ISSN 0857-6084

THAI JOURNAL OF OBSTETRICS AND GYNAECOLOGY

THE OFFICIAL JOURNAL OF
THE ROYAL THAI COLLEGE OF OBSTETRICIANS AND GYNAECOLOGISTS

VOL. 31 NO. 4 JULY - AUGUST 2023

Executive Board
of
The Royal Thai College of Obstetricians and Gynaecologists

PRESIDENT

Prof. V. Titapant, M.D.

PRESIDENT-Elect

Prof. S. Wilailak, M.D.

EXECUTIVE BOARD MEMBERS

Assoc. Prof. A. Jaishuen, M.D.
Assoc. Prof. A. Kamudhamas, M.D., DHS, Ph.D.
Assist. Prof. A. Yantapant, M.D.
Assoc. Prof. B. Chumworathayi, M.D., Ph.D.
Assoc. Prof. C. Wanapirak, M.D.
Assoc. Prof. K. Panyakhamlerd, M.D.
Assoc. Prof. M. Thamkhantho, M.D.
Assist. Prof. N. Israngura Na Ayudhya, M.D.
O. Musigavong, M.D.
Assoc. Prof. P. Ruangvutilert, M.D., Ph.D.
Assoc. Prof. S. Bunyavejchevin, M.D.
S. Khunpradit, M.D.
Assoc. Prof. T. Suntharasaj, M.D.
Assoc. Prof. W. Termrungruanglert, M.D.
C. Matatratip, M.D.

Thai Journal of Obstetrics and Gynaecology

The Official Journal of the Royal Thai College of Obstetricians and Gynaecologists

ISSN 0857-6084 E-ISSN 2673-0871

Editor in Chief

PHUPONG Vorapong

King Chulalongkorn Memorial Hospital, Chulalongkorn University, Thailand

International Editorial Board:

Manager: Prof. Vitaya Titapant, M.D.
Assistant Manager: Arissara Puangmalee, B.A.
Office: 8th Floor, The Royal Golden Jubilee Bldg. 2, Soi Soonvijai, New Petchburi Road, Bangkapi, Bangkok 10310, Thailand
Published by: PIMDEE Co., Ltd. Tel: 091-009-4011

Website: www.tci-thaijo.org, E-mail: vorapong.p@chula.ac.th

Aim and Scope of the Thai Journal of Obstetrics and Gynaecology (Official journal of the Royal Thai College of Obstetricians and Gynaecologists (RTCOG)

Thai Journal Obstetrics and Gynaecology (TJOG) is the official journal of The Royal Thai College of Obstetricians and Gynaecologists (RTCOG). This is a double-blind peer-reviewed journal aiming to promote academic knowledge and provide a forum for publication in Obstetrics and Gynecology. Manuscripts submitted to TJOG will be accepted on the understanding that the author must not have previously submitted the paper to another journal or have published the material elsewhere.

Type of Paper: Special article (invited), Original article, Case report
Frequency: 6 issues per year (January-February, March-April, May-June, July-August, September-October, November-December)
Language: Fulltext in English, Abstract both in Thai and English
Free Access: online
ISSN: 0857-6084 (Since 1989)
E-ISSN: 2673-0871 (Since December 2010)

Direction to contributors. All papers should be sent to Editor, Thai Journal of Obstetrics and Gynaecology, 8th Floor, The Royal Golden Jubilee Bldg. 2, Soi Soonvijai, New Petchburi Road, Bangkapi, Bangkok 10310, Thailand. The editorial board will decide upon the time of publication and retain the right to modify the style and the length of the contribution. However, major changes will be agreed with the authors.

Manuscripts. All manuscripts can be submitted online (http://tci-thaijo.org/index.php/tjog) along with a cover letter, author agreement form and the checklist guideline. A cover letter must include name of the corresponding author, full address, telephone number, fax number, and e-mail address, title and category of the submitted manuscript: original article, case report or review articles. Authors for whom English is a second language may choose to have their manuscript professionally edited before submission to improve the English.

The requirements for manuscripts submitted to Thai Journal of Obstetrics and Gynaecology conform to the UNIFORM REQUIREMENT FOR MANUSCRIPTS SUBMITTED TO BIOMEDICAL JOURNALS established by the international committee of medical journal editor which published in N Engl J Med 1991;324:424-8 and BMJ 1991;302:338-41.

Manuscripts of original work should be arranged in the conventional order of title page, abstract, keywords, introduction, materials and methods, results, discussion, acknowledgments, references, table and figure legends.

Manuscripts of research article, case report and review article (without author's name) will be reviewed by two reviewers. Editor in chief will make the final decision in case of discrepancy of reviewer's opinion. The editorial board has the right to grammatically correct any content and has all right preserved to consider and to publish any article.

All published manuscripts are properties of Thai Journal of Obstetrics and Gynaecology. The content and any opinions in the published papers are the sole responsibility of the authors, not the editorial board.

Title page. The title page should contain the title, which should be concised and informative, the authors' name with the highest academic degree, and address of the authors including the correspondence.

Abstract. A structured abstract, with 250 words or less, is submitted as required for regular articles. The abstract should state the Objective, Materials and Methods, Results, and Conclusions, each with a brief adequate presentation. Abstracts for case reports should not exceed 50 words.

Keyword. Below the abstract list 3 to 5 keywords or short phrases for indexing purposes.

Introduction. State clearly the purpose of the study. Summarize the rationale for the study. Give only strictly pertinent references and it is not necessary to include all the background literature.

Materials and Methods. Describe briefly the plan, patients, procedures, controls and statistical method employed.

Results. Present your results in sequence in the text, tables, and illustrations. Summarize and emphasize only important observations.

Discussion. Comment on your results and relate them to those of other studies. Recommendations may be included.

References. References to the literature should be numbered consecutively and indicated by a superscript in parenthesize. Identify references in the text, tables and legends by arabic numerals within marks. Cite the names of all authors when there are six or fewer; when seven or more list the first six followed by et al. Names of journals should be abbreviated in the style used in *Index Medicus*. Try to avoid using abstracts as references. Unpublished data and personal communication should not be used as references.

Example of references:

Journal article

Phupong V, Aribarg A. Congenital arteriovenous malformations of the uterus. Thai J Obstet Gynaecol 2000;12:67-70.

Book

Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Rouse DJ, Spong CY. Williams Obstetrics. 23rd ed. New York: McGraw-Hill, 2010: 804-31.

Chapter in a Book

Phupong V. Management of PPROM AT 32 to 34 weeks. In: Desai SV, Tank P, eds. Handbbok on preterm prelabor rupture of membranes in a low source setting. New Delhi: Jaypee Brothers Medical Publishers Ltd, 2012: 39-46.

Tables. Tables should present new information rather than duplicating what is in the text. Please supply editable files. A short descriptive title should appear above each table with a clear legend and any foonotes suitably identified below. All units must be included.

Figures. Figures should be high quality (1200 dpi for line art, 600 dpi for gray scale and 300 dpi for colour). Figures should be saved as TIFT or JPEG files. Figures should be completely labelled, taking into account necessary size reduction. Captions should be typed, double - spaced, on a separate sheet.

Ethical consideration. Each author's contribution to the paper is to be quantified. Authors must state that the protocol for the research project has been approved by a suitably constituted Ethics Committee of the institution within which the work was undertaken.

Publication Ethics and Publication Malpractice Statement. The publication ethics is required for publication in *Thai J Obstet Gynaecol*. The publication ethics guidelines are followed the *Committee on Publication Ethics-COPE (http://publicationethics.org/)*.

Editor of Thai Journal of Obstetrics and Gynaecology

1. strive to meet the needs of readers and authors, constantly improve the journal.

2. have processes in place to assure the quality of the material published.

3. give timely and comprehensive feedback to authors.

4. maintain the integrity of the academic record and preclude business needs from compromising intellectual and ethical standards.

5. are willing to publish corrections, clarifications, retractions and apologies when needed.

6. seek the views of authors, readers, reviewers and editorial board members about ways of improving the journal's processes.

7. encourage and being aware of research into peer review and publishing and reassessing the journal's processes in the light of new findings.

8. endeavor to ensure that research published was carried out according to the relevant internationally accepted guidelines (e.g. the Declaration of Helsinki for clinical research, the AERA and BERA guidelines for educational research).

9. seek assurances that all research has been approved by an appropriate body (e.g. research ethics committee, institutional review board).

10. have a duty to act if editors suspect misconduct or if an allegation of misconduct is brought to editors.

11. pursue misconduct for the following reasons in published and unpublished work: plagiarism of other works, data fabrication and falsification, when a submitted manuscript has been found to be under revision elsewhere or published elsewhere, or where there is citation manipulation.

12. make decisions to accept or reject a paper for publication based on the paper's importance, originality and clarity, and the study's validity and its relevance to the remit of the journal.

13. respect requests from authors that an individual should not review their submission, if these are well reasoned and practicable.

Authors who submit articles to TJOG should

1. Report the research conducted in an ethical and responsible manner and comply with all relevant legislation.

2. Present the results clearly, honestly, and without fabrication, falsification or inappropriate data manipulation.

3. Strive to describe the methods clearly and unambiguously so that the findings can be confirmed by others.

4. Adhere to publication requirements that submitted work is original, is not plagiarized, and has not been published elsewhere.

5. Take collective responsibility for submitted and published work.

6. Confirm that the authorship of research publications should accurately reflect individuals' contributions to the work and its reporting.

7. Disclose funding sources and relevant conflicts of interest.

Reviewers of TJOG should

1. Only agree to review manuscripts for which they have the subject expertise required to carry out a proper assessment and which they can assess in a timely manner

2. Respect the confidentiality of peer review and not reveal any details of a manuscript or its review, during or after the peer-review process, beyond those that are released by the journal

3. Declare all potential conflicting interests, seeking advice from the journal if they are unsure whether something constitutes a relevant interest

4. Not allow their reviews to be influenced by the origins of a manuscript, by the nationality, religious or political beliefs, gender or other characteristics of the authors, or by commercial considerations

5. Be objective and constructive in their reviews, refraining from being hostile or inflammatory and from making libelous or derogatory personal comments

6. Acknowledge that peer review is largely a reciprocal endeavor and undertake to carry out their fair share of reviewing and in a timely manner

7. Provide journals with personal and professional information that is accurate and a true representation of their expertise

8. Recognize that impersonation of another individual during the review process is considered serious misconduct.

Article processing charge. To publish in Thai J Obstet Gynaecol, authors are required to pay an article processing charge (APC). The APC for all published papers is $100. Members of RTCOG have 50% discount for APC.

Subscription. Thai Journal of Obstetrics and Gynaecology is published every three months. The annual subscription rate is US$ 50 post free by surface mail. Order for subscription, business correspondences and advertising space should be addressed to the editor.

Thai Journal of Obstetrics and Gynaecology

The Official Journal of the Royal Thai College of Obstetricians and Gynaecologists

ISSN 0857-6084 E-ISSN 2673-0871

Vol. 31 No. 4 JULY - AUGUST 2023

CONTENTS

Thai Journal of Obstetrics and Gynaecology
July 2023, Vol. 31, No. 4, pp. 235.

EDITORIAL

I am pleased to inform the Royal Thai College of Obstetricians and Gynaecologists (RTCOG) members that Thai Journal of Obstetrics and Gynaecology (TJOG) already received the Q3 journal rankings (149/203 journals) with SJR score 0.158 in Obstetrics and Gynaecology category from Scimago Journal & Country Rank 2022. The quality of TJOG has been improved. TJOG has been indexed in many databases: Scopus, TCI, ASEAN Citation Index, DOAJ, EuroPub, and Google Scholar. The journal editorial team would like to thank past RTCOG executive board, past editor in chief, editorial board and staff, reviewers, all members of RTCOG, and all researchers for their kind contribution and support to TJOG.

Editor in Chief and managing staff already attended "Policy, Achievement and Award Ceremony for Outstanding Performance Journals in the TCI-TSRI-Scopus Collaboration Project" on Friday, May 26th, 2023, at the Phayathai 3-4 Rooms, Level 6, Eastin Grand Hotel Phayathai Ratchathewi, Bangkok.

This fourth issue of TJOG 2023 contains many interesting articles. One special article is "Pre-implantation genetic testing for monogenic disorders at Chiang Mai University: 20 Years Experience".

RTCOG Annual Meeting 2023 will be held during 24-27 October 2023 at Dusit Thani Pattaya Hotel, Chonburi, Thailand. The theme of this meeting is "Smart O&G". All RTCOG members are cordially invited to participate this scientific meeting.

Residents who would like to publish their researches in TJOG should submit their works before September 30, 2023. Our editorial team and constructive reviewers will let them know the results before December 31, 2023.

Wish to see you at RTCOG Annual Meeting 2023 at Dusit Thani Pattaya Hotel, Chonburi, Thailand

Prof. Vorapong Phupong, M.D.
Editor in Chief

Thai Journal of Obstetrics and Gynaecology
July 2023, Vol. 31, No. 4, pp. 236-247.

SPECIAL ARTICLE

Pre-implantation Genetic Testing for Monogenic Disorders at Chiang Mai University : 20 Years Experience

Wirawit Piyamongkol, M.D., PhD*,
Sirivipa Piyamongkol, PhD**.

** Department of Obstetrics and Gynaecology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*
*** Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand*

ABSTRACT

Objectives: The prevention and control program for severe thalassemias in Thailand has been very successful. However, at present, some families at risk of having an offspring with severe thalassemias are looking for a better option other than termination of pregnancy. Pre-implantation genetic testing of monogenic disorders (PGT-M) or embryo selection would be the correct answer. This study presents the PGT-M with 20 years experience at Chiang Mai University.

Materials and Methods: The couples at risk of having the offspring with Hemoglobin Bart's (Hb Bart's) disease, beta-thalassemia major, and beta-thalassemia-Hb E disease came in for genetic counselling and PGT-M treatment. PGT-M protocols are based on multiplex fluorescent polymerase chain reaction (PCR) and mini-sequencing. PGT-M protocols for Hb Bart's disease, beta-thalassemia major, and beta-thalassemia-Hb E disease have been developed, tested, and clinically applied.

Results: Since 2003, a total of 168 PGT-M cycles in 125 families have been performed, giving rise to a total of 75 pregnancies (85 healthy babies). A total of 132 clinical PGT-M cycles were performed for 111 couples at risk of having the offspring with Hb Bart's disease, beta-thalassemia major, and beta-thalassemia-Hb E disease giving rise to 66 pregnancies with 76 babies. No misdiagnosis has been detected. Notably, three families were at risk of having the offspring with both Hb Bart's disease and beta-thalassemia-Hb E disease. Two families had already had a affected child with beta-thalassemia-Hb E disease and came in for PGT-M of beta-thalassemia-Hb E disease and human leukocyte antigen (HLA) matching.

Conclusion: The pregnancy rates were 44.6%, however, some PGT-M cycles are still on-going and the embryos are kept frozen. More pregnancies should be obtained when the patients return for embryo transfer. In addition to severe thalassemias, PGT-M for other rare diseases have been done. During the past 20 years, over 20 PGT-M protocols have been developed, tested, and clinically applied. All protocols were novel and home grown.

Keywords: embryo selection, multiplex fluorescent polymerase chain reaction (F-PCR), pre-implantation genetic diagnosis (PGD), pre-implantation genetic testing for monogenic disorders (PGT-M), thalassemias

Correspondence to: *Wirawit Piyamongkol, MD, Ph.D., Department of Obstetrics and Gynaecology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand. E-mail: wirawit.p@cmu.ac.th*

Received: 6 June 2023, **Revised:** 27 June 2023, **Accepted:** 30 June 2023

Introduction

Pre-implantation Genetic Testing (PGT)

Pre-implantation genetic testing (PGT) for embryo selection was first performed for sex determination using PCR to avoid X-linked disorders by Alan Handyside at Hammersmith Hospital, London[1]. For PGT, single blastomeres for PCR analysis were biopsied[2] from embryos generated using in vitro fertilization (IVF) techniques[3]. Since then until 2018, over 100,000 clinical PGT cycles have been performed worldwide resulting in over 20,000 clinical pregnancies and more than 13,000 PGT babies were born according to European Society of Human Reproduction and Embryology (ESHRE) PGT Consortium[4]. Indications for PGT include PGT for monogenic disorders (PGT-M), PGT for aneuploidies (PGT-A) and PGT for structural rearrangements (PGT-SR). Blastocyst biopsy[5] has become the most popular sampling methods instead of cleavage stage embryo biopsy in the early age. Blastocyst biopsy provided more cells, consequently, eases molecular analysis, however, the embryos need to be kept frozen after the biopsy waiting for transfer. Therefore, molecular procedures do not need to rush.

Molecular Genetic Techniques

Due to misdiagnosis of embryo gender in the very first cases from allele drop out (ADO)[6], fluorescent in situ hybridization (FISH) was recommended for sexing[7], numerical chromosomal abnormalities and chromosome translocations[8]. The most popular platform, AneuVysis, limited to 5 chromosomes probes, i.e. chromosomes 21, 18, 13, X and Y. Comparative genome hybridization array (aCGH) replaced FISH in 2011 when BlueGnome could overcome the 72-hour incubation period into 16-hour incubation period. 24Sure could reveal copy number variation (CNV) of 22 chromosomes, X and Y[9]. Since 2015 Next Generation Sequencing (NGS) CGH has become the main platform for PGT-A due to its higher sensitivity and lower cost[10].

- Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR)[11] has been the main tool for PGT-M including Myotonic Dystrophy[12], beta-thalassemia[13], alpha-thalassemia (-SEA)[14], beta-thalassemia-Hb E disease[15], Duchenne muscular dystrophy[16], Marfan's syndrome[17], Oculocutaneous Albinism (OCA)[18], etc. The present standard of blastocyst biopsy provides 5-10 trophectoderm for PCR analysis which is better than the formally cleavage stage embryo biopsy which provides only 1-2 blastomeres for the analysis. Embryo freezing after blastocyst biopsy also reduces the time pressure of the analysis. Prior to embryo freezing techniques, cleavage stage embryo biopsy on day 3 with embryo transfer on day 4, the time frame for PGT was restricted to only less than 24 hours. However, amplification failure, ADO[19] and contamination[20] are still crucial obstacles in PCR procedures. The details of techniques and obstacles for PGT-M were discussed previously[21].

- Fluorescent PCR (F-PCR)

The use of fluorescent PCR (F-PCR)[22] by labelling the primers with fluorochromes helps in increasing the sensitivity and the specificity of PGT-M. The combination of several sets of primers using different fluorescent dyes facilitates multiplex F-PCR[14]. This enables the analysis of more than one locus/gene at a time. PGT-M protocols at Chiang Mai University are based on multiplex F-PCR. The addition

of whole genome amplification (WGA) can increase the DNA templates of low copy number, i.e. samples from embryo biopsy, enormously, as a result, PCR can be performed as many times as required[23].

- Real-time PCR (RT-PCR)

Although real-time PCR are usually employed for quantitative assay, it can also be used as qualitative function. Allele discrimination assay can distinguish between homozygous normal, heterozygous and homozygous affected of single nucleotide substation mutation. This has been demonstrated as an simple, quick and accurate assay for the identification of Hb E disease (c.26G>A)[24].

Thalassemias

Thalassemias and hemoglobinopathy, autosomal recessive conditions, are the most common monogenic disorder. alpha-Thalassemia, beta-thalassemia, and Hb E disease are common in Thailand. Hb Bart's disease (homozygous alpha-thalassemia-1), beta-thalassemia major, and beta-thalassemia-Hb E disease are the most severe forms of thalassemia syndrome[25]. beta-Thalassemia major and beta-thalassemia-Hb E disease patients start having anemia and need blood transfusion since 6 months after birth. The patients also need iron chelators and bone marrow transplantation as indicated. There are more than 200 mutations of beta-thalassemia reported. Families with different mutations need different primers/protocols for PGT-M. It is usual that one family may carry more than one mutation and the affected members are compound heterozygous. This makes molecular diagnosis even more sophisticated. The most severe form of alpha-thalassemia causes homozygous alpha-thalassemia-1 or Hb Bart's hydrop fetalis. Fetuses with Hb Bart's die in utero or soon after birth, however, cause significant maternal morbidity and mortality. Therefore, the aim of Hb Bart's control is to save the mothers.

Prevention and Control of Thalassemias

At present, most of the thalassemias management focuses on supportive measures. Countries with the prevalence of thalassemias develop prevention and control program by genetic counselling, population screening, prenatal diagnosis and the option for termination of affected pregnancy in order to reduce the number of new cases[25]. However, invasive prenatal diagnosis possesses some risk of miscarriage and the choice of termination of pregnancy is unpleasant. Embryo selection gives the couples at risk the chance to start the pregnancy with assuring that the baby is unaffected and the option for termination of pregnancy is omitted.

This study presents the PGT-M protocols for thalassemias with 20 years experience at Chiang Mai University.

Materials and Methods

The couples at risk of having the offspring with Hb Bart's disease, beta-thalassemia major, and beta-thalassemia-Hb E disease came in for genetic counselling and PGT-M treatment. Standard in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) procedures, embryo culture and embryo biopsy were carried out. The biopsied cells were then delivered to the Department of Obstetrics and Gynaecology, Faculty of Medicine, Chiang Mai University, Thailand for PGT-M. DNA extraction was performed using proteinase K/sodium dodecyl sulfate (PK/SDS) protocol as previously described[15]. One µl of 17 mmol/l sodium dodecyl sulfate (SDS, Sigma®) and 2 µl of 125 mg/ml proteinase K (PK, Roche Diagnostics (Thailand) Ltd.) were added into 2 µl of phosphate-buffered saline (PBS, Cell Signaling Technology) with 0.1% polyvinyl alcohol (PVA, Sigma-Aldrich) containing the biopsied cells. The mixtures were incubated at 37°C for 1 h, and at 99°C for 15 min on a thermal cycler (Roche Diagnostics (Thailand) Ltd.). PGT-M protocols for Hb Bart's disease, beta-thalassemia major, and beta-thalassemia-Hb E disease were developed, tested and clinically applied. Informed consent was obtained. The study was approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University, Thailand (study code: OBG-

2564-08660).

PGT-M protocols for alpha-Thalassemia(-SEA) using multiplex F-PCR (PAF protocol)

The extracted DNA was amplified using PAF protocol (Table 1) as gap F-PCR for alpha-thalassemia(SEA) deletion determination(14). D16S475, an short tandemly repeat (STR) linking to alpha-globin gene (HBA), was included for contamination detection and linkage analysis. The primers were labelled with the fluorescent dye 6'FAM® (blue), VIC® (green), PET® (red) and NED® (yellow/black). Primers details, PCR mixtures and thermal cycler programs are demonstrated in Table 1. The PCR products were then analyzed by Fragment Analysis on an automated laser fluorescent sequencer ABI Prism® 3130 (GenePlus Co., Ltd.). Three amplified fragments (288bp 6'FAM® (blue), 130bp VIC® (green), and 110bp PET® (red)) of the normal allele and one (217bp 6'FAM® (blue) & VIC® (green)) of the mutant allele can be identified by F-PCR

Table 1. Pre-implantation genetic testing for monogenic disorders (PGT-M) protocols for thalassemias. PAF is PGT-M protocol for alpha-thalassemia(-SEA). PBF is PGT-M protocol for beta-thalassemia with deletion and insertion using F-PCR i.e. CD41/42 (-TTCT). PEF is PGT-M protocol for beta-thalassemia with single base substitution using mini-sequencing i.e. Hb E disease (c.26G>A), c.17A>T.

Protocols	Primers	Sequences	Labeling Dyes	Mixtures	Thermal Cycles	Interpretations	References
PAF	W1	5'-GAA GGA GGG GAG AAG CTG AG-3'	6'FAM®	• 1 µl of 10X PCR Reaction Buffer with 20 mM MgCl2 • 2 µl of 5X GC-RICH solution • 0.2 mM dNTPs • 0.5 U FastStart™ Taq DNA Polymerase • 200 mM of each primers • Water • 10 µl total volume	• 95°C 4 min • 40 cycles of • 95°C 45 s • 60°C 45 s • 72°C 60 s • 72°C 10 min	6'FAM® (blue) (288bp) normal	(14)
	W2	5'-TGT GGA AAA GTT CCC TGA GC-3'	-			VIC® (green) (130bp) normal	
	W3	5'-TGC ACA CCT ATG TCC CAG TT-3'	-			PET® (red) (110bp) normal	
	W4	5'-TTG AGA CGA TGC TTG CTT TG-3'	VIC®			6'FAM® (blue) & VIC® (green) (217bp) mutant	
	W5	5'-GCC ACT GCC TGC TGG TG-3'	PET®				
	W6	5'-AGG TCA GCA CGG TGC TCA C-3'	-				
	D16S475F	5'-AGG GGT TGA CAG AGT GAG ACT CC-3'	NED®			HBA STR linked marker	
	D16S475R	5'-CAG GAA CAG AAA ATA CTG CAC GG-3'	-				
PBF	bthalw1f	5'-CCT GAG GAG AAG TCT GCC GTT AC-3'	VIC®	• 5 µl of 2X QIAGEN® Multiplex PCR Master Mix • 200 mM of each primers • Water • 10 µl total volume	• 95°C 15 min • 37 cycles of • 94°C 30 s • 60°C 90 s • 72°C 90 s • 72°C 10 min	c.41/42 (-TTCT) VIC® (green) (388bp) normal VIC® (green) (384bp) mutant	(13)
	bthalw1r	5'-GTG CAG CTC ACT CAG TGT GGC-3'	-				
	HUMTH01f	5'-AGG GTA TCT GGG CTC TGG-3'	NED®			HBB STR linked marker	
	HUMTH01r	5'-CTT CCG AGT GCA GGT CAC-3'	-				
PEF				• 0.86 µl of ExoProStar™ 1-Step • 2.14 µL of the PCR products • 3 µl total volume	• 37°C 30 min • 80°C 15 min	(purification step)	(15)
	c.26G>A	5'-ACG TGG ATG AAG TTG GTG GT-3'	-	• 5 µL of SNaPshot® Multiplex Kit • 200 mM of mini-sequencing primer • 3 µL of purified templates • Water • 10 µl total volume	• 25 cycles of • 96°C 10 s • 50°C 5 s • 60°C 30 s	G(blue)>A(green)	(15)
	c.17A>T	5'-CAA CTT CAT CCA CGT TCA CCT-3'	-			Complimentary T(red)>A(green)	

HBA = alpha-globin gene, HBB = beta-globin gene, STR = short tandemly repeat

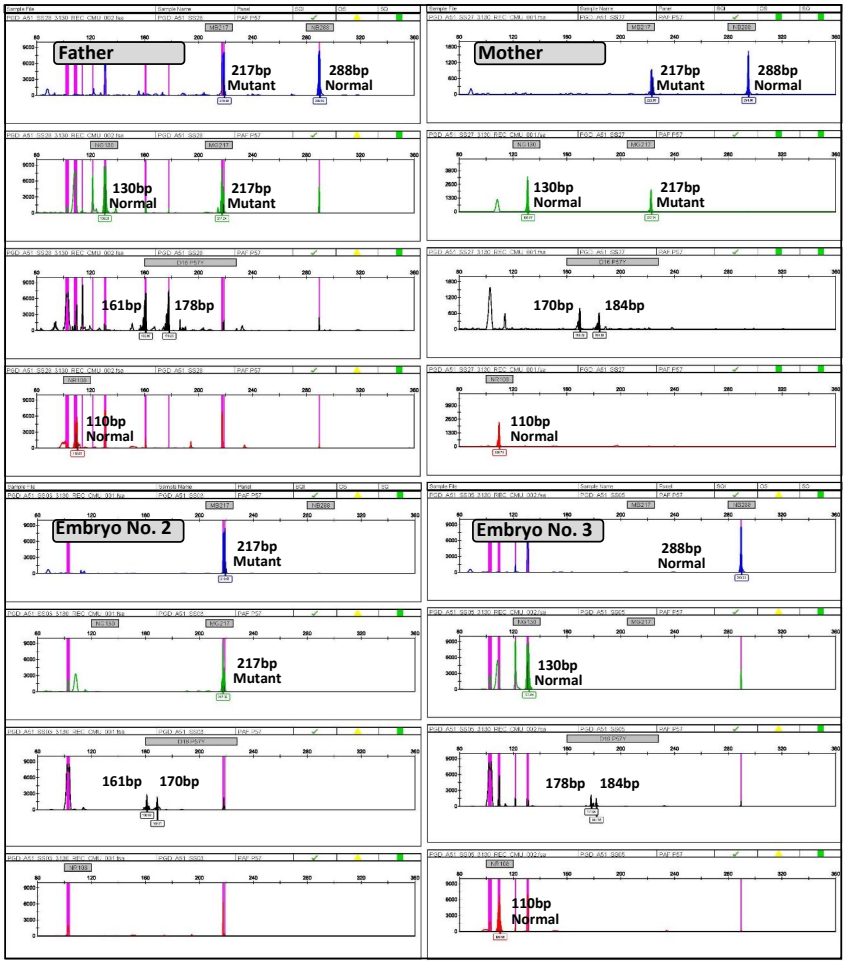

Fig. 1. Pre-implantation genetic testing for monogenic disorders (PGT-M) of Hb Bart's disease (alpha-thalassemia^{-SEA}) using PAF multiplex fluorescent PCR protocol (Table 1) for family A. The fluorograms show PAF analysis of heterozygous genotype of alpha-thalassemia^{-SEA} of the father and the mother (normal 288bp, 6'FAM®, blue; normal 130bp, VIC®, green; normal 110bp, PET®, red; and mutant 217bp 6'FAM®, blue & VIC®, green), Hb Bart's genotype of embryo No. 2 (mutant 217bp 6'FAM®, blue & VIC®, green), and normal genotype of embryo No. 3 (normal 288bp, 6'FAM®, blue; normal 130bp, VIC®, green; and normal 110bp, PET®, red). Short tandemly repeat markers, D16S475, was labeled with NED® (yellow/black).

(Fig. 1).

PGT-M protocols for beta-Thalassemia using multiplex F-PCR (PBF protocol)

The extracted DNA was amplified using PBF protocol (Table 1) as multiplex F-PCR[13]. HUMTH01, an short tandemly repeat (STR) linking to beta-globin gene (HBB), was included for contamination detection and linkage analysis. The primers were labelled with the fluorescent dye 6'FAM® (blue) and NED® (yellow/black). Primers details, PCR mixtures and thermal cycler programs are demonstrated in Table 1. The PCR products were then analyzed by Fragment Analysis on an automated laser fluorescent sequencer ABI Prism® 3130 (GenePlus Co., Ltd.). Mutations with deletions and insertions i.e. c.41/42 (-TTCT) (388bp 6'FAM® (blue) fragment as normal allele and 384bp 6'FAM® (blue) fragment as mutant allele (4bp deleted)) can be identified by F-PCR

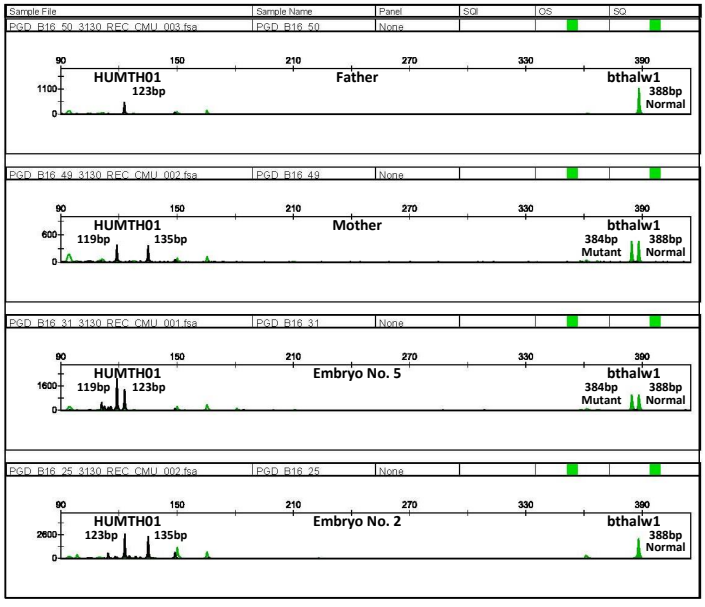

Fig. 2. Pre-implantation genetic testing for monogenic disorders (PGT-M) of beta-thalassemia c.41/42(-TTCT) using PBF multiplex fluorescent PCR protocol (Table 1) for family B. The fluorograms show PBF analysis of normal genotype of beta-thalassemia c.41/42(-TTCT) of the father and embryo No. 2 (388bp, VIC®, green) and heterozygous genotype of the mother and embryo No. 5 (384bp and 388bp, VIC®, green). Short tandemly repeat markers, HUMTH01, was labeled with NED® (yellow/black).

(Fig. 2).

PGT-M protocols for Hb E disease using mini-sequencing (PEF protocol)

The PCR products from PBF protocol was amplified using PEF protocol (Table 1) as mini-sequencing reaction. The amplified products were purified with Exonuclease I/Alkaline Phosphatase using ExoProStar™ 1-Step (Bang Trading 1992 Co., Ltd.) to remove unincorporated primers and dNTPs from previous PCR reactions. 2.14 µL of PCR products were added into 0.2-mL centrifuge tubes containing 0.86 µl of ExoProStar™ 1-Step. The mixtures were incubated at 37°C for 30min and 80°C for 15 min. Mini-sequencing reaction mixture comprised 5.0 µL of SNaPshot® Multiplex Kit (GenePlus Co., Ltd.), 200 mM of the mini-sequencing primers (Table 1), 3.0 µL of the purified templates and distilled deionized water in a total volume of 10 µL. The thermal cycles program was 96°C for 10 s, 50°C for 5 s and 60°C for 30 s for 25 cycles. The mini-seqeuncing products were then analyzed by Fragment Analysis on the automated laser fluorescent sequencer ABI Prism® 3130 (Fig. 3).

Fragment analysis on ABI Prism® 3130

A mixture of 1 µl of fluorescent PCR products, 1 µl Genescan™-500 LIZ® size standard (GenePlus Co., Ltd.), and 10 µl of deionized formamide (GenePlus Co., Ltd.) was prepared and denatured at 95°C for 5 minutes. Denatured samples were subjected to capillary electrophoresis using Performance Optimized Polymer 7 (POP-7®, GenePlus Co., Ltd.; 5 s injection time, 15,000 V, 60°C, 20 min) on the automated laser fluorescent sequencer ABI Prism® 3130. The data were analyzed by GeneMapper® software; version 4.0 (GenePlus Co., Ltd.)[14].

Mini-sequencing analysis on ABI Prism® 3130

A mixture of 1 µL of purified mini-sequencing product, 1 µL of GeneScan™-120 LIZ® (GenePlus Co., Ltd.) size standard, and 10 µL of deionized formamide

was prepared and denatured to 95°C for 5 min. The denatured samples were subjected to capillary electrophoresis using POP-7® (5 s injection time, 15,000 V, 60°C, 24 min) on the automated laser fluorescent sequencer ABI Prism® 3130. The data were analyzed by GeneMapper® software; version 4.0. The color of individual peaks was interpreted as A (Green, dR6G™ dye), C (Yellow/Black, dTAMRA™ dye), G (Blue, dR110™ dye) and T (Red, dROX™ dye)[15].

Statistical Analysis

Analysis was performed by SPSS version 21.0 (IBM Corp. Released 2012; IBM SPSS Statistics for Windows, Armonk, NY). Descriptive data were presented as means or percentage as appropriate. P-values of less than 0.05 was considered significant.

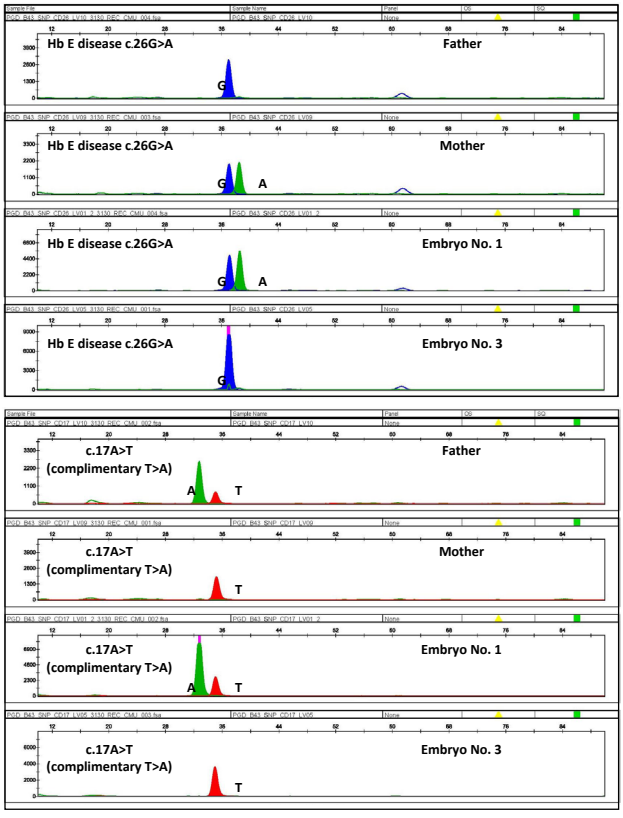

Fig. 3. Pre-implantation genetic testing for monogenic disorders (PGT-M) of beta-thalassemia-Hb E disease (c.26G>A & c.17A>T) using PEF mini-sequencing protocol (Table 1) for family C. The fluorograms of Hb E disease c.26G>A show normal genotypes (G, blue) of the father and embryo No. 3 and heterozygous genotypes (G, blue and A, green) of the mother and embryo No. 1. The fluorograms of beta-thalassemia c.17A>T (complimentary analysis as T>A in this figure) show of normal genotypes (T, red) of the mother and embryo No. 3 and heterozygous genotypes (T, red and A, green) of the father and embryo No. 1. Embryo No. 1 possesses the mutant alleles of both Hb E disease c.26G>A and beta-thalassemia c.17A>T. Therefore, embryo No. 1 is compound heterozygous for beta-thalassemia-Hb E disease.

Results

PGT-M at Chiang Mai University

Since 2003, a total of 168 clinical PGT-M cycles have been done for 125 families. In addition to alpha-thalassemia, beta-thalassemia major and beta-thalassemia-Hb E disease, other monogenic disorders including rare conditions were also included for the PGT-M. Families with affected beta-

thalassemia-Hb E disease offspring who sought for PGT-M in order to having a second healthy child decided to have HLA-matched un-affected embryos as well. Our 20 years experience gives rise to 75 pregnancies with 85 healthy babies. However, the pregnancy outcomes are expected to be higher as some PGT-M cycles are still ongoing and the embryos are kept frozen waiting for transfer. No contamination or misdiagnosis was detected.

PGT-M for Thalassemias

A total of 111 couples at risk of having the offspring with Hb Bart's disease, beta-thalassemia major, and beta-thalassemia-Hb E disease (c.26G>A) came in for genetic counselling and PGT-M treatment. All PGT-M protocols were developed and tested. A total of 132 clinical PGT-M cycles were carried out giving rise to 66 pregnancies with 76 babies (Table 2).

Table 2. Pre-implantation genetic testing for monogenic disorders (PGT-M) at Chiang Mai University.

Conditions	Families (Cycles)	Pregnancies (Babies)	Families (Cycles)	Pregnancies (Babies)
Thalassemias	**111 (132)**	**66 (76)**		
- alpha-Thalassemia				
• Hb Bart's disease (alpha-Thalassemia$^{(-SEA)}$)[14]	58 (68)	32 (36)		
• Hb H-CS	1 (3)	1 (1)		
- beta-Thalassemia major	12 (12)	8 (9)		
• c.17A>T & -28A>G			1 (1)	0
• c.17A>T & c.17A>T			3 (3)	3 (3)
• c.17A>T & c.35C>A			1 (1)	0
• c.41/42(-TTCT) & -28A>G			1 (1)	1 (1)
• c.41/42(-TTCT) & c.41/42(-TTCT)[13]			4 (4)	2 (2)
• c.41/42(-TTCT) & IVS1-nt1G>T			1 (1)	1 (2)
• c.41/42(-TTCT) & c.71/72(+A)			1 (1)	1 (1)
- beta-Thalassemia-Hb E disease	35 (43)	22 (27)		
• Hm c.26G>A & c.17A>T			4 (5)	3 (4)
• Hm c.26G>A & c.41/42(-TTCT)			7 (10)	4 (5)
• c.26G>A & c.17A>T[15]			10 (13)	7 (8)
• c.26G>A & c.30G>C			1 (2)	2 (3)
• c.26G>A & c.41/42(-TTCT)			10 (10)	4 (5)
• c.26G>A & c.71/72(+A)			1 (1)	2 (2)
• c.26G>A & -3.5kb deletion			1 (1)	0
• c.26G>A & unknown deletion			1 (1)	0
- beta-Thalassemia-Hb E disease & HLA matching	2 (3)	1 (1)		
- Hb Bart's disease & beta-Thalassemia-Hb E disease	3 (3)	2 (2)		
Marfan syndrome[17]	**1 (2)**	**1 (1)**		
Karyomapping	**13 (34)**	**8 (8)**		
• Hb Bart's disease (alpha-Thalassemia)			2 (5)	0
• beta-Thalassemia-Hb E disease[22]			2 (10)	4 (4)
• Duchene muscular dystrophy (DMD)[22]			2 (4)	2 (2)
• Polycystic kidney type 1 (PKD1)			2 (4)	0
• Spinal muscular atrophy (SMA)			2 (5)	0
• Oculocutaneous albinism (OCA) type 1[18]			1 (1)	0
• Infantile neuroaxonal dystrophy type 1 (INAD1)			1 (3)	2 (2)
• Usher syndrome (Hearing loss)			1 (2)	0
Total	**125 (168)**	**75 (85)**		

CS = Constant Spring, Hm = homozygous

PGT-M for alpha-Thalassemia$^{(-SEA)}$

Fifty eight alpha-thalassemia families carried^{-SEA} deletion had 68 PGT-M cycles, giving rise to 32 pregnancies with 36 healthy babies. One family were at risk of having offspring with Hb H-Constant Spring. The patient went through 3 PGT-M cycles, and had one un-affected baby. Interestingly, three families were at risk of having offspring with both Hb Bart's disease and beta-thalassemia-Hb E disease, consequently, embryos with either conditions needed to be excluded. Each family had one PGT-M cycle so far, two families succeeded with one baby each (Table 2). Examples of PGT-M for alpha-thalassemia$^{(-SEA)}$ using PAF multiplex F-PCR is demonstrated in Fig. 1.

PGT-M for beta-Thalassemia major and beta-thalassemia-Hb E disease

Twelve families were at risk of having offspring with beta-thalassemia major and 35 families were at risk of beta-thalassemia-Hb E disease (c.26G>A). A total of 55 clinical PGT-M cycles were performed, resulting in 30 pregnancies with 36 babies. beta-Thalassemia mutations of the PGT-M families included -28A>G, c.17A>T, c.30G>C, c.35C>A, c.41/42 (-TTCT), IVS1-nt1G>T, c.71/72(+A), and -3.5kb deletion. Unfortunately, there were 11 families whose one of the spouse was homozygous for Hb E disease, therefore, half of their embryos would be affected. Moreover, beta-thalassemia mutation could not identified in one family at risk of have offspring with beta-thalassemia-Hb E disease. PGT-M was carried out by using STR-based linkage analysis incorporating with mutation analysis of c.26G>A and predictive analysis for the absent of normal c.26G>A allele (Table 2). Examples of PGT-M for beta-thalassemia c.41/42 (-TTCT) using PBF multiplex F-PCR is shown in Figure 2. Examples of PGT-M for beta-thalassemia c.17A>T and c.26G>A using PEF mini-sequencing is demonstrated in Fig. 3.

PGT-M for other monogenic disorders and karyomapping

In addition to PGT-M of thalassemias, one family with Marfan syndrome came through for PGT-M. Two PGT-M cycles were carried out resulting in one baby[17]. Additionally, 34 PGT-M cycles were performed using single nucleotide polymorphism microarray (aSNP) based karyomapping for 13 families resulting in 8 babies. PCR-based protocols were developed, clinically applied and confirmed haplotyping results in all embryos. The rest of the embryos are still kept frozen waiting for transfer. The conditions of the PGT-M karyomapping done included alpha-thalassemia, beta-thalassemia-Hb E disease[23], Duchene muscular dystrophy (DMD)[16], Polycystic kidney type 1 (PKD1), Spinal muscular atrophy (SMA), Oculocutaneous albinism (OCA) type 1[18], Infantile neuroaxonal dystrophy type 1 (INAD1), and Usher syndrome (hearing loss) (Table 2).

Discussion

PGT-M at Chiang Mai University, Thailand started in 2003. The first two cases were performed for beta-thalassemia major c.41/42(-TTCT) using PBF multiplex F-PCR protocol for mutation analysis of 4 bp deletion[13]. Both couples carried the same mutations. During that time, most IVF centers performed cleavage stage day 3 embryo biopsy and transferred the embryos on day 4. Therefore, the results needed to be reported within 24 hours. The laboratory works were carried out under time pressure. Another difficulty was that day 3 embryo biopsy provided only 1-2 cells for PGT-M. Consequently, amplification efficiency, ADO, and contamination could deteriorate the accuracy of the results. These problems became ease when the IVF laboratories switched to do day 5 blastocyst biopsy with embryo freezing. With these changes, more cells and time were available for PGT-M.

PGT-M for alpha-thalassemia^{-SEA} has been available since 2007 when the new set of primers, PAF protocol, for alpha-thalassemia^{-SEA} was developed[14]. The original published gap PCR primers for alpha-thalassemia^{-SEA} was effective on genomic DNA and prenatal samples. However, the efficiency reduced to lower than 50% at the single cell level. The

newly designed PAF primers were effective and accurate for genotyping on genomic DNA, prenatal samples and single cells. All of the PGT-M for alpha-thalassemia were done for $^{-SEA}$ deletion which is the most common mutation in Thailand. Hb Bart's disease has become the most frequent indication for PGT-M here due to its high prevalence in this region.

Novel PCR protocol was developed for genotyping of Hb Constant Spring (CS). PGT-M was successfully carried out. One healthy baby was obtained. Three families were at risk of have offspring with both alpha-thalassemia and beta-thalassemia-Hb E disease. This event was not unusual in Thailand. However, excluding both conditions, only 56.25% of the embryos would be unaffected. One clinical PGT-M cycle was performed for each family, two succeeded in having a pregnancy. The third family will return for their second PGT-M treatment.

PGT-M for beta-thalassemia major and beta-thalassemia-Hb E disease is challenging as there are a wide variety of mutations among the population i.e. there were 10 mutations in this study. Each mutation needed a particular PGT-M protocol for genotyping. As the families who requested for PGT-M already had the mutation reports, PGT-M focused on the particular mutations of each family. It is noticed that one of the spouse of 11 families was homozygous for Hb E disease. Consequently, all of their gametes would have Hb E disease, therefore, half of the embryos would be affected. Previous report on PGT-M of beta-thalassemia using nested PCR with mini-sequencing were in 23 couples with 42 clinical cycles. Four successful pregnancies were resulted[26].

Two families who already had an affected child with beta-thalassemia-Hb E disease came for PGT-M. Their affected children had transfusion dependent anemia and were waiting for donors in order to have bone marrow transplantation treatment. It would be great if their unaffected second child were HLA matched to the affected elder siblings. Therefore, in addition to excluding beta-thalassemia-Hb E disease, STR-based linkage analysis HLA matching was also performed. Three PGT-M cycled had been carried out, resulting in one unaffected baby. The second family will return for the second PGT-M treatment. Successful PGT-M for beta-thalassemia major and beta-thalassemia-Hb E disease with HLA matching has been reported[27]. From 8 families with affected children, one successful pregnancy was obtained. HLA-matched sibling bone marrow transplantation for beta-thalassemia major was then performed.

One family came through for IVF treatment. Family history revealed Marfan syndrome with aortic replacement surgery in the husband and his father. Exome sequencing demonstrated c.3373C>T mutation within the fibrillin-1 (FBN1) gene. Two clinical PGT-M cycles were performed giving rise to one healthy baby[17]. PGT-M for Marfan syndrome is also challenging because Marfan families have different mutations. However, with the present exome sequencing technology, identifying the mutation from the proband is much quicker.

We also experienced PGT-M using karyomapping for 34 cycles in 13 families. Karyomapping employs aSNP technology for SNP-based haplotyping and SNP-based chromosome balance or copy number variation (CNV) information. In addition to alpha-thalassemia and beta-thalassemia-Hb E disease, the additional 6 rare conditions were performed including DMD, PKD1, SMA, OCA1, INAD1, and Usher syndrome. The specific mutation of each family was revealed by exome sequencing. Novel PCR protocols for genotyping and STR-based linkage analysis were developed for each family. PCR-based genotyping confirmed haplotyping results in every embryo. The pregnancy rates reported in Table 2 seems low, however, due to the covid-19 outbreak the patients will return for embryo transfer in the future and there should be more pregnancy outcomes.

Notably, in the karyomapping analysis of one of the PKD1 families, the reference DNA who was the affected sister of the affected husband had identical snp information. Therefore, her DNA was an uninformative reference. However, the aSNP-based haplotyping was proceeded by using one of the affected embryos, which was identified by PCR-based

genotyping, as the reference. It was shown that even with the hardest effort the sophisticated aSNP could go wrong on the day of the analysis. Without PCR-based analysis results, karyomapping analysis would have failed. One pitfall of the karyomapping software was that, with the uninformative reference in this case, the software proceeded with providing the incorrect haplotyping reports. If the operators had not validated the haplotyping with the PCR-based results, there would have been the chance of transfer affected embryo. Even though, karyomapping can be a universal protocol that provides both haplotyping and CNV, PCR-based genotyping should be performed along side to confirm the results.

Conclusion

The prevention and control program for severe thalassemias in Thailand has been done for over 20 years. The program has been very successful. However, at present, some families at risk of having an offspring with severe thalassemias are looking for a better option other than termination of pregnancy. PGT-M or embryo selection would be the correct answer. During the past 20 years, a total of 168 PGT-M cycles in 125 families have been done at Chiang Mai University, resulting in a total of 75 pregnancies (85 healthy babies). The pregnancy rates were 44.6%, whereas, some PGT-M cycles are still on-going and the embryos are kept frozen. More pregnancies should be obtained when the patients return for embryo transfer. PGT-M protocols at Chiang Mai University are based on multiplex F-PCR and mini-sequencing. Over 20 protocols have been developed, tested, and clinically applied for PGT-M. All protocols were novel and home grown. Waiting time for the work up prior to clinical PGT-M is about 2 weeks to 2 months (in case of developing a new protocol). So far, no misdiagnosis has been found.

Acknowledgements

The authors thank all families, the Medical Science Research Equipment Center, Faculty of Medicine, Chiang Mai University, Thailand and the IVF laboratories for their support.

Authors' contributions: WP and SP contributed equally to this work.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature 1990;344:768-70.
2. Handyside AH, Pattinson JK, Penketh RJ, Delhanty JD, Winston RM, Tuddenham EG. Biopsy of human preimplantation embryos and sexing by DNA amplification. Lancet 1989;1:347-9.
3. Edwards RG, Bavister BD, Steptoe PC. Early stages of fertilization in vitro of human oocytes matured in vitro. Nature 1969;221:632-5.
4. Spinella F, Bronet F, Carvalho F, Coonen E, De Rycke M, Rubio C, et al. ESHRE PGT Consortium data collection XXI: PGT analyses in 2018. Hum Reprod Open 2023;2023:hoad010.
5. Dokras A, Sargent IL, Ross C, Gardner RL, Barlow DH. Trophectoderm biopsy in human blastocysts. Hum Reprod 1990;5:821-5.
6. Hardy K, Handyside AH. Biopsy of cleavage stage human embryos and diagnosis of single gene defects by DNA amplification. Arch Pathol Lab Med 1992;116:388-92.
7. Griffin DK, Handyside AH, Harper JC, Wilton LJ, Atkinson G, Soussis I, et al. Clinical experience with preimplantation diagnosis of sex by dual fluorescent in situ hybridization. J Assist Reprod Genet 1994;11: 132-43.
8. Conn CM, Harper JC, Winston RM, Delhanty JD. Infertile couples with Robertsonian translocations: preimplantation genetic analysis of embryos reveals chaotic cleavage divisions. Hum Genet 1998;102: 117-23.
9. Alfarawati S, Fragouli E, Colls P, Wells D. First births after preimplantation genetic diagnosis of structural chromosome abnormalities using comparative genomic hybridization and microarray analysis. Hum Reprod 2011;26:1560-74.
10. Lukaszuk K, Pukszta S, Wells D, Cybulska C, Liss J, Plociennik L, et al. Routine use of next-generation sequencing for preimplantation genetic diagnosis of

blastomeres obtained from embryos on day 3 in fresh in vitro fertilization cycles. Fertil Steril 2015;103: 1031-6.
11. Saiki RK, Walsh PS, Levenson CH, Erlich HA. Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes. Proc Natl Acad Sci U S A 1989;86:6230-4.
12. Piyamongkol W, Harper JC, Sherlock JK, Doshi A, Serhal PF, Delhanty JD, et al. A successful strategy for preimplantation genetic diagnosis of myotonic dystrophy using multiplex fluorescent PCR. Prenat Diagn 2001;21:223-32.
13. Piyamongkol W, Vutyavanich T, Piyamongkol S, Wells D, Kunaviktikul C, Tongsong T, et al. A successful strategy for Preimplantation Genetic Diagnosis of beta-thalassemia and simultaneous detection of Down's syndrome using multiplex fluorescent PCR. J Med Assoc Thai 2006;89:918-27.
14. Piyamongkol W, Vutyavanich T, Sanguansermsri T. Preimplantation genetic diagnosis of alpha-thalassemia-SEA using novel multiplex fluorescent PCR. J Assist Reprod Genet 2012;29:95-102.
15. Lattiwongsakorn W, Jansaka N, Piyamongkol S, Pantasri T, Tongsong T, Suriya W, et al. Successful Strategy of Pre-implantation Genetic Testing for Beta-Thalassemia (c.17A>T Mutation)-Hb E Disease Using Multiplex Fluorescent PCR and Mini-Sequencing. International J Women's Health Reprod Sciences 2023;11:58-64.
16. Mongkolchaipak S, Piyamongkol S, Teekaput C, Sirapat R, Suriya W, Pantasri T, et al. Successful strategy of comprehensive pre-implantation genetic testing for Duchenne muscular dystrophy and chromosome balance using karyomapping and fluorescent PCR. Clin Exp Obstet Gynecol 2021;48:1167-77.
17. Piyamongkol S, Makonkawkeyoon K, Shotelersuk V, Sreshthaputra O, Pantasri T, Sittiwangkul R, et al. Pre-implantation genetic testing for Marfan syndrome using mini-sequencing. J Obstet Gynaecol 2022;42:2846-52.
18. Piyamongkol S, Mongkolchaipak S, Chaidaroon W, Pantasri T, Sirapat R, Suriya W, et al. Pre-implantation genetic testing for oculocutaneous albinism type 1 using karyomapping. Clin Exp Obstet Gynecol 2022;49:1-9.
19. Findlay I, Ray P, Quirke P, Rutherford A, Lilford R. Allelic drop-out and preferential amplification in single cells and human blastomeres: implications for preimplantation diagnosis of sex and cystic fibrosis. Hum Reprod 1995;10:1609-18.
20. Sermon K, Goossens V, Seneca S, Lissens W, De Vos A, Vandervorst M, et al. Preimplantation diagnosis for Huntington's disease (HD): clinical application and analysis of the HD expansion in affected embryos. Prenat Diagn 1998;18:1427-36.
21. Piyamongkol W. Pre-implantation genetic giagnosis of Thalassemias. Thai J Obstet Gynaecol 2018;26:2-9.
22. Hattori M, Yoshioka K, Sakaki Y. High-sensitive fluorescent DNA sequencing and its application for detection and mass-screening of point mutations. Electrophoresis 1992;13:560-5.
23. Piyamongkol S, Mongkolchaipak S, Charoenkwan P, Sirapat R, Suriya W, Pantasri T, et al. The successful strategy of comprehensive pre-implantation genetic testing for beta-thalassaemia-haemoglobin E disease and chromosome balance using karyomapping. J Obstet Gynaecol 2022;42:2433-41.
24. Upanan S, Srichairatanakool S, Piyamongkol S, Piyamongkol W. Accuracy of Hemoglobin E Screening Test Using Allelic Discrimination Assay. Thai J Obstet Gynaecol 2020;28:136-41.
25. Tongsong T, Wanapirak C, Sirivatanapa P, Sanguansermsri T, Sirichotiyakul S, Piyamongkol W, et al. Prenatal control of severe thalassaemia: Chiang Mai strategy. Prenat Diagn 2000;20:229-34.
26. Monni G, Cau G, Usai V, Perra G, Lai R, Ibba G, et al. Preimplantation genetic diagnosis for beta-thalassaemia: the Sardinian experience. Prenat Diagn 2004;24:949-54.
27. Qureshi N, Foote D, Walters MC, Singer ST, Quirolo K, Vichinsky EP. Outcomes of preimplantation genetic diagnosis therapy in treatment of beta-thalassemia: A retrospective analysis. Ann N Y Acad Sci 2005;1054: 500-3.

Thai Journal of Obstetrics and Gynaecology
July 2023, Vol. 31, No. 4, pp. 248-254.

OBSTETRICS

A Study to Compare the Fetomaternal Outcomes of Dinoprostone Gel Administration for Induction of Labor Across Posterior Fornix versus Intracervical Routes

Anusha Devalla, MS, DNB obgy*,
Niranjan Mayadeo, M.D, DGO.*

** Department of Obstetrics and Gynecology, KEM Hospital, Parel, Mumbai, Maharashtra, India*

ABSTRACT

Objectives: Although Dinoprostone (synthetic prostaglandin) gel as a cervical ripening agent for induction of labour has been extensively studied but there has been a paucity in the current literature, employing its use through the intracervical and posterior routes, especially in the Indian setting. The authors aimed to study and compare the fetomaternal outcomes with the use of 0.5 mg dinoprostone gel for induction of labour across intracervical and posterior fornix routes.

Materials and Methods: An observational study was conducted at a tertiary care hospital in Western India. Pregnant women presenting in the Obstetrics and Gynecology department of the institute were recruited in the study (n = 120). They were allowed to choose between the two groups, posterior fornix (PF; n = 60) and intracervical (IC; n = 60) after taking a valid written and inform consent. Primary outcomes were to measure the rates of normal vaginal delivery. Secondary outcomes that were studied included induction-to-delivery interval, rates of operative vaginal deliveries/ need for emergency caesarean section and incidence of maternal complications and adverse fetal outcomes were compared along the two routes of dinoprostone administration.

Results: Both the groups were homogenous in terms of maternal age, gestational age, or other maternal characteristics. Induction of labor was successful to result in a normal vaginal delivery in 45 and 42 women respectively in IC and PF groups. Participants undergoing emergency cesarean deliveries were 15 in IC and 18 in PF groups, respectively (differences not statistically significant).

Conclusion: Our study revealed that either of the routes can be successfully utilized for induction of labour with equal probability of successful vaginal delivery. Dinoprostone gel being relatively cheaper and more widely available can still serve as a potential cervical ripening agent.

Keywords: cervical ripening, dinoprostone, labor, induced, pregnancy, tertiary care centers.

Correspondence to: *Anusha Devalla, MS, DNB obgy, H No 3-11-50/2, HCL Nagar, Mallapur, Hyderabad, Telangana state, India. E-mail: anushadevalla2@gmail.com*

Received: 2 September 2022, **Revised:** 24 January 2023, **Accepted:** 13 February 2023

Introduction

Induction of labor (IOL) is the process whereby the uterus is stimulated by artificial means to initiate labor[1]. Dinoprostone (PGE2) used as a cervical ripening agent is commercially available in various formulations[2,3]. In recent practice, dinoprostone pessaries are the preferred mode of dinoprostone delivery for IOL[4]. However, the availability of dinoprostone gel maintains its status of a lucrative cervical ripening agent[5,6]. Additionally, there is a paucity in recent literature exploring the gel formulation. Fetomaternal outcomes, success of IOL, rate of uncomplicated vaginal deliveries with least number of adverse effects shall govern the route of dinoprostone gel administration[3,7,8]. Previous studies have compared the two routes for the mentioned outcomes; but there is no consensus of evidence on the preferred route of administration[9-14]. The aim of our study was to compare the effects of the two routes of dinoprostone gel instillation on fetomaternal outcomes.

Materials and Methods

Study design: A quasi-experimental study was conducted over a period of 10 months from February 2016 to November 2016 at a tertiary care hospital in India. Participants were recruited from the out patient department (OPD) and emergency room fulfilling the following inclusion criteria.

Inclusion criteria: Gestational age: ≥ 37 weeks and ≤ 40 weeks and six days, intact membranes, singleton pregnancy with vertex presentation, reassuring admission NST, modified Bishop's score ≤ 4.

Exclusion criteria: Gestational age beyond 41 weeks, history of prior uterine surgery, prelabor rupture of membranes, multiple gestations, maternal high-risk factors such as contracted pelvis, gestational diabetes mellitus (DM), hepatic or cardiac conditions, asthma and moderate or severe anaemia, known fetal anomaly and allergy to prostaglandins.

Sample size calculation:

The number of labour inductions done in the institute were determined for a month (prevalence) and the outcome in terms of normal vaginal deliveries (primary outcome) was calculated. Thirty patients were included, where 14 patients were in the (p1) group and 16 patients in the PF (p2) group. Out of the 14 patients in the p1 group, nine delivered vaginally and of the 16 patients in the p2 group, 11 patients delivered vaginally. Hence, the sample size of the index study performed was then calculated as follows:

$$N = \frac{\sqrt{[Z\alpha\sqrt{2p'q'} + Z_\beta \sqrt{p1q1+p2q2)}]}}{(p1-p2)}$$

Where p' = (p1+p2)/2 and q'=100-p'

Substituting the values of $Z\alpha = 1.96$(level of statistical significance) and $Z_\beta = 0.907$ (power of the study), p1 = 9, p2 = 11, q1 = 100-9 = 91, q2 = 100-11 = 89, p' = 10, q' = 90

Sample size, (each group) n = 60 in each group and total number of patients in the study were 120.

We also conducted a post-hoc power analysis of the number of participants included in the study using G*power software[15]. Assuming a low effect size of 0.3 and type I error probability of 0.5, and degree of freedom of one, the power of the study was computed to be 0.907.

Patients fulfilling the criteria were allowed to choose the route of administration of dinoprostone gel after taking a written and informed consent (English/ Hindi/ Marathi) with 1:1 distribution (60 participants in each group). There was no blinding during the study.

Dinoprostone gel was administered either through PF or IC routes with 0.5 mg gel. During the study period, the 2 mg PF gel was unavailable in India, and hence, the available formulation of IC gel (Cerviprime, Astra IDL, Bangalore, India) was administered through both the routes[14]. First dose of gel (at 0.5 mg) was instilled either IC or PF under all aseptic conditions. The same dose was re-instilled along the same route after an interval of six hours as per the Bishop's score. Induction of labor was considered "failed" in the absence of initiation of uterine contractions post 12 hours of instillation of 3rd

dose of dinoprostone gel.

Post-gel administration monitoring: Continuous Electronic fetal and tocodynamometric monitoring was performed for two hours[16] followed by intermittent auscultation for fetal heart rate along with manual observation of contractions. Successful induction was considered as occurrence of two contractions every 15 minutes along with cervical dilatation of 3 cm.

Fetal monitoring: Fetal hypoxia and/or acidemia was assessed in terms of non-reassuring fetal status, according to the National Institute for Health and Care Excellence (NICE) guidelines[17].

Uterine hyperstimulation was defined as more than five contractions in 10 minutes, measured for 30 minutes and/or each lasting for more than 2 minutes[18] for which preparations for medical management were kept ready (Injection terbutaline 0.25 mg).

Primary outcome was to measure the rates of normal vaginal delivery. Secondary outcomes that were studied included induction-to-delivery interval, rates of operative vaginal deliveries/ need for emergency caesarean section and incidence of maternal complications such as prolonged labour, postpartum hemorrhage, need of blood transfusion, febrile morbidity and urinary retention as well as adverse fetal outcomes such as neonatal asphyxia were compared along the two routes of dinoprostone administration. The indications for the emergency caesarean sections were also noted.

Statistical analysis:

All the data were analysed using Statistical Package for the Social Science (SPSS; SSS Inc., Chicago, IL, USA) version 21. Continuous variables were expressed in terms of mean and standard deviation and discrete variables were represented in frequencies and percentages. T-test and Mann-Whitney U test were used for comparison of normally and non-normally distributed continuous variables. Pearson's Chi square and Fischer's exact tests were used to compare categorical variables.

Results

A total of 120 participants were recruited in the study. Demographic characteristics and pre-induction observations of the participants were noted as in Table 1. The differences in maternal age, gestational age and mean Bishop's score (MBS) between the two groups were non-significant.

Table 1. Characteristics of the sample and indications for induction of labour (n = 120).

Characteristic of the sample	Intracervical group (IC) (n = 60)	Posterior fornix group (PF) (n = 60)	p value
Maternal age (years)	24.5 ± 3.6	25.4 ± 4.1	0.476
Gestational age (weeks)	39.9 ± 1.08	39.6 ± 1.21	0.197
Parity	0.7 ± 0.02	0.8 ± 0.02	0.220
Weight (kg)	51.2 ± 5.2	52.4 ± 5.07	0.351
Height (cm)	151.4 ± 5.3	151.4 ± 5.9	0.756
Pre-delivery Haemoglobin (g%)	11.03 ± 1.18	10.95 ± 1.25	0.801
Modified Bishop's score (on admission)	1.66 ± 0.92	1.9 ± 0.87	0.208
Modified Bishop's score (after 1st administration)	4.12 ± 0.73	4.38 ± 0.71	0.140
Indication for IOL			
Post-dates (n, %)	30 (50%)	34 (56.66%)	0.065
Suspicion of fetal growth restriction (n, %)	9 (15%)	6 (10%)	0.092
Severe Oligohydramnios (n, %)	8 (13.33%)	10 (16.66%)	0.378
Others (n, %)#	13 (20%)	10 (16.66%)	0.081

Data presented as mean ± standard deviation or n (%)
indications included intrahepatic cholestasis of pregnancy, decreased fetal movements, Rh negative pregnancy at 40 weeks
IOL: Induction of labor

Post-dated pregnancy occurring in 50.0% (n = 30) and 56.66% (n = 34) of the participants in IC and PF groups, respectively, was the most common indication for IOL; followed by suspicious fetal growth (in 9 participants (15%) in IC and 6 in PF (10%) groups, each) and others. Induction of labor was successful in 45 participants (75%) and 42 (70%) participants in the IC and PF groups, respectively. Induction to delivery interval was 14.4 ± 3.6 hours in IC group and 14.6±3.6 hours in PF group. Spontaneous vaginal deliveries were observed in 42 participants (93.3%) of IC group and 34 participants (80.95%) of PF group (Table 2).

Table 2. The fetomaternal outcomes of IOL in the two groups (n = 120).

Outcome of IOL	Intracervical group (IC) (n = 60)	Post fornix group (PF) (n = 60)	p value
Mode of delivery			
Spontaneous vaginal delivery	42 (93.33%)	34 (80.95%)	0.125
Operative vaginal delivery	3 (6.66%)	8 (19.04%)	0.079
Emergency cesarean section	15 (25%)	18 (30%)	0.290
Induction to delivery interval (hours)	14.4 ± 3.6	14.6 ± 3.6	0.778
Number of successful inductions of labor	45 (75%)	42 (70%)	0.248
Need for 2nd reinstallation of gel	32 (53.33%)	27 (45%)	0.24
Need for 3rd reinstallation of gel	0	0	
Spontaneous rupture of membranes	12 (20%)	15 (25%)	0.901
Oxytocin augmentation	20 (33.33%)	21 (0.35%)	0.517
Abnormal fetal heart tracings	7 (11.66%)	11 (18.33%)	0.182
Stillbirths	0	0	
Weight of newborns (kg)	2.76 ± 0.29	2.81 ± 0.38	0.294
APGAR Score > 7	56 (93.33%)	57 (95%)	0.231
APGAR Score < 7	4 (6.66%)	3 (5%)	0.871
NICU admissions	4 (6.66%)	3 (5%)	0.609
Vaginal lacerations (traumatic PPH)	2(3.33%)	1(1.66%)	0.790
Atonic PPH	0	4(6.66%)	0.009
Urinary retention	1 (1.66%)	0	0.010
Puerperal pyrexia	1(1.66%)	1(1.66%)	0.290
Maternal mortality	0	0	N/A

Data presented as mean + standard deviation or n (%)
IOL: Induction of labor, NICU: neonatal intensive care units, PPH: postpartum hemorrhage.

Total number of emergency cesarean deliveries required in the study was 33 of total 120 deliveries. Out of which, 15 (25% of total deliveries) were required in IC group and 18 (30% of total deliveries) were required in PF group as noted in Table 3.

In our study, none of the participants experienced hyperstimulation. Majority of the newborns were fullterm with mean weights of 2.76 ± 0.29 kg (IC) and 2.81 ± 0.38 kg (PF) in the two groups (p = 0.29). Amongst those induced for suspicious fetal heart rate (FHR), the birth weights ranged between 1.9 to 2.3 kg. Other fetomaternal outcomes across the two groups are tabulated in Table 3.

Table 3. Indications for emergency cesarean section deliveries in the two groups (n = 33).

Indication for emergency cesarean delivery*	Intracervical group (IC) (n = 15)	Posterior fornix group (PF) (n = 18)	p value
Meconium-stained amniotic fluid with fetal distress	6 (40%)	9 (50%)	0.092
Placental abruption	2 (13.33%)	1 (5.5%)	0.128
Arrest of dilatation in first stage of labor	4 (26.6%)	6 (33.3%)	0.099
Arrest of descent in second stage of labor	3 (20%)	2 (11.1%)	0.267

Data presented as n (%)
*statistical test used was Mann Whitney U test.

Discussion

Induction of labor is a commonly used obstetric intervention aimed to mitigate the possible adverse perinatal outcomes. In this study, the authors have attempted to highlight the differences in the fetomaternal outcomes with the use of 0.5 mg dinoprostone gel through IC and PF routes. This is the first of its kind study from the recently published literature, that mainly have observed the differences using dinoprostone vaginal pessary versus intracervical dinoprostone gel. The two groups did not differ significantly in terms of maternal characteristics. The mean initial MBS at the time of admission was 1.66 ± 0.92 in the IC group and 1.9 ± 0.87 in the PF group ($p > 0.05$). In the study by Perry et al, the mean MBS was 2.5 and 3.0 in IC and PF groups, respectively. Similar to this study, neither the initial scores nor the difference between subsequent scores, assessed within six hours of first dose of PGE2 administration, were statistically significant[19].

Post-datism (53.33% in 120 participants) was the most common indication for IOL in our study. Kemp et al observed post-dates pregnancies to be the second most common in their study (32.9%)[20]. The number of vaginal deliveries in the current study were 45 (75%) in IC group as compared to 42 (70%) in the PF group ($p > 0.05$). This was similar to the study by Grignaffini et al, the PF insertion of dinoprostone lead to 67% successful vaginal deliveries as compared to 66% in the IC route of administration. Thus, indicating that both the routes lead to similar proportion of successful vaginal deliveries[21].

The need for emergency cesarean deliveries across the two groups was also not statistically significant. The most common indications have been failure to progress in the studies of Perry et al and Corrado et al and suspected uterine rupture in IC route by Irion et al, but none were found in the current study[22,23].

The induction-to-delivery interval showed no statistically significance between the two groups similar to findings by Corrado et al[22] but contrary to the findings of Grignaffini et al, where they found induction-to-delivery interval to be shorter in IC gel group as compared to slow-release PF insert of dinoprostone (12 h 54 min IC vs 16 h 59 min IV; $p < 0.05$)[21]. This difference could have probably been due to the difference in the sample size included in the latter study.

Of the total successful vaginal deliveries, 71.11% (32 of 45) participants in the IC group and 64.28% (27 of 42) participants in the PF group required re-instillation of gel, the difference not statistically significant.

Rates of instrumental deliveries increased with the use of dinoprostone induction of labour. Instrumentation was required for three deliveries in IC group and eight in PF group, whereas oxytocin augmentation was required for 20 deliveries and 21 deliveries in the IC and PF groups, respectively. These findings were also not significant, similar to the previous studies mentioned earlier[20-22]. No statistically significant differences were seen in the rates of rupture of membranes, abnormal FHR tracings, APGAR scores and NICU admissions across the two groups.

In our study, there was a statistically significant

differences noted in the incidence of atonic postpartum hemorrhage and urinary retention across the two groups.

In a systematic review by Boulvain et al on 7,738 participants, it was found that although the IC application of PGE2 was an effective route for IOL, it offered no advantage over PF[24]. The findings of our study supported these earlier findings.

Contrary to our study findings, the observations made by Ekman et al and the recent study by Reinhard et al concluded statistically significant differences showing IC route to be superior of the two routes[25, 26].

Conclusion

In conclusion, overall, the differences in the two routes were found to be statistically insignificant in our study; but the authors would like to draw important conclusions relevant to the current practice. Dinoprostone 0.5 mg could be used through both the routes with similar efficacy (not previously studied), unlike recent studies which have used vaginal dinoprostone pessaries that are not widely available. Moreover, it is evident that there still lies controversy in the two modes of application and most of the studies comparing the effect were conducted in the late 1900s and there is a dearth of current studies in this domain. Dinoprostone gel 0.5 mg being more readily and widely available at a reduced costs suggests that it still serves as a promising the 0.5 mg dinoprostone gel serves as a promising agent for cervical ripening during induction of labour at term for both the routes. The recently popular vaginal dinoprostone pessary 2 mg tablet was not available widely during the study period in India.

The limitation of the study was that the observations and conclusions drawn from the current study, being a quazi-experimental, need to be tested in randomized controlled trials on a larger number of participants.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Alfirevic Z, Keeney E, Dowswell T, Welton NJ, Medley N, Dias S, et al. Which method is best for the induction of labour? A systematic review, network meta-analysis and cost-effectiveness analysis. NIHR Journals Library; 2016.
2. Viteri OA, Sibai BM. Challenges and limitations of clinical trials on labor induction: A review of the literature. AJP Rep 2018;8:e365-78.
3. Menon M, Ta S, Krishnamurthy V. Comparing various dose of PGE2 gel in induction of labour-maternal and fetal outcome. Int J Reprod Contracept Obstet Gynecol 20174;5:1520-4.
4. Kho EM, Sadler L, McCowan L. Induction of labour: a comparison between controlled-release dinoprostone vaginal pessary (Cervidil) and dinoprostone intravaginal gel (Prostin E2). Aust N Z J Obstet Gynaecol 2008;48:473-7.
5. Kelly AJ, Kavanagh J, Thomas J. Vaginal prostaglandin (PGE2 and PGF2a) for induction of labour at term. Cochrane Database Syst Rev 2003;(4):CD003101.
6. Thomas J, Fairclough A, Kavanagh J, Kelly AJ. Vaginal prostaglandin (PGE2 and PGF2a) for induction of labour at term. Cochrane Database Syst Rev 2014;(6):CD003101.
7. Shakya R, Shrestha J, Thapa P. Safety and efficacy of misoprostol and dinoprostone as cervical ripening agents. JNMA J Nepal Med Assoc 2010;49:33-7.
8. Guerra GV, Cecatti JG, Souza JP, Faúndes A, Morais SS, Gülmezoglu AM, et al. Elective induction versus spontaneous labour in Latin America. Bull World Health Organ 2011;89:657-65.
9. Zeng X, Zhang Y, Tian Q, Xue Y, Sun R, Zheng W, et al. Efficiency of dinoprostone insert for cervical ripening and induction of labor in women of full-term pregnancy compared with dinoprostone gel: A meta-analysis. Drug Discov Ther 2015;9:165-72.
10. Zanconato G, Bergamini V, Mantovani E, Carlin R, Bortolami O, Franchi M. Induction of labor and pain: a randomized trial between two vaginal preparations of dinoprostone in nulliparous women with an unfavorable cervix. J Matern Fetal Neonatal Med 2011;24:728-31.
11. Triglia MT, Palamara F, Lojacono A, Prefumo F, Frusca T. A randomized controlled trial of 24-hour vaginal dinoprostone pessary compared to gel for induction of labor in term pregnancies with a Bishop score < or = 4. Acta Obstet Gynecol Scand 2010;89:651-7.
12. Kho EM, Sadler L, McCowan L. Induction of labour: a comparison between controlled-release dinoprostone vaginal pessary (Cervidil) and dinoprostone intravaginal gel (Prostin E2). Aust N Z J Obstet Gynaecol 2008;48:473-7.

13. Khan ZA, Abdul B, Majoko F. Induction of labour with vaginal prostaglandin tablet vs gel. J Obstet Gynaecol 2011;31:492-4.
14. Singh U, Mehrotra S, Gupta HP, Dhakad A, Jain V. A prospective double blind trial investigating impact of vaginal pH on efficacy of prostaglandin gel for cervical ripening and course of labour. J Obstet Gynaecol 2011;31:217-9.
15. Faul F, Erdfelder E, Lang A-G, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39:175-91.
16. Nuutila M, Kajanoja P. Local administration of prostaglandin E2 for cervical ripening and labor induction: the appropriate route and dose. Acta Obstet Gynecol Scand 1996;75:135-8.
17. Guidelines on fetal monitoring aim to codify normal, abnormal FHR. Available from: https://www.mdedge.com/obgyn/article/63323/obstetrics/guidelines-fetal-monitoring-aim-codify-normal-abnormal-fhr
18. Health (UK) NCC for W and C. Methods of induction of labour [Internet]. Induction of Labour. RCOG Press; 2008. Available from: https://www.ncbi.nlm.nih.gov/books/NBK53608/
19. Perry MY, Leaphart WL. Randomized trial of intracervical versus posterior fornix dinoprostone for induction of labor. Obstet Gynecol 2004;103:13–7.
20. Kemp B, Winkler M, Rath W. Induction of labor by prostaglandin E(2) in relation to the Bishop score. Int J Gynaecol Obstet 2000;71:13-7.
21. Grignaffini A, Soncini E, Anfuso S, Ronzoni E. Dinoprostone: slow release vaginal insert (Propess) and intracervical gel (Prepidil) for the induction of labour with unriped cervix. Minerva Ginecol 2004;56:413-8.
22. Corrado F, Cannata ML, Facciola G, Stella NC. Intravaginal vs. intracervical PGE2 gel first application for labor induction. Int J Gynaecol Obstet 2001;75:195-7.
23. Irion O, Pedrazzoli J, Mermillod B. A randomized trial comparing vaginal and cervical prostaglandin gel for cervical ripening and labor induction. Obstet Gynecol 1998;91:65-71.
24. Boulvain M, Kelly A, Irion O. Intracervical prostaglandins for induction of labour. Cochrane Database Syst Rev 2008;1:CD006971.
25. Ekman G, Forman A, Marsál K, Ulmsten U. Intravaginal versus intracervical application of prostaglandin E2 in viscous gel for cervical priming and induction of labor at term in patients with an unfavorable cervical state. Am J Obstet Gynecol 1983;147:657-61.
26. Reinhard J, Rösler R, Yuan J, Schiermeier S, Herrmann E, Eichbaum MH, et al. Prostaglandin E2 labour induction with intravaginal (Minprostin) versus intracervical (Prepidil) administration at term: randomized study of maternal and neonatal outcome and patient's perception using the osgood semantic differential scales. BioMed Res Int 2014;2014:682919.

Thai Journal of Obstetrics and Gynaecology
July 2023, Vol. 31, No. 4, pp. 255-264.

GYNAECOLOGY

Association between Chemiluminescent Microparticle Immunoassay Signal-to-cutoff Ratio and Active Stage of Syphilis in Thai Pregnant Women

Piyachat Sakunborrirak, M.D.*,
Chenchit Chayachinda, M.D.*,
Jaruda Kobkitjaroen, M.D.**

** Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*
*** Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*

ABSTRACT

Objectives: To demonstrate the association between chemiluminescent microparticle immunoassay (CMIA) signal-to-cutoff (S/CO) ratio and active stage of syphilis as well as adverse perinatal outcomes.

Materials and Methods: A retrospective chart review was conducted in pregnant women with reactive CMIA (CMIA S/CO ratio ≥ 1) as the primary test in the reverse algorithm of syphilis screening. The participants were categorized into three groups: Group 1 CMIA+ venereal disease research laboratory (VDRL)+; Group 2 CMIA+ VDRL- *Treponema pallidum* haemagglutination test (TPHA)+; and Group 3 CMIA+ VDRL- TPHA-. CMIA S/CO ratio and perinatal outcomes were compared. Active stage of syphilis refers to having venereal disease research laboratory (VDRL) titer ≥ 1:8.

Results: Eighty-three out of 8,987 (0.92%) pregnant women who came for antenatal care at Siriraj Hospital between January 2020 and February 2021 were reactive for CMIA. Two twin gestations were excluded. The CMIA S/CO ratio was highest in group 1 (n = 39) at 23.1 ± 5.5, followed by 16.1 ± 5.2 in group 2 (n = 25) and 2.1 ± 3.2 in group 3 (n = 17), $p < 0.001$. Perinatal outcomes were not different among the groups, except for congenital syphilis (CS). All six neonates with CS were born to the participants in group 1 who had CMIA S/CO ratio ≥ 19.9. Most of the participants who delivered neonates with CS were diagnosed with syphilis in third trimester and had VDRL titer ≥ 1:8.

Conclusion: Instances of adverse perinatal outcomes and active stage of maternal syphilis were more frequent in pregnant women with higher CMIA S/CO ratio. The use of CMIA S/CO ratio as an adjunct to clinical evaluation may provide additional benefits to the syphilis screening.

Keywords: chemiluminescent microparticle immunoassay, perinatal outcomes, syphilis, screening, signal-to-cutoff ratio.

Correspondence to: *Jaruda Kobkitjaroen, M.D., Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. E-mail: jaruda.kob@mahidol.ac.th*

Received: 26 May 2022, **Revised:** 5 December 2022, **Accepted:** 21 December 2022

ความสัมพันธ์ระหว่างค่าสัดส่วน chemiluminescent microparticle immunoassay กับระยะแพร่กระจายเชื้อมากของโรคซิฟิลิสในหญิงตั้งครรภ์ไทย

ปิยฉัตร สกุลบริรักษ์, เจนจิต ฉายะจินดา, จารุดา กอบกิจเจริญ

บทคัดย่อ

วัตถุประสงค์: *เพื่อแสดงความสัมพันธ์ระหว่างสัดส่วนค่า chemiluminescent microparticle immunoassay (CMIA) signal-to-cutoff (S/CO) กับระยะแพร่กระจายเชื้อมากของโรคซิฟิลิสในหญิงตั้งครรภ์ไทย และผลต่อทารกแรกเกิด*

วัสดุและวิธีการ: *ทำการศึกษาแบบ retrospective chart review ในหญิงตั้งครรภ์ที่ได้รับการตรวจคัดกรองโรคซิฟิลิสโดยการตรวจเลือดด้วย CMIA เป็นวิธีแรก และได้ผล CMIA เป็นบวก (CMIA S/CO ratio ≥1) จากนั้นจะตรวจยืนยันด้วย Venereal Disease Research Laboratory (VDRL) หากผลไม่ตรงกัน จะทำการตรวจยืนยันอีกครั้งด้วยวิธี Treponemal pallidum hemagglutination (TPHA) มีแบ่งกลุ่มการศึกษาเป็น 3 กลุ่ม ดังนี้ กลุ่ม 1 CMIA+ VDRL+ กลุ่ม 2 CMIA+ VDRL- TPHA+ และกลุ่ม 3 CMIA+ VDRL- TPHA- จากนั้นหาความสัมพันธ์ระหว่างสัดส่วนค่า chemiluminescent microparticle immunoassay (CMIA) signal-to-cutoff (S/CO) กับระยะแพร่กระจายเชื้อมากของโรคซิฟิลิสในหญิงตั้งครรภ์ไทย และผลต่อทารกแรกเกิด โดยที่ระยะแพร่กระจายเชื้อมากของโรคซิฟิลิสหมายถึงกลุ่มที่มีระดับ VDRL titer ≥1:8*

ผลการศึกษา: *จากจำนวนหญิงตั้งครรภ์ที่มาฝากครรภ์และได้รับการตรวจคัดกรองโรคซิฟิลิส ที่โรงพยาบาลศิริราชในช่วงเดือน มกราคม พ.ศ.2563 ถึง เดือนกุมภาพันธ์ พ.ศ.2564 ทั้งหมด 8,987 ราย พบว่ามีหญิงตั้งครรภ์จำนวน 83 ราย (ร้อยละ 0.92) ตรวจพบ CMIA เป็นบวก มีจำนวนหญิงตั้งครรภ์สองรายถูกนำออกจากการศึกษาเนื่องจากเป็นการตั้งครรภ์แฝด ผลการศึกษาพบว่าในกลุ่มที่ 1 (จำนวน 39 คน) มีค่าสัดส่วน CMIA S/CO ที่สูงที่สุด โดยค่าเฉลี่ยคือ 23.1 ± 5.5 รองลงมาคือในกลุ่มที่ 2 (จำนวน 25 คน) และกลุ่มที่ 3 (จำนวน 17 คน) ที่มีค่า 16.1 ± 5.2 และ 2.1 ± 3.2 ตามลำดับ, $p < 0.001$ ผลของทารกแรกเกิดไม่ได้มีความแตกต่างกันอย่างมีนัยสำคัญในทั้งสามกลุ่ม ยกเว้นพบทารกทั้งหมดจำนวน 6 รายที่เป็นโรคซิฟิลิสแต่กำเนิดโดยทั้งหมดเกิดจากมารดาในกลุ่มที่ 1 ที่มีค่าสัดส่วน CMIA S/CO ที่ ≥19.9 และหญิงตั้งครรภ์ส่วนใหญ่ที่คลอดทารกที่ได้รับการวินิจฉัยว่าเป็นโรคซิฟิลิสแต่กำเนิดพบว่าได้รับการวินิจฉัยว่าเป็นซิฟิซิสในไตรมาสที่สาม และตรวจพบว่ามีค่า VDRL titer ≥1:8*

สรุป: *ภาวะแทรกซ้อนของทารกแรกเกิดและระยะแพร่เชื้อมากของโรคซิฟิลิสพบได้มากกว่าในหญิงตั้งครรภ์ที่ตรวจพบว่ามีค่าสัดส่วน CMIA S/CO ที่สูง และการใช้ค่าสัดส่วน CMIA S/CO เสริมในการตรวจคัดกรองโรคซิฟิลิสอาจเป็นประโยชน์ในการดูแลรักษาทางคลินิก*

คำสำคัญ: *chemiluminescent microparticle immunoassay, ผลของทารกแรกเกิด, ซิฟิลิส, สัดส่วน*

Introduction

Syphilis is a destructive infectious disease caused by *Treponema pallidum* subspecies *pallidum*. It is a sexually transmitted infection, spreading mainly through lesion contact. In pregnant women, *T. pallidum* can infect in-utero fetus through placenta causing congenital syphilis (CS). Over the past 10 years, CS in Thailand is on the rise with an increase in the incidence rate from 7.48 per 100,000 live births in 2011 to 161.78 per 100,000 live births in 2021[1]. The risk factors include ineffective syphilis screening methods, failure to complete course of treatment, teenage pregnancy, commencing treatment for less than four weeks before delivery, and reinfection during pregnancy[2].

Diagnosis of syphilis can be done either by detection of the etiologic organism or serological diagnosis[3, 4]. The serological diagnosis of syphilis, which requires both treponemal and nontreponemal tests, is widely applied due to the quick resolution of the clinical presentations. Nontreponemal test detects antibodies to lipoidal antigens being released from damaged host tissues which occur most obviously during early stage of syphilis. Therefore, its titers indicate disease activity. In other words, higher titers relate to the more active stage of syphilis[5]. On the contrary, treponemal tests detect specific antibodies against *T. pallidum*, which remain detectable for life. A positive treponemal test may refer to current or past infection. At the moment, the clinical use of treponemal test is only reactive or non-reactive result, but their antibody titers have no clinical application[3, 4].

Formerly, the screening protocol of syphilis starts with a non-treponemal test which was called the 'traditional sequence'. A treponemal test will be done if only the primary test is reactive. This results in a big missing portion of patients in very early and late stage of syphilis[6-8]. Later, the reverse sequence which switches the order of the tests has shown a greater screening performance. It has been recommended by the World Health Organization (WHO)[9] and the Thai national guideline[10] as a pivotal tool to eliminate CS.

Chemiluminescent microparticle immunoassay (CMIA) is the recommended primary treponemal test in Thailand due to its high sensitivity[11, 12]. If CMIA is positive, a nontreponemal test will be performed to confirm the diagnosis of syphilis, which is either rapid plasma reagin (RPR) or venereal disease research laboratory (VDRL). In case of discordant results, false positive CMIA should be excluded by performing the second treponemal test, such as *Treponema pallidum* hemagglutination (TPHA). A reactive second treponemal test indicates syphilis infection, while a non-reactive one indicates that syphilis is unlikely. CMIA is run by an automated system and reported in a quantitative result with a signal-to-cutoff (S/CO) ratio. This ratio varies with the amount of antibody in a sample. The manufacturer defines reactive CMIA as the CMIA S/CO ratio at ≥1. Some studies reported the optimal cutoff points set at higher S/CO ratio to decrease false positive rate[13, 14]. Moreover, the association of the higher CMIA S/CO ratio and poorer neonatal outcomes were reported[15]. Therefore, the present study aims to demonstrate the association between CMIA S/CO ratio and clinical stage of syphilis during pregnancy and perinatal outcomes of pregnancy with syphilis.

Materials and Methods

This retrospective chart-review study was conducted at Siriraj Hospital, Thailand between January 2020, and February 2021. It was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University. (COA no. Si 938/2021).

Participants

All pregnant women who came for antenatal care (ANC) at Siriraj Hospital between January 2020 and February 2021; and had reactive CMIA were included in the study. Those who delivered at Siriraj Hospital were included for the analysis of the secondary outcomes. Exclusion criterion was multifetal pregnancy.

Laboratory investigations for syphilis screening and management

All laboratory investigations were conducted at the ISO 15189-accredited Laboratory at Siriraj Hospital. They were performed by using commercial reagent and following manufacturer instruction. The CMIA, using ARCHITECT Syphilis TP (Abbott Laboratories, Abbott Park, Illinois, USA), was performed to initially screen for antibodies to *T. pallidum*. CMIA was an immunoassay run by an automated instrument and reported quantitatively using S/CO ratio and considered reactive when the ratio was ≥1 according to manufacturer recommendation. Sera with reactive CMIA were later tested using the Venereal Disease Research Laboratory (VDRL) (Becton, Dickinson and Company, Sparks, Maryland, USA), which is a non-treponemal test. VDRL was a flocculation test performed manually and reported in titers. Those with reactive VDRL (CMIA+, VDRL+) were diagnosed with syphilis (Group 1). Those with discordant results (CMIA+, VDRL-) were further tested by *Treponema pallidum* hemagglutination (TPHA) (Rapid Labs, Colchester, Essex, UK). TPHA was an indirect hemagglutination assay performed manually and reported qualitatively as reactive and non-reactive. Those with reactive TPHA (CMIA+, VDRL-, TPHA+) were diagnosed with late syphilis (Group 2). Those with non-reactive TPHA (CMIA+, VDRL-, TPHA-) were considered as having false positive CMIA (Group 3)[4].

All pregnant women diagnosed with syphilis were treated in accordance with the standard guideline provided by the Center for Disease Control and Prevention and the Thai guideline[3, 16]. In the labor room, their blood was drawn for VDRL testing regardless of previous treatment to compare with that in neonate's blood. All neonates born to pregnant women with syphilis were examined thoroughly for the evidence of congenital syphilis and placentas were sent for pathological examination and detection of *T. pallidum*.

Neonates were considered to have CS if any of the following criteria was met: abnormal physical examination compatible with CS; fourfold higher than maternal titer of VDRL at delivery; or pathological reports representing evidence of *T. pallidum* infection[3]. Those with proven CS received aqueous crystalline penicillin for a total of 10 days.

Outcome measures

The association between CMIA S/CO ratio and activities of disease, including syphilis (Group 1), late syphilis (Group 2) and false positive CMIA (Group 3), was the primary outcome. Also, the CMIA S/CO ratio was divided into three intervals according to previous studies[13] for the comparison, including <9.9, 9.9 to <19.9 and ≥19.9.

Perinatal outcomes including preterm birth, low birth weight (LBW), birth asphyxia and CS were the secondary outcomes. Preterm birth was defined as delivery prior to 37 weeks of gestational age (GA). Low birth weight (LBW) was defined as a neonate birth weight lower than 2,500 grams. Birth asphyxia was defined as having an Apgar score of less than 7 at one or five minutes.

Sample size calculation and statistical analysis

According to a study by Zofkie AC et al[15], which used chemiluminescence immunoassays (CIA), *Treponemal pallidum* particle agglutination (TPPA) and rapid plasma regain (RPR) for screening syphilis in pregnant women, the means and standard deviations of CIA S/CO titers were 18.3 ± 5.4 (CIA+/RPR+/TPPA+), 12.1 ± 5.3 (CIA+/RPR-/TPPA+) and 1.9 ± 0.8 (CIA+/RPR-/TPPA-), respectively. The sample size was calculated by nQuery Advisor. The alpha and power were set at 0.05 and 80%, respectively. Consequently, the minimum sample size required for our study was 75.

Stata program version 12.1 (Statacorp LP, College Station, Texas USA) was used for statistical analysis. Presentation of descriptive data was done with n (%), mean ± standard deviation (SD), and median with interquartile range (IQR). The comparisons between 3 groups were performed using one-way analysis of variance (ANOVA)

pairwise comparison by the Bonferroni for normal continuous data. Non-normal distribution data was analyzed using pairwise comparison Dunn's test's and Kruskal-Wallis test. Comparison of categorical data was done using chi-square test or Fisher's exact test. The t test and Wilcoxon Rank Sum test were used to compare parametric data and non-parametric data, respectively. A p value of < 0.05 was statistically significant.

Results

Between January 2020 and February 2021, there were 8,987 pregnant women who came for ANC at Siriraj Hospital. The total number of reactive CMIA samples was 83 (0.92%). Two participants had multifetal pregnancies and were excluded. The VDRL and TPHA results of 81 participants with reactive CMIA were shown in Fig. 1. Participants with reactive VDRL (CMIA+, VDRL+) were found most in CMIA S/CO ratio ≥19.9 (33/38 or 86.8%). On the contrary, participants with false positive CMIA (CMIA+, VDRL-TPHA-) were found most in CMIA S/CO ratio < 9.9 (19/23 or 82.6%) and not found in CMIA S/CO ratio ≥19.9.

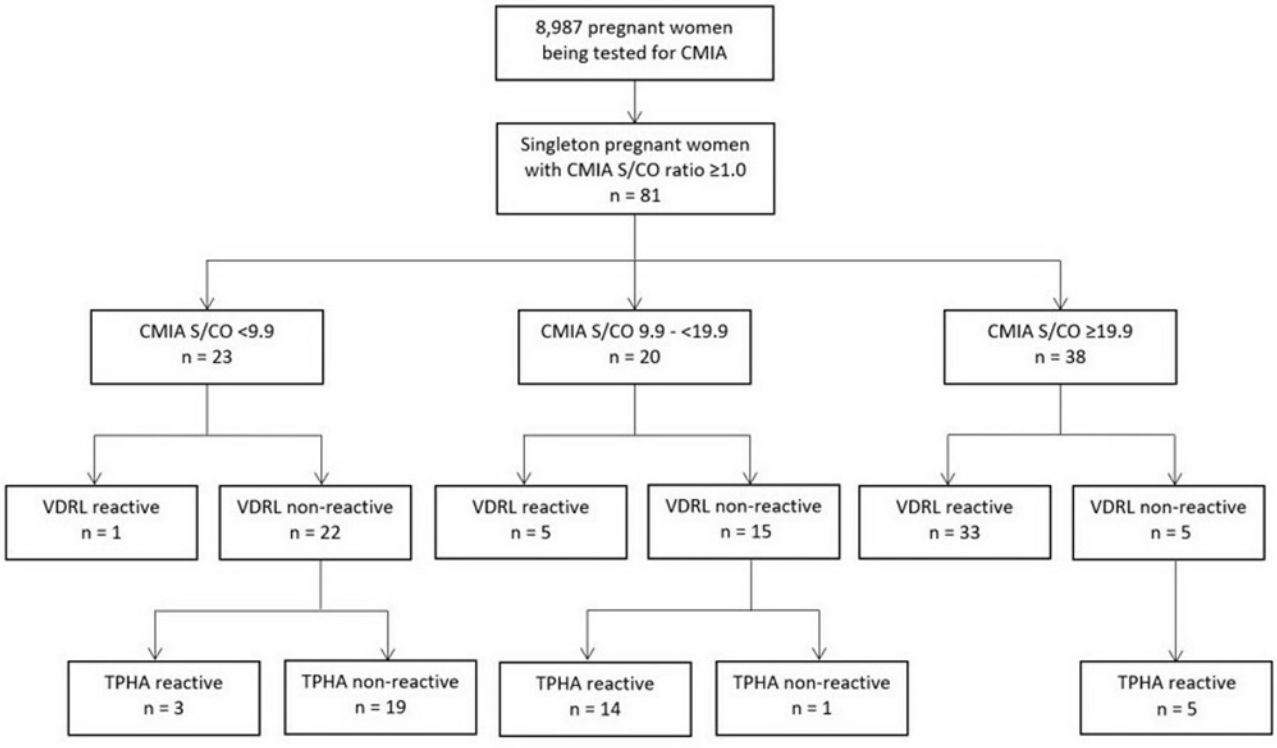

Fig. 1. Diagram of the participants.
CMIA: chemiluminescent microparticle immunoassay, S/CO: signal-to-cutoff ratio, VDRL: venereal disease research laboratory, TPHA: *Treponema pallidum* hemagglutination.

Participants' characteristics were compared among Group 1 (CMIA+ VDRL+), Group 2 (CMIA+ VDRL- TPHA+) and Group 3 (CMIA+ VDRL- TPHA-) as shown in Table 1. The participants in Group 1 were younger and more likely to be primiparous. Three of them had HIV infection. The body mass index (BMI), parity, previous abortion, and GA at diagnosis were comparable. CMIA S/CO ratio was highest in Group 1 at 23.1 ± 5.5, followed by 16.1 ± 5.2 in Group 2 and 2.1 ± 3.2 in Group 3, p < 0.001. (Fig. 2)

Table 1. Characteristics of pregnant women with reactive CMIA (n = 81).

	CMIA+, VDRL+ (n = 39)	CMIA+, VDRL-, TPHA+ (n = 22)	CMIA+, VDRL-, TPHA- (n = 20)	p value
Age (years)	22.7 ± 5.4	26.7 ± 5.5	30.2 ± 5.7	< 0.001
Age (years)				0.013
< 20	9 (23.1)	1 (4.5)	1 (5.0)	
20-30	26 (66.7)	15 (68.2)	10 (50.0)	
> 30	4 (10.3)	6 (27.3)	9 (45.0)	
BMI (kg/m^2)	23.4 ± 3.7	25.5 ± 5.7	24.5 ± 3.9	0.189
Primiparity	18 (46.2)	2 (9.1)	7 (35.0)	0.013
Previous abortion	11 (28.2)	9 (40.9)	4 (20.0)	0.321
GA at diagnosis (weeks)	20.0 ± 10.9	21.3 ± 13.3	16.0 ± 11.3	0.300
GA at diagnosis (weeks)				0.532
< 14	16 (41.0)	10 (45.5)	12 (60.0)	
14-28	12 (30.8)	4 (18.2)	4 (20.0)	
> 28	11 (28.2)	8 (36.4)	4 (20.0)	
HIV infection	3 (7.7)	0 (0)	0 (0)	0.187
CMIA S/CO ratio	23.1 ± 5.5	16.1 ± 5.2	2.1 ± 3.2	< 0.001

Data presented in n (%), mean ± standard deviation (SD) and median ± SD

CMIA: chemiluminescent microparticle immunoassay, VDRL: venereal disease research laboratory, TPHA: *Treponema Pallidum* hemagglutination assay, Rx: treatment, BMI: body mass index, GA: gestational age, HIV: human immunodeficiency virus, S/CO: signal-to-cutoff ratio

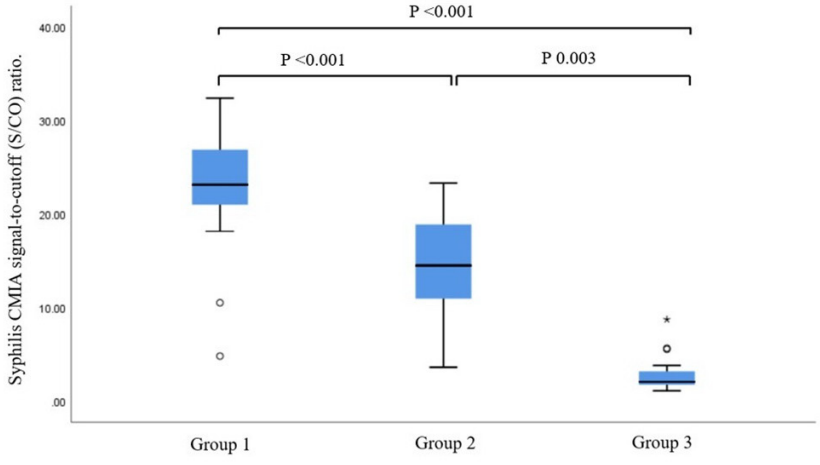

Fig. 2. Chemiluminescent microparticle immunoassay signal to-cutoff ratio by groups. Group 1 CMIA+/VDRL+; Group 2 CMIA+/VDRL-/TPHA+; Group 3 CMIA+/VDRL-/TPHA-.

CMIA: chemiluminescent microparticle immunoassay, VDRL: venereal disease research laboratory, TPHA: *Treponema pallidum* hemagglutination.

Miscarriages occurred in three participants at GA 10, 11, and 21 weeks. The first two cases were diagnosed with false positive CMIA and the last one was early syphilis. Eight participants delivered at other hospitals. Accordingly, 70 participants were included into the analysis of the perinatal outcomes.

The perinatal outcomes were similar among pregnant women with different ranges of CMIA except for the incidence of CS (Table 2). All six neonates diagnosed with CS were born to mothers with CMIA S/CO ratio ≥19.9 and reactive VDRL. The pregnant women who delivered neonates with CS tended to be younger; were diagnosed with syphilis in third trimester and had higher CMIA S/CO ratio and VDRL titer, compared to those who delivered neonates without CS (Table 3).

Table 2. Perinatal outcomes in pregnant women with reactive CMIA (n = 70).

	S/CO ratio ≥ 1 to < 9.9 (n = 17)	**S/CO ratio 9.9 to < 19.9 (n = 19)**	**S/CO ratio ≥ 19.9 (n = 34)**	**p value**
GA at delivery (weeks)	38.2 ± 1.1	38.5 ± 1.6	37.4 ± 2.8	0.251
GA at delivery (weeks)				0.131
< 34	0 (0)	0 (0)	3 (8.8)	
< 37	1 (5.9)	3 (15.8)	6 (17.6)	
≥ 37	16 (94.1)	16 (84.2)	25 (73.5)	0.424
Birth weight (gm)	3,055 ± 274	2,899 ± 296	2,832 ± 595	0.276
< 1500	0 (0)	0 (0)	2 (5.9)	
< 2500	0 (0)	1 (5.3)	5 (14.7)	0.263
Birth asphyxia	1 (5.9)	0 (0)	3 (8.8)	0.678
Congenital syphilis	0 (0)	0 (0)	6 (17.6)	0.040

Data are presented as n (%) or mean ± standard deviation (SD)
CMIA: Chemiluminescent microparticle immunoassay, S/CO: signal-to-cutoff ratio, GA: gestational age

Table 3. Characteristics of pregnant women delivering neonates with and without congenital syphilis (n = 70).

	Congenital syphilis (n = 6)	**No congenital syphilis (n = 64)**	**p value**
Age (years)	23.5 ± 7.6	25.7 ± 6.0	0.407
< 20	2 (33.3)	8 (12.5)	0.377
20-30	3 (50.0)	43 (67.2)	
> 30	1 (16.7)	13 (20.3)	
GA at diagnosis (weeks)	27.7 ± 9.0	19.5 ± 12.0	0.111
Trimester at diagnosis			
First	1 (16.7)	31 (48.5)	0.141
Second	1 (16.7)	15 (23.4)	
Third	4 (66.6)	18 (28.1)	
CMIA S/CO ratio	26.8 ± 4.4	16.0 ± 8.9	0.005
≥ 1 to < 9.9	0	17 (26.6)	0.025
9.9 to < 19.9	0	20 (31.2)	
≥ 19.9	6 (100)	27 (42.2)	
VDRL titer	1:16 [1:2,1:64]	0 [0,1:2]	< 0.001*
VDRL titer ≥ 1: 8	4 (66.6)	7 (10.9)	< 0.001

Data are presented as n(%), mean ± standard deviation (SD), median ± SD or median with interquartile range.
GA: gestational age, CMIA: chemiluminescent microparticle immunoassay, VDRL: venereal disease research laboratory, S/CO: signal-to-cutoff ratio.
*Wilcoxon rank sum test

Discussion

High CMIA S/CO ratio in pregnant women with syphilis relates to the occurrence of congenital syphilis. Both high CMIA S/CO ratio and high VDRL titer favor active stage of maternal syphilis. This supports the fact that early stage of syphilis in pregnant women associates with CS, at 70-100%[16], due to a high level of spirochetemia together with a greater tissue damage. Compatible with previous studies, in order to eliminate CS, the higher CMIA S/CO ratio may be applied as an adjunct to help identify the stage of syphilis and make a prompt decision for management[15, 17].

The median CMIA S/CO ratio from participants with active syphilis in the present study is slightly higher than that from Thai pregnant women with untreated syphilis in the previous study (23.11 vs 21.27)[14]. The higher level of S/CO ratio can be partly explained by the fact that the participants in the present study were younger (26 vs 32 years). As widely known, a recent infection of syphilis leads to marked horizontal and vertical transmission as well as a greater tissue damage. Young women, particularly teenagers, who are not far from their sex debut, thus tended to be in the early stage of syphilis. This results in a higher VDRL titer and, as being shown in the present study, a higher CMIA S/CO ratio.

The false positive CMIA results using S/CO ratio ≥1 as a cutoff point was observed at 11.8-53.8% in previous studies[14, 15, 18]. In this study, the proportion of CMIA results which returned false positive was 24.7% (20 in 81). The false positive CMIA can be lessened by increasing S/CO ratio diagnostic point. In line with the previous study in non-pregnant women reporting a diagnostic specificity of 100% when using CMIA S/CO ratio ≥9.9 as a cutoff point[13], our findings showed that only 1.7% (1 in 58) of pregnant women with CMIA S/CO ratio ≥9.9 were false positive. Additionally, 82.6% of samples with reactive CMIA S/CO ratio < 9.9 were false positive. Because of the difference in population, each laboratory should determine its own optimal CMIA S/CO ratio cutoff point. However, as false positive tests appear more acceptable in terms of screening, CMIA S/CO ratio cutoff point at ≥1 remains for it has high screening performance.

The present study demonstrated that 27.2% (22 in 81) of CMIA-reactive participants had false negative VDRL (CMIA+, VDRL-, TPHA+). The diagnosis of syphilis would have been missed in those participants if the traditional sequence had been used, similar to the previous studies that found a missed diagnosis rate of 24.2-27.1%[19, 20]. The finding underlines the superiority of the reverse sequence in terms of syphilis screening.

Although perinatal outcomes were not different among the three groups of CMIA S/CO ratio in this study, all neonates with CS were born to mothers with high CMIA S/CO ratio and high VDRL titer. Additionally, the participants with younger age and greater GA at diagnosis tended to have neonates with CS. This is consistent with a previous report which showed that 50% of teenage mothers with syphilis gave birth to neonates with CS, and that the first ANC after 20 weeks of gestation was a predictive factor[5]. Late ANC may lead to delayed diagnosis and treatment of syphilis while intrauterine fetuses are increasingly harmed with GA[21]. Information regarding prevention of both sexually transmitted infections and pregnancy, together with proper management once either occurs should be more emphasized in Thai young population. The single study center is the strength in that all laboratory investigations were performed in one qualified center. Therefore, the whole process of the reverse sequence algorithm was completely adhered. Moreover, the good collaboration between the Department of Obstetrics and Gynaecology, that of Pediatrics and that of Clinical Pathology are well-established. The limitation was the lack of long-term follow-up of newborns as late-onset congenital syphilis may develop much later.

Conclusion

Instances of adverse perinatal outcomes, particularly congenital syphilis, and maternal syphilis with VDRL titers ≥1:8 are more frequent in pregnant

women with higher CMIA S/CO ratio. All neonates with CS were born to mothers with CMIA S/CO ≥19.9. The use of CMIA S/CO ratio as an adjunct to clinical evaluation may provide additional benefits to the syphilis screening and raise clinicians' awareness of commencing immediate and proper management for pregnant patients.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Bureau of Epidemiology. Department of Disease Control. National Disease Surveillance (Report 506), Ministry of Public Health, Bangkok; 2012-2021. (Accessed 30 January 2022) Available from: http://doe.moph.go.th/surdata/disease.php?ds=37
2. Rungmaitree S, Mokkhamakkun C, Phongsamart W, Lolekha R, Wittawatmongkol O, Lapphra K, et al. Incidence of and factors associated with congenital syphilis at a tertiary hospital in Bangkok, Thailand. Southeast Asian J Trop Med Public Health 2019;50:1090-101.
3. Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, et al. Sexually transmitted diseases treatment guidelines, 2021. MMWR Recomm Rep 2021;70:1-187.
4. Centers for Disease Control and Prevention. Sexually transmitted infections treatment guidelines, 2021. United States; 2021. (Accessed 3 December 2022) Available from: https://www.cdc.gov/std/treatment-guidelines/syphilis.htm
5. Panchalee T, Chayachinda C, Panyakat W, Chen M, Quinones-Garcia V Z, Pernsoongnern P. Characteristics and neonatal outcomes of teenage pregnant women diagnosed with syphilis at Siriraj Hospital. Siriraj Med J 2018;70:298–301.
6. Centers for Disease Control and Prevention. Syphilis testing algorithms using treponemal tests for initial screening---four laboratories, New York City, 2005--2006. MMWR Morb Mortal Wkly Rep 2008;57:872-5.
7. Centers for Disease Control and Prevention. Discordant results from reverse sequence syphilis screening--five laboratories, United States, 2006-2010. MMWR Morb Mortal Wkly Rep 2011; 60:133–7.
8. Gratrix J, Plitt S, Lee BE, Ferron L, Anderson B, Verity B, et al. Impact of reverse sequence syphilis screening on new diagnoses of late latent syphilis in Edmonton, Canada. Sex Transm Dis 2012;39:528-30.
9. World Health Organization. WHO guideline on syphilis screening and treatment for pregnant women. Geneva: World Health Organization; 2017.
10. Division of AIDS and STIs. Prevention and management of mother-to-child transmission of syphilis guideline. Bangkok: Department of Disease Control, Ministry of Public Health, Bangkok;2021. (Accessed 27 September 2021) Available from: http://klb.ddc.moph.go.th/dataentry/handbook/form/128
11. Bangrak STI Center. National Guideline for Syphilis Testing: Laboratory Manual for Diagnosis and Monitoring. Bangkok, Bangrak STI Center; 2021. (Accessed 27 September 2021) Available from: http://klb.ddc.moph.go.th/dataentry/handbook/form/117
12. Seña AC, White BL, Sparling PF. Novel *Treponema pallidum* serologic tests: a paradigm shift in syphilis screening for the 21st century. Clin Infect Dis 2010;51:700-8.
13. Dai S, Chi P, Lin Y, Zheng X, Liu W, Zhang J, et al. Improved reverse screening algorithm for Treponema Pallidum antibody using signal-to-cutoff ratios from chemiluminescence microparticle immunoassay. Sex Transm Dis 2014;41:29–34.
14. Boonchaoy A, Wongchampa P, Hirankarn N, Chaithongwongwatthana S. Performance of chemiluminescent microparticle immunoassay in screening for syphilis in pregnant women from low-prevalence, resource-limited settings. J Med Assoc Thai 2016;99:119-24.
15. Zofkie AC, Seasely AR, Gaffney D, Rogers VL, Mcintire DD, Roberts SW, et al. Syphilis immunoassay signal strength correlates with active infection in pregnant women. Am J Perinatol 2020;37:671-8.
16. Chayachinda C, Chinhiran K, Chaithongwongwatthana S, Kittiyaowamarn R, Thamkhantho M. Elimination of congenital syphilis: what's new for obstetricians in the 2020 Thai National Guideline? Thai J Obstet Gynaecol 2021:29;2-9.
17. Adhikari EH, Frame IJ, Hill E, Fatabhoy R, Strickland AL, Cavuoti D, et al. Abbott ARCHITECT Syphilis TP chemiluminescent immunoassay accurately diagnoses past or current syphilis in pregnancy. Am J Perinatol 2020;37:112-8.
18. Li Z, Feng Z, Liu P, Yan C. Screening for antibodies against Treponema pallidum with chemiluminescent microparticle immunoassay: analysis of discordant serology results and clinical characterization. Ann Clin Biochem 2016;53:588-92.
19. Tong ML, Lin LR, Liu LL, Zhang HL, Huang SJ, Chen YY, et al. Analysis of 3 algorithms for syphilis serodiagnosis and implications for clinical

management. Clin Infect Dis 2014;58:1116-24.
20. Totten YR, Hardy BM, Bennett B, Rowlinson MC, Crowe S. Comparative performance of the reverse algorithm using Architect Syphilis TP Versus the traditional algorithm using rapid plasma reagin in Florida's Public Health Testing Population. Ann Lab Med 2019;39: 396-9
21. Thornton C, Chaisson LH, Bleasdale SC. Characteristics of pregnant women with syphilis and factors associated with congenital syphilis at a Chicago Hospital. Open Forum Infect Dis 2022;9:ofac169.

Thai Journal of Obstetrics and Gynaecology
July 2023, Vol. 31, No. 4, pp. 265-272.

GYNAECOLOGY

Effects of Caffeine Dose on Bowel Function Recovery Following Gynecologic Cancer Surgery: A randomized double-blind controlled trial

Yaowapa Jirawongprapa, M.D.*,
Chokchi Chotboon, M.D.*,
Srisuda Songthamwat, M.D.*,
Ueamporn Summart, DrPH**,
Metha Songthamwat, M.D., Ph.D.*

** Department of Obstetrics and Gynecology, UdonThani Hospital, UdonThani, Thailand*
*** Faculty of Nursing, Western University, Burirum, Thailand*

ABSTRACT

Objectives: To compare the effects of different caffeine doses on bowel function recovery following abdominal gynecological cancer surgery.

Materials and Methods: A randomized double-blind controlled trial was undertaken. 92 patients were enrolled and allocated to one of two groups: with group 1, 50 mg caffeine (n = 46) or group 2, 100 mg caffeine (n = 46). Both groups being prescribed three times a day in the postoperative period. Surgical staging had been performed on patients who were diagnosed with endometrial, ovarian, and cervical cancer. The primary outcome was to compare the time to first flatus after surgery between patients of each group. The secondary outcomes were to determine the time to first defecation, time to normal bowel movement and time to tolerate a solid diet.

Results: The mean time to first flatus was 36.54 vs 38.39 hours (p = 0.53), time to first defecation was 66.65 vs 67.08 hours (p = 0.92) and time to normal bowel sound was 26.61 vs 29.41 hours (p = 0.16). All of the results in both groups were not significantly shorter in the 100 mg caffeine group than in the 50 mg caffeine group. Furthermore, 15.22% in the 100 mg caffeine group experienced insomnia compared to 2.17% in 50 mg caffeine group (p = 0.03).

Conclusion: Drinking coffee with 50 mg of caffeine after abdominal gynecological cancer surgery did not affect bowel function recovery than 100 mg caffeine. However, it caused fewer adverse effects that could be used as additional treatment in postoperative care.

Keywords: coffee, ileus, gynecologic cancer, postoperative.

Correspondence to: *Metha Songthamwat, M.D., Ph.D., Department of Obstetrics and Gynecology, UdonThani Hospital, UdonThani 41000, Thailand. E-mail: udonhome@yahoo.com*

Received: 30 September 2022, **Revised:** 14 November 2022, **Accepted:** 28 November 2022

ผลของปริมาณคาเฟอีนต่อการฟื้นตัวของการทำงานของลำไส้หลังการผ่าตัดมะเร็งทางนรีเวช: การศึกษาทดลองแบบสุ่มปกปิดสองทาง

เยาวพา จิระวงศ์ประภา, โชคชัย โชติบูรณ์, ศรีสุดา ทรงธรรมวัฒน์, เอื้อมพร สุ่มมาตย์, เมธา ทรงธรรมวัฒน์

บทคัดย่อ

วัตถุประสงค์: *เพื่อเปรียบเทียบผลของปริมาณคาเฟอีนที่แตกต่างกันต่อการฟื้นตัวของการทำงานของลำไส้หลังผ่าตัดมะเร็งนรีเวชทางหน้าท้อง*

วัสดุและวิธีการ: *การศึกษาทดลองแบบสุ่มปกปิดสองทาง โดยผู้ป่วย 92 รายได้รับการลงทะเบียนและถูกจัดสรรให้อยู่ในกลุ่มหนึ่งในสองกลุ่ม ได้แก่กลุ่ม 1 ได้รับคาเฟอีน 50 มิลลิกรัม (n = 46) หรือกลุ่ม 2 ได้รับคาเฟอีน 100 มิลลิกรัม (n = 46) ทั้งสองกลุ่มถูกกำหนดให้ดื่มกาแฟสามครั้งต่อวันหลังผ่าตัด ใน ผู้ป่วยที่ได้รับการผ่าตัดเพื่อกำหนดระยะโรคของมะเร็งเยื่อบุโพรงมดลูก รังไข่ และปากมดลูก ผลลัพธ์หลักคือการเปรียบเทียบเวลาที่ผายลมครั้งแรกหลังผ่าตัดระหว่างผู้ป่วยแต่ละกลุ่ม ผลลัพธ์รองคือเวลาที่ถ่ายอุจจาระครั้งแรก เวลาที่ลำไส้คลื่นไหวปกติและเวลาที่ทนต่อการรับประทานอาหารที่เป็นของแข็ง*

ผลการศึกษา: *เวลาเฉลี่ยของการผายลมครั้งแรกคือ 36.54 ชั่วโมงเทียบกับ 38.39 ชั่วโมง (p = 0.53) เวลาในการถ่ายอุจจาระครั้งแรกคือ 66.65 ชั่งโมงเทียบกับ 67.08 ชั่วโมง (p = 0.92) เวลาที่ได้ยินเสียงลำไส้ปกติคือ 26.61 ชั่วโมงเทียบกับ 29.41 ชั่วโมง (p = 0.16) ผลลัพธ์ทั้งหมดในทั้งสองกลุ่ม ในกลุ่มคาเฟอีน 100 มิลลิกรัมไม่ลดลงอย่างมีนัยสำคัญเมื่อเทียบกับกลุ่มคาเฟอีน 50 มิลลิกรัม นอกจากนี้ ร้อยละ 15.22 ในกลุ่มคาเฟอีน 100 มิลลิกรัมมีอาการนอนไม่หลับเทียบกับร้อยละ 2.17 ในกลุ่มคาเฟอีน 50 มิลลิกรัม (p = 0.03)*

สรุป: *การดื่มกาแฟที่มีคาเฟอีน 50 มิลลิกรัมหลังการผ่าตัดมะเร็งทางนรีเวชทางช่องท้องส่งผลต่อการฟื้นตัวของการทำงานของลำไส้ไม่แตกต่างอย่างมีนัยสำคัญจากการดื่มกาแฟที่มีคาเฟอีน 100 มิลลิกรัม แต่มีผลข้างเคียงน้อยกว่าและสามารถใช้เป็นการรักษาเพิ่มเติมในการดูแลหลังผ่าตัดได้*

คำสำคัญ: *กาแฟ, ลำไส้หยุดทำงาน, มะเร็งทางนรีเวช, หลังผ่าตัด*

Introduction

Postoperative ileus (POI) is defined as a temporary cessation of coordinated bowel motility, which causes the obstruction and intolerance of oral intake following nonabdominal or abdominal surgery, especially after laparotomy for any cancers[1]. The incidence of POI in patients who have undergone hysterectomy and radical hysterectomy with pelvic lymph node dissection for treatment of gynecologic malignancy is 10.5-15.5% and 7.9-20.0%, respectively[2].

Postoperative gastrointestinal dysmotility causes abdominal distension, abdominal pain, nausea, vomiting, and abdominal cramps. Gastrointestinal dysfunction is usually self-resolved[3]. However, when POI is prolonged, it leads to patient discomfort, dissatisfaction, prolonged hospital stays, and other postoperative complications, that impact on health care costs[4,5].

Therefore, to reduce the incidence of POI, several methods are used to prevent and restore intestinal function after surgery such as minimal invasive surgery[6], epidural anesthesia[7], multimodal analgesia[8], other pharmacological drugs, chewing gum[9], and coffee. Coffee is a popular beverage worldwide and has multiple systemic effects on the neuropsychiatric[10], cardiovascular[11], and gastrointestinal system[12]. For most adults, consumption of up to 400 mg of caffeine a day appears to be safe[13].

There have been several studies which have shown the beneficial effects of caffeinated coffee consumption on POI[14-17]. However, from previous studies, it is clear that many different doses of caffeine have been used. Some studies have used moderate amounts of caffeine up to high doses (100 mg to 100 g for consumption three times a day) which may present a risk of serious adverse effects when compared with normal daily coffee that has about 27 to 173 mg per drink. Therefore, we conducted this study to compare the effects of caffeine dose that has been used in normal daily life (instant coffee, 50 mg) on bowel function recovery to determine that the low dose caffeine coffee was effective and safer for improving intestinal function and reducing the incidence of POI after gynecological cancer surgery

Materials and Methods

This was a randomized double-blind controlled trial, conducted in the Gynecological Cancer Unit, Department of Obstetrics and Gynecology, UdonThani Hospital, UdonThani, Thailand, during January to July 2022. The study protocol was approved by UdonThani Hospital Ethical Committee on human research (number 90/2564).

The inclusion criteria were that the patients were aged between 18 and 75 years old who were diagnosed with cervical cancer, endometrial cancer, ovarian cancer, and other cancer (such as uterine sarcoma) and planned for surgical staging (abdominal hysterectomy, salpingo-oophorectomy, pelvic lymph node dissection, omentectomy, peritoneal washing, appendectomy, and other surgical procedure). The exclusion criteria were hypersensitivity or allergy to caffeine, thyrotoxicosis, cardiac arrhythmia, liver disease, chronic kidney disease, irritable bowel disease, chronic constipation (defined as defecation less than or equal to 2 time a week), history of previous bowel surgery, bowel anastomosis, a need for intensive care for more than 24 hours postoperatively, and a need for nasogastric tube drainage postoperative. The study details were explained to all patients and their written informed consent was obtained prior to the patients' participation in this study. The randomization was performed when the patients came to the gynecological oncology unit. Eligible patients were randomly assigned to one of two groups by investigator who consecutively opened sequentially numbered, opaque, sealed envelopes. The randomization was performed using computer

generated numbers with a blocked randomization protocol. Group 1 drank three cups of caffeinated coffee in hot water 100 mL (100 mg caffeine per cup) in one day, Group 2 drank three cups of caffeinated coffee in hot water 100 mL (50 mg caffeine per cup) in one day, beginning on the morning after surgery at 7:00, 12:00, 17:00 o'clock within 30 minutes under the supervision of a nurse or a doctor. The same coffee package in both groups was prepared using conventional coffee (Nescafe® red cup).

A standard protocol for pre-operation was implemented for all participants. All operations were performed by the gynecological oncologist's team. During the postoperative period, nonsteroidal analgesia and antiemetic agents were provided according to the patients' request protocol. Early ambulation was encouraged. Postoperative feeding regimen was standardized, liquid diet was begun on the first postoperative day in the morning and stepped up to a regular diet in 24 hours as the individual patients could tolerate.

The primary outcome measurement was the time to first pass of flatus after surgery. The secondary outcomes were the time to first defecation, time to normal bowel movement after surgery, time to tolerate solid diet, POI symptoms, side effects of postoperative coffee intake, additional antiemetics and analgesics used in postoperative care and the length of hospital stay. The time of first flatus and defecation were recorded based on the patient's own statements. The time to normal bowel movement after surgery was defined as the time to audible bowel sound 5 times in 2 minutes[18]. The time to tolerate solid diet was defined as the patients tolerance to intake solid food without nausea or vomiting within 4 hours after meal. POI symptoms and signs were evaluated 3 times daily. The hospital discharge criteria were stable vital signs for at least 24 hours, ability to ambulation, ability to tolerate a solid diet without nausea and vomiting, normal urination, and the absence of any complication after surgery. All data was collected by investigators who were blinded to the study allocation.

Sample size calculation

The number of samples was calculated based on a Güngördük et al' s study. The formula of non-inferior trial by N4studies application was used. The mean time of the first flatus was 33.28 hours in 100 mg caffeine group (control) with the standard deviation was 10.90. The mean difference was 8 hours with the margin of 2. Considering the power of 80% with and α level 0.01. The calculated sample size was 41 patients in each group. Assuming a 10% dropout rate, 92 patients were included.

Statistical analysis

Statistical analyses were performed using STATA statistical program version 13. Continuous data were reported as the mean and standard deviation. Categorical data were shown as the number and percentage. A t-test for comparison of continuous data, and Pearson chi-square test for categorical data. P values < 0.05 was considered statistically significant.

Results

In this study, 94 patients were enrolled; 47 patients were randomly assigned to the 50 mg caffeine coffee group and 47 patients were randomly assigned to the 100 mg caffeine coffee group. Two patients (one in each group) were excluded after surgery because of an intestinal injury during surgery. Ultimately, 46 patients in the 50 mg caffeine group and 46 patients in the 100 mg caffeine group were analyzed. The reason for exclusion is shown in Fig. 1.

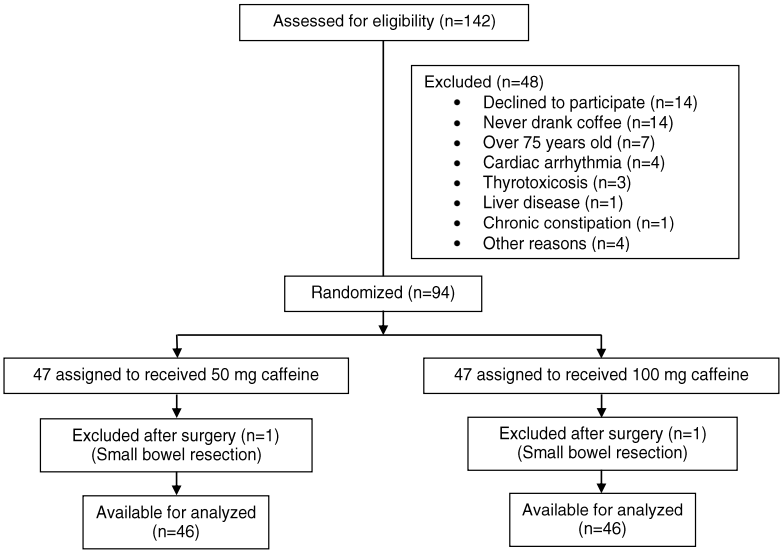

Fig. 1. Consort diagram

The demographic characteristics of the subject are presented in Table 1. In the 50 mg caffeine group, endometrial cancer (43.48%) was the most common indication for surgery than ovarian cancer (39.13%) and cervical cancer (15.22%), respectively. In the 100 mg caffeine group, the most common indication for surgery was ovarian cancer (56.52%) followed by endometrial cancer (32.61%) and cervical cancer (10.87%). There was no statistically significant difference in age, body mass index (BMI), gravida, history of alcohol drinking, and previous abdominal surgery. The surgical characteristics of both caffeine groups are shown in Table 2. The type of operative procedure, duration of operation, duration of anesthesia, estimated blood loss, and blood transfusion showed no significant differences.

Table 1. Baseline characteristics of the patients.

	Coffee 50 gm Group (n = 46)	Coffee 100 gm Group (n = 46)	p value
Age (year)	54.48 ± 9.21	51.35 ± 11.49	0.16
Body mass index (kg/m^2)	25.46 ± 4.97	24.56 ± 4.95	0.39
Gravida	1.61 ± 1.24	1.80 ± 1.45	0.49
Alcohol drinking	1 (2.17)	1 (2.17)	1.00
Hypertension	12 (26.09)	8 (17.39)	0.31
Diabetes mellitus	10 (21.74)	3 (6.52)	0.036
Other disease	21 (45.65)	9 (19.57)	0.008
Indication for surgery			
Endometrial cancer	20 (43.48)	15 (32.61)	0.28
Ovarian cancer	18 (39.13)	26 (56.52)	0.10
Cervical cancer	7 (15.22)	5 (10.87)	0.54
Other cancer	1 (2.17)	0 (0)	0.36
Previous abdominal surgery	23 (50.00)	23 (50.00)	1.00

Data are presented as mean ± standard deviation or number (%)

Table 2. Surgical characteristics of the patients.

	Coffee 50 gm Group (n = 46)	Coffee 100 gm Group (n = 46)	p value
Hysterectomy	43 (93.48)	40 (86.96)	0.29
Salpingectomy and/or oophorectomy	45 (97.83)	43 (93.48)	0.307
Pelvic LN dissection	19 (41.30)	16 (34.78)	0.52
Omentectomy	35 (76.09)	34 (73.91)	0.81
Appendectomy	1 (2.17)	4 (8.7)	0.17
Peritoneal washing	20 (43.48)	22 (47.83)	0.68
Lysis adhesion	36 (78.26)	39 (84.78)	0.42
Other procedure	5 (10.87)	4 (8.70)	0.73
Duration of operation (min)	94.24 ± 26.96	92.41 ± 29.92	0.76
Duration of anesthesia (min)	112.07 ± 28.78	108.70 ± 30.16	0.58
Blood transfusion	3 (6.52)	4 (8.7)	0.69
Estimated blood loss (mL)	95 ± 85.08	128.80 ± 171.14	0.23

Data are presented as mean ± standard deviation or number (%)
min: minutes, LN: lymph node

The primary outcomes and secondary outcomes of the study are shown in Table 3. The mean time to first flatus, the time to first defecation, and time to normal bowel sound in 100 mg caffeine group were all insignificantly shorter than in the 50 mg caffeine group (36.54 vs 38.39 hours, p = 0.53, 66.65 vs 67.08 hours, p = 0.92, 26.61 vs 29.41 hours, p = 0.16, respectively). The mean time to tolerate a solid diet and the length of hospital stay in the 50 mg caffeine group were not significantly shorter than in the 100 mg caffeine group. In the postoperative period, 73.91% of patients in the 100 mg caffeine group and 63.04% in the 50 mg caffeine group required additional analgesic drugs (p = 0.26), 23.91% in the 100 mg caffeine group and 15.22% in the 50 mg caffeine group required additional antiemetic drugs (p = 0.29).

Table 3. Study outcomes.

	Coffee 50 gm Group (n = 46)	Coffee 100 gm Group (n = 46)	p value
Mean time of first flatus (hours)	38.39 ± 14.41	36.54 ± 13.36	0.53
Mean time of first bowel movement (hours)	29.41 ± 11.70	26.61 ± 6.69	0.16
Mean time of first defecation (hours)	67.08 ± 20.18	66.65 ± 20.10	0.92
Additional analgesic	29 (63.04)	34 (73.91)	0.26
Additional antiemetic	7 (15.22)	11 (23.91)	0.29
Postoperative ileus	0 (0)	1 (2.17)	0.32
Side effect of caffeine			
Palpitation	1 (2.17)	2 (4.35)	0.56
Insomnia	1 (2.17)	7 (15.22)	0.03
Time to tolerate diet (days)	2.52 (0.51)	2.54 (0.50)	0.84
Length of hospital stay (days)	4.5 (1.22)	4.89 (1.66)	0.20

Data are presented as mean ± standard deviation or number (%)

In both caffeine consumption groups, 2 patients in the 100 mg caffeine group and 1 patient in the 50 mg caffeine group had palpitation (4.35% vs 2.17%, p = 0.56). Furthermore, 7 patients in the 100 mg caffeine group and 1 patient in the 50 mg caffeine group experienced insomnia (15.22% vs 2.17%, p = 0.03), this was statistically significant. Only one patient in the 100 mg caffeine group had postoperative ileus symptoms. However, no patients required re-admission or re-operation after hospital discharge.

Discussion

This study demonstrated that patients who drank 100 mg caffeine in the postoperative period after abdominal gynecological surgery experienced a shorter; mean time to first flatus, mean time to first defecation and time to normal bowel sound than patients who drank 50 mg caffeinate after surgery, but this was not significantly different. However, in the 50 mg caffeinated coffee group the time to tolerate solid food and length of hospital stay were insignificantly shortened. Furthermore, the need for additional analgesia and/or antiemetics was lower in the 50 mg caffeine group and adverse effects after coffee consumption during the postoperative period were significantly more common in the 100 mg caffeine group than in the 50 mg caffeine group. In this present study, only one patient in the 100 mg caffeine group had POI symptoms.

From the literature review, several studies found that coffee consumption could encourage bowel function recovery and may reduce POI[14-17]. A systematic review and meta-analysis by Gkegkes et al[14] reviewed about the effect of caffeinated coffee intake on postoperative ileus, four randomized studies were enrolled to this meta-analysis (3 studies referred to colorectal procedure and 1 study referred to gynecological procedure). The postoperative administration of caffeinated coffee significantly reduced the time to first bowel movement, the time to first flatus, the time to defecation and time to tolerance of a solid diet. Güngördük et al[16] randomly assigned 114 patients to receive either 100 g caffeinate three times a day or no treatment after abdominal gynecological cancer surgery. The coffee drinkers experienced significantly shorter mean time to flatus, mean time to defecation and mean time to ability to tolerate solid food. Likewise, Koseoglu et al[17] studied patients that had undergone elective cesarean section, randomly assigned 108 patients to receive either 100 mg caffeinate three times a day and no intervention after cesarean section. The mean time to passage of first flatus, the time to defecation and time to tolerate a solid diet were significantly shorter in the coffee group.

This randomized trial tried to demonstrate that 50 mg caffeinated, which is a normal commercial coffee dose, had the similar effect with the 100 mg caffeinate, which is usually in the fresh coffee, in the postoperative period after abdominal surgery. Thus, we suggested that patients diagnosed with gynecological cancer who undergo abdominal surgery should drink coffee with 50 mg of caffeine during the postoperative period, which was effective in restoring intestinal function after surgery. This was not significantly different from drinking coffee with a large amount of caffeine and significantly fewer adverse effects with lower cost.

The strength of this study was a prospective randomized trial that the investigators and patients were blinded. The participants were randomly assigned by computer generated numbers for allocation of two caffeinated coffee groups. Moreover, the two groups had similar demographic and surgical profiles. The limitations of this study were that (1) it did not have a placebo group, (2) the time of flatus and defecation were retrieved from patients and might, in some cases, be inaccurate and (3) many data collectors were employed (doctors or a nurse), which can cause discrepancies in the evaluation such as bowel sound examination. (4) Also, we avoided disturbing the postoperative patients at the night or during sleeping time, therefore some examinations were postponed. (5) Normally, surgical time in oncologic surgery was not less than 150-180 min and blood loss was not less than 500 cc. However, this study was conducted in a single center with only two highly experienced gynecological oncologists, the short duration of operation and less amount of intraoperative blood loss could affect the postoperative bowel function recovery.

Conclusion

Drinking coffee with 50 mg of caffeine after abdominal gynecological cancer surgery could

accelerate bowel function recovery and had statistically significant fewer adverse effects, that could be used as an additional treatment in postoperative care.

Acknowledgments

We gratefully acknowledge gynecological oncologists, gynecological cancer clinic UdonThani Hospital team, the investigators and patients who participated in this trial.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Bederman SS, Betsy M, Winiarsky R, Seldes RM, Sharrock NE, Sculco TP. Postoperative ileus in the lower extremity arthroplasty patient. J Arthroplasty 2001;16:1066-70.
2. Fujita K, Nagano T, Suzuki A, Sakakibara A, Takahashi S, Hirano T, et al. Incidence of postoperative ileus after paraaortic lymph node dissection in patients with malignant gynecologic tumors. Int J Clin Oncol 2005;10:187-90.
3. Miedema BW, Johnson JO. Methods for decreasing postoperative gut dysmotility. Lancet Oncol 2003;4:365-72.
4. Iyer S, Saunders WB, Stemkowski S. Economic burden of postoperative ileus associated with colectomy in the United States. J Manag Care Pharm 2009;15:485-94.
5. Tevis SE, Carchman EH, Foley EF, Harms BA, Heise CP, Kennedy GD. Postoperative ileus—more than just prolonged length of stay? J Gastrointest Surg 2015;19:1684-90.
6. Schwenk W, Haase O, Neudecker JJ, Müller JM. Short term benefits for laparoscopic colorectal resection. Cochrane Database Syst Rev 2005;2005:CD003145.
7. Guay J, Nishimori M, Kopp S. Epidural local anaesthetics versus opioid-based analgesic regimens for postoperative gastrointestinal paralysis, vomiting and pain after abdominal surgery. Cochrane Database Syst Rev 2016;7:CD001893.
8. Helander EM, Webb MP, Bias M, Whang EE, Kaye AD, Urman RD. A comparison of multimodal analgesic approaches in institutional enhanced recovery after surgery protocols for colorectal surgery: pharmacological agents. J Laparoendosc Adv Surg Tech A 2017;27:903-8.
9. Short V, Herbert G, Perry R, Atkinson C, Ness AR, Penfold C, et al. Chewing gum for postoperative recovery of gastrointestinal function. Cochrane Database Syst Rev 2015;2015: CD006506.
10. Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev 1999;51:83-133.
11. Myers MG. Caffeine and cardiac arrhythmias. Ann Intern Med 1991;114:147-50.
12. Murakami K, Okubo H, Sasaki S. Dietary intake in relation to self-reported constipation among Japanese women aged 18–20 years. Eur J Clin Nutr 2006;60: 650-7.
13. Wikoff D, Welsh BT, Henderson R, Brorby GP, Britt J, Myers E, et al. Systematic review of the potential adverse effects of caffeine consumption in healthy adults, pregnant women, adolescents, and children. Food Chem Toxicol 2017;109:585-648.
14. Gkegkes ID, Minis EE, Iavazzo C. Effect of caffeine intake on postoperative ileus: a systematic review and meta-analysis. Dig Surg 2020;37:22-31.
15. Kuo S-C, Mu P-F, Chang L-Y, Chou S-S, Lee M-Y, Su J-Y, et al. Effectiveness of coffee for postoperative ileus in patients following abdominal surgery: a systematic review protocol. JBI Database System Rev Implement Rep 2018;16:2072-9.
16. Güngördük K, Özdemir İA, Güngördük Ö, Gülseren V, Gokçü M, Sancı M. Effects of coffee consumption on gut recovery after surgery of gynecological cancer patients: a randomized controlled trial. Am J Obstet Gynecol 2017;216:145.e1-145.e7.
17. Koseoglu SB, Toker MK, Gokbel I, Celikkol O, Gungorduk K. Can coffee consumption be used to accelerate the recovery of bowel function after cesarean section? Randomized prospective trial. Ginekol Pol 2020;91:85-90.
18. Mealie CA, Ali R, Manthey DE. Abdominal Exam. In: StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2022, StatPearls Publishing LLC.; 2022.

Thai Journal of Obstetrics and Gynaecology
July 2023, Vol. 31, No. 4, pp. 273-284.

OBSTETRICS

Prevalence and Factors Associated with Long-acting Reversible Contraception Initiation in Non-teenage Postpartum Thai Women Attending Siriraj Hospital

Chanakarn Meerod, M.D.*,
Nalinee Panichyawat, M.D.*,
Korakot Sirimai, M.D.*,
Thanyarat Wongwananuruk, M.D.*,
Nichamon Parkpinyo, M.D.*

** Family Planning and Reproductive Health Unit, Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*

ABSTRACT

Objectives: To determine the prevalence of and factors significantly associated with long-acting reversible contraception (LARC) initiation among non-teenage postpartum women attending Siriraj Hospital.

Materials and Methods: This prospective cross-sectional study was conducted at Family Planning and Reproductive Health Unit during June 2021 to January 2022. Thai women aged 20-45 years who requested postpartum contraception within 8 weeks after delivery were eligible for study enrolment.

Results: Three hundred and seven postpartum women were included, but 3 women were excluded from analysis due to incomplete data. The mean age was 29.1 ± 5.6 years. Pregnancy complications were reported in 101 women (33.2%). The prevalence of postpartum LARC initiation was 27.6% (n = 84). The selected LARC method were, as follows: two-rod implant (11.8%), one-rod implant (10.9%), and intrauterine device (4.9%). Presence of pregnancy complications (adjusted odds ratio [aOR] 3.2, 95% confidence interval [CI] 1.21-8.44; p = 0.019) and interest in LARC (aOR 146.60, 95%CI 44.96-478.00; p < 0.001) were the factors independently associated with postpartum LARC initiation. Concern about the insertion procedure and complications or side effects related to LARC were cited as reasons for not requesting LARC initiation.

Conclusion: The prevalence of postpartum LARC initiation among non-teenage Thai women at our centre was higher than the national prevalence of LARC initiation in Thailand. Presence of pregnancy complications and interest in LARC were identified as independent predictors of postpartum LARC use. The barriers to LARC initiation should be evaluated and managed to increase the rate of LARC utilization among interested women, but who harbour concerns about LARC.

Keywords: intrauterine device, IUD, LARC, long-acting reversible contraceptive, postpartum, prevalence, subdermal implant contraceptive.

Correspondence to: *Nalinee Panichyawat, M.D., Family Planning and Reproductive Health Unit, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wanglang Road, Bangkoknoi, Bangkok 10700, Thailand. E-mail: Nalinee.pan@mahidol.ac.th*

Received: 6 September 2022, **Revised:** 23 December 2022, **Accepted:** 9 January 2023

ความชุกและปัจจัยที่มีความสัมพันธ์ต่อการเลือกใช้วิธีคุมกำเนิดชั่วคราวที่ออกฤทธิ์นานของสตรีที่ไม่ใช่วัยรุ่นหลังคลอดบุตรที่โรงพยาบาลศิริราช

ชนกานต์ มีรอด, นลินี พาณิชยวัฏ, กรกฎ ศิริมัย, ธันยารัตน์ วงศ์วนานุรักษ์, ณิชมน ภาคย์ภิญโญ

บทคัดย่อ

วัตถุประสงค์*: เพื่อศึกษาความชุกและปัจจัยที่มีความสัมพันธ์ต่อการเลือกใช้วิธีคุมกำเนิดชั่วคราวที่ออกฤทธิ์นานในสตรีที่ไม่ใช่วัยรุ่นในระยะหลังคลอดที่โรงพยาบาลศิริราช*

วัสดุและวิธีการ*: การศึกษาแบบ cross sectional study ศึกษาในสตรีไทยอายุ 20-45 ปีที่มารับบริการการคุมกำเนิดที่หน่วยวางแผนครอบครัวและอนามัยการเจริญพันธุ์ ในระยะเวลา 8 สัปดาห์หลังคลอด ตั้งแต่เดือนมิถุนายน พ.ศ. 2564 ถึง มกราคม พ.ศ. 2565*

ผลการศึกษา*: มีผู้เข้าร่วมการศึกษาทั้งหมด 307 คน คัดออกจากการวิเคราะห์ข้อมูล 3 คน เนื่องจากข้อมูลไม่สมบูรณ์ อายุเฉลี่ยของผู้เข้าร่วมวิจัยอยู่ในช่วง 29.1±5.6 ปี และผู้เข้าร่วมวิจัย 101 คน (ร้อยละ 33.2) มีภาวะแทรกซ้อนของการตั้งครรภ์ ร้อยละ 27.6 ของผู้เข้าร่วมวิจัย (84 คน) ใช้วิธีคุมกำเนิดชั่วคราวที่ออกฤทธิ์นาน โดยมีผู้เลือกใช้ยาฝังคุมกำเนิดชนิด 2 หลอด ร้อยละ 11.8 ยาฝังคุมกำเนิดชนิด 1 หลอดร้อยละ 10.9 และห่วงคุมกำเนิดร้อยละ 4.9 การมีภาวะแทรกซ้อนของการตั้งครรภ์และมีความสนใจในการใช้วิธีคุมกำเนิดชั่วคราวที่ออกฤทธิ์นานเป็นปัจจัยที่ส่งผลต่อการเลือกใช้วิธีคุมกำเนิดชั่วคราวที่ออกฤทธิ์นานในสตรีที่ไม่ใช่วัยรุ่นในระยะหลังคลอด เหตุผลของผู้เข้าร่วมวิจัยที่ไม่เลือกใช้วิธีคุมกำเนิดชั่วคราวที่ออกฤทธิ์นานเนื่องจากมีความกังวลเกี่ยวกับกระบวนการฝังยาหรือใส่ห่วงคุมกำเนิด และมีความกังวลเกี่ยวกับภาวะแทรกซ้อนหรือผลข้างเคียงจากการใช้วิธีคุมกำเนิดชั่วคราวที่ออกฤทธิ์นาน*

สรุป*: การเลือกใช้วิธีคุมกำเนิดชั่วคราวที่ออกฤทธิ์นานของสตรีที่ไม่ใช่วัยรุ่นหลังคลอดที่โรงพยาบาลศิริราชสูงกว่าข้อมูลของประเทศไทย การมีภาวะแทรกซ้อนของการตั้งครรภ์และความสนใจในการใช้วิธีคุมกำเนิดชั่วคราวที่ออกฤทธิ์นานเป็นปัจจัยที่มีผลต่อการเลือกใช้วิธีคุมกำเนิดชั่วคราวที่ออกฤทธิ์นานอย่างมีนัยสำคัญทางสถิติ อุปสรรคที่มีผลต่อการใช้วิธีคุมกำเนิดชั่วคราวที่ออกฤทธิ์นานควรได้รับการประเมินและแก้ไขเพื่อที่จะช่วยเพิ่มอัตราการใช้ในสตรีที่สนใจใช้วิธีคุมกำเนิดวิธีนี้*

คำสำคัญ*: อุปกรณ์ช่วยพยุงมดลูก, ห่วงอนามัย, LARC, ยาคุมกำเนิดแบบย้อนกลับที่ออกฤทธิ์นาน, หลังคลอด, ความชุก, ยาคุมกำเนิดชนิดฝังใต้ผิวหนัง*

Introduction

Birth spacing or interpregnancy interval (IPI) is a potentially modifiable risk factor that is related to adverse pregnancy outcome[1]. Inappropriate IPI has negative effects on maternal health and increases the risk of adverse maternal, perinatal and infant outcomes[2-5]. The American College of Obstetricians and Gynaecologists (ACOG) recommends that women should be advised to avoid an IPI of less than 6 months, and should be counselled about the risks and benefits of repeat pregnancy sooner than 18 months[1]. To decrease the risk of adverse pregnancy outcomes, the World Health Organization (WHO) recommends at least a 24-month interval following a live birth prior to attempting conception for the next pregnancy[2].

According to the ACOG and WHO, long-acting reversible contraception (LARC) is defined as subdermal contraceptive implant, copper-containing intrauterine device (IUD) and levonorgestrel-releasing IUD[6,7]. LARC is considered to be a highly effective contraceptive method that has few contraindications, is user independent, and should be offered routinely as a contraceptive option for most women[6,8]. Family planning counselling and access to postpartum contraception are the effective strategies for optimizing IPI and preventing unintended pregnancy[1]. Despite the effort to improve contraceptive services, the rate of subdermal contraceptive implant and IUD use in Thailand in 2019 was reported to be only 1.6% and 0.4%, respectively[9].

Previous studies reported the prevalence of postpartum LARC use to vary widely from 5%-40.4% according to the study context[10-16]. The aforementioned studies differed in study design, participant characteristics (teenage and/or non-teenage women), timing of postpartum duration (immediate/delayed postpartum), and healthcare systems. Several factors associated with LARC utilization were identified, but those results also varied among studies. The reported associating factors included age, parity, occupation, education, previous LARC use, intention to use LARC, and type of medical insurance or health care coverage[10-17].

National healthcare policy in Thailand has recently changed from fully covering to not covering the cost of LARC in non-teenage women. Moreover, data specific to the prevalence of and factors associated with postpartum LARC initiation in non-teenage women at our centre, which is the university-based hospital in Thailand, are currently scarce. Accordingly, the aim of this study was to investigate the prevalence of and the factors significantly associated with LARC initiation among non-teenage postpartum Thai women attending Siriraj Hospital - Thailand's national tertiary referral centre. The information and data derived from this study will enhance our understanding of current use, behaviours, perceptions, and opinions relative to LARC, and this information will be used to facilitate improved use of and understanding about LARC among interested non-teenage postpartum women.

Materials and Methods

This cross-sectional study was conducted during June 2021 to January 2022 at the Family Planning and Reproductive Health Unit of the Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. The protocol for this study was approved by the Siriraj Institutional Review Board (COA No. Si. 186/2021) and complied with the principles set forth in the 1964 Declaration of Helsinki and all of it subsequent amendments. Written informed consent was obtained from all included participants in the 6-8 weeks postpartum visit at the unit.

Thai women aged 20-45 years who requested postpartum contraception within 8 weeks after delivery were eligible for inclusion. Individuals

having one or more of the following were excluded: postpartum tubal sterilization or male partner with vasectomy, contraindication for IUD or implant contraception according to the medical eligibility criteria for contraceptive use published by the WHO[18], and/or neonatal death or stillbirth. In this study, LARC was defined as subdermal contraceptive implant or IUD.

According to our centre's standing postpartum follow-up protocol, postpartum women are scheduled to visit our unit 6-8 weeks after delivery for postpartum evaluation and family planning counselling. After both agreeing to participate and providing written inform consent, participants were interviewed to collect baseline information, obstetrics history, previous contraceptive use and interested contraceptive method. All study women received comprehensive counselling by a well-trained medical provider, such as obstetrics and gynaecological residents, registered nurses, or specialized counsellors in family planning - all under supervision of specialist medical staff. Counselling was provided following the standard practice and care provided by our unit, and focused on family planning, contraceptive methods, and the benefits and drawbacks of each method. After participants chose the contraceptive method, they were then interviewed to collect information regarding their reason for choosing that contraception method. Delivery outcomes were extracted from medical and labour records. All women who chosen LARC method received LARC insertion on the postpartum visit day.

Statistical analysis

Data analyses were performed using PASW Statistics version 23.0 for Windows (SPSS, Inc., Chicago, IL, USA). Patient demographic and clinical data were summarized using descriptive statistics. Chi square test and Student's t-test was used to compare categorical data and normally distributed continuous data between groups, respectively. Data were presented as number (n) and percentage (%) for categorical data, and as mean ± standard deviation (SD) for normally distributed continuous data. Univariable analysis was carried out to identify variables potentially associated with the LARC use. Variables with a p value of less than 0.2 from univariate analysis were included in multiple logistic regression analysis to identify factors independently associated with LARC initiation. A p value less than 0.05 was considered to be statistically significant.

Results

Three hundred and seven postpartum women were initially enrolled in this study. Of those, 3 women were excluded from the analysis due to having incomplete data. The baseline demographic and clinical characteristics of 304 women are shown in Table 1. The mean age was 29.1 ± 5.6 years (range: 19 - 44), and the mean body mass index (BMI) was 25.5 ± 4.9 kg/m^2 (range: 15.0 - 46.4). Pregnancy complications, such as pregnancy-induced hypertension, gestational diabetes, etc, occurred in 101 (33.2%) women. Of the 304 participants, 263 women reported prior use of any contraceptive method before the index pregnancy, including combined oral contraceptive (COC) (n = 142, 54%), condom (n = 74, 28.1%), depot medroxyprogesterone acetate (DMPA) (n = 56, 21.3%), contraceptive implant (n = 15, 5.7%) and IUD (n = 2, 0.8%). Approximately 69.4% of women reported an intention of > 2 years of IPI, and 32.6% of participants reported having an interest in LARC use.

Table 1. Patient baseline demographic and clinical characteristics (n = 304).

Characteristics	mean ± SD or n (%)
Age (years)	29.1 ± 5.6
20 - 30	181 (59.5)
31 - 40	120 (39.5)
≥ 41	3 (1.0)
Body mass index (kg/m^2)	25.5 ± 4.9
< 18.5	13 (4.3)
18.5 - 24.9	142 (46.7)
25 - 29.9	99 (32.6)
≥ 30	50 (16.4)
Education	
Primary School	12 (3.9)
High school	118 (38.8)
Bachelor's degree or higher	174 (57.3)
Marital status	
Married	292 (96.0)
Single	9 (3.0)
Divorced	3 (1.0)
Occupation	
Housewife/unemployed	72 (23.7)
Student	5 (1.6)
Employee	165 (54.3)
Government officer	21 (6.9)
Private business owner	41 (13.5)
Income (Thai baht/month)	
< 10,000	28 (9.2)
10,001 - 50,000	228 (75.0)
> 50,000	48 (15.8)
Adequacy of income	
Adequate	245 (80.6)
Inadequate	59 (19.4)
Parity	
1	172 (56.6)
2	93 (30.6)
≥ 3	39 (12.8)
Gestational age at delivery (weeks)	
< 37	31 (10.2)
≥ 37	273 (89.8)
Route of delivery	
Cesarean section	89 (29.3)
Vaginal delivery	215 (70.7)
First ANC	
1st trimester	227 (74.7)
After 1st trimester	77 (25.3)

Table 1. Patient baseline demographic and clinical characteristics (n = 304). (Cont.)

Characteristics	mean ± SD or n (%)
Presence of pregnancy complications**	101 (33.2)
gestational diabetes	35 (11.5)
pregnancy-induced hypertension	47 (15.5)
fetal growth restriction	22 (7.2)
Others*	10 (3.3)
Any prior contraceptive use before index pregnancy***	263 (86.5)
combined oral contraceptive	142 (46.7)
condom	74 (24.3)
depot medroxyprogesterone acetate	56 (18.4)
contraceptive implant	15 (4.9)
IUD	2 (0.7)
Intended interpregnancy interval (years)	
≤ 2	93 (30.6)
> 2	211 (69.4)
Interested in LARC	99 (32.6)
Currently breast feeding	297 (97.7)

SD: standard deviation, ANC: antenatal care, LARC: long-acting reversible contraception
* Others: postpartum haemorrhage, placenta previa, abruptio placenta
**presence of more than one pregnancy complication in some women
*** prior contraceptive use of more than one method in some women

The distribution of postpartum contraception initiation is shown in Fig. 1. The prevalence of postpartum LARC initiation was 27.6% (n = 84). The selected LARC methods included two-rod implant (11.8%), one-rod implant (10.9%), and copper-containing IUD (4.9%). The factors found to be significantly associated with postpartum LARC initiation are shown in Table 2. Multivariate analysis revealed presence of pregnancy complications (adjusted odds ratio [aOR] 3.2, 95% confidence interval [CI] 1.21 - 8.44; p = 0.019) and interest in LARC (aOR 146.60, 95%CI 44.96 - 478.00; p < 0.001) to be factors independently associated with postpartum LARC initiation (Table 3).

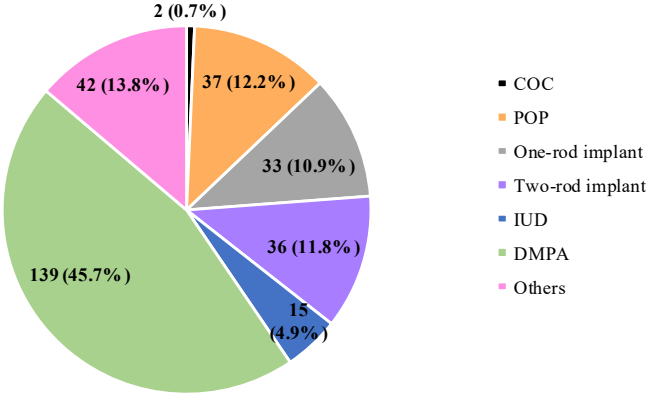

Fig. 1. The distribution of postpartum contraception initiation (n = 304).
(COC: combined oral contraceptive, POP: progestin-only pill, IUD: intrauterine device, DMPA: depot medroxyprogesterone acetate). Others: condom, withdrawal method, no method use

Table 2. Patient baseline demographic and clinical characteristics compared between those choosing for and against LARC initiation (n = 304).

Characteristics	LARC (n = 84) mean ± SD or n (%)	Non-LARC (n = 220) mean ± SD or n (%)	p value
Age (years)	27.3 ± 5.5	29.8 ± 5.2	0.001
20 - 30	60 (71.4)	121 (55.0)	0.029
31 - 40	23 (27.4)	97 (44.1)	
≥ 41	1 (1.2)	2 (0.9)	
Body mass index (kg/m^2)	26.01 ± 5.25	25.25 ± 4.69	0.219
< 18.5	2 (2.4)	11 (5.0)	0.541
18.5 - 24.9	36 (42.9)	106 (48.2)	
25 - 29.9	31 (36.9)	68 (30.9)	
≥ 30	15 (17.9)	35 (15.9)	
Education			0.296
Primary School	2 (2.4)	10 (4.5)	
High school	38 (45.2)	80 (36.4)	
Bachelor's degree or higher	44 (52.4)	130 (59.1)	
Marital status			0.159
Married	78 (92.9)	214 (97.3)	
Single	4 (4.8)	5 (2.3)	
Divorced	2 (2.4)	1 (0.5)	
Occupation			0.072
Housewife/unemployed	23 (27.4)	49 (22.3)	
Student	4 (4.8)	1 (0.5)	
Employee	42 (50.0)	123 (55.9)	
Government officer	6 (7.1)	15 (6.8)	
Private business owner	9 (10.7)	32 (14.5)	
Income (Thai baht/month)			0.021
< 10,000	14 (16.7)	14 (6.4)	
10,001 - 50,000	58 (69)	170 (77.3)	
> 50,000	12 (14.3)	36 (16.4)	
Adequacy of income			0.128
Adequate	63 (75)	182 (82.7)	
Inadequate	21 (25)	38 (17.3)	
Parity			0.057
1	43 (51.2)	129 (58.6)	
2	24 (28.6)	69 (31.4)	
≥ 3	17 (20.2)	22 (10)	
Gestational age at delivery (weeks)			0.543
< 37	10 (11.9)	21 (9.5)	
≥ 37	74 (88.1)	199 (90.5)	
Route of delivery			0.337
Cesarean section	28 (33.3)	61 (27.7)	
Vaginal delivery	56 (66.7)	159 (72.3)	
Presence of pregnancy complications			0.166
No	51 (60.7)	152 (69.1)	
Yes	33 (39.3)	68 (30.9)	
First ANC			0.164
1st trimester	58 (69.0)	169 (76.8)	
after 1st trimester	26 (31.0)	51 (23.2)	

Table 2. Patient baseline demographic and clinical characteristics compared between those choosing for and against LARC initiation (n = 304). (Cont.)

Characteristics	LARC (n = 84) mean ± SD or n (%)	Non-LARC (n = 220) mean ± SD or n (%)	p value
Any prior contraceptive use before index pregnancy			0.104
No	7 (8.3)	34 (15.5)	
Yes	77 (91.7)	186 (84.5)	
Prior LARC use before index pregnancy			0.003
No	74 (88.1)	213 (96.8)	
Yes	10 (11.9)	7 (3.2)	
Intended interpregnancy interval (years)			< 0.001
≤ 2	6 (7.1)	87 (39.5)	
> 2	78 (92.9)	133 (60.5)	
Interested in LARC			< 0.001
No	7 (8.3)	198 (90)	
Yes	77 (91.7)	22 (10)	
Currently breast feeding			0.077
No	4 (4.8)	3 (1.4)	
Yes	80 (95.2)	217 (98.6)	

LARC: long-acting reversible contraception, SD: standard deviation, ANC: antenatal care

Table 3. Univariate and multivariate analysis for factors independently associated with postpartum LARC initiation.

	Crude OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Age ≥ 30 years	0.51 (0.30-0.86)	0.011	1.70 (0.63-4.60)	0.299
Married	0.36 (0.11-1.16)	0.077	0.46 (0.06-3.45)	0.446
Employee, government officer or business owner	0.62 (0.36-1.08)	0.091	0.62 (0.22-1.74)	0.362
Income > 10,000 THB/month	0.34 0.15-0.75)	0.005	0.24 (0.05-1.13)	0.072
Inadequate income	1.60 (0.87-2.92)	0.128	0.66 (0.22-1.99)	0.462
Parity > 2	2.28 (1.14-4.56)	0.017	0.95 (0.30-3.15)	0.939
First ANC after 1st trimester	1.49 (0.85-2.60)	0.164	1.73 (0.63-4.75)	0.286
Presence of pregnancy complications	1.45 (0.86-2.44)	0.166	3.20 (1.21-8.44)	0.019
Any prior contraceptive use before index pregnancy	2.01 (0.85-4.73)	0.104	1.13 (0.27-4.71)	0.866
Prior LARC use before index pregnancy	4.11 (1.51-1.19)	0.003	1.40 (0.30-6.47)	0.666
Currently breastfeeding	0.28 (0.06-1.26)	0.077	2.23 (0.21-24.00)	0.510
Intended interpregnancy interval > 2 years	8.50 (3.55-20.36)	< 0.001	1.10 (0.98-4.07)	0.885
Interested in LARC	99.0 (40.64-241.16)	< 0.001	146.60 (44.96-478.00)	< 0.001

LARC: long-acting reversible contraception, OR: odds ratio, CI: confidence interval, THB: Thai baht, ANC: antenatal care

The reasons given for and against postpartum LARC initiation are listed in Table 4. Convenience, no effect on breastfeeding and good contraceptive efficacy were the most common reasons given among those who chose contraceptive implant, whereas no desire to have more children and health care suggestion were the most common reasons given among those who chose IUD. Concern about complications or side effects related to LARC, such as irregular bleeding, weight gain, and IUD displacement, and the insertion procedure were given as reasons for deciding against LARC use.

There were 22 women who initially expressed interested in LARC, but subsequently decided against LARC. The most commonly given reasons for ultimately deciding against LARC were financial

problem (36.4%), concern about the insertion procedure (31.8%) and concern about complication or side effect of LARC (27.3%) such as irregular bleeding and IUD displacement.

Table 4. Reasons given for and against postpartum LARC initiation.

Reasons for contraceptive implant use (n = 69)	n (%)
No desire to have more children	25 (36.2)
Desire for ≥ 2-year interpregnancy interval	30 (43.5)
Good contraceptive efficacy	43 (62.3)
Long contraceptive duration	42 (60.9)
Easy to use/convenient	55 (79.7)
No effect on breastfeeding	46 (66.7)
Healthcare suggestion	17 (24.6)
Few complications/side effects	8 (11.6)
Health insurance coverage	9 (13.0)
Other	2 (2.9)
Reasons for intrauterine device use (n = 15)	
No desire to have more children	9 (60)
Desire for ≥ 2-year interpregnancy interval	2 (13.3)
Good contraceptive efficacy	5 (33.3)
Long contraceptive duration	6 (40)
Easy to use/convenient	8 (53.3)
No effect on breastfeeding	6 (40)
Healthcare suggestion	9 (60)
Non-hormonal method	6 (40)
Few complications/side effects	4 (26.7)
Health insurance coverage	3 (20)
Reason for non-LARC use (n = 220)	
Unacceptable side effect(s) from previous LARC use	19 (8.6)
Desire for < 2-year interpregnancy interval	70 (31.8)
Concern about complication or side effect of LARC	190 (86.4)
Concern about LARC insertion procedure	107 (48.6)
No health insurance coverage	24 (10.9)

LARC: long-acting reversible contraception

Discussion

Family planning counselling and postpartum contraception are essential components of postpartum care that help to prevent unintended pregnancy and to facilitate appropriate birth spacing. LARC is a highly effective reversible method of contraception that yields several important benefits. However, the utilization rate of LARC in Thailand was reported to be low[9]. The present study was conducted in a tertiary university-based hospital setting that provides family planning counselling and contraception services that are provided by professional healthcare team. The prevalence of LARC initiation in non-teenage women within 8 weeks after their delivery was 27.6%, 22.7% and 4.9% chose contraceptive implant and copper-containing IUD, respectively. We found presence of pregnancy complications and interest in LARC use to be independent predictors of postpartum LARC initiation.

The reported prevalence of postpartum LARC use varied among studies. Our study found a 27.6% prevalence of postpartum LARC initiation. Previous studies conducted in Thailand reported a prevalence of LARC use in postpartum women that ranged from 0.8% - 15.8%[10,11,17,19], while other studies that were conducted in different countries/regions described a

prevalence of postpartum LARC initiation of 9.9% - 36.5%[12-16]. Possible reasons that may explain the difference in prevalence between our study and others included differences in the study design and setting such as hospital-based versus community-based setting; different healthcare systems; diverse socioeconomic status and patient characteristic included in the studies, such as nulliparous or primiparous women[11,17] or adolescent women[10,13-17], and differences in the postpartum period duration[10,13-16].

In this study, most of the women who selected LARC preferred contraceptive implant over IUD, which was similarly reported in previous studies[10-12,14,16] and the Thai national data[9]. However, and in contrast, some studies reported a predominance of IUD use compared to implant[13,15]. These differences between and among studies could be due to difference in duration between methods; concern about the insertion procedure; concern about side effect, such as IUD displacement and bleeding irregularity; differences in beliefs and culture; and differences in national contraceptive use policy.

Identifying the factors that significantly associated with a decision to accept and use LARC is one of the important steps toward optimizing individual contraception use. In the present study, there were two factors that were identified as being independently associated with LARC initiation - presence of pregnancy complications and patient interest in LARC. Women with pregnancy complication were about three times more likely to use LARC compared with their counterparts. The possible reason may be increased awareness of and concern about their health. We also found patient interest in LARC to be an independent predictor of postpartum LARC initiation. This factor was also significantly related to postpartum LARC use in other study[10]. Previous studies identified other factors as being independently associated with postpartum LARC initiation, younger age[13,14,17], being married[14], multiparous women[13,15], lower education level[17], occupation[10,11,16], vaginal delivery in the index pregnancy[15], previous LARC use[12], receiving family planning counselling[12,14,16] and insurance or healthcare coverage[10,13]. This variation in significant factors among studies may be due to differences in study design, setting and patient characteristics. Moreover, some of these previous studies defined DMPA as LARC, which could adversely influence the process of analysing for factors related to LARC use[10,11,17].

The reasons among our cohort for deciding to use contraceptive implant included convenience, not adversely effect on breastfeeding, good contraceptive efficacy, and the reasons for individual who decided to use IUD were complete childbearing and health care suggestion. These mentioned reasons were consistent with previous studies[10,11]. Interestingly, the study women who refused to use LARC or who changed their mind after demonstrating initial interest in LARC expressed concern about complications or side effects related to LARC, the insertion procedure or financial problem. Previous studies reported reason for denying LARC despite showing initial interest that included not currently having sexual intercourse[10] and fear of side effects[12]. Cost was also mentioned as a barrier to using LARC in previous studies[10,20-22].

Since LARC is the most effective reversible contraceptive method, delivery of effective contraceptive counselling and education about LARC should be incorporated into the antenatal and postpartum counselling program to draw attention to and increase interest in LARC. Emphasizing the advantages of LARC and anticipatory counselling about the safety of the insertion procedure and side effects of LARC (mostly minor side effects) could promote both contraceptive implant and IUD use. Future study of effective interventions for promoting LARC acceptance and to assess patient knowledge and attitude towards LARC is warranted. The cost of LARC also appears to be a barrier that limits its use. Individuals should have access to all contraceptive methods, including LARC, when needed with no cost-related barrier that prevents such access. National healthcare policy should be amended to cover these costs for all women, including non-teenagers, in order

to enhance LARC uptake, which will help to reduce unplanned pregnancy and to optimize IPI.

The aim of this study was to investigate postpartum LARC use and associated factors among non-teenage Thai women. We excluded adolescents because they have different characteristics and needs that require special attention to prevent unplanned pregnancy. These differences between teenage and non-teenage women would, therefore, require different counselling processes to educate postpartum women about the important benefits of postpartum contraception. In addition, we did not include DMPA as LARC. Therefore, prevalence, factors, and the reasons related to postpartum LARC initiation in this study are specific to subdermal contraceptive implant use or IUD use, which are the two contraceptive methods defined as LARC by the WHO and ACOG. Compared to DMPA, contraceptive implant and IUD have different properties that confer a longer duration of action, have a higher cost and require an insertion procedure. Additionally, healthcare policy specific to LARC in Thailand has been changed from free-of-charge to all age groups to free-of-charge for only adolescents since November 2021. This factor may have influenced the prevalence of factors associated with, and the reasons for and against postpartum LARC uptake in our study.

This study has some mentionable limitations. First, this study was a cross-sectional hospital-based study that was conducted in a tertiary university hospital setting, so our results may not be generalizable to other care settings. Second, we did not evaluate our patients' attitude toward LARC, their knowledge about LARC, or their partners' sociodemographic characteristics - all of which could influence the decision to use or not use LARC. Furthermore, this study was conducted during the COVID-19 pandemic, which may have to some degree prevented our patients from receiving appropriate contraceptive services; however, our unit focuses specific attention providing comprehensive contraceptive service to postpartum women before their discharge from the hospital.

Conclusion

The prevalence of postpartum LARC initiation among non-teenage Thai women at our centre was higher than the national prevalence of LARC initiation in Thailand. Presence of pregnancy complications and interest in LARC were identified as independent predictors of postpartum LARC use. The barriers to LARC initiation should be evaluated and managed to increase the rate of LARC utilization among interested women, but who harbour concerns about LARC.

Acknowledgements

The authors gratefully acknowledge Miss Julaporn Poolium of the Research Group and Research Network Division, Research Department, Faculty of Medicine Siriraj Hospital, Mahidol University for her assistance with statistical analysis.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. American College of Obstetricians and Gynecologists, Society for Maternal-Fetal Medicine. Obstetric Care Consensus No. 8: Interpregnancy care. Obstet Gynecol 2019;133:e51-e72.
2. Department of Reproductive Health and Research (RHR). Report of a WHO Technical Consultation on Birth Spacing: Geneva, Switzerland 13-15 June 2005. Geneva, Switzerland World Health Organization; 2007. Available from: http://apps.who.int/iris/bitstream/10665/69855/1/WHO_RHR_07.1_eng.pdf. Accessed August 15, 2022.
3. Conde-Agudelo A, Rosas-Bermúdez A, Kafury-Goeta A. Birth spacing and risk of adverse perinatal outcomes. JAMA 2006;295:1809-23.
4. Shree R, Caughey AB, Chandrasekaran S. Short interpregnancy interval increases the risk of preterm premature rupture of membranes and early delivery. J Matern Fetal Neonatal Med 2018;31:3014-20.
5. Hall JA, Benton L, Copas A, Stephenson J. Pregnancy intention and pregnancy outcome: Systematic review and meta-analysis. Matern Child Health J 2017;21:670-704.
6. Committee on Practice Bulletins-Gynecology, Long-Acting Reversible Contraception Work Group. Practice

Bulletin No. 186: Long-Acting Reversible Contraception: Implants and Intrauterine Devices. Obstet Gynecol 2017;130:e251-e69.
7. Selected practice recommendations for contraceptive use. 3rd ed. Geneva: World Health Organization; 2016.
8. Committee on Gynecologic Practice Long-Acting Reversible Contraception Working G. Committee opinion No. 642: Increasing access to contraceptive implants and intrauterine devices to reduce unintended pregnancy. Obstet Gynecol 2015;126:e44-8.
9. National Statistical Office of Thailand. 2020. Thailand Multiple Indicator Cluster Survey 2019, Survey Findings Report. Bangkok, Thailand: National Statistical Office of Thailand.
10. Akesittipaisarn S, Werawatakul Y, Simsin S. Factors associated with long-acting reversible contraception (LARC) use in postpartum women at srinagarind hospital. Thai J Obstet Gynaecol 2018;26:132-9.
11. Chansin W, Manonai J, Wattanayingcharonchai R, Aimjirakul K. Postpartum use of long-acting reversible contraception in primiparous women: Ramathibodi hospital's experiences. Thai J Obstet Gynaecol 2014;22:29-36.
12. Woldu BF, Ermolo TL, Lemu LG, Gejo NG. Long-acting reversible contraception utilization and associated factors among women in extended postpartum period in Hossana town, southern Ethiopia: cross sectional study. Contracept Reprod Med 2020;5:10.
13. Oduyebo T, Zapata LB, Boutot ME, Tepper NK, Curtis KM, D'Angelo DV, et al. Factors associated with postpartum use of long-acting reversible contraception. Am J Obstet Gynecol 2019;221:43 e1- e11.
14. Mesfin Y, Wallelign A. Long-acting reversible contraception utilization and associated factors among women in extended postpartum period in southern Ethiopia. Arch Public Health 2021;79:161.
15. Kang C, Li P, Liu X, Ding Y, Wang X, Zhou H. Use of contraceptives and uptake of long-acting reversible contraception among postpartum women in rural China. BMJ Sex Reprod Health 2018;44:254-9.
16. Kiondo KS, Maro E, Kiwango S, Alloyce JP, Shayo BC, Mahande MJ. Prevalence and factors associated with postpartum use of long-acting reversible contraception in Bukombe District, Geita Region, Tanzania: a community- based study. Contracept Reprod Med 2020;5:24.
17. Chaovisitsaree S, Noi-um S, Kietpeerakool C. Review of postpartum contraceptive practices at Chiang Mai University Hospital: implications for improving quality of service. Med Princ Pract 2012;21:145-9.
18. Medical eligibility criteria for contraceptive use. Fifth Edition 2015. WHO Guidelines Approved by the Guidelines Review Committee. Available from: http://www.who.int/reproductivehealth/publications/family_planning/MEC-5/en/. Accessed August 15, 2022.
19. Jaruamornjit Y, Kaewrudee S, Sothornwit J. Differences in postpartum contraceptive choices and patterns following low- and high-risk pregnancy. Contraception 2022;107:52-7.
20. Zerden ML, Tang JH, Stuart GS, Norton DR, Verbiest SB, Brody S. Barriers to receiving long-acting reversible contraception in the postpartum period. Womens Health Issues 2015;25:616-21.
21. Nelson HN, Thayer E, Bailey C, Leuenberger L, Lehman E, Chuang CH. Factors associated with new uptake of long-actingr reversible contraceptives since the affordable care act among privately insured women in pennsylvania. Womens Health Issues 2019;29: 370-5.
22. Sothornwit J, Lumbiganon P, Saranrittichai K, Sangkomkamhang U, Singhdaeng T, Jampathong N. Barriers and facilitators to implementing immediate postpartum contraceptive implant programs: A formative implementation research. Int J Womens Health 2022;14:945-56.

Thai Journal of Obstetrics and Gynaecology
July 2023, Vol. 31, No. 4, pp. 285-292.

GYNAECOLOGY

Risk Factors Associated with Major Complications of Total Laparoscopic Hysterectomy

Juthamas Chuthong, M.D.*,
Pisutt Srichaikul, M.D.*,
Dittakarn Boriboonhirunsarn, M.D., M.P.H., Ph.D.*

** Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*

ABSTRACT

Objectives: To determine risk factors associated with major complications of Siriraj total laparoscopic hysterectomy (SiTLH) technique.

Materials and Methods: A case-control study was conducted in 275 women who underwent SiTLH at a university-based tertiary care hospital. Cases consisted of 55 women with major intraoperative complications. Controls were 220 women with the uneventful operation, randomly selected from those who underwent SiTLH during the same period as cases. Data were retrieved from medical records, including baseline and operative characteristics, diagnosis and indications, surgeon experience, and characteristics of the complications.

Results: Cases and controls were comparable in terms of baseline characteristics, including age, body mass index, diagnosis, and surgeon's experience. Cases were significantly more likely to have previous abdominal surgery and have preoperative diagnosis of endometriosis. (41.8% vs 25%, p = 0.013 and 47.3% vs 29.5%, p = 0.012, respectively). In addition, cases were significantly more likely to have higher specimen weight, longer operative time, and estimated blood loss (p < 0.001). Among those with major complications, internal organ injuries occurred in 30 cases (54.5%) including injuries to bowel (21.8%), bladder (18.2%), and ureters (16.4%). Conversion to abdominal operation occurred in 32.7%. Multivariate analysis showed that, after adjusting for potential confounders, having had previous abdominal surgery and preoperative diagnosis of endometriosis independently increased risk of major complications (adjusted odds ratio (OR) 2.2, 95% confidence interval (CI) 1.2-4.29, p = 0.015 and adjusted OR 2.1, 95%CI 1.1-4.1, p = 0.019, respectively).

Conclusion: Having had previous abdominal surgery and preoperative diagnosis of endometriosis independently increased the risk of major complications of SiTLH procedure.

Keywords: laparoscopic hysterectomy, complications, abdominal surgery, endometriosis.

Correspondence to: *Pisutt Srichaikul, M.D., Department of obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wanglang Road, Bangkoknoi, Bangkok 10700, Thailand. Email: pisutt.srichaikul@gmail.com*

Received: 29 September 2022, **Revised:** 2 December 2022, **Accepted:** 6 December 2022

ปัจจัยเสี่ยงที่สัมพันธ์กับภาวะแทรกซ้อนที่สำคัญจากการผ่าตัดผ่านกล้องตัดมดลูก

จุฑามาศ ชูทอง, พิสุทธิ์ ศรีชัยกุล, ดิฐกานต์ บริบูรณ์หิรัญสาร

บทคัดย่อ

วัตถุประสงค์: *เพื่อศึกษาปัจจัยเสี่ยงที่สัมพันธ์กับภาวะแทรกซ้อนหลักของการผ่าตัดผ่านกล้องตัดมดลูกโดยวิธี Siriraj total laparoscopic hysterectomy (SiTLH)*

วัสดุและวิธีการ: *การศึกษาแบบ case-control โดยสตรี จำนวน 55 คน ที่มีภาวะแทรกซ้อนหลักจากการผ่าตัด เป็นกลุ่มศึกษาและสตรีที่ไม่มีภาวะแทรกซ้อนหลักจากการผ่าตัด จำนวน 220 คน เป็นกลุ่มควบคุม ข้อมูลจากเวชระเบียน ได้แก่ ข้อมูลพื้นฐานทั่วไป ข้อมูลการผ่าตัด การวินิจฉัยโรค และภาวะแทรกซ้อนจากการผ่าตัด จะนำมาใช้คำนวณเพื่อหาปัจจัยเสี่ยงในการผ่าตัดผ่านกล้องตัดมดลูก*

ผลการศึกษา: *กลุ่มศึกษามี ระยะเวลาในการผ่าตัด และปริมาณการเสียเลือดมากกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ในกลุ่มศึกษาภาวะแทรกซ้อนที่พบมากที่สุดคือการบาดเจ็บต่ออวัยวะข้างเคียงพบ 30 คน (54.5%) ประกอบด้วยการบาดเจ็บต่อลำไส้ (21.8%) กระเพาะปัสสาวะ (18.2%) ท่อไต (16.4%) และเส้นเลือด (3.6%) การศึกษา Multivariate analysis พบว่าการมีประวัติผ่าตัดในช่องท้อง (adjusted odds ratio (OR) 2.2, 95% confidence interval (CI) 1.2-4.29, p = 0.015) และการวินิจฉัยภาวะเยื่อบุโพรงมดลูกเจริญผิดที่ (adjusted OR 2.1, 95%CI 1.1-4.1, p = 0.019) เป็นปัจจัยเสี่ยงที่ทำให้เกิดภาวะแทรกซ้อนหลัก*

สรุป: *ปัจจัยเสี่ยงที่สัมพันธ์กับภาวะแทรกซ้อนหลักจากการผ่าตัดผ่านกล้องตัดมดลูกด้วยวิธี SiTLH คือการมีประวัติผ่าตัดในช่องท้อง และการวินิจฉัยเยื่อบุโพรงมดลูกเจริญผิดที่*

คำสำคัญ: *การผ่าตัดผ่านกล้องตัดมดลูก, ภาวะแทรกซ้อน, การผ่าตัดในช่องท้อง, ภาวะเยื่อบุโพรงมดลูกเจริญผิดที่*

Introduction

Laparoscopic surgery is considered as an alternative surgical approach in gynecologic surgery. The advantages of laparoscopic surgery over laparotomy include a smaller incision, better visualization, less tissue trauma, shorter hospital stay, and less blood loss and fibrosis[1]. Moreover, surgical outcomes after laparoscopic surgery have been reported to be equivalent to or better than laparotomy[2]. Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, has begun laparoscopic hysterectomy since 2004 and developed a novel technique for laparoscopic hysterectomy (SiTLH) in 2006. The objectives of this technique are to reduce surgical complications and blood loss during surgery[3].

The overall complication rate in gynecologic laparoscopic surgery ranged from 0.2-10.3% but higher rates were noted in major laparoscopic operations such as hysterectomy ranging from 0.6-18%[4]. Major complications were identified as death, organ injuries that need the surgical correction, unintended laparoconversion, or massive blood loss, whereas minor complications include complications that have a low impact on patient's quality of life, such as postoperative fever, mild bleeding, postoperative urinary retention[5]. Data from most studies showed possible specific risk factors for laparoscopic surgery, including history of previous laparotomy, presence of adhesions, intra-operative technical difficulty, level of laparoscopic complexity, suspicion of malignancy ,and surgeon's experience[6-9]. However, there were the discrepancies in these results. It possibly due to study design, cohort size, and surgeon's expertise.

Currently, there is no information regarding this specific issue in Siriraj Hospital. Therefore, this study aimed to identify the risk factors of major complications from gynecologic laparoscopic surgery. The results could provide more information to identify those with higher risk and help raise awareness among surgeons to prepare better and further minimize the risk of major complications.

Materials and Methods

After approval from Siriraj Institutional Review Board, a case-control study was conducted on 2,858 women who underwent SiTLH as a major laparoscopic procedure at the Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital between February 2014 and December 2020. Details of the SiTLH surgical technique have been previously reported elsewhere[3]. This technique begins with retroperitoneal dissection, an avascular area, at the beginning of the procedure to locate and ligate the uterine artery to minimize blood loss during surgery and improve surgical exposure. Furthermore, ureters and bladder were clearly identified before hysterectomy began.

Sample size was estimated based on pilot study that 25% and 10% had previous abdominal surgery among those with and without major complications, respectively. At 95% confidence level and 80% power with 4:1 control-to-case ratio, at least 50 cases and 200 controls were required.

Cases were identified as patients with acute major complications that occurred in 30 days postoperative period, including critical blood loss, organ injuries, unintended conversion to laparotomy, death, vaginal cuff dehiscence requiring surgical intervention, wound infection, re-admission, re-operation, and postoperative ureteric stenosis. Critical blood loss was defined as estimated blood loss that required intraoperative blood transfusion or total blood loss of ≥ 1000 mL[3,10]. Organ injuries are defined by Clavien–Dindo classification of grade > 3[11]. Controls were patients who underwent SiTLH and did not have any major complications. Controls were selected from those with uneventful operations during the same period of cases by two controls above and two controls below case as shown in

Fig. 1. All the operations were performed by surgeons who had laparoscopic surgery experiences of more than five years at the time of the operations.

Data were reviewed and extracted from medical records, including baseline and operative characteristics such as age, body mass index (BMI), diagnosis and indications, surgeon's experience, characteristics of the complications, and surgical outcomes. Body weight was categorized as normal weight (BMI 18.5-22.9 kg/m^2), underweight (BMI < 18.5 kg/m^2), overweight (BMI 23-24.9 kg/m^2), and obese (BMI ≥ 25 kg/m^2).

Descriptive statistics, including mean, standard deviation, number, and percentage were used to describe various characteristics as appropriate. Student t-test, Mann-Whitney U test, Chi-square test, and Fisher's Exact test were used to compare characteristics between cases and controls. Odds ratios (OR) and 95% confidence intervals (CI) were estimated to determine the association between major complications and various characteristics. Multivariate logistic regression analysis was performed to evaluate independently associated factors for major complications, adjusted for potential confounders. A p value of < 0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics for Windows®, Version 21.0. Armonk, NY: IBM Corp.

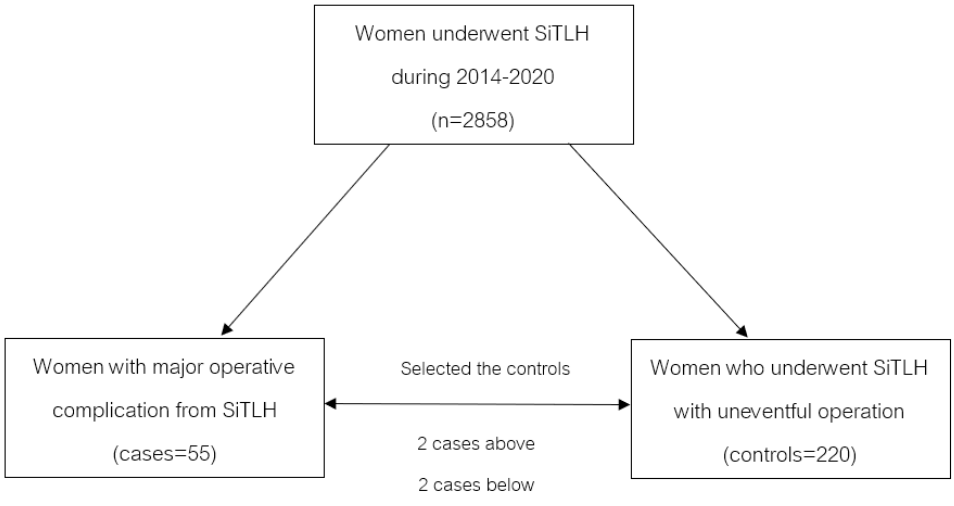

Fig. 1. Flow chart of the study population.

Results

A total of 2,858 women who underwent SiTLH at Siriraj hospital 275 women were enrolled in this study. Cases consisted of 55 women with major complications, and controls consisted of 220 women with uneventful operations. The prevalence of major complications from the SiTLH occurred 1.9%. Baseline characteristics between the two groups were compared, and the results are shown in Table 1. Both groups were comparable concerning age, BMI, diagnosis, primary pathologic site, and surgeon's experience. However, cases were significantly more likely to have previous abdominal surgery (41.8% vs 25.0%, p = 0.013)

Comparisons of operative characteristics between the two groups are shown in Table 2. Cases were significantly more likely to have endometriosis (47.3% vs 29.5%, p = 0.012). Compared with controls, cases had significantly longer operative time (190 vs 150 minutes, p < 0.001) and higher estimated blood loss (150 vs 30 mL, p < 0.001).

Table 1. Comparison of baseline characteristics between the 2 groups.

Characteristics	Cases n = 55	Controls n = 220	p value
Mean age ± SD (years)	44.9 ± 8.2	45.5 ± 10.5	0.709
Mean BMI ± SD (kg/m^2)	23.9 ± 4.6	24.4 ± 4.8	0.550
BMI category			0.897
Normal	31 (56.4%)	123 (55.9%)	
Underweight	5 (9.1%)	14 (6.4%)	
Overweight	13 (23.6%)	57 (25.9%)	
Obese	6 (10.9%)	26 (11.8%)	
Diagnosis condition			1.00
Benign pathology	51 (92.7%)	203 (92.3%)	
Malignant pathology	4 (7.3%)	17 (7.7%)	
Primary pathologic site			0.282
Uterus only	46 (83.6%)	193 (88.9%)	
Uterus with others	9 (16.4%)	24 (11.1%)	
Previous abdominal surgery			0.013
No	32 (58.2%)	165 (75%)	
Yes	23 (41.8%)	55 (25.0%)	
Surgeon's experiences			0.673
≥ 10 years	30 (54.5%)	113 (51.4%)	
5 - 10 years	25 (45.5%)	107 (48.6%)	

SD: standard deviations, BMI: Body Mass Index

Table 2. Comparison of operative characteristics between the 2 groups.

Operative characteristics	Cases n = 55	Controls n = 220	p value
Existence of endometriosis			0.012
No	29 (52.7%)	155 (70.5%)	
Yes	26 (47.3%)	65 (29.5%)	
	Median (IQR)	**Median (IQR)**	
Specimen weight (grams)	250 (160-453)	221 (150-380)	0.191
Operative times (minutes)	190 (150-250)	150 (115-188)	< 0.001
Estimated blood loss (mL)	150 (50-450)	30 (20-73.7)	< 0.001

IQR: interquartile range

Characteristics of major complications are shown in Table 3. Internal organ injuries occurred in 54.5%, including bowel, bladder, ureter, and vessel injuries. Other complications consisted of unintended conversion to laparotomy (32.7%), critical blood loss (16.4%), and vaginal cuff dehiscence (7.3%). Re-operation was required in 30.9% and re-admission was needed in 32.7% which included organ injuries for fourteen patients including ureter injury for nine patients, bladder injury for two patients, bowel injury for two patients, blood vessel injury for one patient, and vaginal cuff dehiscence for four patients. Patients with ureter and bladder injuries presented with abdominal pain and fever or watery discharge from

the vagina. Patients with ureteral injuries underwent ureteral reimplantation for three patients and others were treated with ureteral stenting. Patients with bladder injuries were repaired vaginally. For bowel injury, patients presented with fever and intraabdominal collection. Exploratory laparotomy with repair bowel and colostomy was done in both cases. Patient with blood vessel injury presented with a large vaginal stump hematoma with active bleeding from the vagina and anemia one week postoperatively. Angiographic embolization was successfully done at the vaginal branch of the uterine artery.

Table 3. Characteristics of major complications (n = 55).

Major complications	n (%)
Re-operation	17 (30.9%)
Re-admission	18 (32.7%)
Critical blood loss	9 (16.4%)
Organ injury	30 (54.5%)
Ureter injury	9 (16.4%)
Bladder injury	10 (18.2%)
Bowel injury	12 (21.8%)
Vessel injury	2 (3.6%)
Conversion to TAH	18 (32.7%)
Vaginal cuff dehiscence	4 (7.3%)

TAH: total abdominal hysterectomy

Multivariate logistic regression analysis was performed to determine independently associated factors for major complications of SiTLH, and the results are shown in Table 4. After adjusting for potential confounders, independently associated risks for major complications were previous abdominal surgery (adjusted OR 2.21, 95%CI 1.17-4.17, p = 0.015) and the existence of endometriosis (adjusted OR 2.15, 95%CI 1.13-4.06, p = 0.019).

Table 4. Multivariate logistic regression analysis to determine independent associated factors for major complication of SiTLH, adjusted for potential confounders.

Factors	Adjusted OR	95% CI	p value
Age	1.0	0.97-1.04	0.852
BMI category			
Normal	1.0		
Underweight	1.28	0.40-4.10	0.677
Overweight	0.94	0.45-1.99	0.88
Obesity	1.17	0.39-3.43	0.776
Previous abdominal surgery	2.21	1.17-4.17	0.015
Diagnosis of malignancy	1.06	0.27-4.15	0.929
Existence of endometriosis	2.15	1.13-4.06	0.019
Uterine pathology only	0.52	0.21-1.29	0.156
Surgeon's experience of 5-10 years	0.87	0.46-1.62	0.653

SiTLH: Siriraj total laparoscopic hysterectomy, OR: odds ratio, CI: confidence interval, BMI: body mass index

Discussion

Over the past decades, laparoscopy has been the gold standard for diagnosis and therapeutic purposes. Many patients prefer TLH over total abdominal hysterectomy due to the benefits of TLH. Laparoscopic surgery remains an intra-abdominal procedure. Therefore, it shares all the intraoperative and postoperative risk of laparotomy, such as infection and injury to the adjacent intraabdominal organs. Recognizing risk factors of major complications from TLH is thus important in improving care process and aiding physicians to identify high-risk women and provide proper information to decide mode of surgical access.

The results of this study showed that independently associated risks for major complications of the SiTLH were previous abdominal surgery and the existence of endometriosis. A previous study has also reported that serious complications were significantly more frequent in patients with prior abdominal surgery with a similar adjusted OR.5,9 While the other study focusing on risk of unintended laparoscopic conversion as a major complication also found that patients with previous laparotomy had an increased risk of laparoscopic surgery failure.6 Previous abdominal surgery might increase the chance of abdominopelvic adhesions that could limit visualization during laparoscopic surgery and increase the risk of organ injuries during adhesiolysis procedure. A previous study showed that umbilical adhesion was presented in 0.68% of patients without history of surgery and 19.8% in patients with previous Pfannenstiel and 51.7% with vertical abdominal incision[12].

In this study, the presence of endometriosis also significantly increased the risk of major complications of the SiTLH. A recent study has reported that the independent risk factors for severe TLH complication events was endometriosis, with OR of 3.51[13]. Another study has reported similar results.14 The nature of endometriosis might be explained that it can cause continuous inflammation and scaring that lead to adhesion formation, derangement of normal tissue neovascularization, and anatomical distortion. Furthermore, these can lead to inadequate exposure, uterine immobility, and bleeding during the procedures, especially for large uterus.

Some studies have reported that postoperative hemoglobin drop was an independent risk factor for postoperative morbidity and mortality[14,15]. However, as postoperative hemoglobin is not included as a routine test according to our institutional guideline, such association could not be evaluated. Further studies might be required to determine if hemoglobin is of value in predicting major SiTLH complications. Other risk factors for TLH complications that have been reported including age, obesity, malignant condition, non-uterine pathology as a major surgical indication, and surgeon's experiences[6-8]. This study evaluated these factors but did not reach a statistical significance level in multivariate analysis. The discrepancy might be from the differences in TLH technique in different settings and the surgeon's experiences. The technique used in Siriraj Hospital includes the proper port placement according to body habitus, use of retroperitoneal space, and early ligation of uterine blood supply before initiation of hysterectomy.

Re-admission occurred in as many as 32.7%, possibly because some postoperative complications were unrecognized intraoperatively and required in-patient admission to treat and re-evaluate properly. The bowel, bladder, and ureter were common intraoperatively injured organs. The previous study has also reported that most internal organ injuries occurred in the bowel, especially in case of endometriosis[16].

The strengths of this study included that data were from a single tertiary center where all patients were operated on with similar surgical techniques by the experienced surgeons. All SiTLH-related data were routinely recorded and collected systematically. However, some limitations should be mentioned. Due to retrospective nature of the data, some information was unavailable in detail, such as severity or stage of

endometriosis, number and types of previous abdominal surgical procedures. The results might have limited generalizability due to population characteristics between settings. Further larger studies are needed to evaluate and identify significant associated risks of SiTLH complications and to determine if those complications are related to adverse surgical outcomes in more detail.

Nevertheless, the results of this study provide more insights into the management of women undergoing SiTLH. Understanding the significant risk factors will help physicians identify women at higher risk for the procedure. Awareness of both conditions will make the surgery safer and more effective.

Conclusion

Independently associated risks of major complications of SiTLH included having had previous abdominal surgery and diagnosis of endometriosis.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Crosignani PG, Vercellini P, Biffignandi F, Costantini W, Cortesi I, Imparato E. Laparoscopy versus laparotomy in conservative surgical treatment for severe endometriosis. Fertil Steril 1996;66:706-11.
2. Johnson N, Barlow D, Lethaby A, Tavender E, Curr E, Garry R. Methods of hysterectomy: systematic review and meta-analysis of randomized controlled trials. Br Med J 2005;330:1478-85.
3. Srichaikul P, Chalermchockcharoenkit A, Yahata H, Sirimai K, Sutchritpongsa P. Low complication rate associated with total laparoscopic hysterectomies using the retroperitoneal approach: a series of 1,092 cases in Siriraj Hospital. SMJ 2018;70:191-7.
4. Magrina JF. Complications of laparoscopic surgery. Clin Obstet Gynecol 2002;45:469-80.
5. Fuentes MN, Rodriguez-Oliver A, Naveiro Rilo JC, Paredes AG, Aguilar Romero MT, Parra JF. Complications of laparoscopic gynecologic surgery. JSLS 2014;18:1-9.
6. Sokol AI, Chuang K, Milad MP. Risk factors for conversion to laparotomy during gynecologic laparoscopy. J Am Assoc Gynecol Laparosc 2003;10:469-73.
7. Kaya AC, Radosa MP, Zimmermann JSM, Stotz L, Findeklee S, Hamza A, et al. Intraoperative and postoperative complications of gynecological laparoscopic interventions: incidence and risk factors. Arch Gynecol Obstet 2021;304:1259-69.
8. Chi DS, Abu-Rustum NR, Sonoda Y, Awtrey C, Hummer A, Venkatraman ES, et al. Ten-year experience with laparoscopy on a gynecologic oncology service: Analysis of risk factors for complications and conversion to laparotomy. Am J Obstet Gynecol 2004;191:1138-45.
9. Jansen FW, Kapiteyn K, Trimbos-Kemper T, Hermans J, Trimbos JB. Complications of laparoscopy: a prospective multicentre observational study. Br J Obstet Gynaecol 1997;104:595-600.
10. Shah DK, Vitonis AF, Missmer SA. Association of body mass index and morbidity after abdominal, vaginal, and laparoscopic hysterectomy. Obstet Gynecol 2015;125:589-98.
11. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg 2004;240:205-13.
12. Audebert AJ, Gomel V. Role of microlaparoscopy in the diagnosis of peritoneal and visceral adhesions and in the prevention of bowel injury associated with blind trocar insertion. Fertil Steril 2000;73:631-5.
13. Casarin J, Cromi A, Bogani G, Multinu F, Uccella S, Ghezzi F. Surgical morbidity of total laparoscopic hysterectomy for benign disease: Predictors of major postoperative complications. Eur J Obstet Gynecol Reprod Biol 2021;263:210-5.
14. Uccella S, Marconi N, Casarin J, Ceccaroni M, Boni L, Sturla D, et al. Impact of endometriosis on surgical outcomes and complications of total laparoscopic hysterectomy. Arch Gynecol Obstet 2016;294:771-8.
15. Jadunandan S, Tano R, Vicus D, Callum J, Lin Y. The incidence of perioperative anemia and iron deficiency in patients undergoing gyne-oncology surgery. Can Oncol Nurs J 2022;32:75-80.
16. Johnston K, Rosen D, Cario G, Chou D, Carlton M, Cooper M, et al. Major complications arising from 1265 operative laparoscopic cases: a prospective review from a single center. J Minim Invasive Gynecol 2007;14:339-44.

Thai Journal of Obstetrics and Gynaecology
July 2023, Vol. 31, No. 4, pp. 293-301.

GYNAECOLOGY

The Effect of Cold Gel Pack on Pain Reduction in Patients Undergoing Complete Surgical Staging: A randomized controlled trial

Yanin Srirussamee, M.D.*,
Kitiya Wutthibenjarussamee, M.D.*,
Thumwadee Tangsiriwatthana, M.D.*

** Department of Obstetrics and Gynecology, Khon Kaen Hospital, Khon Kaen, Thailand*

ABSTRACT

Objectives: To study the effectiveness of a cold gel pack for reducing surgical wound pain in patients undergoing complete surgical staging

Materials and Methods: We enrolled women 18 or older with gynecologic malignancy and undergoing complete surgical staging by gynecologic oncologists between November 2021 and May 2022. They were randomly assigned to two groups: the study group – who received a cold gel pack applied to the low midline surgical wound 6 h post-operatively, and the control group – who received standard post-operative care. Post-operative pain at 2, 6, and 12 h were evaluated.

Results: Forty eligible women were enrolled. Post-operative pain at 12 h was lower in the study group albeit not statistically significant (4.95 ± 1.67 vs 5.90 ± 1.65, p = 0.08). Time to first flatus and additional opioids consumption were also not significantly different between groups (2,506.50 ± 85.86 vs 2,473.50 ± 189.69 min, p = 0.2) and (11.80 ± 3.37 vs 12.05 ± 3.52 mg, p = 0.85). No adverse events were observed.

Conclusion: Cold gel pack did not significantly reduce post-operative pain compared with standard post-operative care in patients undergoing complete surgical staging.

Keywords: cold gel pack, post-operative pain, surgical wound pain, complete surgical staging.

Correspondence to: *Yanin Srirussamee, M.D., Department of Obstetrics and Gynecology, Khon Kaen Hospital, Khon Kaen, 40000, Thailand. E-mail: yyyanin.sri@gmail.com*

Received: 30 September 2022, **Revised:** 3 Janurary 2023, **Accepted:** 5 January 2023

ผลของการประคบด้วยถุงเจลเย็นเพื่อลดระดับความเจ็บปวดหลังผ่าตัดประเมินระยะโรคของมะเร็งนรีเวช การศึกษาแบบสุ่ม

ญาณินท์ ศรีรัศมี, กิติยา วุฒิเบญจรัศมี, ทุมวดี ตั้งศิริวัฒนา

บทคัดย่อ

วัตถุประสงค์: *เพื่อศึกษาประสิทธิภาพของถุงเจลเย็นในการลดระดับความเจ็บปวดของแผลผ่าตัดประเมินระยะโรคของมะเร็งนรีเวช*

วัสดุและวิธีการ: *ผู้ป่วยมะเร็งนรีเวชอายุ 18 ปีขึ้นไป ที่ได้รับการผ่าตัดเปิดช่องท้องเพื่อประเมินระยะโรคโดยแพทย์ผู้เชี่ยวชาญสาขามะเร็งนรีเวช ตั้งแต่เดือนพฤศจิกายน 2564 ถึงเดือนพฤษภาคม 2565 โดยแบ่งเป็น 2 กลุ่มโดยการสุ่ม สำหรับกลุ่มทดลองจะได้รับการวางถุงเจลเย็นประคบบริเวณแผลผ่าตัดแนวตั้ง ที่ 6 ชั่วโมงหลังการผ่าตัด และกลุ่มควบคุมได้รับการดูแลหลังการผ่าตัดตามมาตรฐานเพียงอย่างเดียว และทำการประเมินระดับความเจ็บปวดของแผลผ่าตัดที่ 2,6 และ12 ชั่วโมง*

ผลการศึกษา: *ผู้เข้าร่วมการศึกษาจำนวน 40 คน พบว่าระดับความเจ็บปวดของแผลผ่าตัดที่ 12 ชั่วโมงในกลุ่มที่ได้รับการวางถุงเจลเย็นน้อยกว่ากลุ่มควบคุม แต่ไม่มีนัยสำคัญทางสถิติ (4.95 ± 1.67 vs 5.90 ± 1.65, p = 0.08) ระยะเวลาในการผายลมครั้งแรกหลังผ่าตัด การใช้ยาแก้ปวดเพิ่มเติมไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ (2,506.50 ± 85.86 vs 2,473.50 ± 189.69 นาที, p = 0.2) และ (11.80 ± 3.37 vs 12.05 ± 3.52 มิลลิกรัม, p = 0.85) และไม่พบภาวะแทรกซ้อนหรือผลข้างเคียงจากการใช้ถุงเจลเย็น*

สรุป: *การใช้ถุงเจลเย็นประคบบริเวณแผลผ่าตัดในผู้ป่วยที่ผ่าตัดประเมินระยะโรคของมะเร็งนรีเวช ไม่ได้ช่วยลดความเจ็บปวดของแผลผ่าตัดที่ 12 ชั่วโมงหลังการผ่าตัด เมื่อเปรียบเทียบกับการดูแลหลังผ่าตัดตามมาตรฐาน*

คำสำคัญ: *ถุงเจลเย็น, ความเจ็บปวดหลังผ่าตัด, ความเจ็บปวดของแผลผ่าตัด, ผ่าตัดทางมะเร็งนรีเวช, ผ่าตัดประเมินระยะของโรค*

Introduction

Surgery often causes tissue injury and inflammation. In response, nociceptors are activated and transmit nerve signals (i.e., prostaglandins, interleukins, cytokines, and neurotrophins), which travel through the spinal cord towards the brain, where the sensation of pain is recognized. The neurotransmitters initiate a spinal reflex, resulting in increased muscle activity and tonicity at the site of injury. Other skin receptors include (a) thermoreceptors that are activated by changes in skin temperature, and (b) proprioceptors that detect physical changes in tissue pressure and movement – the activation of both inhibit nociceptors resulting in muscle relaxation and enhanced tissue blood flow[1, 2].

Surgery-related morbidities impact patient outcomes and quality of life. Most gynecologic malignancies require surgical management to remove the macroscopic tumor and stage the disease to plan proper adjuvant therapies[3]. Complications often develop when post-operative pain management is inadequate, such as delayed ambulation, bowel ileus, lung atelectasis, venous thromboembolism, and infection, resulting in delayed post-operative recovery and prolonged hospitalization, especially in oncologic patients. Most surgical patients experience post-operative pain, and less than 50% receive adequate pain control management[4].

There are two categories of post-operative pain management, medication and non-medication. Multimodal therapies (multimodal anesthesia plus adjunctive therapy) are usually recommended. As the innervation of the anterolateral abdominal wall arises from the anterior rami of spinal nerves T7 to L1, including intercostal nerves (T7-T11), subcostal nerve (T12), and iliohypogastric and ilioinguinal nerves (L1). Transversus abdominis plane (TAP) blocks are additional local anesthesia widely used to diminish the post-operative pain of the required low midline incision. The target of the TAP block is the space between the internal oblique and transverse abdominis muscle where the nerves are located. The innervation to abdominal skin, muscles, and parietal peritoneum may be blocked, but tissue injury and inflammation are not eliminated[5]. The anesthetic effect of the TAP block ranges from 6 to 36 h[6].

Several studies argue that non-medical pain management could be more appropriate because of fewer side effects, and it could synergize with other medical approaches, including thermal therapy[4].

Cryotherapy is any application that removes heat from the body, causing vasoconstriction, reducing tissue metabolism, oxygen consumption, inflammation, and muscle spasms. Likewise, cryotherapy minimizes the inflammatory processes by activating thermoreceptors and proprioceptors, thereby aiding recovery after soft tissue trauma[1,2]. The most effective cryotherapy is between 10 - 15 ºC within 72 h of injury, for 20 to 30 min, 2 to 4 times per day, or 30 to 45 min every 2 h[7,8]. Using an ice pack or cold gel pack applied to the surgical wound is a simple way to reduce post-operative pain. Other non-medical pain management include (a) transcutaneous electrical nerves stimulation, typically applied at the incision site (note: contraindicated in patients with a pacemaker or implanted defibrillator, lymphedema, and/or broken skin); and (b) cognitive modalities that include guided imagination and relaxation, which often require pre-operative education or training[4].

Previous studies reported that cryotherapy effectively reduced post-operative pain and reduced post-operative additional opioid consumption without serious adverse events after repairing the inguinal hernia, benign gynecologic operations, exploratory laparotomy, episiotomy wound, and cesarean section[9-16] using varies

rationale, for example cold pack applied at 2 h after the procedure for 20 minutes in study of Nuangpho, applied at 2 hours post operative and changed every 2 h for 2 consecutive times in study of Chumkam and Suwannalert. Furthermore, gynecologic malignancy operations—including total abdominal hysterectomy, bilateral salpingo-oophorectomy, partial omentectomy, and bilateral pelvic lymph nodes dissection—frequently have a longer incision length and include more traction force to provide good exposure of the intra-abdominal and pelvic organs. The consequence of these invasive procedures is severe tissue injury, inflammation, and greater post-operative pain.

The current study aimed to evaluate the effects of cryotherapy by applying a cold gel pack to reduce post-operative pain at the surgical wound after completing surgical staging for gynecologic malignancy.

Materials and Methods

This randomized controlled study was performed at the Department of Obstetrics and Gynecology, Khon Kaen Hospital, Thailand. The objective was to study the effectiveness of a cold gel pack in reducing post-operative pain in patients undergoing complete surgical staging compared with standard post-operative care without a cold gel pack. The study was approved by the Khon Kaen Hospital Institute Review Board in Human Research (KEF64017). The study included 40 women, 18 or older, diagnosed with gynecologic malignancy, undergoing complete surgical staging with a midline incision between November 2021 and May 2022. We excluded women who had cold hypersensitivity, active skin disease at the surgical site, received patient-controlled analgesia, or had any perioperative complications (including torn internal organs, drainage tube, or needed post-operative intensive care).

Computer-generated block randomization was performed and allocated using opaque sealed envelopes. Women who met the inclusion criteria were informed, and the gynecologic residents obtained consent after completing the surgical staging surgery and immediate post-operative care in recovery room. Participants were assigned to group 1 or 2 per number in the envelope.

Demographic data were recorded, including age, underlying disease, body weight (BW), height, body mass index (BMI), history of previous abdominal surgeries, and the gynecologic malignancy diagnosis. The physician recorded perioperative data and findings, including time start or surgery open and closed, operative findings, estimated blood loss (EBL), suture materials, surgical incision length measured in centimeters. The histopathological report was recorded when the findings were made available.

The study group received standard post-operative care and a cold gel pack (Nanomed: CSI-CH-I 450), which was refrigerated for 1 h. The gel pack was between 10 and 15ºC when applied for 1 h above the surgical wound at 6 h after surgery by using stopwatch to ensure the exact time for applying the cold gel pack. The control group received standard post-operative care. Standard pre- and post-operative care included: (a) complete surgical staging by gynecologic oncologists; (b) standard general anesthesia with endotracheal tube TAP block using 1% lidocaine with adrenaline and 0.25% bupivacaine, corresponding to a maximum dose of 7 mg/kg lidocaine with adrenaline and 3 mg/kg bupivacaine injected between the transversus abdominis and internal oblique with ultrasound guided; (c) post-operative antibiotics prophylaxis of cefazolin 1 g (2 g if BW > 90 kg) intravenous every 6 h for 24 h or clindamycin 300 mg intravenous every 8 h for 24 h if patients had a drugs allergy; (d) post operative opioids; morphine

sulfate 2 mg (if body weight < 50 kg) intravenous every 4 h or 3 mg intravenous (if body weight > 50 kg) every 4 h for 24 h; (e) additional morphine sulfate to breakthrough pain if pain score ≥ 5; (f) post-operative prokinetic drug - metoclopramide 10 mg intravenous every 8 h if they had nausea or vomiting as the morphine sulfate side effects; and, (g) surgical wound covered with sterile gauze and waterproof patch and wound dressing if there was bleeding or oozing.

The primary outcome of the present study was a post-operative pain score at 12 h. Participants were asked to assess their post-operative pain score using a visual analog scale (VAS) (0 = no pain and 10 = worse pain) at 2, 6, and 12 h post-operatively. Those with a pain score ≥ 5 (severe pain)[4] received additional analgesia (opioids). The secondary outcomes include time to first flatus and opioid consumption in the first 24 h post-operatively. Registered nurses checked and recorded the outcomes and any adverse events.

The sample size was based on the pilot study of 30 women (n = 15 in each group) using two independent mean formula. Mean ± standard deviation (SD) of post-operative pain score in study group and control group were 4.27 ± 1.28 and 6.07 ± 1.98, respectively. The sample size was 40 participants (20 in each group), calculated per a type 1 error of 0.05 and a power of 90%, and dropout rate of 5%. The data were analyzed using STATA statistical software version 13.0. The student's t-test was used for continuous variables and reported as means and SD. The Chi-square and Fisher's exact test for categorical variables were reported as numbers and percentages. A p value < 0.05 was considered statistically significant.

Results

Forty women scheduled for elective complete surgical staging surgery were enrolled in the study, of whom 22 were diagnosed with ovarian cancer, 13 with endometrial cancer, 4 with uterine sarcoma, and 1 with fallopian tube cancer. All participants were randomized into the study group (cold gel pack) and the control group post-operatively. None of the participants was excluded, withdrew, or dropped out from the study (Fig. 1).

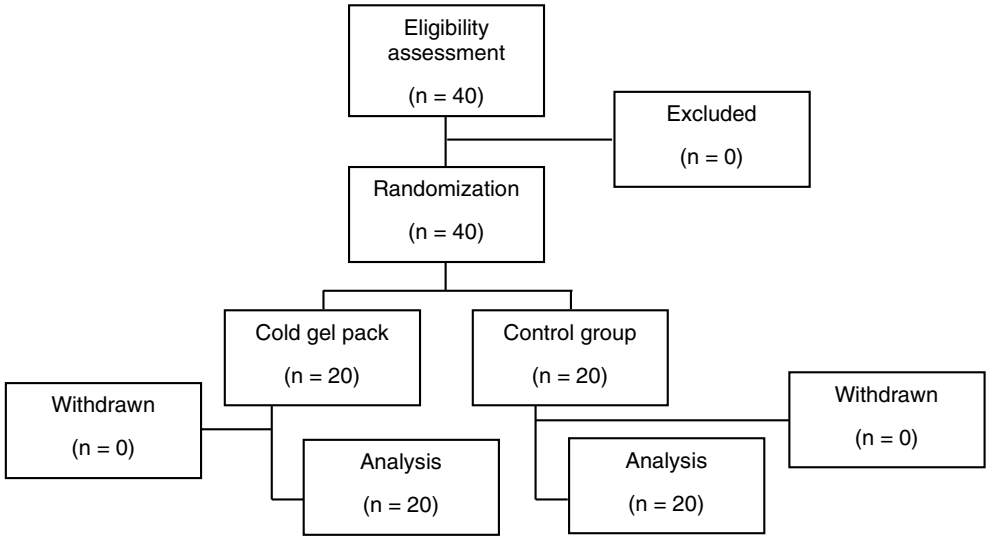

Fig. 1. Study flow diagram.

The demographic data between groups were similar, including ages, BMI, underlying diseases, and prior abdominal surgery. The peri-operative data and findings revealed that EBL was significantly lower in the study group than in the control group (227.50 ml ± 72.95 vs. 347.50 ml ± 96.8, p = 0.01) and there were no significant differences in incision length, operative time, suture materials, and opioids consumption before intervention. The post-operative pain scores at 6 h (before applying the cold gel pack) was higher in study group than control group but were not statistically significant (7.90 ± 2.12 vs 6.65 ± 1.81, p = 0.05) (Table1).

Table 1. Baseline Characteristics.

	Cold gel pack group (n = 20)	**Control group (n = 20)**	**p value**
Ages (years)*	59.80 ± 1.74	57.55 ± 2.07	0.41
(95% CI)	(56.17 - 63.43)	(53.20 - 61.90)	
BMI (kg/m2)*	24.28 ± 1.0	24.30 ± 1.21	0.56
(95% CI)	(22.26 - 26.29)	(21.76 - 26.84)	
Underlying diseases n (%)			
DM, HT	9 (45)	8 (40)	
No	11 (55)	12 (60)	0.37
Prior abdominal surgery n (%)			
Yes	13 (65)	12 (60)	0.74
Length (cm)*	14.58 ± 0.68	12.95 ± 0.41	0.45
(95% CI)	(13.14 - 16.01)	(12.09 - 13.81)	
Operative time (min) *	98.15 ± 5.86	112.40 ± 9.77	0.05
(95% CI)	(85.88 - 110.42)	(91.95 - 132.84)	
EBL (ml)*	227.50 ±72.95	347.50 ± 96.8	0.01
(95% CI)	(74.81 - 380.19)	(144.84 - 550.15)	
Suture materials n (%)			0.53
Nylon	9 (45)	11 (55)	
Staples	11 (55)	9 (45)	
Post-operative diagnosis n (%)			0.09
CA ovary	8 (40)	14 (70)	
CA endometrium	10 (50)	3 (15)	
CA fallopian tube	0 (0)	1 (5)	
Uterine Sarcoma	2 (10)	2 (10)	
Pre-intervention opioids consumption (before 6 h) (mg)	3.30 ± 0.80	3.45 ± 0.94	0.56
Post-operative pain score at 6 h	7.90 ± 2.12	6.65 ± 1.81	0.05

* mean ± standard deviation
BMI: body mass index, DM: diabetes mellitus, HT: hypertension, EBL: estimate blood loss, CI: confidence interval

The results indicated that a cold gel pack reduced post-operative pain score at 12 h in the study group compared to the control group (4.95 ± 1.67 vs. 5.90 ± 1.65, p = 0.08), albeit not statistically significant. In terms of the secondary outcomes, there was no significant difference in time to first flatus between groups (2,506.50 min ± 85.86 vs 2,473.50 min ± 189.69, p = 0.2), or post-operative additional opioid consumption (11.80 mg ± 3.37 vs 12.05 mg ± 3.52, p = 0.85) (Table 2). However, the study group had less additional opioid consumption than the control group. It was noted that there were no post-operative

complications in either group, such as re-exploring laparotomy or infection, and none of the participants had any adverse events after applying the cold gel pack.

Table 2. Outcomes of the study.

	Cold gel pack group (n = 20)	Control group (n = 20)	p value
Primary outcome			
Post-operative pain score at 12 h	4.95 ± 1.67	5.90 ± 1.65	0.08
Secondary outcomes			
Time to first flatus (min)*	2,506.50 ± 85.86	2,473.50 ± 189.69	0.20
(95% CI)	(2,326.78 - 2,686.22)	(2,076.47 - 2,870.53)	
Opioid consumption (mg)*	11.80 ± 3.37	12.05 ± 3.52	0.85
(95% CI)	(9.26 - 15.25)	(9.15 - 17.28)	

* mean ± standard deviation
CI: confidence interval

Discussion

To minimize post-operative pain, multimodalities anesthesia takes more role nowadays. In this study, we used the combined general anesthesia with TAP block in all participants and additional cold gel pack in the study group. While Nuangpho et al and Chumkam et al applied cold gel pack for 20 minutes and 2 h postoperative, then changed every 2 h for 2 consecutive times, respectively. They found that the cold gel pack was effective in reducing post-operative pain without adverse events. However, complete surgical staging takes longer time than in benign gynecologic surgery and cesarean section. Hence, we chose the duration of applying cold gel pack for 1 h in study group for the convenience, avoid adverse event especially frostbite and anticipated to improve the efficacy of cold gel pack. And post-operative pain score at 12 h may least the effect of TAP block. The primary outcome of this study found that the difference in post-operative surgical wound pain at 12 h in women undergoing complete surgical staging trended to be less in the study group than in the control group albeit not statistically significant (Fig. 2).

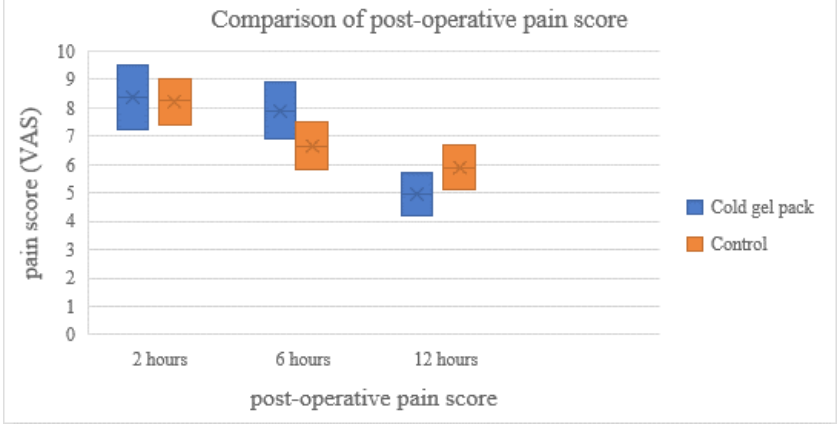

Fig. 2. Comparison post-operative pain scores between groups.

The lesser EBL may reflect the easier procedures and less intraabdominal adhesion but the operative time did not significantly difference between groups, so that the period of traction or tissue injuries were not difference. Moreover, we randomized the participants into 2 groups and baseline characteristics were not statistically difference, this factor might not have a difference effect to the outcome.

Our findings were consistent with Finan et al who reported no effect of cold gel pack in improving post-operative pain control in gynecologic patients undergoing exploratory laparotomy, measured as the amount of post-operative morphine sulfate consumption on the first operative day[11]. To contrast, Ammara et al[9], Nuangpho et al[13], Chumkam et al[14], Suwannalert et al[15], and Siripanthong et al[16] reported that ice packs or cold gel packs significantly lowered post-operative pain scores without any adverse events. The variation across studies might be due to differences in study design (e.g., type of operation and incision, operative time, time to first applying and duration for applying the cold gel pack on the surgical wound, type of cold gel pack, and the anesthesia used general anesthesia with/without additional TAP block). TAP block blocks the innervation at the abdominal wall so that the patients experience less post-operative pain. Type or length of incision wound that in malignant operations might be larger incision and require more traction than benign operations to provide optimized approach. The more traction and larger incision, the more tissue injuries, and inflammation.

Suwannalert et al[15] reported that patients undergoing cesarean section with a low midline incision and receiving post-operative cold gel packs applied at the surgical site have significantly lower post-operative pain scores from 6 h to 24 h. We identified a similar trend in our cold gel group.

Notwithstanding, the decreasing trend in post-operative pain scores at 12 h in the study group could be helpful for designing future studies. It is expected that observing post-operative pain scores for a longer period could provide more insightful data. Cold gel packs may still be helpful in patients who are allergic to analgesic drugs, have adverse events from opioids, or suffer from breakthrough pain. In such cases, cold gel packs could be used to mitigate the pain with a low risk of adverse events.

The strengths of this study were (a) it was a randomized controlled trial, and (b) no participants were lost or dropped out. Limitations included that (a) it was not blinded to both participants and medical workers, and (b) environmental factors may have interfered with temperature measurement of the cold gel pack.

Conclusion

In conclusion, the effect of a cold gel pack was not significant in reducing post-operative pain compared with standard post-operative care in patients undergoing complete surgical staging and receiving regional anesthesia for post-operative pain control.

Acknowledgments

The authors thank (a) the staff, Obstetrics-Gynecology residents, and the registered nurses at Khon Kaen Hospital for their support and help, (b) the participants for their willingness to participate in this study, and (c) Mr. Bryan Roderick Hamman for assistance with the English-language presentation of the manuscript.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Nadler SF, Weingand K, Kruse RJ. The physiologic basis and clinical applications of cryotherapy and thermotherapy for the pain practitioner. Pain Physician 2004;7:395-9.
2. Brennan TJ. Pathophysiology of postoperative pain. Pain 2011;152(3 Suppl):S33-S40.
3. Bogani G, Sarpietro G, Ferrandina G, Gallotta V, DI Donato V, Ditto A, et al. Enhanced recovery after surgery (ERAS) in gynecology oncology. Eur J Surg Oncol 2021;47: 952-9.

4. Chou R, Gordon DB, de Leon-Casasola OA, Rosenberg JM, Bickler S, Brennan T, et al. Management of postoperative pain: a clinical practice gfideline From the American Pain Society, the American Society of Regional Anesthesia and Pain Medicine, and the American Society of Anesthesiologists' Committee on Regional Anesthesia, Executive Committee, and Administrative Council. J Pain 2016;17:131-57.
5. Garcia-Quintero P, Suraci N, D Mello J. TAP blocks: A recent review of anesthetic combinations. J Pain Relief 2018;7:333:1-3
6. Transversus abdominis plane block [Internet]. Medscape 2018 [cited 15 April 2021]. Available from: https://emedicine.medscape.com/article/2000944-periprocedure?reg=1
7. Cryotherapy [Internet]. ChiroACCESS. 2022 [cited 15 April 2021]. Available from: http://www.chiroaccess.com/Articles/Cryotherapy-A-Review-of-the-Literature.aspx?id=0000070
8. Bleakley C, McDonough S, MacAuley D. The use of ice in the treatment of acute soft-tissue injury: a systematic review of randomized controlled trials. Am J Sports Med 2004;32:251-61.
9. Watkins AA, Johnson TV, Shrewsberry AB, Nourparvar P, Madni T, Watkins CJ, et al. Ice packs reduce postoperative midline incision pain and narcotic use: a randomized controlled trial. J Am Coll Surg 2014;219:511-7.
10. Koç M, Tez M, Yoldaş O, Dizen H, Göçmen E. Cooling for the reduction of postoperative pain: prospective randomized study. Hernia 2006;10:184-6.
11. Finan MA, Roberts WS, Hoffman MS, Fiorica JV, Cavanagh D, Dudney BJ. The effects of cold therapy on postoperative pain in gynecologic patients: a prospective, randomized study. Am J Obstet Gynecol 1993;168:542-4.
12. Lu YY, Su ML, Gau ML, Lin KC, Au HK. The efficacy of cold-gel packing for relieving episiotomy pain - a quasi-randomised control trial. Contemp Nurse 2015;50:26-35.
13. Nuangpho W, Srinil S, Tangsiriwatthana T, Siripipattanakul M. Gel pack reduced postoperative pain in benign gynecologic surgery: a randomized controlled trial. Thai J Obstet Gynaecol 2018; 26: 52-8.
14. Chumkam A, Pongrojpaw D, Chanthasenanont A, Pattaraarchachai J, Bhamarapravatana K, Suwannarurk K. Cryotherapy reduced postoperative pain in gynecologic surgery: a randomized controlled trial. Pain Res Treat 2019;2019:2405159.
15. Suwannalert P, Chanthasenanont A, Pongrojpaw D. Effect of applying cold gel pack on reduction of postoperative pain in cesarean section, low midline skin incision: A randomized controlled trial. J Obstet Gynaecol Res 2021;47:2653-8.
16. Siripanthong P, Wuttikonsammakit P, Chamnan P. Efficacy of cold gel pack in reducing postoperative pain in cesarean delivery at Sanpasitthiprasong Hospital: randomized controlled trial. Thai J Obstet Gynaecol 2022;30:15-24.

Thai Journal of Obstetrics and Gynaecology
July 2023, Vol. 31, No. 4, pp. 302-316.

GYNAECOLOGY

Treatments and Outcomes of Endometrial Cancers in Srinagarind Hospital

Pianpun Glaharn, M.D.*,
Bandit Chumworathayi, M.D., Ph.D.*,
Chumnan Kietpeerakool, M.D.*,
Sanguanchoke Luanratanakorn, M.D.*,
Amornrat Temtanakitpaisan, M.D.*,
Apiwat Aue-aungkul, M.D.*,
Naratassapol Likitdee, M.D.*,
Yuwadee Itarat, M.D.*,
Pilaiwan Kleebkaow, M.D.*

** Department of Obstetrics and Gynaecology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*

ABSTRACT

Objectives: Endometrial cancer was the second most common gynecologic cancer. Despite many standard guidelines, variation of management could still occur. This study was done with the objectives to compare treatments in real-life practice to standard guidelines and oncologic outcomes to quality indicators.

Materials and Methods: This retrospective descriptive analytical study was conducted in 316 endometrial cancer patients registered at Srinagarind Hospital, Khon Kaen University, during 2016-2020. Forty-three patients were excluded and 273 were analyzed. Surgical and adjuvant treatments were compared to standard guidelines. Oncological outcomes were compared to quality indicators. Prognostic factors were also analyzed.

Results: Total hysterectomy with bilateral salpingo-oophorectomies was mostly done in all women (96.3%) and some of them were done via minimal invasive surgery (7.3%). Omentectomies were done in 75.1% and all were negative. Bilateral pelvic node dissection or sampling was done in 51.6%, while 84.9% that should be done in group of those were not. About two-thirds (64.5%) of patients received adjuvant therapies, which 91.5% of their waiting time was within 60 days after surgeries. external beam radiation therapy (EBRT) was done via three-dimensional technique 100%. Over- and under-treatment were found in 16.5% (95% confidence interval (CI) 10.9-22.0) and 19.9% (95%CI 13.9-25.8) of all adjuvant therapies. There was no death within 30 days after surgeries. Three-years overall survival (OS) and recurrence-free survival (RFS) rates were 93.4% and 94.9%, respectively. Significant prognostic factors for both kinds of survivals were stage and residual lesion.

Conclusion: Over- and under-treatment of adjuvant therapy were found in 16.5% and 19.9%, respectively. Oncologic outcomes were good and comparable with others' despite of low lymphadenectomy rate. However, according to the quality indicators, we had to increase our

minimal invasive surgery rate.

Keywords: treatments, outcomes, endometrial cancers, quality indicators.

Correspondence to: *Bandit Chumworathayi, M.D., Ph.D., Department of Obstetrics and Gynaecology, Faculty of Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand. E-mail: bchumworathayi @gmail.com*

วิธีและผลการรักษาของโรคมะเร็งเยื่อบุโพรงมดลูกในโรงพยาบาลศรีนครินทร์

เพียรพันธ์ กล้าหาญ, บัณฑิต ชุมวรฐายี, ชำนาญ เกียรติพีระกุล, สงวนโชค ล้วนรัตนากร, อมรรัตน์ เต็มธนะกิจไพศาล, อภิวัฒน์ เอื้อองักูร, นราทัศพล ลิขิตดี, ยุวดี อิฐรัตน์, พิไลวรรณ กลีบแก้ว

บทคัดย่อ

วัตถุประสงค์*: โรคมะเร็งเยื่อบุโพรงมดลูก พบได้บ่อยเป็นอันดับสองของโรคมะเร็งทางนรีเวช แม้จะมีแนวทางการรักษามาตรฐานอยู่แล้วหลายฉบับ การรักษาที่แตกต่างออกไปจากแนวทางมาตรฐานก็ยังคงมีได้ การศึกษานี้จึงทำขึ้นโดยมีวัตถุประสงค์เพื่อเปรียบเทียบวิธีการรักษา ที่เกิดขึ้นในชีวิตจริงกับแนวทางการรักษามาตรฐาน และผลของการรักษามะเร็งชนิดนี้กับดัชนีชี้วัดคุณภาพ*

วัสดุและวิธีการ*: การศึกษาแบบเก็บข้อมูลย้อนหลังเชิงพรรณนาวิเคราะห์นี้ ได้ทำในผู้ป่วยโรคมะเร็งเยื่อบุโพรงมดลูก 316 รายที่ได้รับการวินิจฉัยและรักษาในโรงพยาบาลศรีนครินทร์ มหาวิทยาลัยขอนแก่น ระหว่างปี ค.ศ. 2016-2020 คัดผู้ป่วยออก 43 ราย ตามเกณฑ์ แล้วเหลือเพียง 273 ในการวิเคราะห์ ผู้วิจัยได้เปรียบเทียบวิธีการรักษา ทั้งด้วยการผ่าตัดและการเสริมด้วยรังสีรักษาหรือเคมีบำบัด กับแนวทางมาตรฐาน และเปรียบเทียบผลการรักษา กับดัชนีชี้วัดคุณภาพ รวมไปถึงการวิเคราะห์หาปัจจัยการพยากรณ์โรคที่เกี่ยวข้อง*

ผลการศึกษา*: มีการผ่าตัดเอามดลูกและปีกมดลูกออกทั้งหมดในผู้ป่วยส่วนใหญ่ (ร้อยละ 96.3) โดยในบางรายได้ทำด้วยวิธีการผ่าตัดผ่านกล้อง (ร้อยละ 7.3) มีการตัดมันเปลวออกด้วยร้อยละ 75.1 ซึ่งมีผลการตรวจทางพยาธิวิทยาเป็นปกติทั้งหมด มีการสุ่ม/เลาะต่อมน้ำเหลืองในอุ้งเชิงกรานออกร้อยละ 51.6% ในขณะที่กลุ่มไม่ได้รับการสุ่ม/เลาะ ควรได้รับการสุ่ม/เลาะถึงร้อยละ 84.9 ประมาณสองในสาม (ร้อยละ 64.5) ของผู้ป่วยได้รับการรักษาเสริมโดยร้อยละ 91.5 มีระยะเวลาการรอคอยไม่เกิน 60 วันหลังผ่าตัด รังสีรักษานอกร่างกายใช้วิธีสามมิติร้อยละ 100 การรักษามากเกินไปและน้อยเกินไปพบได้ร้อยละ 16.5 (ช่วงเชื่อมั่นร้อยละ 95, 10.9-22.0) และร้อยละ 19.9 (ช่วงเชื่อมั่นร้อยละ 95, 13.9-25.8) ของการรักษาเสริมทั้งหมด ไม่พบว่ามีการเสียชีวิตภายใน 30 วันหลังผ่าตัด อัตราการอยู่รอดโดยรวมที่สามปีและอัตราการอยู่รอดปลอดโรคเท่ากับร้อยละ 93.4 และ 94.9 ตามลำดับ ปัจจัยการพยากรณ์โรคที่มีนัยสำคัญต่ออัตราการอยู่รอดทั้งสองชนิดคือ ระยะของโรคและการมีรอยโรคหลงเหลืออยู่หลังจากการผ่าตัด*

สรุป*: การรักษามากเกินไปและน้อยเกินไปพบได้ร้อยละ 16.5 และ 19.9 ตามลำดับ ผลการรักษามะเร็งได้ผลดีที่เปรียบ*

เทียบได้กับสถาบันอื่นๆ ทั้งที่มีอัตราการสุ่ม/เลาะต่อมน้ำเหลืองต่ำ อย่างไรก็ตาม หากพิจารณาเปรียบเทียบกับดัชนีชี้วัดคุณภาพ ผู้วิจัยยังจำเป็นต้องเพิ่มอัตราการผ่าตัดผ่านกล้อง

***คำสำคัญ**: วิธีการรักษา, ผลการรักษา, มะเร็งเยื่อบุโพรงมดลูก, ดัชนีชี้วัดคุณภาพ*

Introduction

Cancers are the main cause of deaths worldwide and their prevalence tend to increase every year because of population shifting to elderly society[1]. World Health Organization (WHO) found that there were new 19 million cancer cases diagnosed and nearly 10 million cancer deaths in 2020[2]. In Thailand, breast cancer was the most common cancer in women (22.8%), and endometrial cancer was the second most common gynecologic cancers (4.6%, under cervical cancers) leading to 2.4% deaths[3].

Surgery is cornerstone of endometrial cancer for both staging and treatment to find out any indication for adjuvant therapy. Nowadays, 2009 International Federation of Gynecology and Obstetrics (FIGO) system is commonly used[4]. Surgical staging is consisted of total hysterectomy, bilateral salpingo-oophorectomy (TH/BSO) and lymph node assessment (pelvic and/or para-aortic lymphadenectomy). Omentectomy is considered in high-grade histology. Peritoneal cytology no longer affects FIGO 2009[5].

According to latest version of National Comprehensive Cancer Network (NCCN) 1.2022[6], adjuvant treatments have been mainly consisted of external beam radiation therapy (EBRT), vaginal brachytherapy (VBT) and systemic chemotherapy (CMT), being considered from FIGO stage, histologic grade, lymphovascular invasion (LVSI), age, depth of invasion, and peritoneal washing result of some high-grade histology. Authors considered this as standard guideline. Due to the easiness to follow without any need for molecular profiles data, which were still limited in many places, it was worldwide used, same as our institute. For oncological outcomes, there was also a Thai study[7] reported about survival, pathological and clinical presentation that about 80% of endometrial cancers coming with abnormal uterine bleeding (AUB) and their early stage leading to good prognosis with 5-year overall survival (OS) of 83.6%.

Despite many standard guidelines, variation of management could still occur in any real-life practices. There were substantial differences between those guidelines. Even within the same one, there also were many choices of treatments to choose. There was also a pattern of practice survey done in Australia and New Zealand[8] showing variability in adjuvant RT between VBT, EBRT, or both, particularly in stage II cases where there was lack of randomized data and discrepancies in consensus guidelines. For stage IIIA and IIIC1 cases, the majority suggested EBRT with or without VBT (79% and 77%) and of these, most were combined with CMT (61% and 88%).

The more advanced stage, the more invasive treatment procedures and modalities were recommended, but may lead to unnecessary complications without improving survival. As mentioned above, if there was indicated less aggressive treatment with equivalent outcomes, it would be better for patients. A recent review paper in 2021[9] mentioned that there was available evidence supporting VBT alone for stage I with high-intermediate risk (HIR) features or occult stage II. It provided non-different vaginal control with lower risk of toxicity compared to EBRT. VBT alone was a more non-toxic alternative to combined RT in medium risk. Superiority of VBT combined with 3 cycles of paclitaxel/carboplatin (PT), over standard EBRT in

early-stage endometrioid with HI- and hazard ratio (HR) features, was not demonstrated.

Decisions whether to perform lymphadenectomy and, if done, to what extent (bilateral pelvic node dissection or sampling (BPND/S) and/or para-aortic node dissection or sampling (PAND/S)) could be made based on preoperative and intraoperative findings[10,11]. However, this may be difficult to accurately determine final pathology results. In 2013, Belgian Cancer Registry used Rank method[12] (combination of consensus, review of the literature and panel of experts) to propose 36 quality indicators (QIs) for endometrial cancer management concerning all processes of care in different steps (treatment decision, surgery, adjuvant treatment and outcomes) and included 3 dimensions in quality of care (timeliness, effectiveness and safety). In 2017, a multicenter study has evaluated these QIs in 13 French University institutions and has identified 5 relevant QIs by their measurability (at least 80% of patients were affected by indicator) and improvability (difference between theoretical target and observed rate was below 5%)[13].

These 5 QIs were; QI 1: Proportion of patients who are alive at 3 years after their diagnosis, QI 2: Proportion of patients receiving adjuvant treatment, within a maximum waiting time of 60 days, QI 3: Proportion of patients who received adjuvant EBRT with intensity modulated radiotherapy (IMRT) or three-dimensional conformal radiotherapy (3DCRT) techniques, QI 4: Proportion of patients with clinical stage I cancer who underwent minimally invasive surgery (MIS) laparoscopy or robot-assisted, QI 5: Proportion of patients operated who died within the 30 days after surgery. Two QIs covered dimension in quality of care about adjuvant treatment (QI 2, 3), one about surgical management (QI 4), and two covered outcomes (QI 1, 5). Recently, a study was done in 2019 to evaluate French quality of care using these published relevant indicators[14].

However, it has not been yet explored about treatments and outcomes of practice in Srinagarind Hospital comparing with standard guideline and other country or institution. Despite there was the study already done in Thailand[7], only aspects of outcomes were evaluated, not any about the treatment comparison. Furthermore, the study was done a long time ago. Many things would be changed more or less, especially in demographic data and recommended management. In Thailand, even in different institutions of the same region, they could have their own unique ways of practice which were individualized. Therefore, the merit of conducting this study should be at least the awareness of authors' treatment outcomes and vulnerability for further improvement.

Materials and Methods

Srinagarind Hospital, Khon Kaen University, had been using Heath Object (HO) Program to collect patients' data in electronics' pool, which was paperless based, since January 2014. However, the system was not completely functioning until 2016. Therefore, this study was conducted in endometrial cancers patients diagnosed in Srinagarind Hospital from January 2016 to December 2020 retrieving data from the HO. A total of 316 women were diagnosed as endometrial cancers during that period (Inclusion criteria). Exclusion criteria were as follows: 1) surgeries were done elsewhere, 2) surgeries were not done, 3) final diagnoses were uterine sarcomas, 4) surgeries were done after 2020, and 5) incomplete data collection. This study was approved by the Office of the Khon Kaen University Ethics Committee in Human Research on 14 June 2021 (HE641320). In lieu of a formal ethics committee, the principles of the Helsinki Declaration were followed. After then, this retrospective study was conducted.

The following data were collected: age, parity, marital status, health insurance, Body Mass Index (BMI), other medical illnesses, previous abdominal surgeries, symptoms, mean time to surgeries, stages, histology, myometrial invasion ≥ 50%, isthmus, cervical and serosal involvement, LVSI, precision of endometrial biopsies, co-existing diseases, surgical treatments related procedures, adjuvant treatments and survival according to clinical and pathological features. Categories of BMI were classified into underweight (< 18.5 kg/m^2), normal (18.5 – < 23 kg/m^2), overweight

(23 – < 25 kg/m^2) and obese (≥ 25 kg/m^2). Other medical illnesses were underlying diseases in nervous, cardiovascular, pulmonary, gastrointestinal, renal, endocrine and/or musculoskeletal systems excluding cancers or gynecological diseases.

For adjuvant radiation therapy, the indicated patients were homogeneously scheduled following NCCN guideline[6]. External-beam doses for microscopic disease were 45 – 50 Gy or up to 60 – 70 Gy in macroscopic disease dividing into 25 – 28 fractions. Regimens were 6 – 7 Gy x 3 – 5 fractions for VBT alone and 4 – 6 Gy x 2 – 3 fractions for EBRT boost.

Recurrence-free survival (RFS) and overall survival (OS) were determined. RFS was defined as interval from end of treatment to recurrence time, disease progression or dead. For patients who were lost to follow-up, RFS data was right censored at time of the last evaluation or contact when they were known to be free from recurrence. OS were defined as time from date of diagnosis to deaths from any causes. For ones alive at time of study, survival data were right censored at date of last follow-up visit.

Sample size was calculated using 5-year OS rate of endometrial cancers from the study that was previously done in Thailand[7]. in which was 83.6%. Authors inferred that this study would get the same rate, if appropriate treatment was managed. The sample size was estimated to be about 250. Because no harm would be received from a retrospective study design, authors chose to use all patients' data within the above-mentioned period analyzed for more precision. With those numbers, it could be sufficient to show precise 95% confidence interval (CI) of proportion and rates with alpha error of 5% and test power of 80%.

Descriptive statistics (mean, number, standard deviation and percentage) were used to describe demographic data. Student t-test and Chi-square or Fisher's exact test were used to compare characteristics between groups. OS and RFS were analyzed by Kaplan-Meier method and were compared between groups with log rank test and cox proportional hazard regression model. We considered a p value of < 0.05 as statistically significant. All data analyses were performed using IBM SPSS statistics for Windows, Version 25.0 (Armonk, NY: IBM Corp).

Results

Not all data from 316 patients were analyzed, due to 43 of them were excluded by the criteria mentioned above. Details were described in Fig. 1. Finally, there were 273 fully retrieved datasets for analyses. Baseline characteristics were demonstrated in Table 1. Mean age was 58.6 years. Sixty-one (22.3%) were nulliparity. Most of them were married (85%) and had civil servant medical benefit scheme (CSMBC) and universal coverage (UC) as their health insurances (52.8% and 40.3%, respectively). Mean BMI was 26.3 kg/m2. Over a half of women was overweight or more (54.9%), had at least 1 underlying medical illness (63.7%) and previous abdominal surgery (60.5%).

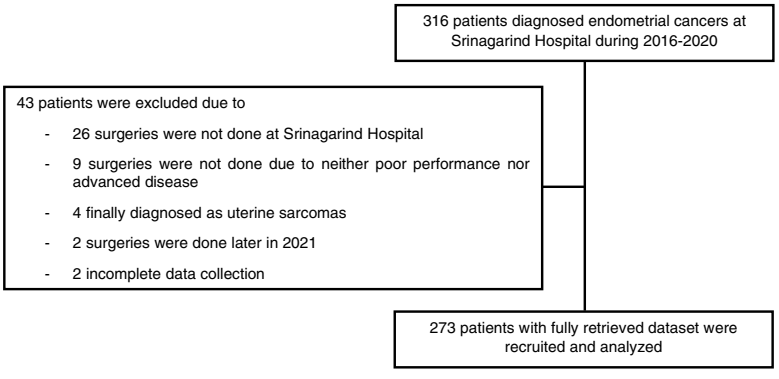

Fig. 1. Participants flow diagram.

Table 1. Baseline characteristics/ demographic data of endometrial cancers (n = 273).

Baseline characteristics/ demographic data	n (%)
Age (years) (mean ± SD)	58.6 ± 9.2
Parity	
0	61 (22.3)
1	31 (11.4)
≥ 2	181 (66.3)
last (years) (mean ± SD)	31.2 ± 9.7
Status	
Single	40 (14.6)
Married	232 (85.0)
Divorced	1 (0.4)
Health insurance	
Civil servant medical benefit scheme (CSMBC)	144 (52.8)
Universal coverage (UC)	110 (40.3)
Social security scheme (SSS)	8 (2.9)
Self-pay	11 (4.0)
BMI categories	
Underweight	13 (4.8)
Normal	110 (40.3)
Overweight	94 (34.4)
Obese	56 (20.5)
BMI (kg/m^2) (mean ± SD)	26.3 ± 5.3
Other medical illnesses	
1	61 (22.3)
≥ 2	113 (41.4)
Previous cancers	19 (7.0)
Previous abdominal surgeries	
1	132 (48.4)
≥ 2	33 (12.1)

SD: standard deviations, BMI: body mass index

Clinical and pathological features were shown in Table 2. Patients came with only one symptom and were found more than multiple complaints (57.1% and 42.9%, respectively). Most of them had abnormal uterine bleeding (AUB) (93.8%) and were in early stages (72.9%). About half were found ≥ 50% myometrial invasion (51.6%), isthmus involvement (50.2%) and positive for LVSI (45.8%). Twenty-four women did not undergo endometrial biopsies (EBs) or did but no malignancy was found (8.8%). Mean time from symptoms to surgeries was 6.4 months and from diagnoses to surgeries was 2.4 months. Endometrioid was the majority of pathology (78%). Half of women who underwent EBs had same results as final ones (53.5%), with quarter of underdiagnosis (28.5%). Common co-existing diseases were myoma uteri (45%) and adenomyosis (39.5%). There were 2.9% of second primary ovarian cancers.

Table 2. Clinical and pathological features of endometrial cancers (n = 273).

Clinical and pathological features	n (%)
Symptoms	
1	156 (57.1)
≥ 2	117 (42.9)
Abnormal uterine bleeding (AUB)	256 (93.8)
Pelvic mass	52 (19)
Abnormal Pap smear[a]	41 (15)
Intrauterine content from imaging	39 (14.3)
Mean time (months) ± SD	
Symptoms to surgeries	6.4 ± 6.7
Diagnoses to surgeries	2.4 ± 2.6
Stages	
I	166 (60.8)
II	33 (12.1)
III	55 (20.1)
IV	19 (7)
Histology	
Endometrioid grade 1 (G1)	103 (37.7)
Endometrioid grade 2 (G2)	65 (23.8)
Endometrioid grade 3 (G3)	45 (16.5)
Serous	14 (5.1)
Clear cell	11 (4)
Mixed carcinoma[b]	20 (7.4)
Carcinosarcoma[c]	15 (5.5)
Myometrial invasion ≥ 50%	141 (51.6)
Isthmus involvement	137 (50.2)
Cervical involvement	78 (28.6)
Serosal involvement	17 (6.2)
Lymphovascular invasion (LVSI)	125 (45.8)
Precision of endometrial biopsies (EB)	256 (93.8)
Correct	137 (53.5)
Incorrect	21 (8.2)
Underdiagnosed	73 (28.5)
Overdiagnosed	25 (9.8)
Co-existing diseases	
Endometrial hyperplasia	61 (22.3)
Endometritis or cervicitis	16 (5.9)
Endometrial or endocervical polyp	27 (9.9)
Myoma uteri	123 (45)
Adenomyosis	108 (39.6)
Tubal lesions (hydro-, haemato- or pyosalpinx)	54 (19.8)
Benign ovarian tumors[d]	23 (8.4)
Other ovarian cancers	8 (2.9)

[a] 17 atypical glandular cell, 11 adenocarcinoma, 7 atypical glandular cell, favor neoplasia, 4 atypical squamous cell of undetermined significance of and 1 each of low grade squamous intraepithelial lesion and high grade squamous intraepithelial lesion.

[b] 5 each of G2 and G3+clear cell, 3 G1+mucinous, 2 each of G3 and clear cell+serous and 1 each of G1+clear cell, G2+serous and G3+mucinous.

[c] 6 G3+leiomyosarcoma, 5 G2+leiomyosarcoma, 2 G1+leiomyosarcoma and 1 each of G2+serous+leiomyosarcoma and serous+leiomyosarcoma.

[d] tubo-ovarian abscess, polycystic ovary, serous or sero-mucinous cystadenoma or borderline ovarian tumors, dermoid, endometrioma, fibro-thecoma and Brenner's tumor

SD: standard deviations.

Table 3 shows surgical treatment procedures. Minimal invasive surgery (MIS) was done in 7.3%. TH/BSO was mostly done in all patients except 4 bilateral salpingectomies (1 bilateral oophorectomy was done later), 3 unilateral SOs, 2 subtotal hysterectomies, and 1 radical hysterectomy (RH). Omentectomies were done in 75.1% and all were negative. Peritoneal washings were done in 15.0% with 7.3% positive. Half of all women (51.6%) underwent BPND/S with mean number of collected nodes was 9.3 (no data in one case) and their sizes ranged from 0.6-1.4 cm (no data in 18 cases and unilateral PND/S in 7 cases). In the group of whom BPND/S was done, nearly half were collected with adequate number of nodes (42.6%). In 9 patients BPND/S could be omitted, authors still did BPND/S (the reasons would be pre-operative EBs were serous and/or intra-operative tumor sizes were almost 2 cm and/or LNs were palpable). In the group of whom BPND/S was not done, there were 84.9% that should be done. Only 8.4% underwent PAND/S with mean number of collected nodes was 3.2 (failed to get tissue in one case) and their sizes ranged from 0.8-1.3 cm. Three patients underwent PAND/S without BPND/S (all positive). Residual tumors were left in 13.6% and post-operative complications occurred in 15% which common ones were bowel events (36.6%) and wound dehiscence (29.3%). Pre-operative neoadjuvant therapies and enoxaparin were given in 4.4% and 2.6%, respectively.

Table 3. Surgical treatments related procedures of endometrial cancers (n = 273).

Surgical treatments related procedures	n (%)
Minimal invasive surgery (MIS)	20 (7.3)
Total hysterectomy (TH) and bilateral salpingo-oophorectomy (BSO)	263 (96.3)
Omentectomy	205 (75.1)
Peritoneal washing	41 (15.0)
- Positive	3 (7.3)
Bilateral pelvic node dissection or sampling (BPND/S)	141 (51.6)
- Total number (mean ± SD)	9.3 ± 5.9
- Size range (Centimeters) (min-max mean ± SD)	min 0.6 ± 0.5 max 1.4 ± 0.9
BPND/S were done in	141 (51.6)
- Could be omitted	9 (6.4)
- Adequate number (≥ 10 collected nodes)	60 (42.6)
BPND/S were not done in	132 (48.4)
- Should be done	112 (84.8)
Para-aortic node dissection or sampling (PAND/S)	23 (8.4)
- Total number (mean ± SD)	3.2 ± 2.4
- Size range (Centimeters) (min-max mean ± SD)	min 0.8 ± 0.8 max1.3 ± 0.8
Other procedures (adhesiolysis, appendectomy, biopsy, etc.)	59 (21.6)
Residual tumors	37 (13.6)
Post-operative complications	41 (15.0)
- Bowel events (evisceration, hernia, tear, etc.)	29 (36.6)
- Wound dehiscence	12 (29.3)
Pre-operative treatments	19 (7.0)
- Neoadjuvant therapy	12 (4.4)
- Enoxaparin	7 (2.6)

SD: standard deviations

Adjuvant treatments were classified in Table 4 depending on stages and histology following NCCN Guidelines Version 1.2022 in Uterine Neoplasms which was the latest update[6]. A total of 176 patients received adjuvant therapies (64.5%). After excluding 3 cases who received only neoadjuvant courses (without further treatment), there were 161 women receiving adjuvant CMTs and/or RTs (91.5%) within 60 days after surgeries (mean waiting time ± SD: 39.8 ± 22.5 days). About 40% of patients who received adjuvant therapies were given EBRT/VBT+CMT which was the most common treatment.

Over- and under-treatments were found in 16.5% (95%CI 10.9-22.0) and 19.9% (95%CI 13.9-25.8), respectively, comparing with NCCN guideline as a standard[6]. In stage IAG1, there was 1 overtreatment receiving VBT alone without neither LVSI nor age ≥ 60 years. CMT was given as neoadjuvant therapy using 4 cycles of PT due to concurrent ovarian cancer (no further adjuvant course). In stage IAG2, there were also 3 patients getting adjuvant CMT PT 6 cycles from their other primary ovarian cancers. Due to no role of EBRT in this group, there was 1 overtreatment. In stage IAG3, because no role of CMT, there were 10 overtreatments in this group (1 received EBRT without neither LVSI nor age ≥ 70 years). There was also 1 case that refused any adjuvant therapy, which was designated as an under-treatment (at least VBT was preferred in this group). In stage IA with high grade histology, there was 1 overtreatment due to no role of EBRT in clear cell without invasion and 4 under-treatments, including 3 no adjuvant therapy and 1 only a cycle of CMT (at least 4 cycles of CMT were preferred in this group). For stage IA, overtreatment group had no recurrence with 1 each of radiation burn and proctitis except 1 case that we found no data after 13 months. In undertreatment group also had no recurrence without any complication.

In stage IBG1, there were 8 overtreatments due to no role of EBRT in this group. One was given CMT from co-existing ovarian cancer. Eight patients did not receive RT, but there were only 6 undertreatments (2 neither LVSI nor age ≥ 60 years could be observed). In stage IBG2, there were 8 overtreatments, because there was no role of combined EBRT/VBT in this group. There were 2 undertreatments from no adjuvant therapy (at least VBT was preferred in this group). In stage IBG3, there were 5 undertreatments because they did not receive adjuvant RT which was at least preferred in this group. In stage IB with high grade histology, there were 3 undertreatments, including 1 without adjuvant therapy, 1 with only 3 cycles of CMT and 1 CS (carcinosarcoma) with only RT (at least CMT was preferred in this histology). For stage IB, there was no recurrence in overtreatment group but 2 cases of radiation proctitis, while 1 case was found no data after 3 months. Two cases without data after 3 and 9 months and a death from disease recurrence were found in undertreatment group.

In stage II endometrioid, there were 3 undertreatments, including 2 no adjuvant therapy and 1 only VBT and CMT (at least EBRT if grade 3 and ≥ 50% myometrial invasion). In stage II with high grade histology, there was 1 undertreatment due to only a cycle of CMT. For stage II, there was no recurrence in the undertreatment group. In stage III, there were 8 undertreatments, including 7 no adjuvant therapy and 1 only RT (at least CMT was preferred). For stage III, in the undertreatment group we found 2 deaths from disease recurrence and 3 cases with no data after 4, 9 and 16 months. In stage IV, there were 2 undertreatments, including 1 VBT alone and 1 no adjuvant therapy (at least CMT was preferred). Both of them were dead from advanced disease.

For treatment outcomes, there were 37 recurrences (13.6%) with mean time ± SD from surgeries to recurrences was 15.8 ± 11.2 months. Recurrent types were locoregional, distant and combined in 10.8%, 37.8%, and 51.4%, respectively. Fifteen patients did not receive any further treatment (40.5%). In those whom received, nearly half of them got CMT (48.6%), some were followed by RT

or given after surgeries, and some received EBRT alone. Survival rates, including 30-day, 1-, 3- and 5-year of OS and RFS were 100%, 93.4%, 81%, 76.2% and 99.6%, 94.9%, 86.2%, 83.6%, respectively. Graphs of survival time were demonstrated in Fig. 2 and 3.

Table 4. Adjuvant treatments of endometrial cancers (n = 176).

Stages	EBRT	VBT	EBRT/VBT	CMT	EBRT/ VBT+CMT	Over-treated	Under-treated
IA (31/105)							
- G1 (2/58)	-	1 (7.7)	-	1 (2.1)	-	1 (3.4)	-
- G2 (6/20)	-	2 (15.4)	1 (2.4)	3 (6.2)	-	1 (3.4)	-
- G3 (12/13)	-	-	3 (7.3)	2 (4.2)	7 (9.9)	10 (34.5)	1 (2.9)
- High grade (11/14)	-	-	-	5 (10.4)	6 (8.5)	1 (3.4)	4 (11.4)
IB (48/61)							
- G1 (13/20)	1 (33.3)	4 (30.8)	7 (17.1)	1 (2.1)	-	8 (27.6)	6 (17.1)
- G2 (14/16)	2 (66.7)	4 (30.8)	8 (19.5)	-	-	8 (27.6)	2 (5.7)
- G3 (11/14)	-	-	4 (9.8)	2 (4.2)	5 (7.0)	-	5 (14.3)
- High grade (10/11)	-	-	2 (4.9)	1 (2.1)	7 (9.9)	-	3 (8.6)
II (31/33)							
- Endometrioid (24/26)	-	1 (7.7)	15 (36.6)	2 (4.2)	6 (8.5)	-	3 (8.6)
- High grade (7/7)	-	-	-	1 (2.1)	6 (8.5)	-	1 (2.9)
III (48/55)	-	-	1 (2.4)	18 (37.5)	29 (40.8)	-	8 (22.9)
IV (18/19)	-	1 (7.7)	-	12 (25)	5 (7.0)	-	2 (5.7)
Total (176/273) n (%)	3 (1.7)	13 (7.4)	41 (23.3)	48 (27.3)	71 (40.3)	29 (16.5)	35 (19.9)

EBRT: external beam radiation therapy, VBT: vaginal brachytherapy, CMT: chemotherapy

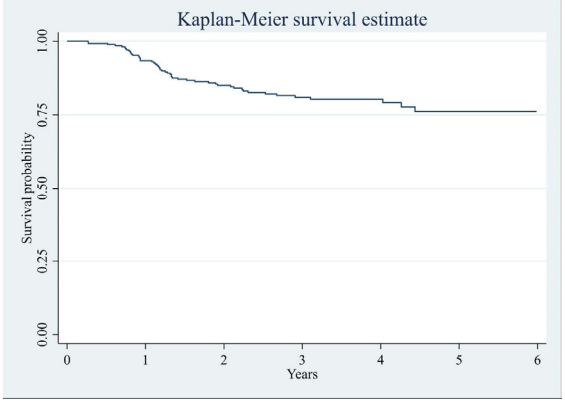

Fig. 2. Overall survival (OS).

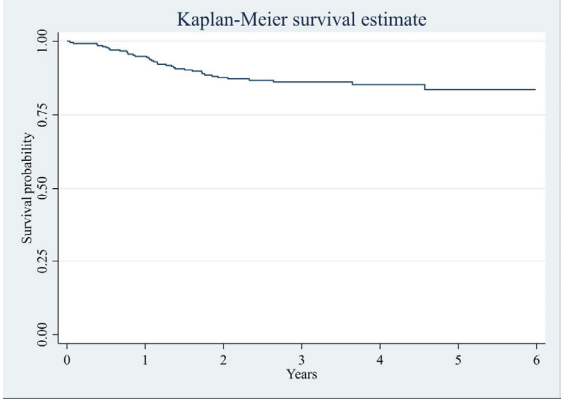

Fig. 3. Recurrence free survival (RFS).

Table 5 lists potential clinical and pathological features as prognostic factors for survival. Univariate cox proportional hazards models were fit for each covariate with both survivals. Factors that had a p value of less than 0.2 were considered feasible for multivariate analysis. Variables were subject to backwards elimination using an alpha of 0.05 for removal. Finally, there were 4 and 3 statistically significant prognostic factors for OS and RFS, consecutively which consisted of advanced stages, residual lesion (for both OS and RFS), non-endometrioid histology, LVSI (for OS only), and underlying diseases ≥ 2 (for RFS only).

Table 5. Survival of patients according to clinical and pathological features (n = 273).

Clinical and pathological features	Overall Survival Time		Recurrence-Free Survival Time	
	HR (95%CI), p	aHR (95%CI, p)	HR (95%CI), p	aHR (95%CI, p)
Age ≥ 60 years	1.094 (0.605, 1.976), 0.767	-	1.013 (0.525, 1.953), 0.969	-
Advanced stage (III – IV)	10.875 (5.383, 21.972), < 0.001	4.190 (1.898, 9.247), < 0.001	8.084 (3.986, 16.396), < 0.001	3.982 (1.742, 9.105), 0.001
Non-endometrioid histology	3.850 (2.137, 6.937), < 0.001	2.544 (1.398, 4.629), 0.002	2.317 (1.177, 4.562), 0.015	-
High grade histology (endometrioid grade 3 and non-endometrioid histology)	3.518 (1.907, 6.487), < 0.001	-	1.793 (0.940, 3.420), 0.077	-
Myometrial invasion ≥ 50%	8.080 (3.188, 20.480), < 0.001	-	4.479 (1.966, 10.203), < 0.001	-
Isthmus involvement	2.904 (1.498, 5.628), 0.002	-	2.222 (1.116, 4.423), 0.023	-
Cervical involvement	4.994 (2.711, 9.198), < 0.001	-	4.294 (2.226, 8.285), < 0.001	-
Serosal involvement	5.745 (2.732, 12.082), < 0.001	-	5.030 (2.198, 11.511), < 0.001	-
Lympho-vascular invasion	8.658 (3.665, 20.453), < 0.001	3.854 (1.551, 9.574), 0.004	3.686 (1.783, 4.618), < 0.001	-
Lymph node dissection	0.707 (0.393, 1.272), 0.247	-	0.901 (0.473, 1.718), 0.752	-
Underlying diseases ≥ 2	0.671 (0.361, 1.249), 0.209	-	0.434 (0.205, 0.919), 0.029	0.393 (0.185, 0.837), 0.015
Previous abdominal surgeries ≥ 2	0.540 (0.167, 1.746), 0.304	-	0.404 (0.097, 1.682), 0.213	-
Residual lesion	7.541 (4.186, 13.586), < 0.001	2.462 (1.286, 4.713), 0.007	10.739 (5.590, 20.628), < 0.001	5.547 (2.582, 11.916), < 0.001

* HR: hazard ratio, aHR: adjusted hazard ratio, CI: confidence interval, p: p value

Discussion

Srinagarind Hospital is one of the largest tertiary-care hospitals in Thailand. Authors found that there were 273 patients coming to treat endometrial cancer during 2016-2020 compared to those 261 patients during 1992-2008 in Vajira Hospital[7], which is at the same level of healthcare. Equal cases occurred in shorter period of time might indicate increasing incidence year by year. Patients' baseline characteristics/ demographic data were quite similar, including age, parity, underlying diseases, previous cancers, stages, histology and symptoms in which most common one was AUB. Three less common ones were pelvic mass, abnormal Pap smear and intrauterine content from imaging.

Over- and under-treatment of adjuvant therapy were found in 16.5% (95%CI 10.9-22.0) and 19.9% (95%CI 13.9-25.8), respectively. For over-treatment, the modality that was given more than suggestions had no survival benefit but risk for unnecessary complications, similar to a report from the study in Korea[15]. A total of 44 patients with mostly stage IIIC (IIIC1 36.4% and IIIC2 59.1%) received complete staging procedures including lymphadenectomy (pelvic, para-aortic and/or supraclavicular) and adjuvant CMT (75%) or chemoradiation (25%). Survival rates were not different (DFS, 81.8% vs 82.1%, p = 0.743; OS, 90.9% vs 95.8%, p = 0.537) between the 2 groups. Incidence rates of grade 2/3 gastrointestinal complications (36.4% vs 0.0%, p < 0.001) and grade 2 lymphedema (36.4% vs 9.1%, p = 0.032) were higher in chemoradiation comparing with CMT[15].

In women who underwent BPND/S (51.6%), 13.5% were upstaged, and in who underwent PAND/S (8.4%), 26.1% were upstaged. The 10 lymph nodes cutoff was chosen based on the Gynecologic Oncology Group (GOG) criteria for adequate lymphadenectomy[16], which authors also found in 42.6%. Comparing with Surveillance, Epidemiology, and End Results (SEER) data from the study including > 80,000 patients in USA during 2000-2011, lymphadenectomy was done in 57.1% which were adequate in 61.5%[17]. Previously, a full standard lymphadenectomy was recommended for all patients in author's institute. However, more selective and tailored approach is now suggested to avoid systematic overtreatment[18]. Systematic lymph node dissection (LND) was recommended in HR but its role in HI- and IR was inconclusive, therefore, there was a study reported in 2019 of more than 5,000 German patients about this topic[19]. LND was performed in 20.2%, 53% and 63.7% within low-, I-/HI- and HR groups (there was 58.5%, 30.1% and 11.4% in each group, respectively). Lymph node involvement was diagnosed in 1.7%, 9.6% and 19.3%, respectively. There was no significant difference in time to local-, lymph node recurrence or distant metastases, between ones with and without LND. After adjusting for age and comorbidity-status, also, no significant difference in OS was found. By the way, from 132 women who did not undergo BPND/S (47.7%), only 15.1% were reasonable for omitting LND[10]. This meant that there were 84.9% of them missing chances to be upstaged and to get their tumor out. More than 4 out of 5 missing the chances in this group was quite a large room for authors' improvement.

In spite of standard guidelines to follow, variation of management would still occur, even in a tertiary-care institute. The low rates of both peritoneal washing and lymphadenectomy of ours could be due to individual multifactorial causes. Although positive cytology did not impact on upstaging[5], it was a personal preference to do peritoneal washing as a standard of surgical staging or not. In the authors' institute, there was multidisciplinary consultation between gynecologic oncologist and radiologist to choose any appropriate adjuvants for each patient. VBT alone was not a common way of ours but boost after EBRT, which would mainly affect rates of overtreatment in stage IB low grade (G1 – 2). These were found over a half as shown in Table 4. From this reason, some surgeons may infer that EBRT and VBT would be anyway given in whom having grossly intraoperative stage IB. Finally, they came up with assumption that omitting lymphadenectomy could

cause less complication. By the way, it was not yet to neither summarize nor suggest skipping nodal dissection with adding EBRT, without more data and details about oncological outcomes and adverse events from this comparison.

Comparing with the study in France[14], authors' results for 4 out of 5 QIs were better except only MIS rate that we had to improve; QI1: Proportion of patients who are alive 3 years after their diagnosis (81% vs 77%), QI 2: Proportion of patients receiving adjuvant treatment, within a maximum waiting time of 60 days (93% vs 47.8%), QI 3: Proportion of patients who received adjuvant EBRT with IMRT or 3DCRT (100% vs 83.3%), QI 4: Proportion of patients with clinical stage I cancer who underwent MIS (7.3% vs 17.3%), and QI 5: Proportion of patients operated who died within the 30 days after surgery (0% vs 0.5%). However, in Thailand, MIS are not available for all patients. They had to pay 10,000-20,000 THB more to get this kind of operation in author's institute.

For treatment outcomes, 5-year OS and RFS (76.2 % and 83.6%) were slightly lower than the study previously done in Thailand (83.6% and 86.5%)[7]. These could be due to shorter follow-up times (5 vs 16 years). Rates of BPND/S and PAND/S, which were much less in our study (51.6% vs 93.1% and 8.4% vs 70.5%), did not seriously affect survivals, and were not the significant prognostic factors, as seen in Table 5. Anyway, for univariate analysis, clinical and pathological features as prognostic factors for survivals were quite similar (as they were not statistically significant different). After multivariate analysis, there were 4 and 3 statistically significant prognostic factors for OS and RFS, consecutively which consisted of advanced stages, residual lesion (for both OS and RFS), non-endometrioid histology, LVSI (for OS only), and underlying diseases ≥ 2 (for RFS only).

Endometrial cancer is histologically classified into type I and type II in relation to estrogen. Clinicopathological analysis revealed that type II (non-endometrioid) is generally more aggressive than type I (endometrioid) and is associated with poorer prognosis even when diagnosed at early stage[20]. Data from study done in Japan found there were 2 times more recurrences of type II than type I (34% VS 17%) in patients without residual tumor[21]. Women with type I were associated with obesity and more likely to die of cardiovascular disease than cancer[22]. A study done in China showed that metabolic syndrome was closely related to OS (HR 2.14, p = 0.032) and RFS (HR 1.80, p = 0.045) by univariate but not multivariate analysis[23]. More specifically, patients with 3 or more components had worse outcome than those with lesser ones. According to what authors mentioned above, the reason why women with underlying diseases ≥ 2 had better RFS than those with one or none, should be that type I patients were included more in this group. Unfortunately, authors found no statistically significant correlation to those 4 significant prognostic factors (advanced stages, residual lesion, non-endometrioid and LVSI, by post-hoc analyses). This might have occurred by chance.

Conclusion

Over- and under-treatment of adjuvant therapy were found in 16.5% (95%CI 10.9-22.0) and 19.9% (95%CI 13.9-25.8), respectively. The oncologic outcomes were good and comparable with others' despite of low lymphadenectomy rate. However, according to quality indicators, authors had to increase minimal invasive surgery rate. The strengths were that this was the second study done in Thailand evaluating outcomes of endometrial cancers and the first study comparing about treatment methods and oncological outcomes to standard guideline and quality indicators. Retrospective design was the limitation of this study, it could be more reliable with prospective design and data about targeted therapy and molecular profiles.

Acknowledgments

Authors are thankful to all supporting staffs and patients at Srinagarind Hospital involving in this study. This study was approved by the Postgraduate

Committee of the Department as a Gynecologic Oncology Fellowship thesis of Dr Glaharn and supported by funding from authors.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Pilleron S, Sarfati D, Janssen-Heijnen M, Vignat J, Ferlay J, Bray F, et al. Global cancer incidence in older adults, 2012 and 2035: A population-based study. Int J Cancer 2019;144:49-58.
2. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, et al. World factsheets: GLOBOCAN 2020 [Internet]. Lyon: International Agency for Research on Cancer/World Health Organization; 2020. [cited 24 January 2021] Available from: https://gco.iarc.fr/today/data/factsheets/populations/900-world-fact-sheets.pdf
3. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, et al. Thailand factsheets: GLOBOCAN 2020 [Internet]. Lyon: International Agency for Research on Cancer/World Health Organization; 2020. [cited 24 January 2021] Available from: https://gco.iarc.fr/today/data/factsheets/populations/764-thailand-fact-sheets.pdf
4. Creasman W. Revised FIGO staging for carcinoma of the endometrium. Int J Gynaecol Obstet 2009;105:109.
5. Mariani A, Dowdy SC, Podratz KC (2009). New surgical staging of endometrial cancer: 20 years later. Int J Gynaecol Obstet 2009;105:110-1.
6. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guideline in Oncology: Uterine Neoplasms (Version 1.2022). Plymouth Meeting: National Comprehensive Cancer Network; 2021. [cited 3 February 2022]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/uterine.pdf
7. Tangjitgamol S, Manusirivithaya S, Srijaipracharoen S, Khunnarong J, Tanvanich S, Katanyu K, et al (2010). Endometrial cancer in Thai women: clinico-pathological presentation and survival. Asian Pac J Cancer Prev 2010;11:1267-72.
8. Lapuz C, Govindarajulu G, Tacey M, Lim A, Johnson C. Adjuvant radiotherapy for endometrial cancer with cervical stromal involvement: A patterns of practice survey in Australia and New Zealand. J Med Imaging Radiat Oncol 2021;65:95-101.
9. Serkies K, Baczkowska-Waliszewska Z. Adjuvant vaginal cuff brachytherapy in surgically treated endometrial carcinoma patients – in view of the recent evidence. J Contemp Brachytherapy 2021;13:221-30.
10. Neubauer NL, Lurain JR. The role of lymphadenectomy in surgical staging of endometrial cancer. Int J Surg Oncol 2011;2011:814649.
11. Milam MR, Java J, Walker JL, Metzinger DS, Parker LP, Coleman RL. Nodal metastasis risk in endometrioid endometrial cancer. Obstet Gynecol 2012;119:286-92.
12. Werbrouck J, Bouche G, de Jonge E, Jacomen G, D'Hondt V, Denys H, et al. Evaluation of the quality of the management of cancer of the corpus uteri--selection of relevant quality indicators and implementation in Belgium. Gynecol Oncol 2013;131:512-9.
13. Chenoz L, Phelippeau J, Barranger E, Bourdel N, Brun JL, Chereau E, et al. Evaluation and Selection of Quality Indicators for the Management of Endometrial Cancer. Int J Gynecol Cancer 2017;27:979-86.
14. Larouzée E, Phelippeau J, Roberti E, Koskas M. Evaluation of the French medical practices in endometrial cancer management by using quality indicators. Eur J Obstet Gynecol Reprod Biol 2019;236:198-204.
15. Kong TW, Jo E, Son JH, Paek J, Chang SJ, Ryu HS. Treatment outcomes and complications in FIGO stage IIIC and IVB endometrioid endometrial cancer patients presenting as nodal spreads following systematic lymphadenectomy and adjuvant therapy. J Obstet Gynaecol Res 2021;47:3322-30.
16. Hill HA, Eley JW, Harlan LC, Greenberg RS, Barrett RJ 2nd, Chen VW. Racial differences in endometrial cancer survival: the black/white cancer survival study. Obstet Gynecol 1996;88:919-26.
17. Rodriguez AM, Schmeler KM, Kuo YF. Lack of improvement in survival rates for women under 50 with endometrial cancer, 2000-2011. J Cancer Res Clin Oncol 2016;142:783-93.
18. Soliman PT, Frumovitz M, Spannuth W, Greer MJ, Sharma S, Schmeler KM, et al. Lymphadenectomy during endometrial cancer staging: practice patterns among gynecologic oncologists. Gynecol Oncol 2010;119:291-4.
19. Pölcher M, Rottmann M, Brugger S, Mahner S, Dannecker C, Kiechle M, et al. Lymph node dissection in endometrial cancer and clinical outcome: A population-based study in 5546 patients. Gynecol Oncol 2019;154:65-71.
20. Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: more than two types. Lancet Oncol 2014;15:e268-78.
21. Miyahara D, Yotsumoto F, Hirakawa T, Yoshikawa K, Shigekawa K, Miyata K, et al. Clinical Features of

Recurrence in Patients Without Residual Tumour in Endometrial Cancer. Anticancer Res 2019;39:4581-8.
22. Ward KK, Shah NR, Saenz CC, McHale MT, Alvarez EA, Plaxe SC. Cardiovascular disease is the leading cause of death among endometrial cancer patients. Gynecol Oncol 2012;126:176-9.
23. Yang X, Li X, Dong Y, Fan Y, Cheng Y, Zhai L, et al. Effects of Metabolic Syndrome and Its Components on the Prognosis of Endometrial Cancer. Front Endocrinol (Lausanne) 2021;12:780769.