect immunofluorescence assay (IFA) with commercial reagent kit containing HSV type 1, 2 antibodies (Bio-Rad Laboratories) and VZV monoclonal antibodies (Merck, Ltd). Specimens scraped from the base of the lesions were fixed in acetone for 15

minutes before adding a primary antibody. The smear was then incubated at 37 degrees Celsius for 30 minutes and rinsed with phosphate-buffered saline; pathogen-specific fluorescein-tagged secondary antibodies were subsequently added, and the mixture was incubated at 37 degrees Celsius for 30 minutes. The smear was examined with an epifluorescence microscope by virology technicians. The test turnaround time was 3 days.

All statistical analyses were undertaken using SPSS Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were presented as numbers or numbers with percentages, while continuous variables were shown as means with standard deviations. The sensitivities and specificities of the two tests were calculated. For the correlation between the tests, Cohen's Kappa coefficient (κ) was reported. A p-value of less than 0.05 was considered to indicate statistical significance.

RESULTS

A total of 440 and 172 specimens from patients with clinically suspicious cutaneous HSV and VZV infections, respectively, were reviewed. The demographic data of the included patients are detailed in Table 1. The mean age of the participants was around 50. The majority of them were tested more than 3 days after onset of the lesions. For cutaneous HSV infections, the main characteristic of the tested lesions was non-vesicle (66.2%) whereas vesicle was the major type of tested lesions in cutaneous VZV infections (70.1%).

TABLE 1. Demographic data and disease characteristics of the included patients.

	HSV N = 440		VZV N = 172	
	N	(%)	N	(%)
Demographic				
Sex, Female	239	(54.3)	103	(59.9)
Age (mean) ± SD	51.4 ± 18.4		56.1 ± 18.2	
Underlying disease				
Hypertension	101	(23.0)	36	(20.9)
Diabetes mellitus	49	(11.1)	20	(11.6)
Autoimmune disease	51	(11.6)	16	(9.3)
Cancer	51	(11.6)	32	(18.6)
HIV (n = 240; n = 62)	45	(18.7)	6	(9.7)
On immunosuppressive drugs	66	(15.0)	26	(15.1)
Disease characteristics				
Onset ≤ 3 days (n = 420; n = 167)	165	(39.3)	75	(44.9)
Taken oral acyclovir before testing	29	(6.6)	21	(12.2)
Site				
Mucosa	233	(53.0)	8	(4.7)
Skin	207	(47.0)	164	(95.3)
Morphology (n = 420; n = 164)				
Vesicle	142	(33.8)	115	(70.1)
Non-vesicle	278	(66.2)	49	(29.9)
Erosion	113	(26.9)	6	(3.7)
Ulcer	105	(25.0)	3	(1.8)
Papule	26	(6.2)	19	(11.6)
Crust	12	(2.9)	12	(7.3)
Erythematous macule	11	(2.6)	8	(4.9)
Verrucous plaque	11	(2.6)	1	(0.6)

Of 440 specimens, 229 (52%) had clinically confirmed diagnosis of HSV, while 127 (73.8%) of 172 specimens had clinically confirmed diagnosis of VZV. Table 2 compares sensitivities, specificities, positive predictive values, and negative predictive values of the Tzanck smear and IFA related to the clinically confirmed cases. For HSV infections, the respective sensitivity and specificity of Tzanck smear were 32.8% and 96.6% whereas those of IFA were 60.7% and 100%. As to VZV infections, the sensitivity and specificity of Tzanck smear were 54.3% and 97.8%, respectively, while the corresponding values of IFA were 71.7% and 100%. The sensitivity and specificity of IFA was substantially higher than those of the Tzanck smear. In addition, the Tzanck smear and IFA showed higher sensitivities in detecting VZV infections than HSV infections. The Kappa agreements between the Tzanck smear and IFA in detecting HSV and VZV infections were moderate, with the values of 0.4 and 0.5, respectively.

A comparison was made on the sensitivity of the Tzanck smear and IFA for cutaneous HSV and VZV infections among various subgroups of patients, durations, and clinical morphologies of lesions.

In cutaneous HSV infections (Table 3), it was found that IFA yielded a greater sensitivity in detecting HSV infections than the Tzanck smear in nearly all subgroups of patients with statistical significance. In terms of disease onset, IFA showed the sensitivity around 60% in both early-onset (≤ 3 days) and late-onset (> 3 days) HSV infections whereas the percentage of HSV detection from the Tzanck smear dropped from 45.2% in early-onset to 24.8% in late-onset HSV infections. Furthermore, the sensitivity rate of Tzanck smear in patients taken oral acyclovir before testing was very low (19%) while IFA in these patients still yielded a sensitivity rate nearly 60%. Clinical morphologies of the lesions also determined the sensitivity rates of both tests. IFA also showed a high sensitivity (around 60%) in detecting HSV in vesicle and non-vesicle lesions. The detection rate of Tzanck smear was only 45.7% in vesicle lesions and very low in non-vesicle lesions (25.4%).

In cutaneous VZV infections (Table 4), even though IFA yielded a greater sensitivity than Tzanck smear but the magnitude of difference was not much as in case of cutaneous HSV infections. Interestingly, the Tzanck smear was not statistically different from the IFA in some situations such as early-onset (≤ 3 days) of infection, non-vesicular lesions and patients who had a history of taking oral acyclovir before testing.

DISCUSSION

The current research demonstrated that both the Tzanck smear and IFA had a higher sensitivity in detecting VZV infections than HSV infections. The comparison of their sensitivities and specificities revealed that the IFA was superior overall to the Tzanck smear, corresponding with earlier findings.[3] The higher sensitivity of IFA was significantly shown in nearly all situations of cutaneous HSV infections.

However, in cutaneous VZV infections, the sensitivity rate of Tzanck smear was not far different from IFA. Our study showed that the Tzanck smear is still comparable to the IFA in some VZV-infection situations, such as the early-onset (≤ 3 days) of infection. This can be explained by the Tzanck smear having a high ability to detect VZV infections[5], as well as by a shorter duration of disease normally resulting in an increase in the sensitivity of the Tzanck smear.[10]

The type of lesions is also an important factor in determining the sensitivity of the two tests. Prior research

TABLE 2. The sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV) for the cutaneous HSV and VZV infections.

HSV infection	% Sensitivity	% Specificity	PPV*	NPV**
Tzanck	32.8	98.6	96	57.5
IFA	60.7	100	100	70.1
VZV infection				
Tzanck	54.3	97.8	98.6	43.1
IFA	71.7	100	100	55.6

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

TABLE 3. Comparison of the sensitivities of detection of the Tzanck smear and IFA for cutaneous HSV infections.

	Positive Tzanck smears N = 229		Positive IFAs N = 229		P-value
	N	(%)	N	(%)	
Demographics					
Sex F	54/139	(38.8)	88/139	(63.3)	< 0.001
M	21/90	(23.3)	51/90	(56.7)	< 0.001
Age ≤ 60 years	46/145	(31.7)	87/145	(60.0)	< 0.001
Age > 60 years	29/84	(34.5)	52/84	(61.9)	< 0.001
Underlying disease					
Hypertension	15/57	(26.3)	33/57	(57.9)	0.001
Diabetes mellitus	9/28	(32.1)	18/28	(64.3)	0.022
Autoimmune disease	6/25	(24.0)	17/25	(68.0)	0.003
Cancer	10/22	(45.5)	14/22	(63.6)	0.388
HIV	8/30	(26.7)	22/30	(73.3)	0.001
On immunosuppressive drugs	9/32	(28.1)	24/32	(75.0)	< 0.001
Disease characteristics					
Onset ≤ 3 days	42/93	(45.2)	59/93	(63.4)	0.003
Onset > 3 days	31/125	(24.8)	76/125	(60.8)	< 0.001
Taken oral acyclovir before testing	4/21	(19.0)	12/21	(57.1)	0.008
Site					
Mucosa	26/102	(25.5)	56/102	(54.9)	< 0.001
Skin	49/127	(38.6)	83/127	(65.4)	<0.001
Morphology					
Vesicle	46/102	(45.1)	67/102	(65.7)	0.001
Non-vesicle	28/116	(24.1)	65/116	(56.0)	<0.001
Ulcer	12/54	(22.2)	29/54	(53.7)	<0.001
Erosion	8/41	(19.5)	24/41	(58.5)	<0.001
Hypertrophic	4/9	(44.4)	6/9	(66.7)	0.625
Crust	2/6	(33.3)	2/6	(33.3)	1.000
Papule	2/4	(50.0)	2/4	(50.0)	1.000
Erythematous macule	0/2	(0.00)	2/2	(100)	-

TABLE 4. Comparison of the sensitivities of detection of the Tzanck smear and IFA for cutaneous VZV infections.

	Positive Tzanck smears N = 127		Positive IFAs N = 127		P-value
	N	(%)	N	(%)	
Demographics					
Sex F	43/76	(56.6)	56/76	(73.7)	0.011
M	25/51	(49.0)	35/51	(68.0)	0.021
Age ≤ 60 years	40/69	(58.0)	50/69	(72.5)	0.031
Age > 60 years	28/58	(48.3)	41/58	(70.7)	0.007
Underlying disease					
Hypertension	16/31	(51.6)	25/31	(80.6)	0.012
Diabetes mellitus	10/16	(62.5)	14/16	(87.5)	0.125
Autoimmune disease	7/12	(58.3)	11/12	(91.7)	0.125
Cancer	10/21	(47.6)	14/21	(66.7)	0.344
HIV	3/5	(60.0)	4/5	(80.0)	1.000
On immunosuppressive drug	15/19	(78.9)	15/19	(78.9)	1.000
Disease characteristics					
Onset ≤ 3 days	40/58	(69.0)	47/58	(81.0)	0.092
Onset > 3 days	27/67	(40.3)	43/67	(64.2)	0.002
Taken oral acyclovir before testing	6/20	(30.0)	10/20	(50.0)	0.344
Site					
Mucosa	1/3	(33.3)	3/3	(100)	–
Skin	67/124	(54.0)	88/124	(71.0)	0.001
Morphology					
Vesicle	55/95	(57.9)	75/95	(78.9)	<0.001
Non-vesicle	8/26	(30.8)	13/26	(50.0)	0.180
Crust	4/10	(40.0)	7/10	(70.0)	0.250
Papule	2/9	(22.2)	3/9	(33.3)	1.000
Erosion	1/3	(33.3)	0/3	(00.0)	-
Erythema	1/2	(50.0)	1/2	(50.0)	1.000
Ulcer	0/2	(00.0)	2/2	(100)	-

has found that vesicles and blisters generally yield higher sensitivities of detection than other types of lesions with these two tests[2,10]; the present study had a similar finding. However, our work determined that there was no statistical difference in the sensitivities of detection of the Tzanck smear and IFA for non-vesicular lesions of VZV. It is possible that the non-vesicular lesions which were usually in the late stage of infection might have a low number of virus and was therefore comparable difficult for both tests to yield the positive result[1], or the number of specimens enrolled in the non-vesicular-VZV group might not be enough to provide a statistically significant difference.

Furthermore, in terms of underlying disease of the patients, the sensitivity of IFA in cutaneous HSV infections was prominently higher with statistical significance compared to Tzanck smear particularly in patients with immunosuppressive conditions including HIV infection and taking immunosuppressive agents. The detection ability of HSV by Tzanck smear in these patients was around 30% which was substantially lower than IFA (above 70%). IFA testing in suspected cutaneous HSV patients with immunosuppressed conditions is recommended. Whether the underlying disease would affect the yield of Tzanck smear or IFA test in cutaneous VZV infections was difficult to conclude. As the majority of underlying diseases or comorbidity subgroups in cutaneous VZV infections contained a small number of patients.

There are some limitations in this study. The reference standard for confirmed cases used in this study was a clinical diagnosis by dermatologists on two separate occasions, rather than a standard laboratory investigation like viral culture or polymerase chain reaction testing. The explanation is that this was a retrospective study conducted at a dermatology outpatient clinic in a developing country and in a real-life clinical setting, where dermatologists need to make prompt diagnosis without the ready utilization of sophisticated laboratory testing. For example, the use of viral culture tends to be avoided because specimens need to be promptly transported on ice to a laboratory, refrigerated-culture media are required, and long turnaround times are involved. Polymerase chain reaction testing, generally recognized as the platinum standard for VZV and HSV infections, has a higher sensitivity and specificity than any other test. Nevertheless, its relatively high cost and limited accessibility are problematic for developing countries.

In addition, the number of patients with morphology of vesicle were substantially higher in VZV (70.1%) than HSV (33.8%). This might affect the overall sensitivity of both tests and was another limitation of our study. However, focusing in subgroup analysis based on morphology of the lesions, Tzanck smear and IFA still yielded higher sensitivity in VZV than HSV in either vesicle or non-vesicle subgroup.

In conclusion, this study revealed the sensitivity and specificity of the Tzanck smear and IFA which could be used as a benchmark in a real-life setting. The tests had a higher sensitivity in detecting VZV infections than HSV infections. Even though IFA had an overall higher sensitivity and specificity than the Tzanck smear, the Tzanck smear is a comparable option to IFA for early-onset VZV infections. This information is valuable, especially in an outpatient dermatologic clinic, where prompt diagnosis of HSV and VZV infections is required.

ACKNOWLEDGMENT

The authors thank Ms. Orawan Supapueng for her assistance with the statistical analyses.

Conflicts of interest: All authors have neither conflicts of interest nor financial support to declare.

Funding: None

REFERENCES

1. Levin MJ, Weinberg A, Schmid DS. Herpes Simplex Virus and Varicella-Zoster Virus. Microbiol Spectr, 2016.p.4.
2. Zirn JR, Tompkins SD, Huie C, Shea CR, Rapid detection and distinction of cutaneous herpesvirus infections by direct immunofluorescence. J Am Acad Dermatol 1995;33: 724-8.
3. Frisch S, Guo AM, Diagnostic methods and management strategies of herpes simplex and herpes zoster infections. Clin Geriatr Med 2013;29:501-26.
4. Stuart-Harris C, The epidemiology and clinical presentation of herpes virus infections. J Antimicrob Chemother 1983;12 Suppl B:1-8.
5. Nahass GT, Goldstein BA, Zhu WY, Serfling U, Penneys NS, Leonardi CL, Comparison of Tzanck smear, viral culture, and DNA diagnostic methods in detection of herpes simplex and varicella-zoster infection. JAMA 1992;268:2541-4.
6. Oranje AP, Folkers E, The Tzanck smear: old, but still of inestimable value. Pediatr Dermatol 1988;5:127-9.
7. Grossman MC, Silvers DN, The Tzanck smear: can dermatologists accurately interpret it? J Am Acad Dermatol 1992; 27:403-5.
8. Fan F, Day S, Lu X, Tang YW, Laboratory diagnosis of HSV and varicella zoster virus infections. Future Virology 2014;9:721-31.
9. Sadick NS, Swenson PD, Kaufman RL, Kaplan MH, Comparison of detection of varicella-zoster virus by the Tzanck smear, direct immunofluorescence with a monoclonal antibody, and virus isolation. J Am Acad Dermatol 1987;17:64-69.
10. Ozcan A, Senol M, Saglam H, Seyhan M, Durmaz R, Aktas E, et al. Comparison of the Tzanck test and polymerase chain reaction in the diagnosis of cutaneous herpes simplex and varicella zoster virus infections. Int J Dermatol 2007;46:1177-9.

Predictors of Mortality among Inter-Hospital Transferred Patients in a Middle-Income Country: a Retrospective Cohort Study

Parin Rattananon, M.D.*, Isara Yenyuwadee, M.D., Thanchanok Dheeradilok, M.D.**, Parichaya Boonsoong, M.D.*, Nintita Sripaiboonkij Thokanit, Dr.PH***, Salinthip Chimdist, M.D.*, Tawin Siwanuwatn, M.D.*, Sidsadeeya Yuwapoom, M.D.*, Paibul Suriyawongpaisal, M.D.******

**Prachuap Khiri Khan Hospital, Ministry of Public Health, Prachuap Khiri Khan 77000, Thailand, **Thap Sakae Hospital, Ministry of Public Health, Prachuap Khiri Khan 77000, Thailand, ***Ramathibodi Comprehensive Cancer Center, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand, ****Department of Community Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand.*

ABSTRACT

Objective: To identify predictors for hospital mortality among inter-hospital transferred patients in low-resource settings of rural hospitals in Thailand.

Methods: We conducted a retrospective cohort study of patients transferred from emergency room(ER) of a community hospital to its designated tertiary care hospital in a western province of Thailand. During March 2018 and February 2019, medical records of 412 patients were reviewed and extracted for potential predictor variables and outcomes. We defined deaths within 72 hrs after a transfer as primary outcome and overall hospital mortality as secondary outcome. Multivariate logistic regression analysis was performed to identify predictors of the outcomes adjusted for potential confounders.

Results: Out of 412 patients, a total of 37 patients (9.0%) died during the stay in receiving hospital and 18 (4.4%) of them died within 72 hrs after transfer. Top ten primary diagnostic categories included road traffic injuries (19.7%), acute appendicitis (9.7%), and acute myocardial infarction (5.1%). Univariate analysis revealed early mortality (<72 hrs) was associated with NEWS2, Emergency Severity Index (ESI), cardiac arrest prior to transfer, use of vasoactive agents, endotracheal intubation and admitting service. Using multiple logistic regression model adjusted for the predictors identified by univariate analysis, we found early mortality was independently associated with NEWS2 ≥ 9 (compared to NEWS2 0-6) with OR= 17.51(95%CI 3.16-97.00) and vasoactive medication use (OR= 5.46, 95%CI 1.39-21.46). Similarly, overall mortality was also independently associated with NEWS2 ≥ 9(OR= 4.76, 95%CI 1.31–17.36) and vasoactive medication use (OR= 7.51,95%CI 2.76 -20.45).

Conclusion: This study identified predictors of early (<72 hrs) hospital mortality and overall hospital mortality among ER patients transferred from a rural community hospital to its designated tertiary care hospital in Thailand, a middle-income country with universal healthcare coverage. The findings might be helpful to inform decision-making dealing with the inter-hospital transfer of ER patients in resource-poor rural settings with similar case-mix.

Keywords: Patient transfer; critical illness; prognosis; mortality (Siriraj Med J 2021; 73: 312-321)

INTRODUCTION

Inter-hospital transfer(IHT) is considered a complex and challenging practice, requiring multiple resources and coordination from varied healthcare providers.[1] The transitional process is vulnerable for discontinuity error, combining with restricted resources outside hospital

Corresponding author: Parin Rattananon
E-mail: parin.rt@gmail.com
Received 15 October 2020 Revised 1 April 2021 Accepted 2 April 2021
ORCID ID: http://orcid.org/0000-0002-1911-8987
http://dx.doi.org/10.33192/Smj.2021.41

settings during transport, IHT patients are at risk of adverse events and unsatisfied outcomes.[2]

Additional to the systemic threats, growing evidence demonstrated higher acute severity, a longer length of stay, higher hospital mortality and higher resources use in IHT patients when compared to non-IHT cases.[3-7] These undesirable outcomes of IHT patients could be due to heterogeneity among IHT patients depending on the diagnosis, presenting a nuanced assessment of this complex care transition.[8] Variability in transfer practices means ambiguity and subjectivity in decision making between transferring physicians and receiving physicians.[9,10] Standardization of the care processes is considered a means to minimize the variability, which is amenable to improving the quality of care among IHT patients.[11]

According to earlier studies, prognostic factors for early death (<72 hrs) included male gender, summer season, admitting service, diagnostic related group level, Charlson Comorbidity Score, insurance type, and major diagnostic category. For overall hospital mortality, prognostic factors included length of stay, medical complication, distance traveled, insurance type, and major diagnostic category.[5,6,8] Application of such knowledge in overcrowded emergency room (ER) settings is a challenge.

As a result several triage systems have been proposed and were found to be significantly related with admission rate and medical resource consumption.[4,5] According to previous reports, triage systems such as Acute Physiology and Chronic Health Evaluation (APACHE) or Sequential Organ Failure Assessment (SOFA) were frequently applied to estimate disease severity in IHT patients.[4,5,12,13] However, some parameters (e.g., arterial oxygenation and blood pH) in these scoring systems may not be available at ER of rural community hospital settings where resources are limited.

In Thailand, many hospitals, especially in rural areas, have no standardized decision-support and communication tool during patient transfer. Even in a similar patient, management decisions may differ as there is variation in clinical practices among physicians. This study intends to identify predictors of IHT patients using basic parameters, which are generally available at ER of rural community hospitals in Thailand. The expected findings might be useful to facilitate patient care during IHT.

MATERIALS AND METHODS

This study was approved by the Office for Research Ethics Committee of Hua Hin Hospital, Prachuap Khiri Khan, Thailand (RECHHH145/2019).

Setting

Our study involved ER patients transferred from a community hospital to its designated tertiary care hospital in a western province of Thailand, a middle-income country with universal healthcare coverage. The community hospital is a 60-bed public hospital (No intensive care bed) staffed with 1 pediatrician, 7 general practitioner physicians, 5 pharmacists, and 54 nurses. Four ambulances equipped with an oxygen tank, suction, blood pressure monitor, and a defibrillator. are available for IHT and Emergency Medical Services. At ER of the community hospital, there are 1 physician, 3 ER nurses, and 2 assistant nurses for each 8-hour shift. The estimated nurse-to-patient ratio in the ER is 1 to 9. The estimated annual number of IHT patients from ER and inpatient care are 750. The receiving hospital is a 278-bed (12 intensive care beds) tertiary hospital staffed with 4 internists, 1 gastroenterologist, 1 nephrologist, 4 general surgeons, 2 neurosurgeons, 3 orthopedic surgeons, 2 ophthalmologists, 3 obstetricians, and 2 pediatricians. The distance between the two hospitals is 43 kilometers, with an average ground transport time of 30 minutes. When a transfer decision is determined, a primary care doctor will contact the transfer operation center in the receiving hospital. After receiving the referral request, the center, operated by registered nurses, will notice the specialist and present all the patient information. The teleconsultant will be provided for initial management. If the referral request is accepted, the patient will be transported to the emergency department (ED) of the tertiary hospital, where the patient's conditions are reevaluated before a decision for hospitalization. ER patients deemed a need for IHT are accompanied by an ambulance staffed with a nurse and a nurse assistant. As there is no clinician accompanies the ambulance, the emergency patient needs to be stabilized enough before transfer.

Study design

A retrospective cohort study was conducted during March 2018 and February 2019. We included adult patients aged 16 or above who were transferred from ER of the transferring hospital and hospitalized at the tertiary care hospital. We excluded obstetric patients, pediatric patients, IHT patients not hospitalized at the receiving hospital and patients with incomplete data. Patients with multiple transfers were considered the same episode.

The authors, working independently in two teams, reviewed all the extracted data from electronic and/or paper-based medical records using a standard data form.

The first team, working as primary care doctor in the community hospital, documented patients' characteristics consisting of demographics, health insurance status, primary diagnosis categories based on the International Statistical Classification of Diseases and Related Health Problems (ICD-10), underlying diseases, past medical history, physiological parameters and severity categories according to the Emergency Severity Index (ESI). The ESI is a five-level triage scale, ranging from level 5 (Non-urgent) to ESI level 1 (Resuscitative), based on patient acuity and resource needs.[14] The ESI system has been used primarily in Thailand for triaging ER patients.[15] National Early Warning Score 2 (NEWS2) for each patient was calculated from the physiological parameters on arrival at the ER to represent acute severity index of IHT patients. This aggregated scoring system is built from six basic parameters including respiratory rate, oxygen saturation, temperature, systolic blood pressure, heart rate, and level of consciousness.[16] Underlying diseases and past medical history were reviewed and calculated into the Charlson's comorbidity score.[17] Apart from those variables, the following were also included: events before the transfer (cardiac arrest, use of vasoactive drugs, and endotracheal intubation); transfer time in minutes (starting from a patient's arrival at the transferring hospital until admission at the receiving hospital). The second team, working as a general practitioner at the receiving hospital, extracted patient outcomes from electronic health records, consisting of diagnosis based on ICD-10, length of stay, and discharge status. Within 72-hour mortality after IHT was considered primary outcome and overall hospital mortality as secondary outcome.

Data analysis

Data analysis was conducted using STATA statistical software version 14. Continuous and categorical variables were presented as means with standard deviation (SD) and as frequencies with percentages, respectively. To identify potential predictors, patient characteristics of those with or without the outcomes were compared using Student's t-test for continuous variables and Chi-square test for categorical variables.

Multivariate logistic regression models using backward stepwise regression for variables selection were developed to identify predictors of the outcomes. Parameters associated with a p-value below 0.25 were included in the initial model. Highly related parameters were removed to diminish multicollinearity. Least significant factors were deleted one by one according to a backward elimination algorithm until reaching the final models. The receiver operating characteristic curve (ROC) was developed with a calculated area under the curve(AUC) to inform model performance. P-values (*p*) less than 0.05 were considered as statistically significant.

RESULTS

There were 519 patients transferred from ER of the community hospital to the designated receiving hospital during the study period (Fig 1). After applying the inclusion and exclusion criteria, 412 patients were entered into the study. Among them, 11 patients revisited ER of the transferring hospital and were re-hospitalized to the tertiary hospital twice, and 3 more patients faced these experiences for three times. Thirty-seven patients (9.0%) died upon discharge, half of them died within

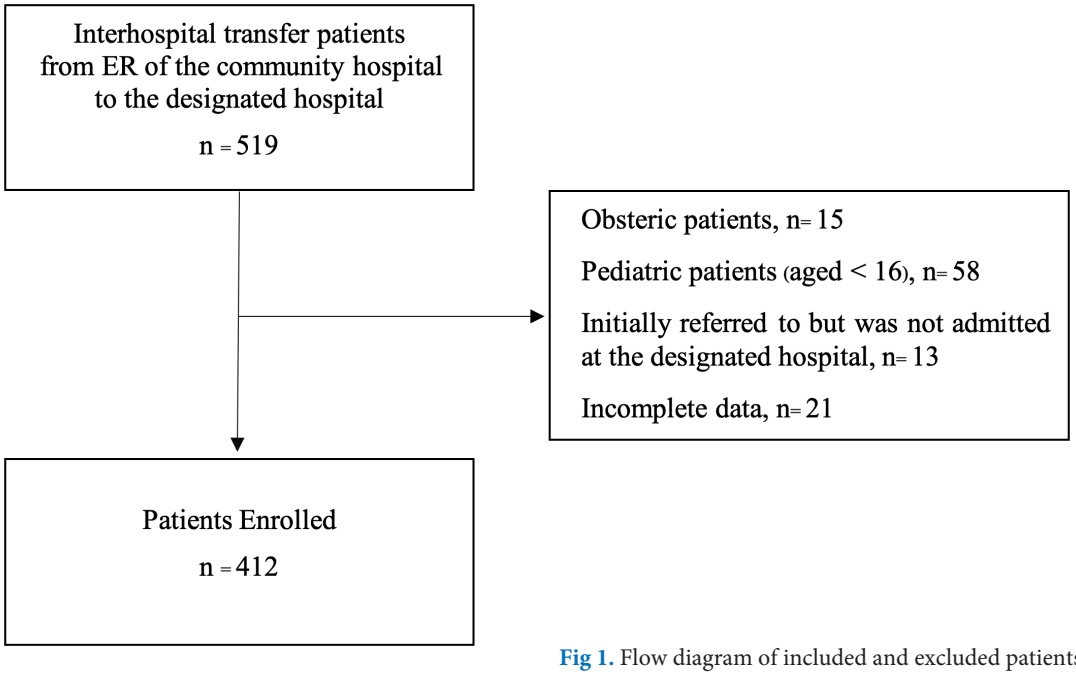


Fig 1. Flow diagram of included and excluded patients.

three days after a transfer). Thirty-eight patients were discharged home or transferred back to the community hospital or transferred to a higher-level hospital within 72 hrs of the admission.

Out of 412 patients, a total of 37 patients (9.0%) died during the stay in receiving hospital and 18 (4.4%) of them died within 72 hrs after transfer (Table 1). Table 2 demonstrates top ten primary diagnostic categories including road traffic injuries (19.7%), acute appendicitis (9.7%), and acute myocardial infarction (5.1%). Univariate analysis (Table 1) reveals early mortality (<72 hrs) was associated with NEWS2, Emergency Severity Index (ESI), cardiac arrest prior to transfer, use of vasoactive agents, endotracheal intubation and admitting service. For overall mortality, univariate analysis identified age and Charlson's co-morbidity score as predictors in addition to those for early mortality. Using multiple logistic regression model adjusted for the predictors identified by univariate analysis (Table 3), we found early mortality was independently associated with NEWS2 ≥ 9 (compared to NEWS2 0-6) with OR= 17.51(95%CI 3.16 – 97.00) and use of vasoactive medication (OR= 5.46, 95%CI 1.39-21.46). Similarly, overall mortality was also independently associated with NEWS2 ≥ 9(OR= 4.76, 95%CI 1.31 – 17.36) and use of vasoactive medications (OR= 7.51,95%CI 2.76 – 20.45) (Table 4). Performance of the multivariate models were validated with AUC 0.91 (95% CI 0.82-0.99) for the first model (Table 3) and 0.88 (95% CI 0.83-0.94) for the second model (Table 4).

DISCUSSION

Applying multiple logistic regression analysis to the cohort data (N=412), we were able to identify two independent predictors for early mortality: NEWS2 score ≥ 9 (OR: 17.51; 95% CI 3.16-97.00, p=0.001) and vasoactive agent use (OR 5.46; 95% CI 1.39-21.46, p=0.015). NEWS2 is used internationally as an early warning score for triaging in ER and monitoring hospitalized patients. From the Royal College of Physicians report, the aggregated score of 7 or more is defined as a threshold for emergency response, and patient transfer to a higher setting facility should be considered.[16] Our findings are comparable with previous studies that reported high acute severity index and events such as cardiac arrest, mechanical ventilation, and vasoactive drug use as mortality predictor in IHT patients.[12,13,18] With ROC 0.91(95% CI 0.82-0.99), our model performs as high as that of other studies in HICs and LMICs, although the results, in this regard, may not be directly comparable given different sets of predictors and study settings.[19,20]

The predictors discovered from our study allow healthcare providers to estimate the severity of the ER patients who might need transfer to other hospitals capable of providing definitive care. Scoring systems such as NEWS2 provided a standardized tool for clinical monitoring and assessment. By combining physiological variables into scores, it reduces variation in assessing patient status among healthcare professionals. Several triage systems, including ESI, have been developed for use in the ER. However, they are not designed to detect deterioration in patients.[21] NEWS can further risk stratifying patients within higher ESI risk categories, both for death and need for admission.[22] Patients with a high NEWS score have not only been identified as being at risk of a poor outcome but have already physiologically deteriorated to the extent where urgent medical review and intervention is required. With a common scoring system between facilities, it also functions as a standard language in communication on patient's clinical acuity.[23]

Out of 412 transfer patients (mean age 53) from the transferring hospital to the receiving hospital (43 km apart), 9.0% died upon discharge with a half died within 72-h after the transfer. We could not identify other studies in a similar setting both in high-income countries (HICs) and low-middle income countries (LMICs) for mortality comparison. Our overall-mortality figure is, at most, one-third of the reported figures from several other studies dealing with intensive care patients.[12,24] This indicates our patients were in much less critical conditions than those in other studies. Finally, similar to findings from other studies[7,8], the patients' profiles of our study were heterogeneous (Table 2).

In our study, we found no association between transfer time and patient mortality, which is compatible with previous similar studies.[12,13] As suggested from many guidelines for the interfacility transport, our finding also supports a "stabilize and shift" approach rather than a "scoop and run" strategy.[25-27] However, even though there is no significant relationship between transfer times and hospital mortality, some studies have demonstrated the benefit of appropriate, timely referrals in lessening complications, length of stay, and morbidity of IHT patients.[28,29] Additionally, certain diseases such as ST-segment elevation myocardial infarction or expanding intracranial hematoma, are considered as time-sensitive emergency conditions.[30,31] Delays to definite treatment in such diseases could result in lethal outcomes. We conclude that, in general, critically ill patients should be resuscitated until achieving possibly maximum stabilization by the referring hospital before the interhospital transport without unnecessary delays.

TABLE 1. Patient characteristics and admitting service categorized by the outcome status.

Variables	All patients (*n* = 412)	Within 72 hrs Alive (*n* = 394)	Dead (*n* = 18)	p-value	Overall Alive (*n* = 375)	In-hospital Death (*n* = 37)	p-value
Patient characteristics							
Age, mean years (±SD)	53 (±20)	53 (±20)	59 (±20)	0.18	52 (±19)	64 (±19)	<0.001
Gender, male, n (%)	245 (59.5)	235 (59.6)	10 (55.6)	0.73	220 (58.7)	25 (67.6)	0.293
Health insurance status, n (%)				0.733			0.204
Universal Coverage	268 (65.1)	254 (64.5)	14 (77.8)		235 (62.7)	30 (81.1)	
Compulsory Motor Insurance	78 (18.9)	75 (19.0)	3 (16.7)		77 (20.5)	4 (10.8)	
Social Security Scheme	17 (4.1)	17 (4.3)	0 (0.0)		17 (4.5)	0 (0.0)	
CSMBS	42 (10.2)	41 (10.4)	1 (5.6)		39 (10.4)	3 (8.1)	
Out-of-pocket	7 (1.7)	7 (1.8)	0 (0.0)		7 (1.9)	0 (0.0)	
Transfer time, mean minutes (±SD)	226 (±97)	227 (±98)	212 (±74)	0.531	226 (±98)	232 (±81)	0.733
Charlson's co-morbidity score, n (%)				0.533	19 (5.1)	0 (0.0)	0.002
0 149 (36.2)	145 (36.8)	4 (22.2)		144 (38.4)	5 (13.5)		
1-2	137 (33.3)	131 (33.3)	6 (33.3)		125 (33.3)	12 (32.4)	
3-4	94 (22.8)	88 (22.3)	6 (33.3)		81 (21.6)	13 (35.1)	
>4	32 (7.8)	30 (7.6)	2 (11.1)		25 (6.7)	7 (18.9)	
NEWS2, mean (±SD)	4 (±4)	3 (±3)	12 (±4)	<0.001	3 (±3)	9 (±4)	<0.001

TABLE 1. Patient characteristics and admitting service categorized by the outcome status. (Continue)

Variables	All patients (*n* = 412)	Within 72 hrs Alive (*n* = 394)	Dead (*n* = 18)	p-value	Overall Alive (*n* = 375)	In-hospital Death (*n* = 37)	p-value
The ESI (Level of urgency), n (%)				<0.001			<0.001
1 (Resuscitative)	35 (8.5)	27 (6.9)	8 (44.4)		22 (5.9)	13 (35.1)	
2 (Emergent)	101 (24.5)	95 (24.1)	6 (33.3)		86 (22.9)	15 (40.5)	
3 (Urgent)	161 (39.1)	157 (39.9)	4 (22.2)		153 (40.8)	8 (21.6)	
4 (Less urgent)	111 (26.9)	111(28.2)	0 (0.0)		110 (29.3)	1 (2.7)	
5 (Non-urgent)	4 (1.0)	4 (1.0)	0 (0.0)		4 (1.1)	0 (0.0)	
Cardiac arrest prior to transfer, yes (%)	8 (1.9)	2 (0.5)	6 (33.3)	<0.001	1 (0.3)	7 (18.9)	<0.001
Any vasoactive agent, yes (%)	32 (7.8)	20 (5.1)	12 (66.7)	<0.001	13 (3.5)	17 (46.0)	<0.001
Endotracheal intubation prior to transfer, yes (%)	68 (16.5)	55 (14.0)	13 (72.2)	<0.001	46 (12.3)	22 (59.5)	<0.001
Admitting service							
Inpatient department, n (%)				0.003			<0.001
Internal Medicine	136 (33.0)	123 (31.2)	13 (72.2)		108 (28.8)	27 (73.0)	
General Surgery	161 (39.1)	159 (40.4)	2 (11.1)		96 (25.6)	5 (13.5)	
Neurosurgery	49 (11.9)	46 (11.7)	3 (16.7)		74 (19.7)	4 (10.8)	
Orthopedic	47 (11.4)	47 (11.9)	0 (0.0)		42 (11.2)	1 (2.7)	
Others*	19 (4.6)	19 (4.8)	0 (0.0)		36 (9.6)	0 (0.0)	

Abbreviations: CSMBS, Civil Servant Medical Benefit Scheme; ESI, Emergency Severity Index; ETT, Endotracheal tube; NEWS2, National Early Warning Score 2; SD, Standard deviation.
* Others include Gynecology, Ophthalmology, and Otorhinolaryngology

Supplementary Table 1. Characteristics of study patients according to mortality status within the same admission after transfer.

Variables	All patients (n = 412)	Alive (n = 375)	In-hospital Death (n = 37)	*p*-Value
Age, mean years (±SD)	53 (±20)	52 (±19)	64 (±19)	<0.001
Gender, male (%)	245 (59.5)	220 (58.7)	25 (67.6)	0.293
Health Insurance status, n (%)				0.234
Universal Coverage	268 (65.1)	238 (63.5)	30 (81.1)	
Compulsory Motor Insurance	78 (18.9)	74 (19.7)	4 (10.8)	
Social Security Scheme	17 (4.1)	17 (4.5)	0 (0.0)	
CSMBS	42 (10.2)	39 (10.4)	3 (8.1)	
Out-of-pocket	7 (1.7)	7 (1.9)	0 (0.0)	
Transfer time, mean minutes (±SD)	226 (±97)	226 (±98)	232 (±81)	0.733
Inpatient department, n (%)				<0.001
Internal Medicine	136 (33.0)	109 (29.1)	27 (73.0)	
General Surgery	161 (39.1)	154 (41.1)	7 (18.9)	
Neurosurgery	49 (11.9)	46 (12.3)	3 (8.1)	
Orthropedic	47 (11.4)	47 (12.5)	0 (0.0)	
Others*	19 (4.6)	19 (5.1)	0 (0.0)	
Charlson's co-morbidity score, n (%)				0.002
0	149 (36.2)	144 (38.4)	5 (13.5)	
1-2	137 (33.3)	125 (33.3)	12 (32.4)	
3-4	94 (22.8)	81 (21.6)	13 (35.1)	
>4	32 (7.8)	25 (6.7)	7 (18.9)	
NEWS2, mean (±SD)	4 (±4)	3 (±3)	10 (±4)	<0.001
ESI scores (Level of urgency), n (%)				<0.001
1 (Resuscitative)	35 (8.5)	22 (5.9)	13 (35.1)	
2 (Emergent)	101 (24.5)	86 (22.9)	15 (40.5)	
3 (Urgent)	161 (39.1)	153 (40.8)	8 (21.6)	
4 (Less urgent)	111 (26.9)	110 (29.3)	1 (2.7)	
5 (Non-urgent)	4 (1.0)	4 (1.1)	0 (0.0)	
Cardiac arrest prior to transfer, yes (%)	8 (1.9)	1 (0.3)	7 (18.9)	<0.001
Any vasoactive agent, yes (%)	32 (7.8)	14 (3.7)	18 (48.7)	<0.001
ETT insertion prior to transfer, yes (%)	68 (16.5)	46 (12.3)	22 (59.5)	<0.001

Abbreviations: CSMBS, Civil Servant Medical Benefit Scheme; ESI, Emergency Severity Index; ETT, Endotracheal tube; NEWS2, National Early Warning Score 2; SD, Standard deviation.

* Others include Gynecology, Ophthalmology, and Otorhinolaryngology.

TABLE 2. Most common primary diagnoses according to ICD-10.

Primary diagnostic categories with ICD-10 All patients (n = 412)	Early mortality*, n (%)		Overall mortality, n (%)	
	Alive (n = 394)	Dead (n = 18)	Alive (n = 375)	Death (n = 37)
C15-C26 Malignant neoplasms of digestive organs (n=9, 2.2%)	9 (2.3)	0 (0.0)	7 (1.9)	2 (5.4)
I21 Acute myocardial infarction (n=21, 5.1%)	19 (4.8)	2 (11.1)	16 (4.3)	5 (13.5)
I61 Intracerebral haemorrhage (n=20, 4.9%)	20 (5.1)	0 (0.0)	20 (5.3)	0 (0.0)
I63 Cerebral infarction (n=16, 3.9%)	16 (4.1)	0 (0.0)	16 (4.3)	0 (0.0)
J12-J18 Pneumonia (n=15, 3.6%)	13 (3.3)	2 (11.1)	11 (2.9)	4 (10.8)
K27 Gastric ulcer with perforation (n=9, 2.2%)	9 (2.3)	0 (0.0)	9 (2.4)	0 (0.0)
K35 Acute appendicitis (n=40, 9.7%)	40 (10.2)	0 (0.0)	40 (10.7)	0 (0.0)
K92.2 Gastrointestinal haemorrhage, unspecified (n=17, 4.1%)	17 (4.3)	0 (0.0)	17 (4.5)	0 (0.0)
S72 Fracture of femur (n=11, 2.7%)	11 (2.8)	0 (0.0)	11 (2.9)	0 (0.0)
V01-V99 Road traffic injuries (n=81, 19.7%)	78 (19.8)	3 (16.7)	77 (20.5)	4 (10.8)
Other diagnoses (n=173, 42.0%)	162 (41.1)	11 (61.1)	151 (40.3)	22 (59.5)

ICD-10, the International Statistical Classification of Diseases and Related Health Problems.
* Defined as death within 72 hrs after an inter-hospital transfer

TABLE 3. Multivariate logistic regression analysis of factors associated with early mortality (< 72 hrs) (n = 412).

Variables	OR	95% CI	*p*
NEWS2			
7-8 vs. 0-6	6.61	0.77-56.62	0.085
≥ 9 vs. 0-6	17.51	3.16-97.00	0.001
Cardiac arrest prior to transfer	5.37	0.79-36.54	0.086
Vasoactive agent use			
Yes vs. No	5.46	1.39-21.46	0.015

Abbreviations: NEWS2, National Early Warning Score 2; OR, Odds ratio; *p*, p-value

TABLE 4. Multivariate logistic regression analysis of factors associated with overall mortality (n = 412).

Variables	OR	95% CI	*p*
NEWS2			
7-8 vs. 0-6	1.49	0.32-6.84	0.608
≥ 9 vs. 0-6	4.76	1.31-17.36	0.018
Age	1.02	1.00-1.05	0.076
Endotracheal intubation prior to transfer	2.28	0.73-7.17	0.158
Vasoactive agent use			
Yes vs. No	7.51	2.76-20.45	<0.001

Abbreviations: NEWS2, National Early Warning Score 2; OR, Odds ratio; *p*, p-value

Another interesting finding from our study is an apparent degree of unplanned ER revisits and re-transfers. These events may be explained either by the nature and severity of individual diseases or inappropriate post-discharge follow-up care. Because most patients would receive follow-up care after discharge at their transferring hospital, appropriateness of discharge communication about a follow-up plan from the receiving hospital could improve the quality of care at the transferring hospital.[32] Future studies should explore deeper to clarify the causes of repeated transfers in our area.

Our present study has three potential limitations which need consideration. Firstly, this study was conducted in a single hospital in a rural area of Thailand and its designated tertiary care hospital. Patient characteristics and performance in transfer practices may be different from other hospital settings. For this reason, external validity is uncertain, so results from this research should be carefully examined before application. Secondly, the number of included patients in the retrospective cohort may not be large enough, as indicated by wide confidence intervals. With a small sample size, the power of tests may not be sufficient to detect a statistically significant association in some clinically relevant parameters. Lastly, we have not accounted for adverse incidents during inter-hospital transport as a predictor variable in our study due to inaccessible data and/or unavailability of data. Those unexpected events are common during transport and could greatly influence the outcomes in critically ill patients.[33] Hence, further studies are needed to explore this key area of healthcare with complexity, which is understudied, especially in LMICs.

CONCLUSION

To our best knowledge, our study may be the first demonstrating outcome predictors of inter-hospital transfer patients in Thailand and low- and middle-income countries. We managed to identify predictors of hospital mortality for transfer patients from a rural hospital ER to a receiving hospital i.e., high NEWS2 scores and use of vasoactive agents. These factors could be used to standardize rationale and clinical care processes in ER patients transferred from rural community hospitals to other hospitals capable of providing definitive care. With NEWS2 included among the predictors, we were able to suggest using NEWS2 as a value-added tool to better monitoring of the patients' status during the transfer and facilitate a mutual agreement between clinicians.

ACKNOWLEDGMENTS

The authors want to express gratitude to Dr. Supaporn Pamonsut, a hospital director at Thap Sakae Hospital, Prachuap Khiri Khan, Thailand, for providing us valued comments. We also would like to thank Mr. Stephen Pinder, a Medical Education/ Medical English specialist at the Department of Clinical Epidemiology and Biostatistics, Ramathibodi Hospital, Mahidol University, for proof reading and grammar correction of our manuscript.

Data availability: The datasets used to support the findings of this study are available upon request.

Conflicts of interest: The authors state that they have no Conflict of Interest (COI).

Funding statement: This research received no external funding.

REFERENCES

1. Kiss T, Bolke A, Spieth PM. Interhospital transfer of critically ill patients. Minerva Anestesiol 2017;83:1101-8.
2. Eiding H, Kongsgaard UE, Braarud AC. Interhospital transport of critically ill patients: experiences and challenges, a qualitative study. Scand J Trauma Resusc Emerg Med 2019;27:27.
3. Sokol-Hessner L, White AA, Davis KF, Herzig SJ, Hohmann SF. Interhospital transfer patients discharged by academic hospitalists and general internists: Characteristics and outcomes. J Hosp Med 2016;11:245-50.
4. Golestanian E, Scruggs JE, Gangnon RE, Mak RP, Wood KE. Effect of interhospital transfer on resource utilization and outcomes at a tertiary care referral center. Crit Care Med 2007; 35:1470-6.
5. Hill AD, Vingilis E, Martin CM, Hartford K, Speechley KN. Interhospital transfer of critically ill patients: demographic and outcomes comparison with nontransferred intensive care unit patients. J Crit Care 2007;22:290-5.
6. Hernandez-Boussard T, Davies S, McDonald K, Wang NE. Interhospital Facility Transfers in the United States: A Nationwide Outcomes Study. J Patient Saf 2017;13:187-91.
7. Mueller SK, Zheng J, Orav J, Schnipper JL. Interhospital Transfer and Receipt of Specialty Procedures. J Hosp Med 2018;13: 383-7.
8. Mueller S, Zheng J, Orav EJ, Schnipper JL. Inter-hospital transfer and patient outcomes: a retrospective cohort study. BMJ Qual Saf 2019;28:e1.
9. Bosk EA, Veinot T, Iwashyna TJ. Which patients and where: a qualitative study of patient transfers from community hospitals. Med Care 2011;49:592-8.
10. Wagner J, Iwashyna TJ, Kahn JM. Reasons underlying interhospital transfers to an academic medical intensive care unit. J Crit Care 2013;28:202-8.
11. Mueller SK, Zheng J, Orav EJ, Schnipper JL. Rates, Predictors and Variability of Interhospital Transfers: A National Evaluation. J Hosp Med 2017;12:435-42.
12. Strauch U, Bergmans DC, Winkens B, Roekaerts PM. Short-term outcomes and mortality after interhospital intensive care transportation: an observational prospective cohort study of 368 consecutive transports with a mobile intensive care unit. BMJ Open 2015;5:e006801.
13. Patel JJ, Kurman J, Al-Ghandour E, Thandra K, Mawari S, Graf J, et al. Predictors of 24-h mortality after inter-hospital transfer to a tertiary medical intensive care unit. J Intensive Care Soc 2018;19:319-25.
14. Gilboy N, Tanabe T, Travers D, Rosenau AM. Emergency Severity Index (ESI): A triage tool for emergency department. Rockville, MD: Agency for Healthcare Research and Quality, 2011.
15. Wachiradilok P, Sethasathien A, Sirisamutr T, Chaiyasit S. A National Survey of Emergency Departments Triage Systems in Thailand. Prehospital and Disaster Medicine. 2017;32(S1):S233-S233.
16. Physicians RCo. National Early Warning Score (NEWS) 2. Standardising the assessment of acute-illness severity in the NHS. Updated report of a working party. RCP London, 2017.
17. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373-83.
18. Durairaj L, Will JG, Torner JC, Doebbeling BN. Prognostic factors for mortality following interhospital transfers to the medical intensive care unit of a tertiary referral center. Crit Care Med 2003;31:1981-6.
19. Haniffa R, Isaam I, De Silva AP, Dondorp AM, De Keizer NF. Performance of critical care prognostic scoring systems in low and middle-income countries: a systematic review. Crit Care 2018;22:18.
20. Knaus WA, Zimmerman JE, Wagner DP, Draper EA, Lawrence DE. APACHE-acute physiology and chronic health evaluation: a physiologically based classification system. Crit Care Med 1981; 9:591-7.
21. Ludikhuize J, Smorenburg SM, de Rooij SE, de Jonge E. Identification of deteriorating patients on general wards; measurement of vital parameters and potential effectiveness of the Modified Early Warning Score. J Crit Care 2012;27:424. e7-13.
22. Alam N, Vegting IL, Houben E, van Berkel B, Vaughan L, Kramer MHH, et al. Exploring the performance of the National Early Warning Score (NEWS) in a European emergency department. Resuscitation 2015;90:111-5.
23. Brangan E, Banks J, Brant H, Pullyblank A, Le Roux H, Redwood S. Using the National Early Warning Score (NEWS) outside acute hospital settings: a qualitative study of staff experiences in the West of England. BMJ Open 2018;8:e022528-e022528.
24. Riviello ED, Kiviri W, Fowler RA, Mueller A, Novack V, Banner-Goodspeed VM, et al. Predicting Mortality in Low-Income Country ICUs: The Rwanda Mortality Probability Model (R-MPM). PLoS One 2016;11:e0155858.
25. Warren J, Fromm RE, Jr., Orr RA, Rotello LC, Horst HM, American College of Critical Care M. Guidelines for the inter- and intrahospital transport of critically ill patients. Crit Care Med 2004;32:256-62.
26. Sethi D, Subramanian S. When place and time matter: How to conduct safe inter-hospital transfer of patients. Saudi J Anaesth 2014;8:104-13.
27. Kulshrestha A, Singh J. Inter-hospital and intra-hospital patient transfer: Recent concepts. Indian J Anaesth 2016;60:451-7.
28. Duke GJ, Green JV. Outcome of critically ill patients undergoing interhospital transfer. Med J Aust 2001;174:122-5.
29. Catalano AR, Winn HR, Gordon E, Frontera JA. Impact of interhospital transfer on complications and outcome after intracranial hemorrhage. Neurocrit Care 2012;17:324-33.
30. Scholz KH, Maier SKG, Maier LS, Lengerfelder B, Jacobshagen C, Jung J, et al. Impact of treatment delay on mortality in ST-segment elevation myocardial infarction (STEMI) patients presenting with and without haemodynamic instability: results from the German prospective, multicentre FITT-STEMI trial. Eur Heart J 2018;39:1065-74.
31. Seelig JM, Becker DP, Miller JD, Greenberg RP, Ward JD, Choi SC. Traumatic acute subdural hematoma: major mortality reduction in comatose patients treated within four hours. N Engl J Med 1981;304:1511-8.
32. Organization WH. Management of health facilities: Referral systems. World Health Organization, 2014.
33. Gray A, Gill S, Airey M, Williams R. Descriptive epidemiology of adult critical care transfers from the emergency department. Emerg Med J 2003;20:242-6.

Accuracy of Third Trimester Ultrasound for Predicting Large-for-Gestational Age Newborn in Women with Gestational Diabetes Mellitus

Pornpimol Ruangvutilert, M.D., Ph.D., Thanapa Rekhawasin, M.D., Chayawat Phatihattakorn, M.D., Dittakarn Boriboonhirunsarn, M.D., Ph.D.

Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

ABSTRACT

Objective: To determine the accuracy of ultrasonography during 32-36 weeks of gestation for predicting a large-for-gestational-age (LGA) newborn in women with gestational diabetes mellitus (GDM).

Materials and Methods: Women with singleton pregnancy, aged ≥ 18 years old and diagnosed with GDM were recruited. They underwent ultrasonography at 32-36 weeks' gestation for fetal biometry, namely, biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL). Estimated fetal weight (EFW) was derived from these 4 parameters by Hadlock formula. Delivery of an LGA newborn in women with the ultrasound finding of LGA fetus was the primary outcome of interest along with determination of predicting factors.

Results: Of 345 studied women, 107 (31%) had an LGA newborn. EFW of ≥ 90th percentile at third trimester ultrasonography was found in 13 women, all of whom had an LGA newborn. It had a positive predictive value (PPV), specificity, sensitivity and negative predictive value (NPV) of 100%, 100%, 12.1% and 71.7% respectively to predict LGA at birth. Considering each fetal parameter individually, AC ≥ 90th percentile and HC ≥ 90th percentile had odds ratios (OR) with 95% confidence intervals of the newborn being LGA of 6.5 (3.3-12.8) and 2.0 (1.0-4.0) respectively while EFW ≥ 85th percentile had the highest OR of 9.3 (1.1-77.9). Lowering cutoff values of EFW to 80th and 70th percentile increased the sensitivity and NPV for prediction of LGA at birth while reducing the PPV and specificity slightly.

Conclusion: EFW derived from the third trimester ultrasonography in women with GDM had high PPV and specificity with low to moderate sensitivity and NPV to predict an LGA newborn in women with GDM.

Keywords: Estimated fetal weight; third trimester ultrasound; large-for-gestational age newborn; gestational diabetes mellitus (Siriraj Med J 2021; 73: 322-329)

INTRODUCTION

Gestational diabetes mellitus (GDM) is a condition diagnosed during pregnancy associated with a lack of tolerance to increased blood glucose level.[1] Approximately 7% of all pregnancies are affected, with a worldwide incidence of more than 200,000 pregnancies annually.[2] During the past decade, the incidence of GDM in Siriraj Hospital, a Thailand national tertiary center, has increased from 2-3% to 10-15%.

GDM can cause adverse maternal and fetal/neonatal outcomes such as the need for cesarean delivery, cephalopelvic disproportion, postpartum hemorrhage,

Corresponding author: Chayawat Phatihattakorn
E-mail: naeobgyn@gmail.com
Received 19 January 2021 Revised 22 February 2021 Accepted 24 February 2021
ORCID ID: http://orcid.org/0000-0002-7433-5386
http://dx.doi.org/10.33192/Smj.2021.42

pregnancy-induced hypertension, large-for-gestational age (LGA) fetus, shoulder dystocia, neonatal hypoglycemia, and jaundice.[3,4] The incidence of LGA fetus in women with GDM was reported in the range of 15-20%.[3,5,6] The ability to diagnose LGA fetus in GDM women in advance would improve the management and outcomes of both women and their babies.

Ultrasonography in the third trimester was proven to be useful for predicting the actual birth weight.[7,8] Ultrasonography has been reported to help guide management and improve pregnancy outcomes in women with GDM.[9] However, to our knowledge, no study has addressed the accuracy of the third trimester ultrasound at 32-36 weeks' gestation, which is the period just after the maximal fetal growth rate, for predicting an LGA newborn in these women.

The current study was performed to determine the accuracy of ultrasound during 32-36 weeks' gestation for predicting LGA newborn in women with GDM.

MATERIALS AND METHODS

This prospective cohort study was performed at Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand during January 2017 to January 2018. Women aged ≥ 18 years with a singleton pregnancy at 32-36 weeks' gestation, diagnosed with GDM, and without known fetal anomalies were included. The study was approved by the Siriraj Institutional Review Board (SIRB) (Si 007/2017). Written informed consent was obtained from all women.

Gestational age was based on either crown-rump length in the first trimester or last menstrual period correlating with BPD in the second trimester. Screening for GDM with 50-g glucose challenge test (50-g GCT) was performed in pregnant women with any of the following risk factors: age ≥ 30 years old, BMI >25 kg/m^2, family history of diabetes mellitus, history of GDM in previous pregnancy, history of dead fetus in utero (DFIU), fetal anomaly or a macrosomic baby in a previous pregnancy[10]. Women with an abnormal 50-g GCT (≥ 140 mg/dl) underwent a 100-g oral glucose tolerance test (OGTT). According to Carpenter-Coustan criteria, GDM was diagnosed when two or more values were abnormal.

The women underwent ultrasound scanning using a machine with a 2-5 MHz curvilinear transabdominal transducer (Voluson E8; GE Healthcare, Zipf, Austria). Fetal biometry, namely, biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL), were measured by an experienced physician. With inappropriate fetal position or acoustic shadows, remeasurement was performed after a short break until standard planes were achieved in all pregnant women. Three measurements were obtained for each parameter and the averages were used to calculate the estimated fetal weight (EFW) by Hadlock formula.[10] EFW percentile was determined and was classified as small-for-gestational age (SGA) if the EFW was ≤ 10th percentile, LGA if the EFW was ≥ 90th percentile, and appropriate-for-gestational age (AGA) if the EFW was in the range between these two limits. Birth weight was classified as LGA (≥ 90th percentile) or SGA (≤ 10th percentile) status based on 2004-2008 WHO Global Survey on Maternal and Perinatal Health (WHOGS) data.[12] Macrosomia was defined when birth weight was 4,000 grams or more.

Body mass index (BMI) was categorized into four groups according to the 2009 Institute of Medicine (IOM)/National Research Council (NRC) guidelines as follows: underweight (BMI < 18.5 kg/m^2), normal (BMI 18.5-24.9 kg/m^2), overweight (BMI ≥ 25.0-29.9 kg/m^2), and obese (BMI ≥ 30.0 kg/m^2). Recommended total weight gain in each group is 13-18 kg, 11-16 kg, 7-11 kg, and 5-9 kg, respectively.[13] Overweight and obese groups were defined as high BMI.

GDM management started wth proper exercise and diet adjustment. Insulin would be added in cases uncontrollable by these two strategies. Glycemic follow-up checks were performed using either fasting blood sugar (FBS) (normal value: < 95 mg/dl) with two-hour postprandial (2-h PP) blood sugar (normal value: < 120 mg/dl) or 2-h PP alone. GDM diagnosed before 24 weeks of gestation was defined as early GDM, and GDM diagnosed after 24 weeks was defined as late GDM.[11]

Maternal complications, including gestational hypertension, preeclampsia, shoulder dystocia, 3rd or 4th degree laceration of birth canal, postpartum hemorrhage, and preterm delivery were recorded. Neonatal outcomes, including birth weight, birth asphyxia, subgaleal hematoma, hypoglycemia, polycythemia, jaundice, respiratory distress syndrome, and NICU admission, were also studied.

Statistical analysis

SPSS Statistics version 21 (SPSS, Inc., Chicago, IL, USA) was used for statistical analyses. Sample size was calculated based on the study of Scifres et al.[14], showing that the accuracy of third trimester ultrasound was 22.6% for predicting LGA newborn in women with GDM. With the error of 30% and loss of data of 10%, the required total sample size was 360.

Demographic data were summarized using descriptive statistics. Data are presented as number and percentage

for categorical variables, and mean ± standard deviation for continuous variables. Student's t-test or Chi-square test was used to compare patient data between groups. Results of multivariate analysis are shown as adjusted odds ratio and 95% confidence interval. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and cut-off value of third trimester ultrasound for predicting LGA newborn in women with GDM were also calculated. P-value of < 0.05 was considered statistically significant.

RESULTS

Of the 360 pregnant women initially recruited, 15 women were lost to follow-up and 345 women were included in the final analysis. The demographic and clinical characteristics of the women are shown in Table 1. Forty percent of the women had a high BMI. Almost two-thirds of women were diagnosed with GDM before 24 weeks' gestation. Approximately 90% of women did not need insulin therapy. The three most common risk factors for GDM were age ≥ 30 years old, BMI > 25 kg/m^2 and family history of diabetes mellitus.

Fetal parameters at 32-36 weeks' gestation are shown in Table 2. BPD, HC and FL of ≥ 90th percentile each accounted for 35.9-38.0% of women, and 20.3% of women had AC of ≥ 90th percentile. EFW of ≥ 90 percentile (LGA) was present in 13 (3.8%) fetuses.

Maternal and neonatal outcomes are described in Table 3. Forty-two percent of women were delivered vaginally while primary cesarean section was performed in 36.5%. Thirty-one percent of the neonates were LGA babies. The percentage of macrosomic newborns was 2.9%.

TABLE 1. Baseline demographic and clinical characteristics of study women (N = 345)

Characteristics	n (%)*
Age (years), mean ± SD	34.4 ± 10.7
BMI (kg/m^2), mean ± SD	24.7 ± 5.0
BMI classification	
Normal (18.5-24.9 kg/m^2)	207 (60.0)
Overweight (≥ 25 kg/m^2)	83 (24.1)
Obese (≥ 30 kg/m^2)	55 (15.9)
Nulliparity	141 (40.9)
GA at GDM diagnosis (weeks), mean ± SD	17.9 ± 9.1
Early GDM diagnosis	218 (63.2)
Well-controlled GDM	
Yes	290 (84.1)
No	55 (15.9)
GDM control	
Diet	308 (89.3)
Diet with insulin	37 (10.7)
Risk factors for GDM	
Age ≥ 30 years	286 (82.9)
BMI > 25 kg/m^2	138 (40.0)
Family history of diabetes mellitus	124 (35.9)
History of GDM in previous pregnancy	17 (4.9)
History of DFIU in previous pregnancy	6 (1.7)
History of fetal anomaly in previous pregnancy	9 (2.6)
History of giving birth to macrosomic newborn	7 (2.0)

*unless stated otherwise

Abbreviations: SD = standard deviation; BMI = body mass index; GA = gestational age; GDM = gestational diabetes mellitus; DFIU = dead fetus in utero

TABLE 2. Fetal parameters at 32-36 weeks' gestation (N=345)

Parameters	Mean ± SD	Percentile, n (%)			
		≤ 10th	>10th-50th	51st-< 90th	≥ 90th
BPD (mm)	83.5 ± 4.2	26 (7.5 %)	74 (21.4 %)	119 (34.5 %)	126 (36.5 %)
HC (mm)	303.0 ± 15.1	34 (9.9 %)	74 (21.4 %)	106 (30.7 %)	131 (38.0 %)
AC (mm)	295.8 ± 20.1	23 (6.7 %)	103 (29.9 %)	149 (43.2 %)	70 (20.3 %)
FL (mm)	62.6 ± 3.5	17 (4.9 %)	61 (17.7 %)	143 (41.4 %)	124 (35.9 %)
EFW (g)	2179.6 ± 375.0	56 (16.2 %)	201 (58.3 %)	75 (21.7 %)	13 (3.8 %)

Abbreviations: BPD = biparietal diameter; HC = head circumference; AC = abdominal circumference; FL = femur length; EFW = estimated fetal weight

TABLE 3. Maternal and neonatal outcomes (N = 345)

Outcomes	n (%)*
GA at delivery (weeks), mean ± SD	38.0 ± 1.2
Birth weight (grams), mean ± SD	3,148 ± 466
Delivery route	
Spontaneous vaginal delivery	139 (40.3)
Instrument-assisted delivery	6 (1.7)
Primary cesarean section	126 (36.5)
Repeat cesarean section	74 (21.4)
LGA newborn	107 (31.0)
Macrosomia	10 (2.9)
Birth asphyxia	10 (2.9)
NICU admission	4 (1.2)

*unless stated otherwise
Abbreviations: GA = gestational age; LGA = large-for-gestational age; NICU = neonatal intensive care unit

Univariate analysis for factors associated with LGA newborn in GDM is shown in Table 4. Women with high BMI and women with any fetal parameter of ≥ 90th percentile at 32-36 weeks' gestation were significantly more likely to deliver an LGA baby. All the 13 fetuses with EFW of ≥ 90th percentile at 32-36 weeks' gestation were LGA at birth, resulting in PPV and specificity of 100%. However, the NPV and sensitivity were 71.7% and 12.2% respectively.

TABLE 4. Univariate analysis for factors associated with LGA newborn in GDM

Variables	No LGA newborn (N=238) n (%)	LGA newborn (N=107) n (%)	*p*-value*
BMI			***0.038***
Normal (18.5-24.9 kg/m^2)	150 (72.5)	57 (27.5)	
Overweight (≥ 25 kg/m^2)	58 (69.9)	25 (30.1)	
Obese (≥ 30 kg/m^2)	30 (54.5)	25 (45.5)	
Gestational weight gain			0.345
Below recommended range	99 (69.7)	43 (30.3)	
Within recommended range	78 (72.9)	29 (27.1)	
Above recommended range	61 (63.5)	35 (36.5)	
Nulliparity	98 (69.5)	43 (30.5)	0.863
Multiparity	140 (68.6)	64 (31.4)	
Early GDM	154 (70.6)	64 (29.4)	0.383
Late GDM	84 (66.1)	43 (33.9)	
GDM control			0.116
Well-controlled	33 (60.0)	22 (40.0)	
Poorly-controlled	205 (70.7)	85 (29.3)	
BPD ≥ 90th percentile	66 (52.4)	60 (47.6)	***<0.001***
< 90th percentile	172 (78.5)	47 (21.5)	
HC ≥ 90th percentile	69 (51.9)	64 (48.1)	***<0.001***
< 90th percentile	169 (79.7)	43 (20.3)	
AC ≥ 90th percentile	19 (26.0)	54 (74.0)	***<0.001***
< 90th percentile	219 (80.5)	53 (19.5)	
FL ≥ 90th percentile	72 (55.4)	58 (44.6)	***<0.001***
< 90th percentile	166 (77.2)	49 (22.8)	
EFW ≥ 90th percentile	0 (0.0)	13 (100)	***<0.001***
< 90th percentile	238 (71.7)	94 (28.3)	

*p-value < 0.05 indicates statistical significance

Abbreviations: LGA = large-for-gestational age; GDM = gestational diabetes mellitus; BMI = body mass index; BPD = biparietal diameter; HC = head circumference; AC = abdominal circumference; FL = femur length; EFW = estimated fetal weight

The comparison between groups was performed using Chi-square test.

Multivariate analysis for factors independently associated with LGA newborn in GDM is shown in Table 5. AC and HC of ≥ 90th percentile were independent predictors of LGA newborn with adjusted odds ratio (OR) of 6.5 and 2.0 respecitvely. We determined the adjusted OR of EFW of ≥ 85th percentile because EFW ≥ 90th percentile would produce a "zero" value in calculation formula. EFW of ≥ 85th percentile was the strongest factor of LGA newborn with the adjusted OR of 9.3.

EFW of ≥ 90th percentile resulted in 100% positive predictive value and 100% specificity for identification of fetuses at risk to be born LGA. However, as this cutoff accounted for 3.8% of the fetuses measured at the third trimester, the benefit was limited. In addition, a number of the actual LGA neonates would be missed. Therefore, we tried lower cutoff percentiles in an attempt to increase the sensitivity of third trimester ultrasonography to predict LGA babies. Table 6 shows performance using various cutoffs. Using cutoff levels at 80th and 70th percentile could increase the sensitivity while slightly reducing the PPV and specificity.

Regarding different timing of ultrasonography, no difference of performance in predicting an LGA baby was observed between examination at 32-34 weeks' and 34-36 weeks' gestation.

TABLE 5. Multivariate analysis for factors independently associated with LGA newborn in GDM

Variables	Adjusted OR (95% CI)	*p*-value*
BMI		
Normal (18.5-24.9 kg/m^2)	1	0.253
Underweight (<18.5 kg/m^2)	0.8 (0.4-1.6)	0.53
Overweight and obese (≥25 kg/m^2)	1.6 (0.7-3.5)	0.20
BPD ≥ 90th percentile	1.3 (0.6-2.7)	0.406
HC ≥ 90th percentile	2.0 (1.0-4.0)	***0.048***
AC ≥ 90th percentile	6.5 (3.3-12.8)	***<0.001***
FL ≥ 90th percentile	1.7 (0.9-3.0)	0.059
EFW ≥ 85th percentile	9.3 (1.1-77.9)	***0.038***

*p-value < 0.05 indicates statistical significance

Abbreviations: BMI = body mass index; BPD = biparietal diameter; HC = head circumference; AC = abdominal circumference; FL = femur length; EFW = estimated fetal weight

TABLE 6. Estimated fetal weight (EFW) cutoff percentile for predicting LGA newborn in GDM

Cutoff percentile	PPV (%)	Specificity (%)	Sensitivity (%)	NPV (%)
EFW ≥ 90th percentile	100	100	12.1	71.7
EFW ≥ 80th percentile	90.3	98.7	26.2	74.8
EFW ≥ 70th percentile	88.0	97.5	41.1	78.6

Abbreviations: LGA = large-for-gestational age; GDM = gestational diabetes mellitus; PPV = positive predictive value

DISCUSSION

This prospective cohort study demonstrated that EFW obtained by ultrasound in the third trimester is useful for predicting LGA newborn in women with GDM, especially when all parameters (BPD, HC, AC, and FL) were measured to calculate EFW. Considereing these parameters individually, HC ≥ 90th percentile and AC ≥ 90th percentile were able to predict LGA newborn, with AC being the stronger associating parameter.

The present study found that EFW of ≥ 90th percentile at 32-36 weeks' gestation yielded a PPV of 100%, a specificity of 100%, a NPV of 71.7%, and a sensitivity of 12.2% in prediction of LGA at birth. Previous studies reported lower PPV and specificity with higher NPV and sensitivity,[9,14] whereas the most recent study showed high specificity and low sensitivity, which is similar to our study.[15] The disparity in findings may be due to differences in study population, risk factors, and GDM screening method. Inclusion criteria and the reference growth chart used in other studies were different from ours. Specifically, one study included only women with early GDM and pregestational diabetes,[9] and another used a United States National Reference for Fetal Growth that was published in 1996.[16] The study period during gestation also varied, with one study performing ultrasound during a gestational age range from 28 to 32[6,7] weeks' gestation.[9] In addition, the previously cited studies used EFW percentile cutoffs of 70th, 75th, and 80th percentile, whereas the 90th percentile was used in this study.

The birth weight percentile used in this study was based on 2004-2008 WHO Global Survey on Maternal and Perinatal Health (WHOGS) data,[12] which recruited pregnant women across most countries worldwide, including Thai women. This study, in addition, could be more applicable in clinical practice in comparison to the previous study[14] as the ultrasound was performed during 32-36 weeks' gestation, just after maximal acceleration of fetal growth.

Among various formulas, Hadlock I and III perform best in estimating fetal weight, with Hadlock I having a lower mean absolute percentage error (MAPE).[11,17] Accordingly, Hadlock I formula was used in this study. The present study reveals EFW as the best predictor of LGA newborn in women with GDM. Among all parameters evaluated in this study for estimating fetal weight, AC was found to be the strongest individual predictor of LGA newborn. This finding was similar to that from a previous study which found that AC was the parameter with highest sensitivity. This may be explained by fat accumulation and liver glycogen storage when fetal weight increases in late pregnancy.[18]

This study suggests that the third trimester ultrasound for fetal biometry should be performed in all women with GDM to identify fetus at risk to be LGA at birth. EFW using all parameters (AC, HC, BPD, and FL) provided high PPV and high specificity. However, measuring only AC may be acceptable when measuring all parameters is not feasible due to improper fetal position or difficult maternal habitus.

Despite a high PPV and a high specificity, EFW ≥ 90th percentile had a low sensitivity and low NPV to predict LGA at birth. Lowering to 80th and 70th percentile cutoff values improved the sensitivity and NPV with a slightly reduced PPV and specificity. Concerning gestational age at examination, performance of ultrasonography to predict an LGA neonate was comparable between performing at 32-24 and 34-36 weeks' gestation.

The strengths of the study include its prospective cohort design, and the fact that the reference of EFW was derived from an international standard. A few limitations were also appreciated. Women's glycemic control was only assessed from the values of FBS and 2-h PP blood sugar at antenatal visits, so blood sugar level trends and fluctuations were not examined. Moreover, GDM management during the remaining time before birth could affect the fetal growth.

This study may guide physicians to give special attention for fetuses diagnosed with LGA from ultrasound at a hospital in rural areas for delivery or referral planning in advance.

CONCLUSION

Estimated fetal weight derived from the fetal biometry measured in the third trimester had a high PPV and specificity with a low to moderate sensitivity in predicting LGA at birth.

ACKNOWLEDGEMENTS

The authors are grateful to medical personnel of the Maternal-Fetal Medicine Unit, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University for assistance with study.

Conflict of interest declaration

All authors declare no conflicts of interest.

REFERENCES

1. Committee on Practice B-O. Practice Bulletin No. 137: Gestational diabetes mellitus. Obstet Gynecol 2013;122:406-16.
2. Mpondo BC, Ernest A, Dee HE. Gestational diabetes mellitus: challenges in diagnosis and management. J Diabetes Metab Disord 2015;14:42.
3. Srichumchit S, Luewan S, Tongsong T. Outcomes of pregnancy

with gestational diabetes mellitus. Int J Gynaecol Obstet 2015;131:251-4.
4. Weissmann-Brenner A, Simchen MJ, Zilberberg E, Kalter A, Weisz B, Achiron R, et al. Maternal and neonatal outcomes of large for gestational age pregnancies. Acta Obstet Gynecol Scand 2012;91:844-9.
5. Johns K, Olynik C, Mase R, Kreisman S, Tildesley H. Gestational diabetes mellitus outcome in 394 patients. J Obstet Gynaecol Can 2006;28:122-7.
6. Boriboonhirunsarn D, Kasempipatchai V. Incidence of large for gestational age infants when gestational diabetes mellitus is diagnosed early and late in pregnancy. J Obstet Gynaecol Res 2016;42:273-8.
7. Eze CU, Ohagwu CC, Abonyi LC, Irurhe NK, Ibitoye ZA. Reliability of Sonographic Estimation of Fetal Weight: A Study of Three Tertiary Hospitals in Nigeria. Saudi J Med Med Sci 2017;5:38-44.
8. Ben-Haroush A, Yogev Y, Bar J, Mashiach R, Kaplan B, Hod M, et al. Accuracy of sonographically estimated fetal weight in 840 women with different pregnancy complications prior to induction of labor. Ultrasound Obstet Gynecol 2004;23:172-6.
9. Nelson L, Wharton B, Grobman WA. Prediction of large for gestational age birth weights in diabetic mothers based on early third-trimester sonography. J Ultrasound Med 2011;30:1625-8.
10. Bunthalarath S, Sunsaneevithayakul P, Boriboohirunsarn D. Risk factors for early diagnosis of gestational diabetes mellitus. J Med Assoc Thai 2004;87 Suppl 3:S50-3.
11. Esinler D, Bircan O, Esin S, Sahin EG, Kandemir O, Yalvac S. Finding the best formula to predict the fetal weight: comparison of 18 formulas. Gynecol Obstet Invest 2015;80:78-84.
12. Mikolajczyk RT, Zhang J, Betran AP, Souza JP, Mori R, Gulmezoglu AM, et al. A global reference for fetal-weight and birthweight percentiles. Lancet 2011;377:1855-61.
13. Rasmussen KM, Catalano PM, Yaktine AL. New guidelines for weight gain during pregnancy: what obstetrician/gynecologists should know. Curr Opin Obstet Gynecol 2009;21:521-6.
14. Scifres CM, Feghali M, Dumont T, Althouse AD, Speer P, Caritis SN, et al. Large-for-Gestational-Age Ultrasound Diagnosis and Risk for Cesarean Delivery in Women With Gestational Diabetes Mellitus. Obstet Gynecol 2015;126:978-86.
15. Simpson KJ, Pavicic M, Lee GT. What is the accuracy of an early third trimester sonogram for identifying LGA infants born to GDM patients diagnosed with the one-step approach? J Matern Fetal Neonatal Med 2018;31:2628-33.
16. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. Obstet Gynecol 1996;87:163-8.
17. Siemer J, Egger N, Hart N, Meurer B, Muller A, Dathe O, et al. Fetal weight estimation by ultrasound: comparison of 11 different formulae and examiners with differing skill levels. Ultraschall Med 2008;29:159-64.
18. Ashimi Balogun O, Sibai BM, Pedroza C, Blackwell SC, Barrett TL, Chauhan SP. Serial Third-Trimester Ultrasonography Compared With Routine Care in Uncomplicated Pregnancies: A Randomized Controlled Trial. Obstet Gynecol 2018;132:1358-67.

Sonographic Lower Uterine Segment Thickness to Predict Cesarean Scar Defect in Term Pregnancy

Nichapat Pahirah, M.D.*, Wipada Laosooksathit, M.D.*, Kittipong Kongsomboon, M.D., Ph.D., Maethaphan Kitporntheranunt, M.D.***

**Department of Obstetrics and Gynecology, **Department of Preventive and Social Medicine, Faculty of Medicine, Srinakharinwirot University, Nakhon Nayok, 26120, Thailand.*

ABSTRACT

Objective: To study the validity of abdominal sonographic lower uterine segment (LUS) thickness in predicting intraoperative cesarean scar defect (CSD) and thin incision-site uterine wall thickness in term pregnancy.

Methods: This was a cross-sectional study involving 111 full-term pregnant women who were scheduled for repeat cesarean delivery from April, 2019 to January, 2020. The abdominal sonographic myometrial LUS thickness was measured prior to surgery. The cesarean scar was assessed using the morphologic classification system as either grade 1 (a normally formed LUS), grade 2 (a thin LUS, but without visible content), or grade 3 (a thin LUS with visible content). Then, the ophthalmic calipers was used to measure the incision-site uterine wall thickness. The correlations between the abdominal sonographic measurements and intraoperative findings were reported. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated.

Results: There were two cases (1.8%) of grade 3 CSD. The overall correlation between the abdominal sonographic and intraoperative incision-site uterine wall thickness showed r=0.559 with p value < 0.001. The sonographic cut-off value of 1.5 mm could predict CSD and a thin incision-site uterine wall thickness with sensitivity, specificity, PPV, NPV of 50.0%, 90.8%, 9.1%, 99.0%, and 37.5%, 94.6%, 54.5%, 90.0%, respectively. A receiver operating characteristic curve was generated to determine the optimum cut-off value at 2.5 mm with a sensitivity of 76.5% and a specificity of 73.3%. The area under the curve was 0.8 (a 95% confidence interval, 0.718-0.885).

Conclusion: Abdominal sonography is a valuable tool for the preoperative predicting of CSD. A myometrial LUS thickness of more than 1.5 mm is associated with a lower likelihood of cesarean scar dehiscence.

Keywords: Sonography; lower uterine segment; cesarean scar; term pregnancy (Siriraj Med J 2021; 73: 330-336)

INTRODUCTION

Uterine rupture is a devastating complication of cesarean scar defect (CSD). Several factors influence cesarean scar healing, such as the suturing technique, the suture materials, the anatomical site, and the apposition of the myometrium.[1,2] The prevalence of a niche in a cesarean scar at six-weeks postpartum was 64.5% and continued rising.[3] Previous studies demonstrated CSD by using various methods, such as vaginal sonography[3], 3-dimensional (D) abdominal sonography[4], and pelvic magnetic resonance imaging.[5] But these methods are inconvenient, expensive, and require expertise on the part of the operators. Two-dimensional (2D) abdominal sonography is a simple, less invasive, more affordable and readily available method.

There was a high relationship between the sonographic LUS thickness and the risk of CSD.[6] Earlier researchers measured the entire layer of the LUS, including the

Corresponding author: Maethaphan Kitporntheranunt
Email: mtp_swu@hotmail.com
Received 1 December 2020 Revised 29 March 2021 Accepted 1 April 2021
ORCID ID: http://orcid.org/0000-0002-5794-9810
http://dx.doi.org/10.33192/Smj.2021.43

bladder wall, uterine scar fibrosis, the myometrial layer, and the chorio-amniotic membranes, without a good comparator.[7,8] Sen, et al.[9] reported that a cut-off value of 2.5 mm full LUS thickness is associated with uterine dehiscence. Recent studies focused only the myometrial layer of the LUS, with a higher accuracy in detecting CSD.[10] There still is no consensus on using antepartum LUS thickness to evaluate CSD.[11] So, the objective of this study was to assess the validity of 2D abdominal sonography in predicting CSD and the thin incision-site uterine wall thickness in term pregnancy.

MATERIALS AND METHODS

This cross-sectional study was conducted at HRH Princess Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University, Thailand from April, 2019 to January, 2020. The study was approved by the Institute's Ethical Review Board (SWUEC/F 386/2018) and registered to Thai Clinical Trials Registry number 20190718001. Participants were the singleton term pregnant women with at least a prior cesarean section, aged 18 years and up, and who were scheduled for repeat cesarean delivery between 38 and 40 weeks of gestation. The exclusion criteria were women who had labor symptoms, abnormal placentation, leiomyoma at the LUS, and prior classical uterine incision. Consent was obtained from all the participants. Their maternal age, gestational age, body mass index, parity number, miscarriage number, and number of previous cesarean sections were recorded.

Preoperative LUS sonography

Two-dimension abdominal sonography was performed by a well-trained sonographer (NP) within 24 hours before the operation. An Accuvix XG (Samsung Medison Co Ltd., Seoul, Korea) ultrasound machine was used in the study. The participants were prepared in a supine position with a full bladder. The convex probe (frequency of 1-4 MHz) was placed at the suprapubic area in the midsagittal plane. A two-layer structure between the urinary bladder and uterine cavity was identified, consisting of a hyperechogenic layer (bladder wall) and a hypoechogenic layer (myometrium).[7] The area of interest was magnified to occupy up to two-thirds of the screen. For the myometrial-thickness measurement, the first marker was placed at the interface between the urinary bladder wall and the myometrial layer. The second marker was placed at the interface between the myometrial layer and the amniotic membranes (or fetal scalp) (Fig 1).[10] The same procedures were repeated at 1 cm laterally apart from the first measurement on both sides. Three values were calculated into an average value. The thin sonographic myometrial LUS was defined as having thickness < 1.5 mm.[12]

Intraoperative LUS assessment

In the operative field, cesarean scar morphology was classified into three groups by direct visualization (Fig 2): grade 1= normal-formed LUS; grade 2= thin LUS without visible content; and grade 3= thin LUS with visible content or an absence of LUS continuity.[13] The cesarean scar dehiscence was defined as cesarean scar morphologic grade 3. Then, a low-transverse uterine incision was made until the amniotic membrane was exposed. The CASTROVIEJO ophthalmic calipers was applied to the upper uterine flap at the midpoint of the uterine incision site for measurement, and reapplied at 1 cm apart on both sides (Fig 3). The average value of the incision-site uterine wall thickness was calculated. A thin LUS was defined as a uterine wall thickness equal to or less than 1.0 mm.[10] All obstetricians were trained for the caliper measurement and blinded from the preoperative results. If this procedure could not be accomplished, they were excluded from the study.

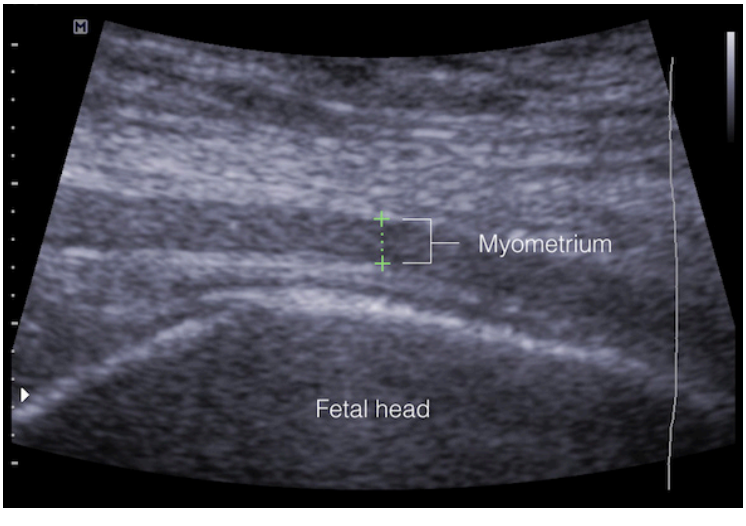
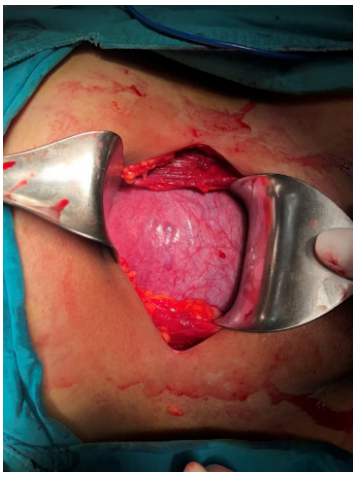
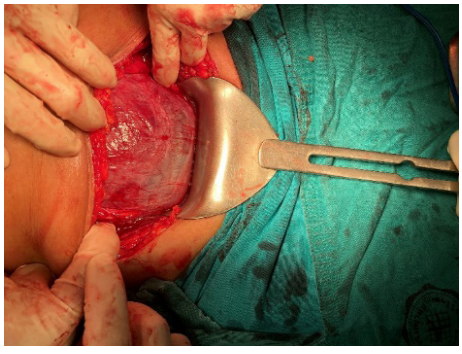


Fig 1. The longitudinal abdominal sonogram showing the myometrial LUS thickness measurement.
Abbreviation: LUS: lower uterine segment



2A



2B

Fig 2. The Intraoperative cesarean scar morphology: grade 1 normal LUS (A) and grade 3 thin LUS with visible content (B)
Abbreviation: LUS: lower uterine segment

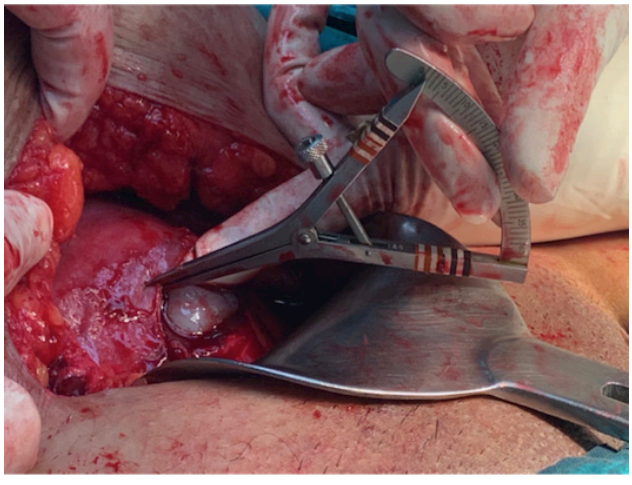


Fig 3. The intraoperative incision-site uterine wall thickness measurement using the CASTROVIEJO ophthalmic calipers.

Statistical analysis

The sample-size calculation was done by using a prevalence of CSD at 8.5%[6] with an expected sensitivity of 90%, a confidence interval at 95%, and an allowable number of errors of 15%. The number of participants needed in this study was 92 pregnant women.

The baseline characteristics were analyzed using descriptive statistics - namely, mean ± standard deviation (SD), median with an interquartile range, and percentage, as appropriate. The calculation for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were made. The authors used the Chi-square test to find the relationship between the two parameters. A p-value of less than 0.05 was considered statistically significant.

RESULTS

120 pregnant women were enrolled in this study. Nine were excluded from the study (two women had labor pain and seven couldn't go through the intraoperative assessment). The baseline characteristics of the 111 participants are presented in Table 1.

There was a statistically significant correlation between the sonographic LUS thickness and the cesarean scar morphology in grade 1, grade 2, and all grades (Table 2). There were two cases in the grade 3 CSD group that did not correlate with the incision-site uterine wall thickness.

A cut-off level at 1.5 mm for the abdominal sonographic myometrial LUS could predict uterine dehiscence (grade 3 morphology) and a thin incision-site uterine wall thickness with validity, as shown in Table 3. Based on our data, a receiver operating characteristic graph was generated (Fig 4). The authors suggested that using a cut-off point at 2.5 mm sonographic LUS thickness could predict an intraoperative thin-incision site with a sensitivity of 76.5% and a specificity of 73.3%.

DISCUSSION

The CSD is an abnormal finding manifested during a repeat cesarean section. The CSD spectrum can present with scar dehiscence or a uterine scar rupture during labor. This devastating complication can be prevented. To do so, a good screening tool for the early detection of CSD is needed.

TABLE 1. Patient's characteristics (n=111).

Characteristics	Values
Age (years) (mean±SD)	29 ± 6
Gestational age (weeks) (mean±SD)	38.5 ± 0.6
BMI (kg/m^2) (number (%))	
< 18.5	9 (8.1)
18.5 - 22.9	45 (40.5)
23.0 - 24.9	13 (11.7)
25.0 - 29.9	29 (26.1)
> 30	15 (13.5)
Parity (number (%))	
1	93 (83.8)
2	16 (14.4)
> 3	2 (1.8)
Miscarriages (number (%))	
0	80 (72.1)
1	28 (25.2)
> 2	3 (2.7)
Number of previous cesarean sections (number (%))	
1	101 (91.0)
> 1	10 (9.0)

Data are presented as mean ± standard deviation (SD), or number (%)
Abbreviation: BMI, body mass index

TABLE 2. The correlations between the abdominal sonographic LUS thickness and the incision-site uterine wall thickness in each cesarean-scar morphologic grading.

		LUS thickness (mm) measured by			
Cesarean scar morphology	Number	Sonography	Ophthalmic calipers	Correlation coefficient	P-value
Grade 1	55	3.1 ± 1.0	3.3 ± 0.8	r = 0.559*	0.001
Grade 2	54	2.3 (1.8-2.9)	2.3 (1.7-2.3)	r = 0.407**	0.002
Grade 3	2	Case A= 1.4 Case B= 3.4	Case A=1.2 Case B=0.7	-	-
Overall	111	2.6 ± 1.0	2.5 ± 1.1	r = 0.559*	0.001

Data are presented as mean±standard deviation or median (interquartile range).
*Pearson correlation, **Spearman correlation
Abbreviation: LUS, lower uterine segment.

TABLE 3. The validity of the abdominal sonographic LUS thickness to detect cesarean scar dehiscence (grade 3 morphology) and thin incision-site uterine wall thickness.

		Sonographic LUS thickness(mm)		Total	Sensitivity		Specificity		PPV	NPV
		<1.5	>1.5	number	(%)	95%CI	(%)	95%CI	(%)	(%)
Cesarean scar	Yes	1	1	2	50.5	40-59	90.8	85-96	9.1	99.0
dehiscence	No	10	99	109						
Total number		11	100	111						
Thin incision-site	Yes	3	5	8	37.5	4-71	92.2	87-94	27.3	95.0
uterine wall	No	8	95	103						
thickness										
Total number		11	100	111						

Abbreviations: LUS: lower uterine segment; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value

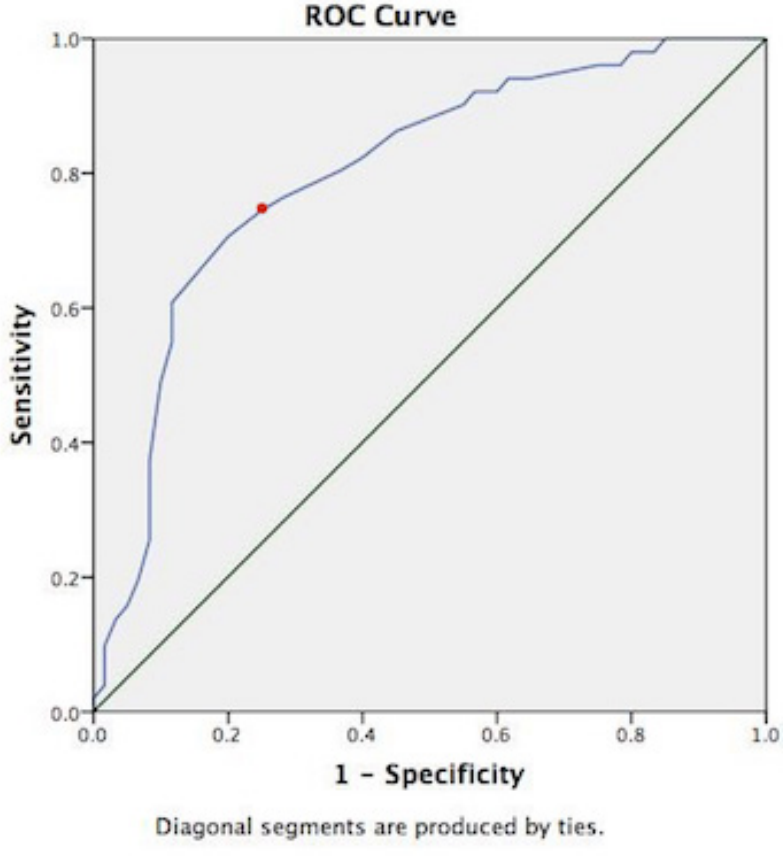


Fig 4. Receiver operating characteristic curve showing the optimal cut-off value for thin LUS (2.5 mm): the area under the curve was 0.8 (95% CI, 0.718-0.885).
Abbreviations: LUS: lower uterine segment; CI: confidence interval

The 2D abdominal sonography is a simple, noninvasive, widely used, and readily available device in most hospitals. The abdominal sonographic LUS thickness can be used for antepartum CSD screening. Theoretically, a thinner LUS will result in a more severe degree of CSD.[14] In this study, the authors evaluated only the myometrial layer of the LUS, which directly represents the uterine scar's integrity. The overall mean sonographic myometrial LUS thickness was 2.6+1.0 mm, which is comparable to the Tazion, et al study.[15] Other studies have reported a thinner sonographic measurement.[10,12] The differences involving the sonographic LUS thickness may be caused by the variation of participants' characteristics, gestational age, uterine-closure techniques, scar-fibrosis formation, uterine healing process, and the sonographic protocol used. However, the number of grade 3 CSD found in this study was 1.8%, which is comparable to what was reported in those' studies.[10,12]

There is a significant correlation between the overall sonographic LUS thickness and the incision-site uterine wall thickness, and that is consistent with a prior study[6] which had a high level of correlation. Surprisingly, one case in the grade 3 group had a sonographic LUS thickness of 3.4 mm, while the incision-site uterine wall thickness was only 0.7 mm. This unexpected result may be caused by a poor imaging technique used on the thick abdominal wall; less urine in the bladder; or abnormal focal myometrial thickening. The authors intend to use the specific sonographic protocol and three-point measurement technique to maximize the correspondence between the sonographic area of interest and the cesarean scar site, but mislocation may still occur.

With regard to any prediction of scar dehiscence, the use of a sonographic myometrial LUS thickness of less than 1.5 mm had a sensitivity of 50.0% and a specificity of 90.8%, which is quite different from what Gizzo, et al.[12] Specifically, they reported a high sensitivity of 100% and a specificity of 85%. A possible reason for the differences is the different characteristics of the participants, especially the higher number of previous cesareans. The thicker sonographic LUS in this study results in a lower number of positive tests, which can lead to less sensitivity and more specificity. Our results showed a high NPV of 99.0%; thus, when the sonographic LUS thickness is more than 1.5 mm, it is less likely to have CSD.

With regard to the detection of a thin incision-site uterine wall thickness, a sonographic myometrial LUS thickness of less than 1.5 mm had a sensitivity of 37.5%. So, there is a need to redefine the optimum cut-off point. Based on this study, the authors suggest a cut-off level at 2.5 mm.

Strengths and limitations

The strengths of this study are that only one sonographer was used, so as to minimize interobserver variations[16]; all assessors were blinded from the sonographic results; and an ophthalmic calipers was employed for objective measurement.

The limitation of this study was the small number of cesarean scar dehiscence cases. Also, there was a possible error during the incision-site uterine wall thickness measurement, as the ophthalmic calipers jaws have to grasp a certain amount of tissue deep from the incisional edge, which may result in abnormally thick uterine walls. There are different types of ophthalmic calipers used for LUS measurement, such as Castroviejo ophthalmic calipers[6] or Vernier calipers[10], and this may affect the results.

Further study with more participants and a longer duration of follow-up should be carried out to achieve the most accurate method for antepartum CSD prediction.

CONCLUSION

Preoperative abdominal sonography is a simple tool for CSD prediction. A myometrial LUS thickness of more than 1.5 mm is associated with a lower likelihood of cesarean scar dehiscence.

ACKNOWLEDGMENTS

This study got funding from HRH Princess Maha Chakri Sirindhorn Medical Center, Faculty of Medicine, Srinakharinwirot University (Contract No.256/2019).

Conflict of interest: No potential conflict of interest regarding this article was reported.

REFERENCES

1. Sholapurkar SL. Etiology of cesarean uterine scar defect (Niche): detailed critical analysis of hypotheses and prevention strategies and peritoneal closure debate. J Clin Med Res 2018;10:166-73.
2. Başbuğ A, Doğan O, Ellibeş Kaya A, Pulatoğlu Ç, Çağlar M. Does suture material affect uterine scar healing after cesarean section? results from a randomized controlled trial. J Invest Surg 2019;32:763-9.
3. Verma U, Chandra M, Nagrath A, Singh S, Agrawal R. Assessment of cesarean section scar strength: still a challenge? Indian J Clin Pract 2014;24:974-7.
4. Basic E, Basic-Cetkovic V, Kozaric H, Rama A. Ultrasound evaluation of uterine scar after cesarean section. Acta Inform Med 2012;20:149-53.
5. Satpathy G, Kumar I, Matah M, Verma A. Comparative accuracy of magnetic resonance morphometry and sonography in assessment of post-cesarean uterine scar. Indian J Radiol Imaging 2018;28:169-74.
6. Fukuda M, Fukuda K, Shimizu T, Bujold E. Ultrasound assessment of lower uterine segment thickness during pregnancy, labour,

and the postpartum period. J Obstet Gynaecol Can 2016;38:134-40.
7. Jastrow N, Vikhareva O, Gauthier RJ, Irion O, Boulvain M, Bujold E. Can third-trimester assessment of uterine scar in women with prior cesarean section predict uterine rupture? Ultrasound Obstet Gynecol 2016;47:410-4.
8. Bujold E, Jastrow N, Simoneau J, Brunet S, Gauthier RJ. Prediction of complete uterine rupture by sonographic evaluation of the lower uterine segment. Am J Obstet Gynecol 2009;201:320.e1-6.
9. Sen S, Malik S, Salhan S. Ultrasonographic evaluation of lower uterine segment thickness in patients of previous cesarean section. Int J Gynecol Obstet 2004;87:215-9.
10. Cheung VYT, Constantinescu OC, Ahluwalia BS. Sonographic evaluation of the lower uterine segment in patients with previous cesarean delivery. J Ultrasound Med 2004;23:1441-7.
11. Kok N, Wiersma IC, Opmeer BC, de Graaf IM, Mol BW, Pajkrt E. Sonographic measurement of lower uterine segment thickness to predict uterine rupture during a trial of labor in women with previous cesarean section: a meta-analysis. Ultrasound Obstet Gynecol 2013;42:132-9.
12. Gizzo S, Zambon A, Saccardi C, Patrelli TS, Di Gangi S, Carrozzini M, et al. Effective anatomical and functional status of the lower uterine segment at term: estimating the risk of uterine dehiscence by ultrasound. Fertil Steril 2013;99:496-501.
13. Jha NNS, Maheshwari S, Barala S. Ultrasonographic assessment of strength of previous cesarean scar during pregnancy. Int J Reprod Contracept Obstet Gynecol 2018;7:1458-63.
14. Seliger G, Chaoui K, Lautenschläger C, Riemer M, Tchirikov M. Technique of sonographic assessment of lower uterine segment in women with previous cesarean delivery: a prospective, pre/intraoperative comparative ultrasound study. Arch Gynecol Obstet 2018;298:297-306.
15. Tazion S, Hafeez M, Manzoor R, Rana T. Ultrasound predictability of lower uterine segment cesarean section scar thickness. J Coll Physicians Surg Pak 2018;28:361-4.
16. Jastrow N, Antonelli E, Robyr R, Irion O, Boulvain M. Inter- and intraobserver variability in sonographic measurement of the lower uterine segment after a previous cesarean section. Ultrasound Obstet Gynecol 2006;27:420-4.

Selective Arterial Embolization of Renal Angiomyolipoma: Efficacy, Tumor Volume Reduction, and Complications

Walailak Chaiyasoot, M.D., Jirawadee Yodying, M.D., Thanita Limsiri, M.D.

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

ABSTRACT

Objective: To evaluate the efficacy and complications of selective arterial embolization in renal angiomyolipoma and to identify predictive factors for tumor rupture.

Materials and Methods: Twenty-one patients with 25 renal angiomyolipoma (AML) underwent selective arterial embolization (SAE) between January 2008 and June 2019, 15 lesions involving prophylaxis embolization of a tumor >4 cm diameter and 10 involving embolization for a ruptured tumor. Multidetector computed tomography (MDCT) was performed pre- and post-SAE, using the 2D tumor diameter measurement in the ruptured AMLs. Three-dimensional volumetry and density histogram were performed for determining the total tumor volume, fat, and angiomyogenic component reduction in the unruptured AMLs. The predictive factors for tumor rupture, the treatment outcome and complications were analyzed.

Results: The clinical success rate was 84% (21/25 lesions) and the technical success rate was 96% (24/25 lesions). The 3D volume post-SAE within 1-3 months showed a greater decrement of the enhanced angiomyogenic component than the fat component, with median percentages of -62.2% and -18.4%, respectively (p-value = 0.333). Minor complications were post-embolization syndrome (5 lesions, 20%) and minimal renal infarction (4 lesions, 16%). Renal abscesses were the major complications (3 lesions, 12%). A factor associated with tumor rupture was the presence of an intra-tumoral aneurysm (p-value < 0.05).

Conclusion: SAE is an effective treatment for renal AML with a high technical and clinical success rate and limited complications. Three-dimensional volumetry and density histogram analysis might be better tools than two-dimensional CT to evaluate post-SAE response. The presence of an intra-tumoral aneurysm is a significant predictive factor associated with tumor rupture.

Keywords: Renal angiomyolipoma; selective arterial embolization (Siriraj Med J 2021; 73: 337-343)

INTRODUCTION

Renal angiomyolipoma (AML), a benign neoplasm accounting for 0.3-3% of all renal tumors[1], composed of dysmorphic blood vessels, fat, and smooth muscle.[2] Eighty percent of renal AML is a sporadic group, found among women in their 4th - 5th decade, usually presented as a solitary AML. The remainder has a female predilection, usually symptomatic with multiple bilateral AMLs associated with tuberous sclerosis complex (TSC).[3-5] Renal AMLs can potentially grow substantially and cause many complications[5,6] which the major fatal complication is a retroperitoneal bleeding.[3,4,7] Previous studies have

Corresponding author: Jirawadee Yodying
E-mail: jirawadee.yod@gmail.com
Received 15 January 2021 Revised 8 February 2021 Accepted 10 February 2021
ORCID ID: http://orcid.org/0000-0002-2369-9008
http://dx.doi.org/10.33192/Smj.2021.44

proposed predictive factors for tumor rupture, including tumor size, aneurysm formation, associated TSC[3,8,9], the size of an intra-tumoral aneurysm and a proportion of angiogenic component.[10,11] The bleeding tendency of the tumor might be come from an irregular shape appearance of the intra-tumoral aneurysm.[12]

Computed tomography (CT) and magnetic resonance imaging (MRI) are important tools to diagnose renal AML based on the tumors' fat component to be differentiated from a renal cell carcinoma.[13,14] The treatment modalities for asymptomatic renal AML are surgery, selective arterial embolization (SAE), tumor ablation, and the use of Mamalian Target of Rapamycin (mTOR^R) inhibitors.[5] Recently, SAE has been accepted as the first-line treatment of renal AML, either for prophylaxis in tumor >4 cm or treatment in acute hemorrhage patients with hemodynamic instability.[15,16] However, from the literature review, there is no research concerning the efficacy of SAE in renal angiomyolipoma in Thailand.

Objectives

The primary objective of our study aimed to evaluate the efficacy of transarterial embolization using multidetector CT (MDCT) measurement of total tumor volume, quantification of the fat and angiomyogenic component reduction post-SAE. The secondary objectives were to analyze the post-procedural complications and to identify the predictive factors for tumor rupture.

MATERIALS AND METHODS

Population

The study protocol was approved by the Institutional Review Board (IRB) of Siriraj Hospital, Mahidol University (Si 051/2020). Totally 161 patients with renal AML, we retrospectively analyzed 54 patients (56 lesions) who had CT diagnosis as renal AML (Fig 1) and undergone SAE during January 2008 to June 2019. Thirty-three patients were excluded due to unavailable CT studies, lost on follow-up or expired. This left 21 patients enrolled in the study.

Embolization procedure

Of the 21 patients with renal AMLs, 12 patients were embolized electively and 9 patients had emergency embolization. Most procedures were performed under local anesthesia, only 3 cases needed general anesthesia due to unstable vital signs. Selective renal angiogram was performed using a 5 Fr catheter, followed by superselective catheterization using a microcatheter to spare the normal renal parenchyma. A coaxial system comprised of a microcatheter; a 2.7 Fr Progreat® (Terumo, Tokyo, Japan) were performed in 14 lesions, a 1.98 Fr tip Masters Parkway® (Asahi Intecc USA, Inc.) in 7 lesions, and a 2.8 Fr Renegade HI-FLO® (Boston Scientific, Natick, MA, USA) in 1 lesion. Three lesions used only 5 Fr selective catheters because of large arterial feeders. Several embolic materials were selected depend on each operator, including polyvinyl alcohol, PVA (Contour®, Boston Scientific, Ireland), absolute ethyl alcohol (Siriraj Hospital), N-butyl cyanoacrylate (NBCA) or glue (Histoacryl®, Braun, Spain), interlocking coil (Interlock® Boston Scientific, Ireland), and thrombin (Thrombin-JMI®, Pfizer, United States). Technical success was defined as stasis of tumoral blood flow and lack of contrast opacified renal AMLs on post-embolization angiogram.[17]

Imaging studies

A diagnostic CT scan (120 kVp; 115-500 mA; section thickness, 1.25-3 mm; pitch, 0.992:1 and 1.375:1) was conducted on a 64-slice and a 256-slice MDCT. Contrast-enhanced CT was performed using non-ionic iodinate contrast medium (320-370 mg I/ml) at a dose of 1.5-2 ml/kg.

All the patients had pre- and post-procedural MDCT. The most recent pre-procedural CT (median, 29 days; range, 0-219 days) and all post-procedural follow-up CT (median, 2 months; range, 1–76 months) were reviewed by a radiology resident and an interventional radiology (IR) staff including maximal 2D diameter of the ruptured tumor, the presence of an intra-tumoral aneurysm, the aneurysm size and post-procedural complications. In unruptured cases, we analyzed changes in the tumor volume, enhanced angiomyogenic and fat component of renal AMLs. The data analysis was performed using an Advantage Workstation from Diagnostic Imaging (ADW 4.6, GE Healthcare). The tumor volume was calculated by drawing a region of interest (ROI) covering the tumor on axial pre- and post-contrast (80-100 sec) images and converting to 3D volumetry. The ROI of the AML was then converted to a density histogram. The enhanced angiomyogenic component volume was calculated using the difference between the area under the curve of the density histogram with a density >100 HU on pre- and post-contrast MDCT.[18] The fat component volume was defined as the area under the curve of the density histogram with a density <-20 HU on pre-contrast phase (Fig 2). The percentage reduction was compared between the pre- and post-procedural CT.

Clinical success was defined as no recurrence, no new bleeding episode or complication related to SAE within 30 days, and no further surgery or re-embolization.[19] Complications were categorized as major and minor

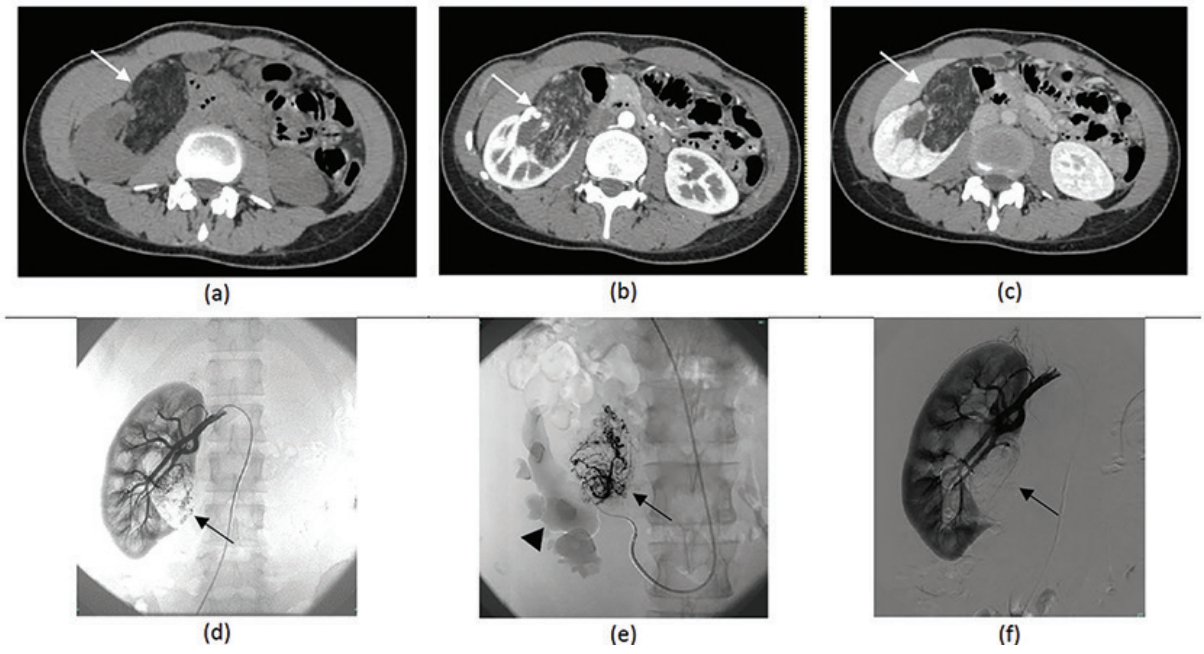


Fig 1. Typical CT and angiographic features of renal angiomyolipoma in the same patient (lesion no. 21)
(a) Pre-contrast axial phase CT showed a well-defined macroscopic fat-containing lesion at right kidney *(arrow)*
(b) Arterial phase CT demonstrated tortuous blood vessels *(arrow)* in the lesion
(d) Post-contrast phase CT revealed a heterogeneously enhanced fat-containing lesion *(arrow)*
(a) Right renal angiogram revealed a renal mass at interpolar region *(arrow)*
(b) Superselection into inferior segmental branch of right renal artery revealed a neovascularized and hypervascularized tumor *(arrow)*.
Note contrast excretion into dilated right renal pelvis, indicating hydronephrosis *(arrowhead)*
(c) Post-embolization angiogram showed arterial occlusion supplying the tumor *(arrow)* with preservation of normal renal parenchymal blood supply

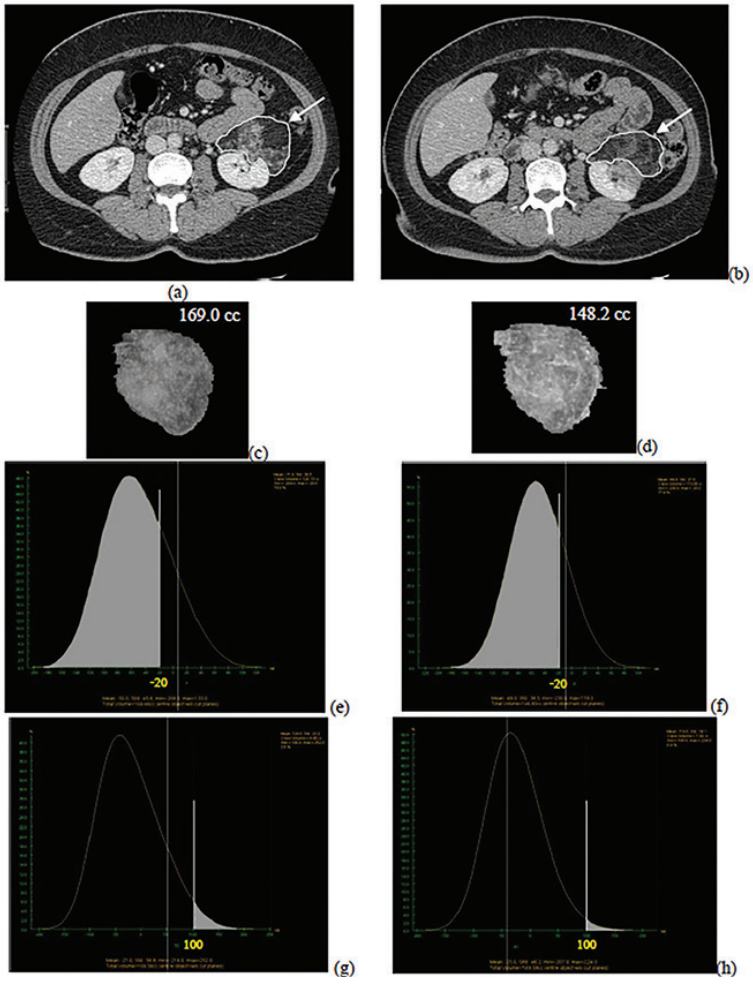


Fig 2. Three-dimensional (3D) volumetry and density histogram comparing between pre- (a, c, d, g) and 1-month post-procedural CT (b, d, f, h)
(a-b) Axial post-contrast CT showed a right renal AML *(arrows)* containing macroscopic fat. The region of interest (ROI) was drawn encircling the mass
(c-d) 3D volumetry of a total tumor volume measured 169.0 and 148.2 cc, respectively (12.3% reduction)
(e-f) Density histogram of the fat component volume (attenuation <- 20 HU) measured 126.1 cc and 113.7 cc, respectively (9.8% reduction)
(g-h) Density histogram of the enhanced angiomyogenic component volume (attenuation >100 HU) measured 4.48 cc and 1.34 cc, respectively (69.8% reduction)

complications according to the Society of Interventional Radiology Clinical Practice Guidelines.[20] The patients' medical records and MDCT findings were reviewed for the predictive factors associated with tumor rupture.

Statistical analysis

Data were analyzed using PASW Statistics 21.0 (SPSS Inc., Chicago IL USA). Patients' demographic data and the lesions' characteristics were recorded as the mean±SD (range) and median (P_{25}, P_{75}) for the quantitative variables, while numbers and percentages were summarized for the qualitative variables. Comparisons between the percentage reduction of fat and enhanced angiomyogenic component were calculated using the Wilcoxon Signed Rank test. Fisher's exact test, 2-sample T-test, and Mann-Whitney test were used to identify predictive factors associated with tumor rupture. A p-value of <0.05 was considered as a statistically significant difference.

RESULTS

Patients' demographic data

A total of 21 patients (16 female, 5 male) and 25 lesions were analyzed. The mean patient's age at the diagnosis was 47 years old (range, 9-68 years) and during the treatment was 50 years old (range, 22-68 years). Four patients (19%) had underlying tuberous sclerosis complex. Nine asymptomatic lesions were incidentally found renal AMLs from prior check-up ultrasound. Fourteen lesions presented with abdominal pain, 5 with anemia, and 1 with hematuria. Single renal AML was found in 12 patients (57.1%), and multiple AMLs in 9 patients (42.9%). Bilateral and unilateral lesions were found in 13 cases (61.9%) and 8 cases (38.1%), respectively. The mean renal AML diameter before SAE was 8.93.3 cm (range, 3.8-18.4 cm). Among 25 lesions, 10 were ruptured AMLs (40%) and 15 were unruptured AMLs (60%).

Embolization and outcome

The embolic materials and outcomes of SAE are shown in Table 1. The most common embolic material was PVA particles (13 lesions, 54%) and the second common was combined materials (4 lesions, 17%).

The technical success rate of SAE was 96% (24/25) and a clinical success rate of 84% (21/25) including 9 lesions in asymptomatic patients, who had no complication within 30 days post SAE and no re-intervention. Four lesions had clinical failure and one lesion had technical failure. Fifteen unruptured AML patients had imaging follow-up intervals. Almost 19/21 patients had long-term clinical follow-up period (range 14-128 months, mean 63 months) and all were well without requiring re-intervention. Two patients died from the other non-related diseases.

Post-embolization syndrome found in 5 lesions characterized by fever, nausea, and abdominal pain. Four lesions had a minimal renal infarction which did not contribute to renal impairment during the follow-up period (mean, 41.3 months; range, 16-70 months). Two lesions with renal abscesses post-SAE required percutaneous drainage and conservative treatment. Another lesion with infected hematoma underwent percutaneous drainage.

Imaging comparison between pre- and post-SAE

Three-dimensional (3D) volumetry and the density histogram showed the total tumor volume, fat, and angiomyogenic component reduction after SAE during the follow-up period (1 to >12 months) (Table 2). The median percentage of fat reduction was -18.4% while the median percentage of enhanced angiomyogenic reduction was -62.2% at 1-3 months follow-up, with a p-value of 0.333.

The analysis of predictive factors for tumor rupture showed that the presence of an intra-tumoral aneurysm was statistically significantly associated with tumor rupture (p-value = 0.015). The tumor size and aneurysm size were also associated with tumor rupture but did not show a significant difference (p-value = 0.071 and 0.154, respectively) (Table 3).

DISCUSSION

Recently, SAE has become widely accepted as a first-line treatment for symptomatic renal AMLs or an AML sized >4 cm.[15,16] Planché et al[18] also suggested that SAE is effective, especially on the angiomyogenic component. Our study showed a high technical success (96%) and clinical success rate (84%) of SAE, agreed with Bardin et al[10] who reported a 95.6% technical success rate of SAE in 34 cases of symptomatic and asymptomatic renal AMLs over a mean follow-up period of 20.5 months. Ramon et al[8] found a clinical success rate of 91% in 48 symptomatic renal AMLs or renal AML >4 cm. over a mean follow-up period of 58 months.

The total tumor size reduction in our study measured by 3D volumetry at 1-3 months, 6-12 months, and >12 months follow-up were -7.1%, -48.9%, and -65.3%, respectively, corresponding with the study by Planché et al[18], which showed a mean total volume reduction of -54% and -81% during 1-12 months and >12 months follow-up period, respectively.

Previous studies reported that the size of the intra-tumoral aneurysm and a proportion of the angiogenic

TABLE 1. Embolic materials and outcomes.

Embolic material used (n=24 lesions*)	
Particles (PVA†)	13 (54%)
Alcohol	3 (13%)
Glue	2 (8%)
Coil	1 (4%)
Thrombin	1 (4%)
Combined	4‡ (17%)
Treatment success (n=25 lesions)	
Technical success	24/25 (96%)
Clinical Success	21/25 (84%)
Complication§ (n=25 lesions)	
Minor complications	
Post-embolization syndrome	5/25 (20%)
Non-target Embolization	4/25 (16%)
Major complications	3/25 (12%)
Renal abscess	Conservative
Renal abscess	Percutaneous drainage
Infected hematoma	Percutaneous drainage

* Twenty-four lesions were embolized and one lesion was not embolized due to failure selection into arterial pedicle
† PVA = Polyvinyl alcohol
‡ Combined particles and glue (3 lesions) and combined coil and glue (1 lesion)
§ Categorized followed Society of Interventional Radiology Guidelines

TABLE 2. Total tumor volume, fat component volume and angiomyogenic component volume in pre-treatment, post-treatment and %reduction during follow-up.

	Follow-up period		
	1-3 months	6-12 months	>12 months
Total tumor volume (ml): median (P_{25}, P_{75})			
Lesion (n)*	10	4	6
Pre-treatment	146.9 (52.3, 177.2)	140.2 (76.7, 315.7)	65.2 (61.5, 76.7)
Post-treatment	142.2 (40.5, 154.8)	67.2 (37.5, 298.3)	27.8 (9.3, 64.3)
% Reduction	-7.1 (-26.6, +0.6)	-48.9 (-58.9, -8.1)	-65.3 (-85.7, -11.4)
Fat component volume (ml): median (P_{25}, P_{75})			
Lesion (n)*	10	4	6
Pre-treatment	127.1 (46.0, 164.0)	115.0 (73.5, 274.0)	63.4 (53.3, 73.5)
Post-treatment	107.0 (34.8, 129.5)	59.0 (34.5, 274.6)	24.8 (8.8, 58.0)
% Reduction	-18.4 (-32.3, -3.8)	-48.2 (-56.7, -5.6)	-66.2 (-86.0, -10.0)
Angiomyogenic component volume (ml): median (P_{25}, P_{75})			
Lesion (n)*	10	3†	3†
Pre-treatment	1.4 (0.4, 4.0)	1.5 (0.4, 3.9)	0.5 (0.4, 1.8)
Post-treatment	0.7 (0.2, 1.4)	0.4 (0.2, -)	1.5 (0.3, -)
% Reduction	-62.2 (-72.8, +13.5)	-82.8 (-85.5, -)	-13.1 (-49.0, -)

*Number of lesions are depended on post-treatment MDCT availability during follow-up period
†Only 3/4 lesions and 3/6 lesions had post-contrast MDCT for angiomyogenic component volume analysis in 6-12 months and >12 months follow-up period, respectively

TABLE 3. Predictive factors associated with tumor rupture.

Factors	Unruptured (n=15)	Ruptured (n=10)	p-value
TSC			
Related	3 (60%)	2 (40%)	1.000*
Not related	12 (60%)	8 (40%)	
Lesion size (cm)			
Mean±SD	7.9±2.7	10.3±3.7	0.071†
Aneurysm			
Present	4 (33.3%)	8 (66.7%)	0.015*
Not present	11 (84.6%)	2 (15.4%)	
Aneurysm size (mm)			
Median (P_{25}, P_{75})	5.3 (3.2, 8.0)	15.7 (4.8, 29.0)	0.154‡

*Fisher's Exact Test
†2-Sample T-Test
‡Mann-Whitney Test

component were the main causes of tumor rupture.[9,10-12,21] Therefore, a reduction of the total tumor size might not represent the treatment endpoint of SAE. Planché et al[18] and Han et al[21] suggested that the angiomyogenic component disappeared faster with a higher percentage of decrement than the fat component. Correspond to our study that a median percentage of fat reduction was -18.4% while the median percentage of enhanced angiomyogenic reduction was - 62.2% within 1-3 months follow-up post-SAE.

There were 4/25 lesions (16%) of clinical failure. The first lesion was a ruptured AML with an increased tumoral size containing hemolyzed blood on 2 months follow-up CT. This patient underwent surgical nephrectomy 9 months later. The second was also a ruptured AML with 30% decreased tumoral size plus resolving hematoma on CT 3 months follow-up post-SAE. Six-month later, this patient received surgical tumor removal. In these two lesions, the associated perirenal hematoma might limit the accuracy of the tumor measurement, resulting in an unnecessary surgery. The third lesion was an unruptured AML locating at renal collecting system (lesion no. 21) (Fig 1) which showed decreased total tumor size, fat, and enhanced angiomyogenic components on follow-up CT at 1 and 29 months. However, the tumor gradually increased causing obstructive left hydronephrosis on follow-up CT at 75 months, then it was surgically removed 6 years later.

The last clinical failure lesion was the same as a technical failure lesion (1/25, 4%). This was a 7.5 cm unruptured AML receiving a 2nd SAE due to an inadequate decreased size (38.7%) on follow-up CT at 6 months after the 1st SAE. The 2nd SAE was unsuccessful due to the inability to catheterize into the arterial feeder, this patient subsequently received surgery. However, our retrospectively 3D-volumetry and density histogram showed a significant reduction of the total tumor volume (41%), fat component (44%), and enhanced angiomyogenic component (53%) on follow-up CT at 6 months after the 1st SAE. This could imply that 3D measurement and density histogram might be more precise than 2D measurement to evaluate post-treatment response, thus avoiding further unnecessary treatment.

Post-embolization syndrome, a common minor complication of SAE[10] found in 5/25 lesions (20%), all were improved after conservative treatment. Four lesions (16 %) had limited renal infarction without impact on renal function. There were only 3 lesions (12%) of major complications, consisting of renal abscesses. These results suggested that SAE had low rate of major complication, in agreement with other studies.[10,15,18]

We used several embolic materials depend on the operator and the lesions. For devascularization distally, we usually used particles. But for an intra-tumoral aneurysm, we preferred glue injection or microcoil placing at the proximal arteries feeding the aneurysm.

Many prior studies have reported predictive factors associated with ruptured renal AMLs, including the tumor size, aneurysm formation, and the presence of TSC.[3,8,9] Similar to our study that found statistically significantly of an intra-tumoral aneurysm associated with tumor rupture. The tumor size and aneurysm size were also associated with tumor rupture but TSC failed to show the association.

Our study had some limitations. First, only symptomatic or large renal AMLs are indicated for SAE, leading to a small sample size. Second, the retrospective study design could make the selection bias. Third, we did not have a standard protocol of MDCT after SAE, resulting in different interval and imaging follow-up.

CONCLUSION

SAE is an effective treatment for renal AML with a high technical and clinical success rate and limited major complications. Three-dimensional volumetry and density histogram analysis might be better tools than 2D CT measurement for evaluation of post-SAE response. The presence of an intra-tumoral aneurysm is a significant predictive factor associated with tumor rupture.

ACKNOWLEDGEMENTS

The authors would like to express our gratitude to all IR staffs at Siriraj Center of Interventional Radiology for their suggestion and sincerely thanks Asst. Prof. Chulaluk Komoltri for the statistical analysis.

Conflict of interest: No potential conflicts of interest relevant to this article are reported.

REFERENCES

1. Fujii, Y, Ajima J, Oka K, Tosaka A, Takehara Y. Benign renal tumors detected among healthy adults by abdominal ultrasonography. Eur Urol 1995;27:124-7.
2. Caliò A, Warfel KA, Eble JN. Pathological features and clinical associations of 58 small incidental angiomyolipomas of the kidney. Hum Pathol 2016;58:41-6.
3. Nelson CP, Sanda MG. Contemporary diagnosis and management of renal angiomyolipoma. J Urol 2002;168:1315-25.
4. Steiner MS, Goldman SM, Fishman EK, Marshall FF. The natural history of renal angiomyolipoma. J Urol 1993;150:1782-6.
5. Flum AS, Hamoui N, Said MA, Yang XJ, Casalino DD, McGuire BB, et al. Update on the diagnosis and management of renal angiomyolipoma. J Urol 2016;195(4):834-46.
6. Prasad T, Singh A, Das CJ, Seth A. An unusually large renal angiomyolipoma peeping into the right atrium. BMJ Case Rep 2016:bcr2016215673.
7. Soulen MC, Faykus MH Jr, Shlansky-Goldberg RD, Wein AJ, Cope C. Elective embolization for prevention of hemorrhage from renal angiomyolipomas. J Vasc Interv Radiol 1994;5:587-91.
8. Ramon J, Rimon U, Garniek A, Golan G, Bensaid P, Kitrey ND, et al. Renal angiomyolipoma: long-term results following selective arterial embolization. Eur Urol 2009;55:1155-62.
9. Yamakado K, Tanaka N, Nakagawa T, Kobayashi S, Yanagawa M, Takeda K. Renal angiomyolipoma: relationships between tumor size, aneurysm formation, and rupture. Radiology 2002;225:78-82.
10. Bardin F, Chevallier O, Bertaut A, Delorme E , Moulin M, Pottecher P, et al. Selective arterial embolization of symptomatic and asymptomatic renal angiomyolipomas: a retrospective study of safety, outcomes and tumor size reduction. Quant Imaging Med Surg 2017;7:8-23.
11. Rimon U, Duvdevani M, Garniek A, Golan G, Bensaid P, Ramon J, et al. Large renal angiomyolipomas: digital subtraction angiographic grading and presentation with bleeding. Clin Radiol 2006;61:520-6.
12. Eble JN. Angiomyolipoma of kidney. Semin Diagn Pathol 1998;15:21-40.
13. Sasiwimonphan K, Takahashi N, Leibovich BC, Carter RE, Atwell TD, Kawashima. A Small (< 4 cm) renal mass: differentiation of angiomyolipoma without visible fat from renal cell carcinoma utilizing MR imaging. Radiology 2016;280:653.
14. Song S, Park BK, Park JJ. New radiologic classification of renal angiomyolipomas. Eur J Radiol 2016;85:1835-42.
15. Kiefer RM, Stavropoulos SW. The role of interventional radiology techniques in the management of renal angiomyolipomas. Curr Urol Rep 2017;18:36.
16. Akhavan Rezayat A, Aslezare M, Ahmadnia H, Soltani S, Asadpour A. Recent Strategies for the Management of Renal Angiomyolipoma: A Review of Diagnostic and Therapeutic Approaches. Nephro-Urol Mon 2017;9:e14251.
17. Bishay VL, Crino PB, Wein AJ, Malkowicz SB, Trerotola SO, Soulen MC, et al. Embolization of giant renal angiomyolipomas: technique and results. J Vasc Interv Radiol 2010;21: 67-72.
18. Planché O, Correas JM, Mader B, Joly D, Méjean A, Hélénon O. Prophylactic embolization of renal angiomyolipomas: evaluation of therapeutic response using CT 3D volume calculation and density histograms. Vasc Interv Radiol 2011;22:1388-95.
19. Andersen PE, Thorlund MG, Wennevik GE, Pedersen RL, Lund L. Interventional treatment of renal angiomyolipoma: immediate results and clinical and radiological follow-up of 4.5 years. Acta Radiol 2015;4:2058460115592442.
20. Sacks D, McClenny TE, Cardella JF, Lewis CA. Society of Interventional Radiology Clinical Practice Guidelines. J Vasc Interv Radiol 2003;14:S199-S202.
21. Han YM, Kim JK, Roh BS, Song HY, Lee JM, Lee YH, et al. Renal angiomyolipoma: selective arterial embolization--effectiveness and changes in angiomyogenic components in long-term follow-up. Radiology 1997;204:65-70.

Efficacy of Lenalidomide plus Low-Dose Dexamethasone in Thai Patients with Relapsed and/or Refractory Multiple Myeloma

Chutima Kunacheewa, M.D., Noppadol Siritanaratkul, M.D.

Department of Medicine, Faculty of Medicine Siriraj Hospital, Bangkok 10700, Thailand.

ABSTRACT

Objective: Lenalidomide is an immunomodulatory agent with proven efficacy in the treatment of multiple myeloma. In large global clinical studies, lenalidomide plus dexamethasone has demonstrated significant improvements in the overall response rate and overall survival in patients with relapsed and/or refractory multiple myeloma, compared with a placebo and dexamethasone. This is the first study to report lenalidomide plus low-dose dexamethasone administered in Thai patients.

Methods: The aim of this phase II, single-center, single-arm study was to evaluate the efficacy and safety of lenalidomide and low-dose dexamethasone in patients with relapsed and/or refractory multiple myeloma. The primary endpoint was the overall response rate at the fourth treatment cycle. Secondary endpoints included depth of response, time to response, and adverse events.

Results: In total, 15 patients with a median age of 61 years old (range 23-74 years old) who had received at least one prior anti-myeloma therapy were enrolled in the study and administered 4-week cycles of lenalidomide 25 mg/day (days 1-21) and dexamethasone 40 mg/week. Patients continued in the study until the occurrence of disease progression or serious adverse events. The overall response rate was 86% and 73.3% at the fourth and from all treatment cycles, respectively (median number of treatment cycles, 10.25), and the median dose for patients aged >60 years old was 15 mg/day. The overall response rate at the fourth cycle in patients who had received prior novel agents (bortezomib and/or thalidomide) was 81.82% compared with 100% in those who had received prior conventional therapy ($p = 0.15$). The most common adverse events reported were anemia and neutropenia, which were both manageable.

Conclusion: Lenalidomide and low-dose dexamethasone was highly effective in Thai patients with relapsed and/or refractory multiple myeloma, with a manageable adverse event profile. These findings suggest that lenalidomide 15 mg/day is a safe and effective dose for Thai patients aged ≥60 years old.

Keywords: Relapsed multiple myeloma; refractory multiple myeloma; lenalidomide; adverse events (Siriraj Med J 2021; 73: 344-353)

INTRODUCTION

Multiple myeloma is a plasma cell disorder that, to date, remains incurable.[1] Patients with relapsed and treatment-refractory multiple myeloma require effective salvage therapies to prolong disease-free progression. The introduction of autologous stem cell transplantation, and newer agents for the treatment of multiple myeloma, has substantially improved the options available for

Corresponding Author: Noppadol Siritanaratkul
E-mail: sinoppadol@gmail.com
Received 29 May 2020 Revised 4 January 2021 Accepted 6 January 2021
ORCID ID: http://orcid.org/0000-0001-8624-5516
http://dx.doi.org/10.33192/Smj.2021.45

patients who do not respond well to initial therapy. Novel agents including immunomodulatory drugs (thalidomide, lenalidomide), proteasome inhibitors (bortezomib, carfilzomib, and ixazomib), and monoclonal antibodies (elotuzumab and daratumumab) in combination with other agents have all demonstrated favorable results in terms of response, progression-free survival, and also overall survival, compared to established treatments for refractory disease, such as melphalan-based regimens or alkylating agents.[1-4]

Currently, worldwide practice uses a combination of newer novel agents, such as carfilzomib/lenalidomide/dexamethasone, daratumumab/lenalidomide/dexamethasone, or elotuzumab/lenalidomide/dexamethasone, in relapsed refractory multiple myeloma.[2-4] However, in Thailand, economic limitations have led to these new novel agents being generally unavailable for this group of patients. Although, lenalidomide plus dexamethasone has been a standard treatment in Western countries in the past decade, for developing countries, like Thailand, this combination only now represents a new hope for myeloma patients.

In phase III clinical trials (MM-009[5], MM-010[6], the use of lenalidomide plus dexamethasone in relapsed/refractory multiple myeloma patients produced improvements in overall survival and event-free survival compared with high-dose dexamethasone alone.[5,6] However, in these and other studies, lenalidomide was shown to be associated with a higher rate of grade 3-4 hematologic toxicity and a high incidence of thromboembolic events compared with dexamethasone alone.[5-7] In a randomized, controlled trial of patients with newly diagnosed multiple myeloma, the combination of lenalidomide with either high- or low-dose dexamethasone as an initial therapy resulted in high rates of treatment response and event-free survival.[8] Lenalidomide with low-dose dexamethasone was associated with significantly higher rates of overall survival at 1 year, and lower rates of thromboembolic events than lenalidomide with high-dose dexamethasone.[6-8]

There are few published data on the efficacy and safety of lenalidomide in the treatment of refractory/relapsed multiple myeloma patients in Asia. This study is the first to prospectively evaluate the administration of lenalidomide for multiple myeloma in Thailand. The aim of the study was to investigate the efficacy and safety of lenalidomide plus low-dose dexamethasone in Thai patients with refractory/relapsed multiple myeloma.

MATERIALS AND METHODS

Patients

Patients were eligible for inclusion in the study if aged ≥18 years old and if they presented with progressive multiple myeloma after at least one previous treatment regimen (e.g., vincristine, adriamycin, dexamethasone [VAD]; liposomal doxorubicin, vincristine, dexamethasone; high-dose dexamethasone; cyclophosphamide plus dexamethasone; cyclophosphamide plus prednisolone; bortezomib plus dexamethasone [VD]; thalidomide plus dexamethasone; thalidomide, cyclophosphamide, dexamethasone; bortezomib, thalidomide, dexamethasone; VD plus panobinostat; dexamethasone, cyclophosphamide, etoposide, cisplatin [DCEP]; or melphalan plus prednisolone).[9,10] Patients were required to have adequate hematologic and organ function, as demonstrated by an absolute neutrophil count ≥1,500/μL, platelet count ≥75,000/μL, hemoglobin ≥7.5 g/dL, serum creatinine <2.0 mg/dL, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels <3x the upper limit of normal, all obtained 21 days prior to enrolment. Additionally, patients were eligible for the study if they had an Eastern Cooperative Oncology Group (ECOG) performance status ≤2. Women with childbearing potential were eligible if they agreed to use contraception and had a negative pregnancy test before enrolment and took monthly pregnancy tests thereafter. Exclusion criteria for this study were dexamethasone intolerance or an allergy to any of the study mediations; inadequate liver or renal function at screening; ≥grade 2 peripheral neuropathy within 14 days prior to screening; the diagnosis or treatment of another malignancy within 2 years prior to screening (with the exception of patients with non-melanoma skin carcinoma who had undergone complete resection); ongoing or active hepatitis B virus , hepatitis C virus or HIV infection; uncontrolled comorbid cardiovascular conditions within 6 months prior to screening; an inability to take oral medication, or unwillingness to comply with the drug administration requirements, or have undergone a gastrointestinal procedure that could interfere with oral absorption or tolerance of treatment; and pregnancy. This study was approved by the Ethics Review Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University (Si 650/2010).

Study design

This was a phase II, single-center, single-arm, open-label study. Patients received oral lenalidomide 25 mg/day on days 1–21 of a 28-day cycle and dexamethasone 40 mg once weekly. The lenalidomide dose was adjusted according to patients' creatinine clearance level, absolute neutrophil count, and platelet count as recommended by the European Myeloma Network.[11] Treatment was continued until disease progression, as defined below.

Thromboembolic prophylaxis with aspirin 81 mg daily was administered to patients with at least one risk factor for thrombosis according to the International Myeloma Working Group (IMWG) guidelines for the prevention of thalidomide- and lenalidomide-associated thrombosis in myeloma.[12]

Complete blood count, blood chemistry and physical examination were conducted every 15 days in the first treatment cycle and every 4 weeks thereafter.

Response criteria

Patient disease response and progression were assessed according to the IMWG guidelines[10] and the European Group for Blood and Marrow Transplant[9] criteria for multiple myeloma. A partial response was defined as a reduction of M protein by at least 50% in the serum and 90% in urine, or both.[9,10] A complete response was defined as the complete disappearance of M protein in serum and urine by immunofixation and <5% plasma cell presence in the marrow. A very good partial response (VGPR) was defined as a >90% reduction of M protein in the serum and urine.[9,10] In patients with light chain MM, the IMWG 2011 response criteria was used. A >90% reduction of difference in involved and uninvolved serum FLC was classified as VGPR and the CR criteria require a normal serum FLC ratio in addition to CR criteria defined above.[13]

Progressive disease was defined as a ≥25% increase in serum M protein from best response, or an absolute increase in serum M protein of >500 mg/dL compared to the nadir value, or the appearance of a new bone lesion or plasmacytoma that was increasing in size.[9,10]

All toxicities were graded and attributed according to the National Cancer Institute Common Toxicity Criteria for Adverse Events, version 3.

Statistical analysis

The primary endpoint was the overall response rate (ORR) at the end of the fourth treatment cycle. Secondary endpoints included response to therapy across all cycles (limited to eight cycles), toxicity, dose adjustment due to toxicity, and time to progression (TTP). Descriptive continuous data were summarized using mean (SD), median (range) according to their distribution and categories data were demonstrated as percentage. Response to therapy was evaluated using the chi-square test to compare treatment response between patients who did or did not receive novel agents prior to enrolment. The Mann–Whitney test was used to compare the appropriate lenalidomide dose (the mean effective dose following adjustment for adverse events) in patients aged <60 and ≥60 years old. All patients were included for analysis ORR and toxicities.

RESULTS

Patient characteristics

In total, 15 patients were enrolled in this study between January 2011 and March 2012 at Siriraj Hospital, Bangkok. The median age was 61 years old (range 23-74 years old). Among these patients, 11 had received a novel agent in a prior treatment regimen, with a median of two prior treatment regimens (range 1-7). Other baseline characteristics and laboratory findings are summarized in Tables 1 and 2, respectively.

Treatment administration

Patients received a median of 10.25 treatment cycles (range 1.8-15); nine received eight complete cycles and were eligible for evaluation in this study. Two patients progressed before the fourth treatment cycle (1 of 2 them previously underwent transplantation) and were excluded from the study to receive another salvage therapy; two patients progressed at the fifth and seventh cycles, respectively, after achieving a partial response at the fourth cycle; one of these patients died as a result of infection without neutropenia after achieving a very good partial response at the fourth cycle. Two patients underwent autologous stem cell transplantation after achieving a complete response. Fig 1 illustrates the treatment pathway of the enrolled patients.

The lenalidomide dose was adjusted according to toxicity. In total, 105 doses of lenalidomide were administered. Nine of the 15 patients received a reduced lenalidomide dose, as shown in Table 2. The median lenalidomide dose was 25 mg for patients aged ≤60 years old and 15 mg for patients >60 years old (p = 0.101) (Table 3).

Aspirin 81 mg/day was administered as thromboprophylaxis for two patients (one patient with diabetes mellitus, and one patient who was immobilized due to plasmacytoma-related spinal cord compression) for the duration of lenalidomide therapy, when their platelet count was >50,000 µL. Another patient who developed bilateral edema in the legs after one cycle of lenalidomide treatment also started aspirin 81 mg/day, but this was stopped when no deep vein thrombosis was detected by compression ultrasonography. However, after complete 8 cycles of the treatment, all patients who had continued the treatment received aspirin 81 mg/day.

TABLE 1. Patient demographics and clinical characteristics at baseline.

Characteristic	All patients (n = 15) N (%)
Age (years); Median (min–max)	61 (23–74)
Gender: Male	5 (33)
ISS staging	
I	1 (20)
III	4 (80)
M protein isotype	
Immunoglobulin G	7 (47)
Immunoglobulin A	2 (13)
Light chain	6 (40)
Plasmacytoma	
Present	2 (13)
Number of previous treatment regimens	
Median (min–max)	2 (1–7)
Prior regimen	
Bortezomib	10 (67)
Thalidomide	7 (47)
Novel agent (bortezomib and/or thalidomide)	11 (73)
Stem cell transplantation	1 (7)
Laboratory	
Hemoglobin, g/dL; Median (min–max)	10.1 (7.5–11.9)
Creatinine, mg/dL; Median (min–max)	0.9 (0.5–1.8)
LDH, U/L; Median (min–max)	383 (227–864)
β-2-microglobulin, mg/L; Median (min–max)	4.75 (2.28–19.3)

Abbreviations: ISS, international staging system; LDH, lactate dehydrogenase.

TABLE 2. Lenalidomide-dose adjustment during the study.

Reasons for dose adjustment	n (%)
No. cycles administered	105
No. dose-adjusted cycles	14 (13.3)
No. patients with dose reduction (%)	9 (60)
Reason for dose reduction, n (%)	
Constitutional symptoms	4 (44.4)
Neutropenia	3 (33.3)
Renal insufficiency	3 (33.3)
Infection	2 (22.2)
Anemia	1 (1.1)
Thrombocytopenia	1 (1.1)

TABLE 3. Lenalidomide-dose adjustment according to patient age.

	Lenalidomide dose (mg/day)		
	≤60 years old	>60 years old	*p* value
Median (min–max)	25 (15–25)	15 (7.5–25)	0.101

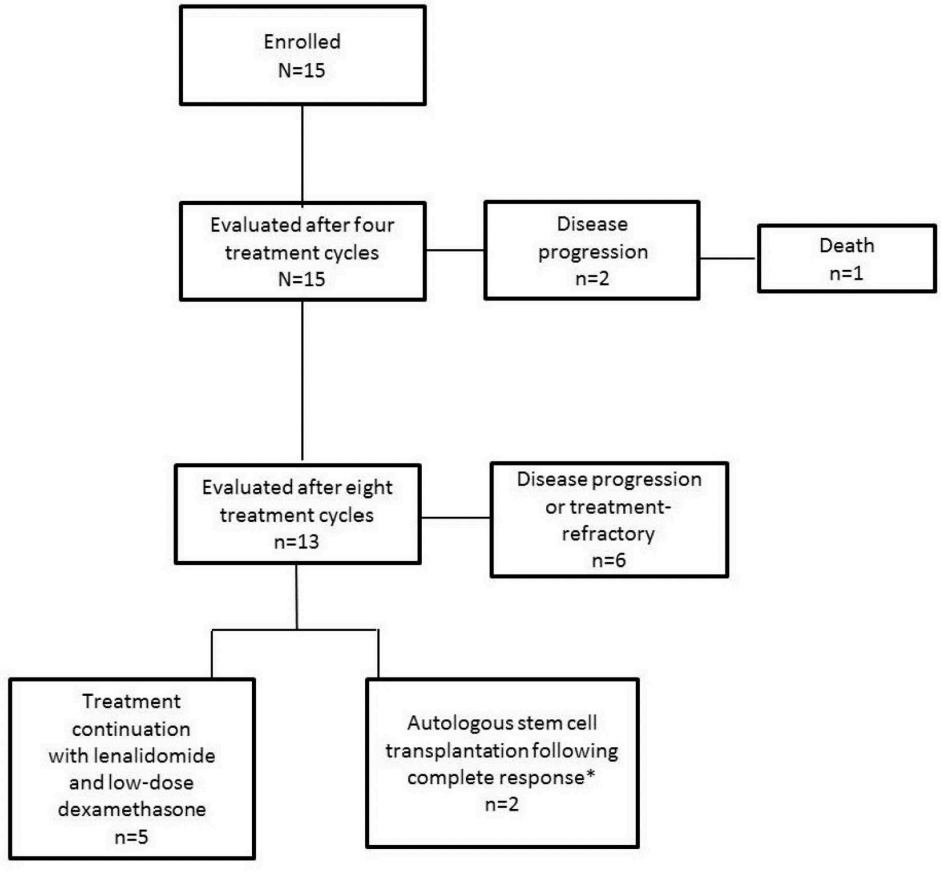


Fig 1. Treatment pathway and progression of patients during the study

Response to treatment

The median follow-up to treatment was 41 weeks (range 7-60 weeks). The ORR was 86% and 73.3% at the fourth treatment cycle and from all cycles, respectively. Seven patients (46.7%) achieved at least a very good partial response (VGPR) to treatment (Table 4). The ORR of patients with prior regimen ≤2 was trend to be better than those who received >2 prior line of therapy, 62% versus 39%, p=0.065. The ORR in patients who had received prior bortezomib or thalidomide compared with those who had not received prior novel therapy was 81.82% versus 100% (p = 0.15) and 63.6% versus 100% (p = 0.13) at the fourth and from all cycles, respectively (Table 5). The median time to response in patients who achieved a response was 0.93 months (range 0.93–2.8).

To date, five patients continue to receive lenalidomide with low-dose dexamethasone. Of the remaining patients, two underwent autologous stem cell transplantation, one patient died from septic pneumonia without neutropenia, two patients were refractory to this regimen, and five patients were considered to have progressive disease. The median time to progression (TTP) for these seven treatment-refractory patients was 8.9 months (range 1.8-14 months).

TABLE 4. Treatment response after four treatment cycles, and after all cycles.

Response	All patients (n = 15)		Prior bortezomib and/or thalidomide (n = 11)		Prior bortezomib (n = 10)		Prior thalidomide (n = 7)	
	Fourth treatment cycle	All cycles	Fourth treatment cycle	All cycles	Fourth treatment cycle	All cycles	Fourth treatment	All cycles cycle
ORR, n (%)	13 (86.7)	11 (73.3)	9 (81.8)	7 (63.6)	8 (77.8)	6 (60.0)	5 (71.4)	4 (57.1)
CR, n (%)	1 (6.7)	4 (26.7)	0	1 (9.1)	0	1 (10)	0	0
VGPR, n (%)	7 (46.7)	6 (40.0)	4 (36.4)	5 (45.5)	3 (30)	4 (40)	2 (28.6)	3 (42.9)
PR, n (%)	5 (33.3)	1 (6.7)	5 (45.5)	1 (9.1)	5 (50)	1 (10)	3 (42.9)	1 (14.3)
PD, n (%)	2 (13.3)	4 (26.7)	2 (18)	4 (36.4)	2 (20)	4 (40)	2 (28.6)	3 (42.9)

Abbreviations: CR, complete response; ORR, Overall response rate; PR, partial response; PD, progressive disease; VGPR, very good partial response

TABLE 5. Comparison of treatment responses between patients who received novel agents and those who received conventional therapy prior to lenalidomide administration.

Treatment group	Overall response rate per treatment group, n (%)			
	Fourth cycle (n = 15)	*p* value	All cycles* (n = 13)	*p* value
No prior bortezomib or thalidomide therapy	4 (100)	0.15	4 (100)	0.13
Prior bortezomib or thalidomide therapy	9 (81.8)		7 (63.6)	
No prior bortezomib only	5 (100)	0.08	5 (100)	0.15
Prior bortezomib only	8 (80)		6 (60)	
No prior thalidomide only	8 (100)		7 (87.5)	0.12
Prior thalidomide only	5 (71.43)		4 (57.1)	
No prior SCT	13 (92.9)	0.2	11 (78.6)	0.6
Prior SCT	0		0	

* Median number of treatment cycles = 10.25. SCT, stem cell transplantation

Stem cell harvest and transplantation

The two patients who underwent stem cell transplantation received lenalidomide plus low-dose dexamethasone for seven and 10 cycles, respectively. Both patients could successfully collect stem cell with high-dose cyclophosphamide and 10 microgram/kilogram of G-CSF. The first patient, a 64-year-old male, achieved a complete response at the sixth cycle and stem cells were harvested successfully after one procedure; his total CD34+ cell count was 4.3×10^6 cells/kg following 2 days of stem cell collection. The patient received melphalan 200 mg/m^2 as a conditioning regimen for 1 day, and their response was re-evaluated 3 months after stem cell transplantation. This patient achieved a complete response

1 month after the transplantation. The second patient, a 36-year-old female, received two stem cell harvesting procedures because her initial overall CD34+ cell count was 1.5×10^6 cells/kg following 3 consecutive days of stem cell collection. She then received melphalan 200 mg/m^2 as a conditioning regimen and was admitted for autologous stem cell transplantation. This patient also achieved a complete response, 3 months after stem cell transplantation.

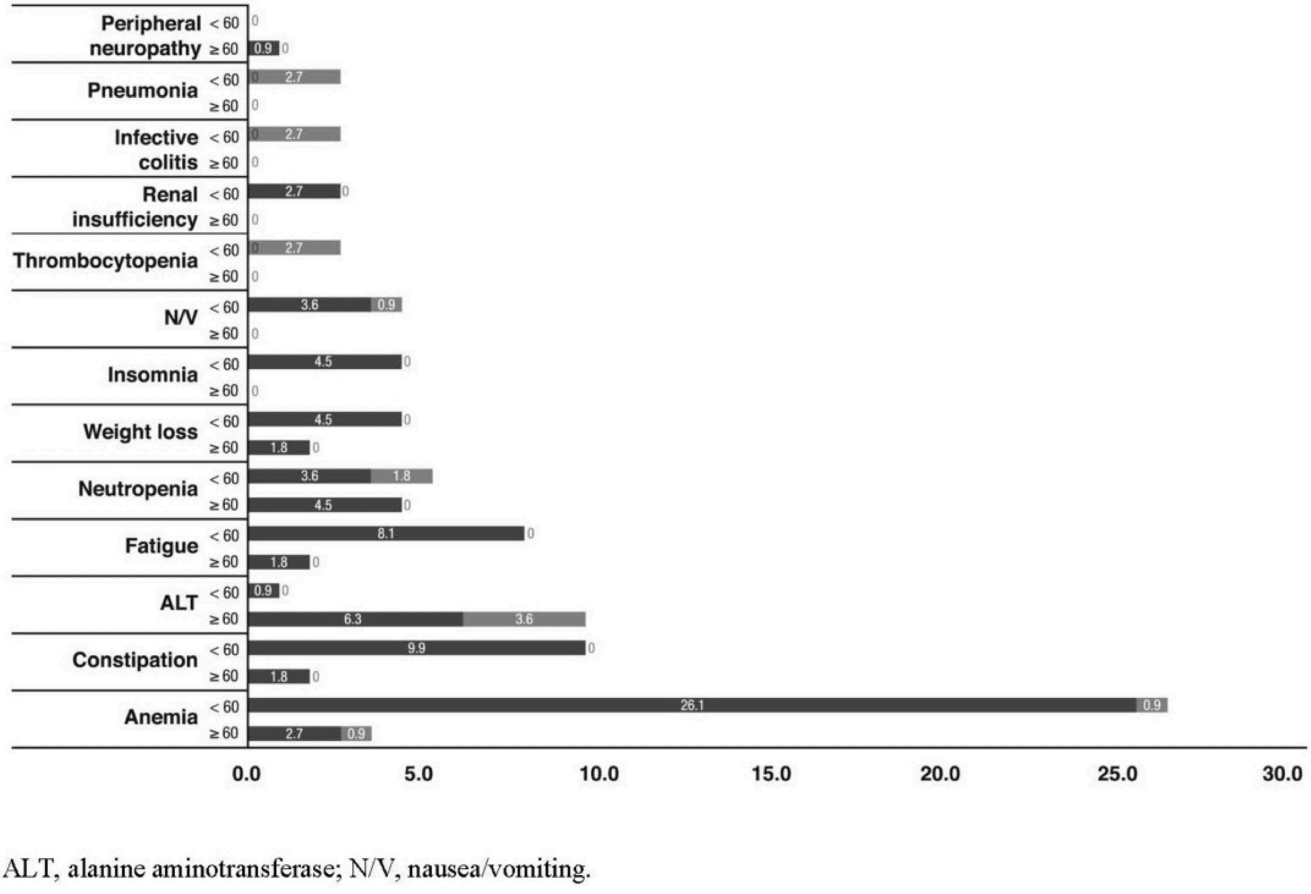
Treatment toxicity

The most common treatment-related toxicities were hematologic events. Overall, 50% of patients had at least one episode of hematologic toxicity, anemia, and/or neutropenia. However, none of the patients reported grade 3 or 4 neutropenia. The most common non-hematologic toxicity was fatigue. There was no thrombosis events. Other adverse events in patients aged <60 and ≥60 years old are shown in Fig 2. The distribution of adverse events was similar in both age groups, with notable differences shown in the frequency of grade 1-2 anemia, elevated ALT, and constipation between the two groups (2.7%, 6.3%, and 1.8% versus 26.1%, 0.9%, and 9.9%, respectively; Fig 2). The overall frequency and grade of toxicities across the 105 cycles of treatment administered are shown in Fig 3.

One patient who achieved a stringent complete response after eight cycles reported progressive disease with meningeal involvement following the twelfth treatment cycle.

DISCUSSION

Lenalidomide plus dexamethasone has demonstrated clinical efficacy in both relapsed/refractory multiple myeloma and newly diagnosed myeloma.[5-8] This is the first study to evaluate the use of lenalidomide plus low-dose dexamethasone in relapsed/refractory multiple myeloma patients in Thailand. The ORR reported here (86.7%) is consistent with those reported in prior multinational phase II and phase III trials (MM-009, MM-010) using this regimen.[5,6] Despite failing prior therapy with novel agents such as bortezomib and/or thalidomide, these patients demonstrated a positive response to lenalidomide plus low-dose dexamethasone.



ALT, alanine aminotransferase; N/V, nausea/vomiting.

Fig 2. Frequency and grade of adverse events/toxicities by age group.

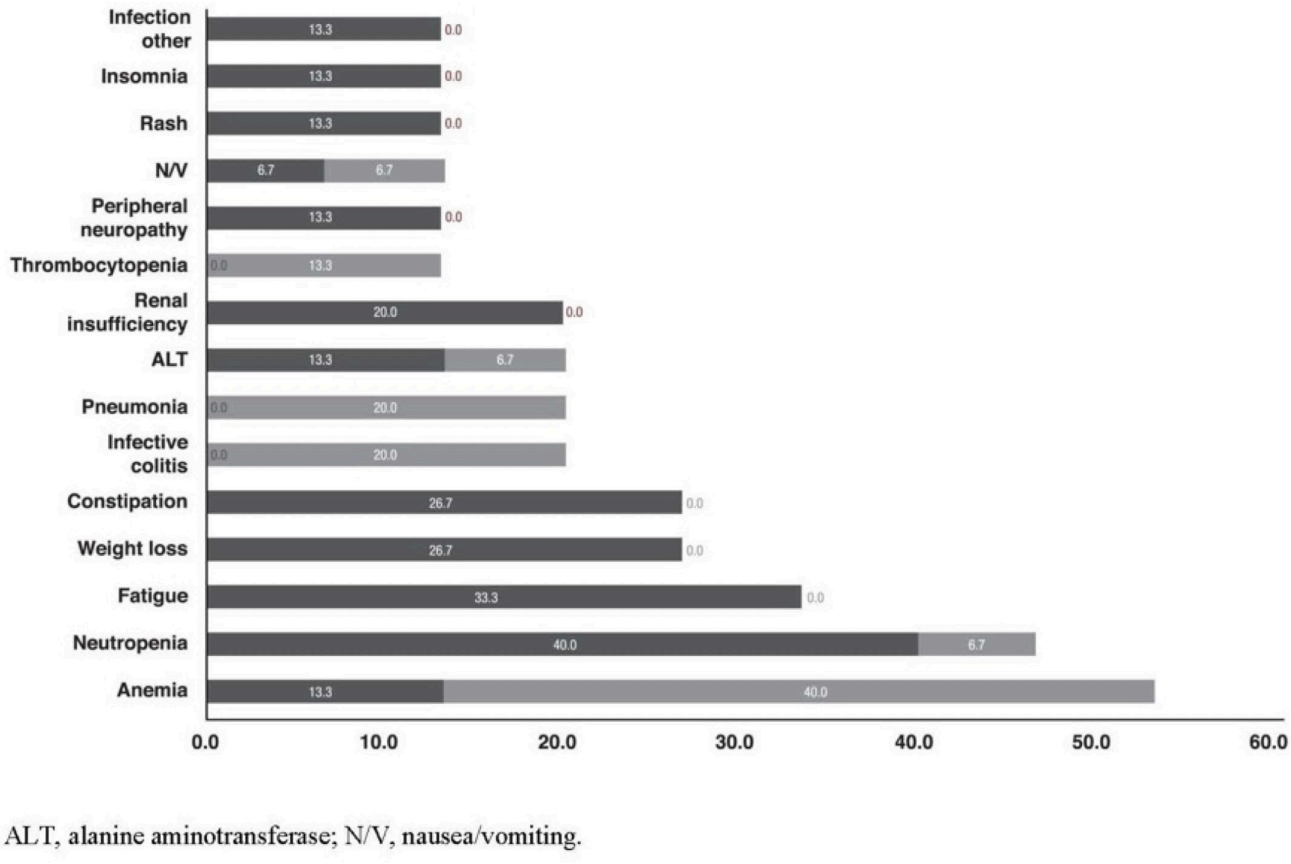


Fig 3. Frequency and grade of adverse events/toxicities across all treatment cycles.

There are few studies in the English literature investigating the efficacy of novel regimens in treatment-refractory multiple myeloma in Asia, with even fewer studies investigating lenalidomide in these patients. At our hospital in Thailand, patients with newly diagnosed multiple myeloma typically receive immunomodulatory (IMiD)- or bortezomib-based regimens, or a combination of both; if patients achieve a complete response, they then receive approval for stem cell transplantation. For patients with relapsed/refractory disease, the initial treatment regimen may be switched; if patients are candidates for transplantation, melphalan-based combinations are not used. The majority of patients receive bortezomib or IMiDs with cyclophosphamide-based conventional chemotherapy, such as VAD or DCEP. The introduction of lenalidomide further increases the treatment choice for multiple myeloma, warranting its evaluation for safety and efficacy in Thai patients.

Bortezomib- and thalidomide-based salvage therapies have demonstrated efficacy in Korean patients, with ORR of 88% - 90% reported in one clinical study.[14] Similarly high response rates (100%) were observed in an open-label study of Japanese patients with relapsed/refractory multiple myeloma receiving a combination of lenalidomide plus dexamethasone.[15] A retrospective study investigating the use of thalidomide plus high-dose dexamethasone in Thailand in newly diagnosed and treatment-refractory multiple myeloma patients reported an ORR of 92%, which is similar only to that reported in our study of treatment-refractory multiple myeloma patients.[16] The high response rates reported here support the available data in the literature and confirm the efficacy of lenalidomide in treating refractory multiple myeloma in an Asian population. However, heavily pretreated patients showed lower response when compared with patients who received ≤2 lines. In addition, the only patient who exposed to transplantation did not response well with this regimen.

Hematologic toxicities were the most common treatment-related adverse events reported in this study. However, in contrast to those reported in other studies, the most frequently reported toxicity was anemia rather than neutropenia.[5-8,16,17] Both anemia and neutropenia were manageable using transfusion and dose-reduction strategies.

The median dose of lenalidomide in patients aged >60 years old was 15 mg/day. Patients received dose reductions from the initial 25 mg/day primarily because of fatigue, anemia, and neutropenia. Following lenalidomide-dose adjustment, the toxicity profile improved in patients

aged ≥60 years old, although most of these patients reported disease progression after responding to therapy at the fourth treatment cycle. Renal impairment was another important factor leading to dose reduction. One such patient developed grade 2 neutropenia, which was successfully managed with dose reduction and appropriate correction for the renal impairment.

Our study is limited by its open-label, single-arm design, and the small size of the patient population. While the focus of our study was on treatment response, analyses incorporating progression-free and overall survival may have provided further insights into the efficacy of lenalidomide in treatment-experienced patients. Despite these limitations, the findings support the use of lenalidomide in treatment-refractory multiple myeloma, particularly in an Asian population. Our findings are consistent with data from multinational studies and also those of other Asian studies.[5,6,14-16] A larger scale, long-term randomized clinical trial would further confirm the safety and efficacy of lenalidomide for multiple myeloma in Thai patients.

Novel agents can significantly improve progression-free survival and overall survival in multiple myeloma patients. However, health insurance in Thailand does not cover the use of lenalidomide, except government health coverage. Our study showed excellent outcomes in this group of patients. In addition, this regimen is an outpatient-based regimen. Therefore, a socioeconomic study is important for the further adaptation of this regimen into all health coverage for patients' benefit.

In conclusion, the regimen of lenalidomide and low-dose dexamethasone was found to be highly effective in Thai patients with relapsed and/or refractory multiple myeloma; adverse events were manageable with an acceptable toxicity profile. Our findings suggest that lenalidomide 15 mg/day is a safe and effective dose for Thai patients older than 60 years old. This combination could be a new standard treatment in relapsed/refractory multiple myeloma in Thailand.

ACKNOWLEDGMENTS

We are grateful to all participating patients and our clinical and laboratory colleagues for their collaboration and support throughout this trial. The study was supported by the Thai Society of Hematology and the Faculty of Medicine of Siriraj Hospital, Bangkok. Lenalidomide for use in this study was donated by Celgene Corporation. Medical editorial assistance was provided by Timothy Stentiford of MediTech Media Asia. Medical editorial assistance was supported by Celgene Corporation.

Conflicts of interest: There are no conflicts of interest to declare.

REFERENCES

1. Kunacheewa C, Orlowski RZ. New Drugs in Multiple Myeloma. Annu Rev Med 2019;70:521-47.
2. Stewart AK, Rajkumar SV, Dimopoulos MA, Masszi T, Špička I, Oriol A, et al. Carfilzomib, Lenalidomide, and Dexamethasone for Relapsed Multiple Myeloma. New Engl J Med 2014;372:142-52.
3. Lonial S, Dimopoulos M, Palumbo A, White D, Grosicki S, Spicka I, et al. Elotuzumab Therapy for Relapsed or Refractory Multiple Myeloma. New Engl J Med 2015;373:621-31.
4. Dimopoulos MA, Oriol A, Nahi H, San-Miguel J, Bahlis NJ, Usmani SZ, et al. Daratumumab, Lenalidomide, and Dexamethasone for Multiple Myeloma. New Engl J Med 2016;375:1319-31.
5. Weber DM, Chen C, Niesvizky R, Wang M, Belch A, Stadtmauer EA, et al. Lenalidomide plus dexamethasone for relapsed multiple myeloma in North America. New Engl J Med 2007;357:2133-42.
6. Dimopoulos M, Spencer A, Attal M, Prince HM, Harousseau JL, Dmoszynska A, et al. Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. New Engl J Med 2007;357:2123-32.
7. Dimopoulos MA, Chen C, Spencer A, Niesvizky R, Attal M, Stadtmauer EA, et al. Long-term follow-up on overall survival from the MM-009 and MM-010 phase III trials of lenalidomide plus dexamethasone in patients with relapsed or refractory multiple myeloma. Leukemia 2009;23:2147-52.
8. Rajkumar SV, Jacobus S, Callander NS, Fonseca R, Vesole DH, Williams ME, et al. Lenalidomide plus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone as initial therapy for newly diagnosed multiple myeloma: an open-label randomised controlled trial. Lancet Oncol 2010;11:29-37.
9. Blade J, Samson D, Reece D, Apperley J, Bjorkstrand B, Gahrton G, et al. Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation. Myeloma Subcommittee of the EBMT. European Group for Blood and Marrow Transplant. Br J Haematol 1998;102:1115-23.
10. Durie BG, Harousseau JL, Miguel JS, Blade J, Barlogie B, Anderson K, et al. International uniform response criteria for multiple myeloma. Leukemia 2006;20:1467-73.
11. Dimopoulos MA, Palumbo A, Attal M, Beksac M, Davies FE, Delforge M, et al. Optimizing the use of lenalidomide in relapsed or refractory multiple myeloma: consensus statement. Leukemia 2011;25:749-60.
12. Palumbo A, Rajkumar SV, Dimopoulos MA, Richardson PG, San Miguel J, Barlogie B, et al. Prevention of thalidomide- and lenalidomide-associated thrombosis in myeloma. Leukemia 2008;22:414-23.
13. Rajkumar SV, Harousseau JL, Durie B, Anderson KC, Dimopoulos M, Kyle R, et al. Consensus recommendations for the uniform reporting of clinical trials: report of the International Myeloma Workshop Consensus Panel 1. Blood 2011;117:4691-5.
14. Ahn JS, Yang DH, Jung SH, Park HC, Moon JH, Sohn SK, et al. A comparison of bortezomib, cyclophosphamide, and dexamethasone (Vel-CD) chemotherapy without and with

thalidomide (Vel-CTD) for the treatment of relapsed or refractory multiple myeloma. Ann Hematol 2012;91:1023-30.

15. Iida S, Chou T, Okamoto S, Nagai H, Hatake K, Murakami H, et al. Lenalidomide plus dexamethasone treatment in Japanese patients with relapsed/refractory multiple myeloma. Int J Hematol 2010;92:118-26.

16. Niparuck P, Sorakhunpipitkul L, Atichartakarn V, Chuncharunee S, Ungkanont A, Aungchaisuksiri P, et al. Treatment outcome of thalidomide based regimens in newly diagnosed and relapsed/refractory non-transplant multiple myeloma patients: a single center experience from Thailand. J Hematol Oncol 2010;3:1.

17. Dimopoulos MA, Hussein M, Swern AS, Weber D. Impact of lenalidomide dose on progression-free survival in patients with relapsed or refractory multiple myeloma. Leukemia 2011;25:1620-6.

Changes in HbA_2 Levels May Signify Hemoglobin Defects in Infants

To the editor:

The hemoglobin profile has been utilized as a clue for the diagnosis of thalassemia and hemoglobinopathy. Fetal hemoglobin (HbF, $\alpha_2\gamma_2$) is highly expressed as the major functional hemoglobin during the fetal stage. Following hemoglobin switching, HbF declines to less than 1% of the total hemoglobin within one year of age. Adult hemoglobin (HbA, $\alpha_2\beta_2$) therefore predominates over total hemoglobin in combination with 2-3% of HbA_2 ($\alpha_2\delta_2$) and trace amounts of HbF throughout adult life. Hemoglobin can routinely be analyzed using chromatography or electrophoresis. Relative alterations of these hemoglobin types have been shown to be associated with abnormal globin production in adults. For instance, hemoglobin E (HbE, $\alpha_2\beta_2^{26Glu\rightarrow Lys}$), the hallmark hemoglobinopathy of Southeast Asia, exists in a range from 25–90% of the total hemoglobin in adults with HbE inheritance; however, HbE levels present less than 25% of total hemoglobin in heterozygous HbE patients who co-inherit with α-thalassemia. Moreover, HbA_2 is markedly raised between 3.5 and 9.9% in adult heterozygous β-thalassemia. In contrast, relative changes in hemoglobin types are unnoticeable by routine hemoglobin analysis in adults with one or two α-globin gene defects.[1] To broaden the knowledge and illustrate the effects of globin disorders on hemoglobin profile in infants, leftover blood samples from 142 unrelated individuals aged between 8 and 12 months with a mean corpuscular volume (MCV) less than 80 fL were subjected to thalassemia screening and diagnosis. Hemoglobin analysis was performed regarding the Bio-Rad Variant II Hemoglobin Testing System with β-Thalassemia Short Program (BioRad, Hercules, CA). The diagnosis of thalassemia was performed according to standard diagnostic guidelines used in Thailand.[2-4] In addition, common deletion types of α^+-thalassemia ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) and α^0-thalassemia ($--^{SEA}$ and $--^{THAI}$) were genotyped as described in previous studies.[5-6] The protocols in this study were approved by the ethics committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand (ethical approval reference number AMSEC-63EM-001). The results revealed that 59 (41.5%) were negative for the common thalassemias (hereafter so called normal),37 (26.1%) were heterozygous for β-thalassemia, 19 (13.4%) were heterozygous for HbE, 18 (12.7%) were heterozygous for α-thalassemia 1, and 9 (6.3%) were deletional HbH disease caused by compound heterozygous α-thalassemia 1 with α-thalassemia 2 (Table 1). Complete blood count (CBC) showed hypochromic microcytic anemia with reduced total Hb level, Hct, MCV, and mean corpuscular hemoglobin (MCH) (Table 1). On average, the hemoglobin profile of normal infants was similar to that of normal adults, suggesting that the hemoglobin switching had completed in these subjects. Conversely, a significant increase in HbA_2 and HbF levels was observed in infants with heterozygous β-thalassemia and heterozygous HbE (Table 1). The elevated HbA_2 levels were similar to those of adults with heterozygous β-thalassemia. Regardless of age, this suggested that increased HbA_2 levels determine the inheritance of β-thalassemia. Interestingly, elevated HbF levels were absent in adults with either heterozygous β-thalassemia or heterozygous HbE,[7-8] suggesting a developmental stage-specific effect. This finding is comparable to previous studies in which the delayed HbF to HbA switching is remarkably shown in infants with β-globin defects.[9,10] Although the mechanisms underlining HbF to HbA switching are unclear, the prolonged HbF levels in infants with a β-globin defect maybe due to the primary compensation of β-like globin gene expression and total hemoglobin during development in affected infants. Similar to those of adults with homozygous β^0-thalassemia and compound heterozygous β^0-thalassemia with HbE disease, the increase in γ-globin gene expression has been shown to associate with milder clinical manifestations as it is able to substitute for the inadequate β-globin gene expression and yields increased HbF levels. In contrast to β-globin gene defects, heterozygous α^0-thalassemia demonstrated comparable hemoglobin types to the normal in our finding. Despite insignificance, infants with two α-globin gene defects displayed reduced HbA_2 and modestly increased HbF levels in the previous study.[9] The decrease in HbA_2 levels was clearly noticed in infants with three α-globin gene defects or deletional HbH disease in our study. Together, the results suggested that HbA_2 levels may be considered as valuable markers for the inheritance of globin gene defects in infants.

TABLE 1. Hematological data of the infants participated in this study.

Characteristic	Age (Month), Mean ± SD	Sex (No.), Boy/Girl	RBC (10^{12}/L), Mean ± SD	Hb (g/dL), Mean ± SD	Hct (%), Mean ± SD	MCV (fL), Mean ± SD	MCH (pg), Mean ± SD	MCHC (g/dL), Mean ± SD	HbA2/E (%), Mean ± SD	HbF (%), Mean ± SD	HbA (%), Mean ± SD	Hb Bart's	HbH
Normal (n = 59)	11.6 ± 1.0	27/32	5.1 ± 0.5	9.9 ± 1.2	32.0 ± 3.5	62.5 ± 4.4	19.3 ± 2.1	30.9 ± 1.6	2.6 ± 0.4	1.1 ± 0.8	91.5 ± 5.8	Absent	Absent
Heterozygous β-thalassemia (n = 37)	12.0 ± 0.2	21/16	5.6 ± 0.5	10.0 ± 0.7	31.9 ± 2.4	56.8 ± 3.0	17.9 ± 1.1	31.5 ± 1.4	5.5 ± 0.5	6.1 ± 3.8	83.3 ± 6.8	Absent	Absent
Heterozygous HbE (n = 19)	11.9 ± 0.2	11/8	5.0 ± 0.6	10.6 ± 2.2	31.8 ± 4.9	64.1 ± 6.1	21.3 ± 3.4	33.1 ± 3.8	24.7 ± 4.2	4.2 ± 2.4	66.7 ± 7.1	Absent	Absent
Heterozygous α^0-thalassemia ($--^{SEA}/\alpha\alpha$) (n = 18)	12.0 ± 0.0	10/8	6.0 ± 0.5	10.4 ± 1.2	34.0 ± 3.6	57.2 ± 6.2	17.5 ± 2.2	30.5 ± 1.3	2.5 ± 0.3	1.2 ± 0.8	92.7 ± 5.5	Absent	Absent
HbH disease ($--^{SEA}/-\alpha^{3.7}$) (n = 9)	12.0 ± 0.0	4/5	5.8 ± 0.6	9.1 ± 0.7	30.8 ± 2.7	53.0 ± 2.9	15.7 ± 1.5	29.5 ± 1.8	1.6 ± 0.5	1.4 ± 1.1	92.7 ± 3.5	Present	Present

Abbreviations: RBC, red blood cell; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

REFERENCES

1. Akhavan-Niaki H, Youssefi Kamangari R, Banihashemi A, Kholghi Oskooei V, Azizi M, Tamaddoni A, et al. Hematologic features of alpha thalassemia carriers. Int J Mol Cell Med 2012;1:162-7.
2. Fucharoen G, Sanchaisuriya K, Sae-ung N, Dangwibul S, Fucharoen S. A simplified screening strategy for thalassaemia and haemoglobin E in rural communities in south-east Asia. Bull World Health Organ 2004;82:364-72.
3. Sanchaisuriya K, Fucharoen S, Fucharoen G, Ratanasiri T, Sanchaisuriya P, Changtrakul Y, et al. A reliable screening protocol for thalassemia and hemoglobinopathies in pregnancy: an alternative approach to electronic blood cell counting. Am J Clin Pathol 2005;123:113-8.
4. Karnpean R, Fucharoen G, Pansuwan A, Changtrakul D, Fucharoen S. A proficiency testing program of hemoglobin analysis in prevention and control of severe hemoglobinopathies in Thailand. Clin Chem Lab Med 2013;51:1265-71.
5. Liu YT, Old JM, Miles K, Fisher CA, Weatherall DJ, Clegg JB. Rapid detection of alpha-thalassaemia deletions and alpha-globin gene triplication by multiplex polymerase chain reactions. Br J Haematol 2000;108:295-9.
6. Pornprasert S, Wiengkum T, Srithep S, Chainoi I, Singboottra P, Wongwiwatthananukit S. Detection of alpha-thalassemia-1 Southeast Asian and Thai type deletions and beta-thalassemia 3.5-kb deletion by single-tube multiplex real-time PCR with SYBR Green1 and high-resolution melting analysis. Korean J Lab Med 2011;31:138-42.
7. Yamsri S, Sanchaisuriya K, Fucharoen G, Sae-Ung N, Fucharoen S. Genotype and phenotype characterizations in a large cohort of beta-thalassemia heterozygote with different forms of alpha-thalassemia in northeast Thailand. Blood Cells Mol Dis 2011;47:120-4.
8. Sae-ung N, Srivorakun H, Fucharoen G, Yamsri S, Sanchaisuriya K, Fucharoen S. Phenotypic expression of hemoglobins A(2), E and F in various hemoglobin E related disorders. Blood Cells Mol Dis 2012;48:11-16.
9. Kingchaiyaphum B, Sanchaisuriya K, Fucharoen G, Chaibunruang A, Hess SY, Hinnouho GM, et al. Hemoglobins F, A2 , and E levels in Laotian children aged 6-23 months with Hb E disorders: Effect of age, sex, and thalassemia types. Int J Lab Hematol 2020;42:277-83.
10. Winichagoon P, Svasti S, Winichagoon P, Chitchumroonchokchai C, Fucharoen S. Expression of betaE and gamma-globin genes in infants heterozygous for hemoglobin E and double heterozygous for hemoglobin E and alpha-thalassemia. Haematologica 2007; 92:702-3.

Pinyaphat Khamphikham, Ph.D.*, Manoo Punyamung, B.Sc., Sakorn Pornprasert, Ph.D.***

**Division of Clinical Microscopy, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200, Thailand, **Clinical Service Center, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200, Thailand.*

Corresponding author: Sakorn Pornprasert
E-mail: sakornmi001@gmail.com

Received 20 January 2021 Revised 8 March 2021 Accepted 8 March 2021

ORCID ID: http://orcid.org/0000-0003-0280-5569

http://dx.doi.org/10.33192/Smj.2021.46