ISSN 0857-6084

THAI JOURNAL OF OBSTETRICS AND GYNAECOLOGY

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

VOL. 32 NO. 1 JANUARY - FEBRUARY 2024

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.
C. Matatratip, M.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.

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.B.A. (Management)
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

ISSN : 0857-6084. The Official Journal of the Royal Thai College of Obstetricians and Gynaecologists

Reviewer acknowledgement 2024

The editors would like to publicly acknowledge the people listed below who served as reviewers on the journal during 2023. Without their efforts, the quality of the journal could not be sustained. We appreciate their time and effort in evaluating papers and express our sincere thanks for their hard work and support.

Alok Kumar Dwivedi	Texas Tech University Health Sciences Center	United States
Arb-aroon Lertkhachonsuk	Mahidol University	Thailand
Areepan Sophoonsritsuk	Mahidol University	Thailand
Boonsri Chanrachakul	Mahidol University	Thailand
Chanakarn Suebthawinkul	Chulalongkorn University	Thailand
Chenchit Chayachinda	Mahidol University	Thailand
Chuenkamon Charakorn	Mahidol University	Thailand
Dittakarn Borriboonhiransan	Mahidol University	Thailand
Gul Nihal Buyuk	Ankara Zekai Tahir Burak Education and Research Hospital	Turkey
Jin Huang	Peking University Third Hospital	China
Jitti Hanprasertpong	Prince of Songkla University	Thailand
Jittima Manonai Bartlett	Mahidol University	Thailand
Katika Nawapun	Mahidol University	Thailand
Kittipat Charoenkwan	Chiang Mai University	Thailand
Lunan Ji	Columbia University Medical Center	United States
Lunthaporn Puttanavijarn	Rajavithi Hospital, College of medicine, Rangsit University	Thailand
Mahmoud Alalfy	National Research Centre (Egypt), Aljazeerah Hospital	Egypt
Mahsa Imanian	Jahrom University of Medical Sciences	Iran
Mingxing Xie	Tongji Hospital (Tongji Medical College)	China
Natacha Phoolcharoen	Chulalongkorn University	Thailand
Nisarat Yamaphai	Mahidol University	Thailand
Nitikorn Phoosuwan	Uppsala University	Sweden
Ounjai Kor-anantakul	Prince of Songkla University	Thailand
Panyu Panburana	Mahidol University	Thailand
Pakhee Aggarwal	All India Institute of Medical Sciences	India
Panchampreet Kaur	Indian Institute of Technology Delhi	India
Pornpimol Ruangvutilert	Mahidol University	Thailand
Pornswan Ngamprasertwong	Cincinnati Children's Hospital Medical Center	United States
Porntip Sirayapiwat	Chulalongkorn University	Thailand
Rabi'at Muhammad Aliyu	Ahmadu Bello University Teaching Hospital	Nigeria
Rabiul Islam	Department of Pharmacy, University of Asia Pacific	Bangladesh
Rachel Y K Cheung	The Chinese University of Hong Kong	Hong Kong
Ruangsak Lertkhachonsuk	Chulalongkorn University	Thailand
Shashikala K Bhat	Manipal Academy of Higher Education	India
Shina Oranratanaphan	Chulalongkorn University	Thailand
Snehamay Chaudhuri	NRS Medical College	India
Somsook Santibenchakul	Chulalongkorn University	Thailand
Sukanya Chaikittisilpa	Chulalongkorn University	Thailand
Sumonmal Manusirivithaya	Navamindradhiraj University	Thailand
Suthee Panichkul	Phramongkutklao College of Medicine	Thailand
Suvit Bunyavejchevin	Chulalongkorn University	Thailand
Thanapan Choobun	Prince of Songkla University	Thailand
Tharangrut Hanprasertpong	Srinakharinwirot University	Thailand
Theera Tongsong	Chiang Mai University	Thailand
Thiti Snabboon	Chulalongkorn University	Thailand
Thitima Suntharasaj	Prince of Songkla University	Thailand
Tsia-Shu Lo	Chang Gung Memorial Hospital International Medical Center	China
Varsha Huddar	KLE's Institute of Physiotherapy	India
Vorapong Phupong	Chulalongkorn University	Thailand
Wirawit Piyamongkol	Chiang Mai University	Thailand
Worasak Keeyapaj	Stanford University School Medicine	United States
Xuhong Li	Central South University	China
Yenrudee Poomtavorn	Thammasat University	Thailand
Ying Ying Ng	Universiti Sains Malaysia	Malaysia

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. 32 No. 1 JANUARY - FEBRUARY 2024

CONTENTS

Thai Journal of Obstetrics and Gynaecology
January 2024, Vol. 32, No. 1, pp. 1.

EDITORIAL

At the beginning of New Year 2024, it's time for beginning good things. May this year bring happiness, new inspirations and new success to all members of Royal Thai College of Obstetricians and Gynaecologists (RTCOG).

The quality of Thai Journal of Obstetrics and Gynaecology (TJOG) has been improved. In year 2023, TJOG received the Q3 journal rankings (149/203 journals) with SJR score 0.158 in Obstetrics and Gynaecology category from Scimago Journal & Country Rank 2022. TJOG has been indexed in many databases: Scopus, TCI, ASEAN Citation Index, DOAJ, EuroPub, and Google Scholar.

For the New Year 2024, we would like to extend our warmest wishes to RTCOG members, editorial board, reviewers, authors and families. We sincere thank to all the authors, readers, reviewers, and editors for your contributions to TJOG the past year and look forward to receiving your invaluable contributions in 2024.

This first issue of TJOG 2024 contains many interesting articles. One special article is "Acute vaginal candidiasis: another step forward to the deeper understanding". The contents include factors impacting vaginal balance, diagnosis, treatment and patient guidance.

Happy New Year 2024

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

Thai Journal of Obstetrics and Gynaecology
January 2024, Vol. 32, No. 1, pp. 2-12.

SPECIAL ARTICLE

Acute Vaginal Candidiasis: Another Step Forward to the Deeper Understanding

Chenchit Chayachinda, M.D.*,
Kittipoom Chinhiran, M.D.**,
Payaow Aneklap***,
Porntip Rachapromma***,
Sunisa Sonwicha, M.D.*,
Chanon Neungton, M.D.*

** Unit of Infectious Diseases, Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*
*** Bangrak STIs Center, Division of AIDS and STIs, Department of Disease Control, Ministry of Public Health, Bangkok, Thailand*
**** Department of Nursing, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*

ABSTRACT

Vaginal candidiasis (VC) arises from an imbalance in the vaginal milieu influenced by intrinsic and extrinsic factors, including diet, hormonal fluctuations, and genital hygiene. The predominant causative organisms are *Candida* spp., with *C. albicans* being the most prevalent. *Candida* spp. are part of the normal flora residing on the vaginal mucosa and other mucous membranes throughout the body. The human body employs numerous mechanisms to maintain equilibrium between these fungi and commensal bacteria. Any disruption of this equilibrium can result in *Candida* overgrowth, manifesting as symptoms such as profuse vaginal discharge or burning and itching of the vulvar and vaginal region. In 2022, the Royal Thai College of Obstetricians and Gynaecologists issued treatment guidelines for reproductive-age women presenting with abnormal vaginal discharge. These guidelines recommend various pharmacological regimens and behavior modifications to minimize future recurrence. Nevertheless, experts in real-world practice often suggest adaptations to these regimens to enhance therapeutic outcomes. The Siriraj Female Sexually Transmitted Infections Clinic regularly manages VC cases and implements a 2-week follow-up protocol for all patients. Given that VC is the most common diagnosis for abnormal vaginal discharge at the clinic, significant expertise has been accumulated in treating the condition. The collected perceived insights can be invaluable for fellow professionals, potentially broadening service perspectives and catalyzing further research.

Keywords: vaginal candidiasis, reproductive-aged, treatment, alternative, pap smear.

Correspondence to: *Chenchit Chayachinda, M.D., Unit of Infectious Diseases, Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*
E-mail: chenchit.cha@mahidol.ac.th

Received: 5 September 2023, **Revised:** 22 December 2023, **Accepted:** 25 December 2023

Introduction

Vaginal imbalances are commonplace and can lead to abnormal proliferation of vaginal organisms. These imbalances can result in conditions such as an overgrowth of fungi (fungal vaginitis) and a surge in anaerobic bacteria (bacterial vaginosis; BV), aerobic bacteria (aerobic vaginitis; AV), or *Lactobacilli* (cytolytic vaginitis; CV)[1]. The most prevalent conditions are bacterial vaginosis and fungal vaginitis[2]. The most common type of fungal vaginitis is caused by the genus *Candida*, and it is often referred to as vaginal candidiasis (VC). This condition is caused by an increase in *Candida* spp., predominantly *C. albicans*, a component of the vaginal flora[3]. The symptoms of VC include a profuse, curd-like discharge that might separate into a watery, yogurt-like consistency, vulvar and vaginal itching, redness, swelling, abrasiveness, dysuria, and dyspareunia[1]. Despite the previous report showing that untreated VC in pregnant women was associated with an increased risk of preterm labor[4], recent evidence reveals some controversy for this issue[5].

Affecting women of all ethnicities, VC is estimated to occur in 75% of women at some point in their lives[6]. Studies indicate that 10%–20% of asymptomatic, nonpregnant women harbor VC[3], with the prevalence rising to 20% in second-trimester pregnant women and up to 52% in the third trimester[7, 8]. Understanding the mechanisms driving the transition from a normal balance of organisms to a disease state, alongside the available treatment modalities and their limitations, is crucial. VC is typically categorized into acute and recurrent types[9]. Recurrent VC is defined as having at least 4 episodes, including the present one, in prior one year; and requires a much longer course of treatment.

In 2022, the Royal Thai College of Obstetricians and Gynaecologists issued guidelines for the management of reproductive-age women with abnormal vaginal discharge. These guidelines recommend various drug regimens and behavioral modifications to minimize the recurrence risk. However, some experts, including our staff members, argue for adjusting specific regimens to optimize therapeutic outcomes[10]. Consequently, the Siriraj Female Sexually Transmitted Diseases Clinic has expanded its medication offerings for treatment. In the past three years, all patients were advised to return for re-evaluation. Those without cure received secondary treatment and few needed the third or fourth regimen. This article focuses primarily on acute vaginal candidiasis, the most prevalent type, and addresses the challenge of managing patients with fungi detected in Pap smears—a frequent issue in medical practice.

Candida spp. and Immunopathology

Candida spp. are fungi from the family Saccharomycetaceae, a family of yeasts that reproduce by budding and prefer carbohydrate-rich environments, on which they feed. The *Candida* genus comprises more than 30 pathogenic species, with *C. albicans* being the most prevalent. Notably, *C. albicans* can morph between the yeast and pseudohyphae forms. Cellularly, *C. albicans* is characterized outside by a cell wall, followed by a plasma membrane, cytoplasm, and various organelles. The wall has a core skeleton composed of β-glucan and chitin, providing structural strength and ensuring shape retention. The outermost layer of the wall is coated with mannan, which exhibits low permeability. The innermost layer of the cell wall, just before the plasma membrane, is made of chitin, which is particularly robust[11]. The plasma membrane itself is a bilipid layer comprising ergosterol and phospholipids. The external structures of *Candida* spp. serve as targets for antifungal drugs[12].

C. albicans is a component of the normal flora that inhabits the mucous membranes of the intestines, oral cavity, and vagina. However, when these mucosal barriers weaken and local immunity is compromised, *Candida* spp. multiply and induce disease. The pathogenesis of this fungus arises from a combination of several virulence factors. Polymorphism allows the fungi to assume various shapes. Their invasive capabilities permit cell entry via induced endocytosis and active penetration. This intrusion leads to an accumulation of the toxin called candidalysin on the

surface resulting in inflammatory mucosal damage that later destroys host's innate immune cells. Furthermore, the capacity of *Candida* spp. for adhesion and biofilm formation enables them to bind to both biotic (living) and abiotic (nonliving) surfaces, such as catheters and intrauterine devices (IUDs). These biofilms not only provide a protective habitat for the fungi, but also protect them from external threats. Genetic and metabolic plasticity endows *Candida* spp. with significant metabolic flexibility, facilitating environmental adaptation and rapid evolutionary modification. This plasticity allows the fungi to survive pressure and stress, especially from antifungal drugs and host defense responses. Furthermore, the robust structure of the cell wall equips it with resilience against external factors and offers mechanisms to evade host immune detection[13].

A healthy vagina sustains a balanced mix of bacteria and fungi, and *Lactobacilli* playing a pivotal role in the preservation of this equilibrium. *Lactobacilli* generate lactic acid and hydrogen peroxide that neutralize other organisms and form a biofilm on the vaginal walls[14]. A reduction in *Lactobacillus* levels allows the proliferation of resident fungi, leading to disease. The progression of fungal vaginitis entails 3 stages: adhesion, blastopore germination, and invasion. Notably, *C. albicans* exhibits a superior adhesive capability[15]. The presence of estrogen potentiates this adhesion by increasing the surface exposure of glycoprotein complexes, which act as receptors[16]. Moreover, *Lactobacilli* secrete bacteriocin, thereby impeding fungal germination, and compete with *C. albicans* for limited nutrients[17].

When fungal hyphae extend, they attempt to infiltrate the vaginal wall. In response, the body deploys neutrophils, macrophages, and dendritic cells, leading to the release of proinflammatory cytokines and chemokines that further recruit macrophages and neutrophils. Simultaneously, fungi secrete candidalysin, inflicting direct damage on the epithelium. The epithelium retaliates by releasing antimicrobial peptides that restrict fungal growth, damage-associated molecular patterns that amplify inflammation, and additional chemokines and cytokines. Although neutrophils can eliminate *Candida* through phagocytosis, the fungi's ability to morph from yeast to hyphae complicates this process. After phagocytosis, the fungus continues its yeast-to-hyphae transformation, resulting in neutrophil death. Another contributing factor involves the formation of neutrophil extracellular traps, leading to further neutrophil death. Following these events, adaptive immunity is activated, prompting T cells to target fungi and promoting dendritic cell maturation to enhance fungal elimination. However, fungi are also capable of adaptation, thus increasing their resilience to external conditions. This dual mechanism of inflammation and cellular destruction provides fungi with additional nutrients from host cells, which supports their proliferation[13].

The regulation of *Candida* populations is location dependent. For instance, oral mucosal *Candida* levels are modulated by T cells (Th17 immunity), whereas the vaginal environment is controlled by neutrophil-mediated immunopathology. This difference becomes apparent in patients living with human immunodeficiency virus (HIV): a direct impact on T cells leads to increased oropharyngeal candidiasis, but the incidence of VC remains stable[13].

Factors Impacting Vaginal Balance

Vaginal imbalance, induced by both intrinsic and extrinsic factors, can persistently occur and eventually culminate in VC. Intrinsic factors are sex hormone levels and menstrual blood. Specifically, estrogen plays a pivotal role in *Candida* pathogenesis[16], while menstruation disrupts vaginal balance by diminishing *Lactobacillus* populations. Contraceptive methods that induce spotting or elevate estrogen levels can likewise foster *Candida* proliferation. Moreover, vaginal douching can disrupt *Lactobacillus* activity and amplify VC risks[18], with a higher incidence of nonalbicans *Candida* noted[19].
A diet abundant in sugary foods promotes fungal growth[20]. Our experience has shown that the decreased intake of a sugar-rich diet results in an increase in the culture-based cure rate[3]. Pregnancy,

characterized by fluctuating blood sugar levels and escalated estrogen, fosters VC development—a scenario also seen in individuals with diabetes. Long-term antibiotic use can dramatically reduce *Lactobacillus* numbers, precipitating VC. Although tight clothing has not been directly associated with VC[21], it should be avoided during symptomatic periods. Additional extrinsic factors include stress[22], iron deficiency anemia, and excessive genital cleaning[9].

Diagnosis

The diagnosis of VC necessitates laboratory confirmation, such as microscopic examination of the vaginal discharge diluted with a saline solution. When this is combined with a 10% potassium hydroxide solution, pseudohyphae become visible, as the solution is unable to degrade chitin, a crucial cell wall component. Nevertheless, relying solely on visual examination of vaginal discharge yields limited diagnostic sensitivity[23]. Culture is beneficial for recurrent VC or cases where symptoms are present without detectable pseudohyphae under microscopy. Conversely, molecular testing offers limited value[9].

The differential diagnoses of excessive discharge combing with vaginal itching are trichomoniasis, cytolytic vaginitis, and aerobic vaginitis[1]. They can be differentiated by wet preparation (proportion of leukocytes and epithelial cells) and vaginal pH[24]. The Siriraj Female Sexually Transmitted Infections Clinic has provided a 5-minute long educational tool on wet preparation for medical students and healthcare providers (Fig. 1). Our experience has shown that briefly reviewing the online tool has a great impact on the recall to perform effective wet preparation[25].

Fig. 1. QR code of an on-line educational tool on wet preparation

Source: Unit of Infectious Diseases, Department of Obstetrics and Gynaecology, Faculty of Medicine, Siriraj Hospital, Mahidol University

When primary symptoms are itching in the external genitalia, the differential diagnoses expand to four possible groups; chronic inflammatory dermatoses, preinvasive lesions, infections and secondary causes[26]. Chronic inflammatory dermatoses include lichen sclerosus, lichen planus, contact dermatitis, eczema, and psoriasis. Preinvasive lesions include Paget's disease, melanoma in situ, and vulvar intraepithelial neoplasia. Infections include herpes genitalis, genital wart and fungal infection. Secondary causes include allergy, diabetes mellitus, shaving, tight clothing and psychological cause (vulvodynia). Interestingly, our previous report showed that 10% of women presenting with anogenital warts concurrently had VC[27].

Coinfections with other conditions are common, particularly with bacterial vaginosis[28], due to a similar reduction in *Lactobacilli*. In response to environmental stressors, anaerobes and *Candida* collaboratively form a mixed biofilm that provides protection. *Candida*'s low-oxygen biofilm enables rapid growth of anaerobes, even in the presence of potentially higher oxygen levels outside the biofilm[28]. These biofilms play an important role in therapeutic

response and recurrence, with biofilms dominated by multiple *Candida* strains further complicating treatment strategies[29].

Treatment

In line with the treatment guidelines provided by the Center for Disease Control and Prevention and the British Association for Sexual Health and HIV[9, 30], the Royal Thai College of Obstetricians and Gynaecologists has established guidelines for the treatment of reproductive-age women with abnormal vaginal discharge. The guidelines were informed by practices from multiple countries, and they factored in drugs used in Thailand. They include recommendations for acute VC treatment (Table 1)[24].

Table 1. The Royal Thai College of Obstetricians and Gynaecologists' Treatment Guidelines for Reproductive-Age Women with Acute Vaginal Candidiasis

Recommended treatment regimens	Caution
- Fluconazole 150-200 mg orally single dose - Itraconazole 200 mg orally twice daily for 1 day - Clotrimazole 500 mg vaginal suppository single dose - Clotrimazole 200 mg vaginal suppository daily for 3 days - Clotrimazole 100 mg vaginal suppository daily for 6-7 days - Miconazole 100 mg vaginal suppository daily for 7 days - Miconazole 200 mg vaginal suppository daily for 3 days	Pregnant women with vaginal candidiasis cannot take oral medications because fluconazole is a pregnancy category C. There was a report showing an association between congenital heart defect and maternal intake of fluconazole 400-800mg/d during the first trimester(31).

Notes: - Putting gentian violet in the vagina will help the early improvement of the vaginal itching.
- Nystatin 100 000 units (Gynecon®) vaginal suppositories for 14 days or dequalinium chloride 10 mg vaginal suppositories for 6 days are alternative regimens for azole-resistant individuals.
- Sertaconazole 300mg is available in Thailand and has an acceptable efficacy.
Reference: Royal Thai College of Obstetricians and Gynaecologists[24]

The azole group, comprising imidazoles (clotrimazole, miconazole, ketoconazole, sertaconazole, econazole) and triazoles (itraconazole, fluconazole), functions by inhibiting 14α-sterol-demethylase. The production of ergosterol, a vital component of the plasma membrane of *Candida* spp., is thereby reduced. This inhibition results in cell death. Triazoles, which minimally affect sterol synthesis in other body parts, can be administered orally. However, they may present cytochrome P450-dependent enzyme side effects such as vomiting, liver enzyme abnormalities, and QT prolongation[33]. Miconazole also operates by augmenting reactive oxygen species within cells, thus enhancing the fungicidal activity of miconazole compared to other drugs[34]. The predominant mechanism of drug resistance is mutations in ERG11, the gene coding for 14α-sterol-demethylase[33].

Nystatin, a polyene, binds directly to ergosterol, triggering plasma membrane perforation, cytoplasmic leakage, acidification, cell lysis, and ultimately cell death. Potential side effects are rash, localized pain, and itching[35]. In Thailand, combination drugs such as nystatin 100 000 IU + diiodohydroxyquinoline 100 mg + benzalkonium chloride 7 mg (brand name: Gynecon®) are available. Gynecon® is packaged in a 7 once-daily-tablet box, which can be used as the primary treatment. However, the antiseptic drugs being combined cause local irritation resulting in their lower popularity. Two boxes of Gynecon® are recommended for those who fail many azole regimens or are suspicious of azole resistance. The long regimen of 14 days can be interrupted by the menstrual period.

Dequalinium chloride, a quaternary ammonium salt, demonstrates antimicrobial and antimycotic activity by increasing cell permeability, leading to a loss of mitochondrial ATP synthesis[36]. Its multipurpose may fit health professionals who are not keen on wet preparation. Nonetheless, follow-up must not be neglected. A study on Thai women reported that the treatment efficacy of dequalinium chloride was non-

significantly inferior to that of clotrimazole[37].

The adjunct treatment using topical gentian violet is also recommended as it significantly lessen time-to-cure[3]. Gentian violet is a permanently stained violet solution. It has been used to treat both skin and mucosa fungal infections in both children and adults. One mL of gentian violet is pushed into the vaginal cavity during pelvic examination, preferably after removal of curd-like discharge. A sanitation napkin should always be provided after this application. In addition, all patients must be informed that violet vaginal discharge will be evident for a few more days without serious side effects.

Patient Guidance

Health professionals must ensure that patients comprehend the nature of their ailment, its causes, the prescribed treatment, and necessary self-care measures. They must clearly communicate that VC is not a sexually transmitted disease; therefore, treating sexual partners is not advised. Health professionals should encourage patients to adopt long-term self-care strategies to mitigate risks, as the condition predominantly emerges from vaginal imbalance and is often associated with certain behaviors. VC can be managed with oral medications or vaginal suppositories. However, vaginal suppositories may only be suitable for sexually active individuals. Medical providers should always inquire about the patients' experiences and comfort with suppositories. Moreover, oral medications are contraindicated in pregnancy. The Siriraj Female Sexually Transmitted Infections Clinic has developed educational resources to help patients understand how to use vaginal suppositories properly and maintain overall vaginal health[38].

Other factors, such as certain nutrients and stress, are associated with VC. Carbohydrates fuel the vaginal fungus. A survey of sugary drink consumption among Thai women found high consumption rates (=more than five glasses of sugary drink per week) in both a VC group (39.4%) and a healthy group (59.5%). The two groups also reported consuming sweets at least three times a week (61.7% and 71.6%, respectively[39]. Individuals with VC are advised to curtail sweet intake, and, if being irresistible, preferably consume a small amount of sugar-rich diet in the morning or before physical activity. Screening for diabetes at suitable intervals is also advised. Additionally, evidence suggests that iron deficiency can increase the risk of VC[9]. Hence, iron supplementation is often prescribed for symptomatic patients, particularly those with frequent episodes of VC.

Treatment Experience at the Siriraj Female Sexually Transmitted Infections Clinic

Our attending physicians customize medication protocols in clinical practice based on their experience, often in conjunction with guidance involving other drugs or medical supplies. Their experiences using various drug regimens among nonpregnant women are consolidated in Tables 2.

Table 2. Therapeutic Strategies for Acute Vaginal Candidiasis in Nonpregnant Women.

	FLU 1 (n=90)	FLU 1,4 (n=48)	FLU 1,4,7 (n=47)	FLU+CLO cream (n=37)	FLU+SER (n=48)	SER (n=17)	CLO[1] 500 (n=25)	CLO[2] 500 (n=14)	CLO 100 (n=25)	NPF (n=24)	FLU+ Fluomizin (n=24)
Age (years)	32.9±8.6	33±9.1	31.6±8.9	34.1±9.6	33±9.1	33.1±10.8	31.5±10.6	32±9.2	33.6±8.2	32.3±9.7	30.9±8.7
BMI (kg/m^2)	23.1±4.3	22.7±4.9	22.7±4.9	21.8±6.4	22.7±4.9	22.6±4.0	24.7±4.7	22.2±4.4	23.5±4.7	20.8±2.5	23.2±5.4
Clinical cure	67 (74.4)	36 (75.0)	32 (68.1)	24 (64.9)	36 (75.0)	15 (88.2)	18 (72.0)	11 (78.6)	16 (64)	19 (79.2)	18 (75)
Microscopic cure	–	44 (91.7)	39 (83.0)	31 (83.8)	44 (91.7)	14 (82.4)	16 (64)	12 (85.7)	19 (82.6)	20 (83.3)	22 (91.7)
Time-to-cure (days)	4.0±1.8	2.9±2.0	2.2±0.6	3.3±2.2	2.9±2.0	3.7±3.0	2.7±1.3	3.8±2.6	2.3±2.8	3.2±2.4	2.1±2.5

CLO= clotrimazole, FLU=fluconazole, NPF=Neo-Penotrans Forte, SER=sertaconazole

FLU1 =a single dose fluconazole 200mg; FLU 1,4=fluconazole 200mg on first and fourth day; FLU 1,4,7= fluconazole 200mg on first, fourth and seventh day, CLO1= original clotrimazole (Bayer, Thailand); CLO2=generic 500mg clotrimazole (Kenet, Patar Lab (2517), Thailand); SER= sertaconazole 300mg (Pacific Health Care, Thailand)

Source: Unit of Infectious Diseases, Department of Obstetrics and Gynaecology, Faculty of Medicine, Siriraj Hospital, Mahidol University

Treatment of Acute Vaginal Candidiasis in Nonpregnant Women

A previous study conducted at the Clinic found that the therapeutic efficacy of a single dose of fluconazole 200 mg taken orally was 74.4%. The average onset of symptomatic improvement occurred approximately 4 days post-treatment initiation[3]. Since 2020, 309 individuals were treated for acute VC. All presented with abnormal vaginal discharge, with 76.3% reporting itching, 37.3% experiencing foul-smelling discharge during the preceding month, and 5.7% having concurrent fungal infections elsewhere on the body. Among the cohort, 58.6% consumed at least 5 glasses of sweetened beverages per week. The vast majority (89.6%) had experienced abnormal vaginal discharge during the previous year, with 36.8% and 42.8% having symptoms once and twice, respectively. Furthermore, 98% were sexually active, 44.1% used sanitary napkins outside the menstrual cycle, 88.8% used specialized genital cleaning products, 78.2% had a history of vaginal douching, and 65.7% utilized tampons.

In light of expert recommendations which suggest that augmented drug regimens expedite patient recovery and bolster treatment efficacy[10], the Clinic has expanded its drug protocols. Per symptomatic and microscopic evaluations, the clinical cure and microscopic cure of oral fluconazole 200 mg on the first and fourth days were 75% and 91.7%, respectively. However, when the regimen was expanded to 600 mg (3 capsules) and administered on the first, fourth, and seventh days, there was a decline in treatment efficacy. Unlike the study by Quereux et al in which the addition of azole cream increased the cure rate[40], combining oral fluconazole 200 mg and topical clotrimazole cream did not improve outcomes. Conversely, combining fluconazole 200 mg with sertaconazole suppositories enhanced treatment efficacy and hastened symptom resolution, paralleling results observed with 2 doses of fluconazole. Among all treatments, suppositories, especially those containing sertaconazole, proved highly effective, leading to symptom resolution in 88.2% of cases.

Vaginal clotrimazole suppository has been used to treat women with VC for around 50 years and has shown high treatment efficacy and the most acceptable safety[41]. When comparing the original clotrimazole (Canesten®, Bayer, Thailand) in the form of single dose and multiple doses, we found that multiple doses led to a lower clinical cure but higher microscopic cure. The local-made clotrimazole had good performance in treatment outcomes that were comparable to the original one. All of the single-dose clotrimazole suppositories being used in the Clinic were applied by residents or experienced gynaecologists in order to minimize inappropriate insertion technique. Accordingly, our findings are applicable for patients who are well-trained for vaginal suppository before the prescription.

The optimal follow-up time is two weeks because, based on our experience, many women become asymptomatic at the end of the first week and start to develop symptoms after that. Normally, patients' symptoms should be at least 50% subsided in the first week and become absent at the end of two weeks. Recurrence mostly occurs within two months post-treatment, in particular those who refuse lifestyle modification. When any participant fails the first regimen, the alternative regimens will be administered. However, drug compliance should be the first issue of concern. Inability to perform vaginal insertion is not an uncommon condition. Therefore, our third-line regimen is usually a two or three doses of fluconazole 200 mg being given in a three-day interval.

Treatment of Acute Vaginal Candidiasis in Pregnant Women

For pregnant women, vaginal suppositories remain the sole viable treatment modality. Most treatment guidelines recommend the use of suppositories over multiple days[9]. However, pregnant women often find suppository administration challenging due to their enlarged abdomen and constraints against lying supine. Our Clinic has therefore documented the therapeutic effects of various regimens. They are a single dose of

clotrimazole 500 mg vaginal suppository, a single dose of sertaconazole 300 mg vaginal suppository, and the daily administration over 7 days of a combination of metronidazole 750 mg with miconazole 200 mg vaginal suppositories (brand name: Neo-Penotran Forte®). Two weeks after treatment, symptomatic improvement was found in 50% (24/48), 62.5% (30/48), and 83.7% (31/43) of the patients for each regimen, respectively. Regarding microscopic cures, the corresponding rates were 62.5% (30/48, 47.9% (23/48), and 72.1% (31/43) of the patients. Satisfaction with treatment outcomes was high across all groups. In order to lessen drug administration and gain prolonged treatment effects, an on-going trial in the Clinic is to compare a single dose of sertaconazole 300mg and two doses of seven-day-interval sertaconazole 300mg.

A small drawback of Neo-Penotran Forte usage is vaginal irritation. Of the patients, 27% experienced symptoms during the initial 1–2 days, 11% had persisting symptoms up to days 3–4, and 8% reported symptoms lasting beyond 4 days. However, all patients were able to complete the treatment regimen. The use of lubricants can mitigate symptoms. In accordance with a previous study in Russian pregnant women that an additional dose of sertaconazole 300mg at a seven-day interval increased cure rate[41], in cases where symptoms persist or fungi are still detectable microscopically, the Clinic recommends adding therapeutic drugs. If initiated with a single vaginal suppository, treatment should be repeated weekly with microbial and symptomatic evaluations every 2–4 weeks until resolution. Despite being asymptomatic, 1 patient consistently exhibited fungal presence upon microscopic evaluation at each follow-up. Consequently, the Clinic recommends continuing weekly vaginal suppository administration of a single-dose azole regimen for the duration of the pregnancy in case of no treatment response following the third regimen.

Chronic Vaginal Candidiasis and Recurrent Episode

Treatment outcomes of VC can be divided into being cured, being not cured, chronic VC and recurrent VC[9]. Chronic VC is a subset of being not cured in that women who belong to chronic VC have no response to many consecutive treatment regimens. In contrast, recurrent VC is a subset of being cured, but there are at least three following VC episodes in 12 months. Since 2020 that we started to set up the follow-up protocol for all women presenting with VC, we telephoned them for asking about their symptoms and recurrent episodes.

Table 3 shows that, among those who could be contacted, recurrent episodes in two years were not common. Health education on lifestyle modification and disease awareness, which is the strength of the Siriraj Female STI Clinic, may be the best explanation. Therefore, we encourage healthcare professionals to focus on this part, not less than the antimycotic one. Furthermore, our findings support the high impact of VC for pregnant women as it was seen that many of them tended to have chronic VC.

Table 3. Thai women presenting with acute vaginal candidiasis; being treated with a single dose vaginal suppository; and being followed up to two years

	Being non-pregnant during diagnosis (n=18)	Diagnosis during first trimester (n=7)	Diagnosis during second trimester(n=25)	Diagnosis during third trimester(n=6)
Microscope cure at 2 weeks	15 (83.3)	3 (42.9)	13 (52)	4 (66.7)
No microscopic cure at 2 weeks	3 (16.7)	4 (57.1)	12 (48)	2 (33.3)
Chronic VC*	0 (0)	3/4 (75)	6/12 (50)	2/2 (100)
Recurrence** in 2 years	1 (5.6)	0	4 (16)	0 (0)

Data presented in n(%)

*Chronic VC = women who had no microscopic cure at 2 week follow-up; and received second single dose, anti-fungal, vaginal suppository; and still had no microscopic cure at the following two weeks. **Recurrence = At least one recurrent episodes being evaluated by telephone call

Source: Unit of Infectious Diseases, Department of Obstetrics and Gynaecology, Faculty of Medicine, Siriraj Hospital, Mahidol University

Managing Cases with Fungal Detection in Cervical Cancer Screening Results

Cervical cancer screening, particularly cytological examination, often detects *Candida* spp. in the form of yeast or pseudohyphae. *C. albicans* is the most frequently identified pathogen and is often present in pseudohyphae form during the symptomatic period.

A review of cervical cancer test results from 82 patients presenting with acute vaginal candidiasis at the Clinic between June 2020 and May 2021 revealed that approximately 30% had evidence of a fungal infection in their latest Pap test. Intriguingly, these patients did not display typical curd-like vaginal discharge or complain of itching on the testing day. As a result, from June 2022, cervical cancer test reports sent to patients have been updated to indicate the presence of fungus, urging those identified to seek further examination.

Data from 56 individuals with detection of fungus in their latest, they came to the Clinic for pelvic examination ranging from 2 weeks to 2 months. Pseudohyphae were detected microscopically in 30.4% (17/56) of these patients, and 47.1% (8/17) reporting vaginal itching on the day of their consultation. Conversely, 62.5% (35/56) did not exhibit pseudohyphae under microscopic examination, yet 17.7% (6/35) of this group reported vaginal itching and received appropriate treatment. Four cases (7.1%) had self-medicated prior to consultation due to the discomfort from vaginal itching. This observation underscores the importance of follow-up examinations for enhancing diagnostic accuracy; and probably alleviates client anxiety.

Conclusions

Vaginal candidiasis, resulting from a vaginal imbalance, is prevalent. The dissemination of information on proper healthcare practices is vital to prevent the condition. When symptoms manifest, multiple treatment options are available. Physicians should assess each patient's circumstances, monitor their treatment progress, and provide comprehensive information regarding the limitations and efficacy of each drug. It is also crucial to address the issue of frequent recurrence and to emphasize the importance of adhering to the prescribed medication regimen to mitigate the risk of drug resistance. Patients should be encouraged to seek medical attention if they are uncertain of their symptoms or abnormal Pap test, given the propensity for this condition to cooccur with other infections.

References

1. Chayachinda C, Chinhiran K, Kittiyaowamarn R, Chaithongwongwatthana S, Teeratakulpisarn N. The Thai 2022 sexually transmitted infections treatment guideline: abnormal vaginal discharge. Thai J Obstet Gynaecol 2022;30:222-33.
2. Chayachinda C, Thamkhantho M, Chalermchockcharoenkit A, Neungton C, Thipmontree W. Characteristics of clients at the Siriraj Female STD Clinic during 2011-2015. Siriraj Med Bull 2018;11:182-9.
3. Chayachinda C, Thamkhantho M, Ngamsakulringroj P, Leeyaphan C, Tulyaprawat O. Effect of intravaginal gentian violet for acute vaginal candidiasis treated with a single dose oral fluconazole: a randomised controlled trial. J Obstet Gynaecol 2022;7:1-7.
4. Roberts C, Algert C, Rickard K, Morris J. Treatment of vaginal candidiasis for the prevention of preterm birth: a systematic review and meta-analysis. Syst Rev 2015;4:31.
5. Blomberg L, Backman K, Kirjavainen P, Karvonen A, Harju M, Keski-Nisula L. Vulvovaginal yeast infections, gestational diabetes and pregnancy outcome. BMC Pregnancy Childbirth 2023;23:70.
6. Sobel JD. Vulvovaginal candidosis. Lancet 2007;369:1961-71.
7. Kiss H, Petricevic L, Husslein P. Prospective randomised controlled trial of an infection screening programme to reduce the rate of preterm delivery. BMJ 2004;329:371.
8. Roberts C, Rickard K, Kotsiou G, Morris J. Treatment of asymptomatic vaginal candidiasis in pregnancy to prevent preterm birth: an open-label pilot randomized controlled trial. BMC Pregnancy Childbirth 2011;11:18.
9. British Association for Sexual Health and HIV. British Association for Sexual Health and HIV national guideline for the management of vulvovaginal candidiasis (2019)2019 28 July 2020. Available from: https://www.bashhguidelines.org/media/1223/vvc-2019.pdf.

10. Chalermchockcharoenkit A. (24 August 2022) A novel topical alternative treatment for treating women with acute vaginal candidiasis: Sertaconazole 300 mg [Webinar]. Pacific Health Care, Thailand.
11. Garcia-Rubio R, de Oliveira HC, Rivera J, Trevijano-Contador N. The fungal cell wall: Candida, Cryptococcus, and Aspergillus Species. Front Microbiol 2020;10:1-13.
12. Lv Q, Yan L, Jiang Y. The synthesis, regulation, and functions of sterols in Candida albicans: Well-known but still lots to learn. Virulence 2016;7:649-59.
13. d'Enfert C, Kaune A, Alaban L, Chakraborty S, Cole N, Delavy M, et al. The impact of the Fungus-Host-Microbiota interplay upon Candida albicans infections: current knowledge and new perspectives. FEMS Microbiol Rev 2021;45:fuaa060.
14. Amabebe E, Anumba DOC. The Vaginal Microenvironment: The physiologic role of Lactobacilli. Front Med (Lausanne) 2018;5:181.
15. King R, Lee J, Morris A. Adherence of Candida albicans and other Candida species to mucosal epithelial cells. Infect Immun 1980;27:667-74.
16. Ferrer J. Vaginal candidosis: epidemiological and etiological factors. Int J Gynaecol Obstet 2000;71:S21-7.
17. Narayanan T, Rao G. Beta-indoleethanol and beta-indolelactic acid production by Candida species: their antibacterial and autoantibiotic action. Antimicrob Agents Chemother 1976;9:375-80.
18. Heng L, Yatsuya H, Morita S, Sakamoto J. Vaginal douching in Cambodian women: its prevalence and association with vaginal candidiasis. J Epidemiol 2010;20:70-6.
19. Shaaban O, Abbas A, Moharram A, Farhan M, Hassanen I. Does vaginal douching affect the type of candidal vulvovaginal infection? Med Mycol 2015;53:817-27.
20. Rachapromma P, Chayachinda C, Kerdklinhom C, Iamvijarn W, Thanasakthitiku T. Nursing care for women with acute vaginal candidiasis. Siriraj Med Bull 2022;15: 107-13.
21. Elegbe I, Elegbe I. Quantitative relationships of Candida albicans infections and dressing patterns in Nigerian women. Am J Public Health. 1983;73: 450-2.
22. Padgett DA, Glaser R. How stress influences the immune response. Trends Immunol 2003;24:444-8.
23. Chayachinda C, Rekhawasin T, Thamkhantho M, Aneklap P. Acute vaginal candidiasis: A review of treatment guidelines and Siriraj experience. Thai J Obstet Gynaecol 2021;29:306-12.
24. Royal Thai College of Obstetrics and Gynaecologists. Management of abnormal vaginal discharge in reproductive-aged women. Siriraj Med Bull 2023;16:187-29.
25. Tuangrattanasirikun D, Chayachinda C, Rachapromma P, Sonwicha S. Effect of on-line vs on-site class on medical students' competency in vaginal wet mount. Siriraj Med Bull 2023;16:269-75.
26. Woelber L, Prieske K, Mendling W, Schmalfeldt B, Tietz H, Jaeger A. Vulvar pruritus-causes, diagnosis and therapeutic approach. Dtsch Arztebl Int 2020;116:126-33.
27. Chayachinda C, Panchalee T, Thamkhantho M. A Never-ending story of anogenital warts: review article. J Med Assoc Thai 2020;103:614-9.
28. Qi W, Li H, Wang C, Li H, Zhang B, Dong M, et al. Recent advances in presentation, diagnosis and treatment for mixed vaginitis. Front Cell Infect Microbiol 2021;11:759795.
29. Olson M, Jayaraman A, Kao K. Relative abundances of Candida albicans and Candida glabrata in in vitro coculture biofilms impact biofilm structure and formation. Appl Environ Microbiol 2018;84: e02769-17.
30. Workowski K, Bachmann L, Chan P, Johnston C, Muzny C, Park I, et al. Sexually transmitted infections treatment guidelines 2021. MMWR Recomm Rep 2021;70:1-187.
31. Budani M, Fensore S, Di Marzio M, Tiboni G. Maternal use of fluconazole and congenital malformations in the progeny: A meta-analysis of the literature. Reprod Toxicol 2021;100:42-51.
32. Ghannoum M, Rice L. Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clin Microbiol Rev 1999;12:501-17.
33. Regidor P, Thamkhantho M, Chayachinda C, Palacios S. Miconazole for the treatment of vulvovaginal candidiasis. In vitro, in vivo and clinical results. Review of the literature. J Obstet Gynaecol 2023;43:2195001.
34. Fan S, Liu X, Wu C, Xu L, Li J. Vaginal nystatin versus oral fluconazole for the treatment for recurrent vulvovaginal candidiasis. Mycopathologia 2015;179:95-101.
35. Merianoss J. Quaternary ammonium antimicrobial compounds. In: Block S, editor. Disinfection, sterilization, and preservation. Fourth ed. Philadelphia: Lea and Febiger; 1991. p. 225-55.
36. Thamkhantho M, Chayachinda C. Vaginal tablets of dequalinium chloride 10 mg versus clotrimazole 100 mg for vaginal candidiasis: a double-blind, randomized study. Arch Gynecol Obstet 2021;303:151-60.

37. Chayachinda C, Chinhiran K, Aneklap P, Rachapromma P, Sornwicha S. Bacterial Vaginosis: a comprehensive approach to management in reproductive-aged women. Thai J Obstet Gynaecol 2023;31:318-25
38. Hosiriphon K, Chayachinda C, Keawpoonsub K, Taibowornpitak K, Tuangrattanasirikun D. A Survey of daily genital care practices among reproductive-aged female personnel at Siriraj Hospital. Siriraj Med J 2023;75:259-65
39. Quereux C, Gelas B, Chevallier T, Petit F, Micheletti M. Evaluation of the efficacity and speed of action of sertaconazole nitrate suppository and cream combined treatment for vulvovaginal candidiasis. Gynecol Obstet Fertil 2000;28:238-44.
40. Mendling W, Atef El Shazly M, Zhang L. Clotrimazole for vulvovaginal candidiasis: more than 45 years of clinical experience. Pharmaceuticals 2020;13:274.
41. Egorova AT, Bazina MI, Savitskaya EA. The experience of treatment of Candida vulvovaginitis in pregnant women with 'Zalain'. Russian Bulletin of Obstetrician-Gynaecologist 2010;4:35-8.

Thai Journal of Obstetrics and Gynaecology
January 2024, Vol. 32, No. 1, pp. 13-21.

OBSTETRICS

Comparison between Lidocaine Spray and Cryotherapy for Pain Reduction from Amniocentesis in Second Trimester Pregnancy; A randomized controlled trial

Janissta Khunprabaht, M.D.*,
Densak Pongrojpaw, M.D.*,
Athita Chanthasenanont, M.D.*,
Sawanya Benchahong, M.D.*,
Junya Pattaraarchachai, Ph.D**,
Komsun Suwannarurk, M.D.*

** Department of Obstetrics and Gynecology, Faculty of Medicine Thammasat University, Pathum Thani, Thailand*
*** Chulabhorn International College of Medicine, Thammasat hospital, Pathum Thani, Thailand*

ABSTRACT

Objectives: To compare the pain level from amniocentesis between lidocaine spray, cryotherapy prior to the procedure and control groups in second trimester pregnant women.

Materials and Methods: This was a prospective randomized-control trial study. It was conducted at Maternal and Fetal Medicine clinic at Thammasat University Hospital, Pathum Thani, Thailand between July 2021 and December 2021. Participants were pregnant women undergoing amniocentesis at gestational ages between 15 and 20 weeks. They were divided into three groups namely lidocaine, cryotherapy, and control. Subjects in lidocaine or cryotherapy groups received an administration of 8 spritzes of 10% lidocaine (80 mg) spray or cold gel packs (-18 to -24 degrees Celsius) onto the marked puncture site for five minutes before amniocentesis, respectively. The control group underwent amniocentesis in the same manner without any analgesia. Anticipated pain (Te), pain during the procedure (T0), 15 and 30 minutes after the procedure (T15 and T30) were evaluated based on 10-cm visual analog scale (VAS).

Results: A total of 330 pregnant women were recruited and allocated (110 cases per group). Mean maternal age was 36.1 years old. The demographic characters of the three groups were comparable. Pregnant women who received lidocaine had significantly less pain than control at T0, T15 and T30 (3.00 ± 2.18 vs 3.97 ± 2.27, p = 0.001, 0.95 ± 1.41 vs 1.95 ± 1.75, p < 0.001, 0.48 ± 1.11 vs 0.95 ± 1.28, p = 0.004, respectively). Those who received cryotherapy had significantly less pain than control at T0 and T15 (3.39 ± 1.84 vs 3.97 ± 2.27, p = 0.038 and 1.48 ± 1.49 vs 1.95 ± 1.75, p = 0.032, respectively). Lidocaine had less pain level than cryotherapy group at T15 and T30 (0.95 ± 1.41 vs 1.48 ± 1.49, p = 0.008 and 0.48 ± 1.11 vs 0.93 ± 1.20, p = 0.005, respectively).

Conclusion: Participants in both the lidocaine spray and cryotherapy groups had comparable

pain levels during the procedure. In contrast, at 15 and 30 minutes after the procedure, the lidocaine spray group had less pain than the cryotherapy group.

Keywords: lidocaine spray, cryotherapy, amniocentesis, pain.

Correspondence to: *Densak Pongrojpaw, M.D., Department of obstetrics and gynecology, Thammasat hospital, Pathum Thani 12120, Thailand. E-mail: pongrojpaw@gmail.com*

Received: 2 October 2022, **Revised:** 10 April 2023, **Accepted:** 26 June 2023

การทดลองแบบสุ่มเปรียบเทียบประสิทธิภาพระหว่างสเปรย์ลิโดเคนและการประคบเย็นก่อนการเจาะตรวจน้ำคร่ำเพื่อลดความเจ็บปวดขณะเจาะตรวจน้ำคร่ำในสตรีตั้งครรภ์ไตรมาสที่สอง

จณิสตา ขุนพระบาท, เด่นศักดิ์ พงศ์โรจน์เผ่า, อธิตา จันทเสนานนท์, สวรรยา เบ็ญจหงษ์, จรรยา ภัทรอาชาชัย, คมสันต์ สุวรรณฤกษ์

บทคัดย่อ

วัตถุประสงค์: *เพื่อเปรียบเทียบอาการปวดจากการเจาะน้ำคร่ำระหว่างการฉีดสเปรย์ลิโดเคน การประคบเย็น และกลุ่มควบคุมในสตรีตั้งครรภ์ไตรมาสสอง*

วัสดุและวิธีการ: *การศึกษาทดลองแบบสุ่มไปข้างหน้าในสตรีตั้งครรภ์ที่มารับการเจาะตรวจน้ำคร่ำทางพันธุกรรม ณ หน่วยเวชศาสตร์มารดาและทารกในครรภ์ โรงพยาบาลธรรมศาสตร์ ระหว่างเดือน กรกฎาคม พ.ศ. 2564 ถึงเดือนธันวาคม พ.ศ. 2564 อาสาสมัครหญิงตั้งครรภ์ อายุครรภ์ 15-20 สัปดาห์ที่เข้ารับการเจาะตรวจน้ำคร่ำได้รับการแบ่งแบบสุ่มเป็นสามกลุ่ม คือ กลุ่มลิโดเคน กลุ่มประคบเย็น และกลุ่มควบคุม กลุ่มลิโดเคนได้รับการพ่นร้อยละ 10 ของสเปรย์ลิโดเคน 8 พัฟต่อเนื่องกัน (80 มิลลิกรัม) บริเวณหน้าท้องที่จะเจาะน้ำคร่ำ กลุ่มประคบเย็นได้รับการประคบเจลความเย็น (อุณหภูมิ -18 ถึง -24 องศาเซลเซียส) บริเวณหน้าท้องที่จะเจาะน้ำคร่ำเป็นเวลานาน 5 นาที ก่อนทำการเจาะน้ำคร่ำทั้งสองกลุ่ม และกลุ่มควบคุมได้รับการดูแลตามมาตรฐานปกติ บันทึกข้อมูลระดับความเจ็บปวดที่คาดหวัง ขณะเจาะน้ำคร่ำ (T0) หลังเจาะ 15 (T15) และ 30 นาที (T30) โดยคะแนนความเจ็บปวด (VAS) 0-10 คะแนน*

ผลการศึกษา: *สตรีตั้งครรภ์จำนวน 330 ราย (กลุ่มละ 110 ราย) อายุเฉลี่ย 36.1 ปี กลุ่มที่ได้รับสเปรย์ลิโดเคนสามารถลดความเจ็บปวดขณะการเจาะน้ำคร่ำ หลังการเจาะ 15 และ 30 นาที ได้ดีกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ($p = 0.001$, < 0.001 และ 0.004 ตามลำดับ) กลุ่มที่ได้รับการประคบเย็นสามารถลดความเจ็บปวดขณะการเจาะน้ำคร่ำหลังการเจาะ 15 นาที ได้ดีกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ($p = 0.038$ และ 0.032 ตามลำดับ) และผลของสเปรย์ลิโดเคนดีกว่าการประคบเย็นในการลดความเจ็บปวด ที่เวลา 15 และ 30 นาทีหลังการเจาะอย่างมีนัยสำคัญทาง*

สถิติ (p = 0.008 และ 0.005 ตามลำดับ)

***สรุป**: สเปรย์ลิโดเคนและการประคบเย็นสามารถลดความเจ็บปวดขณะการเจาะตรวจน้ำคร่ำทางพันธุกรรมและหลังการเจาะที่เวลา 15 นาทีได้อย่างมีนัยสำคัญเมื่อเทียบกับกลุ่มควบคุม และหลังการเจาะที่เวลา 15 และ 30 นาที สเปรย์ลิโดเคนสามารถลดความเจ็บปวดได้ดีกว่าการประคบเย็นอย่างมีนัยสำคัญ*

***คำสำคัญ**: สเปรย์ลิโดเคน, การประคบเย็น, การเจาะน้ำคร่ำ, ความเจ็บปวด*

Introduction

Prenatal diagnosis is an investigation to detect genetic or structural abnormalities of a fetus in utero. Early detection, proper management, fetal surveillance and scheduled appropriate date of pregnancy termination depended on the prenatal diagnosis result. Prenatal diagnosis may be performed by means of invasive or non-invasive procedures[1]. Amniocentesis is an invasive diagnostic procedure where a needle is inserted through the abdominal wall into the uterus and used to extract a small sample of amniotic fluid for further analysis. Amniocentesis is accepted as a standard for detecting chromosomal anomalies in the second trimester of pregnancy and is easily conducted with low risk of maternal and fetal complications. The level of patients' pain perception is a hindrance to patient cooperation. Unfortunately, the standard protocol for amniocentesis does not utilize local anesthetics or pain relievers. Factors that affected pain perception were number and the age of gestation, body mass index, and a history of previous abdominal surgery with an incision near the amniocentesis site[2].

Lidocaine is one of the most widely used anesthetic agents either for local or neuraxial application. It can be administered in major or minor surgery for pain reduction. Lidocaine can be applied topically in the form of gel or spray. It works by stabilizing the neuronal membrane through inhibition of the ionic fluxes required for the initiation and the conduction of neural impulses, thereby affecting local anesthetic action[3].

Cryotherapy has been used for pain reduction for many years in localized tissue trauma. A reduction in soft tissue temperature by 10 to 15 degrees Celsius can show pain reduction efficacy by slowing local metabolic activity and decreasing oxygen requirement. Reduction in tissue swelling, bleeding, bruising, and local pain were benefits of soft tissue temperature reduction via vasoconstriction[4].

Homkrun and coworkers reported a significant pain reduction by the application of lidocaine spray before amniocentesis[5]. Benchahong and colleagues showed that cold therapy before and after amniocentesis was the most effective in pain reduction during amniocentesis compared to only exclusively cold therapy before or after amniocentesis[6]. Similar to Hanprasertpong's study which demonstrated that cryoanalgesia prior to amniocentesis could significantly alleviate pain from the procedure[7].

Contrarily, Gordon reported in 2007 that there was no significant pain difference between 1% lidocaine local infiltration and non-analgesia during amniocentesis[8]. Similarly, Wax and coworkers showed no significant difference in procedural pain between amniocentesis using a needle chilled at -14 degrees Celsius as compared to a needle at the room temperature[9]. Supportively to Gordon's and Wax's literatures, Pongrojpaw in year 2007 reported that the application of 1 gm of lidocaine-prilocaine cream and placebo cream had similar pain results during amniocentesis[10].

From previous literature, local lidocaine spray and cryotherapy were both effective for pain management and convenience for use during

amniocentesis. Hence, the aim of this study was to compare the pain levels between local lidocaine spray, cryotherapy and control groups during second trimester amniocentesis.

Materials and Methods

This was a prospective randomized-control trial study. It was conducted at the Maternal and Fetal Medicine clinic, Thammasat University Hospital, Pathum Thani, Thailand between July 2021 and December 2021. The study was approved by the Human Research Ethics Committee of Thammasat University and was registered in Thai Clinical Trials Registry (TCTR20210331005).

The participants in this study were pregnant women undergoing amniocentesis at a gestational age between 15 and 20 weeks during the study period. The exclusion criteria were multifetal pregnancies, known fetal abnormalities detected by ultrasonography, threatened abortion, alteration in amniocentesis site after administration of intervention subsequently from fetal repositioning, more than one attempt of amniocentesis, allergy to lidocaine, infection of the abdominal wall, underlying maternal psychiatric conditions, and refusal to participate in the study.

According to Homkun's[5] and Hanprasertpong's studies[7], pain reduction differences were calculated to compare lidocaine spray and cryotherapy, post-amniocentesis pain measured at 2.3 ± 2.9 vs 3.2 ± 1.6 (mean ± SD), respectively, $\alpha = 0.01$, $\beta = 0.10$. This study was an open-label trial and aimed at an intention-to-treat analysis. The minimal sample sizes that made the statistic significant were 94 cases per group. Ten percent addition for to account for potential lost cases were applied. The sample in the current study was 110 cases per group. The treatment assignment ratio of the study was 1:1:1 in each group.

After the details of the procedure were disclosed to the participants and the consent forms were signed, all eligible participants were consecutively allocated into three groups: lidocaine, cryotherapy, and control. The randomization process was carried out by a random manual draw system without stratification. Demographic data of all participants were recorded, namely maternal age, the number of gestations, gestational age, body mass index (BMI), history of abdominal surgery, and the results of standardized ultrasonographic examination of fetal biometry and anomaly screening.

All amniocentesis procedures were performed by maternal-fetal medicine (MFM) staff under Thammasat University Hospital protocols, by which an ultrasound was used to locate the injection site before and during the procedure. The skin was disinfected with 10% povidone-iodine. A 22-gauge needle was used for amniocentesis with the collection of approximately 15-20 ml amniotic fluid per sample. Participants in the control group underwent the standard amniocentesis procedure without analgesia. Participants in the lidocaine group received 8 sprays of 10% lidocaine (80 mg) onto the marked puncture site five minutes prior to an amniocentesis. As for the cryotherapy group, the cold gel packs were stored at -24 to -18 degree Celsius for at least one hour. The cold gel pack was wrapped with 2mm thick cotton cloth sleeves. Then, the cold gel pack was placed at the marked puncture site for five minutes and amniocentesis was promptly performed in the same manner as lidocaine and control group. Once placed onto the participant's abdomen, the gel temperature was measured regularly in one-minute intervals, maintaining the gel temperature between 10 to 15 degrees Celsius. Participants underwent bed rest for thirty minutes to observe for complications. Routine ultrasonographic examinations for fetal heart rate and umbilical artery blood flow evaluation were performed before participants' discharge.

The goal of this study was to assess the effectiveness of local lidocaine spray and cryotherapy in decreasing the pain perception during and after amniocentesis. Participants estimated their anticipated pain level (expected pain: Te) before amniocentesis, and recorded level of pain during the amniocentesis process (T0), at 15 minutes (T15) and 30 minutes (T30) after the procedure in printed 10 - cm visual analog scale forms. The lowest and highest pain scores were 0 and 10 cm, respectively.

Demographic and clinical data were compiled

and electronically organized into a record form. The data was then processed into frequencies and percentages using Statistical Package for the Social Science (SPSS Inc., Chicago, IL USA) for Windows version 23. Continuous and category data were analyzed for statistical differences using analysis of variance (ANOVA) with repeated measurement and chi square or Fisher exact test when clinically applicable, respectively. A p value of less than 0.05 indicates a statistically significant difference.

Results

A total of 330 pregnant women who underwent amniocentesis during the study period were recruited. They were allocated into three groups equally, namely control, cryotherapy and lidocaine groups (Fig. 1). Mean maternal age was 36.1 years old. The average gestational age of participants was 16.8 weeks. Average BMI was 25.1 kg/m^2. Half of participants had education level at the bachelor level or higher. Two-thirds of the participants were employees. Less than 10 percent of participants had prior experience of amniocentesis. There were no statistically significant differences between the groups in terms of maternal age, BMI, parity, education, occupation, history of abdominal surgery at randomization (Table 1).

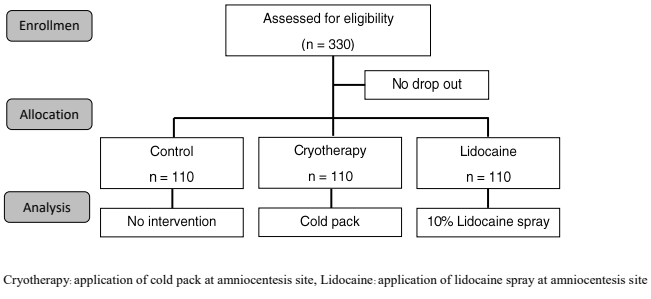


Fig. 1. Flow chart of this study.

Table 1. Demographic characteristics of amniocentesis cases for each group (n = 110 cases per group).

	Control*	Cryotherapy*	Lidocaine*	p value
Age (years)**	36.42 ± 3.99	36.03 ± 4.27	35.79 ± 4.67	0.55‡
BMI (kg/m^2) **	24.51 ± 3.96	24.92 ± 3.85	25.32 ± 5.05	0.08‡
Nulliparity	37 (33.6)	37 (33.6)	60 (54.5)	0.74†
Education level				0.06††
≤ Primary	3 (2.7)	8 (7.3)	6 (5.4)	
Secondary	62 (56.4)	49 (44.5)	42 (38.2)	
≥ Bachelor	45 (40.9)	53 (48.2)	62 (56.4)	
Occupation				0.729†
Government officer	14 (12.7)	12 (10.9)	14 (12.7)	
Business owner	12 (10.9)	21(19.1)	15 (13.7)	
Employee	75 (68.2)	69 (62.7)	70 (63.6)	
Others	9 (8.2)	8(7.3)	11 (10)	
No history of surgery	70 (63.6)	71 (64.6)	74 (67.3)	0.918†
History of amniocentesis	8 (7.3)	10 (9.1)	7 (6.4)	0.74†

Control: no intervention before amniocentesis, Cryotherapy: application of cold gel pack at amniocentesis site, Lidocaine: application of lidocaine spray at amniocentesis site
BMI: body mass index, No history of surgery: no history of abdominal surgery, C/S: cesarean delivery
*n (%), **mean ± standard deviation (SD), ‡ analysis of variance, † Chi-square test, †† Fisher exact test

Table 2 represents the indications of amniocentesis in this study. The majority of cases were of advanced maternal age (85%) followed by family history of abnormal chromosomes with an average of 4.86% among the groups while patient's desires were the least common indications. Abnormal prenatal screening accounted for an average of 4.8%.

Table 2. Indications for amniocentesis in each group (n = 110 cases per group).

	Control*	Cryotherapy*	Lidocaine*
Advanced maternal age	94 (85.6)	92 (83.6)	95 (86.4)
Family history of chromosome abnormality	7 (6.4)	6 (5.5)	3 (2.7)
Abnormal prenatal screening	5 (4.5)	5 (4.5)	6 (5.4)
Patient's desires	1 (0.9)	0 (0)	1 (0.91)
Previous child with chromosome abnormality	3 (2.7)	7 (6.4)	5 (4.5)

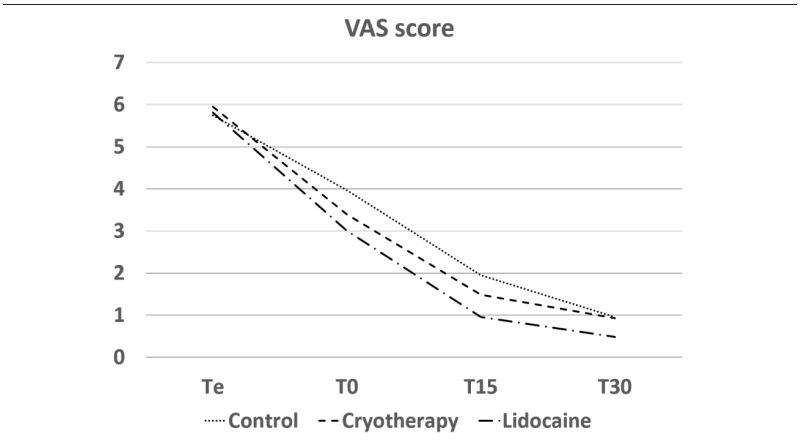
Control: no intervention before amniocentesis, Cryotherapy: application of cold pack at amniocentesis site, lidocaine: application of lidocaine spray at amniocentesis site, Advanced maternal age: maternal age ≥ 35 years old, n (%)

Comparison of pain score visual analog scale (VAS). during amniocentesis at timely manner: expected pain before amniocentesis (Te), during amniocentesis (T0), 15 minutes (T15) and 30 minutes after amniocentesis (T30) were presented in Table 3 and Fig. 2. The expected pain of amniocentesis before the procedure among three groups of participants were comparable. Patients with cryotherapy administration experienced a significantly lower pain level compared to the control group during amniocentesis and 15 minutes after procedure (3.39 ± 1.84 vs. 3.97 ± 2.27, p = 0.038 and 1.48 ± 1.49 vs 1.95 ± 1.75, p = 0.032, respectively). Nevertheless, the pain level at 30 minutes post amniocentesis was not different between the two aforementioned groups (0.93 ± 1.20 vs 0.95 ± 1.28, p = 0.871). In a similar manner, local lidocaine spray significantly reduced the pain score at T0 and T15 after amniocentesis compared to the control group (3.00 ± 2.18 vs 3.97 ± 2.27, p = 0.001 and 0.95 ± 1.41 vs 1.95 ± 1.75, p < 0.001, respectively). Surprisingly, at 30 minutes post procedure, local lidocaine spray still had a significant impact on the pain reduction compared to the control group (0.48 ± 1.11 vs 0.95 ± 1.28, p = 0.004). The comparison of pain reduction efficacy from local lidocaine spray and cryotherapy was also taken into consideration. At T0, the patients with local lidocaine spray and cryotherapy administration had comparable pain scores (3.00 ± 2.18 vs 3.39 ± 1.84, p = 0.154). However, lidocaine spray had a higher efficacy on pain reduction than cryotherapy at 15 and 30 minutes post amniocentesis (0.95 ± 1.41 vs 1.48 ± 1.49, p = 0.008 and 0.48 ± 1.11 vs 0.93 ± 1.20 p = 0.005, respectively).

Table 3. Comparison of pain score visual analog scale (VAS) from amniocentesis among participants in control, cryotherapy and lidocaine groups (n = 110 cases per group).

	Control*	Cryotherapy*	Lidocaine*	p value‡		
				Con vs Cryo	Con vs Lido	Cryo vs Lido
Te	5.75 ± 2.09	5.95 ± 1.84	5.81 ± 2.12	0.453	0.823	0.612
T0	3.97 ± 2.27	3.39 ± 1.84	3.00 ± 2.18	0.038	0.001	0.154
T15	1.95 ± 1.75	1.48 ± 1.49	0.95 ± 1.41	0.032	< 0.001	0.008
T30	0.95 ± 1.28	0.93 ± 1.20	0.48 ± 1.11	0.871	0.004	0.005

VAS: visual analog scale(range 0-10), Control: no intervention before amniocentesis, Cryotherapy: application of cold pack at amniocentesis site, Lidocaine: application of lidocaine spray at amniocentesis site, Te: expected pain before amniocentesis, T0: pain during amniocentesis, T15: pain at 15 minutes after amniocentesis, T30: pain at 30 minutes after amniocentesis, Con vs Cryo: between control and cryotherapy, Con vs Lido: between control and lidocaine group, Cryo vs Lido: between cryotherapy and lidocaine group
* mean ± standard deviation (SD), ‡ post hoc test (LSD)



VAS: visual analog scale(range 0-10), Control : no intervention before amniocentesis, Cryotherapy: application of cold pack at amniocentesis site, Lidocaine: application of lidocaine

Fig. 2. Comparison of pain score (visual analog scale score) during amniocentesis at timely manner among participants in control, cryotherapy and lidocaine group.

Discussion

Amniocentesis is a type of prenatal diagnostic that is particularly invasive in nature, inciting some degree of concern such as the magnitude of procedural pain or possible complications towards the patient and the fetus which may follow. Lidocaine and cryotherapy were methods of interest for pain reduction in amniocentesis. Various methods of lidocaine application either with topical or injection were reported from previous literature.

From previous studies, Gordon[8] and Elimian[11] investigated the pain reduction among pregnant women who underwent amniocentesis. Gordan and Elimian used similar needle size for amniocentesis, 22-gauge vs 20- or 22-gauge spinal needle respectively. However, the needle sizes for lidocaine injection were different in these 2 studies. Gordon utilized a 27-gauge needle for an intradermal injection then switched to a 21-gauge needle for a deeper injection of lidocaine. On the other hand, Elimian performed lidocaine injection using a 21-gauge needle in one simultaneous step. Elimian reported that the local infiltration of lidocaine could reduce pain from amniocentesis while Gordon did not demonstrate any pain reduction.

Pongrojpaw reported that lidocaine cream application before amniocentesis could not reduce pain[10]. Another study, Homkrun and colleagues reported that lidocaine spray application at the amniocentesis site had significant pain reduction from the procedure[5]. Their work reported that the efficacy of lidocaine spray for pain reduction was during and immediately after the procedure. From the current study, local lidocaine spray at the puncture site before amniocentesis was effective in reducing pain during amniocentesis in the second trimester. Moreover, the pain reduction effect persisted until 30 minutes after the procedure. The current study supports Homkrun's study that an administration of lidocaine spray before amniocentesis could reduce the pain level.

Wax and coworkers stated that subfreezing needle (-14 degrees Celsius) for amniocentesis could not reduce pain from the procedure[9]. The use of cryotherapy for reducing pain during amniocentesis was reported by Benchahong[6] and Hanprasertpong[7]. Both studies demonstrated that cryoanalgesia application before amniocentesis could reduce pain. Benchahong's study showed that cryotherapy could reduce pain for up to 30 minutes. The present study supports Benchahong's and Hanprasertpong's works that cryotherapy could reduce pain from amniocentesis for up to 15 minutes after the procedure. Telapol reported that ethyl chloride spray before amniocentesis

could not reduce pain during the procedure[12].

From the current study, application of cryotherapy and local lidocaine spray at the puncture site before amniocentesis were effective in reducing pain during amniocentesis in the second trimester. Both lidocaine spray and cryotherapy applications before amniocentesis had comparable pain reduction during the procedure. While at 15 and 30 minutes, lidocaine spray was significantly more effective in pain reduction than cryotherapy. Hence, it seems that lidocaine spray had longer duration of pain reduction than cryotherapy. Comparison of the current study to previous literatures was summarized and presented in Table 4.

Table 4. Comparison of previous literature of pain reduction method in amniocentesis.

	Wax[9]	Pongrojpaw[10]	Gordon[8]	Hanprasertpong[7]	Elimian[11]	Telapol[12]	Homkrun[5]	Benchahong[6]	Present
Years	2005	2007	2007	2012	2013	2018	2019	2021	2023
Country	USA	THA	USA	THA	USA	THA	THA	THA	THA
Cases (n)	62	120	204	372	76	148	570	480	330
Method	FN	LC	LI	Cryo	LI	ES	LS	Cryo	LS/Cryo
Time (min)		30		5			1	5	5
Pain	N	N	N	Y	Y	N	Y	Y	Y

THA: Thailand, Cryo: cryotherapy, LS: lidocaine spray, LI: lidocaine injection, LC: lidocaine-prilocaine cream, FN: freezing needle, ES: Ethyl chloride spray, Pain: pain reduction, Y: pain reduction efficacy, N: no pain reduction efficacy

Conclusion

Participants in both groups who received lidocaine spray and cryotherapy before the amniocentesis had comparable pain levels during the procedure. In contrast, at 15 and 30 minutes after the procedure, the lidocaine spray group had less pain than the cryotherapy group. Lidocaine spray was an easy, convenient, and non-invasive technique. Local lidocaine spray application before amniocentesis followed by cryotherapy after the procedure might be in the next investigation.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Cunningham FG, Lenovo KJ, editors. Williams Obstetrics. 26th ed. New York: McGraw-Hill Education 2022:332-51.
2. Suntornlimsiri W, Naunkeaw K. Clinical correlates of pain with second-trimester genetic amniocentesis. J Med Assoc Thai 2009;92:1567-72.
3. Derry S, Moore RA. Topical lidocaine for neuropathic pain in adults. Cochrane Database Syst Rev 2014;2014:CD010958.
4. Oliveira SM, Silva FM, Riesco ML, Latorre Mdo R, Nobre MR. Comparison of application times for ice packs used to relieve perineal pain after normal birth: a randomised clinical trial. J Clin Nurs 2012; 21: 3382-91.
5. Homkrun P, Tongsong T, Srisupundit K. Effect of Xylocaine spray for analgesia during amniocentesis: a randomized controlled trial. Prenat Diagn 2019;39: 1179-83.
6. Benchahong S, Pongrojpaw D, Chanthasenanont A, Limpivest U, Nanthakomon T, Lertvutivivat S, et al. Cold therapy for pain relief during and after amniocentesis procedure: A randomized controlled trial. J Obstet Gynaecol Res 2021;47:2623-31.
7. Hanprasertpong T, Kor-Anantakul O, Prasartwanakit V, Leetanaporn R, Suntharasaj T, Suwanrath C. Efficacy of cryoanalgesia in decreasing pain during second trimester genetic amniocentesis: a randomized trial. Arch Gynecol Obstet 2012;286:563-6.
8. Gordon MC, Ventura-Braswell A, Higby K, Ward JA. Does local anesthesia decrease pain perception in women undergoing amniocentesis? Am J Obstet Gynecol 2007;196:55.e1-4.
9. Wax JR, Pinette MG, Carpenter M, Chard R, Blackstone J, Cartin A. Reducing pain with genetic amniocentesis-A randomized trial of subfreezing versus room temperature needles. J Matern Fetal Neonatal Med 2005;18:221-4.

10. Pongrojpaw D, Somprasit C, Chanthasenanont A. The efficacy of lidocaine-prilocaine cream to reduce pain in genetic amniocentesis. J Med Assoc Thai 2007;90: 1992-6.
11. Elimian A, Goodman JR, Knudtson E, Wagner A, Wilson P, Williams M. Local anesthesia and pain perception during amniocentesis: a randomized double blind placebo-controlled trial. Prenat Diagn 2013; 33:1158-61.
12. Telapol K, Bumrungphuet S, Israngura Na Ayudhya N. Effect of ethyl chloride spray for pain reduction during amniocentesis: A non-blinded randomized controlled trial. Thai J Obstet Gynaecol 2018;26: 75-82.

Thai Journal of Obstetrics and Gynaecology
January 2024, Vol. 32, No. 1, pp. 22-32.

GYNAECOLOGY

Comparison of Tissue Adequacy from Office-based Endometrial Sampling between Manual Vacuum Aspiration and the Wallach Endocell in Premenopausal and Perimenopausal Women with Abnormal Uterine Bleeding: A randomized controlled trial

Chayanis Apirakviriya, M.D.*,
Somsook Santibenchakul, M.D., PhD. MPH.*,
Patou Tantbirojn, M.D.*,
Noppadol Chaiyasit, M.D.*,
Shina Oranratanaphan, M.D.*,
Nipon Khemapech, M.D.*,
Surasith Chaithongwongwatthana, M.D., MSc.*,
Unnop Jaisamrarn, M.D., MHS.*

** Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

ABSTRACT

Objectives: To investigate the tissue adequacy rate of endometrial biopsy devices (Wallach Endocell® and manual vacuum aspiration [MVA]), focusing on premenopausal and perimenopausal women, in an outpatient setting.

Materials and Methods: In this randomized controlled trial, 290 premenopausal or perimenopausal women with abnormal uterine bleeding who planned to undergo an endometrial biopsy were randomized to Ipas MVA Plus® (n = 146) and Wallach Endocell® (n = 144). The primary outcome was tissue adequacy for pathological examination, which was evaluated by two pathologists. Secondary outcomes were the amount of endometrial tissue, postoperative pain scores, immediate complications of the procedure, patient satisfaction, and operator satisfaction.

Results: The mean and standard deviation of participants' age were 42.8 ± 5.9 and 43.6 ± 5.8 years in the MVA and Endocell groups, respectively. Tissue adequacy for pathological examination in the MVA and Endocell groups was 95.2% and 95.8%, respectively. Both methods did not show differences in tissue adequacy (p = 1.00). The median amount of endometrial tissue was slightly higher in the MVA group than in the Endocell group (2.1 g vs 1.8 g, p = 0.02). The pain score during the procedure was slightly higher in the MVA group than in the Endocell group (p < 0.01). Patient satisfaction and operator satisfaction did not differ between groups. The complications that we found were pelvic pain and pelvic infection,

which did not differ between groups (1.4% vs 2.8%, p = 0.4).

Conclusion: There was no evidence that the efficacy of endometrial tissue biopsy by MVA or Endocell was different in terms of tissue adequacy for pathological diagnosis. Both devices showed a very high percentage of tissue adequacy and very low adverse effects.

Keywords: abnormal uterine bleeding, endocell, endometrial biopsy, manual vacuum aspiration.

Correspondence to: *Somsook Santibenchakula, M.D., PhD. MPH., Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. E-mail: Somsook.S@chula.ac.th*

Received: 29 March 2023, **Revised:** 18 April 2023, **Accepted:** 26 June 2023

การศึกษาแบบสุ่มเปรียบเทียบความเพียงพอของชิ้นเนื้อจากการดูดตัวอย่างเยื่อบุโพรงมดลูกด้วยเครื่องมือดูดสุญญากาศมือถือเทียบกับ Wallach Endocell ในสตรีวัยเจริญพันธุ์และวัยใกล้หมดประจำเดือนที่มีภาวะเลือดออกผิดปกติจากโพรงมดลูก

ชญานิศ อภิรักษ์วิริยะ, สมสุข สันติเบ็ญจกุล, พฐู ตันฑ์ไพโรจน์, นพดล ไชยสิทธิ์, ชินา โอฬารรัตนพันธ์, นิพนธ์ เขมะเพชร, สุรสิทธิ์ ชัยทองวัฒนา, อรรณพ ใจสำราญ

บทคัดย่อ

วัตถุประสงค์*: เพื่อศึกษาอัตราความเพียงพอของชิ้นเนื้อจากการดูดตัวอย่างเยื่อบุโพรงมดลูกด้วยเครื่องมือดูดสุญญากาศมือถือเทียบกับ Wallach Endocell ในสตรีวัยเจริญพันธุ์และวัยใกล้หมดประจำเดือนที่มีภาวะเลือดออกผิดปกติจากโพรงมดลูก ในการตรวจแบบผู้ป่วยนอก*

วัสดุและวิธีการ*: การศึกษานี้เป็นการศึกษาทดลองแบบสุ่มในสตรีวัยเจริญพันธุ์และวัยใกล้หมดประจำเดือนที่มีภาวะเลือดออกผิดปกติจากโพรงมดลูกจำนวน 290 คนที่จำเป็นต้องได้รับการตรวจวินิจฉัยด้วยการดูดชิ้นเนื้อจากเยื่อบุโพรงมดลูก แบ่งกลุ่มโดยการสุ่มตัวอย่าง สตรี 146 คนได้รับการตรวจด้วยเครื่องมือดูดสุญญากาศมือถือและสตรี 144 คน ได้รับการตรวจด้วย Endocell วัตถุประสงค์หลักเพื่อประเมินอัตราความเพียงพอของชิ้นเนื้อในการตรวจทางพยาธิวิทยา ซึ่งประเมินโดยพยาธิแพทย์สองคน วัตถุประสงค์รองคือ ปริมาณชิ้นเนื้อที่ได้ อาการปวด ภาวะแทรกซ้อน รวมทั้งความพึงพอใจของผู้ป่วยและแพทย์ผู้ทำหัตถการ

ผลการศึกษา*: อายุเฉลี่ยของสตรีที่ได้รับการตรวจด้วยเครื่องมือดูดสุญญากาศมือถือและ Endocell เท่ากับ 42.8 และ 43.6 ปีตามลำดับ อัตราความเพียงพอของชิ้นเนื้อในการตรวจทางพยาธิวิทยาเท่ากับร้อยละ 95.2 และ 95.8 ตามลำดับ สำหรับค่ากลางของปริมาณชิ้นเนื้อที่ได้คือ 2.1 กรัมในกลุ่มที่ตรวจด้วยเครื่องมือดูดสุญญากาศมือถือและ 1.8 กรัมในกลุ่ม Endocell (p = 0.02) อาการปวดที่เกิดจากการตรวจพบมากกว่าในกลุ่มที่ได้รับการตรวจด้วยเครื่องมือดูดสุญญากาศมือถือ*

(p < 0.01) สำหรับความพึงพอในของผู้ป่วยและแพทย์ผู้ทำหัตถการไม่แตกต่างกัน ภาวะแทรกซ้อนที่พบ ได้แก่ การติดเชื้อ อาการปวดท้อง พบว่าอัตราการเกิดไม่แตกต่างกันในทั้งสองกลุ่ม (1.4% เทียบกับ 2.8%, p = 0.40)

***สรุป**: ยังไม่พบความแตกต่างในแง่ประสิทธิภาพของวิธีการดูดชิ้นเนื้อจากโพรงมดลูกด้วยเครื่องมือดูดสุญญากาศมือถือ และ Endocell ในแง่ความเพียงพอของชิ้นเนื้อในการตรวจทางพยาธิวิทยา เครื่องมือทั้งสองชนิดมีประสิทธิภาพสูงในการเก็บชิ้นเนื้อและมีภาวะแทรกซ้อนต่ำมาก*

***คำสำคัญ**: เลือดออกผิดปกติจากโพรงมดลูก, endocell, การดูดตัวอย่างชิ้นเนื้อเยื่อบุโพรงมดลูก, เครื่องมือดูดสุญญากาศ*

Introduction

Abnormal uterine bleeding (AUB) is a common problem in clinical practice that leads women to visit gynecologists. The prevalence of approximately 3-30% among reproductive-aged women[1]. Approximately one-third of women are affected at some point in their life[2, 3]. The pattern of bleeding and age of the patient can help assess bleeding etiology. The initial uterine evaluation in women with abnormal uterine bleeding after detailed history taking and physical examination are transvaginal ultrasound (TVUS) in women with enhanced risk for structural abnormality or endometrial sampling in women with enhanced risk of endometrial hyperplasia[1]. Selection for endometrial sampling is based on a combination of risk factors for the presence of premalignant or malignant changes, comprising a combination of age, personal and genetic risk factors, and transvaginal ultrasound (TVUS) screening for endometrial echocomplex thickness. In cases that endometrial sampling cannot retrieve adequate endometrial tissue or suspected the focal uterine cavity lesion, the standard method for evaluating endometrial pathology is hysteroscopy[1]. Hysteroscopy can be performed in an office setting or in an operating theater setting, with or without general anesthesia. However, hysteroscopic procedures require complex instruments, trained personnel, and specialized gynecologists. Office-based endometrial sampling using vacuum aspirator devices is increasingly used because of several advantages. For example, the setting is in an outpatient clinic; the procedure does not require anesthetic; they require a shorter operative time and less cost and equipment. The adequacy of the samples is essential because the use of aspiration techniques implies that the pathology is global rather than focal. However, a previous study revealed that tissue adequacy was 85.6% when using the Wallach Endocell® device (CooperSurgical, Inc., Trumbull, Connecticut, USA)[4], and the detection rates for endometrial carcinoma in postmenopausal and premenopausal women were 99.6% and 91.0%, respectively[5]. Therefore, office-based endometrial aspiration sampling techniques can be used as initial steps to diagnose endometrial pathology.

Office-based endometrial sampling techniques are divided into two categories based on the equipment used: 1) low-pressure devices, such as Pipell® (CooperSurgical, Inc., Trumbull, Connecticut, USA) and Wallach Endocell®, 2) high-pressure devices, such as Vabra aspirator® (Sterylab, Rho, Milan, Italy) and manual vacuum aspiration (MVA). High-pressure devices are superior in terms of endometrial cell volume collection, and the use of such devices should result in a better pathological diagnosis. The Wallach Endocell® is disposable while MVA is a reusable device. MVA has not been widely used for endometrial biopsy, but it is often used in abortions. Therefore, Thai gynecologists are not familiar with the use of MVA to detect endometrial

pathologies.

Endometrial sampling with Endocell®[4,5] revealed that tissue adequacy and detection rate of endometrial carcinoma and atypical hyperplasia are within an acceptable range and that the direct medical cost of endometrial sampling from the patient's perspective is much less than that of hysteroscopy. However, there is limited information on the tissue adequacy, detection rate, and cost-effectiveness of MVA in evaluating endometrial pathology. The characteristics of MVA, which can create a higher negative pressure than Wallach Endocell®, may provide a greater amount of endometrial tissue in which it enhances the better pathological evaluation. This study aimed to investigate the tissue adequacy between Wallach Endocell® and MVA, focusing on premenopausal and perimenopausal women in an outpatient setting.

Materials and Methods

This was a randomized controlled trial, a parallel study, retrospectively registered in the Thai Clinical Trials Registry (study ID TCTR20160225001) and approved by the Chula IRB 35-55. Eligible participants were premenopausal and perimenopausal women with AUB who planned to undergo an endometrial biopsy at King Chulalongkorn Memorial Hospital between November 2012 and October 2013. Inclusion criteria were age over 30 years, premenopausal or perimenopausal status, having an indication for endometrial sampling, and providing written informed consent. We excluded women with active pelvic inflammatory disease, unstable hemodynamic status, or pregnancy. Baseline characteristics, such as parity, number of vaginal deliveries, body mass index, underlying disease, and pattern of AUB were collected. The study participants were randomized into two groups using a block of four randomization techniques. A biostatistician, who was not involved in data analysis, conducted a computer-generated randomization sequence. To ensure concealment, the allocation was placed inside of opaque envelopes and sealed. A 1:1 allocation ratio was used for the MVA and Endocell group. The allocation sequence was concealed until the endometrial biopsy procedure. Participants underwent endometrial sampling according to the assigned protocol. Participants and pathologists were blinded to the sampling method. The sample size was calculated based on the results of a previous study[5] (85.6% of tissue adequacy rate using Endocell endometrial biopsy) and the expected tissue adequacy using MVA was 95.6%. A total of 290 participants were included, and the calculation was based on a statistical significance level of 0.05% to yield a power of 90%.

The endometrial biopsy was performed in the outpatient clinic on the same day as the participants enrolled in the study. Endometrial biopsy was performed in the following steps. First, a bimanual pelvic examination was performed to identify the size, shape, and orientation of the uterus. Then, a bivalve speculum or vaginal retractor was inserted, and the vagina and cervix were cleaned with 10% povidone-iodine solution. According to the protocol, group A was assigned to Ipas MVA Plus® (Ipas, Chapel Hill, NC, USA) with an Ipas 3-mm cannula, while group B was assigned to the Wallach Endocell® as the endometrial biopsy device. An Ipas 3 mm cannula and Ipas MVA Plus® were reusable devices. The Ipas 3-mm cannula was 23 cm in length and 3-mm in outer diameter. After insertion of the Ipas 3-mm cannula, the cannula was attached to a 60-ml double-valve syringe, which was locked to create negative pressure. Endometrial tissue was aspirated into the syringe when the valve was turned on. The Wallach Endocell® was a disposable flexible plastic cannula diameter (24.3-cm in length and 3.1-mm in outer diameter). After insertion of the cannula, the plunger was smoothly drawn back to the stop, creating a negative pressure within the sheath. In cases where the cannula could not be inserted, a tenaculum was used to straighten the cervico-uterine angle in patients by grasping the anterior lip and gently drawing outward in direction. If the uterine cavity could not be accessed, misoprostol (400 mcg) was inserted into

the vagina for 3 hours, and the procedure was repeated. Endometrial tissue was collected with at least four complete back-and-forth passes with the device from the fundus to the internal os. Each specimen was expelled into a formalin container. Before removing the device, the depth of the uterus was checked and recorded. The amount of endometrial tissue, postoperative pain scores, operative time, and immediate complications of the procedure were recorded. Tissue adequacy of the procedure was defined as the presence of both adequate endometrial glands and stroma to identify endometrial histology, as reported by two pathologists who were blinded to the endometrial sampling procedure. In case of a discrepancy in pathological results, the final pathological diagnosis was made based on the consensus of both pathologists after reviewing the specimens together. Patient and doctor satisfaction were collected on the same day using a visual analog scale. Data were analyzed using STATA version 17 (StataCorp LLC, College Station, TX, USA). Continuous variables were represented as mean (standard deviation [SD]) or median (interquartile range), while categorical variables were represented as numbers and percentages. The unpaired t-test was used to compare normally distributed continuous variables, and the Wilcoxon rank-sum test was used for a skewed distribution. Categorical variables were compared using Fisher's exact test.

Results

This study enrolled 290 women with AUB required endometrial biopsy. A total of 146 women were assigned to receive endometrial biopsy using Ipas MVA Plus®, and 144 women were assigned to using Wallach Endocell®. Each participant underwent an endometrial biopsy with an instrument as assigned. The study flow is shown in Fig. 1. The mean age ± SD was 42.8 ± 5.9 and 43.6 ± 5.8 years in the MVA and Endocell groups, respectively. The baseline characteristics are shown in Table 1. The majority of the participants in both groups were parous and had at least one vaginal delivery. The characteristics of abnormal bleeding did not differ between the two groups.

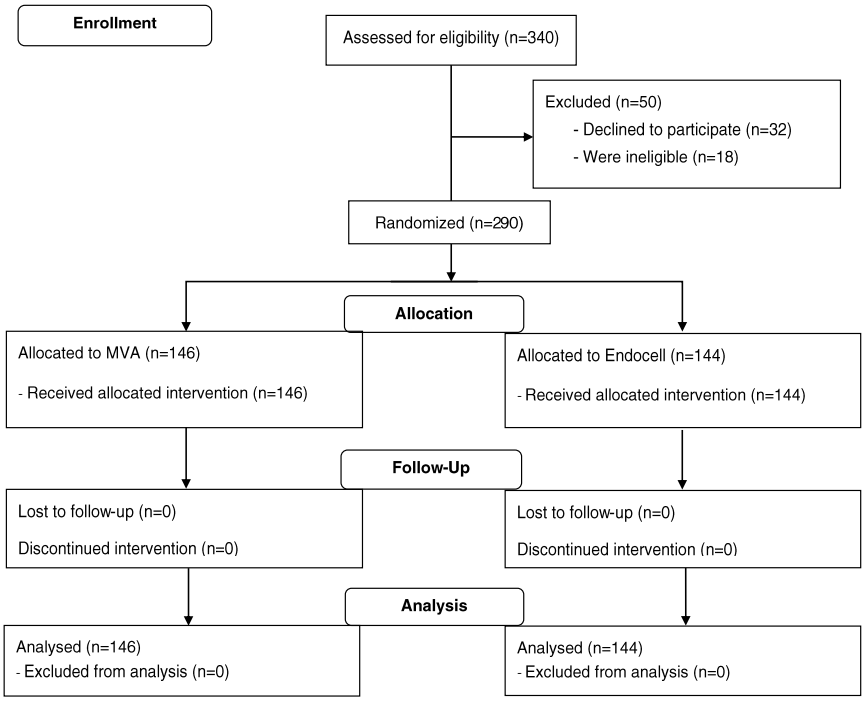


Fig. 1. CONSORT flow diagram

Table 1. Baseline characteristics of the groups

Characteristics	MVA (n = 146)	Endocell (n = 144)
Age (years), n (%)		
< 35	15 (10.3)	10 (6.9)
35 - 49	114 (78.1)	111 (77.1)
> 50	17 (11.6)	23 (16.0)
Parity, n (%)		
0	32 (21.9)	45 (31.3)
1	42 (28.8)	40 (27.8)
2	51 (34.9)	44 (30.6)
> 3	21 (14.4)	15 (2.4)
Vaginal birth, n (%)		
0	74 (50.7)	76 (52.8)
1	27 (18.5)	31 (21.5)
2	25 (17.1)	28 (19.4)
> 3	20 (13.7)	9 (6.3)
Duration of a recent episode of AUB (days), median (IQR)	16 (7-40)	14 (7-21)
Years since the last child, median (IQR)	11 (4-17)	11 (0-19)
Menarche (years), n (%)		
≤ 11	15 (10.3)	15 (10.4)
12-15	114 (78.1)	103 (71.5)
≥ 16	17 (11.6)	26 (18.1)
BMI (kg/m^2), n (%)		
< 18.5	8 (5.5)	4 (2.8)
18.5-22.9	45 (30.8)	54 (37.5)
23-24.9	20 (13.7)	22 (15.3)
25-29.9	55 (37.7)	42 (29.2)
≥ 30	18 (12.3)	22 (15.3)
AUB type, n (%)		
Intermenstrual bleeding	47 (32.2)	40 (27.8)
Heavy menstrual bleeding	47 (32.2)	51 (35.4)
Heavy menstrual bleeding and intermenstrual bleeding	36 (24.7)	39 (27.1)
Others[a]	16 (11.0)	14 (9.7)
DM, n (%)		
No	138 (94.5)	135 (93.8)
Yes	8 (5.5)	9 (6.3)
HT, n (%)		
No	136 (93.2)	129 (89.6)
Yes	10 (6.9)	15 (10.4)
Dyslipidemia, n (%)		
No	139 (95.2)	138 (95.8)
Yes	7 (4.8)	6 (4.2)
Anemic symptoms, n (%)		
No	85 (58.2)	85 (59.3)
Yes	60 (41.1)	59 (41.0)

MVA: manual vacuum aspiration, AUB: abnormal uterine bleeding, BMI: body mass index, DM: diabetes mellitus, HT: hypertension, IQR: interquartile range
[a] Others, e.g., oligomenorrhea.

An endometrial biopsy could be performed in all participants in whom no other sedation was required; the biopsy procedure was mostly performed by the attending physicians in both groups (76.3% vs 70.8%, p = 0.35). In the MVA group, the tenaculum was needed to facilitate the cannula insertion more than in the Endocell group, but the requirement for misoprostol to ripen the cervix before cannula insertion was not different. The only complication of misoprostol was pelvic pain, with an incidence of 22.2%. The pain score during the procedure was slightly higher in the MVA group than in the Endocell group, which was statistically significant. The mean difference in pain score during the procedure was 1.13 (0.53-1.74), as shown in Table 2. Participants in the MVA group needed more pain medication immediately after the procedure compared to the Endocell group (32.9% vs 17.4%, p < 0.01). Patient satisfaction and operator satisfaction did not differ between the two groups. Complications that we observed after the procedure were pelvic pain and pelvic infection, which did not differ between the two groups.

Table 2. Details of endometrial sampling procedure

	MVA (n = 146)	Endocell (n = 144)		p value
Type of operator, n (%)				
Resident	35 (24.0)	42 (29.2)		0.35[a]
Attending	111 (76.0)	102 (70.8)		
Uterine depth, median (IQR)	7.5 (7-8)	7 (7-8)		0.38[b]
Tenaculum used, n (%)				
No	50 (34.5)	71 (49.3)		0.01[a]
Yes	96 (67.5)	73 (50.7)		
Misoprostol used, n (%)				
No	140 (95.9)	141 (97.9)		0.50[a]
Yes	6 (4.1)	3 (2.1)		
Pain score (VAS), mean (SD)	5.6 (2.7)	4.5 (2.6)	MD 1.13 95%CI (0.53-1.74)	
Use of a pain killer immediately after the procedure				
No	98 (67.1)	119 (82.6)		< 0.01[a]
Yes	48 (32.9)	25 (17.4)		
Patient satisfaction, mean (SD)	4.4 (0.6)	4.4 (0.6)	MD 0.02 95%CI (-0.12–0.15)	0.83[c]
Operator satisfaction, mean (SD)	4.2 (0.7)	4.3 (0.6)	MD -0.04 95%CI (-0.20–0.12)	0.63[c]
Complications within 2 weeks				
No	144 (98.6)	140 (97.2)		0.40[a]
Yes, e.g., pelvic pain, pelvic infection	2 (1.4)	4 (2.8)		

MVA: manual vacuum aspiration, IQR: interquartile range, MD: mean difference, VAS: visual analog scale, SD: standard deviation
[a] Fisher's exact rest, [b] Wilcoxson rank-sum test, [c] unpaired t-test.

Tissue adequacy for pathological examination in the MVA and Endocell groups was 95.2% and 95.8%, respectively. Both methods did not show differences in tissue adequacy. The median amount of endometrial tissue was slightly higher in the MVA group than in the Endocell group (2.1 g vs 1.8 g, p = 0.02), but this difference did not affect the adequacy of the pathological diagnosis. The mean difference in endometrial tissue was 0.59 g (0.01-1.08, p = 0.02). Age, parity, and vaginal birth did not affect the adequacy of endometrial tissue between biopsy procedures. However, the tissue adequacy rate tended to be higher in younger women, as shown in Table 3. Among the 13 women who had inadequate tissue, five women underwent uterine curettage, and one woman underwent a hysterectomy, while five women did not

undergo further investigation. The clinical signs of AUB improved after 6-month follow-up, and two women were lost to follow-up. The histopathological findings of women with adequate tissue are shown in Table 4.

Table 3. Tissue adequacy of the groups in all participants and stratified by age, parity, and number of vaginal births

	MVA (n = 146)	Endocell (n = 144)	p value[a]
Participant, n (%)	139 (95.2)	138 (95.8)	1.00
Age group (years), n(%)			
< 35	15 (100)	10 (100)	1.00
35 - 49	109 (95.6)	107 (96.4)	1.00
≥ 50	15 (88.2)	21 (91.3)	1.00
Parity, n (%)			
0	29 (90.6)	44 (97.8)	0.30
1	41 (97.6)	39 (97.5)	1.00
≥ 2	69 (95.8)	55 (93.2)	1.00
Vaginal birth, n (%)			
0	69 (93.2)	72 (94.7)	0.74
1	27 (100)	29 (93.6)	0.49
≥ 2	43 (95.6)	37 (100)	0.49

MVA: manual vacuum aspiration, [a] Fisher's exact test.

Table 4. Pathological diagnosis of the groups

	MVA	Endocell	p value
Amount of endometrial tissue (gram), median (IQR)	2.1 (1.2 - 3.6)	1.8 (1.1 - 2.9)	0.02[a]
Physiological results (n = 173), n (%)			
Proliferative endometrium	57 (67.1)	63 (71.6)	0.14[b]
Secretory endometrium	17 (20.0)	21 (23.9)	
Menstruation	1 (1.2)	2 (2.3)	
Inactive endometrium	8 (9.4)	2 (2.3)	
Atrophic endometrium	2 (2.4)	0	
Pathological results (n = 77), n (%)			
Endometrial polyp	33 (76.7)	22 (62.9)	0.59[b]
Persistent proliferative phase	8 (18.6)	10 (28.6)	
Submucous myoma	1 (2.3)	1 (2.9)	
Endometritis	1 (2.3)	2 (5.7)	
Endometrial hyperplasia (n = 26), n (%)			
Simple hyperplasia without atypia	8 (72.7)	12 (80.0)	0.40[b]
Complex hyperplasia without atypia	1 (9.1)	0	
Simple hyperplasia with atypia	1 (9.1)	3 (20.0)	
Complex hyperplasia with atypia	1 (9.1)	0	
CA endometrium (n = 1), n (%)	0	1 (100)	

MVA: manual vacuum aspiration, IQR: interquartile range
[a] Wilcoxson rank-sum test; [b] Fisher's exact test.

Discussion

This study showed very high percentages of tissue adequacy from both endometrial tissue biopsy devices, MVA and Endocell. The tissue adequacy

using MVA device was 95.2% and using Endocell device was 95.8%. The degree of negative suction pressure from the MVA affects the amount of collected endometrial tissue; as shown in our study, the amount of endometrial tissue from the MVA device was significantly higher than that from the Endocell device. However, the mean difference in the collected tissue was small, only 0.59 g and did not affect the pathological diagnosis. The tissue adequacy rate in our study was higher than Wanijasombutti's study[4], which showed an 83.3% tissue adequacy rate using the MVA device and an 81.1% tissue adequacy rate using the Endocell device. However, the tissue adequacy rate was comparable between the two devices, similar to our study. Another study[6] showed that the tissue adequacy rate using MVA endometrial sampling was 87.8%. For Endocell endometrial biopsy devices, previous studies[4,7] reported a tissue adequacy rate of 72.7-85.6%. Previous studies[8,9] on Karman endometrial aspiration showed a tissue adequacy rate of 76.4-86.7%. Our study showed a higher tissue adequacy rate compared to previous studies[4, 6, 7]. The factor that may have affected this outcome was the menopausal status of the participants. Our study included only premenopausal and perimenopausal women, but previous studies[4, 6, 7] included both premenopausal and postmenopausal women. The effect of estrogen level, which impacts endometrial growth, also plays an important role in endometrial thickness and tissue adequacy for pathological examination. As in our study, a higher tissue adequacy rate was found in the younger age group. Even in the premenopausal and perimenopausal age group, our study showed that MVA and Endocell devices still provided a comparable tissue adequacy for pathological examination.

Our study found that the endometrial pathology in patients with AUB of both endometrial biopsy devices was not different. Pathological examination revealed 62.5% physiologic endometrium, 27.8% pathological endometrium, 9.4% endometrial hyperplasia, and 0.4% endometrial carcinoma. The most common benign pathologic endometrium was endometrial polyp (71.4%), followed by persistent proliferative endometrium (23.4%), endometritis (3.9%), and submucous myoma (2.6%). The sequence of endometrial pathological findings was similar to that of a previous study[10].

The pain score was slightly higher in the MVA group than in the Endocell group, which was statistically significant. This result is mainly related to the degree of negative suction pressure of the 60 ml MVA syringe. Furthermore, the MVA group also needed a tenaculum to facilitate cannula insertion more than the Endocell group, which also affected the pain. However, the patient satisfaction score did not differ between the groups. Our study found cervical stenosis in 3.1% of the participants which was not different between groups. After insertion of 400 µg misoprostol transvaginally for 3 h, we were able to access the uterine cavity in all participants without the need for analgesia. Cervical priming may be an optional way to overcome cervical stenosis without requiring cervical dilatation or analgesia, which is less practical in an outpatient setting. Previous studies[11,12] also showed the effectiveness of misoprostol in facilitating cervical dilatation, decreasing the duration of the endometrial biopsy procedure, and lowering the pain score compared to placebo. The only side effect we found from misoprostol insertion was pelvic pain. Other side effects are rare and fully reversible. The following symptoms may occur: diarrhea, shivering, nausea, vomiting, increased body temperature, and skin rash[13]. Endometrial sampling with both devices showed a small risk of complications. The rate of complications was not different between the two groups. Severe pelvic pain is the most common complication, but can be resolved with non-steroidal anti-inflammatory drugs, and hospital admission or opioid analgesia are not necessary. Pelvic infection was found in 0.7% in our study. Routine administration of antibiotic prophylaxis in endometrial biopsies is not recommended[14,15]. A careful history and pelvic examination for recent cervicitis or pelvic infection should be performed in every woman before performing an endometrial biopsy; if there are any

signs of cervical or pelvic infection, the procedure should be postponed.

Our data showed that tissue adequacy for pathological diagnosis from both endometrial biopsy devices were not different. The side effects from both procedures were very low and there was no difference. However, the pain associated with procedure was less in the endocell group than the MVA group. Therefore, in terms of the discomfort associated with the procedure, Endocell may be a good option.

This was a prospective, randomized, parallel study. Both participants and certified gynecologic pathologists were blinded to the procedure. In our clinical practice, the initial uterine evaluation in women with abnormal uterine bleeding after detailed history taking and physical examination are transvaginal ultrasound (TVUS) in women with enhanced risk for structural abnormality or endometrial sampling in women with enhanced risk of endometrial hyperplasia[1]. Therefore, not all participants were evaluated by TVUS before endometrial biopsy. Another limitation of our study is that participants with inadequate tissue for pathological diagnosis did not undergo a hysteroscopic procedure to obtain a definite diagnosis of the cause of AUB. However, we followed-up with all of these participants, and no further endometrial pathologies or cancers were detected. MVA is a reusable device that can reduce the cost for the patient who receives an endometrial biopsy. The personnel who process the instruments need to be well-trained in cleaning and disinfecting the instrument. Otherwise, the instrument will be damaged.

Conclusion

There was no evidence that the efficacy of endometrial tissue biopsy by MVA or Endocell was different in terms of tissue adequacy for pathological diagnosis. Both devices showed a very high percentage of tissue adequacy and very low adverse effects.

Acknowledgments

We acknowledge the contribution and cooperation of Research assistants Ms. Somtawin Pojjanasopanakun, Ms. Rachanee Wongwathanavikrom, Ms. Surapeee Suntavaruk, Ms. Chalalai Amatayakul, Ms. Nantana Thongrod, Ms. Kangsadal Amatayakul, and all staff at the Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Munro MG, Critchley HOD, Fraser IS, FIGO Menstrual Disorders Committee. The two FIGO systems for normal and abnormal uterine bleeding symptoms and classification of causes of abnormal uterine bleeding in the reproductive years: 2018 revisions. Int J Gynaecol Obstet 2018;143:393-408.
2. Oehler MK, Rees MC. Menorrhagia: an update. Acta Obstet Gynecol Scand 2003;82:405-22.
3. Liu Z, Doan QV, Blumenthal P, Dubois RW. A systematic review evaluating health-related quality of life, work impairment, and health-care costs and utilization in abnormal uterine bleeding. Value Health 2007;10:183-94.
4. Wanijasombutti P, Imruetaicharoenchok A, Tangjitgamol S, Loharamtaweethong K, Phuriputt N, Phaloprakarn C. Comparison of tissue adequacy for histologic examination from Ipas MVA plus and Wallach Endocell in women with abnormal uterine bleeding. J Obstet Gynaecol Res 2015;41:1246-54.
5. Dijkhuizen FP, Mol BW, Brölmann HA, Heintz AP. The accuracy of endometrial sampling in the diagnosis of patients with endometrial carcinoma and hyperplasia: a meta-analysis. Cancer 2000;89:1765-72.
6. Kitiyodom S. The adequacy of endometrial sampling: comparison between manual vacuum aspiration and metal curettage method. J Med Assoc Thai 2015;98: 523-7.
7. Kunaviktikul K, Suprasert P, Khunamornpong S, Settakorn J, Natpratan A. Accuracy of the Wallach Endocell endometrial cell sampler in diagnosing endometrial carcinoma and hyperplasia. J Obstet Gynaecol Res 2011;37:483-8.
8. Tansathit T, Chichareon S, Tocharoenvanich S, Dechsukhum C. Diagnostic evaluation of Karman endometrial aspiration in patients with abnormal uterine bleeding. J Obstet Gynaecol Res 2005;31: 480-5.

9. Zutshi V, Gupta M, Kaur P, Malik A, Zaheer S, Gambhir P. Office endometrial sampling: a comparison between Endosampler and Karman cannula number 4. Int J Reprod Contracept Obstet Gynecol 2018;7:4.
10. Doraiswami S, Johnson T, Rao S, Rajkumar A, Vijayaraghavan J, Panicker VK. Study of endometrial pathology in abnormal uterine bleeding. J Obstet Gynaecol India 2011;61:426-30.
11. Maneesorn W, Chanthasenanont A, Bhamarapravatana K, Suwannarurk K. Misoprostol for cervical ripening prior to manual vacuum aspiration (MVA) in abnormal uterine bleeding: double blinded randomized controlled trial. J Med Assoc Thai 2013;96:1525-30.
12. Ghosh A, Chaudhuri P. Misoprostol for cervical ripening prior to gynecological transcervical procedures. Arch Gynecol Obstet 2013;287:967-73.
13. Fiala C, Gemzell-Danielsson K, Tang OS, von Hertzen H. Cervical priming with misoprostol prior to transcervical procedures. Int J Gynaecol Obstet 2007;99 (Suppl. 2):S168-71.
14. Van Eyk N, van Schalkwyk J, Infectious Disease Committee. Antibiotic prophylaxis in gynaecologic procedures. J Obstet Gynaecol Can 2012;34:382-91.
15. ACOG Practice Bulletin No. 195. ACOG Practice Bulletin No. 195: Prevention of infection after gynecologic procedures. Obstet Gynecol 2018;131:e172-89.
16. WHO/IASO, IOTF. The Asia-Pacific Perspective: Redefining obesity and its treatment health communications, Australia; Melbourne, 2000

Thai Journal of Obstetrics and Gynaecology
January 2024, Vol. 32, No. 1, pp. 33-44.

GYNAECOLOGY

Cut-off Levels of Visceral Adiposity Index for Determining Hyperandrogenemia in Women with Polycystic Ovary Syndrome

Suchada Indhavivadhana, M.D.*,
Saranya Pattaranutaporn, M.D.*,
Thanyarat Wongwananuruk, M.D.*,
Panicha Chantrapanichkul, M.D.*,
Kitirat Techatraisak, M.D., Ph.D.*,
Nutchaya Sa-nga-areekul, B.N.S*.

** Gynecologic Endocrinology Unit, Department of Obstetrics and Gynecology Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*

ABSTRACT

Objectives: To evaluate the optimum cut-off values and AUC of visceral adiposity index (VAI) and lipid accumulation product (LAP) to predict hyperandrogenemia in Thai polycystic ovary syndrome (PCOS) women and to identify factors that associated with these values.

Materials and Methods: This prospective cross-sectional study recruited 102 Thai PCOS women, aged 18-45 years. All participants were measured for anthropometric data, lipid, carbohydrate and androgen profiles. VAI, LAP and free testosterone were calculated. The receiver operating characteristics (ROC) curve was performed to evaluate the optimum cut-off values of VAI and LAP in predicting hyperandrogenemia, and also to identify factors associated with VAI and LAP.

Results: The mean ± standard deviation of age was 26.9 ± 5.7 years. Prevalence of hyperandrogenemia was 49%. The optimal cut-off values of VAI and LAP in predicting hyperandrogenemia were ≥ 1 (AUC = 0.755) and ≥ 16.5 (AUC = 0.756) respectively. VAI was positively correlated with LAP, hip circumference (HC), waist-to-hip ratio (WHR), the waist-to-height ratio and low density lipoprotein-cholesterol. LAP was significantly correlated with HC, waist circumference, and WHR. Both LAP and VAI were significantly correlated with free testosterone and free androgen index (FAI), but not correlated with total testosterone and dehydroepiandiosterone sulfate.

Conclusion: Both VAI and LAP were significantly correlated with free testosterone and FAI. A VAI value of ≥1 and a LAP value of ≥ 16.5 were determined to be indicators for predicting hyperandrogenemia in PCOS women.

Keywords: hyperandrogenemia, lipid accumulation product, polycystic ovary syndrome, visceral adiposity index.

Correspondence to: *Suchada Indhavivadhana, M.D., Gynecologic Endocrinology Unit, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. E-mail: suchada.ind@mahidol.ac.th*

Received: 17 February 2023, **Revised:** 1 June 2023, **Accepted:** 15 June 2023

การศึกษาค่าจุดตัดของ visceral adiposity index เพื่อทำนายภาวะฮอร์โมนเพศชายสูงในหญิงกลุ่มอาการถุงน้ำรังไข่หลายใบ

สุชาดา อินทวิวัฒน์, ศรัณยา ภัทรนุธาพร, ธันยารัตน์ วงศ์วนานุรักษ์, ปณิชา จันทราพานิชกุล, กิติรัตน์ เตชะไตรศักดิ์, ณัฐชญา สง่าอารีย์กุล

บทคัดย่อ

วัตถุประสงค์: *เพื่อหาค่าจุดตัดที่เหมาะสมและค่า AUC ของ visceral adiposity index (VAI) และ lipid accumulation product (LAP) ในการทำนายภาวะฮอร์โมนเพศชายสูง ของหญิงไทยที่มีกลุ่มอาการถุงน้ำรังไข่หลายใบร่วมกับหาปัจจัยเสี่ยงที่สัมพันธ์กับค่าเหล่านี้*

วัสดุและวิธีการ: *การวิจัยแบบภาคตัดขวาง (cross section study)ในหญิงไทยที่มีกลุ่มอาการถุงน้ำรังไข่หลายใบจำนวน 102 คน ที่มีอายุ 18-45 ปี ผู้เข้าร่วมวิจัยจะได้รับการตรวจวัด ข้อมูลพื้นฐานและตรวจเลือดเพื่อนำมาใช้ในการคำนวณ VAI, LAP และฮอร์โมนเพศชายอิสระ (Free testosterone) และหลังจากนั้นนำมาคำนวณาจุดตัดที่เหมาะสม รวมถึงปัจจัยที่มีผลต่อ VAI และ LAP*

ผลการศึกษา: *อายุเฉลี่ยของผู้เข้าร่วมวิจัย 26.9 ± 5.7 ปี พบความชุกของภาวะฮอร์โมนเพศชายสูงร้อยละ 49 จุดตัดที่เหมาะสมของค่า VAI และ LAP ในการทำนายภาวะฮอร์โมนเพศชายสูง คือ ≥ 1 (AUC = 0.755) และ ≥ 16.5 (AUC = 0.756) ตามลำดับ การศึกษาพบว่าค่า VAI สัมพันธ์กับ LAP เส้นรอบสะโพก (hip circumference) สัดส่วนรอบเอวต่อรอบสะโพก (waist-to-height ratio) และค่าไขมันคลอเรสเตอรอลชนิด LDL นอกจากนี้ยังพบว่าค่า VAI และ LAP มีความสัมพันธ์อย่างมีนัยสำคัญกับระดับฮอร์โมนเพศชายอิสระและดัชนีฮอร์โมนเพศชายอิสระ (free androgen index)*

สรุป: *ค่า VAI และ LAP สัมพันธ์กับกับระดับฮอร์โมนเพศชายอิสระและดัชนีฮอร์โมนเพศชายอิสระ (free androgen index) อย่างมีนัยสำคัญและค่า VAI ที่ตั้งแต่ 1 ขึ้นไปและ LAP ตั้งแต่ 16.5 ขึ้นไป สามารถทำนายภาวะ ฮอร์โมนเพศชายสูงในหญิงไทยที่มีกลุ่มอาการถุงน้ำรังไข่หลายใบ*

คำสำคัญ: *ภาวะไขมันในเลือดสูง, lipid accumulation product, กลุ่มอาการถุงน้ำรังไข่หลายใบ, visceral adiposity index*

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among women of reproductive age, approximately 10-15% worldwide[1]. Clinical manifestations of PCOS are irregular menstruation, anovulation, hirsutism, acne, male pattern alopecia and infertility[2]. Furthermore, PCOS is strongly associated with central obesity, insulin resistance and metabolic syndrome, all of which increase metabolic cardiovascular risk[3].

Among the diagnostic criteria, hyperandrogenism is a key feature of PCOS[2]. An androgen excess in PCOS is primarily produced by ovaries; other causes are the adrenal glands and peripheral conversion. The mechanism of androgen excess results from an increase in LH pulse frequency, leading to an increase in theca cell volume and androgen synthesis. In addition, insulin and abdominal visceral adiposity contribute to the development of androgen excess in PCOS by increasing lipolysis of visceral adiposity, followed by the release of more free fatty acids and the production of more adipocytokines. The deterioration in insulin sensitivity causes hyperinsulinemia and, eventually, increases ovarian androgen production and decreases hepatic sex hormone-binding globulin production. Furthermore, in PCOS the insulin receptor increases phosphorylation of serine, which inhibits the insulin signaling pathway, causing insulin resistance. Elevated levels of serum free fatty acids also cause androgen overproduction by increasing in serine phosphorylation of P450c17. Consequently, chronic androgen excess results in abdominal visceral obesity in a vicious cycle[1, 3-5].

Abdominal visceral fat surrounds internal abdominal organs such as the liver, pancreases and intestines[6, 7]. Abdominal visceral fat has been found to be associated with several metabolic conditions, including impaired glucose, insulin resistance, poor lipid metabolism, metabolic syndrome, and hyperandrogenism in PCOS[3]. The gold standard to directly evaluate visceral and subcutaneous adipose tissue is by imaging, including computerized tomography (CT) and magnetic resonance imaging (MRI); nevertheless, imaging is not recommended as a routine procedure due to its high cost and complicated, technical nuances. As a result, indirect methods have been progressively introduced to determine visceral adipose tissue. They include anthropometric data (body mass index (BMI), waist circumference (WC) and waist-to-hip ratio (WHR)), the visceral adiposity index (VAI), and the lipid accumulation product (LAP). While BMI and WC cannot distinguish between subcutaneous and visceral fat[6-9]. VAI and LAP are reliable indirect methods for determining abdominal visceral tissue.

In the recent years, many studies have investigated the association between VAI and various features of PCOS, such as the severity of anovulation, insulin resistance, hyperandrogenemia and metabolic syndrome in PCOS women[10-13]. Aboelnaga et al[14] studied the correlation of VAI with the Rotterdam criteria in Egyptian PCOS women. They reported that total testosterone was significantly correlated with VAI, WC, BMI and weight. Furthermore, Androulakis et al in 2014[12], reported that VAI correlation with free testosterone level was significantly positive.

Currently, there is insufficient evidence to established definitive values for hormonal levels or clinical presentations of hyperandrogenism in case of hyperandrogenemia. Accurate methods of free testosterone measurement (such as equilibrium dialysis, gas or liquid chromatography-mass spectrometry) are technically complex, costly and not widely available[2]. In this study, we set out to establish measurements that could be easily and cost-effectively used to screen for hyperandrogenemia in Thai PCOS women. Accordingly, the aim of the study was to ascertain the cut-off points for VAI and LAP values that could be used to predict hyperandrogenemia in Thai PCOS women, and to identify factors that are significantly associated with those values in the PCOS population. Data from this study will improve screening; in turn, this will result in an increase in definite diagnoses of hyperandrogenemia and a reduction in healthcare costs related to using the testosterone cut-off.

Materials and Methods

This prospective cross-sectional study was performed from October 2019 to February 2020 at the Gynecologic Endocrinology Clinic, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj hospital, Mahidol University, Thailand. The study was conducted in accordance with the principle of the International Conference on Harmonization in Good Clinical Practice (ICH-GCP), Declaration of Helsinki, the Belmont Report and The Council for International Organizations of Medical Sciences (CIOMS) Guidelines. The protocol of this study was approved by the Siriraj Institutional Review Board (SIRB No.561/2562). All participants were informed and written consent to participate in this study was obtained.

In all, the study recruited 102 Thai PCOS women, who had been diagnosed using the 2003 Rotterdam criteria[15]. Eligible participants were registrants who were 18-45 years of age; were not pregnant; and during the 3 months preceding their participation, had not taken any hormones, hormonal contraceptive drugs, steroids, or other medications which could interfere with their serum lipid profiles or insulin and androgen levels (for example, niacin, corticosteroid, beta-blockers, calcium channel blocker, and lipid-lowering medications). Patients were excluded if they had a severe medical disease, such as a liver or renal disease, that may cause an abnormal liver function. After the diagnosis of PCOS was made, all patients were registered to this project. After their written informed consent was obtained, their personal and medical history was obtained in a structured interview. Their medical history records in the hospital database were also reviewed by the investigators to see if any of the exclusion criteria were met. Baseline characteristics were collected and recorded in case record form. Physical examinations measured height (cm), weight (kg), waist and hip circumference (WC and HC; cm), and blood pressure (mmHg). The values were subsequently used to calculate BMI, WHR, and waist-to-height ratio (WHtR). The following were also recorded: signs of clinical hyperandrogenism using modified Ferriman-Gallwey score (mFG), carbohydrate metabolic profiles (fasting glucose and insulin, 2-hr glucose and insulin post 75-gram oral glucose loading or oral glucose tolerance tests (OGTT), and calculated homeostatic measurement assessment-insulin resistance or homeostatic model assessment for insulin resistance (HOMA-IR), lipid profiles (total cholesterol, triglyceride (TG), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C)) and androgens (dehydroepiandrosterone sulfate (DHEAS); total testosterone and calculated free testosterone) were recorded. Details of measurements and biochemical assays were present in our previous report[13].

Laboratory assays

All laboratory assays for carbohydrate profiles were performed using automatic analyzers, Cobas 8000 with ISO 15189 certification. Serum total testosterone was measured using electrochemiluminescence immunoassay (ECLIA) on a Roche Cobas 8000 c602 instrument (Roche Diagnostic, Germany) with intra-assay coefficient of variation (CV) of 1.57% - 2.26%, and inter-assay CV of 2.92% - 4.32%. DHEA-S and sex hormone binding globulin (SHBG) were analyzed by measured using ECLIA on a Roche Cobas 8000 modular analyzer.

Visceral adiposity index (VAI) and Lipid accumulation product (LAP)[8, 9]

VAI is a gender specific, mathematical model which combines anthropometric data (BMI and WC) and functional parameter (TG and HDL-C). VAI was calculated by using the following formula.

VAI (women) = WC/[(36.58+(1.89×BMI)]× TG/0.81×1.52/HDL

LAP is a simple, sex specific formula that combines waist circumference and triglyceride concentration. LAP was calculated by using the following formula.

LAP women = (WC-58) x TG concentration

WC is expressed in centimeter, BMI in kg/m^2, TG in mmol/L and HDL-C in mmol/L.

Definition of hyperandrogenemia

Clinical hyperandrogenism was diagnosed when a modified Ferriman-Gallwey score ≥ 8[16]. Hyperandrogenemia was diagnosed when the serum level of least one androgen was higher than the recommended cutoff, namely, total testosterone > 0.8 ng/mL; free testosterone > 0.006 ng/mL; or DHEAS > 350 µg/dL[17]. Free testosterone level was calculated using method described by Vermeulen et al[18] which includes total testosterone, SHBG and albumin level in the calculation.

Definition of metabolic syndrome

Metabolic syndrome was defined according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III criteria, in Asian[19], metabolic syndrome is present if three or more of the following five criteria are met included WC ≥ 80 cm, elevated blood pressure (systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥ 85 mmHg), fasting TG level ≥ 150 mg/dL, fasting HDL-C level < 50 mg/dL and impaired glucose tolerance (fasting plasma glucose ≥ 100 mg/dL).

Statistical analysis

From the previous study by Hossein et al[20], the optimal cut-off point of anthropometric measurement to predict insulin resistance has an area under the curve (AUC) of 0.75. Since there is no previous study that assesses the cut-off point for VAI and LAP to predict hyperandrogenemia in PCOS patients, we hypothesized that VAI could predict hyperandrogenemia with sensitivity at 75%. At the error level of 12%, the calculation formula with p = 0.75 and α = 0.05 was applied. We need 51 PCOS with hyperandrogenemia to test this hypothesis. According to statistic records at the Gynecologic Endocrinology Clinic, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, the prevalence of hyperandrogenemia was found to be 50%. Therefore, we needed a total 102 patients to be enrolled in our study.

Descriptive characteristics were presented in mean and standard deviation (SD), median and interquartile range (IQR), number (n) and percent (%), or odds ratio (OR) and 95% confidence interval (CI). Pearson correlation coefficient (r) was used to analyze factors associated with VAI and LAP. Comparisons between hyperandrogenemia and non-hyperandrogenemia, also metabolic and non-metabolic syndrome groups were used student's t-test or Mann-Whitney U test for continuous data and chi-square test or Fisher's exact test for categorical data. All tests were two-sided, and the cut-off levels of VAI and LAP for determining hyperandrogenemia were used in receiver operating characteristics (ROC) curve analysis. All statistical analysis was performed using SPSS version 22.0. A p value < 0.05 was considered to be statistically significant.

Results

In all, 102 PCOS women were enrolled. Their baseline characteristics are detailed in Table 1 and 2. The mean age was 26.9 ± 5.7 years; the mean BMI was 26.1 ± 6.7 kg/m^2. Among the participants, 41.2 % were classified as normal weight, while 33.3% were categorized as being overweight according to Asian BMI reference. Clinical hyperandrogenism was found in 15.7% of the participants. The median level of TG was 73 (54-102.3) mg/dL, and 11% of all were defined as having hypertriglyceridemia. The mean HDL-C was 61.3 ± 16.1 mg/dL, with 17.6% of the participants having an HDL-C levels less than 50 mg/dL. Metabolic syndrome was diagnosed in 17.6% of the 102 participants. The prevalence of hyperandrogenemia (defined by an excess of total testosterone, free testosterone, or DHEAS) was 49% (50/102). In the hyperandrogenemia PCOS group, free testosterone was > 0.006 ng/mL for 98% of the group (49/50), serum total testosterone was > 0.8 ng/mL for 10% (5/50) and DHEAS was > 350 µg/mL for 22% (11/50).

Table 1. Characteristics of Thai women with polycystic ovary syndrome.

Characteristics	mean ± SD or n (%) or median [interquartile range]
Age (years)	26.9 ± 5.7
Body mass index (kg/m^2)	26.1 ± 6.7
≥ 23.5	60 (58.8%)
≥ 30	26 (25.5%)
Hip circumference (cm)	100.6 ± 12.4
Waist circumference (cm)	84.4 ± 14.6
≥ 80	63 (61.8%)
Waist/hip ratio	0.84 ± 0.06
Waist/height ratio	0.53 ± 0.09
Systolic blood pressure (mmHg)	118 ± 13
≥ 130	24 (23.5%)
Diastolic blood pressure (mmHg)	73 ± 10
≥ 85	15 (14.7%)
Modified Ferriman-Gallwey score	4.0 [3.0-6.0]
≥ 3	83 (81.4%)
≥ 8	16 (15.7%)

Data are mean ± SD, or median [interquartile range], or number (%), Data were analyzed using Student t-test or Mann-Whitney U test for continuous data, or Chi-square test or Fisher's exact test for categorical data. WC: waist circumference

Table 2. Biochemical laboratory assays of Thai polycystic ovary syndrome women.

Laboratory	mean ± SD or n (%) or median [interquartile range]
Carbohydrate profiles	
Fasting blood glucose	87.8 ± 25
≥ 100 mg/dL	5 (4.9%)
Fasting insulin	10.8[6.9-19.5]
2-hour 75 gm OGTT	120 ± 53
≥ 140 mg/dL	21(20.6%)
Fasting glucose/insulin ratio	8.1 [4.7-12.2]
HOMA-IR	1.36 [0.86-2.44]
Lipid profiles	
Triglyceride	73 [54-102.3]
≥ 150 mg/dL	11 (10.8%)
HDL-C	61.3 ± 16.1
< 50 mg/dL	76 (74.5%)
Metabolic syndrome	18 (17.6%)
Androgens	
Total testosterone (ng/mL)	0.41 ± 0.19
> 0.8	5 (4.9%)
Free testosterone (ng/mL)	0.0056[0.0034-0.0099]
> 0.006	49 (48%)
DHEAS (µg/dL)	227.0 ± 100.3
> 350	11 (10.8%)
Free androgen Index	0.97 [0.5-1.86]
Hyperandrogenemia*	50 (49%)
VAI	0.96 [0.6-1.67]
LAP	19.02 [10.28-38.85]

Data are mean ± SD, or median [interquartile range], or number (%), Data were analyzed using Student t-test or Mann-Whitney U test for continuous data, or Chi-square test or Fisher's exact test for categorical data. OGTT: oral glucose tolerance test, HOMA-IR: Homeostatic Model assessment approximates insulin resistance, HDL-C: high density lipoprotein cholesterol, DHEAS: dehydroepiandrosterone sulphate, VAI: visceral adiposity index, LAP: lipid accumulation product. * Hyperandrogenemia was defined as serum of at least 1 of androgen higher than recommended cut-off (Total testosterone > 0.8 ng/mL, Free testosterone > 0.006 ng/mL, DHEAS > 350 µg/dL)

Factors associated with VAI and LAP in Thai PCOS women are demonstrated in Table 3. A significantly strong positive correlation was revealed between VAI and LAP ($r = 0.885$, $p < 0.001$). VAI was also found to positively correlate with HC, WHR, WHtR, blood pressure, fasting blood sugar, 75-gm oral glucose tolerance test, fasting insulin, HOMA-IR, LDL-C, free testosterone, and free androgen index (FAI). On the other hand, VAI was not correlated with total testosterone, DHEA-S, or mFG score. LAP was significantly and strongly correlated with HC, WC, WHR, fasting insulin, and HOMA-IR. Both LAP and VAI were significantly correlated with free testosterone and FAI, but not with total testosterone or DHEA-S.

Table 3. Factors associating with visceral adiposity index and lipid accumulation product in Thai polycystic ovary syndrome women.

Characteristics	VAI		LAP	
	r	p value	r	p value
VAI	1		0.885	< 0.001
LAP	0.885	< 0.001	1	
Clinical				
Age (years)	0.124	0.216	0.130	0.194
Body mass index (kg/m^2)	N/A	N/A	0.813	< 0.001
Hip circumference (cm)	0.533	< 0.001	0.791	< 0.001
Waist circumference (cm)	N/A	N/A	N/A	N/A
Waist/hip ratio	0.447	< 0.001	0.589	< 0.001
Waist/height ratio	0.583	< 0.001	0.830	< 0.001
Systolic blood pressure (mmHg)	0.405	< 0.001	0.471	< 0.001
Diastolic blood pressure (mmHg)	0.380	< 0.001	0.427	< 0.001
Modified Ferriman-Gallwey score	0.177	0.076	0.207	0.037
Carbohydrate profiles				
Fasting blood glucose	0.422	< 0.001	0.522	< 0.001
Fasting insulin	0.679	< 0.001	0.773	< 0.001
2-hour glucose	0.480	< 0.001	0.468	< 0.001
Fasting glucose/insulin ratio	- 0.642	< 0.001	- 0.730	< 0.001
HOMA-IR	0.688	< 0.001	0.787	< 0.001
Lipid profiles				
Triglyceride	N/A	N/A	N/A	N/A
HDL-C	N/A	N/A	-0.684	< 0.001
Cholesterol	0.170	< 0.001	0.202	0.042
LDL-C	0.345	< 0.001	0.340	< 0.001
Androgens				
Total testosterone	0.039	0.693	0.064	0.521
Free testosterone (ng/mL)	0.407	< 0.001	0.443	< 0.001
Free androgen index	0.502	< 0.001	0.532	< 0.001
DHEAS (µg/dL)	0.007	0.947	0.021	0.835

Data were analyzed using Pearson correlation, N/A = not applicable. VAI: visceral adiposity index, LAP: Lipid accumulation product, WC: waist circumference, OGTT: oral glucose tolerance test, HOMA-IR: Homeostatic Model assessment approximates insulin resistance, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol DHEAS: dehydroepiandrosterone sulphate

Table 4 details the factors associated with hyperandrogenemia in Thai PCOS women. There were significant differences in the median VAI and LAP levels of the hyperandrogenemia and non-hyperandrogenemia groups (VAI = 0.72, with 95% CI of 0.55–1.04; and LAP = 1.23, with 95% CI of 0.83-2.28, $p < 0.001$). There were also significant statistical differences in the 2 groups' values for other laboratory data: fasting insulin, 75-gm oral glucose tolerance test, fasting glucose/insulin ratio, HOMA-IR, TG, and HDL-C. This is consistent with their respective prevalences of metabolic syndrome. The

hyperandrogenemia women were also significantly different from the non-hyperandrogenemia women by way of a younger mean age, a higher mean BMI, and a higher WC ($p < 0.001$). Moreover, some components of metabolic-diastolic blood pressure, WC, and TG-were significantly higher in the hyperandrogenemia group than in the non-hyperandrogenemia group ($p < 0.05$ for each component).

Table 4. Factors associating with hyperandrogenemia in polycystic ovary syndrome Thai women.

Characteristics	Hyperandrogenemia mean ± SD or n (%) or median [interquartile range]		p value
	No n = 52	Yes n = 50	
VAI	0.72 [0.55-1.04]	1.23 [0.83-2.28]	< 0.001
LAP	13.86 [6.81-21.42]	31.67 [17.41-54.0]	< 0.001
Clinical			
Age (years)	28.3 ± 5.7	25.5 ± 5.3	0.013
Body mass index (kg/m^2)	23.9 ± 6.4	28.4 ± 6.4	0.001
≥ 23.5	21 (40%)	39 (78%)	< 0.001
≥ 30	8 (15%)	18 (36%)	
Hip circumference (cm)	96.4 ± 11.8	105 ± 11.5	< 0.001
Waist circumference (cm)	79.4 ± 13.4	89.6 ± 14.1	< 0.001
≥ 80	22 (42.3%)	41 (82%)	< 0.001
Waist/hip ratio	0.82 ± 0.07	0.85 ± 0.06	0.026
Waist/height ratio	0.49 ± 0.08	0.56 ± 0.09	< 0.001
Systolic blood pressure (mmHg)	116 ± 12	120 ± 14	0.057
≥ 130	10 (19.2%)	14 (28%)	0.350
Diastolic blood pressure (mmHg)	71 ± 10	75 ± 10	0.037
≥ 85	6 (11.5%)	9 (18%)	0.411
MFG	3 [2-5]	5 [4-8]	< 0.001
≥ 3	37 (71.2%)	46 (92%)	0.01
≥ 8	1 (1.9%)	15 (30%)	< 0.001
Carbohydrate profiles			
Fasting blood glucose	83.3 ± 8.9	92.6 ± 34.1	0.062
≥ 100 mg/dL	1 (1.9%)	4 (8%)	0.200
Fasting insulin	7.15 [5.59-10.68]	15.39 [10.83-22.43]	< 0.001
2-hour 75 gm OGTT	105.5 ± 27.6	136.9 ± 66.4	0.002
≥ 140 mg/dL	5 (9.6%)	16 (32%)	0.007
Fasting glucose/insulin ratio	10.98 [8.12-14.44]	5.59 [3.94-8.08]	< 0.001
HOMA-IR	0.90 [0.71-1.36]	2.01 [1.38-2.92]	< 0.001
Lipid profiles			
Triglyceride	63 [51.3-76.75]	85 [59.5-130.25]	0.001
≥ 150 mg/dL	2 (3.8%)	9 (18%)	0.027
HDL-C	68.5 [57.25-82]	54 [45-60]	< 0.001
< 50 mg/dL	44 (84.6%)	32 (64%)	0.023
Metabolic syndrome	4 (7.7%)	14 (28%)	0.009

Data are mean ± SD, or median [interquartile range], or number (%). * Data were analyzed using Student t-test or Mann-Whitney U test for continuous data, or Chi-square test or Fisher's exact test for categorical data. MFG: modified Ferriman-Gallwey score, OGTT: oral glucose tolerance test, HOMA-IR: Homeostatic Model assessment approximates insulin resistance, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol DHEAS: dehydroepiandrosterone sulphate

The ROC curve analyses for the optimal VAI and LAP levels needed to predict hyperandrogenemia in PCOS are presented in Table 5. The VAI cut-off value yielding the highest sensitivity (74%), the highest negative predictive value (75%), and the highest accuracy (74.5%) was 1 or more. The prevalence of

hyperandrogenemia using VAI ≥ 1 was 74% (37/50), and the AUC was 0.755 (95% CI, 0.66–0.85) (Fig. 1). As to the optimal cut-off point of LAP, it was 16.5; the prevalence and AUC were 66.7% and 0.756 (95% CI, 0.66–0.85). Diagnostic performances for the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of LAP were 80%, 61.5%, 66.7%, 76.2%, and 70.5%, respectively (Fig. 2).

Table 5. Diagnostic performance of visceral adiposity index and lipid accumulation product in predicting hyperandrogenemia in polycystic ovary syndrome.

Androgen, cut-off point	Prevalence (n(%))	Performance (%)					ROC-AUC (95% CI)
		Sens.	Spec.	PPV	NPV	Acc.	
VAI							
≥ 1	37/50 (74%)	74	75	74	75	74.5	0.755 [0.66-0.85]
≥ 0.8	41/64 (64%)	82	55.8	64	76	68.6	
≥ 0.73	43/67 (64%)	86	53.8	64.2	80	69.6	
LAP							
≥ 12.5	43/70 (61.4%)	86	48.1	61.4	78.1	66.7	0.756 [0.66-0.85]
≥ 16.5	40/60 (66.7%)	80	61.5	66.7	76.2	70.5	
≥ 17.5	38/56 (67.9%)	76	65.4	67.9	73.9	70.6	

VAI: visceral adiposity index, LAP: lipid accumulation product, Acc: accuracy, NPV: negative predictive value, PPV: positive predictive value, ROC- AUC: receiver operator characteristics area under the curve, Sens: sensitivity, Spec: specificity, CI: confidence interval

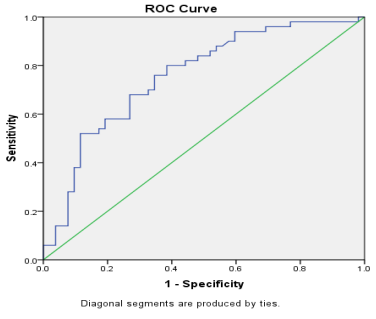


Fig. 1. Receiver operating characteristics curve of visceral adiposity index in predicting hyperandrogenemia in polycystic ovary syndrome.

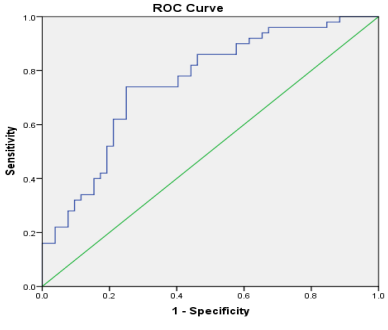


Fig. 2. Receiver operating characteristics curve of lipid accumulation product in predicting hyperandrogenemia in polycystic ovary syndrome.

Both VAI and LAP were found to be significantly associated with metabolic syndrome ($p < 0.01$) (Table 6). Furthermore, we found that 78% of the participants diagnosed with metabolic syndrome also had hyperandrogenemia; this was significantly different from the situation for the non-metabolic syndrome group.

Table 6. Factors associating with metabolic syndrome in polycystic ovary syndrome Thai women.

Characteristics	metabolic syndrome mean ± SD or n (%) or median [interquartile range]		p value
	No n = 84	Yes n = 18	
VAI	0.83 [0.58-1.20]	2.53 [1.64-4.54]	< 0.001
LAP	16.61 [8.13- 26.35]	58.39 [40.04-76.63]	< 0.001
Hyperandrogenemia**	36 (43%)	14 (78%)	0.007
Clinical			
Age (years)	26.7 ± 5.59	28.0 ± 6.07	0.380
Body mass index (kg/m^2)	24.7 ± 6.00	32.3 ± 6.53	< 0.001
≥ 23.5	43 (51%)	17 (94%)	< 0.001
≥ 30	15 (18%)	11 (61%)	
Hip circumference (cm)	98.6 ± 11.89	110.3 ± 10.21	< 0.001
Waist circumference (cm)	81.6 ± 13.42	97.7 ± 12.76	< 0.001
≥ 80	45 (54%)	18 (100%)	< 0.001
Waist/hip ratio	0.83 ± 0.06	0.88 ± 0.06	< 0.001
Waist/height ratio	0.51 ± 0.08	0.61 ± 0.78	0.001
MFG	4 [3-6]	4 [3.75-7.25]	0.181
≥ 3	65 (77%)	18 (100%)	0.025
≥ 8	12 (14%)	4 (22%)	0.401

Data are mean ± SD, or median [interquartile range], or number (%)

* Data were analyzed using Student t-test or Mann-Whitney U test for continuous data, or Chi-square. Test or Fisher's exact test for categorical data.

VAI: visceral adiposity index, LAP: lipid accumulation product, MFG: modified Ferriman-Gallwey score, DHEAS: dehydroepiandrosterone sulphate. **Hyperandrogenemia was defined as serum of at least 1 of androgen higher than Recommended cut-off (Total testosterone > 0.8 ng/mL, Free testosterone > 0.006 ng/mL, DHEAS > 350 µg/dL)

Discussion

Hyperandrogenism can be defined by either clinical (e.g., hirsutism, acne, or male pattern alopecia) or biochemical manifestations (serum testosterone, free testosterone, or FAI). In the case of the clinical manifestation of hyperandrogenism, the mFG score is the most widely used tool to assess terminal hairs in order to diagnose hirsutism[2]. In our study, we found that the prevalence of clinical and biochemical hyperandrogenism did not correlate with biochemical hyperandrogenism (15.6% and 49%, respectively). This finding was consistent with other studies that reported that there was no correlation between clinical and biochemical hyperandrogenism[21, 22]. It could be that the development of hirsutism might depend on not only the level of circulating androgen, but also the concentration of the hormone and the degree of androgen exposure to androgen receptors at the target organ. Although the mFG score is the most common visual assessment tool for the evaluation of terminal hairs, it may not be applicable due to variation in ethnicity or overestimation. It is also a subjective assessment tool: bias needs to be considered. For the present study, we therefore decided to evaluate the cut-off values of VAI and LAP to predict hyperandrogenemia, not hyperandrogenism, since those cut-off values were deemed likely to be more objective and reliable.

Previous studies have reported that obesity, insulin resistance, metabolic syndrome, and an excessive accumulation of abdominal visceral fat influence the development of hyperandrogenism in PCOS women[11-13]. Similar to this study, the

hyperandrogenemia PCOS group had a larger waist circumference, a higher BMI, lower insulin sensitivity, a greater degree of dyslipidemia and higher median VAI, and LAP than the non-hyperandrogenemia PCOS group.

VAI is a gender-specific, mathematical model which combines anthropometric data (BMI and WC) and functional parameters (TG and HDL-C). LAP is a simple, sex-specific formula that combines waist circumference and triglyceride concentration. Abdominal visceral fat was found associated with metabolic syndrome and its components including obesity, impaired glucose, insulin resistance, and abnormal lipid metabolism. While VAI and LAP are the specific mathematical formula that uses anthropometric data and lipid parameter. Therefore, the two models were the indirect methods to identify abdominal visceral tissue and the differences in anthropometric data and functional laboratory parameters may affect VAI and LAP values. The mean VAI (0.96 [0.6-1.67]) revealed in the present study was lower than the value of 4.05 ± 3.59 reported for Thai PCOS women by Techatraisak et al in 2015[13]. These differences might be explained by the higher prevalence of metabolic syndrome in our population, which had lower WC and TG levels and a higher HDL value-all components of the VAI formula-than the cohort investigated by Techatraisak and colleagues.

VAI and LAP in the present study were significantly and positively correlated with free testosterone and FAI (VAI: r = 0.4 and 0.5, respectively, with $p < 0.001$; LAP: r = 0.4 and 0.5, respectively, with $p < 0.001$), but total testosterone and DHEAS were not correlated with either VAI or LAP. However, these results were consistent with those of another study[14], which also reported that no correlation between VAI and total testosterone was demonstrated in a regression model. Total testosterone is comprised of bound and free forms, which could interfere with many factors; free testosterone interferes less than total testosterone and might therefore be more representative of the actual action of testosterone[4]. However, hyperandrogenemia in this study was mainly diagnosed by high levels of free testosterone.

This is the first study to evaluate the optimal cut-off values for VAI and LAP to predict hyperandrogenemia in PCOS women. From our findings, we suggest that VAI ≥ 1 (AUC = 0.755, p = 0.66-0.85) is the most appropriate point to use to predict hyperandrogenemia in PCOS. Due to the best accuracy and proper sensitivity, specificity, NPV, and PPV (74.5%, 75%, 75%, and 74% respectively) for LAP, we selected LAP ≥ 16.5 as the optimal cut-point value. Although LAP value of ≥ 17.5 showed the best accuracy, LAP ≥ 16.5 was better in terms of sensitivity and NPV, which are more important in terms of clinical screening.

Our strength of this study was the homogenous Asian population; its results may therefore reflect the Thai or Asian population. In addition, we compared VAI and hyperandrogenemia, which is a biochemical presentation that can avoid some bias or variations from ethnicity and over/under-estimation of hyperandrogenism. The limitation of our study was that it was conducted at a single center, which meant that it could not represent all of Thai PCOS population. A further multicenter study would be able to confirm the cut-off levels that we have proposed for use with the Thai PCOS population.

Conclusion

Both VAI and LAP significantly and positively correlated with free testosterone and the FAI. A VAI ≥ 1 or an LAP ≥ 16.5 can be used to predict hyperandrogenemia in PCOS women at Siriraj Hospital. In addition, the change of those values may be used to monitor responsiveness to treatment for hyperandrogenemia in PCOS women.

Acknowledgments

The authors gratefully acknowledge Miss Nichamon Pingkul and the staff members of the Gynecologic Endocrinology Unit, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University for their contribution to the success of this study.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): The hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr Rev 2016;37:467-520.
2. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod 2018;33:1602-18.
3. Escobar-Morreale HF, San Millan JL. Abdominal adiposity and the polycystic ovary syndrome. Trends Endocrinol Metab 2007;18:266-72.
4. Fritz M, Speroff L. Hormone biosynthesis, metabolism, and mechanism of action. In: Fritz M, Speroff L, editors. Clinical gynecologic endocrinology and infertility. 8th ed. Philadelphia: Lippincott: Williams & Wilkins 2011. 29-104.
5. Pasquali R. Obesity and androgens: facts and perspectives. Fertil Steril 2006;85:1319-40.
6. Shuster A, Patlas M, Pinthus JH, Mourtzakis M. The clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis. Br J Radiol 2012;85:1-10.
7. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev 2000;21:697-738.
8. Amato MC, Giordano C. Visceral adiposity index: an indicator of adipose tissue dysfunction. Int J Endocrinol 2014;2014:730827.
9. Kahn HS. The "lipid accumulation product" performs better than the body mass index for recognizing cardiovascular risk: a population-based comparison. BMC Cardiovasc Disord 2005;5:26.
10. Agrawal H, Aggarwal K, Jain A. Visceral adiposity index: simple tool for assessing cardiometabolic risk in women with polycystic ovary syndrome. Indian J Endocrinol Metab 2019;23:232-7.
11. Amato MC, Guarnotta V, Forti D, Donatelli M, Dolcimascolo S, Giordano C. Metabolically healthy polycystic ovary syndrome (MH-PCOS) and metabolically unhealthy polycystic ovary syndrome (MU-PCOS): a comparative analysis of four simple methods useful for metabolic assessment. Hum Reprod 2013;28:1919-28.
12. Androulakis, II, Kandaraki E, Christakou C, Karachalios A, Marinakis E, Paterakis T, et al. Visceral adiposity index (VAI) is related to the severity of anovulation and other clinical features in women with polycystic ovary syndrome. Clin Endocrinol (Oxf) 2014;81:426-31.
13. Techatraisak K, Wongmeerit K, Dangrat C, Wongwananuruk T, Indhavivadhana S. Measures of body adiposity and visceral adiposity index as predictors of metabolic syndrome among Thai women with PCOS. Gynecol Endocrinol 2016;32:276-80.
14. Aboelnaga MM, Elshahawy H. Visceral adiposity index correlation with Rotterdam criteria in patients with polycystic ovary syndrome. Int J Reprod Contracept Obstet Gynecol 2016;5:2553-60.
15. Rotterdam EA-SPcwg. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41-7.
16. Hohl A, Ronsoni MF, Oliveira M. Hirsutism: diagnosis and treatment. Arq Bras Endocrinol Metabol 2014;58:97-107.
17. Speroff L, Fritz M. Hirsutism. In: Speroff L, Fritz M, editors. Clinical gynecologic endocrinology and infertility. 7th ed. Philadelphia: Lippincott: Williams & Wilkins 2005. 499-530.
18. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666-72.
19. Tan CE, Ma S, Wai D, Chew SK, Tai ES. Can we apply the National Cholesterol Education Program Adult Treatment Panel definition of the metabolic syndrome to Asians? Diabetes Care 2004;27:1182-6.
20. Hatami H, Montazeri SA, Hashemi N, Ramezani Tehrani F. Optimal cutoff points for anthropometric variables to predict insulin resistance in polycystic ovary syndrome. Int J Endocrinol Metab 2017;15:e12353.
21. Leerasiri P, Wongwananuruk T, Indhavivadhana S, Techatraisak K, Rattanachaiyanont M, Angsuwathana S. Correlation of clinical and biochemical hyperandrogenism in Thai women with polycystic ovary syndrome. J Obstet Gynaecol Res 2016;42:678-83.
22. Pasquali R, Zanotti L, Fanelli F, Mezzullo M, Fazzini A, Morselli Labate AM, et al. Defining hyperandrogenism in women with polycystic ovary syndrome: A challenging perspective. J Clin Endocrinol Metab 2016;101: 2013-22.

Thai Journal of Obstetrics and Gynaecology
January 2024, Vol. 32, No. 1, pp. 45-56.

OBSTETRICS

Development and Validation of a Prediction Score for Spinal-anesthesia Induced Hypotension in Cesarean Delivery: A prospective cohort study

Wipada Tingthanathikul, M.D.*,**,
Chamnan Tanprasertkul, M.D., Ph.D.***,****,
Ketchada Uerpairojkit, M.D., MSc.*,
Kanya Kumwilaisak, M.D.*,
Paweena Paarporn, M.D.*,
Titipon Payongsri, M.D.*,
Pongpol Sirilaksanamanon, M.D.*,
Sumaporn Bunyalesniruntr, M.D.*,
Uakarn Petchpanya, M.D.*

** Department of Anesthesiology, King Chulalongkorn Memorial Hospital, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
*** Department of Clinical Epidemiology, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand*
**** Department of Obstetrics and Gynaecology, Faculty of Medicine, Thammasat University, PathumThani, Thailand*
***** Thammasat University Research Unit in Advanced Clinical Data Statistics Analysis and Innovative Research in Obstetrics and Gynecology Group, Pathum Thani, Thailand*

ABSTRACT

Objectives: Spinal anesthesia-induced hypotension is the most common complication in cesarean delivery, which can impede uteroplacental blood flow and may deteriorate maternal and fetal welfare. A good predictor for hypotension can help individualized prophylactic treatment. There is currently no simple and good prediction score for spinal hypotension. We conducted a study to develop and internally validate a risk scoring scheme to predict spinal anesthesia-induced hypotension in cesarean delivery.

Materials and Methods: We performed a prognostic clinical prediction model in a prospective cohort design. The parturients who underwent cesarean delivery using spinal anesthesia were included. The outcome was spinal anesthesia-induced hypotension. Predictors included patients' baseline characteristics, pregnancy details, and preoperative hemodynamic results. Multivariable logistic regression was used for score derivation. Model discrimination and calibration were assessed. The risk score was categorized into low-, moderate-, and high-risk groups.

Results: For 712 parturients who underwent cesarean delivery, a risk score was developed from three predictors: stroke volume index, baseline heart rate, and uterine contraction. The area under the receiver operating characteristic curve was 0.715 (95% confidence interval 0.676-0.754). The risk scores ranged from 0 to 7. When the scores were classified into low- (< 2.5),

moderate- (2.5-4.5), and high- (> 4.5) risk groups, the probability of developing hypotension increased from 21.88% in low-risk to 79.95% in the high-risk group.

Conclusion: A risk score developing from stroke volume index, baseline heart rate, and uterine contraction may help predict spinal hypotension in cesarean delivery and guide individualized prophylactic therapy.

Keywords: prediction score, spinal-anesthesia induced hypotension, cesarean delivery.

Correspondence to: *Wipada Tingthanathikul, M.D., Department of Anesthesiology, King Chulalongkorn Memorial Hospital, Bangkok 10330, Thailand. E-mail: wipada@chula.md*

Received: 22 April 2023, **Revised:** 11 July 2023, **Accepted:** 31 July 2023

เกณฑ์การทำนายการเกิดภาวะความดันโลหิตต่ำจากการฉีดยาชาเข้าช่องไขสันหลังสำหรับการผ่าตัดคลอดทางหน้าท้อง

วิภาดา ติงธนาธิกุล, ชำนาญ แท่นประเสริฐกุล, เกศชาดา เอื้อไพโรจน์กิจ, กัญญา คำวิลัยศักดิ์, ปวีณา ภาอาภรณ์, ฐิติพล พยงค์ศรี, ปองพล ศิริลักษณมานนท์, สุมาภรณ์ บุญญเลสนิรันตร์, เอื้อการย์ เพชรปัญญา

บทคัดย่อ

วัตถุประสงค์*: เพื่อหาเกณฑ์การทำนายของการเกิดภาวะความดันโลหิตต่ำจากการฉีดยาชาเข้าช่องไขสันหลังสำหรับการผ่าตัดคลอดทางหน้าท้อง*

วัสดุและวิธีการ*: ทำการศึกษาแบบ prospective cohort ในหญิงตั้งครรภ์ที่มารับการผ่าตัดคลอดทางหน้าท้องโดยใช้วิธีให้ยาระงับความรู้สึกด้วยการฉีดยาชาเข้าช่องไขสันหลัง ตัวแปรทำนาย ได้แก่ ข้อมูลพื้นฐานและhemodynamic parameters ของหญิงตั้งครรภ์ ผู้วิจัยใช้ Noninvasive ultrasound cardiac output monitoring (USCOM) ในการวัด stroke volume index (SVI), cardiac index (CI), and systemic vascular resistance index (SVRI) ข้อมูลที่ได้นำไปวิเคราะห์โดยใช้ multivariable logistic regression model ในการสร้างคะแนนเพื่อใช้เป็นเกณฑ์การทำนาย จากนั้นนำคะแนนที่ได้ไปแบ่งหญิงตั้งครรภ์เป็นกลุ่มความเสี่ยงระดับต่างๆ*

ผลการศึกษา*: จากข้อมูลของหญิงตั้งครรภ์ที่มารับการผ่าตัดคลอดทางหน้าท้องโดยใช้วิธีให้ยาระงับความรู้สึกด้วยการฉีดยาชาเข้าช่องไขสันหลังจำนวน 712 ราย สามารถพัฒนาคะแนนการทำนายการเกิดภาวะความดันโลหิตต่ำจากการฉีดยาชาเข้าช่องไขสันหลังได้จากสามปัจจัยซึ่ง ได้แก่ stroke volume index อัตราการเต้นของหัวใจพื้นฐานและภาวะการหดรัดตัวของกล้ามเนื้อมดลูก ค่า area under the receiver operating characteristic curve ของคะแนนการทำนายอยู่ที่ 0.715 (95% confidence interval 0.676-0.754) โดยค่าคะแนนการทำนายอยู่ระหว่าง 0 ถึง 7 คะแนน ซึ่งสามารถแบ่งตามระดับความเสี่ยงของการเกิดภาวะความดันโลหิตต่ำเป็นน้อย (คะแนนรวมน้อยกว่า 2.5) ปานกลาง (คะแนน 2.5 ถึง 4.5) และสูง (คะแนนมากกว่า 4.5) โอกาสของการเกิดภาวะความดันโลหิตต่ำจะเพิ่มจากร้อยละ 21.88 ในกลุ่มเสี่ยงต่ำเป็นร้อยละ*

79.95 ในกลุ่มเสี่ยงสูง

***สรุป**: เกณฑ์การทำนายที่ประกอบด้วย stroke volume index อัตราการเต้นของหัวใจพื้นฐานและภาวะการหดรัดตัวของกล้ามเนื้อมดลูกสามารถช่วยทำนายการเกิดภาวะความดันโลหิตต่ำจากการฉีดยาชาเข้าช่องไขสันหลังสำหรับการผ่าตัดคลอดทางหน้าท้อง และอาจช่วยชี้แนะวิสัญญีแพทย์ในการใช้ยาเพิ่มความดันเพื่อป้องกันการเกิดภาวะความดันโลหิตต่ำในผู้ป่วยที่มารับการผ่าตัดคลอดทางหน้าท้องตามระดับความเสี่ยงที่คำนวณได้ในแต่ละคน*

***คำสำคัญ**: ภาวะความดันโลหิตต่ำ, การผ่าตัดคลอดทางหน้าท้อง, การฉีดยาชาเข้าช่องไขสันหลัง*

Introduction

Spinal anesthesia is a common anesthetic technique for cesarean delivery owing to its safety for maternal and fetal aspects[1, 2]. However, the most common complication of this technique is spinal anesthesia-induced hypotension (SIH), which occurs in approximately 30 - 80% of cases. Hypotension before delivery can impede uteroplacental blood flow and may deteriorate maternal and fetal welfare. Despite the widespread use of fluid co-loading and prophylactic vasopressors, the risk of SIH in cesarean delivery cannot be completely eliminated[3]. Additionally, reactive maternal hypertension may occur[4]. While phenylephrine is the vasopressor of choice to prevent SIH, it may not be available in all hospitals. Other vasopressors, such as norepinephrine and metaraminol, have not been widely recommended as first-line drugs for preventing SIH. Therefore, the consideration of using these alternative vasopressors as a replacement for phenylephrine is necessary.

The prediction of SIH can assist anesthesiologists in selecting appropriate parturients for prophylactic vasopressor use and to avoid reactive hypertension. Several studies have identified predictors of SIH in cesarean deliveries. These include demographic data[5] (e.g., body mass index (BMI), maternal weight gain during pregnancy), baseline hemodynamic parameters (e.g., baseline heart rate[6], baseline blood pressure), baseline sympathovagal balance indices (e.g., maternal heart rate variability[7], pulse rate variability), postural stress testing (e.g., supine stress test[8, 9], peripheral perfusion indices (e.g., perfusion index[10], cerebral oxygen saturation[11], blood volume and fluid responsiveness indices (e.g., inferior vena cava collapsibility index[12], Pleth variability index[13], passive leg raising test[14], and genetic polymorphism[5]. However, most studies have small sample sizes, and the predictive power varies. Additionally, some parameters are not suitable for prediction because the elapsed time between prediction and the onset of hypotension is too short.

Currently, there is no single simple tool that serves as a good predictor of SIH. Combining multiple predictors into a prediction score may aid in developing a more effective prediction tool. Bishop et al introduced the pulse rate, age, and mean arterial pressure (PRAM) score, which used only preoperative risk factors to predict hypotension following obstetric spinal anesthesia[15], However, the predictive ability of the PRAM score was found to be poor (area under the receiver operating characteristic curve (AUROC) 0.626).

We conducted a study to develop and internally validate a risk scoring scheme, called the spinal anesthesia-induced hypotension (SIH) score, to predict SIH during cesarean delivery. The predictors considered in the score included maternal baseline characteristics, preoperative physical examination results, and other relevant details associated with the development of SIH.

Since blood pressure is directly influenced by cardiac output and systemic vascular resistance, we included physiological parameters as predictors, namely stroke volume index (SVI), cardiac index (CI), systemic vascular resistance index (SVRI), and flow time corrected (FTc). To obtain these parameters, we utilized noninvasive ultrasound cardiac output monitoring (USCOM). USCOM has been commonly employed in obstetric research for measuring hemodynamic indices. It is a simple, noninvasive, and cost-effective method. The learning curve for USCOM is steep, and good inter-rater reliability can be achieved after a short training period[16].

Materials and Methods

This prospective cohort study was conducted at King Chulalongkorn Memorial Hospital, which is an academic tertiary care center in Bangkok, Thailand. The study protocol was approved by the Chulalongkorn University Institutional Review Board (Med Chula IRB 1543/2560). Written informed consent was obtained from all the participants. This study was conducted between August 2017 and August 2018. Reporting and analysis of study results were conducted according to the transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD) checklist[17].

Study Population

Parturients scheduled for cesarean delivery under spinal anesthesia at King Chulalongkorn Memorial Hospital during the daytime shift (8.00-16.00) were invited to participate. Participants were aged 18 years or older, had a gestational age of 36 weeks or more, and were capable of giving informed consent. Exclusion criteria included parturients with cardiovascular disease or severe systemic disease, hypertensive disorders, extreme body weight (less than 40 kg or greater than 120 kg), extreme height (less than 145 cm or greater than 180 cm), emergency cesarean delivery without sufficient time for data collection, likelihood of excessive blood loss during the pre-delivery period, compromised fetus, and situations where data could not be collected from USCOM. Parturients with incomplete or failed spinal anesthesia who required additional intravenous sedation or general anesthesia before clamping the umbilical cord were excluded from the analysis.

Data collection

After confirming patient eligibility, data collectors who were not involved in anesthetic management recorded the details of predictor variables.

Predictor variables included (1) baseline characteristics (age, body weight, height, BMI, underlying disease), (2) pregnancy details (gestational age, maternal weight gain, fetal presentation, single or multiple pregnancies, head engagement, uterine contraction, emergency or elective surgery, fasting duration, intravenous fluid administration), (3) preoperative assessment (baseline systolic blood pressure, heart rate, supine stress test, USCOM parameters [SVI, SVRI, and CI]), and (4) other details (experience of anesthesiologists).

Baseline systolic blood pressure (SBP) and heart rate (HR) were measured after a 10-minute rest in a supine position with a wedge under the right lumbar area. SBP and HR were calculated as the means of three consecutive measurements, with values not differing by more than 10%. GE® CARESCAPE B650 monitors were used with a blood pressure cuff placed on the right arm, opposite the intravenous catheter site.

The supine stress test (SST) followed a methodology from a previous study[9]. Baseline blood pressure and heart rate were measured after a 10-minute rest in the left lateral position. Parturients were then moved to the supine position for five minutes, with blood pressure measured every minute and continuous heart rate monitoring.

SST is considered positive if any of the following criteria are met:

1. Maternal heart rate increases by more than 10 beats per minute compared to baseline for at least one consecutive minute.
2. Systolic arterial blood pressure decreases

by more than 15 mmHg compared to baseline for at least two consecutive minutes.

3. Signs of hypotension associated with the supine position, such as hip flexion and crossing of legs, are observed.

4. Symptoms of hypotension related to the supine position, such as nausea, vomiting, and dizziness, necessitate a change in position.

Hemodynamic parameters from USCOM were measured by five trained anesthesiologists (WT, KK, PP, TP, PS) with experience in at least 50 cases, as recommended[16]. USCOM was routinely used in their practice. SVI, CI, and SVRI were measured using the ultrasound non-invasive cardiac output monitor (USCOM®, USCOM Ltd, Sydney, Australia) with the suprasternal notch approach. During the measurement period, patients were positioned in supine with a wedge placed under the right lumbar area.

The spinal anesthetic technique and management followed our institute's protocol. An 18G intravenous catheter was inserted in the left hand or arm. Co-loading with warm-acetated Ringer's solution (10 ml/kg) was performed. Spinal anesthesia was induced in the left lateral position at lumbar interspace L2-3 or L3-4 using a Quincke No 27G needle. Hyperbaric bupivacaine (11 mg) and 0.2 mg morphine were used as local anesthetics. Bupivacaine dosage was adjusted based on maternal height (< 150 cm: 10 mg, > 165 cm: 12 mg). Supplemental oxygen was administered when maternal SpO_2 dropped below 95%.

Following intrathecal injection, the parturient assumed a supine position with a wedge supporting the right lumbar area, creating a 15-degree left uterine tilt. Noninvasive blood pressure was measured at one-minute intervals until umbilical cord clamping. Sensory block levels were assessed at post-injection. Continuous monitoring included ECG, HR, and SpO_2. Incidences of hypotension and bradycardia were recorded. Anesthesiologists who performed the spinal anesthesia were considered inexperienced during their first year of residency.

The primary outcome was SIH which was defined as either "systolic blood pressure < 80% of baseline value" or "systolic blood pressure < 100 mmHg with signs or symptoms of hypoperfusion such as nausea, vomiting, and dizziness"[18]. The assessment of SIH focused on the period from intrathecal injection to umbilical cord clamping.

Standard treatment protocols were implemented for hypotension and bradycardia: Intravenous administration of 100 mcg phenylephrine for hypotension with HR ≥ 60 bpm, 6 mg ephedrine for hypotension with HR < 60 bpm, and 0.6 mg atropine for hypotension with HR < 50 bpm. Anesthetic management after umbilical cord clamping was determined by the primary anesthesiologist in charge of the patient.

Sample size estimation

We aimed to include 20 parameters in the model based on the "10 events per parameter rule of thumb"[19]. The observed incidence of SIH at our institute ranged from 30% to 60%. Considering a dropout rate of 10%, the recommended minimum sample size should have been 734 participants. However, a total of 744 participants were enrolled in this study.

Statistical analysis

Baseline characteristics were compared using appropriate statistical tests (e.g., Student's t-test, Fisher's exact test). Discrimination was assessed using the AUROC.

To develop, derive scores, and validate the model, the following steps were taken: initial univariable logistic regression analysis to explore predictor variables' relationship with SIH, selecting predictors with a p value < 0.1 for inclusion in the multivariable logistic regression model while assessing multicollinearity. Backward elimination logistic regression was performed on the multivariable model, retaining variables with a p value < 0.05. Regression coefficients from the final model were transformed into scores to establish the predictive model. Each parturient received a risk score (SIH score) based on

this model. Performance assessment included discrimination using AUROC and calibration using the Hosmer-Lemeshow tests.

Risk stratification was conducted, classifying patients into low-, moderate-, and high-risk groups based on the clinical risk score classification.

Internal validation was conducted using bootstrapping with stepwise variable selection, which involved generating 1,000 random samples with replacement from the original dataset to estimate optimism. To handle the small amount of missing data, complete case analysis was employed. Statistical analyses were carried out using STATA-15 software.

Results

Among the 1,281 initially eligible parturients, 744 were enrolled in the study after excluding certain participants. Out of these, 32 cases (4.3%) encountered failed or incomplete spinal anesthesia, requiring additional sedation or conversion to general anesthesia. Consequently, the final analysis involved 712 parturients, and the recruitment process and study flow are illustrated in Fig. 1.

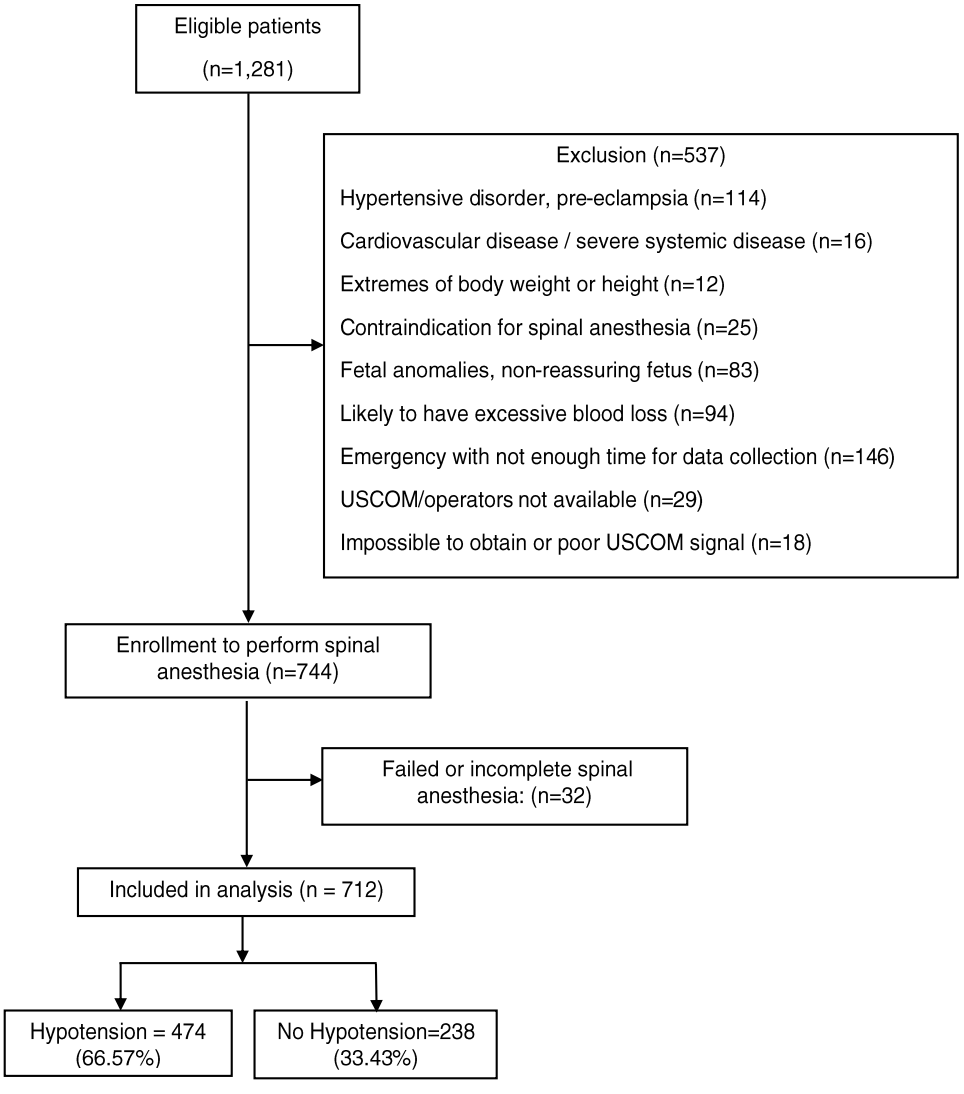


Fig. 1. Study flow diagram.

Of the included participants, 474 parturients (66.57%) developed hypotension, while 238 (33.43%) did not. Additionally, 12 patients (1.69%) developed bradycardia. No severe neonatal adverse events were reported. Among the cases, only five (0.7%) had an Apgar score below 7 at one minute, but all of them improved to a score of 7 or higher at five minutes.

The predictor variables included the baseline characteristics of the parturients, pregnancy details, preoperative assessment, and the inexperienced anesthesiologist were demonstrated with details in Table 1.

Table 1. Patient characteristics.

Characteristics	Hypotension n = 474 (66.57%) Mean (SD)	No hypotension n = 238 (33.43%) Mean (SD)	p value	AUROC (95%CI)
Demographics				
Age (years)	33.30 (4.98)	32.70 (5.02)	0.129	0.53 (0.48 to 0.57)
Maternal weight (kg)	73.07 (11.39)	70.40 (9.93)	0.002	0.57 (0.52 to 0.61)
Body mass index (kg/m^2)	28.98 (4.25)	27.89 (3.79)	< 0.001	0.57 (0.53 to 0.62)
Underlying DM/GDM n (%)	60 (12.66%)	14 (5.88%)	0.006	0.53 (0.51 to 0.55)
Pregnancy information				
Weight gain during pregnancy (kg)	14.17 (5.21)	14.73 (4.90)	0.172	0.47 (0.42 to 0.51)
Multiple pregnancy n (%)	18 (3.8%)	14 (5.88%)	0.249	0.49 (0.47 to 0.51)
Cephalic presentation n (%)	426 (89.87%)	206 (86.55%)	0.186	0.52 (0.49 to 0.54)
GA ≥ 38 weeks n (%)	390 (82.49%)	173 (73.11%)	0.003	0.55 (0.51 to 0.58)
Head engagement n (%)	51 (10.76%)	35 (14.71%)	0.129	0.48 (0.45 to 0.51)
Emergency surgery n (%)	83 (17.51%)	96 (40.34%)	< 0.001	0.61(0.58 to 0.65)
Uterine contraction n (%)	70 (14.80%)	84 (35.29%)	< 0.001	0.61(0.57 to 0.64)
Positive SST n (%)	79 (16.67%)	28 (11.76%)	0.095	0.52 (0.50 to 0.55)
IV fluid before anesthesia n (%)	120 (25.42%)	98 (41.18 %)	< 0.001	0.58 (0.54 to 0.62)
Duration of fasting (min)	654.68 (195.51)	612.79 (255.08)	0.028	0.53 (0.48 to 0.57)
Baseline hemodynamic parameters				
Baseline SBP (mmHg)	113.51 (9.77)	112.94 (10.43)	0.475	0.52 (0.48 to 0.57)
Baseline heart rate (bpm)	82.98 (11.08)	79.85 (10.71)	< 0.001	0.58 (0.54 to 0.63)
SVI (ml/m^2)	45.53 (7.98)	51.01 (8.57)	< 0.001	0.60 (0.57 to 0.64)
CI (L/min/m^2)	3.671 (0.62)	3.87563 (0.67)	< 0.001	0.59 (0.54 to 0.63)
SVRI (dynes · sec/cm^5)	1776.97 (357.39)	1678.96 (325.43)	< 0.001	0.58 (0.53 to 0.62)
FTc (ms)	375.77 (34.74)	369.20 (38.37)	0.022	0.55 (0.50 to 0.59)
Intraoperative detail				
Inexperienced anesthesiologist n (%)	301 (65.15%)	158 (67.52 %)	0.533	0.51 (0.47 to 0.55)

SD: standard deviation, GDM: gestational diabetes mellitus, DM: diabetes mellitus, GA: gestational age, SST: supine stress test, IV: intravenous, SBP: systolic blood pressure, SVI: stroke volume index, CI: cardiac index, SVRI: systemic vascular resistance index, FTc: flow time corrected, AUROC: area under the receiver operating characteristic curve.

Predictors showing statistical significance ($p < 0.1$) in univariable analysis were included in the subsequent multivariable analysis. These predictors include BMI, underlying diabetes mellitus/gestational diabetes mellitus (DM/GDM), gestational age, emergency surgery, positive supine stress test, uterine contraction, intravenous injection fluid administration before anesthesia, fasting duration, baseline heart rate, SVI, CI, and SVRI.

The final multivariable model in Table 2 included three predictors: uterine contraction, baseline heart rate > 80 bpm, and SVI. The model demonstrated an AUROC of 0.715. Coefficients and score transformation details are also provided in Table 2. SIH scores ranged from 0 to 7 (Table 3). Fig. 2 illustrates the distribution of cases across different score points for hypotension and no hypotension. The AUROC of the SIH score was 0.715 (95% confidence interval (CI) 0.676-0.754), demonstrating acceptable discriminative ability. This was significantly better ($p < 0.001$) than the model with only SVI (AUROC 0.643) shown in Fig. 3. The Hosmer-Lemeshow test yielded a p value of 0.639, indicating good model fit. The bootstrapped ROC of the SIH score was 0.660 (95%CI 0.601-0.702%). Fig. 4 highlights how the SIH score corresponds to the actual risk of hypotension.

In this study, SIH scores were classified into three groups (Table 3) based on two cutoff points for the SIH risk score, determining low, moderate, and high-risk categories: scores 0-2 (low-risk), scores 2.5-4.5 (moderate-risk), and scores 5-7 (high-risk). The probabilities of hypotension were 21.88% (low-risk), 54.05% (moderate-risk), and 79.95% (high-risk), showing statistically significant differences ($p < 0.001$). These findings demonstrated the association between the SIH risk score and hypotension likelihood, emphasizing its utility for identifying patients at varying risk levels and informing clinical decision-making.

Table 2. Multivariable risk predictors, logit coefficients, and score transformation.

Predictor	Odds ratio	95%CI	p value	Coefficient	Score
Uterine contraction					
Yes					0
No	3.226	2.187 to 4.758	< 0.001	1.171	2
Heart rate (bpm)					
≤ 80					0
> 80	1.702	1.208 to 2.399	0.002	0.532	1
Stroke volume index (ml/ m^2/ beat)					
> 60					0
45 to 60	3.758	1.881 to 7.913	< 0.001	1.350	2.5
< 45	8.785	4.128 to 18.696	< 0.001	2.173	4

CI: confidence interval

Table 3. Distribution of hypotension vs. no hypotension in low, moderate, and high probability categories.

Probability category	Risk score	Hypotension (n = 474) n (%)	No hypotension (n = 238) n (%)	p value
Low	0-2	7 (21.88)	25 (78.13)	< 0.001
Moderate	2.5-4.5	160 (54.05)	136 (45.95)	
High	5-7	307 (79.95)	77 (20.05)	

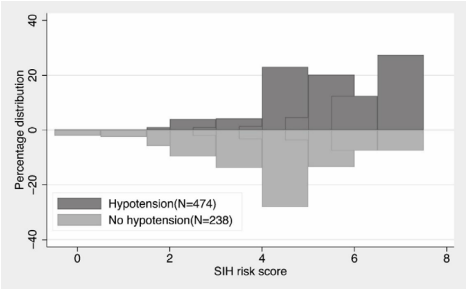


Fig. 2. Percentage distribution of spinal anesthesia-induced hypotension (SIH) risk scores categorized by hypotension and no hypotension.

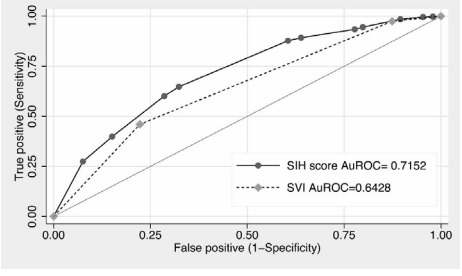


Fig. 3. Receiver operating characteristic curve for the prediction of spinal anesthesia-induced hypotension, comparison of the spinal anesthesia-induced hypotension (SIH) score, and stroke volume index (SVI) obtained from ultrasound cardiac output monitoring (USCOM).

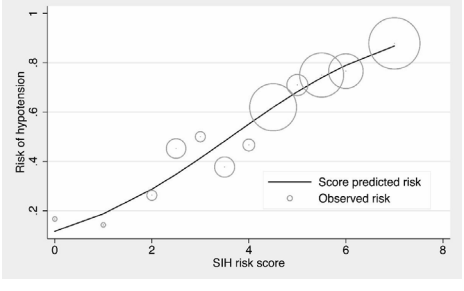


Fig. 4. Observed risk for each score point (circle) versus predicted risk (solid line) of spinal anesthesia-induced hypotension (SIH). The size of the circle represents the number of patients at each score point.

Discussion

Predicting SIH is difficult because of the complex mechanisms of SIH during cesarean delivery. Several previous studies failed to identify a simple good predictor[5]. In our study, we incorporated noninvasive USCOM hemodynamic monitoring to directly measure the mechanism of hypotension, going beyond preoperative risk factors. The best predictor among the various USCOM parameters was the SVI, but its predictive power was relatively poor (AUROC = 0.643). On the other hand, the SIH risk score, derived from a combination of three predictors: SVI,

uterine contraction, and baseline heart rate demonstrated acceptable predictive performance, with an AUROC of 0.715.

In our study, the baseline SVI emerged as the most prominent predictor. SVI reflects preload, which is a crucial component of blood pressure. This finding underscores the continued significance of fluid status in predicting SIH.

Although CI shows significance in both the univariable and multivariable logistic models, it is highly correlated with SVI. To address the issue of multicollinearity, we decided to exclude CI from the model and instead include SVI. SVI was chosen because it is directly obtained from USCOM. Additionally, CI values are typically very small and often expressed in decimal points, making it challenging to categorize CI effectively. Furthermore, the categorization of CI may not have significant clinical relevance due to its small value.

Baseline HR has been identified as a predictor of SIH[6, 15]. Higher baseline HR is believed to indicate increased sympathetic tone and a higher risk of developing hypotension after sympathectomy[6]. In our study, we found that a baseline HR cutoff point of 80 bpm was the best for predicting SIH. Interestingly, uterine contractions during spinal anesthesia were associated with a lower incidence of hypotension, acting as a protective predictor. Autotransfusion during uterine contractions in laboring women has been suggested as a potential mechanism for this protective effect[20]. The release of catecholamines and stress hormones during labor progression[21] may also contribute to this protective mechanism.

Our study did not find the supine stress test (SST) to be a significant predictor, despite its previous association with SIH in cesarean delivery[8, 9]. Unlike previous studies that focused on elective surgeries, our study included both elective and emergency cases. In emergency patients, factors like pain from uterine contractions may impact the accuracy of SST results, even though we attempted to perform the test during non-contraction periods. The increase in stress hormones due to pain could potentially affect the SST results. Consequently, SST may not reliably predict outcomes in emergency cesarean deliveries.

Systemic vascular resistance index (SVRI) did not emerge as a significant predictor in our study. This may be attributed to the fact that SVRI values obtained from USCOM are calculated rather than directly measured. Additionally, the absence of central venous pressure measurement in our data collection could introduce errors in the determination of SVRI values. The three predictors in our model, namely SVI, baseline heart rate, and uterine contraction, reflect fluid status and maternal sympathetic tone, both of which play a direct role in blood pressure regulation. Consequently, the other predictors that did not demonstrate significance are likely indicative of the same underlying mechanism as our three predictors, albeit with weaker correlations. As a result, these predictors were unable to exhibit significance in the multivariable model.

The PRAM score, developed by Bishop et al, utilizes preoperative risk factors to predict obstetric spinal hypotension[15]. However, despite its simplicity, the PRAM score exhibits poor discriminative ability (AUROC = 0.626). Additionally, the PRAM score has several limitations. The original study assessed hypotension until 15 minutes after neonatal delivery, potentially confounded by factors such as bleeding and adverse effects of uterotonic agents. Furthermore, the PRAM study allowed anesthesiologists to decide whether to administer prophylactic phenylephrine, which could impact the outcome variable. This discrepancy may explain the substantial difference in hypotension incidence between the PRAM study (30.36%) and our study (66.57%).

To the best of our knowledge, there is currently no existing predictive score with good or acceptable predictive power for hypotension in cesarean delivery. In our study, we developed a SIH risk score based on three parameters, which demonstrated acceptable predictive power (AUROC = 0.715). One of the strengths of our study is the prospective development of the risk score, utilizing a large number of patients and predictors. Furthermore, we combined data from

both maternal characteristics and hemodynamic monitoring, enhancing the comprehensiveness of our approach.

Our study has several limitations. Firstly, it was conducted in a single academic hospital, and the validation was only performed internally. This raises the possibility of overfitting the model, highlighting the importance of external validation in different settings. Secondly, the use of USCOM to obtain the SVI poses challenges due to equipment availability and the need for experienced operators. Although USCOM is a simple, noninvasive, and cost-effective method, a minimum of 50 cases is required for training to achieve the learning curve and ensure good inter-rater reliability Thirdly, our study included a population of normal healthy parturients, raising questions about its applicability to extreme or unhealthy populations. Developing separate prediction scores for different populations may be more appropriate. Additionally, our study was restricted to daytime shifts, leading to potential data loss during out-of-office hours, particularly in emergency cases. Nonetheless, we included a sufficient number of emergency surgery cases (179 cases, 25.14%). Finally, the discrimination power of our risk score did not reach a very high level. Future studies should explore the complex mechanisms of SIH and identify better predictors for improved risk assessment.

In a resource-limited setting where routine prophylactic therapy with phenylephrine for SIH is unavailable, tailoring prophylactic therapy based on individual risk assessment can contribute to the efficient utilization of resources. This approach can help guide decision-making in selecting patients for prophylactic therapy, prioritizing the high-risk group over the low-risk group.

Further studies should be conducted to evaluate the prophylactic therapy regimen for each individual risk group. For instance, consideration should be given to a higher dose of vasopressor medication or the utilization of combined colloid coloading[22] in the high-risk group, which has a significant likelihood of developing hypotension (79.95%). Conversely, in the low-risk group, a lower dose of prophylactic therapy should be considered to mitigate the risk of side effects, such as reactive hypertension.

Conclusion

Our study developed a scoring system for predicting SIH in cesarean delivery, which included three predictors: SVI, baseline heart rate > 80 bpm, and the presence of uterine contractions. Categorizing patients into low-, moderate-, and high-risk groups based on their SIH scores can assist in prioritizing the use of prophylactic therapy in resource-limited settings.

Acknowledgments

The authors thank Professor Jayanton Patumanond, M.D., MSc., DSc., PhD. for statistical consultation and the anesthetic personnel in the obstetric operating room for facilitating data collection.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Eltzschig HK, Lieberman ES, Camann WR. Regional anesthesia and analgesia for labor and delivery. N Engl J Med 2003;348:319-32.
2. Koković JT, Radunovic N, Filimonović D, Nejković L, Arsenijević L, Mirković LJ, et al. Maternal hemodynamic influence on uteroplacental oxygen distribution during cesarean section. Clin Exp Obstet Gynecol 2015;42:610-3.
3. Butwick AJ, Columb MO, Carvalho B. Preventing spinal hypotension during Caesarean delivery: what is the latest. Br J Anaesth 2015;114:183-6.
4. Fu F, Xiao F, Chen W, Yang M, Zhou Y, Ngan Kee WD, et al. A randomised double-blind dose-response study of weight-adjusted infusions of norepinephrine for preventing hypotension during combined spinal-epidural anaesthesia for Caesarean delivery. Br J Anaesth 2020;124:e108-e114.
5. Yu C, Gu J, Liao Z, Feng S. Prediction of spinal anesthesia-induced hypotension during elective cesarean section: a systematic review of prospective observational studies. Int J Obstet Anesth

2021;47:103175.
6. Frölich MA, Caton D. Baseline heart rate may predict hypotension after spinal anesthesia in prehydrated obstetrical patients. Can J Anaesth 2002;49:185-9.
7. Bishop DG, Cairns C, Grobbelaar M, Rodseth RN. Heart rate variability as a predictor of hypotension following spinal for elective caesarean section: a prospective observational study. Anaesthesia 2017;72:603-8.
8. Erango M, Frigessi A, Rosseland LA. A three minutes supine position test reveals higher risk of spinal anesthesia induced hypotension during cesarean delivery. An observational study. F1000Res 2018;7:1028.
9. Dahlgren G, Granath F, Wessel H, Irestedt L. Prediction of hypotension during spinal anesthesia for Cesarean section and its relation to the effect of crystalloid or colloid preload. Int J Obstet Anesth 2007;16:128-34.
10. Duggappa DR, Lokesh M, Dixit A, Paul R, Raghavendra Rao RS, Prabha P. Perfusion index as a predictor of hypotension following spinal anaesthesia in lower segment caesarean section. Indian J Anaesth 2017;61:649-54.
11. Sun S, Liu NH, Huang SQ. Role of cerebral oxygenation for prediction of hypotension after spinal anesthesia for caesarean section. J Clin Monit Comput 2016;30:417-21.
12. Elbadry AA, El Dabe A, Abu Sabaa MA. Preoperative Ultrasonographic evaluation of the internal jugular vein collapsibility index and inferior vena cava collapsibility index to predict post spinal hypotension in pregnant women undergoing caesarean section. Anesth Pain Med 2022;12:e121648.
13. Yokose M, Mihara T, Sugawara Y, Goto T. The predictive ability of non-invasive haemodynamic parameters for hypotension during caesarean section: a prospective observational study. Anaesthesia 2015;70:555-62.
14. Meirowitz N, Katz A, Danzer B, Siegenfeld R. Can the passive leg raise test predict spinal hypotension during cesarean delivery? An observational pilot study. Int J Obstet Anesth 2012;21:324-8.
15. Bishop DG, Cairns C, Grobbelaar M, Rodseth RN. Obstetric spinal hypotension: Preoperative risk factors and the development of a preliminary risk score - the PRAM score. S Afr Med J 2017;107:1127-31.
16. Hodgson LE, Venn R, Forni LG, Samuels TL, Wakeling HG. Measuring the cardiac output in acute emergency admissions: use of the non-invasive ultrasonic cardiac output monitor (USCOM) with determination of the learning curve and inter-rater reliability. J Intensive Care Soc 2016;17:122-8.
17. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD Statement. Br J Surg 2015;102:148-58.
18. Klöhr S, Roth R, Hofmann T, Rossaint R, Heesen M. Definitions of hypotension after spinal anaesthesia for caesarean section: literature search and application to parturients. Acta Anaesthesiol Scand 2010;54: 909-21.
19. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol 1996;49:1373-9.
20. Clark RB. Hypotension and Caesarean section. Br J Anaesth 2008;101:882-3.
21. Bonapace J, Gagné GP, Chaillet N, Gagnon R, Hébert E, Buckley S. No. 355-Physiologic Basis of pain in labour and delivery: An wvidence-based approach to its management. J Obstet Gynaecol Can 2018;40: 227-45.
22. Ripollés Melchor J, Espinosa Á, Martínez Hurtado E, Casans Francés R, Navarro Pérez R, Abad Gurumeta A, et al. Colloids versus crystalloids in the prevention of hypotension induced by spinal anesthesia in elective cesarean section. A systematic review and meta-analysis. Minerva Anestesiol 2015;81:1019-30.

Thai Journal of Obstetrics and Gynaecology
January 2024, Vol. 32, No. 1, pp. 57-67.

GYNAECOLOGY

Effect of Preoperative Walking Exercise on Postoperative Bowel Function in Patients with Major Gynecological Surgery: A randomized clinical trial

Aticha Patinavin, M.D.*,
Yuthana Khongthip, M.D.*

** Department of Obstetrics and Gynecology, Chonburi Hospital, Chonburi, Thailand*

ABSTRACT

Objectives: To investigate the relationship between preoperative walking exercise and postoperative bowel function in patients undergoing major gynecological surgery.

Materials and Methods: This randomized trial was conducted between July 2022 and January 2023 and included patients who underwent major gynecological surgery. All patients received a standard of care followed by enhanced recovery after surgery protocols. In addition, patients in the exercise group performed 30 minutes of mild intensity walking exercise (at 30% of their maximal heart rate) twice before surgery.

Results: Of the 42 enrolled patients, 17 and 18 patients from the exercise and control group were analyzed, respectively. There were no significant differences in characteristics between the groups. Time to first tolerance of an oral diet did not differ significantly between the groups (22.0 ± 5.9 hours in the exercise group vs 26.3 ± 10.3 hours in the control group, p = 0.144), and neither did time to first achievement of normoactive bowel sound or length of hospital stay. However, patients with an estimated blood loss greater than 1,000 mL had benefit from the intervention, with a shorter time required to tolerate an oral diet (20.0 ± 1.4 hours vs 45.5 ± 3.5 hours, p = 0.011).

Conclusion: There is still insufficient data to encourage routine preoperative walking exercise before surgery in patients with benign gynecological conditions. However, preoperative walking exercise may be beneficial for patients who are at high risk of extensive blood loss during surgery.

Keywords: preoperative exercise, walking, postoperative, ileus, bowel function.

Correspondence to: *Aticha Patinavin, M.D., Department of Obstetrics and Gynecology, Chonburi Hospital, Chonburi 20000, Thailand. E-mail: aticha12347@gmail.com*

Received: 8 May 2023, **Revised:** 29 May 2023, **Accepted:** 12 June 2023

ผลการเดินออกกำลังกายก่อนผ่าตัดต่อการฟื้นตัวของการทำงานของลำไส้ในผู้ป่วยหลังการผ่าตัดใหญ่ทางนรีเวช, การทดลองทางคลีนิคแบบสุ่ม

อธิชา ผาตินาวิน, ยุทธนา ของทิพย์

บทคัดย่อ

วัตถุประสงค์: *เพื่อศึกษาความสัมพันธ์ระหว่างการเดินออกกำลังกายก่อนผ่าตัด และการฟื้นตัวของทางเดินอาหารในผู้ป่วยที่เข้ารับการผ่าตัดใหญ่ทางนรีเวช*

วัสดุและวิธีการ: *วิจัยนี้เป็นการทดลองแบบสุ่มและมีกลุ่มควบคุม ทำการทดลองในเดือนกรกฎาคม 2022 ถึงเดือนมกราคม 2023 ผู้ป่วยทั้งหมดเข้ารับการผ่าตัดใหญ่ทางนรีเวช ผู้ป่วยในกลุ่มควบคุมจะได้รับการดูแลตามมาตรฐานตามมาตรการฟื้นตัวไว ในขณะที่กลุ่มออกกำลังกายจะเดินในคืนก่อนผ่าตัดช่วงละ 30 นาที ทั้งหมดสองช่วงที่ความหนักระดับอ่อน (ร้อยละ 30ของอัตราเร็วหัวใจเต้นสูงสุด)*

ผลการศึกษา: *จากผู้ป่วยเข้าร่วมงานวิจัยทั้งหมด 42 ราย ผู้ป่วยในกลุ่มเดินออกกำลังกาย 17 ราย และในกลุ่มควบคุม 18 ราย ถูกนำมาวิเคราะห์ พบว่าไม่มีความแตกต่างทางข้อมูลประชากรในทั้งสองกลุ่ม สรุปผลว่าระยะเวลาที่ผู้ป่วยสามารถเริ่มทานอาหารได้หลังการผ่าตัดไม่แตกต่างกัน (22.0 ± 5.9 ชั่วโมง ในกลุ่มเดินออกกำลังกาย vs 26.3 ± 10.3 ชั่วโมง ในกลุ่มควบคุม, p = 0.144) ระยะเวลาที่เสียงการทำงานของลำไส้กลับมาเป็นปกติ และระยะเวลาการนอนโรงพยาบาลไม่มีความแตกต่างเช่นกัน ผู้ป่วยที่มีการเสียเลือดระหว่างผ่าตัดมากกว่า 1,000 มิลลิลิตร ได้รับประโยชน์จากการเดินออกกำลังกาย โดยมีระยะเวลาที่ผู้ป่วยสามารถเริ่มทานอาหารได้หลังการผ่าตัดน้อยกว่า (20.0 ± 1.4 ชั่วโมง ในกลุ่มเดินออกกำลังกาย vs 45.5 ± 3.5 ชั่วโมง ในกลุ่มควบคุม, p = 0.011)*

สรุป: *ไม่มีหลักฐานสนับสนุนการเดินออกกำลังกายก่อนการผ่าตัดในกลุ่มผู้ป่วยที่ไม่ใช่โรคมะเร็งนรีเวชทุกคน อย่างไรก็ตาม การเดินออกกำลังกายก่อนผ่าตัดในกลุ่มผู้ป่วยที่มีความเสี่ยงที่จะเสียเลือดมากกว่า 1,000 มิลลิลิตร สามารถช่วยกระตุ้นการฟื้นตัวของทางเดินอาหารได้*

คำสำคัญ: *การออกกำลังกายก่อนผ่าตัด, การเดิน, หลังการผ่าตัด, ลำไส้อืด, การทำงานของลำไส้*

Introduction

Postoperative bowel ileus is a serious healthcare issue that lengthens hospitalization time and increases the risk of postoperative complications, as well as public health expenditures[1, 2]. Bowel ileus is defined as the inability to tolerate an oral diet, a delay in defecation or flatulence lasting more than four days after surgery[3]. The incidence of bowel ileus has been reported to range from 2.9% to 30% in women undergoing laparotomy gynecological surgery[4-7], with factors such as resection surgery type, operative time, opioid analgesic usage, nasogastric catheter insertion, fluid balance, receipt of blood component transfusion, and postoperative abdominopelvic complications being associated with postoperative bowel function recovery[5, 8].

Various interventions have been introduced to prevent postoperative bowel ileus, including the enhanced recovery after surgery (ERAS) protocol, which involves early feeding, coffee consumption, gum chewing, euvolemia, early ambulation, and multimodal analgesia[9]. Preoperative walking exercise has also been shown to improve postoperative bowel function, with researchers investigating the association between walking exercise and bowel function since 1989 and finding that mild intensity walking exercise had the greatest effect on gastric emptying time[10,11].

Walking exercise is employed in various healthcare settings. A randomized controlled trial demonstrated that walking exercise during bowel preparation in patients undergoing colonoscopy is safe and can improve bowel cleansing without significant patient discomfort[12]. However, a systematic literature review conducted using MeSH terms such as ileus, preoperative exercise, walking and/or ambulation, gynecology, and surgery, yielded only one randomized study by Ozdemir in 2019, which showed that preoperative walking exercise significantly reduced the time taken to tolerate solid food, time to first flatus, time to first defecation, and the incidence of postoperative paralytic ileus[10]. Nevertheless, since the study was only conducted in gynecological malignant patients, there is insufficient information to demonstrate the benefits to benign gynecological patients, who comprise the majority of those scheduled for elective major gynecological surgery. Additionally, the report did not highlight the limitations and complications associated with the intervention. The objective of this study was to examine the impact of preoperative walking exercise on postoperative bowel function in patients undergoing major gynecological surgery, as well as to identify any associated intervention related complications.

Materials and Methods

This study was a single center, non-blind, balanced randomization (1:1), standard treatment-controlled, parallel-group study conducted at Chonburi Hospital, a tertiary healthcare center in eastern Thailand. After obtaining approval from the institute ethics committee (reference number 17/65/R/h3), this randomized trial was conducted from July 1, 2022, to January 1, 2023. All enrolled patients provided written informed consent, and the study was registered in the Thai Clinical Trial Registry (TCTR20230103003).

The study recruited patients between the ages of 18 and 65 who were scheduled for elective major gynecological surgery and expected to have intrabdominal operative time longer than 30 minutes. The exclusion criteria included patients scheduled for laparoscopic surgery or spinal anesthesia, patients with an initial pain score of more than 3 points on the numeric rating scale, a history of abnormal bowel movement (constipation, diarrhea, or excessive straining) or inflammatory bowel disease, bowel obstruction, previous bowel surgery or planning of bowel resection, bowel preparation, inability to walk without assistance, abnormal laboratory investigation, cardiovascular disease, pulmonary disease, or osteoarticular disease. Other exclusion criteria were serious adverse events during exercise, a maximal heart rate of more than 220 minus age in years beats per minute, or less than 94% of pulse oximetry during exercise, bowel injury during surgery, and transfer to an intensive care unit or another hospital.

Patients admitted for gynecological services

were randomized into two groups using a computer-generated randomization sequence. Concealed block randomization was employed with a block size of six, and sequentially numbered sealed envelopes were utilized for allocation. The study was not blinded following the assignment of interventions.

All patients received standard care following the ERAS protocol. In the exercise group, patients participated in supervised walking exercise sessions lasting 30 minutes, conducted twice at a mild intensity corresponding to 30% of their maximum heart rate determined by the Karvonen formula[13]. These exercise sessions were supervised by physicians and took place between 16:00 and 22:00 on the day before surgery. Patients were instructed to adjust their walking speed to ensure their heart rate fell within the target range or to slow down if their heart rate exceeded the target. A one-hour break was recommended between exercise sessions. Patients were advised to promptly report any complications experienced during the intervention to their supervisor. Additionally, a pulse oximeter was attached to the middle finger of each participant.

After registration, the ERAS protocol was implemented. Prior to anesthesia, all patients followed a fasting period of up to 8 hours for light meals and up to 2 hours for clear fluids, without mechanical bowel preparation. Prophylactic intravenous antibiotics were administered to patients 15 minutes before the surgical incision.

Following the surgery, the nasogastric tube (if used during the procedure) was removed, and all patients received standardized postoperative care. This care included the ability to drink clear fluids (ranging from 50 to 800 mL) four hours after surgery. Pain management involved the administration of 50 mg of intravenous pethidine every 4 hours, along with regular oral paracetamol. Additional opioid or non-steroidal analgesics were given as needed, while metoclopramide was prescribed to address any instances of nausea or vomiting.

Patients were allowed to resume their liquid and solid diets once they showed no signs of adverse symptoms. Discharge from the hospital occurred when patients had stable vital signs, were free from fever for a minimum of 24 hours (defined as a body temperature ≥ 38°C in one measurement or ≥ 37.8°C in two measurements), were able to walk without assistance, could tolerate solid food without experiencing vomiting, had normal urination, and did not have any other postoperative complications.

The primary outcome measure in this study was the duration (in hours - hr) required for patients to tolerate a solid diet that necessitated chewing, providing more than 5 kcal/kg/meal. This criterion aligns with the guideline criteria outlined by the European Society for Clinical Nutrition and Metabolism[14] for discontinuing intravenous fluid or parenteral nutrition. The measurement period commenced at the conclusion of the surgical procedure, and the outcome assessors calculated the calorie content per meal using a weighing machine and measuring spoon.

After the surgery, the bowel sounds of each patient were monitored every six hours using a stethoscope until the first bowel sound was auscultated. Patients were instructed to promptly notify their doctor, nurses, or researcher upon experiencing their initial passage of flatus and feces. Furthermore, the researcher maintained weekly contact with the patients to assess postoperative complications and ascertain their readmission status.

Secondary outcomes included the time required for the first instance of normoactive bowel sounds (1-2 bowel sounds within 1 minute, as auscultated by stethoscope), the time for the first passage of flatus and stool, diagnosis of pathologic ileus according to Vather et al definition as mentioned above[3], occurrence of adverse events during walking exercise, postoperative complications, length of hospital stays, additional antiemetic, intravenous analgesic requirements, and readmission rate within one month. Based on previous research conducted by Ozdemir et al in 2019[10], the mean time to first tolerance of a solid diet in the exercise group and control group was 81.6 (±16.8) and 103.2 (±19.2) hours, respectively. To

achieve 90% study power with an α level of 0.05 and an assumed 20% dropout rate, a total of 42 patients were enrolled.

The normal distribution of variables was assessed using the Shapiro-Wilk tests. Categorical variables were compared using chi-square tests, while independent t-test was used to compare normally distributed continuous variables, and Welch's t-test was employed to compare unequal variables. The statistical analyses were performed using Stata (version 16). An intention-to-treat protocol was applied, with subgroup analysis conducted on affecting factors such as intraoperative procedure, operative time, amount of blood component transfusion, and estimated blood loss. A p value of less than 0.05 was considered statistically significant.

Results

A total of 45 patients were assessed for eligibility, out of which 42 were enrolled during the trial period, with 21 assigned to the walking exercise group and 21 to the control group (Fig. 1). Among the walking exercise group (E group) , two patients suffered from small bowel injuries, one required an appendectomy, and another was transferred to the surgical intensive care unit. In the control group (C group), two patients also experienced small bowel injuries, and one underwent an appendectomy due to an abnormal intraoperative finding. As anticipated, no individuals with bowel or pelvic adhesions were included in our study. Ultimately, 17 patients from the walking exercise group and 18 patients from the control group were included in the analysis.

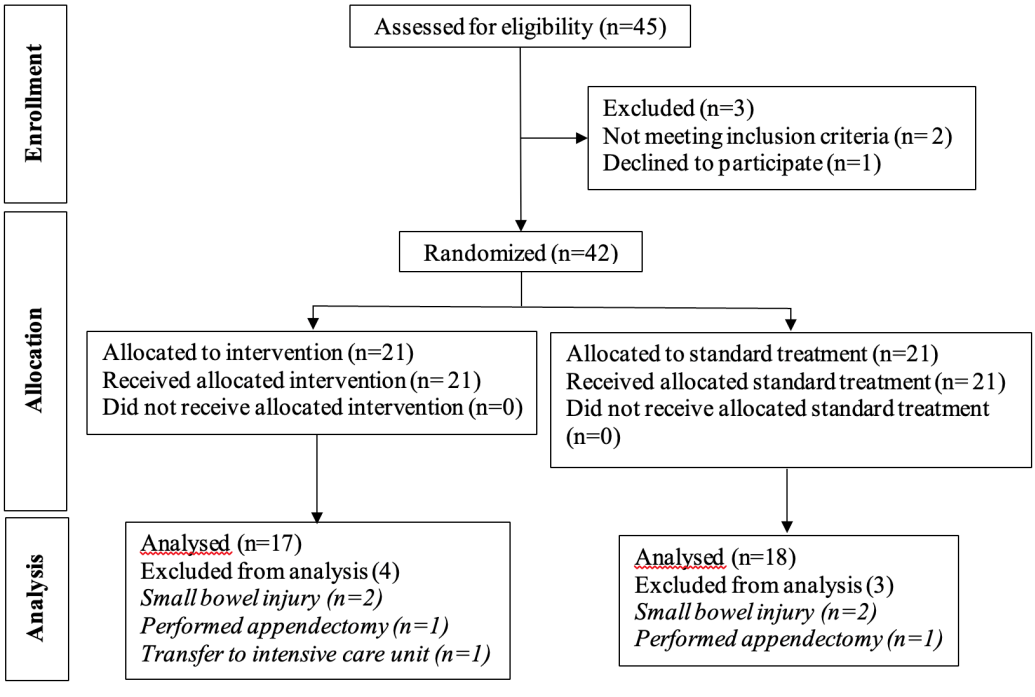


Fig. 1. Flow diagram.

The percentage of patients who walked and reached their target heart rate was 88.2% (n = 15). Patient characteristics of each group were comparable (Table 1). In the E group, 47.1% of patients had prior abdominopelvic surgery, while 50.0% in the control group had the same history. One patient in the E group was on antidepressant medication, and another used an anticholinergic substance. However, none of the patients in the C group used either of these medications.

Table 1. Baseline characteristics.

Baseline Characteristics	Control (n = 18)	Walking exercise (n = 17)
Age (years); mean ± SD	44.3 ± 8.4	39.8 ± 7.6
BMI (kg/m2); mean ± SD	24.8 ± 5.4	26.2 ± 5.2
Race; n (%)		
Thai	16 (88.9)	16 (94.1)
Other*	2 (11.1)	1 (5.9)
Parity; mean ± SD	1.1 ± 1.0	1.7 ± 1.3
Prior abdominopelvic surgery; n (%)	9 (50.0)	8 (47.1)
Cesarean delivery	6 (33.3)	2 (11.8)
Cystectomy	3 (16.7)	8 (47.1)
Salpingectomy	1 (5.6)	0 (0)
Previous abdominal surgical scar; n (%)		
Low midline incision	4 (22.2)	3 (17.6)
Pfannenstiel incision	4 (22.2)	3 (17.6)
Infraumbilical incision	1 (5.6)	2 (11.8)
Alcohol drinking; n (%)	1 (5.6)	1 (5.9)
Tobacco use; n (%)	0 (0)	2 (11.8)
Coffee drinker; n (%)	10 (55.6)	9 (52.9)
Medical condition; n (%)		
Diabetes mellitus	1 (5.6)	2 (11.8)
Hypertension	1 (5.6)	1 (5.9)
Other comorbid disease**	3 (16.7)	5 (29.4)
Medication; n (%)		
Antidepressant	0 (0)	1 (5.9)
Anticholinergic agents	0 (0)	1 (5.9)
Other bowel effected medication***	5 (27.8)	5 (29.4)
Preoperative hemoglobin (g/dL); mean ± SD	12.0 ± 1.6	12.6 ± 1.6

SD: standard deviation, BMI: body mass index, Kg: kilogram, m: meter, g: gram, dL: deciliter

*In control group, one was Cambodian, and one was Lao. In walking exercise group, one was Burmese.

**In control group, two with human immunodeficiency virus infection and one who had well-controlled thyroid disease. In the walking exercise group, two with human immunodeficiency virus infection, one with occasional migraine, one with major depressive disorder, and one who had allergic rhinitis.

***In control group, two were taking tenofovir disoproxil fumarate/ lamivudine/ dolutegravir sodium, one was taking ferrous fumarate, one was taking metformin, and one was taking amlodipine. In walking exercise group, two were taking tenofovir disoproxil fumarate/ lamivudine/ dolutegravir sodium, two were taking metformin, one was taking ergotamine and one was taking amlodipine.

The pathology of surgical lesions in this study was predominantly benign (Table 2). Hysterectomy was the most frequent intraoperative procedure performed in both groups (58.8% in the E group and 55.6% in the C group). The operative time, type of anesthesia, estimated blood loss, volume of perioperative intravenous crystalloid, insertion of postoperative closed-suction percutaneous drain, dosage of perioperative morphine, and usage of anticholinergic drugs were comparable between the two groups.

Table 2. Surgical characteristics.

Surgical characteristics	Control (n = 18)	Walking exercise (n = 17)
Pathology of surgical lesion; n (%)		
Benign	16 (88.9)	14 (82.4)
Malignancy	2 (11.1)	3 (17.6)
Intraoperative procedure; n (%)		
Adnexal surgery	6 (33.3)	6 (35.3)
Hysterectomy	10 (55.6)	10 (58.8)
Surgical staging*	2 (11.1)	1 (5.9)
Operative time (minutes); mean ± SD	117.2 ± 48.4	115.4 ± 56.9
Type of anesthesia; n (%)		
General Anesthesia	18 (100)	16 (94.1)
Combine (Spinal block and General anesthesia)	0 (0)	1 (5.9)
Estimated blood loss (mL); mean ± SD	403.3 ± 337.8	363.5 ± 371.8
Postoperative closed-suction percutaneous drain; n (%)	0 (0)	1 (5.9)
Perioperative morphine used (mg); mean ± SD	10.0 ± 2.1	9.0 ± 1.6
Perioperative anticholinergic agent used (mg); mean ± SD		
Succinylcholine	52.8 ± 56.2	63.5 ± 57.4
Cisatracurium	10.3 ± 6.0	12.6 ± 5.7
Blood component transfusion (mL); mean ± SD	74.1 ± 149.6	39.7 ± 163.5
Perioperative intravenous crystalloid (mL); mean ± SD	1,391.7 ± 581.6	1,511.8 ± 788.7

mL: milliliter, mg: milligram, SD: standard deviation

*In control group, one was performed radical hysterectomy with bilateral salpingo-oophorectomy with bilateral pelvic lymph node dissection, and one was performed transabdominal simple hysterectomy with bilateral pelvic lymph node dissection with omental biopsy with peritoneal washing. In walking exercise group, one was performed radical hysterectomy with bilateral salpingo-oophorectomy with bilateral pelvic lymph node dissection

Table 3 presents the primary and secondary outcomes of the study. The time to first tolerance of an oral diet did not differ between the E group (22.0 ± 5.9 hours) and the C group (26.3 ± 10.3 hours) (p = 0.144). Similarly, the time to first achievement of a normoactive bowel sound and the time to first passage of stool were comparable between the two groups. However, the time to the first passage of flatus was shorter in the control group. The length of hospital stays, readmission rate, additional antiemetic requirement, and additional intravenous analgesia requirement did not differ between the two groups.

No patient in either group was diagnosed with pathologic ileus. One patient in the walking exercise group complained of mild abdominal discomfort with a pain score of 3 out of 10 on the visual analog rating scale after finishing the walking session, which alleviated spontaneously after bed rest. There were no serious adverse events. One patient in the E group had a postoperative complication and was readmitted within one month due to vaginal stump dehiscence.

In the subgroup analysis, patients with an estimated blood loss greater than 1,000 mL had benefit from the intervention as indicated by a shorter time required to tolerate the oral diet (20.0 ± 1.4 in E group vs 45.5 ± 3.5 in C group, p = 0.011) (Table 4). However, no difference in the primary outcome was observed when patients were divided into groups based on the intraoperative procedure, amount of blood component transfusion, and operative time.

Table 3. Study outcomes.

Study outcomes	Control (n = 18)	Walking exercise (n = 17)	p value
Time to first tolerance of an oral diet (hours)*	26.3 ± 10.3	22.0 ± 5.9	0.144
Time to first achieve normoactive bowel sound (hours)*	10.3 ± 4.5	8.1 ± 3.6	0.121
Time to first passage of flatus (hours)*	30.0 ± 8.4	45.4 ± 23.3	0.018
Time to first passage of stool (days)*	4.1 ± 1.6	4.5 ± 1.7	0.510
Diagnosis of pathologic ileus; n (%)	0	0	N/A
Postoperative complication; n (%)	0 (0)	1 (5.9)	0.296
Length of hospital stays (hours)*	46.1 ± 11.8	52.9 ± 20.8	0.239
Readmission to hospital within 1 month; n (%)	0 (0)	1 (5.9)	0.296
Additional antiemetic requirement (mg)*	1.7 ± 3.8	2.9 ± 5.9	0.450
Additional intravenous analgesic requirement (mg)*	191.7 ± 49.3	185.3 ± 34.3	0.662

* mean ± standard deviation

Table 4. Subgroup analysis of effecting factors for postoperative bowel ileus.

Study outcomes	Time to tolerate oral diet		p value
	Control (n = 18)	Walking exercise (n = 17)	
Intraoperative procedure*			
Adnexal surgery	22.0 ± 2.8	21.8 ± 2.1	0.909
Hysterectomy	27.8 ± 12.1	22.4 ± 7.7	0.254
Surgical staging	31.5 ± 16.3	19.0	0.643
Blood component transfusion*			
Yes	40.5 ± 8.7	19.0	0.113
No	22.2 ± 6.5	22.2 ± 6.1	0.997
Estimate blood loss *			
< 500 mL	20.3 ± 2.1	22.7 ± 7.0	0.294
500 – 1,000 mL	31.6 ± 11.1	20.7 ± 2.1	0.151
> 1,000 mL	45.5 ± 3.5	20.0 ± 1.4	0.011
Operative time*			
< 90 min	21.2 ± 1.9	26.2 ± 9.1	0.216
90-180 min	26.1 ± 10.3	20.2 ± 3.4	0.126
> 180 min	37.0 ± 14.9	20.3 ± 2.3	0.190

mL: milliliter, min: minutes

* mean ± standard deviation

Discussion

Walking exercise has been demonstrated to reduce gastric emptying time by increasing bowel motility via various neuro-hormonal mechanisms[11]. A previous study revealed the benefits of intervention in shortening the time to recovery of bowel motility

and the time taken to tolerate solid food in patients with gynecological cancer[10]. However, our randomized controlled trial showed no effects from preoperative walking exercise on postoperative bowel function, as represented by the time taken to tolerate an oral diet in patients undergoing major gynecological surgery. We did not use the rate of bowel ileus as the primary outcome because most of the definitions are composite outcome measurements and are difficult to measure objectively. Additionally, the ERAS protocol encourages oral intake as tolerated regardless of bowel sound.

In the previous trial, all patients were diagnosed with gynecological cancer and scheduled for surgical staging. In contrast, our trial included patients with various generally benign diagnoses and undergoing less invasive procedures, such as adnexal surgery and/or hysterectomy. Only a minority of patients in our study underwent surgical staging. Additionally, the operative time in the previous study was considerably longer than in our study (4.2 hours in the previous study vs 1.9 hours in our study). Therefore, the population in the previous study was at a higher risk of postoperative bowel ileus than the population in our study, which was reflected in the incidence of postoperative bowel ileus. The previous study reported a 28% prevalence of pathologic ileus, whereas no patient in our study was diagnosed with this condition.

Our study had no incidence rate of postoperative bowel ileus, which was undoubtedly lower than the incidence rates reported in previous studies of benign gynecologic surgery (2.9% - 9.2%)[6, 7]. Li et al[6] reported an incidence of 9.2% of postoperative ileus, defined as the absence of flatus and defecation for more than 2 days with the presence of one or more of the following symptoms: nausea, vomiting, and abdominal distention. In contrast, our study used a different definition, which considered a diagnosis of pathologic bowel ileus at 4 days postoperatively. About half (57%) of our patients underwent hysterectomy, while all of Li's population had hysterectomy performed. The routine implementation of the ERAS protocol in our institute may also play an important role in the lower rate of postoperative pathologic bowel ileus. However, given the smaller population in our study, the effect of the intervention on bowel ileus remains unclear.

The precise mechanism underlying the impact of preoperative walking on postoperative bowel function remains unclear. Numerous studies have indicated that different forms of exercise can improve postoperative bowel function. For instance, Peng et al[15] conducted a study revealing the benefits of a preoperative rehabilitation-based enhanced recovery protocol encompassing exercises targeting the upper and lower extremities, thoracic and abdominal breathing, as well as abdominal muscles, in facilitating gastrointestinal function recovery among patients undergoing colorectal surgery. Similar potential benefits may exist for gynecological patients; however, further prospective research is warranted to explore this aspect in more detail.

Other secondary outcomes in the study were collected due to their impact on laboratory investigations, treatment, and hospital costs, including time to first achievement of normoactive bowel sound, time to the first passage of stool, postoperative complications, length of hospital stay, readmission rate within one month, and additional antiemetic or intravenous analgesic requirements, which were all similar in both groups. The only significant difference was the shorter time to the first passage of flatus in the control group. Flatulence was one of the diagnostic criteria for pathologic bowel ileus, but it had to occur in conjunction with additional symptoms such as nausea or vomiting, inability to tolerate an oral diet, abdominal distension, or the presence of bowel ileus radiographic features[3]. However, we concluded that this outcome was not clinically significant, especially since the other outcomes were not different and the symptoms did not cause any discomfort to the patients.

Our study revealed that the intervention had specific benefits for patients who encountered intraoperative hemorrhage surpassing 1,000 mL, as

they faced an elevated risk of postoperative bowel ileus. Literature has previously documented variable rates of hemorrhage following abdominal hysterectomy, ranging from 0.2% to 3.7%[16].

In clinical practice, we advocate for preoperative walking exercise among patients scheduled to undergo elective gynecological cancer surgery, particularly those identified as being at risk of significant intraoperative hemorrhage. Although accurately predicting blood loss before surgery poses challenges, certain factors have been associated with a higher probability of increased blood loss in benign gynecological surgery. These factors encompass an operation duration exceeding 180 minutes, an American society of Anesthesiologists (ASA) class IV, the presence of anemia, prior transfusion history, and surgery performed for fibroids[17].

This study had several strengths. It was the first prospective randomized design to include a largely benign patient population, which represents the majority of gynecological department patients. The demographics and surgical characteristics of the two groups were similar in this study. Furthermore, our primary outcome was objective and clinically relevant, and there was no missing data due to loss to follow-up. Because the trial involved the rational recruitment of participants with appropriate inclusion and exclusion criteria, the time to first toleration of an oral diet in the control group patients was similar to that in the control group of the other study (26.3 ± 10.3 in our study vs 27.4 ± 11.0 in the other study)[18].

However, our study had several limitations. Firstly, the study design did not allow for blinding, which may have influenced the findings. Secondly, the surgical operation was not performed by the same surgical team, which could have introduced variability in the surgical techniques and outcomes. Thirdly, we could not completely restrict walking in the control group during the night before surgery, which could have potentially affected the results. Finally, the study was conducted in a single center, and the findings may not be generalizable to other settings or populations.

Conclusion

Our randomized controlled trial showed no effects from preoperative walking exercise on postoperative bowel function, as represented by the time taken to tolerate an oral diet in patients undergoing major gynecological surgery. Therefore, there is still insufficient data to encourage routine preoperative walking exercise before surgery in patients with benign gynecological conditions. However, preoperative walking exercise may be beneficial for patients who are at high risk of extensive blood loss during surgery.

Acknowledgments

The researcher wishes to express gratitude and acknowledge the invaluable contribution of the nurses and dietitian from the Department of Gynecology at Chonburi Hospital, for their exceptional assistance and unwavering cooperation throughout the course of this research.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Johnson MD, Walsh RM. Current therapies to shorten postoperative ileus. Cleve Clin J Med 2009;76:641-8.
2. Boeckxstaens GE, de Jonge WJ. Neuroimmune mechanisms in postoperative ileus. Gut 2009;58: 1300-11.
3. Vather R, Trivedi S, Bissett I. Defining postoperative ileus: results of a systematic review and global survey. J Gastrointest Surg 2013;17:962-72.
4. 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.
5. Bakkum-Gamez JN, Langstraat CL, Martin JR, Lemens MA, Weaver AL, Allensworth S, et al. Incidence of and risk factors for postoperative ileus in women undergoing primary staging and debulking for epithelial ovarian carcinoma. Gynecol Oncol 2012;125:614-20.
6. Li ZL, Zhao BC, Deng WT, Zhuang PP, Liu WF, Li C,

et al. Incidence and risk factors of postoperative ileus after hysterectomy for benign indications. Int J Colorectal Dis 2020;35:2105-12.
7. Wolff BG, Viscusi ER, Delaney CP, Du W, Techner L. Patterns of gastrointestinal recovery after bowel resection and total abdominal hysterectomy: pooled results from the placebo arms of alvimopan phase III North American clinical trials. J Am Coll Surg 2007;205:43-51.
8. Ay AA, Kutun S, Ulucanlar H, Tarcan O, Demir A, Cetin A. Risk factors for postoperative ileus. J Korean Surg Soc 2011;81:242-9.
9. Nelson G, Bakkum-Gamez J, Kalogera E, Glaser G, Altman A, Meyer LA, et al. Guidelines for perioperative care in gynecologic/oncology: Enhanced Recovery After Surgery (ERAS) Society recommendations-2019 update. Int J Gynecol Cancer 2019;29:651-68.
10. Özdemir İA, Comba C, Demirayak G, Gülseren V, Erdogan SV, Aslanova F, et al. Impact of pre-operative walking on post-operative bowel function in patients with gynecologic cancer. Int J Gynecol Cancer 2019;29:1311-6.
11. Neufer PD, Young AJ, Sawka MN. Gastric emptying during walking and running: effects of varied exercise intensity. Eur J Appl Physiol Occup Physiol 1989;58: 440-5.
12. Kim HS, Park DH, Kim JW, Jee MG, Baik SK, Kwon SO, et al. Effectiveness of walking exercise as a bowel preparation for colonoscopy: a randomized controlled trial. Am J Gastroenterol 2005;100: 1964-9.
13. Karvonen MJ, Kentala E, Mustala O. The effects of training on heart rate; a longitudinal study. Ann Med Exp Biol Fenn 1957;35:307-15.
14. Singer P, Blaser AR, Berger MM, Alhazzani W, Calder PC, Casaer MP, et al. ESPEN guideline on clinical nutrition in the intensive care unit. Clin Nutr 2019;38:48-79.
15. Peng LH, Wang WJ, Chen J, Jin JY, Min S, Qin PP. Implementation of the pre-operative rehabilitation recovery protocol and its effect on the quality of recovery after colorectal surgeries. Chin Med J 2021;134:2865-73.
16. Harris WJ. Early complications of abdominal and vaginal hysterectomy. Obstet Gynecol Surv 1995;50:795-805.
17. Jansen SM, Ghatalia D, Mayo A, Lokke A, Petersen TR, Serna-Gallegos T, et al. Factors associated with increased estimated blood loss and factors associated with utilization of type and screen in benign gynecology: A retrospective chart review. Urogynecology (Phila) 2023;29:195-201.
18. Jernigan AM, Chen CC, Sewell C. A randomized trial of chewing gum to prevent postoperative ileus after laparotomy for benign gynecologic surgery. Int J Gynaecol Obstet 2014;127:279-82.

Thai Journal of Obstetrics and Gynaecology
January 2024, Vol. 32, No. 1, pp. 68-75.

OBSTETRICS

Evaluation of the Episiotomy Scissors Attached with an Adjusted Angle Plate for Mediolateral Episiotomy on the Occurrence of Obstetric Anal Sphincter Injuries

Phiangjit Wiruchpongsanon, M.D.*,
Sunida Chaitikul, M.N.S.*

** Department of Obstetrics and Gynecology, Taksin Hospital, Bangkok, Thailand*

ABSTRACT

Objectives: To assess the rate of obstetric anal sphincter injuries (OASI) and the angle after repair following mediolateral episiotomy performed with episiotomy scissors attached with an adjusted angle plate

Materials and Methods: The study group comprises 153 primiparous women at 37-42 weeks of gestation delivered between December 2022 and July 2023 in the labor room, Taksin Hospital. Scissors with a 60-degree adjusted angle plate were applied. Suture angles were recorded on transparent sheet and analyzed. Before suturing, a rectal exam was done to check for OASI.

Results: Of 153 primiparous women were recruited into the present study, the mean gestational age was 38.56 ± 1.06 weeks, the mean birthweight was 2,990.82 ± 327.39 grams. The mean post-suturing episiotomy angles were 44.92 ± 5.88 degrees (95 % confidence interval 44-45.9). The change in angle decreased from before cutting by 15.07±5.88 degrees. No cases of OASI were detected.

Conclusion: During childbirth, mediolateral episiotomy is performed using scissors attached to a 60-degree adjusted angle plate to cut the perineum. The mean perineal angle after suture repair was 44.9 degrees, a decrease of 15 degrees. No anal sphincter injury was detected.

Keywords: episiotomy scissors, adjusted angle plate, mediolateral episiotomy, obstetric anal sphincter injuries.

Correspondence to: *Phiangjit Wiruchpongsanon, M.D., Department of Obstetrics and Gynecology, Taksin Hospital, Bangkok, Thailand. E-mail: wphiangjit@gmail.com*

Received: 10 August 2023, **Revised:** 15 September 2023, **Accepted:** 18 September 2023

การประเมินผลของการใช้กรรไกรตัดฝีเย็บที่ติดแผ่นปรับมุมในการตัดฝีเย็บจากกึ่งกลางไปด้านข้างต่อการเกิดการฉีกขาดของหูรูดทวารหนักทางสูติกรรม

เพียงจิตต์ วิรัชพงศานนท์, สุนิดา ชัยติกุล

บทคัดย่อ

วัตถุประสงค์*: เพื่อศึกษาอัตราการฉีกขาดของหูรูดทวารหนักทางสูติกรรมและมุมหลังเย็บซ่อมฝีเย็บจากการใช้กรรไกรตัดฝีเย็บที่ติดแผ่นปรับมุม*

วัสดุและวิธีการ*: สตรีตั้งครรภ์เดี่ยวอายุครรภ์ระหว่าง 37 ถึง 42 สัปดาห์ ที่ไม่เคยคลอดบุตรทางช่องคลอดมาก่อน จำนวน 153 ราย ที่มาคลอดที่ห้องคลอดโรงพยาบาลตากสินระหว่างเดือนธันวาคม 2565 ถึงเดือนกรกฎาคม 2566 จะได้รับการตัดมุมฝีเย็บ 60 องศา แบบเฉียงออกไปด้านข้างด้วยกรรไกรที่ปรับทำมุมไว้แล้ว วัดมุมหลังเย็บซ่อมฝีเย็บด้วยแผ่นพลาสติกใสเพื่อนำไปวิเคราะห์ และทำการตรวจทางทวารหนักเพื่อประเมินการฉีกขาดของหูรูดทวารหนักก่อนเย็บทุกราย*

ผลการศึกษา*: สตรีตั้งครรภ์ 153 ราย ที่ได้รับการตัดฝีเย็บด้วยมุม 60 องศา พบว่ามีอายุครรภ์เฉลี่ยอยู่ในช่วง 38.56 ± 1.06 สัปดาห์ น้ำหนักทารกแรกเกิดเท่ากับ 2,990.82 ± 327.39 กรัม ค่าเฉลี่ยของมุมฝีเย็บหลังเย็บซ่อมแซมเท่ากับ 44.92±5.88 องศา (95% confidence interval 44-45.9) มีการเปลี่ยนแปลงของมุมลดลงจากก่อนตัดฝีเย็บ 15.07±5.88 องศา ไม่พบสตรีตั้งครรภ์ที่มีการฉีกขาดของหูรูดทวารหนักทางสูติกรรม*

สรุป*: การตัดฝีเย็บขณะคลอดด้วยกรรไกรที่ติดแผ่นปรับทำมุม 60 องศา ให้เฉียงออกไปด้านข้างจากกึ่งกลาง พบค่าเฉลี่ยของมุมฝีเย็บหลังเย็บซ่อมแซมเท่ากับ 44.9 องศา ลดลง 15 องศา ไม่พบการเกิดการบาดเจ็บของกล้ามเนื้อหูรูดทวารหนัก*

คำสำคัญ*: กรรไกรตัดฝีเย็บ, แผ่นยึดติดที่ปรับมุมได้, การตัดฝีเย็บเฉียงออกไปด้านข้างจากกึ่งกลาง, การบาดเจ็บของกล้ามเนื้อหูรูดทวารหนักทางสูติกรรม*

Introduction

Obstetric anal sphincter injuries (OASI) are reported in 0.5-15% of vaginal deliveries[1, 2], especially operative vaginal delivery, which increases the risk of OASI[3, 4]. As a result, 30-50 percent of these women are afflicted with anal incontinence, fecal urgency, dyspareunia, and perineal pain[1, 5-8]. There were also studies indicating that Asian women were statistically significant to have OASI than Caucasian women, and fourth-degree OASI births were significantly higher even though they bore smaller newborns[9, 10]. Asian races are considered an independence risk to oasis[9, 10]. This could be attributed to various factors such as having a shorter perineal body, a smaller pelvic inclination and different perineal muscle support[11]. Another contributing factors could be racial differences in skin thickness and resistance to mechanical stretch[12]. Therefore, prevention is important.

If the incision angle is adequate, mediolateral episiotomy is linked to a lower risk of OASI[10]. Eogan M, et al[13] conducted a case-control study in primiparous women who had undergone right mediolateral episiotomy 3 months previously. The mean angle of episiotomy scar from the midline in cases with OASI was 30 degrees, while the controls without OASI were 38 degrees. An analysis revealed that for every 6 degrees away from the midline, there was a 50% relative decrease in the risk of experiencing a third-degree tear[13].

During delivery, the perineal tissue will expand and swell, resulting in a greater incisional angle for mediolateral episiotomy compared to the suture angle after repair. In 2008, Vladimir K, et al[14] revealed that the angle of a mediolateral episiotomy formed by the midline and the incision was 40 degrees and the median angle of the suture line after delivery was found to be 20 degrees.

A study conducted by Wiruchpongsanon P, et al[15] in 2013 focused on 70 primiparous women who had a mediolateral episiotomy of 60 degrees during the crowning of their baby's head. The results showed that the average suture angle was 42 degrees with a change of angle of 18 degrees.

According to Stedenfeldt, et al[16] scarred episiotomy with angle range of 30-60 degrees are significantly associated with less risk of OASI. But observed by van Dillen, et al[17], the mean angle of episiotomy was 38.6° ± 7.8° immediately after delivery and 31.2° ± 11.5° at the postnatal check-up. That means the mean suture angle immediately after delivery should be about 40-70 degrees or the angle of mediolateral episiotomy during the crowning of the baby's head should be at least 60-90 degrees. Therefore, Episcissors-60 are invented to achieve a mediolateral cut at 60 degrees to the perineal midline. Based on a systematic review of five studies[18], it has been discovered that using Episcissors-60 can decrease the occurrence of OASI by 50%. Furthermore, in all of the studies, the angle measured after repair was greater than 40 degrees. Unfortunately, the cost of £400 for the Episcissors-60 is too high for a developing country.

In Thailand, Thanapongpibul, et al[19] invented Episioguide to assist in cutting the perineum at a 60-degree angle. They conducted a randomized controlled trial and found that the mean postsuture angle was 34.636° ± 9.445° in the Episioguide group, while it was 27.614° ± 9.267° in the group that followed the standard procedure.

At Taksin Hospital, midwives, nurse teachers, and medical students handle uncomplicated deliveries using routine mediolateral episiotomy. Obstetricians only handle cases requiring obstetric procedures. A new adjusted angle plate has been developed by researcher that can be attached to standard perineal scissors used during childbirth. This plate can be adjusted to angles between 60 and 90 degrees, making it useful for nurses, medical students, nursing teachers, and obstetricians during deliveries. It is particularly useful in situations where an obstetric procedure is required, and there is a higher risk of OASI. The perineum needs to be cut at an angle greater than 60 degrees in such cases. The research study set the cutting angle at 60 degrees, which is considered the least risky angle for obstetric rectal sphincter tearing compared to higher angles. This

study aimed to evaluate the use of perineal scissors fitted with a 60° adjusted angle plate to evaluate the incidence of OASI and whether the post-suture angle is in a safe range or not.

Materials and Methods

This uncontrolled experimental study was approved by Bangkok Metroplitan Administration (BMA) Human Research Ethics Committee (certificate number: S013h/65). The sample size was calculated according to the estimated prevalence of OASI of 10% with acceptable error of 5% at 95% significant level. Allowing for 10% missing data, we had to recruit a total of 153 women. Data was collected from December 2022 to July 2023.

The inclusion criteria were for uncomplicated singleton pregnancies of nulliparous women, 20 years old or older, between 37-42 weeks of gestation, with the fetus in cephalic presentation and vaginal delivery planned. The exclusion criteria for our study were women who had undergone operative vaginal delivery, women with prior surgical scarring of the perineum, women who declined to participate, and fetuses in a persistent occipitoposterior position.

The episiotomy was performed by nurse with at least 3 years of experience in the delivery room of Taksin Hospital. They were trained to use an adjusted angle plate.

Researcher collaborated with engineers and technicians computer numerical control (CNC) to create the adjusted angle plate. It was crafted from stainless steel 304, a material commonly used in medical equipment due to its high resistance to corrosion and ability to withstand temperatures up to 1,400 degrees Celsius without rusting. The adjusted angle plate will have an axis for plugging in and selecting the desired angle, ranging from 60 to 90 degrees. To use the adjusted angle plate, first, align the circle point on the plate with the pivot point of the perineal scissors. After that, lock the handle of the adjusted angle plate to the handle of the scissors handle. (Fig. 1, 2)

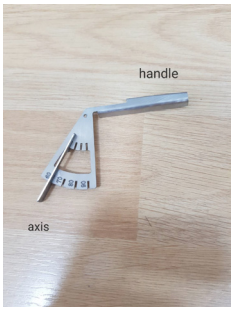


Fig.1. The adjusted angle plate

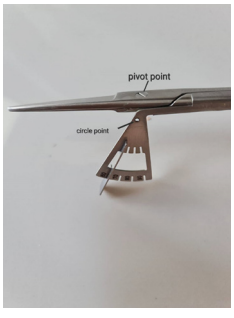


Fig. 2. Align the circle point on the plate with the pivot point of the perineal scissors

In perineal cutting, the axis of the angle-adjusting plate is the reference point with the end of the axis pointing towards the anus. The scissors will be at the angle that we have adjusted. (Fig. 3)

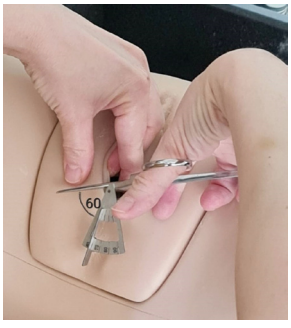


Fig. 3. The axis of the angle-adjusting plate is the reference point with the end of the axis pointing towards the anus.

Perineum was cut when crowning of the baby's head. The adjusted angle plate is located on the outer part of the scissors, which ensures that pregnant women's perineum is not harmed during use.

Only one researcher performed a perineum examination after delivery to detect OASI before suturing. Examination consists of inspection of perineum with labial parting, the distal posterior vagina and for a third-degree tear behind an intact perineum. Palpation is done with the examiner's index inserted in the anus, and the ipsilateral thumb in the vagina. The 2 fingers then palpate with a "pill-rolling" motion to assess thickness. OASI is defined as any type of injury to the anal sphincter muscle, including 3A, 3B, 3C, and 4th-degree perineal tears, based on the classification originally described by Sultan AH, et al[20].

First degree: laceration of the vaginal epithelium or perineal skin only

Second degree: involvement of the perineal muscles but not the anal sphincter

Third degree: disruption of the anal sphincter muscles, further subdivided into:

3a: < 50 percent thickness of external sphincter torn

3b: > 50 percent thickness of external sphincter torn

3c: internal sphincter torn also

Fourth degree: a third-degree tear with disruption of the anal epithelium

Following the repair, while the patient was in the lithotomy position with their leg flexed at the hip joints at an angle ranging from 90 to 100 degrees. Another nurse, who was not involved in the delivery of the baby, used a translucent plastic sheet to draw a line indicating the angle with the midline and the length of the incision. The researcher used a protractor to measure all variables.

Data was entered into a Microsoft Excel database and analyzed to compute basic statistics including mean, standard deviation, and 95% confidence interval.

Results

A total 153 uncomplicated, singleton, nulliparous pregnant women, 20 years old or older between 37-42 weeks of gestation were enrolled. The characteristics are summarized in Table 1.

All episiotomies were performed at 60 degrees. Mean episiotomy and perineal length were 3.13 and 3.59 cm. The mean post-suturing episiotomy angles were 44.92 ± 5.88 degrees (95% confidence interval 44-45.9). The change in angle decreased from before cutting by 15.07 degrees. No cases of OASI were detected (Table 2).

Table 1. Baseline characteristics of 153 women who underwent 60 degrees mediolateral episiotomy.

Characteristics	mean ± standard deviation
Mean maternal age (years)	24.19 ± 3.61
Mean gestational age (weeks)	38.56 ± 1.06
Median duration of the 2nd stage of labor (min)	19.80 ± 11.78
Mean birth weight (g)	2,990.82 ± 327.39

Table 2. Outcome of 153 women who underwent 60 degrees mediolateral episiotomy.

Outcome	mean ± standard deviation
Suture angle of episiotomy	44.92 ± 5.88
Change of angle	15.07 ± 5.88
Length of the episiotomy (cm)	3.13 ± 0.45
Distance from anal canal (cm)	3.59 ± 0.78

Discussion

In this study, the perineum was cut at a 60-degree angle with the aid of an angle adjustment plate. The results indicated that the mean angle after suturing was 44.9 degrees, which was similar to the findings of using the Episcissor-60[21-23]. As we know, the accuracy of the episiotomy angle is important for the prevention of OASI. Based on a study conducted by Eogan M, et al[13], it was found that the group without OASI had a suture angle of 38 degrees. Additionally, there was a minimum incidence of OASI at 0.5% when the suture angle was 43 degrees. Additionally, a study by Stedenfeldt M, et al[16] found that scarred episiotomy with an angle range of 30-60 degrees were significantly associated with a lower risk of OASI. The angle obtained from this study falls within the range that can prevent OASI.

To minimize measurement bias, another nurse, who was not involved in the delivery of the baby, measured the suture angle using a translucent plastic sheet. The researcher then measured the angle according to standard measurement practices using a protractor, after placing the translucent plastic sheet on a flat surface.

One limitation of this study was the absence of randomized controlled trials comparing it with standard episiotomy scissors. Although this study did not report any cases of OASI, it did not provide evidence of a decrease in the rate of OASI.

The reason why a comparison group with conventional perineal scissors was not included in this study was due to the ethics committee's concern for the safety of pregnant women and their potential risk for OASI exposure. A previous study by Andrews V, et al[23] revealed that only 22% of doctors performed mediolateral episiotomy at the recommended angle of 40 to 60 degrees, and none of the midwives did. The researchers also observed that delivery room nurses performed a suture angle of no more than 25 degrees.

In comparison to the Episioguide study[19], which was a randomized controlled trial, the lack of a control group in this study reduced its reliability. This study was performed in primiparous women, eliminating the effect of scar on repair suture angle, which was an advantage over the Episioguide study performed in singletons. When using the Episioguide, it is important to place it on the perineum. However, this may cause difficulties for individuals with a short perineal body or if the perineum is swollen, as the

Episioguide's shank is 4 cm long. Additionally, the instrument cannot adjust to the perineum's extension caused by the child's head pushing. The adjusted angle plate used in this study is easy to operate. All you need to do is adjust the handle to fit the size of the outer scissors handle used at each hospital. Then you can use it to cut like normal perineal scissors. Upon comparison of the two studies, it was discovered that the angle obtained after perineum suture repair using the adjusted angle plate was 44.9 degrees, which was better than the 34.6 degrees achieved using episioguide. In terms of pricing, the reusable Episioguide costs 400 baht, while the prototype of the adjusted angle plate is priced at 450 baht per piece. However, subsequent orders will be priced at 225 baht per piece. The plate can be sterilized using an autoclave and reused multiple times. In addition, the adjusted angle plate can be set to any angle between 60 and 90 degrees, making it more versatile and cost-effective.

According to our research, the utilization of an adjusted angle plate permitted post-suture angles to vary between 40 and 60 degrees. Women can prevent physical and emotional health issues and avoid OASI, which saves costs. In the UK, fixing an OASI can cost up to £48.75 million per year[20]. This instrument is appropriate for usage in hospitals that perform mediolateral episiotomy. It is appropriate for medical students and nurses, especially obstetricians who must perform operative vaginal deliveries because procedures carry a higher risk of OASI, but research has shown that there is a 50% relative decrease in the risk of experiencing a third-degree tear for every 6 degrees away from the midline[13]. The tool may also be used to practice doing an episiotomy in order to learn and become accustomed to the proper incision angle. After practicing with the tool and conducting further studies on the results, if they are good, it will be promoted for use in other hospitals. In the future, a video demonstrating the use of an adjusted angle plate for perineal cutting may be sent to multiple hospitals. If any hospital is interested in ordering an adjustable angle plate, they can contact us. It should be approached from a different angle of mediolateral episiotomy in future research and in a wider range of demographics, such as normal deliveries and operative vaginal deliveries, including evaluating patient pain and user satisfaction.

Conclusion

Based on the study, it was discovered that scissors with an adjusted angle plate can be highly effective. The study showed that the mean angle after suturing was 44.9 degrees for mediolateral episiotomy without OASI. It is also an affordable option.

Acknowledgments

The authors wish to thank Clinical Prof. Dr. Dittakarn Boriboonhirunsarn, Department of Obstetrics and Gynecology, Siriraj Hospital, Mahidol University, for supporting and sharing the discussion.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Sultan AH, Kamm MA, Hudson CN, Bartram CI. Third degree obstetric anal sphincter tears: risk factors and outcome of primary repair. BMJ 1994;304:887-91.
2. Walsh CJ, Mooney EF, Upton GJ, Motson RW. Incidence of third-degree perineal tears in labour and outcome after primary repair. Br J Surg 1996;83:218-21.
3. Andrews V, Sultan AH, Thakar R, Jones PW. Risk factors for obstetric anal sphincter injury: a prospective study. Birth 2006;33:117-22.
4. Klokk R, Bakken KS, Markestad T, Holten-Anderson MN. Modifiable and non-modifiable risk factors for obstetric anal sphincter injury in a Norwegian Region: a case-control study. BMC Pregnancy Childbirth 2022;22:277-83.
5. Fitzpatrick M, Behan M, O'Herlihy C. A randomized clinical trial comparing primary overlap with approximation repair of third-degree obstetric tears. Am J Obstet Gynecol 2000;183:1220-4.
6. de Leeuw JW, Vierhout ME, Struijk PC, Hop WCJ, Wallenberg HCS. Anal sphincter damage after vaginal delivery: functional outcome and risk factors for fecal

incontinence. Acta Obstet Gynecol Scand 2001;80: 830-4.
7. Williams A. Third-degree perineal tears: risk factors and outcome after primary repair. J Obstet Gynecol 2003;23:611-4.
8. Dee EF, Becky G, Pavna B, Lorri M, John OLD. Fecal and urinary incontinence after vaginal delivery with anal sphincter disruption in an obstetrics unit in the United States. Am J Obstet Gynecol 2003;189:1543-50.
9. Baruch Y, Gold R, Eisenberg H, Amir H, Reicher L, Yogev Y. High incidence of obstetric anal sphincter injuries among immigrant women of Asian ethnicity. J Clin Med 2023;12:1044-50.
10. Albar M, Aviram A, Anabusi S, Huang T, Tunde-Byass M, Mei-Dan E. Maternal ethnicity and the risk of obstetrical anal sphincter injury: a retrospective cohort study. Obstetrics 2021;43:469-73.
11. Rizk DE, Czechowski J, Ekelund L. Dynamic assessment of pelvic floor and bony pelvis morphologic condition with the use of magnetic resonance imaging in a multiethnic, nulliparous, and healthy female population. Am J Obstet Gynecol 2004;191:83–9.
12. Muizzuddin N, Hellemans L, Van Overloop L, Corstjens H, Declercq L, Maes D. Structural and functional differences in barrier properties of African American, Caucasian and East Asian skin. J Dermatol Sci 2010;59:123–8.
13. Eogan M, Daly L, O'Connell PR, O'Herlihy C. Does the angle of episiotomy affect the incidence of anal sphincter injury? BJOG 2006;113:190-4.
14. Vladimir K, Karbanova J, Horak M, Lobovsky L, Kralickova M, Rokyta Z. The incision angle of mediolateral episiotomy before delivery and after repair. IJGO 2008;103:5-8.
15. Wiruchpongsanon P, Maharungruengrat A. Incidence of obstetric anal sphincter injuries by 60 degrees for the incision angle of mediolateral episiotomy before delivery. Thai J Obstet Gynaecol 2013;21:95-100.
16. Stedenfeldt M, Pirhonen J, Blix E, Wilsgaard T, Vonen B, Oian P. Episiotomy characteristics and risks for obstetric anal sphincter injuries: a case-control study. BJOG 2012;119:724-30.
17. van Dillen J, Spaans M, van Keijsteren W, van Dillen M, Vredevoogd C, van Huizev M, et al. A prospective multicenter audit of labor-room episiotomy and anal sphincter injury assessment in the Netherlands. Int J Gynecol Obstet 2010;108:97-100.
18. Cole J, Lacey L, Bulchandani S. The use of Episcissors-60 to reduce the rate of obstetric anal sphincter injuries: a systematic review. Eur J Obstet Gynecol Reprod Biol 2019;237:23-7.
19. Thanapongpibul C, Suksamarnwong M. Effects of Episioguide - a 60º mediolateral episiotomy guide device – on post suture episiotomy angle: a randomized controlled trial. Thai J Obstet Gynaecol 2022;30:7-14.
20. Sultan AH. Obstertric perineal injury and anal incontinence. Clin Risk 1999;5:193-6.
19. Freeman RM, Hollands, Barron LF, Kapoor DS. Cutting a mediolateral episiotomy at the correct angle: evaluation of a new device, the Episcissors-60. Med Devices Evid Res 2014;7:23-8.
21. Patel RP, Ubale SM. Evaluation of the angled Episcissors-60 episiotomy scissors in spontaneous vaginal deliveries. Med Devices Evid Res 2014;7:253-6.
22. Cole J, Lacey L, Bulchandani S. The use of Episcissors-60 to reduce the rate of obstetric anal sphincter injuries: a systematic review. Eur J Obstet Gynecol Reprod Biol 2019;237:23-7.
23. Andrews V, Thakar R, Sultan AH, Jones PW. Are mediolateral episiotomies actually mediolateral? BJOG 2005;112:220-4.

Thai Journal of Obstetrics and Gynaecology
January 2024, Vol. 32, No. 1, pp. 76-84.

GYNAECOLOGY

mRNA Expression Profiling in Hydatidiform Mole: Comparison between Pre-Gestational Trophoblastic Neoplasia Moles and Remission Moles

Wilasinee Areeruk, M.D.*,
Patou Tantbirojn, M.D.*,
Shanop Shuangshoti, M.D.**,***,
Chinachote Teerapakpinyo, Ph.D.***,
Ruangsak Lertkhachonsuk, M.D.*

** Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
*** Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
**** Chula GenePRO Center, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

ABSTRACT

Objectives: To explore potential mRNAs that can predict malignant transformation and identify mRNAs expression profile in complete hydatidiform mole.

Materials and Methods: A case-control study was conducted between complete hydatidiform moles that turned out to be postmolar gestational trophoblastic neoplasia (postmolar GTN) and complete hydatidiform moles that regressed spontaneously after evacuation (remission mole). We quantitatively assessed the expression of 770 human cancer genes from formalin-fixed paraffin embedded (FFPE) specimens or fresh frozen tissues using Nanostring nCounter. The differentially expressed genes between postmolar GTN group and remission mole group were analyzed.

Results: There were 12 cases recruited in this study: 6 remission moles and 6 postmolar GTN. Seven hundred and seventy genes were analyzed showing 29 genes that were significantly different in GTN moles compared to the remission moles. Nine of these genes (*JUN, COL4A6, SOCS3, PLA2G10, NFKBIZ, FGFR3, CACNA1D, FGF7 and PLAU*) were significantly different for more than 2 folds. The JUN had the highest different ratio and the lowest p value when compared between 2 groups (3.26 folds, p = 0.003). After reviewing their functions, JUN plays a role in several cancer initiations. These genes are promising biomarkers for prediction of postmolar GTN.

Conclusion: The analysis of mRNA profiles can distinguish between complete hydatidiform moles that will remission from those that will turn out to postmolar GTN. We identified 29 genes that were differentially expressed between the two groups. The results lead to further investigations on candidate genes and could probably explain the mechanism of malignant transformation to postmolar GTN.

Keywords: gestational trophoblastic neoplasia, postmolar GTN, mRNA, nanostring.

Correspondence to: *Ruangsak Lertkhachonsuk, M.D., Placental Related Diseases Research Unit, and Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. E-mail: ruangsak@chula.md*

Received: 31 June 2023, **Revised:** 12 July 2023, **Accepted:** 27 July 2023

เปรียบเทียบการแสดงออกของ mRNA ในครรภ์ไข่ปลาอุกชนิดที่กลายเป็นมะเร็งครรภ์ไข่ปลาอุกและชนิดที่หายขาด

วิลาสินี อารีรักษ์, พฐู ตัณฑ์ไพโรจน์, ชนพ ช่วงโชติ, ชินโชติ ธีรภัคภิญโญ, เรืองศักดิ์ เลิศชจรสุข

บทคัดย่อ

วัตถุประสงค์*: เพื่อศึกษาหา mRNA ที่มีแนวโน้มจะใช้พยากรณ์การกลายเป็นมะเร็งครรภ์ไข่ปลาอุก และศึกษาการแสดงออกของ mRNA ในครรภ์ไข่ปลาอุก*

วัสดุและวิธีการ*: การศึกษาแบบมีกลุ่มควบคุมเปรียบเทียบระหว่างครรภ์ไข่ปลาอุกชนิดที่กลายเป็นมะเร็งครรภ์ไข่ปลาอุกและชนิดที่หายขาด ผู้เข้าร่วมการศึกษาจะได้รับการตรวจวัดปริมาณการแสดงออกของ mRNA 770 ชนิด ที่พบในมนุษย์ โดยตรวจจากชิ้นเนื้อจากพาราฟินบล็อก หรือชิ้นเนื้อแช่แข็ง ด้วยวิธี Nanostring nCounter จากนั้นทำการแปลผลของ mRNA ที่มีความแตกต่างกันระหว่างทั้งสองกลุ่ม*

ผลการศึกษา*: ผู้เข้าร่วมการศึกษาทั้งหมด 12 ราย แบ่งเป็นครรภ์ไข่ปลาอุกชนิดที่หายขาด 6 ราย และชนิดที่กลายเป็นมะเร็ง 6 ราย จาก mRNA 770 ชนิดที่ตรวจวัดปริมาณ พบว่ามี 29 ชนิดที่มีระดับการแสดงออกแตกต่างกันอย่างมีนัยสำคัญทางสถิติ โดย 9 ชนิดมีระดับการแสดงออกแตกต่างกันเกิน 2 เท่า ได้แก่ JUN, COL4A6, SOCS3, PLA2G10, NFKBIZ, FGFR3, CACNA1D, FGF7 และ PLAU ยีนที่มีการแสดงออกแตกต่างระหว่างสองกลุ่มมากที่สุด ได้แก่ JUN ซึ่งมีการแสดงออกในกลุ่มที่หายขาดเป็น 3.26 เท่าของกลุ่มที่กลายเป็นมะเร็ง (p = 0.003) โดยพบว่า JUN มีบทบาทสำคัญในการเกิดของมะเร็งหลายชนิด mRNA เหล่านี้จึงมีความน่าสนใจในแง่ของแนวโน้มที่จะใช้พยากรณ์การกลายเป็นมะเร็งครรภ์ไข่ปลาอุก*

สรุป*: การแสดงออกของ mRNA มีความแตกต่างกันระหว่างครรภ์ไข่ปลาอุกชนิดที่กลายเป็นมะเร็งครรภ์ไข่ปลาอุกและชนิดที่หายขาด จากการศึกษานี้พบว่ามี 29 ชนิดที่มีระดับการแสดงออกแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ผลจากการศึกษานี้จะสามารถนำไปสู่การศึกษาเพิ่มเติมเฉพาะใน mRNA บางชนิด ซึ่งอาจช่วยอธิบายการกลายเป็นมะเร็งของครรภ์ไข่ปลาอุก*

คำสำคัญ*: ครรภ์ไข่ปลาอุก, มะเร็งครรภ์ไข่ปลาอุก, mRNA, nanostring*

Introduction

Gestational trophoblastic disease (GTD) is a pregnancy-related disorder originating from abnormal proliferation of the placental trophoblasts. It can be classified as a benign disorder or hydatidiform mole and a malignant disorder or gestational trophoblastic neoplasia (GTN), of which the latter includes the invasive mole, choriocarcinoma, placental-site trophoblastic tumor and epithelioid trophoblastic tumor[1, 2]. Generally, hydatidiform moles would regress after evacuation, but about 15% of complete hydatidiform moles (CHMs) and 0.5-1% of partial hydatidiform moles (PHMs) could progress into postmolar GTN[2]. The risk of developing postmolar GTN varies among different regions in the world. In Thailand, there is a higher rate of developing postmolar GTN which was approximately 26%[3].

In order to detect postmolar GTN, several human chorionic gonadotropin (hCG) regression models have been used[4]. However, identifying patients who would have postmolar GTN at the time of evacuation would be of greater value because intensive follow-up monitoring can be planned, and in some cases, prophylactic chemotherapy can be given to prevent malignant transformation[5].

Several molecular markers have been studied in GTD. Tumor protein p53 (p53), p21, retinoblastoma gene (Rb) and mouse double minute 2 homolog (mdm^2) showed stronger expression in complete hydatidiform mole and choriocarcinoma than in partial mole[6]. There are few studies of panel genes expression in GTD. Kato et al[7] used microarray analysis to investigate the expression profiles of 589 genes committed to cell growth control in order to characterize the regulatory circuitry for cell proliferation in complete moles. A total of 57 genes were significantly upregulated in complete moles. These involved the Ras-mitogen activated protein kinase III (Ras-MAPKIII), janus kinase-signal transducer and activator of transcription 5 (JAK-STAT5) and Wnt signal pathways, implicating growth factor or cytokine-mediated signal pathways in the trophoblastic hyperplasia of complete moles. Several genes associated with anti-apoptosis, cell structuring and/or cell attachment were also upregulated in complete moles. In contrast, relatively fewer genes were downregulated and these involved insulin growth factor binding proteins (IGFBPs), versican, interleukin-1, tumor necrosis factor receptor, CD44 and Rad 52.

There is limited data on gene profiling in GTD, especially comparison between non cancer hydatidiform moles (remission moles) and moles that turn to be GTN (GTN moles). Therefore, we assessed the messenger ribonucleic acids (mRNAs) that were differentially expressed between CHMs that turned out to be GTN (GTN moles) and CHMs that regressed spontaneously after evacuation (remission moles), and also studied the profiles of mRNA expression in CHMs.

Materials and Methods

This study was a case-control study conducted at the Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Ethical approval was obtained from the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University. The medical records of all patients with pathologically confirmed CHMs diagnosed between January 2007 and December 2016 were reviewed. Cases of CHMs with formalin-fixed paraffin embedded (FFPE) or fresh frozen specimens kept at the division of Gynecologic Pathology at King Chulalongkorn Memorial Hospital who completed clinical follow-up periods of at least one year were included in the study. After the GTN moles were selected, the remission moles were matched to the GTN moles using the same year of specimen retrieval with the nearest gestational age at diagnosis. All specimens were pathologically reassessed by two pathologists. Cases with low quality specimens or inadequate tumor tissues for

RNA extraction were excluded from the study.

RNA extraction

Total RNA was extracted from formalin fixed, paraffin embedded (FFPE) or fresh frozen tissue using RNeasy FFPE kit according to the manufacturer's instruction (Qiagen, Germantown, MD) and performed at Chula GenePRO Center, Faculty of Medicine, Chulalongkorn University. The lesions of interest (villous tissues) were reviewed and selected by the pathologist. Three to five ribbons of 10-µm FFPE tissue section were used for total RNA extraction. Then, RNA concentration and quality were determined by using nanoDropTM 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The RNA was stored at -80^{O}C until mRNA expressions were analyzed.

mRNA expression analysis

Analysis of mRNA expression was performed using nCounter Analysis System and PanCancer Human Pathways Assay Kit according to the manufacturer's instruction (nanoString®, Seattle, WA). One hundred nanograms of each total RNA sample were mixed with the probes and incubated at 65^{O}C for 16 hours. Then probe-hybridized samples were processed on the nanoString prep station. The processed cartridges were then transferred to the nanoString® digital analyzer and scanned on HIGH mode for 280 fields of view per sample. The nSloverTM Analysis software 3.0 (nanoString Technologies, Seattle, WA) was used to perform background subtraction, spike-in-control normalization and reference genes normalization.

Twelve FFPE specimens of CHMs (6 remission moles and 6 GTN moles) were done to discover the differentially expressed mRNA candidates between remission moles and GTN moles. Sample size calculation was based upon the mean difference and standard deviation of the count of mRNA candidate discovered from this study. Statistical analysis was performed using SPSS version 22 (SPSS Inc, Chicago, IL, USA). Baseline clinical data were presented as mean, median and percentage. Level of expression of each mRNA was presented as median count. The differentially expressed mRNAs between GTN moles and remission moles were analyzed using the Mann-Whitney U test. A p value of < 0.05 was considered as statistically significant.

Results

In this study, 6 remission moles and 6 GTN moles were analyzed. Baseline clinical data are shown in Table 1. The age and gestational age at diagnosis of molar pregnancy in both groups were similar. Pretreatment βhCG was higher in the GTN moles group [525,020 mIU/ml (238,759.75, 1,000,000) vs 179,973.5 mIU/ml (150,330.25, 217,952.75)]. The patients in both groups were treated mostly with suction curettage (66.7%).

Among 770 human mRNAs evaluated, 29 mRNAs were found to be differentially expressed between the two groups ($p < 0.05$). Table 2 shows the median count of each of the differentially expressed 29 mRNAs found in this study.

The largest differences (more than 2 folds downregulations) were seen in 9 mRNAs: JUN, COL4A6, SOCS3, PLA2G10, NFKBIZ, FGFR3, CACNA1D, FGF7 and PLAU. Clustering analysis of these 9 mRNAs demonstrated a tree with obvious distinction between remission moles and GTN moles. (Fig. 1.).

Among these 9 mRNAs, JUN demonstrated the largest difference with a median count of 35.62 in GTN moles vs 115.96 in remission moles ($p = 0.003$). COL4A6 had 3.18 folds different in median count between GTN moles and remission moles ($p = 0.041$), and SOCS3 had 3.09 folds different in median count between GTN moles and remission moles ($p = 0.012$).

This study used 10 samples of FFPE specimen and 2 samples of fresh specimen. The amount of mRNAs count was not different between these 2 sources of specimens.

Table 1. Demographic data.

	GTN (n = 6)	Remission (n = 6)
Age (years)	27 (19, 47.5)	31.5 (24.75, 44.75)
GA (weeks)	10 (7.5, 14.5)	12.5 (10.5, 16)
Pretreatment βhCG (mIU/ml)	525,020 (238,759.75, 1,000,000)	179,973.5 (150,330.25, 217,952.75)
Treatment		
• Suction curettage	4 (66.7%)	4 (66.7%)
• Hysterectomy	2 (33.3%)	2 (33.3%)
Duration to normal βhCG (days)		70.5 (51.25, 87.0)
Duration to GTN (days)	49 (19.25, 55.5)	
hCG at Dx GTN (mIU/ml)	7469 (1380, 118553.5)	
Stage		
Stage I	3 (50%)	
Stage II	0 (0%)	
Stage III	2 (33.3%)	
Stage IV	0 (0%)	
Missing	1 (16.7%)	
Risk score		
Low risk	5 (83.3%)	
High risk	0 (0%)	
Missing	1 (16.7%)	
Chemotherapy regimen		
Methotrexate	3 (50%)	
Actinomycin D	1 (16.7%)	
None (Surgical treatment – hysterectomy)	1 (16.7%)	
Missing	1 (16.7%)	

Data are presented as median (interquartile range) and number (percentage)

GTN: gestational trophoblastic neoplasia, GA: gestational age, hCG: human chorionic gonadotropin, Dx: diagnosis

Table 2. Significantly different expressed mRNAs between gestational trophoblastic neoplasia moles group and the remission moles group ($p < 0.05$).

mRNA	GTN (n = 6)	Remission (n = 6)	Fold change	p value
JUN	35.62	115.96	-3.26	0.003
COL4A6	5.71	18.17	-3.18	0.041
SOCS3	89.13	275.76	-3.09	0.012
PLA2G10	18.72	6.22	3.01	0.033
NFKBIZ	52.54	131.7	-2.51	0.009
FGFR3	100.73	43.91	2.29	0.020
CACNA1D	3.18	7.3	-2.29	0.025
FGF7	5.57	12.63	-2.27	0.033
PLAU	32.09	71.8	-2.24	0.023
CCNB1	126.75	64.34	1.97	0.046
MYC	37.25	72.69	-1.95	0.028
SUV39H2	61.71	31.84	1.94	0.006
CACNA1G	8.99	16.97	-1.89	0.021
SFRP4	3.85	7.24	-1.88	0.034
PRKACB	4.83	8.85	-1.83	0.039
WNT3	9.07	16.64	-1.83	0.044
LEPR	18.47	31.76	-1.72	0.028
LAMC3	20.06	33.35	-1.66	0.009
BMP4	17.38	28.73	-1.65	0.031
NFKBIA	203.76	321.85	-1.58	0.049
NFE2L2	40.96	64	-1.56	0.019
DUSP6	37.79	58.35	-1.54	0.042
GNG12	260.05	171.16	1.52	0.047
SOCS2	49.86	75.05	-1.51	0.045
ABL1	43.86	63.94	-1.46	0.015
CCND3	57.82	83.63	-1.45	0.048
SOS1	53.66	75.69	-1.41	0.023
CREB3L1	92.74	127.79	-1.38	0.047
ARID1B	74.03	98.66	-1.33	0.042

Data are presented as median

JUN: Jun proto-oncogene, COL4A6: collagen type IV alpha-6, SOCS3: suppressor of cytokine signaling 3, PLA2G10: phospholipase A2 group X, NFKBIZ: nuclear factor kappa-B inhibitor zeta, FGFR3: fibroblast growth factor receptor 3, CACNA1D: calcium voltage-gated channel subunit alpha-1D, FGF7: fibroblast growth factor 7, PLAU: plasminogen activator urokinase, CCNB1: cyclin B1, MYC: MYC proto-oncogene, SUV39H2: suppressor of variegation 3-9 drosophila homolog of 2, CACNA1G: calcium voltage-gated channel subunit alpha-1G, SFRP4: secreted frizzled-related protein 4, PRKACB: protein kinase cAMP-activated catalytic subunit beta, WNT3: wingless-type MMTV integration site family member 3, LEPR: leptin receptor, LAMC3: laminin gamma-3, BMP4: bone morphogenetic protein 4, NFKBIA: nuclear factor kappa-B inhibitor alpha, NFE2L2: nuclear factor erythroid 2-like 2, DUSP6: dual-specificity phosphatase 6, GNG12: guanine nucleotide-binding protein gamma 12, SOCS2: suppressor of cytokine signaling 2, ABL1: ABL proto-oncogene 1, CCND3: cyclin D3, SOS1: SOS Ras/Rac guanine nucleotide exchange factor 1, CREB3L1: cAMP responsive element binding protein 3 like 1, ARID1B: AT-rich interaction domain 1B

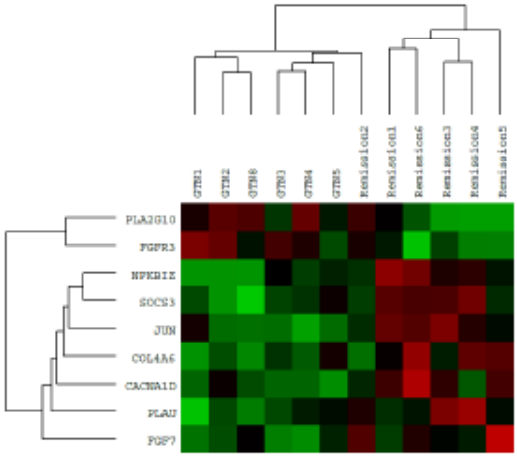


Fig. 1. Heatmap basic analysis showing the levels of mRNAs in gestational trophoblastic neoplasia moles group and the remission moles group.

Discussion

The molecular basis in GTD is not clearly understood. Evaluation of the molecular pathway driving the malignant risk in CHM is challenging. In the current study, we aimed to evaluate mRNA as a new biomarker for the prediction of postmolar GTN.

This study clearly demonstrated the differences in the profile of mRNA expression between GTN moles and remission moles. Our preliminary analysis showed that 29 mRNAs were significantly different expressed in GTN moles compared with remission moles, of which, 9 mRNAs (*JUN, COL4A6, SOCS3, PLA2G10, NFKBIZ, FGFR3, CACNA1D, FGF7* and *PLAU*) demonstrated > 2 folds differences. Therefore, we reviewed literature on the functions of these 9 mRNAs and their associations with malignancy.

JUN (c-Jun, AP-1) is a proto-oncogene. The Jun-family of proteins are critical transcription factors that act as co-activators of the androgen receptor (AR) or form activator protein 1 with fos proto-oncogene (Fos) to regulate the transcription of androgen-regulating genes. Activated c-Jun in the stromal cells plays key roles in stromal-epithelial interactions[8]. Thakur et al identified a novel binding site for activated c-Jun in the promoter of the Snail1 gene, which triggered transforming growth factor-beta (TGFβ)-induced invasion of human prostate cancer cells[9].

Next, *COL4A6* is a member of the type IV collagen family, which is a major component of the basement membrane. The basement membrane is important for confinement of the tumor microenvironment[10]. It was shown that the expression of *COL4A6* is downregulated in basal cell carcinoma, breast cancer, and colorectal carcinoma[11, 12]. Moreover, Dehan et al reported that there is a nearly complete loss of *COL4A6* protein in prostate cancer samples, but not in benign prostate hyperplasia samples using immunohistochemistry techniques[13].

SOCS3 has been studied with HER2 and STAT3 in ovarian cancer tissues. The results suggested that the increased expression of HER2 and STAT3 could promote the formation of tumor cells, and SOCS3 might inhibit tumor differentiation[14]. The outcome correlated with finding from this study which *SOCS3* had 3.09-fold higher in remission mole compared to GTN mole.

The strength of our study was the use of Nanostring nCounter technology to analyze mRNA expression. In order to detect mRNAs in the FFPE specimens, this technology has its advantages over other methods. We can digitally count mRNAs in FFPE specimens without the need for RNA amplification.

Over 770 mRNAs can be assessed in a single reaction that is suitable for cases of GTN which no known mRNA candidate was made before this study. Also, this technology has been proven to accurately assess the expression of mRNAs in both fresh frozen tissues and FFPE specimens[15-17].

Limitations of our study were a small sample size and clinical heterogeneity of the cases in both groups. However, due to the rarity of the diseases, limited studies have been performed in this field. Therefore, we primarily aimed to do this study as preliminary research to gather information on the profiles of mRNA in the GTN moles group and the remission moles group which can provide data for further replicative studies in this field.

Conclusion

In conclusion, the analysis of mRNA pancancer gene profiles can distinguish between complete hydatidiform mole that will remission from those that will turn to postmolar GTN. We identified 29 genes that were differentially expressed between the two groups. The result from this study leads to further investigations on candidate genes and could probably explain the mechanism of malignant transformation to postmolar GTN.

Acknowledgements

This research was supported by Ratchadapiseksompotch Fund, Faculty of Medicine, Chulalongkorn University (grant number: RA60/033) and Placental Related Diseases Research Unit. We acknowledge the medical staff and scientists at Chula GenePRO Center, Faculty of Medicine, Chulalongkorn University, for their assistance in extracting RNA and assessing the mRNA expressions.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Froeling FE, Seckl MJ. Gestational trophoblastic tumours: an update for 2014. Curr Oncol Rep 2014;16:408.
2. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. Lancet 2010;376:717-29.
3. Wairachpanich V, Limpongsanurak S, Lertkhachonsuk R. Epidemiology of Hydatidiform moles in a tertiary hospital in Thailand over two decades: Impact of the national health policy. Asian Pac J Cancer Prev 2015;16:8321-5.
4. Zhao P, Wang S, Zhang X, Lu W. A Novel prediction model for postmolar gestational trophoblastic neoplasia and comparison with existing models. Int J Gynecol Cancer 2017;27:1028-34.
5. Wang Q, Fu J, Hu L, Fang F, Xie L, Chen H, et al. Prophylactic chemotherapy for hydatidiform mole to prevent gestational trophoblastic neoplasia. Cochrane Database Syst Rev 2017;9:CD007289.
6. Fulop V, Mok SC, Genest DR, Gati I, Doszpod J, Berkowitz RS. p53, p21, Rb and mdm2 oncoproteins expression in normal placenta, partial and complete mole, and choriocarcinoma. J Reprod Med 1998;43:119-27.
7. Kato HD, Terao Y, Ogawa M, Matsuda T, Arima T, Kato K, et al. Growth-associated gene expression profiles by microarray analysis of trophoblast of molar pregnancies and normal villi. Int J Gynecol Pathol 2002;21:255-60.
8. Niu YN, Wang K, Jin S, Fan DD, Wang MS, Xing NZ, et al. The intriguing role of fibroblasts and c-Jun in the chemopreventive and therapeutic effect of finasteride on xenograft models of prostate cancer. Asian J Androl 2016;18:913-9.
9. Thakur N, Gudey SK, Marcusson A, Fu JY, Bergh A, Heldin CH, et al. TGFbeta-induced invasion of prostate cancer cells is promoted by c-Jun-dependent transcriptional activation of Snail1. Cell Cycle 2014;13:2400-14.
10. Tanjore H, Kalluri R. The role of type IV collagen and basement membranes in cancer progression and metastasis. Am J Pathol 2006;168:715-7.
11. Nakano S, Iyama K, Ogawa M, Yoshioka H, Sado Y, Oohashi T, et al. Differential tissular expression and localization of type IV collagen alpha1(IV), alpha2(IV), alpha5(IV), and alpha6(IV) chains and their mRNA in normal breast and in benign and malignant breast tumors. Lab Invest 1999;79:281-92.
12. Ikeda K, Iyama K, Ishikawa N, Egami H, Nakao M, Sado Y, et al. Loss of expression of type IV collagen alpha5 and alpha6 chains in colorectal cancer associated with the hypermethylation of their promoter

region. Am J Pathol 2006;168:856-65.
13. Dehan P, Waltregny D, Beschin A, Noel A, Castronovo V, Tryggvason K, et al. Loss of type IV collagen alpha 5 and alpha 6 chains in human invasive prostate carcinomas. Am J Pathol 1997;151:1097-104.
14. Shang AQ, Wu J, Bi F, Zhang YJ, Xu LR, Li LL, et al. Relationship between HER2 and JAK/STAT-SOCS3 signaling pathway and clinicopathological features and prognosis of ovarian cancer. Cancer Biol Ther 2017;18:314-22.
15. Chen X, Deane NG, Lewis KB, Li J, Zhu J, Washington MK, et al. Comparison of Nanostring nCounter(R) data on FFPE Colon cancer samples and affymetrix microarray data on matched frozen tissues. PLoS One 2016;11:e0153784.
16. Tam S, de Borja R, Tsao MS, McPherson JD. Robust global microRNA expression profiling using next-generation sequencing technologies. Lab Invest 2014;94:350-8.
17. Ragulan C, Eason K, Fontana E, Nyamundanda G, Tarazona N, Patil Y, et al. Analytical Validation of multiplex biomarker assay to stratify colorectal cancer into molecular subtypes. Sci Rep 2019;9:7665.

Thai Journal of Obstetrics and Gynaecology
January 2024, Vol. 32, No. 1, pp. 85-90.

CASE REPORT

Ovarian Tumour Presenting as an Inguinal Hernia in a Postmenopausal Woman with Mullerian Agenesis: A case report

Aruna Rangasamy, MRCOG*,
Maherah Kamarudin, MObGyn*,**,
Lee Saw Joo, MObGyn GO*,
Aruku Naidu Apana, FRCOG CU.*

** Department of Obstetrics & Gynaecology, Hospital Raja Permaisuri Bainun, Jalan Raja Ashman Shah, Perak, Malaysia*
*** Department of Obstetrics & Gynaecology, Faculty of Medicine, University Malaya, Jalan Profesor Ungku Aziz, Kuala Lumpur, Malaysia*

ABSTRACT

Although inguinal hernias are common with inguinal hernia repairs being a common surgical procedure, ovarian inguinal hernias are rare. A 67-year-old postmenopausal woman presented with a 10-year history of right inguinal swelling on a background of primary amenorrhoea. Clinical examination revealed a 10 cm right irreducible hernia. Ultrasound and computed tomographic imaging confirmed an ovarian mass in the inguinal hernia with absent uterus and right kidney in the pelvis. Mullerian agenesis with ovarian hernia was diagnosed, however malignancy was considered due to tumour size and raised cancer antigen 125 (CA125). She underwent diagnostic laparoscopy, bilateral salpingo-oopherectomy, partial omentectomy, and inguinal hernia repair. Histopathological reports confirmed ovarian fibroma. Ovarian hernia is rare in postmenopausal women but must be considered in those presenting with inguinal masses. It can occur together with Mullerian abnormalities. CA125 can be elevated in various benign conditions, making interpretation and diagnosis difficult. Multidisciplinary approach is vital to ensure the best outcome for the patient.

Keywords: inguinal hernia, ovarian tumour, mullerian agenesis, MRKH (Mayer-Rokitansky-Kuster-Hauser) syndrome.

Correspondence to: *Aruna Rangasamy, MRCOG, Department of Obstetrics & Gynaecology, Hospital Raja Permaisuri Bainun, Jalan Raja Ashman Shah, 30450 Ipoh, Perak, Malaysia. E-mail: arunagill82@gmail.com*

Received: 13 May 2023, **Revised:** 24 August 2023, **Accepted:** 7 September 2023

Introduction

Inguinal hernia is defined as protrusion of mainly small bowels or omentum through the internal and external rings of the inguinal canal[1]. It is reported that 60% of the inguinal hernias occur on the right side, 30% on the left, and 10% bilaterally[2]. It is more common in the males compared to the females, with a ratio of 10:1. Rarely, 2.9% of inguinal hernias may contain ovaries and fallopian tubes[3]. This is mostly found in the paediatric age group and is commonly in tandem with other genital tract abnormalities[4]. Such presentation is rare in women of reproductive age group and more so in elderly women. Herein we present a case of an elderly woman with underlying Mullerian agenesis who presented with right inguinal swelling which turns out to be an incarcerated inguinal hernia with an ovarian fibroma.

Case Report

A 67-year-old nulliparous lady with underlying decompensated congestive cardiac failure, diabetes, hypertension, and thrombocytopenia, presented with a 10-year history of right inguinal swelling which was gradually increasing in size and subsequently became irreducible. She had no obvious symptoms except for occasional pain in the right inguinal region, which was worse on movement and coughing. She felt her movement had been restricted, affecting her daily activities. She also had loss of appetite and had lost 13 kg in 2 months. She denied any changes of bowel habits. Further history revealed that she never had her menses. She married late at the age of 54. However, the marriage was not consummated in the 2 years when she then lost her husband. There was no significant past surgical history.

On examination, vital signs were normal. Abdomen was soft and non-tender with a 3x3 cm umbilical hernia. There was a hard, well defined mobile mass with smooth borders measuring 9x10 cm at the right inguinal region and labia majora which was irreducible (Fig. 1). Hymen was intact with a short blind ended vagina.

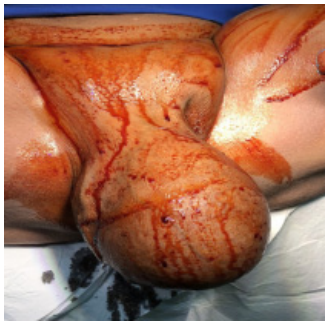


Fig. 1. Right inguinal mass

Full blood count was normal except for platelet count of 70 x 109/L. Liver function test was deranged with a total bilirubin of 30.5 µmol/L, alkaline phosphatase 235 U/L, and aspartate transferase (AST) 82 U/L. Tumour markers were taken which revealed an increased CA125 of 981 U/ml. Renal function, carcinoembryonic antigen (CEA), cancer antigen 19-9 (CA19-9), and alpha fetoprotein (AFP) levels were all normal. Ultrasound of the abdomen and pelvis was done, which revealed an absent uterus. Ovaries were not visualized clearly. The right renal fossa was empty, with the possibility of an ectopic kidney. Ultrasound of the inguinal area showed a large right inguinal mass with soft tissue and calcified component possibly a teratoma, however unable to rule out malignancy. Computed tomography scan of the abdomen and pelvis confirmed the presence of a right inguinal hernia, with a well-defined heterogenous enhancing mass

measuring 11 x 11 x 13 cm within the hernia sac (Fig. 2a and 2b). The blood supply of the mass was arising from the abdominal aorta. These features, along with the raised CA 125, suggested a likely ovarian tumour. Subcentimetre para-aortic and paracaval nodes were present, but there was no ascites. The left ovary was normal, but normal uterus was not seen. The left kidney was normally located, whereas the right kidney was seen in the right pelvic region. There was a small right paraumbilical hernia containing omental fat. There was also liver cirrhosis with splenomegaly and gastro-esophageal varices. A diagnosis of suspected Mullerian agenesis with paraumbilical and ovarian hernia unable to exclude malignant transformation was made. Referral was made to the gastroenterology team for further management of liver cirrhosis.

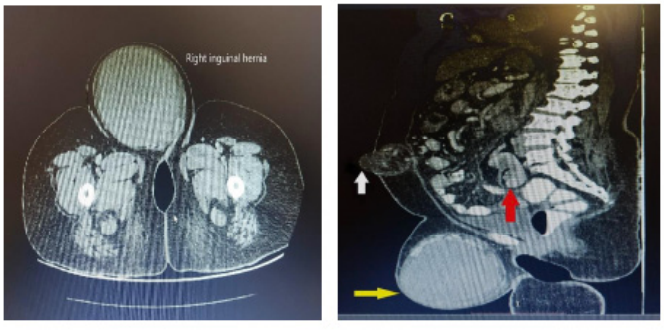


Fig. 2a (left): Axial view of computerized tomography (CT) scan showing right inguinal hernia. **Fig. 2b** (right): Sagittal view of CT scan showing right inguinal hernia containing ovarian tumour (yellow arrow) and umbilical hernia (white arrow); right ectopic kidney noted (red arrow)

The patient underwent diagnostic laparoscopy, bilateral salpingo-oophorectomy, partial omentectomy, and inguinal hernia repair. The surgery was performed together with the surgical team. Intraoperatively, diagnostic laparoscopy revealed absent right rudimentary uterine bud. Right ovary, infundibulopelvic ligament and vessels, as well as omentum were found herniated through the right inguinal canal. The left uterine bud was present with normal left fallopian tube and ovary. There was no ascites and no suspicious tumour nodules seen. Left salpingo-oopherectomy was done laparoscopically. From below, the right inguinal mass was excised (Fig. 3a). The skin defect was refashioned. The skin was closed interruptedly with a drain (Fig. 3b). The patient recovered uneventfully. The histology was consistent with right ovarian fibroma, a benign ovarian tumour (Fig. 4).

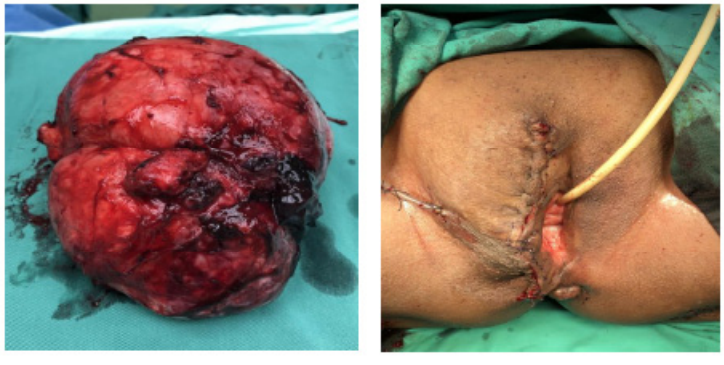


Fig. 3a (left): Right ovarian tumour. **Fig 3b** (right): Skin refashioned and closed interrupted with drain in situ.

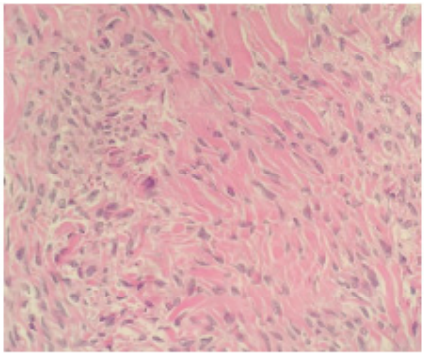


Fig. 4. Spindle shaped cells arranged in fascicles and haphazard pattern. No cellular atypia, necrosis or mitosis

Discussion

The case presented is unique due to the multiple factors which made diagnosis challenging. Firstly, our patient was unfortunate enough to have double pathology, which is the Mullerian agenesis and an ovarian tumour manifesting as an inguinal hernia. The common link between Mullerian abnormalities and ovarian hernias is the embryogenic structure called gubernaculum. Paired gubernaculum attaches to the caudal part of the gonads and helps to guide the descent of the gonads to their proper place in the developing fetus. In females, the upper part of the gubernaculum, together with the ovarian artery and vein, becomes the suspensory ligament of the ovary which helps to suspend the ovary to the pelvic side wall. The lower part of the gubernaculum gives rise to the round ligament and the ovarian ligament. The round ligament then courses through the inguinal canal and attaches to the labia majora (Fig. 5). Gubernaculum dysfunction seems to be related to pathologies arising from the round ligament and inguinal hernia, as well as to Mullerian duct abnormalities[5].

Furthermore, it is suspected that there is underlying weakness of the ligaments that hold the ovaries in place to result in herniation[6]. Weakening and lengthening of the supportive ligaments are exacerbated in multiparous women and those with conditions that result in frequently increased intra-abdominal pressures, such as chronic cough[6]. There is also hypothesis that failure of fusion of the Mullerian ducts lead to excessive mobility of the ovaries and the uterine cornua, making it more likely for these structures to herniate through the inguinal canal[7], as in the case of our patient.

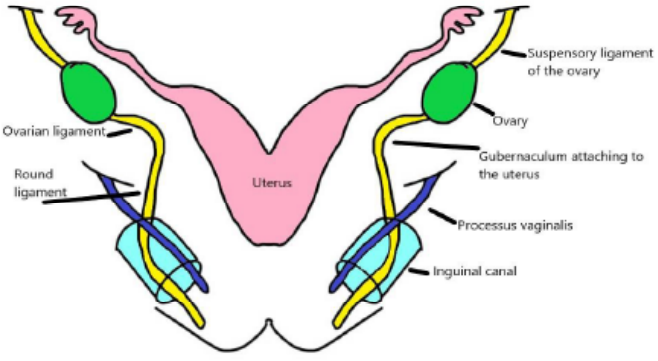


Fig. 5. Gubernaculum and its derivatives

Ovarian fibromas are solid tumours belonging to sex cord stromal cell tumours of the ovary, comprising of spindle shaped fibroblastic cells and abundant collagen[8]. They are the most common benign solid tumours of the ovary, occurring mainly in women in the 5th decade of life. In a small number of cases, ovarian fibromas can also result in raised CA 125 levels[8].

It is well known that raised CA 125 is not diagnostic of ovarian malignancy. It can be raised in many benign gynaecological and non-gynaecological conditions as well. Cirrhosis of the liver is one of the most common disorders associated with increased levels of CA125, occurring in 85% of cases[9]. This could explain the reason behind the raised CA 125 as well as the thrombocytopenia. Many patients with cirrhosis also experience fatigue, anorexia and weight loss[10], mimicking constitutional symptoms of malignancy, as demonstrated with our patient. This highlights the complexity of interpretating CA 125 in the presence of liver disease.

There have been other reported unusual contents discovered in inguinal hernias. Shetty et al[11] report a case of a 66-year-old who presented with a 3-year history of a slowly enlarging right inguinal mass. Ultrasound and computed tomography scan were done prior to surgery. Intraoperatively, the right inguinal hernia was found to contain omentum, caecum, and an ovarian cyst, which turned out to be a mature cystic teratoma. Other atypical inguinal hernia contents include uterus[12] and even endometriosis[13,14], although the latter is mainly found in the premenopausal age group.

One must always consider the possibility of malignancy in such cases too. Although extremely rare, there have been reported cases of ovarian malignancy presenting as inguinal ovarian hernia. Burke et al[15] describe an 89-year-old lady presenting with bilateral inguinal hernias diagnosed to have metastatic ovarian carcinoma and was managed conservatively due to extensive disease and advanced age. Hung et al[16] report a 48-year-old woman with stage 4 ovarian carcinoma with carcinomatosis presenting initially as bilateral inguinal hernias. In this case, the diagnosis was only made after histopathological examination of the excised hernial sac revealed an adenocarcinoma with unknown primary. Further investigations revealed the ultimate diagnosis of ovarian carcinoma and patient responded well to chemotherapy. This highlights the importance of imaging when dealing with inguinal masses. Most of the time, the diagnosis of inguinal hernia is made clinically. However, as these cases have demonstrated, there must be a high index of suspicion as to what the inguinal hernia might contain and if possible, efforts made to identify the contents before surgery. In our case, possible malignancy was a reasonable preliminary diagnosis to make given her age, size of tumour, presence of constitutional symptoms, and markedly raised CA 125 levels.

Conclusion

Ovarian hernia is rare in postmenopausal women but must be considered in those presenting with inguinal masses. It can occur together with Mullerian abnormalities. CA125 can be elevated in various benign conditions, making interpretation and diagnosis difficult. Multidisciplinary approach is vital to ensure the best outcome for the patient.

Acknowledgements

We thank NB Baharudin (MPath), consultant pathologist at the Department of Pathology, Hospital Raja Permaisuri Bainun for providing the histopathological image in Fig. 4.

We also thank G Rangasamy [BEng (Hons)], computer and electronics engineer for illustrating the schematic diagram of gubernaculum and its derivatives in Fig. 5.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Christine R. Inguinal hernias and hydroceles. In: Wylie R, Hyams J, Kay M, eds. Pediatric gastrointestinal

and liver disease. 5th ed. Philadelphia: Elsevier 2015:614.
2. Aiken J. Inguinal hernias. In: Kliegman R, Geme JS, eds. Nelson textbook of pediatrics. 21st ed. Philadelphia: Elsevier 2019:2064-70.
3. Ozkan OV, Semerci E, Aslan E, Ozkan S, Dolapcioglu K, Besirov E. A right sliding indirect inguinal hernia containing paraovarian cyst, fallopian tube, and ovary: A case report. Arch Gynecol Obstet 2009; 279:897-9.
4. Lee J, Scampoli N. Incarcerated ovarian inguinal hernia in a 10-month-old girl. CMAJ 2015;187: 96-8.
5. Acién P, Sánchez Del Campo F, Mayol MJ, Acién M. The female gubernaculum: Role in the embryology and development of the genital tract and in the possible genesis of malformations. Eur J Obstet Gynecol Reprod Biol 2011;159:426-32.
6. Okada T, Sasaki S, Honda S, Miyagi H, Minato M, Todo S. Irreducible indirect inguinal hernia containing uterus, ovaries, and fallopian tubes. Hernia 2012; 16:471-3.
7. Thomson GR. Complete congenital absence of the vagina associated with bilateral herniae of uterus, tubes and ovaries. Br J Surg 1948;36:99.
8. Parwate NS, Patel SM, Arora R, Gupta M. Ovarian Fibroma: A Clinico-pathological study of 23 cases with review of literature. J Obstet Gynecol India 2016; 66:460-5.
9. Edula RG, Muthukuru S, Moroianu S, Wang Y, Lingiah V, Fung P, et al. CA-125 significance in cirrhosis and correlation with disease severity and portal hypertension: A retrospective study. J Clin Transl Hepatol 2018;6:241-6.
10. Wolf DC. Cirrhosis [Internet]. Medscape. 2020 [cited 2023 Jun 15]. Available from: https://emedicine.medscape.com/article/185856-overview#a1
11. Shetty NS, Vallabhaneni S, Patil A, Babu MM, Baig A. Unreported location and presentation for a parasitic ovarian dermoid cyst in an indirect inguinal hernia. Hernia 2013;17:263-5.
12. Ueda J, Yoshida H, Makino H, Maruyama H, Yokoyama T, Hirakata A, et al. Right inguinal hernia encompassing the uterus, right ovary and fallopian tube in an elderly female: Case report. J Nippon Med Sci 2016;83:93-6
13. Mehdizadeh A, Chaichian S, Mirgaloybayat S, Rokhgireh S, Tahermanesh K, Kadivar M, et al. Right-side inguinal canal endometriosis at ultrasound: A case report. Int J Reprod Biomed 2022;20:59-64
14. Yu M, McKay G. Inguinal endometriosis; diagnostic considerations & management. Aust N Z J Obstet Gynaecol 2018;58: 88
15. Burke TP, Waters P, Khan W, Barry K. Bilateral saccular inguinal hernias in an elderly woman presenting with advanced ovarian cancer. BMJ Case Rep 2014;2014:bcr2013202337.
16. Hung YH, Hsu CT, Chang CC. Ovarian carcinomatosis presenting as bilateral inguinal hernia: A brief report. Eur J Gynaecol Oncol 2010;31:345-6.