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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 (invited) article, Original article, Case report
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EDITORIAL

This second issue of Thai Journal of Obstetrics and Gynaecology 2020 contains many interesting articles. One special article is "**Coronavirus Disease-19 and Pregnancy**".

Editor in Chief and managing staff already attended "**The 8th TCI-TRF-Scopus Collaboration Project**" on Thursday 9th January 2020 at the Jupiter 4-5 Rooms, IMPACT Challenger, Muang Thong Thani, Nonthaburi, Thailand. The aims of the meeting were to report progress on the project TCI-TRF-Scopus Collaboration Project and know the criteria and guidelines for evaluating quality after entering Scopus database (also known as Scopus Re-evaluations), guidelines for quality development and impact of academic journals to raise the quartile of the journals as well details of the international publication ethics.

Editor in Chief and managing staff also attended "**The 13th TCI Symposium on Thai Scholarly Journals on the 4th Re-evaluation Results of Thai Journals and Impacts / Visibilities of Fast-Track Indexing System**" on Friday 10th January 2020, at the Royal Jubilee Ballroom, Challenger 2 Building, Impact Muang Thong Thani, Nonthaburi, Thailand. The aims of the meeting were to announce the results of the Quality Assessment of the academic journals in the 4th round of the TCI database, to clarify the details of the evaluation and guidelines for quality development for group promotion, and to offer a fast track data import to the TCI database.

We are pleased to inform the members that Thai Journal of Obstetrics and Gynaecology already received the results of the Quality Assessment of the academic journals in the 4th round of the TCI database. Thai Journal of Obstetrics and Gynaecology has been included in the **Tier 1** journal group of the TCI database.

The quality of Thai Journal of Obstetrics and Gynaecology has been improved. We are pleased to inform members that Thai Journal of Obstetrics and Gynaecology has been indexed in the **EuroPub** database since 24 Jan 2020.

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

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SPECIAL ARTICLE

Coronavirus Disease-19 and Pregnancy

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ABSTRACT

Coronavirus disease-19 (COVID-19) is the name of the disease caused by a 2019 novel coronavirus that has been identified as the cause of the outbreak of respiratory disease that began on 31 December 2019. It was first detected in Wuhan, Hubei Province, China. The symptoms include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. A confirm diagnosis of COVID-19 is made by collecting specimens for SARS-CoV-2 testing by reverse transcription polymerase chain reaction. There is no current evidence from randomized controlled trials to recommend any specific anti-novel-coronavirus treatment for patients with suspected or confirmed COVID-19. Prevention is the best way to COVID-19. The prevention methods for COVID-19 are the same as for other coronavirus infections. The standard recommendations for the prevention of infection spread include regular hand washing, covering the mouth and nose when coughing and sneezing, thoroughly cooking meat and eggs, and avoiding close contact with anyone showing symptoms of respiratory illness such as coughing and sneezing. Regarding COVID-19 in pregnancy, the clinical characteristics of COVID-19 in pregnant women are the same as those of non-pregnant adults in the general population. Two studies with a small number of cases indicated that there is currently no evidence of vertical transmission in women who had COVID-19 in late pregnancy.

Keywords: COVID-19, corona virus, infection, pregnancy, SARS-Co-V2, Wuhan.

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Coronavirus disease-19 (COVID-19) is the name of the disease caused by a 2019 novel coronavirus (2019-nCoV) that has been identified as the cause of the outbreak of respiratory disease that began on 31 December 2019. It was first detected in Wuhan, Hubei Province, China. The virus is also known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a new strain of coronavirus that is pathogenic to humans. COVID-19 is a highly infectious disease. Thus, the World Health Organization (WHO) has declared the COVID-19 outbreak a public health emergency of international concern, after an emergency meeting on Thursday January 30, 2020. The WHO announced the official name: coronavirus disease 2019 (abbreviated "COVID-19") on February 11, 2020. The WHO has made the assessment that COVID-19 can be "characterized as a pandemic" on 11 March 2020[1].

2019-nCoV is one type of coronavirus. Coronavirus

is one of the main pathogens that causes respiratory infection in humans. Two other highly pathogenic viruses, severe acute respiratory syndrome coronavirus (SARS-CoV) and middle east respiratory syndrome coronavirus (MERS-CoV), cause severe respiratory syndrome in humans, and four other human coronaviruses (HCoV-OC43, HCoV-229E, HCoV-NL63, HCoV-HKU1) cause mild upper respiratory disease[2]. The sequence of 2019-nCoV is relatively diffe rent from the six other coronavirus subtypes, but it can be classified as a betacoronavirus. SARS-CoV and MERS-CoV can be transmitted directly to humans from civets and dromedary camels, respectively. Both viruses originate in bats, but the origin of 2019-nCoV needs further investigation[2].

Many patients in the outbreak in Wuhan, China reported that they had some link to a local Huanan seafood and animal market, suggesting animal-to-person spread. However, many patients reported that they had not been exposed to animal markets, indicating that person-to-person spread occurs[1, 2]. As of March 13, 2020, the cumulative number of confirmed cases in mainland China has reached 80,981 cases, with 3,173 (3.9%) deaths. The cumulative number of confirmed cases in the world (from 123 countries) has reached 136,895 cases, with 5,077 (3.7%) deaths. However, the cumulative number of confirmed cases in Thailand has reached 75 cases, with 1 (1.3%) death[3].

Symptoms and signs

The incubation period for COVID-19 is 2-14 days[4]. 2019-nCoV has been found to infect more males than females, similar to the pattern observed for MERS-CoV and SARS-CoV[2]. 2019-nCoV is also more likely to infect older adult males with chronic comorbidities (such as cardiovascular and cerebrovascular diseases and diabetes) as a result of the weaker immune functions of these patients[2].

Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, renal failure and even death[4]. The reported clinical manifestations are shown in Table 1.

Table 1. Clinical manifestation of coronavirus disease-19 in the general population and in pregnant women[2, 5, 6].

	General population (n = 99)	Pregnant women (n = 18)
Age (years)	55.5 ± 13.1	30.4 ± 4.0
Male: female	67 : 32	0 : 18
Underlying chronic diseases	50 (51%)	0
Clinical manifestation		
Fever	82 (83%)	15 (83.3%)
Cough	81 (32%)	8 (44.4%)
Shortness of breath	31 (31%)	0
Muscle aches	11 (11%)	3 (33.3%)
Confusion	9 (9%)	0
Headache	8 (8%)	0
Sore throat	5 (5%)	3 (16.7%)
Rhinorrhea	4 (4%)	0
Chest pain	2 (2%)	0
Diarrhea	2 (2%)	1 (5.6%)
Nausea and vomiting	1 (1%)	0
Malaise	0	2 (11.1%)
Complications		
Acute respiratory distress syndrome	17 (17%)	0
Death	11 (11%)	0

Diagnosis

Suspected COVID-19 cases include[7]:

A. Patients with severe acute respiratory infection (having fever and cough and requiring admission to the hospital), with no other etiology that fully explains the clinical presentation, and at least one of the following:

• a history of travel to or residence in the city of Wuhan, Hubei Province, China in the 14 days prior to symptom onset, or

• the patient is a health care worker who has been working in an environment where severe acute respiratory infections of unknown etiology are being cared for.

B. Patients with any acute respiratory illness and at least one of the following:

• close contact with a confirmed or probable case of COVID-19 in the 14 days prior to illness onset, or

• visit to or worked in a live animal market in Wuhan, Hubei Province, China in the 14 days prior to symptom onset, or

• worked at or attended a health care facility in the 14 days prior to onset of symptoms where patients with hospital-associated COVID-19 infections have been reported.

Specimens from both the upper respiratory tract (nasopharyngeal and oropharyngeal) and lower respiratory tract (expectorated sputum, endotracheal aspirate, or bronchoalveolar lavage) are obtained for SARS-CoV-2 testing by reverse transcription polymerase chain reaction (RT-PCR) to confirm the diagnosis of COVID-19[7]. The Centers for Disease Control and Prevention (CDC) has developed a new laboratory test kit for use in testing patient specimens for SARS-CoV-2, the virus that causes COVID-19. The test kit is called the "Centers for Disease Control and Prevention (CDC) 2019-Novel Coronavirus (2019-nCoV) Real-Time Reverse Transcriptase (RT)-PCR Diagnostic Panel"[8].

Treatment

There is no current evidence from randomized control trials to recommend any specific anti-2019-nCoV treatment for patients with suspected or confirmed COVID-19[7]. Cases with COVID-19 should have early supportive therapy and monitoring[7].

Prevention

Prevention of COVID-19 is the same for other coronavirus infections. The standard recommendations for the prevention of infection spread include regular hand washing, covering the mouth and nose when coughing and sneezing, thoroughly cooking meat and eggs, and avoiding close contact with anyone showing symptoms of respiratory illness such as coughing and sneezing[9].

COVID-19 and pregnancy

Previous studies found that SARS-CoV-2 has a similar receptor binding domain structure to that of SARS-CoV-1. This suggested that COVID-19 might have a similar pathogenesis to SARS-CoV-1 infection. Thus, the risk of vertical transmission might be as low as that of SARS-CoV-1[10, 11]. There has been two studies from China regarding COVID-19 infection in eighteen pregnant women[5, 6]. They found that pregnant women with COVID-19 infection had few adverse maternal and neonatal outcomes. All cases tested positive for SARS-CoV-2 by using quantitative RT-PCR. Fifteen cases had a cesarean section and two cases had normal delivery in the third trimester. The symptoms and signs among these women included fever (83.3%), cough (44.4%), myalgia (16.7%), sore throat (16.7%), malaise (11.1%), lymphopenia (27.8%), and increased aminotransferase (16.7%). None of the pregnant women had severe pneumonia or died. Fetal distress was found in 8 (44.4%) cases, and 19 (100%) live births were delivered. There was no neonatal asphyxia. From one study[5], amniotic fluid, cord blood, neonatal throat swab, and breast milk samples from six women were tested for SARS-CoV-2. The results were negative for the virus. While neonatal pharyngeal swab for 2019-nCoV test in 9 neonates were negative from one study[6]. These studies demonstrated that the clinical characteristics of COVID-19 in pregnant

women were the same as those of non-pregnant adults in the general population (Table 1). There is no evidence of vertical transmission in women who had COVID-19 in late pregnancy from these small studies.

Regarding SARS and MER-Co-V infection in pregnant women from previous studies[12, 13], these viruses were associated with a high incidence of maternal and neonatal complications such as spontaneous abortion, preterm delivery, intrauterine growth restriction (IUGR), need for endotracheal intubation, intensive care unit admission, renal failure and disseminated intravascular coagulopathy. It seems that COVID-19 in pregnant women has fewer adverse maternal and neonatal complications than SARS-CoV-1 infection in pregnant women. A summary of SARS-CoV-1, MERS-Co-V and COVID-19 infection during pregnancy is shown in Table 2.

Table 2. Summary of SARS-CoV-1, MERS-Co-V and COVID-19 infection during pregnancy[5, 6, 12-18].

	COVID-19 (n = 18)	**SARS-CoV-1 (n = 12)**	**MERS-CoV (n = 11)**
Country	China	Hong Kong	Saudi Arabia, Jordan, United Arab Emirates, South Korea
Age (years)	25-40	22-44	27-39
GA (weeks)	31-39^{+4}	3-32	6-38
Maternal complications			
DIC	0	3 (25%)	0
Renal failure	0	3 (25%)	1 (9.1%)
ARDS	0	4 (33.3%)	5 (45.5%)
Sepsis	0	2 (16.7%)	1 (9.1%)
Spontaneous abortion	0	4 (33.3%)	0
Preterm labor	4 (22.2%)	4 (33.3%)	0
IUGR	0	2 (16.7%)	0
Fetal distress	8 (44.4%)	2 (16.7%)	0
PROM	5 (27.8%)	0	0
Placental abruption	0	0	1 (9.1%)
Maternal death	0	3 (25%)	3 (27.3%)
Neonatal complications	(n = 19)	(n = 12)	(n = 11)
Fetal death	0	0	3 (27.3%)
Live births	19 (100%)	5 (41.7%)	8 (72.7%)
Neonatal viral infection	0	0	0

SARS-CoV: severe acute respiratory syndrome coronavirus, MERS-CoV: Middle East respiratory syndrome coronavirus, COVID-19: coronavirus disease-19, GA: gestational age, DIC: disseminated intravascular coagulopathy, ARDS: acute respiratory

SARS-CoV-1 infection during pregnancy was associated with high maternal morbidity and mortality, high incidence of spontaneous abortion, preterm delivery, and IUGR. MERS-CoV infection during pregnancy was associated with maternal mortality and a high incidence of fetal death. COVID-19 during late

pregnancy had more favorable maternal and fetal outcomes than SARS-CoV-1 and MERS-CoV infection except fetal distress. The risk of vertical transmission was low with both SARS-CoV-1 and COVID-19 infection during pregnancy. There was no vertical transmission from SARS-CoV-1 and COVID-19.

Conclusions

The clinical characteristics of COVID-19 in pregnant women were the same as those of non-pregnant adults in the general population. COVID-19 during late pregnancy had more favorable maternal and fetal outcomes than SARS-CoV-1 and MERS-CoV infection in pregnancy except fetal distress. There is currently no evidence of vertical transmission in women who had COVID-19 in late pregnancy from two studies with small cases. There is no current evidence from randomized controlled trials to recommend any specific anti-2019-nCoV treatment for patients with suspected or confirmed COVID-19. Prevention is the best way for avoiding COVID-19. Prevention of COVID-19 is the same as for other coronavirus infection.

Potential conflicts of interest

The author declares no conflict of interest.

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GYNECOLOGY

The Association between Herbal Substances and Endometrial Neoplasia in Thai Women with Postmenopausal Bleeding: A case-control study at Maharat Nakhon Ratchasima Hospital

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ABSTRACT

Objectives: The purpose was to study the association of herbal-medicine users on the endometrial pathology of patients who were diagnosed with postmenopausal bleeding.

Materials and Methods: This research was a retrospective case control study conducted on 170 patients with postmenopausal bleeding who received treatment and underwent endometrial biopsy for pathological examination at Maharat Nakhon Ratchasima Hospital during September 1, 2016 to September 30, 2017. Data were collected from medical records and telephone interviews to obtain information about their baseline characteristics and history of herbal medicine use.

Results: Regarding the age of the onset of postmenopausal bleeding, there were statistically different between the two groups: patients with pathological diagnosis of endometrial neoplasia had a mean age of 59 years, which was higher than those with pathological diagnosis of other benign conditions that had a mean age of 56 years. In addition, the mean age at menopause of patients with pathological diagnosis of endometrial neoplasia was 52 years, which was significantly higher than patients with pathological diagnosis of other benign conditions that had a mean age at menopause of 50 years. With respect to body mass index (BMI), it was evident that there was a larger number of patients with endometrial neoplasia who had a BMI of over or equal to 30 kg/m^2 than patients with other benign conditions, with statistical significance. After controlling for BMI and age at menopause, patients with pathological diagnosis of endometrial neoplasia had 4.11 times higher rates of herbal medicine user than patients with pathological diagnosis of other benign conditions (95% confidence interval 1.76-9.59).

Conclusion: Patients who were diagnosed with endometrial neoplasia had 4.11 times higher rate of herbal medicine use than those with pathological diagnosis of other benign conditions after controlling for BMI and age at menopause.

Keywords: herbal medicines, postmenopausal bleeding, endometrial hyperplasia, endometrial cancer.

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ความสัมพันธ์ของการรับประทานยาสมุนไพรต่อเยื่อบุโพรงมดลูกในสตรีที่มีเลือดออกหลังหมดประจำเดือนในโรงพยาบาลมหาราชนครราชสีมา

พิชญ์ วิโสจสงคราม, สิรยา กิติโยดม

บทคัดย่อ

วัตถุประสงค์: *เพื่อศึกษาผลทางพยาธิวิทยาเยื่อบุโพรงมดลูกของสตรีที่มีภาวะเลือดออกหลังหมดประจำเดือน ในสตรีที่ใช้และไม่ใช้ยาสมุนไพรสำหรับสตรี*

วัสดุและวิธีการ: *เป็นการศึกษาแบบ retrospective case control study design ในสตรีที่มีภาวะเลือดออกทางช่องคลอดหลังหมดประจำเดือนที่เข้ารับการตรวจหรือรักษาในโรงพยาบาลมหาราชนครราชสีมา และต้องได้รับการตรวจวินิจฉัยเก็บเยื่อบุโพรงมดลูกเพื่อส่งตรวจทางพยาธิวิทยา ตั้งแต่วันที่ 1 กันยายน 2559 ถึงวันที่ 30 กันยายน 2560 จำนวน 170 คน โดยใช้ข้อมูลจากเวชระเบียนโรงพยาบาลและการสัมภาษณ์ทางโทรศัพท์ตามแบบสอบถาม เกี่ยวกับข้อมูลพื้นฐานต่างๆ รวมถึงประวัติการใช้ยาสมุนไพรสำหรับสตรี*

ผลการศึกษา: *ในสตรีที่มีภาวะเลือดออกทางช่องคลอดหลังหมดประจำเดือนพบว่า อายุขณะที่มีอาการเลือดออกทางช่องคลอดหลังหมดประจำเดือนในกลุ่มที่มีผลของพยาธิวิทยาเยื่อบุโพรงมดลูกตั้งแต่ระดับ hyperplasia ขึ้นไปเฉลี่ย 59 ปี ซึ่งมากกว่าสตรีที่มีผลของพยาธิวิทยาเยื่อบุโพรงมดลูกเป็นอย่างอื่นเฉลี่ย 55 ปี อย่างมีนัยสำคัญทางสถิติ นอกจากนี้พบว่าอายุที่หมดประจำเดือนนั้นเฉลี่ย 52 ปี ในกลุ่มสตรีมีผลของพยาธิวิทยาของเยื่อบุโพรงมดลูกตั้งแต่ระดับ hyperplasia ขึ้นไปมากกว่าสตรีที่ผลของพยาธิวิทยาของเยื่อบุโพรงมดลูกเป็นอย่างอื่นเฉลี่ย 50 ปี อย่างมีนัยสำคัญทางสถิติ อีกทั้งยังพบว่าสตรีที่มีผลของพยาธิวิทยาของเยื่อบุโพรงมดลูกร้ายแรงตั้งแต่ระดับ hyperplasia ขึ้นไป มีการใช้ยาสมุนไพรสำหรับสตรีมากกว่าสตรีที่มีผลของพยาธิวิทยาของเยื่อบุโพรงมดลูกเป็นอย่างอื่นถึง 4.11 เท่า (95% confidence interval 1.76-9.59) เมื่อควบคุมปัจจัยด้านดัชนีมวลกายและอายุที่สตรีในกลุ่มตัวอย่างหมดประจำเดือน*

สรุป: *การศึกษาพบว่า ผลชิ้นเนื้อทางพยาธิวิทยาของเยื่อบุโพรงมดลูกในสตรีที่มีภาวะเลือดออกทางช่องคลอดหลังหมดประจำเดือนเป็น hyperplasia ขึ้นไป มีประวัติการใช้ยาสมุนไพรสำหรับสตรีมากเป็น 4.11 เท่า อย่างมีนัยสำคัญทางสถิติ หากควบคุมปัจจัยด้านดัชนีมวลกายและอายุที่สตรีในกลุ่มตัวอย่างหมดประจำเดือน เมื่อเปรียบเทียบกับสตรีที่มีผลชิ้นเนื้อทางพยาธิวิทยาของเยื่อบุโพรงมดลูกเป็นอย่างอื่น*

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Introduction

Endometrial cancer is a type of cancer that affects female reproductive organs and is the third most common gynecologic cancer in Thailand. According to WHO, the incidence rate of endometrial cancer in Thailand is 4.3 per 100,000 female population[1]. Based on the data obtained from Maharat Nakhon Ratchasima Hospital[2], there were 479 female patients diagnosed with postmenopausal bleeding during October 1, 2008 to September 30, 2011, whereby endometrial cancer and endometrial hyperplasia were found in 13.6% and 8.6% of the patients, respectively.

It is widely known that the risk factors for endometrial neoplasia are associated with age (above 50 years), obesity, diabetes, nulliparity, menstruation span, and intake of estrogen[3-5]. An intake of estrogen without progesterone affects the regeneration and growth of the endometrium, which subsequently increases the risk of endometrial cancer[6-8].

On the contrary, estrogen deficiency affects emotions and contributes to decreased bone mass, vaginal dryness, metabolic dysfunction, and amenorrhea[9]. In Thailand, there are a variety of herbal medicines available in the market, many of which claim to nourish the body and alleviate menopause symptoms, as well as brighten the skin and treat hormonal imbalances. Nonetheless, it is possible that these herbal medicines contain environmental estrogenic contaminants. A previous study[10] found that the uterus of oophorectomized mice receiving extracts of Thai herbs was heavier than of their counterparts.

Materials and Methods

This research was a retrospective case control study aimed to examine the association of herbal-medicine users on the endometrial pathology of female patients who were diagnosed with postmenopausal bleeding.

The research was conducted on a sample of 170 female patients with postmenopausal bleeding who were diagnosed or treated at Maharat Nakhon Ratchasima Hospital during September 1, 2016 to September 30, 2017 and had been approved by the Research Ethics Committee of Maharat Nakhon Ratchasima Hospital. The sample was randomly selected by the order of the date of visit. Data were collected from the medical records of the hospital. In addition, telephone interviews were conducted with patients upon their consent and according to the designed questionnaire. To minimize bias, the interviews were performed by one interviewer. Data pertaining to baseline characteristics and risk factors for endometrial neoplasia, such as age, age at menarche, age at menopause, gravidity, diabetes mellitus, use of oral contraceptive pills, and history of herbal medicine use, were obtained. Herbal medicines selected for this research were those that claimed to treat menopausal symptoms (such as irritability, skin dullness, bone loss, mild uterine prolapse, low libido, vaginal dryness, and sagging of breasts) and contained safflower, oriental motherwort, sappanwood, Java ginger, Szechuan lovage, pink and blue ginger, and Molineria latifolia as the main ingredients. A previous study[11] found that these herbs are the essential ingredients of herbal medicines for women.

The inclusion criteria of this research were: 1) patients with postmenopausal bleeding who were diagnosed or treated at Maharat Nakhon Ratchasima Hospital and 2) patients with pathological results obtained from endometrial sampling or curettage. In addition, the exclusion were 1) patients who underwent hormone replacement therapy; 2) patients with postmenopausal bleeding of which the causes were not related to endometrial abnormalities, such as vaginal injuries or inflammation, uterine leiomyomas, cervical cancer, vulvar cancer, thyroid disorders, and coagulopathy; 3) patients who had difficulty communicating or responding to the interview questions, such as those with cognitive impairment; 4) patients who were not willing to participate in the research; 5) patients who did not have pathological information; 6) patients whose treatment could not be monitored continuously; and 7) patients who discontinued the use of herbal medicines for more than 1 year.

The sample size was determined from the pilot study conducted during December 1, 2016 to March

31, 2017 and was calculated using a power and sample size calculation software. The two independent proportions were selected as the proportion type, and Fisher's exact test was employed as the statistical test. In addition, the parameters were set on the basis of the low incidence rate of endometrial neoplasia in postmenopausal bleeding patients, as follows: α = 0.05, power = 80%, and control: case ratio = 4:1. According to the pilot study conducted on 100 patients, the prevalence of exposure among controls and cases were 0.03 and 0.2, respectively.

Upon calculation, the minimum sample size was found to be 170, whereby the sample was divided into 34 patients with pathological diagnosis of endometrial neoplasia and 136 patients with pathological diagnosis of other benign conditions. Based on the pilot study and the assumption that 30% of prospective participants cannot be reached or are not willing to participate in the telephone interview, a minimum of 221 patients were required for the research.

The statistical tests employed in this research consisted of Fisher's exact test for dichotomous variables and student's t-test for continuous variables. Moreover, Stata Version 12.0 was used for statistical data analysis. The results were considered statistically significant when p value < 0.05.

Results

At the time of study, there were a total of 300 patients diagnosed with postmenopausal bleeding during September 1, 2016 to September 30, 2017. There were 69 patients who did not meet the sampling criteria, comprising 46 patients who could not be reached, 1 patient who did not have pathological information, 19 patients with postmenopausal bleeding of which the causes were not related to endometrial abnormalities, and 3 patients who were not willing to provide information. Thus, a total of 231 patients were included in the research. The sample was further divided into two groups: 40 patients with endometrial neoplasia and 191 patients with other benign conditions. After that, the sample was randomly selected by the order of the date of visit until 34 patients with endometrial neoplasia and 136 patients with other benign conditions were obtained (Fig. 1).

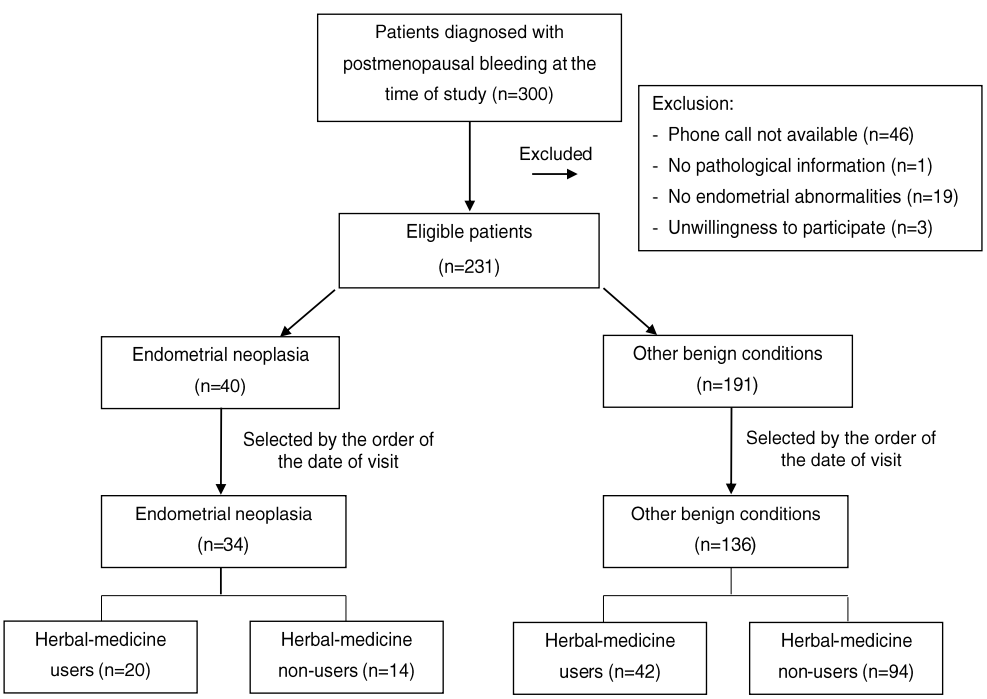

Fig. 1. Participant enrollment.

Regarding the demographic characteristics of the sample, it was found that there were statistically different between the two groups in terms of age, age at menopause and body mass index (BMI). The mean age of patients with pathological diagnosis of endometrial neoplasia was 59 years (±8, range 49 - 79 years), which was higher than patients with pathological diagnosis of other benign conditions that had a mean age of 56 years (±8.8, range 44-89 years) with p value = 0.047. With respect to the age at menopause, patients with pathological diagnosis of endometrial neoplasia had a mean age at menopause of 52 years (±3.6, range 47-63 years), which was higher than patients with pathological diagnosis of other benign conditions that had a mean age at menopause of 50 years (±3.3, range 43-6o years) with p value < 0.001. Moreover, across patients with pathological diagnosis of endometrial neoplasia, 23 patients had BMI ≥ 30 kg/m^2 (67.6%). This was statistically significantly higher than patients with pathological diagnosis of other benign conditions, of which 62 patients were found to have BMI ≥ 30 kg/m^2 (45.6%) with p value = 0.034. Alternatively, there were no statistically significant differences between the two groups in terms of age at menarche, gravidity, diabetes mellitus, and use of oral contraceptive pills (Table 1).

Table 1. Demographic Characteristics.

Characteristics	Endometrial neoplasia (n = 34)	Other benign conditions (n = 136)	p value
Age (years)	59.1 ± 8.0	55.8 ± 8.8	0.047
Age at menarche (years)	11.9 ± 1.7	11.9 ± 2.6	0.936
Age at menopause (years)	52.1 ± 3.6	50.0 ± 3.3	< 0.001
BMI			
< 30 kg/m^2	11 (32.4)	74 (54.4)	0.034
≥ 30 kg/m^2	23 (67.6)	62 (45.6)	
Gravidity			
0-1	8 (23.5)	25 (18.4)	0.823
2	14 (41.2)	59 (43.4)	
≥ 3	12 (35.3)	52 (38.2)	
DM	9 (26.5)	20 (14.7)	0.126
History of OCP use			
None	21 (61.8)	84 (61.8)	0.614
< 5 years	12 (35.2)	41 (30.1)	
≥ 5 years	1 (3)	11 (8.1)	

Data were presented as mean ± standard deviation and n (%)
BMI: body mass index, DM: diabetes mellitus, OCP: oral contraceptive pills

Concerning the duration of herbal medicine use, the mean duration of herbal medicine use in patients with pathological diagnosis of endometrial neoplasia was 4.35 years (± 3.17, range 1 - 15 years).

Upon endometrial biopsy, it was found that there were 34 patients with pathological diagnosis of endometrial neoplasia, 20 of whom had a history of herbal medicine use. Meanwhile, there were 136 patients with pathological diagnosis of other benign conditions, of which 42 patients had a history of herbal medicine use. The endometrial pathology of patients was demonstrated in Table 2.

Table 2. Histology of endometrium of 170 women with postmenopausal bleeding.

Pathology	Herbal medicine users (n = 62)	Herbal medicine non-users (n = 108)
Benign endometrial condition		
Atrophic endometrium	9 (14.5)	25 (23.1)
Proliferative pattern	6 (9.7)	23 (21.3)
Secretory pattern	5 (8.1)	21 (19.4)
Endometritis	5 (8.1)	9 (8.3)
Stromal or glandular breakdown	8 (12.9)	6 (5.6)
Endometrial polyp	5 (8.1)	6 (5.6)
Hormonal effect	4 (6.5)	4 (3.7)
Endometrial neoplasia		
Simple hyperplasia without atypia	2 (3.2)	7 (6.5)
Complex hyperplasia with atypia	2 (3.2)	1 (0.9)
Endometrioid carcinoma	8 (12.9)	4 (3.7)
Adenocarcinoma	4 (6.5)	1 (0.9)
Clear cell carcinoma	1 (1.6)	1 (0.9)
Endometrial stromal sarcoma	1 (1.6)	0 (0.0)
Serous carcinoma	1 (1.6)	0 (0.0)
Mixed serous and endometrioid carcinoma	1 (1.6)	0 (0.0)

Data were presented as n (%)

Upon analysis of data with multiple logistic regression and after controlling for BMI and age at menopause, it was evident that patients with pathological diagnosis of endometrial neoplasia had 4.11 times higher rate of herbal medicine use than patients with pathological diagnosis of other benign conditions (95% confidence interval (CI) 1.76-9.59) (Table 3).

Table 3. Relationship between herbal medicine use and endometrial pathology of postmenopausal bleeding.

Herbal medicine use	Neoplasia	Other benign Conditions	Unadjusted ORs (95%CI)	Adjusted ORs (95%CI)
Use (%)	20 (58.8)	42 (30.9)	3.67 (1.64 - 8.20)	4.11 (1.76-9.59)
Non-use (%)	14 (41.2)	94 (69.1)	1.00 (reference)	1.00 (reference)

[#]Adjusted for body mass index, and age at menopause
ORs: odds ratio, CI: confidence interval

Discussion

This research applied fundamental knowledge on factors that contribute to the incidence of endometrial neoplasia and, importantly, the continuous use of estrogen[6-8]. The research was conducted under the assumption that the herbal medicines available in Thailand may be environmentally estrogen contaminated and carry putative claims of health benefits. This was consistent with previous studies[12,13], conducted on healthy postmenopausal women who were given

Pueraria mirifica pills at a daily dose of 20, 30, and 50 mg for a period of 24 weeks. After comparing the results with the placebo group, it was evident that Pueraria mirifica was able to treat vaginal dryness, dyspareunia, and vaginal atrophy, as well as reducing the level of bone-specific alkaline phosphatase and the rate of bone turnover. Moreover, previous studies suggested that Pueraria mirifica may be effective in inhibiting bone resorption by acting as an antiresorptive agent.

This research found that, after controlling for BMI and age at menopause, the historical use of herbal medicines amongst patients with postmenopausal bleeding was 4.11 times higher in patients with pathological diagnosis of endometrial neoplasia than in patients with pathological diagnosis of other benign conditions (95%CI 1.76-9.59). Regarding the duration of herbal medicine use, the mean duration of herbal medicine use in patients with pathological diagnosis of endometrial neoplasia was 4.35 years (± 3.17, range 1-15 years). These results were consistent with a previous randomized, double-blind, placebo-controlled study[14], which found that an intake of phytoestrogen had an endometrial effect on women and contributed to the formation of endometrial hyperplasia with statistical significance.

In the aspect of the patients' age, there were statistically different between the two groups: patients with pathological diagnosis of endometrial neoplasia had a mean age of 59 years (± 8, range 49-79 years), which was significantly higher than patients with pathological diagnosis of other benign conditions that had a mean age of 56 years (± 8.7, range 44-89 years) ($p = 0.047$). Moreover, the mean age at menopause of patients with pathological diagnosis of endometrial neoplasia was 52 years (± 3.6, range 47-63 years), which was significantly higher than those with pathological diagnosis of other benign conditions that had a mean age at menopause of 50 years (± 3.3, range 43-60 years) with $p < 0.001$. These results conformed to previous studies[15-18], which found that aging and late-onset menopause were correlated with the incidence of endometrial cancer with statistical significance.

Regarding the BMI of patients with endometrial neoplasia, there were 23 patients with BMI ≥ 30 kg/m^2 (67.6%) and 11 patients with BMI < 30 kg/m^2 (32.4%). Meanwhile, across patients with other benign conditions, 62 had BMI ≥ 30 kg/m^2 (45.6%) and 74 had BMI < 30 kg/m^2 (54.5%). Accordingly, it can be inferred that there were statistically significant higher number of patients with BMI ≥ 30kg/m^2 in the endometrial neoplasia group at the p value of 0.034, which conforms to the previous study[19]. On the contrary, there were no statistically significant differences between the two groups in terms of age at menopause, gravidity, diabetes mellitus and contraceptive use.

Concerning the limitations of this research, there were some underlying errors in connection with the patients' responses to research questions, particularly when they were inquired of their past events. Moreover, patients may provide inaccurate responses to shorten the length of time spent in the telephone interview or attempt to provide responses that correspond to the interviewer's expectations, which subsequently leads to response bias. According to previous studies[20,21], close-ended questions that are short, concise, and straightforward were found to give more accurate responses than open-ended questions. Hence, close-ended questions were employed in the telephone interview to minimize errors in this research and the interview were performed by one interviewer. Furthermore, the sample was selected at the time of the patients' most recent visit to Maharat Nakhon Ratchasima Hospital, whereby patients with more than 1-year withdrawal from herbal-medicine use and patients with intellectual disability were excluded from the research. Although this research was a preliminary study, the findings of this research could be used as a guideline for future researches on the use of herbal medicines.

Conclusion

There are multiple risk factors for endometrial neoplasia, one of which includes the intake of estrogens. As far as this research is concerned, herbal medicines available in Thailand may contain an unknown amount

of phytoestrogens, which may contribute to the increased risk of endometrial neoplasia. Based on the results of this research, female patients with pathological diagnosis of endometrial neoplasia had a significantly higher rate of herbal medicine use than those with pathological diagnosis of other benign conditions. Therefore, the safety aspects of long-term herbal medicine use amongst postmenopausal women should be taken into consideration.

Potential conflicts of interest

The authors declare no conflict of interest.

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GYNECOLOGY

Immunohistochemistry Staining for the Mismatch Repair Proteins in Endometrial Cancer Patients

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ABSTRACT

Objectives: Lynch syndrome (LS) increases the lifetime risks of endometrial cancer by approximately 40-60%. Although universal screening with immunohistochemistry (IHC) for mismatch repair (MMR) proteins has been recommended, it is not yet common in Thailand. This study aimed to evaluate the prevalence of MMR deficiency and identify patients who may be at risk for LS.

Materials and Methods: IHC for MMR proteins, including mutL homolog 1 (MLH1), mutS homolog 2 (MSH2), mutS homolog 6 (MSH6), and PMS1 homolog 2 (PMS2), were tested in 156 endometrial cancer patients who underwent primary surgery between 2013-2015. This study also screened for the revised Bethesda guidelines, using age at diagnosis and personal and family history of LS-related cancers as variables.

Results: Fifty-seven of 156 (35.9%) patients had MMR deficiency; 42 experienced losses of MLH1 and PMS2, 10 experienced losses of MSH2 and MSH6, and 5 experienced a loss of MSH6 expression. Only 36 patients (23.1%) met the revised Bethesda guidelines; 29 patients (18.6%) were diagnosed earlier than age 50; 10 patients (6.4%) had synchronous colon or ovarian cancer; and only 13 patients (8.3%) possessed a family history of LS-related cancers. It was possible to detect MMR deficiency in 41/120 patients (34.2%) who did not meet the revised Bethesda guidelines.

Conclusion: MMR deficiency as a result of IHC can be detected in 35.9% of endometrial cancer patients. However, it was still possible to detect MMR deficiency in at least one-third of patients who did not meet the Bethesda guidelines. Screening endometrial cancer patients for MMR IHC should be considered, with the aim of diagnosing and preventing LS-related cancers in both patients and their relatives.

Keywords: endometrial cancer, lynch syndrome, hereditary nonpolyposis colorectal cancer, immunohistochemistry, mismatch repair proteins.

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การย้อมอิมมูโนพยาธิวิทยาโปรตีนมิสแมชรีแพร์ในผู้ป่วยมะเร็งเยื่อบุโพรงมดลูก

พิมพ์พิชชา พวงศรีเจริญ, ธาริณี แม่นชนะ, ชัย อริยศรีวัฒนา, สุรางค์ ตรีรัตนชาติ

บทคัดย่อ

วัตถุประสงค์: *ลินช์ซินโดรม (Lynch Syndrome) เพิ่มความเสี่ยงต่อการเป็นมะเร็งเยื่อบุโพรงมดลูก 40-60% ในต่างประเทศโดยเฉพาะในองค์กรณ์ใหญ่ๆ แนะนำให้ตรวจด้วยเทคนิคทางอิมมูโนพยาธิวิทยาโปรตีนมิสแมชรีแพร์ในผู้ป่วยมะเร็งลำไส้หรือมะเร็งเยื่อบุโพรงมดลูก อย่างไรก็ตามในประเทศไทยยังไม่ได้มีการนำเทคนิคการย้อมอิมมูโนพยาธิวิทยาโปรตีนมิสแมชรีแพร์มาใช้แพร่หลายในผู้ป่วย งานวิจัยนี้ทำขึ้นเพื่อศึกษาอัตราการย้อมไม่ติดของชิ้นเนื้อด้วยเทคนิคทางอิมมูโนพยาธิวิทยาดังกล่าวในผู้ป่วยมะเร็งเยื่อบุโพรงมดลูกในประเทศไทย*

วัสดุและวิธีการ: *ศึกษาในผู้ป่วยมะเร็งเยื่อบุโพรงมดลูกที่ทำการผ่าตัดที่โรงพยาบาลจุฬาลงกรณ์ ระหว่างปี 2556-2558 รวมทั้งสิ้น 156 ราย นำบล็อกชิ้นเนื้อเยื่อบุโพรงมดลูกตัดลงสไลด์ และทำการย้อมชิ้นเนื้อด้วยเทคนิคทางอิมมูโนพยาธิวิทยาโปรตีนมิสแมชรีแพร์ (mismatch repair) ได้แก่ MLH1, MSH2, MSH6, PMS2 อ่านผลอัตราการย้อมไม่ติดสีโดยพยาธิแพทย์ทางนรีเวช 2 ท่าน รวมถึงคัดกรองประวัติตาม Revised Bethesda guideline ได้แก่ อายุที่ได้รับการวินิจฉัย ประวัติมะเร็งของผู้ป่วยและครอบครัว จากเวชระเบียนและจากผู้ป่วย*

ผลการศึกษา: *ตรวจพบอัตราการย้อมไม่ติดอิมมูโนพยาธิวิทยาของโปรตีนมิสแมชรีแพร์ทั้งสิ้น 57 ราย จากผู้ป่วย 156 ราย (35.9%) แยกเป็นย้อมไม่ติดของ MLH1/PMS2 42 ราย MSH2/MSH6 10 ราย และ MSH6 5 ราย การคัดกรองโดยใช้ Revised Bethesda guideline พบว่ามีผู้ป่วยที่เข้าได้ตามเกณฑ์ทั้งสิ้น 36 ราย (23.1%) โดยพบผู้ป่วยที่ได้รับวินิจฉัยเป็นมะเร็งก่อนอายุ 50 ปี จำนวน 29 ราย (18.6%) ผู้ป่วย 10 ราย (6.4%) พบมะเร็งลำไส้หรือมะเร็งรังไข่ร่วมกับมะเร็งเยื่อบุโพรงมดลูก ผู้ป่วย 13 ราย (8.3%) มีประวัติครอบครัวเข้าได้กับ Lynch syndrome อย่างไรก็ตามผู้ป่วย 120 ราย ที่ประวัติไม่ตรงตามเกณฑ์ Revised Bethesda guideline ตรวจพบการย้อมไม่ติดอิมมูโนพยาธิวิทยาของโปรตีนมิสแมชรีแพร์ 41 ราย คิดเป็น 34.2%*

สรุป: *อัตราการย้อมไม่ติดของยีนในกลุ่มมิสแมชรีแพร์ในผู้ป่วยมะเร็งเยื่อบุโพรงมดลูกคิดเป็น 35.9% ในผู้ป่วยที่ไม่พบประวัติความเสี่ยงที่เข้าได้กับ Bethesda guideline สามารถตรวจพบการย้อมไม่ติดของยีนในกลุ่มมิสแมชรีแพร์ได้ถึง 1 ใน 3 ดังนั้นการตรวจคัดกรองทางอิมมูโนพยาธิวิทยาควรพิจารณาทำในผู้ป่วยมะเร็งเยื่อบุโพรงมดลูกทุกราย ผู้ป่วยรายนั้นจะได้รับการปรึกษาทางพันธุกรรม (genetic counseling) เพื่อรับการตรวจทางพันธุกรรมต่อไป รวมถึงให้คำปรึกษากับญาติผู้ป่วยที่มีความเสี่ยง*

คำสำคัญ: *มะเร็งเยื่อบุโพรงมดลูก, อิมมูโนพยาธิวิทยาโปรตีนมิสแมชรีแพร์, ลินช์ซินโดรม*

Introduction

Endometrial cancer is the most common gynecologic malignancy in developed countries[1]. In Thailand, it is the third most common gynecologic malignancy, following cervical cancer and ovarian cancer, which appear in 13.4% and 4.4% of the population, respectively[2]. Approximately 3-5% of endometrial cancer can be attributed to Lynch syndrome (LS), previously known as hereditary nonpolyposis colorectal cancer (HNPCC)[1]. This syndrome is an autosomal dominant disease caused by germline mutations of mismatch repair (MMR) genes (mutL homolog 1 (MLH1), mutS homolog 2 (MSH2), mutS homolog 6 (MSH6), PMS1 homolog 2 (PMS2), and epithelial cell adhesion molecule (EPCAM)). LS patients have a 40-60% lifetime risk of getting endometrial cancer and colon cancer[1, 3].

In general, personal and family history of cancer is used as a screening tool. The Amsterdam criteria were developed in 1990 and have subsequently been modified and become the Amsterdam II criteria. These criteria exhibit high specificity, but low sensitivity. In 1997 the Bethesda guidelines were developed; They were then revised in 2004. In contrast to the Amsterdam criteria, the Bethesda guidelines possess a high degree of sensitivity[1]. However, screening LS using both criteria will still result in a misdiagnosis of at least 30% of patients[4].

Molecular tumour testing, such as immunohistochemistry (IHC) for MMR genes expression, microsatellite instability testing, and MLH1 promoter methylation testing, have been endorsed for universal screening use in all endometrial cancer patients[1, 5]. However, IHC for MLH1, MSH2, MSH6, and PMS2 expression is the most cost-effective, and it is widely available in most pathology laboratories[6]. This screening test is not yet recommended in Thailand, however. In addition, In Thailand, there is no published data about the prevalence of MMR deficiency in endometrial cancer patients. This study may be the first study in Thailand that aimed to identify endometrial cancer patients at risk of LS.

Materials and Methods

Immunohistochemistry screening with MMR proteins was performed in endometrial cancer patients who had undergone primary surgery at King Chulalongkorn Memorial Hospital in Bangkok, Thailand between January 2013 and December 2015. Demographic data, such as age at diagnosis, parity, menopausal status, body mass index (BMI), family or personal history of cancer, pathological data, and received treatment, were retrieved from the medical records. Endometrial cancer patients who met the revised Bethesda guidelines were as follows; less than 50 years old at time of diagnosis; synchronous or metachronous ovarian, colon or other LS-related cancer at any age; first degree relative with LS-related cancer who was diagnosed before 50 years of age or two or more relatives with LS-related cancer at any age[7]. This study was approved by the Institutional Review Board, Faculty of Medicine, at Chulalongkorn University (IRB015/60).

Immunohistochemistry for MMR proteins includes MLH1, MSH2, MSH6, and PMS2. Leica RM2245, a semi-motorized rotary microtome, was used to place a 5-mm-thick, formalin-fixed, paraffin-embedded section onto a 2-micron tissue slide. Primary monoclonal antibodies against MLH1 (clone G168-15; Zytomed system; Berlin, Germany), MSH2 (clone FEE11; Zytomed system; Berlin, Germany), MSH6 (clone SPM525; Zytomed System; Berlin, Germany), and PMS2 (clone EPR3947; Zytomed system; Berlin, Germany) were applied to 2-micron tissue slides. Antigen retrieval was performed using Dako autostainer Link48's proprietary antigen retrieval solution at pH 8.0 (MLH1, MSH2, MSH6, and PMS2). All slides were reviewed by two pathologists. Normal expression is defined as nuclear staining within tumour cells, using the nuclei of infiltrating lymphocytes and/or normal stromal cells as positive internal control. Negative expression is defined as the complete absence of nuclear staining within tumour cells, but the presence of staining in normal endometrial and stromal cells. MMR deficiency is defined as the negative or loss expression of at least one of the four MMR proteins.

(Fig.1). Mutual agreement had been made between two pathologists.

A statistical analysis was conducted using SPSS version 22.0 (IBM Corp., Armonk, N.Y., USA). Categorical variables were calculated using the chi-square or Fisher exact tests. Continuous variables were tested using the student t-test. Statistical significance was achieved when the p value was less than 0.05.

Results

A total of 156 endometrial cancer patients were included. Demographic data and pathological data are presented in Table 1. The mean age is 57.1 ± 11.0 years (range 20-83 years). Most patients (73.7%) were in stage I. Stage II, III, and IV were account for 7.1%, 17.3%, and 1.9% of patients, respectively. Thirty-six patients (23.1%) met the revised Bethesda guidelines; 29 patients (18.6%) were diagnosed at an age of less than 50 years; 13 patients (8.3%) had a family history of LS related-cancer (2 patients had first degree relative with LS-related cancer diagnosed before 50 years and the remaining 11 patients had two or more relatives with LS-related cancer at any age); and 10 patients (6.4%) had synchronous endometrial and ovarian or colon cancers.

Fifty-seven of 156 patients (35.9%) had an MMR deficiency; 42 experienced a loss of MLH1 and PMS2 (26.9%); 10 experienced a loss of MSH2 and MSH6 (6.4%); and 5 experienced a loss of MSH6 expression (3.2%). There was no significant difference in the clinicopathological characteristics between patients with and without MMR deficiency, except family history of LS-related cancers. More patients in the MMR deficient group possessed a family history of LS-related cancers: 15.8% and 4%, respectively (p=0.02). Sixteen of the 36 patients (44.4%) who met the revised Bethesda guidelines exhibited a loss of MMR expression. In contrast, MMR deficiency was still detected in 41 of 120 patients (34.2%) who did not meet the revised Bethesda guidelines. MMR deficiency was detected in less than half of the patients when the following criteria were used: younger than 50 years old when diagnosed and presence of synchronous cancers; 44.8% (13/29 patients) and 40% (4/10 patients), respectively.

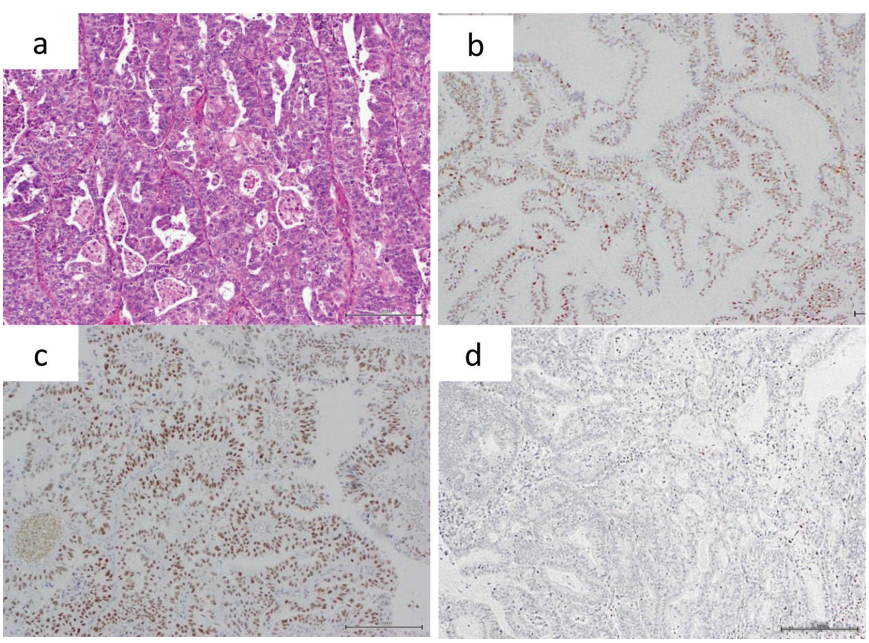

Fig. 1. H&E slide on tumour cells. (a) Tumour cells express nuclear MLH1 (b) and PMS2. (c) Tumour cells do not express MSH6. (d)

Table 1. Demographic data and pathological findings.

	All cases (N=156)	MMR deficiency (N=57)	MMR proficiency (N=99)	p value
Age, mean ± SD (years)	57.1 ± 11.0	56.0 ± 11.0	57.7 ± 11.1	0.35
Age ≤ 50 years	29 (18.6%)	13 (22.8%)	16 (16.2%)	0.39
Nulliparous	62 (39.7%)	21 (36.8%)	41 (41.4%)	0.61
Menopause	103 (66.0%)	35 (61.4%)	68 (68.7%)	0.38
BMI, mean ± SD (kg/m^2)	26.9 ± 6.6	27.6 ± 6.5	26.5 ± 6.7	0.31
Family history of cancers	13 (8.3%)	9 (15.8%)	4 (4.0%)	0.02
Bethesda guidelines	36 (23.1%)	16 (28.1%)	20 (20.2%)	0.32
Stage				0.65
I	115 (73.7%)	45 (78.9%)	70 (70.7%)	
II	11 (7.1%)	4 ((7.0%)	7 (7.1%)	
III	27 (17.3%)	7 (12.3%)	20 (20.2%)	
IV	3 (1.9%)	1 (1.8%)	2 (2.0%)	
Histology				0.41
Endometrioid carcinoma	149 (95.5%)	55 (96.5%)	94 (94.9%)	
Mixed adenocarcinoma	5 (3.2%)	1 (1.8%)	4 (4.0%)	
Papillary serous carcinoma	1 (0.6%)	1 (1.8%)	0 (0%)	
Carcinosarcoma	1 (0.6%)	0 (0%)	1 (1.0%)	
Tumor grade				0.14
G1	86 (55.1%)	28 (49.1%)	58 (58.6%)	
G2	32 (20.5%)	10 (17.5%)	22 (22.2%)	
G3	38 (24.4%)	19 (33.3%)	19 (19.2%)	
Myometrial invasion				0.49
< 50%	97 (62.2%)	33 (57.9%)	64 (64.6%)	
≥ 50%	59 (37.8%)	24 (42.1%)	35 (35.4%)	
LVSI (N=100)	43 (43.0%)	17/35 (48.6%)	26/65 (40.0%)	0.62
Lower uterine segment involvement	100 (64.1%)	37 (64.9%)	63 (65.6%)	1.00
Pelvic node metastasis (N=141)	16 (11.3%)	4/53 (7.5%)	12/88 (13.6%)	0.38
Paraaortic node metastasis (N=80)	5 (6.3%)	2/33 (6.1%)	3/47 (6.4%)	0.46
Synchronous endometrial and ovarian and/or colon cancers	10 (6.4%)	4 (7.0%)	6 (6.1%)	1.00
Adjuvant treatment				0.46
None	64 (41.0%)	19 (33.3%)	45 (45.5%)	
Pelvic radiation with brachytherapy	28 (17.9%)	13 (22.8%)	15 (15.2%)	
Concurrent chemoradiation	24 (15.4%)	8 (14.0%)	16 (16.2%)	
Brachytherapy	21 (13.5%)	10 (17.5%)	11 (11.1%)	
Pelvic radiation then adjuvant chemotherapy	1 (0.6%)	1 (1.8%)	0 (0%)	
Chemotherapy then pelvic radiation	4 (2.6%)	1 (1.8%)	3 (3.0%)	
Chemotherapy	14 (9.0%)	5 (8.8%)	9 (9.1%)	

MMR: mismatch repair, SD: standard deviation, LVSI: lymphovascular invasion.

Discussion

Universal screening with IHC for MMR proteins in endometrial cancer is recommended in many countries. The loss rate of MMR IHC expression was reported between 24-34%[8-12]. Our study demonstrated a loss rate of 35.9%. This finding is consistent with Spanish studies, which found approximately 34%[8]. Studies from USA (20-25%)[9,12] and China (24%)[10], reported lower rates of MMR deficiency. Thus, different ethnicities might influence the findings. The MLH1/PMS2 proteins exhibited loss of expression most often. Our study reported approximately 73.6% loss of expression; this rate is similar to previous studies that reported between 72-81%[9,12]. The revised Bethesda guidelines remain the current clinical criteria for the identification of endometrial patients at risk of having LS in Thailand. In our study, MMR deficiency was detected in one-third of the patients who did not meet the Bethesda guidelines. Recent published data indicates that approximately 41% of patients can be undiagnosed using traditional LS screening[13]. There is much evidence to support universal screening for LS with microsatellite instability (MSI) and/or IHC for MMR proteins in all colorectal carcinoma[7,14]. It appears that MSI is less sensitive than IHC in detecting MSH6 mutation carriers, which exhibit a higher lifetime risk of developing endometrial cancer than colorectal cancer[4]. Although there is evidence to confirm that both MSI and IHC possess excellent sensitivity and specificity for identifying patients with LS, IHC is sufficient for determining MMR deficiency in endometrial cancer. The concordant rate between these two techniques was approximately 94-100%[15-17]. However, IHC is more advantageous than MSI in many aspects: it is less expensive, it is widely available in most pathological centres, and it can guide specific MMR genes that are most likely to have a germline mutation. Nevertheless, improper tissue fixation can result in weak or equivocal staining patterns or be less reliable[14].

Identification of MMR deficient status is beneficial for several reasons. First, an assessment of the molecular classification in comparison to pathological risk groups can be used as a prognostic factor, especially in early stage endometrial cancer. Stage 1 endometrial cancer patients with intermediate to high risk factors, combined with MMR deficiency, exhibited a higher recurrence rate than those with MMR proficiency. It may reduce over-treatment in favourable cases and select unfavourable cases for more intensive treatment[18,19]. Second, it can guide adjuvant treatment, as patients with MMR deficiency may respond to immunotherapy[17]. Third, further genetic testing should be offered to confirm LS. This information may be helpful for guiding further investigation and treatment. LS increases the risk of synchronous and metachronous malignancies compared with the general population. It has been well established that endometrial cancer often precedes colorectal and other LS-related malignancies. In more than half of the patients, gynecologic cancer occurred before the diagnosis of other LS-related cancers with a median duration of 11 years[20]. Therefore, comprehensive cancer surveillance and risk-reducing surgeries should be considered. If LS was established at the time of endometrial biopsy, this information may influence the treatment options, especially in young women. There is a trend of increasing the incidence of endometrial cancer in the young. In this study, 19% of patients were younger than 50 years and 10% were younger than 40 years[21]. Conservative medical treatments or fertility sparing surgery to conserve ovaries should be discussed. Patients should also realize and weigh the risks of synchronous ovarian cancer and worsening prognosis by delaying surgery[13]. Because of its hereditary basis, a genetics evaluation should be offered to patients' family members to identify those who may be at risk and recommend LS-related cancer surveillance.

MMR deficiency by IHC was detected in 35.9% of endometrial cancer patients in our study. Tumour testing with IHC is inexpensive and available in most pathology laboratories. If expression of an MMR protein is absent in any gene, the patients should be offered genetic counselling and further genetic testing. If all four MMR proteins are expressed, the presence of LS is unlikely. Further study to confirm germline MMR mutation in patients with MMR deficiency is ongoing. This study might be the first study to evaluate the

incidence of LS in Thai patients with endometrial cancer. MMR deficiency was still detected in at least one-third who did not meet the revised Bethesda guidelines. Screening endometrial cancer patients for MMR IHC should be considered to diagnose and prevent LS-related cancers in both patients and their relatives.

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Potential conflicts of interest

The authors declare no conflict of interest.

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Potential Factors Associated with Pelvic Lymph Node Metastasis in Endometrioid Endometrial Carcinoma

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ABSTRACT

Objectives: To explore factors that associated with pelvic lymph node metastasis in endometrioid endometrial carcinoma

Materials and Methods: Two hundred and ninety-three patients with endometrioid endometrial carcinoma, who received surgical staging in Siriraj Hospital during 2008-2016, were studied. The relationship between pelvic lymph node metastasis and these data: demographic factors, biochemical markers, preoperative and intraoperative tumor characteristics were analysed by using a logistic regression model.

Results: From multivariate analysis, associated factors were grade of tumor, platelet count, deep myometrial invasion (deep MI: more than half of myometrial invasion) and size of tumor. Platelet count and size of tumor were re-calculated. Thrombocytosis (platelet count more than or equal to 380,000/mm^3) and large tumor (tumor size more than or equal to 6 centimeters) were statistically significant for cut-off point.

Conclusion: Grade of tumor, platelet count, more than half of depth of myometrial invasion and size of tumor, were associated with pelvic lymph node metastasis in endometrioid endometrial carcinoma.

Keywords: endometrial carcinoma, risk factors, pelvic lymph node metastasis.

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ปัจจัยที่สัมพันธ์กับการแพร่กระจายไปต่อมน้ำเหลืองในอุ้งเชิงกรานในมะเร็งเยื่อบุมดลูกชนิด endometrioid

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บทคัดย่อ

วัตถุประสงค์: *เพื่อค้นหาปัจจัยที่สัมพันธ์กับการแพร่กระจายไปต่อมน้ำเหลืองในอุ้งเชิงกรานในมะเร็งเยื่อบุมดลูกชนิด endometrioid*

วัสดุและวิธีการ: *การศึกษาย้อนหลังโดยนำข้อมูลจากผู้ป่วยมะเร็งเยื่อบุมดลูกชนิด endometrioid 293 ราย ที่ได้รับการผ่าตัดประเมินระยะของโรคในโรงพยาบาลศิริราช ระหว่างปี พ.ศ.2551-2560 มาศึกษาความสัมพันธ์ระหว่างการกระจายของมะเร็งไปยังต่อมน้ำเหลืองอุ้งเชิงกรานและปัจจัยต่างๆ ได้แก่ ข้อมูลพื้นฐานของผู้ป่วย ค่าผลเลือด การประเมินคุณลักษณะของเนื้องอกก่อนผ่าตัดและระหว่างผ่าตัด โดยนำไปวิเคราะห์ข้อมูลผ่านการวิเคราะห์ถดถอยโลจิสติกส์ จากนั้นนำตัวแปรที่มีนัยสำคัญมาให้คะแนนถ่วงน้ำหนักเป็นจำนวนเต็ม โดยอ้างอิงจากค่า odds ratio ที่ใกล้เคียงที่สุดของแต่ละตัวแปร ในที่สุดจะนำผลรวมมาคำนวณเพื่อทำนายโอกาสที่จะเกิดการกระจายไปยังต่อมน้ำเหลืองอุ้งเชิงกราน*

ผลการศึกษา: *จากการวิเคราะห์พหุตัวแปร ปัจจัยที่มีนัยสำคัญ ได้แก่ ระดับทางพยาธิวิทยาของเนื้องอก การเพิ่มขึ้นของจำนวนเกล็ดเลือด การลุกลามเข้ากล้ามเนื้อมดลูกชั้นลึก (กำหนดให้เกินครึ่งหนึ่งของชั้นกล้ามเนื้อทั้งหมด) และเนื้องอกขนาดใหญ่ สำหรับจำนวนเกล็ดเลือด และขนาดเนื้องอกได้รับการคำนวณอีกครั้งเพื่อหาจุดตัดที่เหมาะสม ผลลัพธ์คือการเพิ่มขึ้นของจำนวนเกล็ดเลือดตัดที่มากกว่า เท่ากับ 380,000 เซลล์ต่อลูกบาศก์มิลลิเมตร ส่วนขนาดเนื้องอก 6 เซนติเมตร ขึ้นจัดเป็นเนื้องอกขนาดใหญ่*

สรุป: *ระดับทางพยาธิวิทยาของเนื้องอก, การเพิ่มขึ้นของจำนวนเกล็ดเลือด, การลุกลามเข้ากล้ามเนื้อมดลูกชั้นลึก และเนื้องอกขนาดใหญ่ เป็นปัจจัยที่สัมพันธ์กับการแพร่กระจายไปต่อมน้ำเหลืองในอุ้งเชิงกรานในมะเร็งเยื่อบุมดลูกชนิด endometrioid*

คำสำคัญ: *มะเร็งเยื่อบุมดลูก, ปัจจัยเสี่ยง, การแพร่กระจายของต่อมน้ำเหลืองในอุ้งเชิงกราน*

Introduction

Endometrial carcinoma is the most common gynaecologic malignancy in developed countries. The incidence is increasing in developing countries including Thailand. From national cancer institute, endometrial carcinoma is currently the third most common gynaecologic cancer in Thailand[1]. Approximately eighty percent of cases are endometrioid type which usually presents in early stage and suitable for surgical staging: total hysterectomy, bilateral salpingo-oophorectomy and lymph node evaluation. Adjuvant managements will be tailored according to the uterine lesion and lymph node status[2, 3].

Endometrioid carcinoma patients are usually old age, obese and related with medical condition such as diabetes mellitus or hypertension[2]. Two randomized trials concluded that no survival benefit from lymph node removal in endometrial carcinoma patients[4, 5]. However, SEPAL study showed therapeutic benefit of pelvic and para-aortic lymph node dissection in endometrial carcinoma patients[6]. This procedure increased blood loss, operative time and sometime complicated by lymphocele and lymphedema. Most of the guidelines suggested selective pelvic and para-aortic lymph node dissection in the high risk cases such as high grade, large tumor, deep myometrial invasion or extrauterine disease[2, 7, 8]. Advanced imaging did not have good sensitivity to detect lymph node metastasis in endometrial carcinoma. Aleediman, et al recently showed the preoperative serum inflammatory response associate with lymph node metastasis in endometrial carcinoma including lower haemoglobin level, higher neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), monocyte-lymphocyte multiplication (MNM) and platelets count[9].

In Thailand, there were no studies that indicate significant factors associated with pelvic lymph node metastasis. The objective of this study was to explore factors that associated with pelvic lymph nodes metastasis in endometrioid endometrial carcinoma patients. This may help to define which cases may be able to omit lymph node dissection or which cases should have lymph node dissection to avoid those side effects.

Materials and Methods

We conducted the case-control study for this objective. After approved by Siriraj Institutional Review Board, data from Medical Statistic Unit was retrospectively reviewed. Patients with endometrioid endometrial carcinoma underwent surgical staging, including pelvic lymph node dissection at Siriraj Hospital during 2008-2016 were included. Incomplete pathologic and laboratory data were excluded. Case was defined as endometrioid endometrial carcinoma that had positive pelvic lymph node metastasis. Control was endometrioid endometrial carcinoma that had no pelvic lymph node metastasis. From pilot study of 30 cases, the ratio of case and control was 1:3. Sample size was calculated using variables that associated with pelvic lymph node metastasis such as age, grade of tumor, size of tumor and more than half of depth of myometrial invasion. Age yielded the highest number of 72 cases and 216 controls. Totally 293 patients were enrolled. Cases were all pelvic lymph node metastasis patients during that period. Three controls were randomly selected from negative pelvic lymph node patients at the same 3 months' time period of each case.

Preoperative data included age, body mass index (BMI) at the date of surgery, grade of tumor from endometrial biopsy data and complete blood count (CBC) within 3 months before surgery. CBC data including, hemoglobin (Hb), hematocrit (Hct), absolute neutrophil count (ANC), platelet count, NLR and PLR were collected. Intraoperative data included tumor size, depth of myometrial invasion, cervical stromal involvement and ovarian involvement were collected. Pathological examination pelvic lymph nodes were divided patients into case and control group.

The data was analysed by the application of SPSS statistics 21. T-test was used for continuous data while Pearson chi-square test and Mann-

Whitney test were used to analyse the association between each categorical variables and pelvic lymph node metastasis. Logistic regression analysis was performed to determine independent associated factors for pelvic lymph node metastasis adjusting for potential confounders.

Results

The total of 293 patients were recruited, 72 cases and 221 controls. Mean age was similar in case and control group, 57.46 and 57.78 years respectively. In univariate analysis; mean BMI, hemoglobin, hematocrit, platelet count, NLR and PLR, high grade of tumor, tumor size, more than half of myometrial invasion and cervical stromal involvement increased risk for pelvic lymph node metastasis (Table 1). The median number of lymph node obtained was 13 (13.5 in case and 13 in control group). The ratio of positive LN in patient obtained less than 13 lymph nodes and more than or equal to 13 lymph nodes were 25% (33/132) and 24.22% (39/161).

Table 1. Unadjusted variables comparing between pelvic LN metastasis and no pelvic LN metastasis.

Characteristic	Pelvic LN metastasis (n = 72)	No pelvic LN metastasis (n = 221)	p value
Mean age (years)	57.46	57.78	0.82
Mean BMI (kg/m^2)	25.57	27.97	< 0.001
Hemoglobin (g/dL)	12	12.6	0.001
Hematocrit (%)	36.36	38.63	< 0.001
NLR	3.66	2.22	< 0.001
ANC (/mm^3)	4800	4340	0.05
PLR	159.21	123.37	< 0.001
Platelet (/mm^3)	304,500	270,000	0.002
Grade of tissue sampling			
- Grade 1	16 (22.2%)	119 (53.9%)	
- Grade 2	30 (41.7%)	75 (33.9%)	
- Grade 3	26 (36.1%)	27 (12.2%)	
Median size of lesion (cm) (range)	8 (1-10)	3 (0-8)	< 0.001
> 50% depth of myometrial invasion	50 (69.4%)	58 (26.2%)	< 0.001
Cervical stromal involvement	16 (22.2%)	11 (5.0%)	< 0.001
Ovarian involvement	7 (9.7%)	8 (3.6%)	0.06
Median number of LN (range)	13.5 (1-36)	13 (1-48)	0.509

LN = lymph node
BMI = body mass index
NLR = neutrophil-lymphocyte ratio
ANC = absolute neutrophil count
PLR = platelet-lymphocyte ratio

Logistics regression model had been used for multivariate analysis. Factors associated with pelvic lymph node metastasis were higher grade of tumor, more than half of myometrial invasion, platelet count

and tumor size (Table 2). We explored the cut-off points for both platelet count and size of lesion by using receiver operating characteristics (ROC) curve. The proper cut-off point for platelet count was greater than or equaled to 380,000/mm^3 with the area under ROC curve of 0.624 (Fig. 1a). The proper cut-off point of tumor size was larger than or equaled to 6 centimeters with the area under ROC curve of 0.711 (Fig. 1b). Logistic regression was recalculated using platelet count greater than or equaled to 380,000/mm^3 and tumor size larger than or equaled to 6 centimeters as two new categorical variables. Factors associated with pelvic lymph node metastasis were higher grade of tumor, more than half of myometrial invasion, platelet count greater than 380,000/mm^3 and tumor size larger than 6 centimeters (Table 3).

Table 2. Odds ratios from logistic regression model with dependent variables and pelvic LN metastasis.

	Adjusted ORs	p value
BMI (kg/m^2)	0.948	0.072
Grade	5.974	< 0.001
Hemoglobin (g/dL)	0.863	0.703
Hematocrit (%)	1.028	0.834
NLR	1.047	0.523
PLR	1.002	0.653
Platelet (/mm^3)	1.000	0.005
Size of tumor (cm)	1.142	0.006
> 50% depth of myometrial invasion	5.156	< 0.001
Cervical stromal involvement	1.063	0.906
Ovarian involvement	1.35	0.699

LN: lymph node
ORs: odds ratio
BMI: body mass index
NLR: neutrophil-lymphocyte ratio
PLR: platelet-lymphocyte ratio

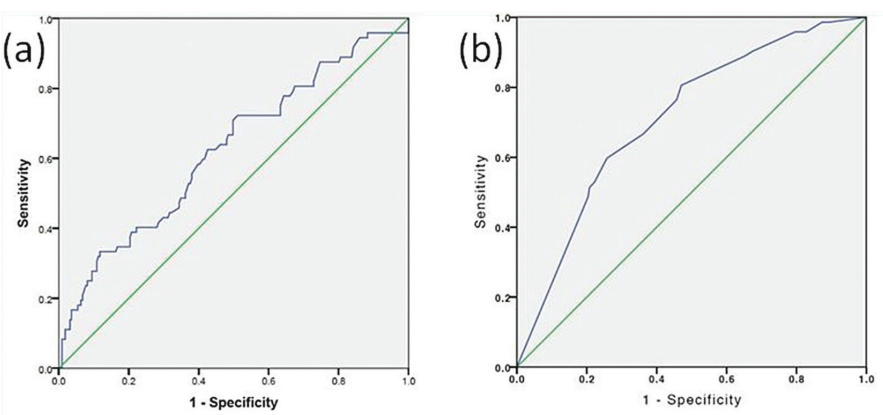

Fig. 1. Receiver operating characteristic curves of factors prediction of pelvic lymph node metastasis (a) platelet count (b) size of tumor.

Table 3. Odds ratios from logistic regression model with dependent variables and pelvic LN metastasis after new cut-off point of platelets and size of tumor.

	Adjusted ORs	p value
BMI (kg/m^2)	0.951	0.092
Grade	5.654	< 0.001
Hemoglobin (g/dL)	0.878	0.738
Hematocrit (%)	1.016	0.902
NLR	1.068	0.553
PLR	1.001	0.809
Platelet > 380,000/mm^3	3.306	0.004
Size of tumor > 6 cm	2.213	0.020
> 50% depth of myometrial invasion	5.435	< 0.001
Cervical stromal involvement	1.083	0.879
Ovarian involvement	1.411	0.657

LN: lymph node
ORs: odds ratio
BMI: body mass index
NLR: neutrophil-lymphocyte ratio
PLR: platelet-lymphocyte ratio

Discussion

Pelvic lymph node dissection is one of the important steps of surgical staging for endometrioid endometrial carcinoma and affects further treatments. However, this procedure can lead to numerous complications. The survival benefit is still inconclusive. Selective pelvic lymph node dissection is widely practiced but there are no definite data to support surgeons' judgments. The median number of lymph node was 13, similar to our previous data[10]. We did not exclude the patient with lymph node obtained less than 13 because the ratio of pelvic lymph node metastasis was comparable with the group of lymph node more than or equal to 13, 25% and 24.22%, respectively.

This study found that there were four factors associated with pelvic lymph node metastasis: higher grade of tumor, depth of myometrial invasion, larger tumor size and higher platelet count. There are no doubt in higher tumor grade and deep myometrial invasion to be indications for pelvic and para-aortic lymph node dissection as suggest in most of the guideline[2, 7, 8]. According to previous data, there were some different details for tumor size and platelet count. Previous data suggested that lesions larger than 2 centimeters were associated with pelvic lymph node metastasis[11, 12]. Our study found that the tumor size of 6 centimeters was the most proper cut-point in multivariate analysis.

Shah, et al studied the relationship of tumor size and lymph node metastasis. The result showed that only tumor size greater than or equal to 8 centimeters conferred a significant risk of lymph node metastasis[13]. However, in multivariate logistic regression, tumor size did not show the statistically significance with the odds ratio of 1.3 (95% CI, 1.0-1.6). This result may be affected by including of the non-endometrioid tumor which has high metastatic nature even with the small tumor. The only 3 variables that remained significant independent predictors were clear cell or papillary serous, grade 3 histology and lymphovascular space invasion. Our study did not include non-endometrioid histology which had the higher risk of lymph node metastasis and already recommended by Society of Gynecologic Oncology to undergo lymphadenectomy in every

patient[14]. The lymphovascular space invasion data were mostly obtained from hysterectomy specimen, barely mentioned in endometrial sampling specimen. We did not include this postoperative factor in our predictors. The grade 3 histology obviously showed the same result as ours.

Teixeira, et al found that grade of tumor, tumor extension (International Federation of Gynecology and Obstetrics stage) and lower uterine segment involvement were significant factors for lymph node metastasis on multivariate analysis[15]. These data were supported our data on grade 3 histology and deep myometrial invasion. However, size of tumor was not a significant factor. The size of tumor was obtained from pathology report which may alter the real size of tumor. Our study reported the median size of tumor at 3 and 8 centimeters in no pelvic lymph node metastasis group and pelvic lymph node metastasis group, respectively. While Teixeira, et al found median size of tumor at 3.5 and 5 centimeters. Another possible reason, multicenter sites may have different method in tumor size collection. Zhang, et al., also reported the clinicopathological factors associated with pelvic lymph node metastasis. Only deep myometrial invasion and lymphovascular space invasion were independent risk factors, based on multivariate analysis[16]. Unfortunately, most of interested factors were obtained from postoperative data. This may not help to decide who should undergo lymph node dissection.

For platelet count, Luomaranta indicated thrombocytosis from average Finnish female population as > 360,000/mm^3[17], Aleediman used ≥ 297,500/mm^3 as cut-point by computing from ROC curve[9]; while our study found that 380,000/mm^3 was the most proper cut-point for thrombocytosis, also calculated from ROC curve. Moreover, although other biomarkers were announced as significant data responsible for host immune response: leukocytosis in Luomaranta's study and lower Hb level, higher NLR, PLR in Aleediman's study[9, 17]; our study found no significant associations between those biomarkers and pelvic lymph node metastasis except thrombocytosis.

Compared with previous studies, especially in Thailand, this research had covered more aspects including demographic data, preoperative histopathological examination, preoperative immune-response biomarkers, intraoperative gross tumor size, and extrauterine evaluation. Moreover, these data were collected from a reliable system which the data can be traced back for more than a decade. Using routine data such as preoperative endometrial biopsy result and complete blood count did not increase any cost. However, there were some limitations from retrospective data collection. We excluded patients who did not undergo pelvic lymph node dissection. These patients could also be in very early stage or very advanced stage of disease. We plan to use the data from logistic regression model for calculation the risk scoring formula, then prove the formula and absolutely comparing with pre-existing formula in a further prospective study.

Conclusion

Factors associated with pelvic lymph node metastasis in endometrioid endometrial carcinoma were high grade tumor, thrombocytosis (more than 380,000/mm^3), tumor size larger than 6 centimeters and deep myometrial invasion.

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Potential conflicts of interest

The authors declare no conflict of interest.

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OBSTETRICS

Relationship between Antenatal Maternal Neutrophil-to-Lymphocyte Ratio and Early Onset Neonatal Sepsis in Preterm Neonates

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ABSTRACT

Objectives: To determine the relationship between maternal neutrophil to lymphocyte ratio (NLR) and early onset neonatal sepsis (EONS) in preterm neonates.

Materials and Methods: Between 2012 to 2016, a total of 485 cases of preterm delivery were retrospectively reviewed. Study group consisted of 97 neonates diagnosed with EONS and other 388 without EONS were randomly selected as a comparison group (1:4 ratio). Data were extracted from medical records, including baseline characteristics, obstetric and delivery data. Maternal NLR was calculated from laboratory results within 72 hours prior to delivery.

Results: Neonates with EONS were significantly more likely to deliver at < 34 weeks ($p < 0.001$), had preterm premature rupture of membranes ($p = 0.043$), and had maternal fever ($p = 0.016$). White blood cell and neutrophil counts were significantly higher in EONS group while lymphocyte counts were comparable. Median NLR was significantly higher in EONS group (4.7 vs. 4.1, $p = 0.005$). NLR of > 5 significantly increased the risk of EONS (26.8% vs. 16.4%, $p = 0.007$). Logistic regression analysis showed that delivery at < 34 weeks and maternal fever were independently associated with EONS (adjusted odds ratio (ORs) 11.5, 95% confidence interval (CI) 6.7-19.7, and 3.4, 95% CI 1.1-11.3, respectively). Subgroup analysis showed that NLR of ≥ 5 independently increased the risk of EONS in those delivered at < 34 weeks (adjusted ORs 3.5, 95% CI 1.4-9.1) and maternal fever independently increased the risk of EONS in those delivered at ≥ 34 weeks (adjusted ORs 6.1, 95% CI 1.8-20.3).

Conclusion: Maternal NLR was significantly associated with EONS in preterm neonates, especially those delivered at < 34 weeks.

Keywords: preterm neonates, early onset neonatal sepsis, neutrophil to lymphocyte ratio.

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ความสัมพันธ์ระหว่างอัตราส่วนเม็ดเลือดขาวชนิดนิวโตรฟิลล์ต่อลิมโฟไซต์ของมารดากับการเกิดภาวะติดเชื้อแรกเกิดระยะแรกในทารกคลอดก่อนกำหนด

ฐิติพรรณ ชยวงศ์รุ่งเรือง, จิรพร เหลืองเมตตากุล, อังสุมาลิน ศรีหล้า

บทคัดย่อ

วัตถุประสงค์: *เพื่อหาความสัมพันธ์ระหว่างอัตราส่วนเม็ดเลือดขาวชนิดนิวโตรฟิลล์ต่อลิมโฟไซต์ของมารดากับการเกิดภาวะติดเชื้อแรกเกิดระยะแรกในทารกคลอดก่อนกำหนด*

วัสดุและวิธีการ: *การศึกษาย้อนหลังโดยศึกษาสตรีที่คลอดก่อนกำหนดในช่วงปี พ.ศ.2555 จนถึง ปี พ.ศ.2559 โดยพิจารณาค่าผลเลือดอัตราส่วนเม็ดเลือดขาวชนิดนิวโตรฟิลล์ต่อลิมโฟไซต์ของมารดาก่อนคลอด 72 ชั่วโมง กับการเกิดภาวะติดเชื้อแรกเกิดระยะแรกของทารก กลุ่มประชากร ได้แก่ ทารกคลอดก่อนกำหนดที่มีภาวะติดเชื้อแรกเกิดระยะแรกจำนวน 97 คน และทารกคลอดก่อนกำหนดที่ไม่มีภาวะติดเชื้อแรกเกิดระยะแรกจำนวน 388 คน (อัตราส่วน 1 ต่อ 4)*

ผลการศึกษา: *การเพิ่มขึ้นของอัตราส่วนเม็ดเลือดขาวชนิดนิวโตรฟิลล์ต่อลิมโฟไซต์ของมารดาก่อนคลอดสัมพันธ์กับการเกิดภาวะติดเชื้อแรกเกิดระยะแรกในทารกที่คลอดก่อนกำหนดอย่างมีนัยสำคัญทางสถิติ โดยค่ามัธยฐานของอัตราส่วนเม็ดเลือดขาวชนิดนิวโตรฟิลล์ต่อลิมโฟไซต์ของมารดาในกลุ่มที่ทารกมีภาวะติดเชื้อแรกเกิดระยะแรกเท่ากับ 4.7 ในขณะที่ค่ามัธยฐานของอัตราส่วนดังกล่าวของกลุ่มมารดาที่ทารกไม่มีภาวะติดเชื้อแรกเกิดระยะแรกเท่ากับ 4.1 ($p = 0.005$) ปัจจัยสำคัญที่ทำให้เกิดภาวะการติดเชื้อแรกเกิดระยะแรก ได้แก่ อายุครรภ์คลอดที่คลอดน้อยกว่า 34 สัปดาห์ ($p < 0.001$) ภาวะน้ำเดินก่อนเจ็บครรภ์คลอด ($p = 0.043$) และการที่มารดามีไข้ก่อนคลอด ($p = 0.016$) และเมื่อพิจารณาค่าการตรวจนับเม็ดเลือดอย่างสมบูรณ์ของมารดาพบว่า กลุ่มมารดาที่ทารกมีภาวะติดเชื้อแรกเกิดระยะแรกจะมีจำนวนเม็ดเลือดขาวทั้งหมด และจำนวนเม็ดเลือดขาวชนิดนิวโตรฟิลล์ก่อนคลอดสูงกว่า ($p = 0.041$ และ 0.001 ตามลำดับ) แต่จำนวนเม็ดเลือดขาวชนิดลิมโฟไซต์ไม่ต่างกันในสองกลุ่ม นอกจากนี้ความเสี่ยงที่ทารกแรกเกิดที่คลอดก่อนกำหนดจะมีภาวะติดเชื้อแรกเกิดระยะแรกเพิ่มมากขึ้น เมื่อค่าอัตราส่วนเม็ดเลือดขาวชนิดนิวโตรฟิลล์ต่อลิมโฟไซต์ของมารดาก่อนคลอด ≥ 5 (ร้อยละ 26.8 vs 16.4, $p = 0.007$) การวิเคราะห์ถดถอยแบบโลจิสติก พบว่าปัจจัยที่สัมพันธ์กับการเกิดภาวะการติดเชื้อแรกเกิดระยะแรกคือการคลอดที่อายุครรภ์ที่น้อยกว่า 34 สัปดาห์ และการที่มารดามีไข้ก่อนคลอด (adjusted ORs 11.5, 95% CI 6.7-19.7 และ 3.4, 95% CI 1.1-11.3 ตามลำดับ) และเมื่อวิเคราะห์แยกกลุ่มพบว่าอัตราส่วนดังกล่าวของมารดาที่ ≥ 5 จะเพิ่มความเสี่ยงของภาวะการติดเชื้อแรกเกิดระยะแรกในกรณีที่ทารกคลอดก่อน 34 สัปดาห์ (adjusted ORs 3.5, 95% CI 1.4-9.1) และการที่มารดามีไข้ก่อนคลอดจะเพิ่มความเสี่ยงของการเกิดภาวะการติดเชื้อแรกเกิดระยะแรกในทารกที่คลอดตั้งแต่ 34 สัปดาห์ขึ้นไป (adjusted ORs 6.1, 95% CI 1.8-20.3)

สรุป: *ค่าอัตราส่วนเม็ดเลือดขาวชนิดนิวโตรฟิลล์ต่อลิมโฟไซต์ของมารดาก่อนคลอดสัมพันธ์กับการเกิดภาวะการติดเชื้อแรกเกิดระยะแรกในทารกที่คลอดก่อนกำหนด โดยเฉพาะกรณีที่คลอดก่อน 34 สัปดาห์*

คำสำคัญ: *ทารกคลอดก่อนกำหนด, ภาวะการติดเชื้อแรกเกิดระยะแรก, อัตราส่วนเม็ดเลือดขาวชนิดนิวโตรฟิลล์ต่อลิมโฟไซต์*

Introduction

Early onset neonatal sepsis (EONS) is an acquired infection according to peripartum vertical mother-to-newborn transmission[1]. The most important neonatal factor of mortality from such condition is prematurity or low birth weight (LBW). According to National Institute of Child Health and Human Development neonatal research network data, the incidence rate of infection is 3 to 10-fold higher in LBW infant comparing to full term normal weight infant and their consequence complications are more hazardous such as respiratory distress syndrome, intraventricular hemorrhage, necrotizing enterocolitis or even death[2]. Current evidence suggests that intraamniotic or intrauterine infection is the major cause of preterm labor accounting for 25-40% of preterm birth with intact membranes[3]. Although the inflammation is subclinical or occurs without clinical signs of chorioamnionitis, there are histological evidence of inflammation in the fetal membranes, umbilical cord or decidua. In order to identify definite intrauterine or placental inflammation, invasive procedures, such as amniocentesis or cordocentesis are needed. Several maternal serum biomarkers have been investigated for their use in predicting in-utero inflammation, including white blood cell (WBC) count, C-reactive protein (CRP), procalcitonin, interleukin (IL)-6, IL-8, IL-10, interferon gamma and others[4-6].

Neutrophil to lymphocyte ratio (NLR) is a marker of inflammatory disease and prognostic value in several types of cancers or cardiovascular diseases[7-11]. It is a simple, inexpensive and safe test which can be obtained from maternal peripheral blood. However, information on the use of NLR in women with preterm delivery to predict or identify neonates at risk for EONS is limited and controversial. Therefore, the primary objective of this study was to determine the relationship between antenatal maternal NLR and EONS in preterm neonates. Secondary objective was to identify factors associated with EONS among preterm neonates. In addition, appropriate cut-off value of NLR to predict EONS was also evaluated.

Materials and Methods

After approval of ethics committee of Medical Service Department, Bangkok Metropolitan Administration, a study was conducted in 485 preterm neonates who were born between 2012 to 2016 in Chareonkrung Pracharak Hospital, Bangkok, Thailand. Inclusion criteria were singleton, preterm infants who delivery between 24 and 36^{+6} weeks of gestation and had maternal complete blood count data within 72 hours prior to delivery. Exclusion criteria were neonates with chromosomal anomalies or metabolic syndrome, mother with preeclampsia, chorioamnionitis, hepatitis, cardiovascular disease, rheumatoid disease, cancer, polycystic ovary syndrome, connective tissue disease, Crohn's disease and history of cervical excision procedure or cervical cerclage.

This study was a retrospective study. Sample size was estimated from data of pilot study that showed NLR in mothers of EONS of 6.8 ± 4.8 and 5.2 ± 4.5 in those without EONS. At 95% confidence level (CI) and 80% power and 1:4 ratio, at least 87 neonates with EONS and 348 without EONS were required.

Preterm neonate was defined as neonate who were born between 24 and 36^{+6} weeks of gestation. EONS was defined as the presence of confirmed or suspected sepsis within 72 hours after birth which was diagnosed by one of the following criteria 1.) positive culture in peripheral blood or cerebrospinal fluid 2.) absence of positive culture but consisting of two or more following: 2.1) body temperature < 36.5°C or >37.5°C; 2.2) respiratory rate > 60/min or grunting or retraction or nasal alar flaring or partial pressure of oxygen (PaO_2) < 70 mmHg; 2.3) heart rate < 100 beats per minute or > 180 beats per minute or capillary refill more than 3 seconds or blood pressure less than 2 time standard deviation at that age; 2.4) perfusion abnormalities; altered mental status or oliguria or acidosis (venous blood pH < 7.25); 2.5) immature to total neutrophil ratio ≥ 0.2 or neutropenia or thrombocytopenia; platelet < 150,000/mm^3 or CRP > 5 mg/mL after birth 6 hours or more[12, 13].

Maternal demographic data including antepartum, intrapartum, mode of delivery and neonatal outcome

data were extracted from medical records. The clinical data included baseline characteristics, obstetric data, labor and delivery data, treatment, and neonatal outcomes. Complete blood count data were retrieved from the latest laboratory results within 72 hours prior to delivery using sysmex XE-5000 and XT-20001 automatic analyzer in all patients. The neutrophil to lymphocyte ratio (NLR) was defined as the ratio of absolute neutrophil count and absolute lymphocyte count.

All statistical analyses were achieved using SPSS version 14.0 software (SPSS Inc, Chicago, IL, USA). Descriptive statistics including mean, standard deviation, number, and percentages were used to describe various characteristics as appropriate. Comparisons of various characteristics between cases and controls were performed with student's t test, chi-squared test, and Mann-Whitney U test as appropriate. NLR was compared between groups to determine its association with EONS, both as continuous data and categorical data with selected cut-off. Logistic regression analysis was used to determine independent associated factors of EONS, adjusting for potential confounders. A p value of < 0.05 was considered statistical significance.

Results

A total of 485 preterm neonates were included, 97 with EONS and 388 without EONS. Various baseline characteristics were compared between the 2 groups and the results are shown in Table 1. Mothers whose neonates had EONS were significantly more likely to have preterm premature rupture of membranes (34% vs. 24%, $p = 0.043$), received antenatal corticosteroids (53.6% vs. 14.4%, $p < 0.001$), received tocolytics (26.8% vs. 7.7%, $p < 0.001$), and had maternal fever (7.2% vs. 2.3%, $p = 0.016$).

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

Clinical characteristics	Early onset neonatal sepsis		p value
	Present (n = 97)	Absent (n = 388)	
Mean maternal age ± SD (years)	27.36 ± 7.3	27.27 ± 6.7	0.904*
Mean BMI ± SD (kg/m^2)	23.07 ± 6.39	24.88 ± 5.06	0.020*
Nulliparity, n (%)	37 (38.1%)	160 (41.2%)	0.579**
Previous preterm birth, n (%)	13 (13.4%)	31 (8.0%)	0.097**
Preterm PROM, n (%)	33 (34.0%)	93 (24.0%)	0.043**
Antenatal corticosteroid use, n (%)	52 (53.6%)	56 (14.4%)	< 0.001**
Antenatal antibiotic use, n (%)	92 (94.8%)	370 (95.4%)	0.831**
Tocolytic use, n (%)	26 (26.8%)	30 (7.7%)	< 0.001**
Maternal fever, n (%)	7 (7.2%)	9 (2.3%)	0.016**

BMI: body mass index; SD: standard variation; PROM: premature rupture of membranes; n: number of patients. * = independent samples t test, ** = chi-squared test.

Table 2 showed comparisons of neonatal outcomes between the 2 groups. Neonates with EONS significantly had worse outcomes than those without EONS, including lower gestational age (GA) at delivery, early preterm birth (< 34 weeks), lower birth weight, lower Apgar score, and higher rate of neonatal intensive care unit admission.

Maternal complete blood count results were compared between the 2 groups and the results are shown in Table 3. Mothers of EONS neonates had significantly lower hemoglobin and hematocrit levels. On the other hand, they had significantly higher mean WBC count, platelet counts, and neutrophil counts while mean lymphocytes count was comparable.

Comparison of NLR between the 2 groups are also demonstrated in Table 3. Neonates with EONS had significantly higher NLR than those without EONS [Median (interquartile range): 4.69 (3.32, 7.49) vs. 4.07 (2.95, 5.55), p = 0.005, respectively]. Using the cut-off value of 5 for NLR, the results showed that those with EONS were significantly more likely to have NLR ≥ 5 than those without EONS (46.4% vs. 31.7%, p = 0.007).

Table 2. Comparison of pregnancy outcomes between the 2 groups.

Characteristics	Early onset neonatal sepsis		p value
	Present (n = 97)	Absent (n = 388)	
Mean gestational age at delivery ± SD (days)	229.88 ± 22.48	249.29 ± 10.30	< 0.001*
Preterm birth			< 0.001**
Early preterm birth (< 34weeks), n (%)	54 (55.7%)	37 (9.5%)	
Late preterm birth (≥ 34weeks), n (%)	43 (44.3%)	351 (90.5%)	
Mean birth weight ± SD (grams)	1,985.13 ± 661.70	2,587.39 ± 444.64	< 0.001*
Apgar scores < 7			
At 1 min, n (%)	23 (23.7%)	11 (2.8%)	< 0.001**
At 5 min, n (%)	10 (10.3%)	1 (0.3%)	< 0.001**
Cesarean delivery, n (%)	33 (34.0%)	94 (24.2%)	0.050**
NICU admission, n (%)	52 (53.6%)	25 (6.4%)	< 0.001**

NICU: neonatal intensive care unit; n: number of patients. * = independent samples t test, ** = chi-squared test.

Table 3. Comparison of maternal laboratory results between the 2 groups.

Laboratory results	Early onset neonatal sepsis		p value
	Present (n = 97)	Absent (n = 388)	
Mean hemoglobin ± SD (g/dL)	11.51 ± 1.70	11.93 ± 1.35	0.026*
Mean hematocrit ± SD (%)	34.27 ± 4.51	35.61 ± 3.67	0.008*
Median white blood cell count (IQR) (cells/μL)	13,210 (10,710, 17,000)	11,930 (9,840, 14,000)	0.041**
Median neutrophil count (IQR) (cells/μL)	9,829 (7,679, 13,663)	8,827 (7,097, 10,752)	0.001**
Median lymphocyte count (IQR) (cells/μL)	2,068 (1,453, 2,719)	2,127 (1,687, 2,635)	0.417**
Median platelet count (IQR) (cells/μL)	262,000 (215,000, 301,500)	238,000 (205,000, 284,000)	0.018**
Median NLR (IQR)	4.69 (3.32, 7.49)	4.07 (2.95, 5.55)	0.005*
NLR category			0.007***
NLR < 5, n (%)	52 (53.6%)	265 (68.3%)	
NLR ≥ 5, n (%)	45 (46.4%)	123 (31.7%)	

SD: standard variation; g/dL: gram/deciliter; IQR: interquartile range; NLR: neutrophil to lymphocyte ratio; n: number of patients. * = independent samples t test, ** = Mann-Whitney U test, *** = chi-squared test.

Subgroup analysis was performed according to GA at delivery and the results are shown in Table 4. NLR ≥ 5 significantly increased the risk of EONS only among early preterm birth at < 34 weeks (72.7% vs. 46.8%, p = 0.012). On the other hand, such association was not observed among late preterm birth (10.5% vs. 11.1%, p = 0.853).

Table 5 showed the results of logistic regression analysis of all cases. The only 2 factors that independently associated with EONS were early preterm birth (< 34 weeks) with adjusted OR 11.53 (95% CI 6.74-19.73, p < 0.001) and maternal fever with adjusted OR 3.45 (95%CI 1.07-11.13, p = 0.038).

Table 4 . Subgroup analyses of relationship between maternal NLR and EONS according to gestational age at delivery.

Characteristics		Early onset neonatal sepsis		p value
		Present (n = 97)	Absent (n = 388)	
GA < 34 weeks	NLR < 5, n (%)	22 (22.7%)	25 (6.4%)	0.012*
	NLR ≥ 5, n (%)	32 (33.0%)	12 (3.1%)	
GA 34-36^{+6} weeks	NLR < 5, n (%)	30 (30.9%)	240 (61.9%)	0.853*
	NLR ≥ 5, n (%)	13 (13.4%)	111 (28.6%)	

GA: gestational age
NLR: neutrophil to lymphocyte ratio
EONS: early onset neonatal sepsis
n: number of patients
* = independent samples t test

Table 5. Logistic regression analyses to determine independent factors associated with EONS.

Variables	Adjusted ORs	95% Confidence Interval	p value
NLR ≥ 5	1.48	0.87 - 2.50	0.146
Gestational age < 34 weeks	11.53	6.74 - 19.73	< 0.001
PPROM	1.65	0.94 - 2.88	0.081
Antenatal antibiotics use	0.87	0.26 - 2.92	0.820
Maternal fever	3.45	1.07 - 11.13	0.038

EONS: Early onset neonatal sepsis
NLR: Neutrophil to lymphocyte ratio
PPROM: Preterm premature rupture of membranes
ORs: Odds ratio.

Logistic regression analysis was also performed separately between those with early and late preterm birth and the results are shown in Table 6. The only independent associated factor of EONS among early preterm birth was NLR ≥ 5 (adjusted OR 3.51, 95% CI 1.36-9.05, p = 0.009), while the only independent associated factor of EONS among late preterm birth was maternal fever (adjusted OR 6.06, 95% CI 1.80-20.34, p = 0.004).

Table 6. Logistic regression analyses to determine independent factors associated with EONS, stratified by gestational age at delivery.

Variables		Adjusted ORs	95% Confidence Interval	p value
GA < 34 weeks	NLR ≥ 5	3.51	1.36-9.05	0.009
	PPROM	1.91	0.70-5.20	0.204
	Antenatal antibiotics use	1.50	0.21-10.83	0.686
	Maternal fever	0.33	0.04-2.73	0.302
GA 34-36^{+6} weeks	NLR ≥ 5	1.01	0.50-2.04	0.986
	PPROM	1.37	0.67-2.80	0.389
	Antenatal antibiotics use	0.82	0.18-3.76	0.799
	Maternal fever	6.06	1.80-20.34	0.004

EONS: early onset neonatal sepsis
GA: gestational age
NLR: neutrophil to lymphocyte ratio
PPROM: preterm premature rupture of membranes

Discussion

In this study, increased maternal serum NLR was significantly increased in preterm neonates with EONS. NLR ≥ 5 was the only independent factor associated with EONS among early preterm birth (< 34 weeks) while maternal fever was the only independent factors associated with EONS among late preterm birth (≥ 34 weeks).

Intrauterine infection was one of the major causes of infection associated preterm labor or preterm premature rupture of membranes even in asymptomatic patient. EONS, by the definition, was the presence of confirmed or suspected sepsis within 72 hours after birth referred to acquired infection before or during delivery (vertical mother-to-child transmission). The fetus exposing to microorganism in utero may cause fetal inflammatory response syndrome, including funisitis[6]. Therefore, several studies have attempted to evaluate prenatal diagnosis of such intrauterine infection or funisitis. Previously reported tests included Gram staining and culture of amniotic fluid via amniocentesis, the measurement of leukoattractants, glucose concentration, WBC count, IL-6 and matrix metalloproteinase-8 (MMP-8) in amniotic fluid[5], cordocentesis for evaluation fetal plasma IL-6 concentration, and tumor necrotic factor-α. However, such procedures are invasive and difficult to achieve. Amniocentesis is related to a risk of fetal loss of approximately 0.2-2.9%[14, 15], and can possibly cause infection by itself. Moreover, histological study of chorioamnionitis can be done only after birth and delay diagnosis.

Many studies have investigated more rapid, noninvasive testing, especially inflammation markers, for evaluation of the infection in effort to predict the EONS. These markers included WBC count, CRP, procalcitonin, IL-6, IL-8, IL-10, interferon gamma and others. Lee, et al., found that maternal serum CRP was significantly associated with funisitis and EONS[6]. Cetin, et al., also studied the prediction of EONS from antenatal non-invasive biomarkers and found that maternal blood procalcitonin level were superior to CRP and WBC count[4].

From a previous study, Kim, et al reported that NLR had a better overall diagnostic performance than maternal serum CRP levels in predicting placenta inflammatory response and distinguishing histologic chorioamnionitis from funisitis[16]. Once the microorganism invaded into intrauterine cavity or placental membranes, neutrophils were recruited into

the site of infection guided by extracellular chemoattractant, proinflammatory factors and several cytokines leading to neutrophilia in maternal blood circulation[3, 17, 18]. Contrary, both T and B lymphocytes were downregulated possibly because of the need to prevent priming of graft-versus-host-type reactivity, which could result in lymphocytopenia.

Therefore, the neutrophil to lymphocyte ratio (NLR) has been proposed to be a parameter of systemic inflammation and prognostic marker in various diseases, including oncologic diseases, septic shock, cardiovascular diseases, and many obstetrics conditions[9-11, 19, 20]. NLR was found to be increased in hyperemesis gravidarum, gestational diabetes, preeclampsia and spontaneous preterm delivery[21-25]. Importantly, NLR has been evaluated as a predictive marker for placental inflammatory response in preterm births[16].

The results of the present study showed that maternal NLR significantly increased among neonates with EONS. However, subgroup analysis showed that NLR of ≥ 5 significantly increased the risk of EONS only among early preterm birth (< 34 weeks) by 3.5 times. This was similar to previous reports by Kim, et al., and Hamiel, et al., which also reported that NLR alone or combined with absolute neutrophil count was a good tool for identification of serious bacterial disease, especially in neonates[16, 26]. On the other hand, no significant association was observed in late preterm birth (≥ 34 weeks) and only maternal fever was significantly increased the risk of EONS by 6 times. In terms of diagnostic performance of NLR, the cut-off value of NLR ≥ 5 had only 46% sensitivity, 68% specificity, 27% positive predictive value and 84% negative predictive value. In subgroup analyses in early preterm birth, NLR ≥ 5 had 59% specificity, 68% specificity, 73% positive predictive value and 68% negative predictive value.

This study was among a few studies determining the relationship between antenatal maternal NLR and EONS in preterm neonates. The results provided some additional information on the usefulness of NLR in preterm delivery. However, there were some limitations to be addressed. Some data might be incomplete due to the retrospective nature of the study. Sample size for subgroup analysis might be inadequate. The value of NLR could vary by GA and individually. Timing and severity of possible infections or inflammations could not be definitely determined and related to NLR. The cut-off value of NLR used in this study is arbitrary and the most appropriate one is still unknown. Further, larger studies are needed to elucidate the use of NLR in predicting EONS and other adverse outcomes in various obstetric conditions.

Nonetheless, from this study, NLR could possibly be useful in guiding the management and decision among women with early preterm labor where conservative treatment to prolong gestational period are commonly practiced. Awareness should be raised among obstetricians and paediatricians that high NLR could be a marker of intrauterine infections and increase the risk of EONS in the neonates. Appropriate management that offered in a timely manner could help reducing maternal and neonatal morbidities and mortalities.

Conclusion

In conclusion, maternal serum NLR was significantly associated with EONS in preterm neonates, especially those delivery at GA less than 34 weeks. It could be considered in clinical practice as a marker for development of EONS in preterm neonates.

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Potential conflicts of interest

The authors declare no conflict of interest.

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OBSTETRICS

The Effect of Single Dose Antenatal Dexamethasone in Reducing Respiratory Complications in Late Preterm

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ABSTRACT

Objectives: To determine the effect of a single dose of dexamethasone in reducing respiratory complications in late preterm newborns.

Materials and Methods: This was a prospective cohort study. Pregnant women who were at risk of preterm births at $34^{0/7}$ - $36^{6/7}$ weeks of gestation were prospectively observed. The first group was given single dose of antenatal dexamethasone, while the second group received only the standard treatment without dexamethasone as the control group. The main outcomes are rate of respiratory complications in newborns.

Results: A total of 205 pregnant women who were enrolled with complete data for analysis. Sixty-eight and 137 were in the single dose and control groups, respectively. The rates of respiratory complications in all gestational ages between the two groups were not significantly different (22.1% and 32.8%, respectively: $p = 0.11$). Multivariate logistic regression model was utilized to adjust confounders for independent factors of respiratory complications in late preterm newborns. A single dose of dexamethasone and gestational age at $36^{0/7\text{-}6/7}$ weeks became significant factors in order to decrease respiratory complication rate and received an adjusted odds ratio of 0.45 and 0.29 at $p = 0.03$ and $p = 0.001$, respectively.

Conclusion: Administration of a single dose of dexamethasone is a factor in reducing rate of respiratory complications in late preterm newborns without any seriously adverse effects.

Keywords: late preterm, respiratory complications, corticosteroids, dexamethasone.

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ผลของการได้ยาเด็กซาเมทธาโซนก่อนคลอดเพียงหนึ่งครั้งในการลดภาวะแทรกซ้อนทางระบบทางเดินหายใจในทารกที่คลอดก่อนกำหนดระยะท้าย

อดิศักดิ์ ไวเขตการณ์, จรินทร์ทิพย์ สมประสิทธิ์, ชำนาญ แท่นประเสริฐกุล

บทคัดย่อ

วัตถุประสงค์: *เพื่อศึกษาผลของการได้รับยาเด็กซาเมทธาโซน 1 ครั้ง ในการลดภาวะแทรกซ้อนทางระบบทางเดินหายใจในทารกที่คลอดก่อนกำหนดระยะท้าย*

วัสดุและวิธีการ: *เป็นการศึกษาเชิงเปรียบเทียบแบบไปข้างหน้า โดยทำการติดตามศึกษาในสตรีตั้งครรภ์ที่มีอายุครรภ์ระหว่าง 34 สัปดาห์ ถึง 36 สัปดาห์ 6 วัน ที่มีภาวะเสี่ยงต่อการคลอดก่อนกำหนด โดยกลุ่มแรกได้รับยาเด็กซาเมทธาโซน 1 ครั้งก่อนคลอด ในขณะที่กลุ่มที่สองได้รับการดูแลตามมาตรฐาน และไม่ได้รับยาเป็นกลุ่มควบคุม โดยทำการศึกษาเปรียบเทียบผลการลดการเกิดภาวะแทรกซ้อนทางระบบทางเดินหายใจในทารก*

ผลการศึกษา: *อาสาสมัคร 205 คน ที่ข้อมูลครบถ้วนเพียงพอต่อการวิเคราะห์ เป็นกลุ่มที่ได้รับยาเด็กซาเมทธาโซน 68 คน และไม่ได้รับยา 137 คน เมื่อเปรียบเทียบการเกิดภาวะแทรกซ้อนทางระบบทางเดินหายใจโดยรวมพบว่า ไม่แตกต่างกันระหว่างทั้งสองกลุ่ม (ร้อยละ 22.1 และร้อยละ 32.8 ตามลำดับ, p = 0.11) แต่เมื่อวิเคราะห์โดยความสัมพันธ์ถดถอยแบบพหุปัจจัย โดยการกำจัดปัจจัยรบกวนพบว่า การได้รับยา 1 ครั้ง และการคลอดที่อายุครรภ์ 36 สัปดาห์ ถึง 36 สัปดาห์ 6 วัน เป็นปัจจัยอิสระที่ทำให้ทารกแรกเกิดมีภาวะแทรกซ้อนทางระบบทางเดินหายใจลดลง อย่างมีนัยสำคัญทางสถิติ โดยมีอัตราความน่าจะเป็นที่ปรับแล้ว เท่ากับ 0.45 และ 0.29 ที่ค่า p = 0.03 และ 0.001 ตามลำดับ*

สรุป: *การให้ยาเด็กซาเมทธาโซน 1 ครั้งก่อนคลอด ในสตรีตั้งครรภ์ที่มีความเสี่ยงต่อการคลอดก่อนกำหนดระยะท้าย เป็นปัจจัยที่สามารถช่วยลดภาวะแทรกซ้อนทางระบบทางเดินหายใจในทารกได้ โดยไม่พบผลข้างเคียงที่รุนแรง*

คำสำคัญ: *คลอดก่อนกำหนดระยะท้าย, ภาวะแทรกซ้อนทางระบบทางเดินหายใจ, คอร์ติโคสเตียรอยด์, ยาเด็กซาเมทธาโซน*

Introduction

Antenatal corticosteroids have been recommended to pregnant women who were diagnosed of possible preterm births before 34 weeks of gestation for fetal lung maturity acceleration[1-3]. However, in pregnant women who were diagnosed of preterm birth at gestational age more than 34 weeks, the administration of antenatal corticosteroids was in controversy[3].

In Thailand, some obstetricians prescribed antenatal corticosteroids in pregnant women with preterm labor at gestational age more than 34 weeks while some obstetricians did not prescribe it. A single course of betamethasone (antenatal corticosteroids) for pregnant women between $34^{0/7}$ and $36^{6/7}$ weeks was recommended by The Society for Maternal-Fetal Medicine in year 2016[4] and the American College of Obstetricians and Gynecologists (ACOG) in year 2017[1]. However, betamethasone is not widely available in Thailand. Intramuscular 6 mg dexamethasone every 12 hours for 4 consecutive doses were alternative used. The Cochrane database review demonstrated that betamethasone was not superior to dexamethasone for accelerating lung maturation for women at risk of preterm birth[5]. At Thammasat University Hospital, most late preterm cases did not receive a complete course of dexamethasone per protocol. Advanced progression of labor and lack of recommendations being made for inhibiting labor in late preterm cases were still controversial. The practice of antenatal corticosteroids administration in these cases heavily depended on the personal preference of individual obstetricians. The aim of this study was to evaluate the effects of a single dose of antenatal dexamethasone in late preterm cases in reducing respiratory complications in newborns.

Materials and Methods

The prospective cohort study was conducted in the labor room and newborn-care unit of Thammasat University Hospital, a tertiary medical care center outskirt of Bangkok. We enrolled singleton pregnant women at $34^{0/7}$ weeks to $36^{6/7}$ weeks of gestation who had preterm labor pain and received only one dose of dexamethasone before delivery between November 2016 and May 2018. Preterm labor is defined as a regular uterine contraction of at least 4 times in 20 minutes or 8 times in 60 minutes with at least 1 cm. cervical dilatation, and /or at least 80% effacement. Their gestational ages (GA) were confirmed by ultrasonography during their first trimesters or by their last menstrual periods on the basis of certain dates. Participants who had fetuses with growth restriction or fetal anomalies and patients who received corticosteroids during current pregnancy, advanced cervical dilatation more than 8 cm., pregnancy complications such as pre-gestational diabetes mellitus (DM) gestational diabetes mellitus (GDM), pregnancy-induced hypertension or placenta previa, or evidence of maternal or fetal infection were excluded from the study. All eligible participates consecutively enrolled in the study during the studied period and were divided into two groups based on physicians' decisions. The study group was the pregnant women who received only a single dose of 6 mg dexamethasone intramuscularly before delivery. Those who were not given dexamethasone were defined as the control group. The standard care for pregnant women with preterm labor was applied by working up for specific causes of preterm labor such as infection or premature ruptured of membranes. The assessment of fetal well-beings and maternal conditions were conducted in every patients. Tocolytic medications were not utilized in these groups of patients.

The research protocol was reviewed and approved by the Institutional Review Board of Faculty of Medicine at Thammasat University, Thailand (IRB: MTU-EC-OB-2-167/59). The inform consent was obtained from all participants. Demographic data including maternal age, parity, body mass index, number of antenatal visits and gestational age at admittance and delivery were recorded. Primary outcomes consisted of respiratory complications and its prevalence. It included the respiratory-distress

syndrome (RDS) defined as tachypnea development, the chest wall retracts and expiration accompanied by grunting and nostril flaring, the chest radiograph shows a diffuse reticulogranular infiltrate and air bronchogram[6]. Transient tachypnea of the newborn (TTNB) is a clinical diagnosis. Chest radiograph finding were used to look at increased lung volumes with flat diaphragms, mild cardiomegaly, prominent vascular markings in a sunburst pattern originating at the hilum, fluid often seen in the interlobar fissures, possible pleural effusions, and possibility of alveolar edema appeared as fluffy densities. Tachypnea is defined as respiratory rate > 60 breaths per minute[7]. Grunting and retraction of newborns who need ventilators or oxygen support after birth as diagnosed by neonatologist on the basis of standard criteria were also included. The secondary outcomes were prevalence of neonatal hypoglycemia and length of hospital stay.

The sample size was calculated based on result of an earlier study by Balci et al[8]. By testing two independent proportions with two-tailed test, with an α error of 5%, 80% power and ratio at 2:1. The number of participants in study and control group were approximately 67 and 134, respectively. For statistical analysis, baseline characteristics were analyzed and presented as frequency, percentage, mean with standard deviation or median with interquartile range. Continuous data were compared among groups using unpaired T-test or Wilcoxon rank-sum test. Chi- squared or Fisher exact tests were used to compare categorical data. Each variable was analyzed for outcome of respiratory complications, all significant factors were then included in the multivariate logistic regression.

Results

During the period of study, 210 cases of pregnant women were enrolled. Of these, 140 women not receiving antenatal dexamethasone were in the control group. Seventy participants who received single dose dexamethasone were classified as the study group. In the control group, 3 participants had incomplete data, 137 participants had enough data for the analysis. In the study group 2 participants had incomplete data. As a result, 68 participants were providing complete data for further analysis (Fig. 1).

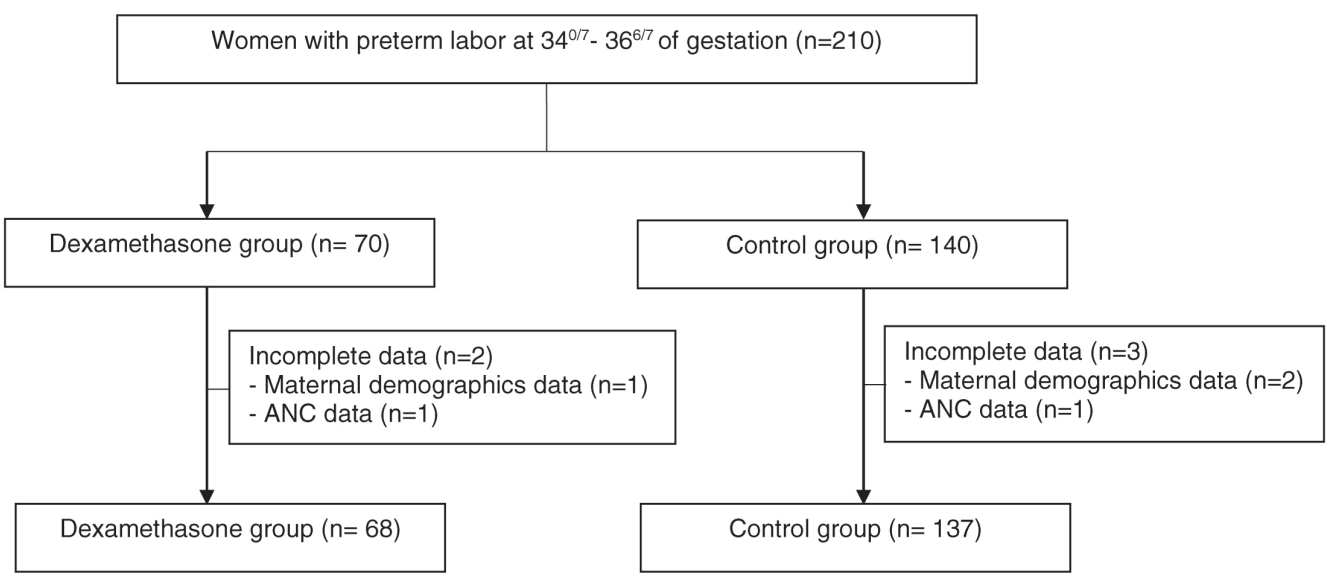

Fig. 1. Participant flow diagram.

As shown in Table 1, demographic data consisted of maternal age, parity, pre-pregnancy body mass index (BMI) and number of total antenatal visits (ANC). The two groups revealed no significant differences in these baseline clinical characteristics. Most of the participants in both groups were nulliparous and had more than 4 antenatal visits. However, the gestational age at delivery between both groups were statistically different ($p < 0.001$).

Table 1. Clinical characteristics of patients participating.

	Study (n=68)	Control (n=137)	p value
Age (year)*	28.3±6.5	28.7±6.9	0.53
Parity**			
Nulliparous	43 (63.2)	74 (54.0)	
Multiparous	25 (36.8)	63 (46.0)	
BMI (kg/m^2)*	26.2±3.9	26.8±5.0	0.39
ANC (time)**			0.29
< 4	15 (22.1)	22 (16.1)	
≥ 4	53 (77.9)	115 (83.9)	
GA at delivery (week)**			< 0.001
340/7-6/7	14 (20.6)	17 (12.4)	
350/7-6/7	28 (41.2)	28 (20.4)	
360/7-6/7	26 (38.2)	92 (67.2)	
all***	35 (35,36)	36 (35,36)	< 0.001

* mean±SD (standard deviation), ** n(%), *** median (iqr).

As shown in Table 2, the rate of respiratory complications in all gestational ages between the two groups were not significantly different (22.1% and 32.8%, respectively: p=0.11). When stratified to each gestational age, respiratory complications between the two groups at $34^{0/6-6/7}$ and $35^{0/6-6/7}$ weeks gestational age were not different. However, at $36^{0/6-6/7}$ weeks of gestational age, the rate of respiratory complications was 7.7 and 28.3% which was significantly different (p=0.047). The mean newborn birth weight (BW), mode of delivery, hypoglycemia and length of hospital stay (LOS) of newborn of both groups were not significantly different.

Factors related with respiratory complications in newborns were analyzed by multivariate logistic regression. After adjusting the confounders, a gestational age of $36^{0/7-6/7}$ weeks and the administering of a single dose of dexamethasone before delivery could independently decreased the risk of respiratory complications by 55%. The adjusted odd ratios were 0.29 and 0.45 with p-values at 0.001 and 0.03, respectively as showed in Table 3.

Table 2. Comparison of neonatal and maternal outcomes.

	Study (n=68)	Control (n=137)	p value
Respiratory			
Complications**			
All	15 (22.1)	45 (32.8)	0.11
$34^{0/7-6/7}$	5/14 (35.7)	9/17 (52.9)	0.55
$35^{0/7-6/7}$	8/28 (28.6)	10/28 (35.7)	0.68
$36^{0/7-6/7}$	2/26 (7.7)	26/92 (28.3)	0.047
Mode of delivery**			
Normal labor	49 (72.1)	94 (68.6)	
Cesarean section	19 (27.9)	43 (31.4)	
BW (grams)*	2,606±312.4	2,668.2±478.7	0.45
Time (hour)*	6.47±2.0	6.56±4.81	0.27
Hypoglycemia**	11 (16.2)	18 (13.1)	0.56
LOS (days)***	3 (3)	3 (3)	0.48

* mean±SD, ** n(%), *** median (iqr), Time: Time from injection of dexamethasone to delivery.

Table 3. Independent factors of respiratory complications by multivariate logistic regression analysis.

Factors	Adjusted odd ratio	p value	95% CI
Parity			
Nulliparous	1.00 (ref)		
Multiparous	0.77	0.27	0.48-1.23
Mode			
Normal labor	1.00		
Cesarean section	1.2	0.124	0.95-1.52
GA (week)			
$34^{0/7-6/7}$	1.00 (ref)		
$35^{0/7-6/7}$	0.59	0.19	0.27-1.3
$36^{0/7-6/7}$	0.29	0.001	0.14-0.62
Dexamethasone (dose)			
0	1.00 (ref)		
1	0.45	0.03	0.22-0.93

* mean±SD, ** n(%), *** median (iqr), Time: Time from injection of dexamethasone to delivery.

Discussion

Respiratory complications rate in control group was 32.8%. The result of this finding is similar to data from the Consortium on Safe Labor that reported

respiratory morbidity rate in late preterm infants at nearly 40%[9]. The respiratory complications rate progressively decreased with an increasing gestational age. Our data showed that half of this complications occurred in the 34th weeks of gestational age, then continuously decreased to 28.3% at the 36th weeks of gestational in the control group. This finding was consistent with the study of Porto et al[10]. In their study, Porto and coworkers demonstrated that respiratory morbidity was substantially reduced according to gestational age, at the rates of 47, 21 and 18% in newborns at $34^{0/7\text{-}6/7}$, $35^{0/7\text{-}6/7}$ and $36^{0/7\text{-}6/7}$ weeks, respectively. Their data strongly supported that increased gestational age as the main factor leading to less newborn respiratory complications. The more advanced the gestational age of newborn, the less are the chances of neonatal morbidity. It was estimated that there was a 23% decrease in adverse outcomes with each additional week of GA[11].

In our study, the rates of respiratory complications was rather high. The reason is that our data combined all severity of respiratory problems that needed oxygen support or monitoring, including such problems as RDS, TTNB, tachypnea or grunting. The rational for the effect of the use of dexamethasone associated with non-severe respiratory complications such as tachypnea or grunting are that corticosteroids could stimulate pneumocyte type 2 and decrease lower alveolar surface tension including the prevention of alveolar collapse[3]. This proposed mechanism could possibly be used to explain the benefit of steroid to the reduction of non-severe respiratory problems.

Despite the fact that some of these complications were non-severe, they could still have effects on long-term growth and the development of infants. All these respiratory complications required meticulous care by pediatricians. Indeed, our data showed low rates of severe respiratory complications after 36 weeks of gestation, as established in the previous literature[9].

Our study demonstrated that the overall rates of respiratory complications in late preterm newborns between the study and control group were comparable. However it is in contrast to the data from the meta-analysis work done by Saccone et al[12]. Their work demonstrated the decrease neonatal respiratory morbidity rate of near term fetus when corticosteroid was used. Because our study consisted of single dose antenatal corticosteroid and it was observational study. The practice of whether to give dexamethasone to late preterm patients was not in anyone's control due to the controversial issue of late preterm management[3].

The National Institute of Health (NIH) panel recommended the use of antenatal corticosteroids prophylaxis with inhibition of labor for deliveries that were anticipated prior to the 34th week of gestation. It would have the most beneficial effects on patients the sooner they received this medication more than 24 hours before the time of delivery[2]. However, ACOG stated that the tocolytic medication's usage is tentative in late preterm[13]. In our study, most patients had delivery within 12 hours after administration of dexamethasone. This is because this study did not include the administering of the tocolytic drug in the standard care of late preterm.

Consequently, we evaluated the benefits of administering a single dose of dexamethasone for reducing respiratory complications in late preterm infants. Our multivariable logistic regression model showed a single dose of dexamethasone before delivery and gestational age $36^{0/7\text{-}6/7}$ weeks were the protective factors against respiratory complications in late preterm newborns after adjustment of other confounders. By the administration of a single dose of dexamethasone, the risk of respiratory complications could be decreased similar to the data from Attawattanakul et al[14] and Balci et al[9] that demonstrated the benefit of antenatal corticosteroids which had decreased the rates of respiratory distress and the need for ventilator support after birth in these late preterm newborns. The benefit of receiving one shot of corticosteroids during late preterm could be explained as involving part of the maturation effects of the fetal lung. There was evidence that it stimulated a surge of endogenous steroids in term newborns when their mothers had gone into spontaneous labor[15].

Neonatal hypoglycemia was the most common of neonatal complications. It was a serious concern. The newborn condition was closely monitored following antenatal corticosteroids use. In our study, neonatal hypoglycemia did not increase in both groups. Our findings were similar to those of studies carried out by Bannerman et al[16] and Attawattanakul et al[14].

The strength of this study is based on the prospective data that dexamethasone usage, which was more available than betamethasone in many countries. The current study was conducted on the basis of standard practice, in which no tocolytic drugs were used as a means of delaying delivery. There are several limitations of this study. Firstly, causes of preterm were unknown in most cases. These could be important baseline characteristics and may possibly be confounder. Secondly, this study lacks randomization and has bias results. The confounding bias is adjusted by multivariate logistic regression analysis. However, there is still some selection bias from corresponding physicians and the underlying diseases of the pregnant women that lead the attending physicians to use corticosteroids.

Conclusion

In conclusion, our study demonstrated the benefit of receiving one dose dexamethasone in reducing rates of respiratory complications in late preterm newborns without any serious neonatal adverse outcomes. Still larger trials with randomization are warranted and ought to be conducted. Also, a study into the long-term effects are needed in order to produce more substantial evidence of these possible beneficial effects.

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Potential conflicts of interest

The authors declare no conflict of interest.

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GYNECOLOGY

Variation of Genital Appearance in Thai Women

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ABSTRACT

Objectives: To study the normal variation of Thai female genitalia and the difference in premenopausal and postmenopausal age groups.

Materials and Methods: One hundred and fifty five Thai women having the annual pelvic examination at King Chulalongkorn Memorials Hospital from May 2017 to April 2018 were recruited. The inclusion criteria were: age between 20-70 years, satisfied with the external genitalia as classified by Thai version of Genital Appearance Satisfaction questionnaires and not seeking for genital cosmetic surgery. The height, weight, body mass index, parity, route of prior delivery, contraception method, age at menopause, and the used of postmenopausal hormonal therapy were recorded. The genital appearance measurements included 10 parameters (length of clitoris, clitorial gland width, clitoro-urethral length, labia minora length and width, labia majora length, perineal length, protusion of labia minora, and the appearance of the perineum).

Results: The median (interquartile range) of left and right labia minora width were 10.46 (6.61, 14.12) and 9.69 (6.25, 14.62) mm. Most women had the darker color of the perineum than the skin of inner thigh (47.7%). Forty four women (28.4%) reported of having ridge at the labia majora. When comparing the genital appearances in premenopausal and postmenopausal group, the clitorial length, clitoro-urethral length and labia minora length were statistically different ($p < 0.001$).

Conclusion: We found the wide range of the variation of female genital appearances in Thai women who were satisfied with their own genital appearance. This information would be useful for the preoperative counseling for Thai women who are not satisfied with her own appearance and seek the genital cosmetic surgery without medical indication to avoid the regret after surgery.

Keywords: genital appearance, female genitalia, female genital cosmetic surgery.

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การศึกษาลักษณะอวัยวะเพศภายนอกของสตรีไทย

ธัญสิตา ชินกังสดาร, ปุริม เรือนภู่, สุวิทย์ บุญยะเวชชีวิน

บทคัดย่อ

วัตถุประสงค์*: เพื่อศึกษาลักษณะปกติของอวัยวะเพศภายนอกของสตรีไทย และศึกษาความแตกต่างระหว่างอวัยะเพศภายนอกของสตรีกลุ่มวัยก่อนหมดประจำเดือน และสตรีวัยหมดประจำเดือน*

วัสดุและวิธีการ*: หญิงไทยที่มาตรวจภายในประจำปีที่ รพ.จุฬาลงกรณ์ ในระหว่างเดือนพฤษภาคม พ.ศ. 2560 ถึงเมษายน พ.ศ. 2561 จำนวน 155 คน จะได้รับการเชิญให้เข้าร่วมการศึกษา โดยมีเงื่อนไขคืออายุระหว่าง 20-70 ปี และมีความพึงพอใจในอวัยวะเพศภายนอกของตนเองตามแบบทดสอบความพึงพอใจต่อลักษณะอวัยวะเพศภายนอกแบบฉบับภาษาไทย จากนั้นประวัติข้อมูลกายภาพของผู้เข้าร่วมวิจัยทุกคน ได้แก่ น้ำหนัก ส่วนสูง ค่าดัชนีมวลกาย ประวัติการคลอดบุตร การคุมกำเนิด อายุที่เข้าสู่วัยหมดประจำเดือน และประวัติการใช้ฮอร์โมนทดแทนจะถูกบันทึกไว้ แล้วจึงทำการตรวจวัดขนาดและลักษณะของอวัยวะเพศภายนอก ได้แก่ ความกว้างความยาวของคริตอริส แคมเล็ก และแคมใหญ่ ระยะห่างระหว่างคริตอริสถึงรูเปิดท่อปัสสาวะ ความยาวของกล้ามเนื้อเพอริเนียล ความยื่นยาวของแคมเล็กที่ยาวพ้นจากแคมใหญ่ และลักษณะภายนอก ได้แก่ สี และความเรียบเนียนของอวัยวะเพศภายนอก*

ผลการวิจัย*: ค่ามัธยฐาน (ค่าพิสัยควอไทล์) ของแคมเล็กข้างซ้ายและขวา คือ 10.46 (6.61, 14.12) และ 9.69 (6.25, 14.62) มม. ตามลำดับ สตรีไทยจำนวนมากมีสีผิวของบริเวณอวัยวะเข้มกว่าสีผิวบริเวณต้นขาด้านใน (ร้อยละ 47.7) มีสตรีจำนวน 44 คน (ร้อยละ 28.4) มีลักษณะผิวของของแคมใหญ่มีรอยย่น ไม่เรียบ และสตรีกลุ่มวัยก่อนหมดประจำเดือนมีแนวโน้มที่จะมีความยาวของคริตอริส และระยะห่างระหว่างคริตอริสและรูเปิดท่อปัสสาวะยาวกว่าสตรีวัยหมดประจำเดือนอย่างมีนัยสำคัญทางสถิติ ($p < 0.001$)*

สรุป*: ลักษณะอวัยะเพศภายนอกของสตรีไทยมีความหลากหลายแตกต่างกันไปในแต่ละบุคคล ซึ่งข้อมูลที่ได้รับจากการศึกษานี้สามารถนำไปใช้ประกอบการให้คำแนะนำก่อนผ่าตัดแก่ผู้ที่ไม่มีความพึงพอใจ ในอวัยวะเพศของตนเองและเข้ามาปรึกษาเพื่อเข้ารับการผ่าตัดศัลยกรรมตกแต่งอวัยวะเพศโดยไม่มีข้อบ่งชี้ทางการแพทย์*

คำสำคัญ*: ลักษณะอวัยวะเพศภายนอก, อวัยวะเพศสตรี, การผ่าตัดศัลยกรรมตกแต่งอวัยวะเพศ*

Introduction

The incidence of women undergone the female genital cosmetic surgery has been increasing. Many studies reported of more than 80% of the women (teenage to menopause) seeking the female genital aesthetic such as the labial reduction or labioplasty to improve their looking, confident and sexual function although they are asymptomatic[1, 2]. This trend also happens in Thailand and many Southeast-Asian countries[3, 4].

The Royal College of Obstetricians and Gynaecologists, Royal Australian College of General Practitioners and American College of Obstetricians and Gynecologists have launched the awareness that this kind of surgery have no guidelines and any recommendations because there is lacking of reliable evidence about the safety and efficacy[5, 7]. Those organizations suggest that all gynecologists have to advise all patients who want to undergone the female genital cosmetic surgery about the variation of female genital organs and also balance the risks and benefits of the procedures to avoid the unnecessary procedures and complication. Up to now, there are a few studies or information about female genital appearance. There are only scanty data about the vulvar morphology appears in gynecologic or anatomical textbook[8]. There is report of high variation of the female genitalia in European and Australian women[9-11]. There has been only one study about the variation of the female genitalia in Chinese[12]. The authors reported a big difference in genital appearance of Chinese women who requested for cosmetic surgery[12]. Up to now, there has been no study in Thai or any Southeast-Asian women. This study aimed to determine the variation of Thai female external genitalia, and compare the differences of female genitalia between pre and postmenopausal women.

Materials and Methods

Patient selection and setting

After IRB approval, 155 Thai women attending the out-patient clinic for annual gynecologic examination at King Chulalongkorn Memorial Hospital were recruited. All women had to sign the consent form. The inclusion criteria were: aged 20 to 70 years, and satisfied with the external genitalia as classified by Thai version of Genital Appearance Satisfaction (GAS) questionnaires and not seeking for genital cosmetic surgery. The Thai version of GAS Scale questionnaire were tested in 50 Thai women for internal validity (Chronbach's Alpha = 0.672) and test retest reliability (Intraclass correlation (r) = 0.965). Only Thai women who answered the GAS questionnaire on the satisfaction questionnaire with the scale = 0 (most satisfied) were included in the study. The exclusion criteria were: history pelvic reconstructive surgery or vulvovaginal cosmetic surgery, prior vaginal birth with known history of mediolateral episiotomy or obviously noticed mediolateral episiotomy scar, and known congenital mullerian anomaly. Participants with previous vaginal delivery with median episiotomy or no episiotomy or natural perineal tear could be included

The characteristics of participants were recorded. The body weight and height, gynecologic history, menarche, menstruation, hormonal use, parity, route of delivery, and history of smoking or drinking were recorded. The menopause was defined as a woman who had amennorhea more than one year at the visited date.

Measurements

The anatomical measurements were performed in lithotomy position with minimal stretching technique. All the parameters were measured by digital vernia caliper: Mitutoyo® model 500-196-20 (Mitutoyo corporation, Kawasaki, Kanagawa Prefecture, Japan) by the first author to minimize the inter-rater variability. This digital caliper had the accuracy of 0.001mm with the reading of the upper and lower scale of 0.0005 and 0.01 mm, respectively. Each parameter measurement was repeated 3 times and the average value was used for data analysis.

The parameters to be measured were:

1. Length of clitoris; the length from the crest of the skin at the base to the end of clitoris.

2. Clitorial gland width; the greatest with of the clitorial gland.

3. Clitoro-urethral length; the length from the tip of the gland-clitoris to the opening of urethra.

4. Labia minora length and width.

5. Labia majora length.

6. Perineal length (distance between the posterior fourchette to the central of anal canal).

7. Protrusion of labia minora (the length of the labia minora that protruded over the labia majora).

8. The appearance of the perineal color (defined as same or darker skin tone of genital area compared with surrounding skin of inner thigh).

9. Rugorsity (smooth or ridge).

Sample size estimation and distribution

The sample size estimation of 155 cases, was done by calculation from the formula for infinite population mean (estimated standard deviation (0.63) of the mean of labia minora width from pilot study in 20 cases. The acceptable error was 1 mm).

Number of participants in each age group was recruited according to the age distribution of 2010 Population and housing census of Thailand from the National Statistical Office of Thailand.

Statistical analysis

The population's characteristics were described in number (%), mean ± standard deviation (SD) or median (interquartile range (IQR)). The difference between pre and postmenopausal women were compared by the independent sample student's t test (parametric variables) and Mann–Whitney U test (non-parametric variables). Student's t test for paired samples and Wilcoxon signed rank test were used to compare right- and left-side measurements among groups. Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). The statistical significance level was set at 0.05.

Results

The mean±SD of body mass index and parity were 24.18 ± 5.04 kg/m^2 and 1.5 ± 1.0, respectively (Table 1).

Table 1. Population's characteristics (N= 155).

Characteristics	
	Mean ± SD
Weight (kg)	59.2 ± 13.2
Height (cm)	156.34 ± 5.7
Body mass index (kg/m^2)	24.18 ± 5.04
Parity	1.5 ± 1.0
	n (%)
Age (year)	
20 - 30	37 (23.9)
31 - 40	39 (25.2)
41 - 50	34 (21.9)
51 - 60	27 (17.4)
61 - 70	18 (11.6)
Prior vaginal delivery*	74 (47.7)
Prior cesarean section	51 (32.9)
Contraception	
None	67 (62)
Condom	2 (1.9)
Oral contraceptive pills	9 (8.3)
DMPA	5 (4.6)
Implants	21 (13.6)
Intrauterine device	6 (5.6)
Tubal resection	9 (5.8)
Postmenopausal hormonal therapy	3 (6.4)

DMPA: depot medroxyprogesterone acetate, * prior vaginal delivery with previous medial episiotomy or no episiotomy or natural perineal tear

Table 2. Female genital appearance (n = 155).

Genital appearance		
	Mean ± SD	**Min - Max**
Clitorial length (mm)	23.35 ± 6.93	9.51 - 47.94
Clitorial width (mm)	6.70 ± 1.42	2.96 - 12.19
Clitoro-urethral length (mm)	18.18 ± 5.60	6.36 - 38.82
Left labia majora length (mm)	81.76 ± 13.08	43.90 - 22.48
Right labia majora length (mm)	82.31 ± 13.37	43.19 - 131.37
Perineal length (mm)	27.79 ± 6.22	3.28 - 46.44
	Median (interquartile range)	
Left labia minora width (mm)	10.46 (6.61, 14.12)	6.75 - 32.91
Right Labia minora width (mm)	9.69 (6.25, 14.62)	6.61 - 27.30
Left Labia minora length (mm)	30.93 (24.30, 42.73)	7.71 - 87.33
Right Labia minora length (mm)	30.91 (22.26, 41.42)	8.85 - 85.73
Protusion of labia minora (mm)	0 (0, 5.63)	0 - 15.81
	n (%)	
Color		
- Same	81 (52.3%)	
- Darker	74 (47.7%)	
Rugorsity		
- Smooth	111 (71.6%)	
- Ridge	44 (28.4%)	

The median (IQR) of left and right labia minora width were 10.46 (6.61, 14.12) and 9.69 (6.25, 14.62) mm (Table 2). The perineal length was 27.79 ± 6.22 mm (Table 2). Most women had the darker color of the perineum than the skin of inner thigh (47.7%). Forty-four women (28.4%) reported of having ridge at the labia majora. When comparing the genital appearances in premenopausal and postmenopausal group, the clitorial length, clitoro-urethral length, and labia minora length were statistically different ($p< 0.001$) (Table 3).

Table 3. Female genital appearance in Thai premenopausal (n = 108) and postmenopausal women (n = 47).

	Premenopause	Postmenopause	p value
	Mean ± SD		
Clitorial length (mm)	24.26 ± 6.41	21.26 ± 7.66	0.013*
Clitorial width (mm)	6.61 ± 1.38	6.89 ± 1.48	0.252
Clitoro-urethral length (mm)	19.41 ± 5.74	15.20 ± 3.97	< 0.001*
Left labia majora length (mm)	82.74 ± 12.22	79.49 ± 14.76	0.156
Right labia majora length (mm)	82.77 ± 12.33	81.24 ± 15.59	0.514
Perineal length (mm)	27.90 ± 6.45	27.53 ± 5.71	0.74
	Median (interquartile range)		
Left labia minora width (mm)	11.13 (6.83, 13.92)	8.89 (5.54, 14.22)	0.347
Right labia minora width (mm)	10.54 (6.44, 14.99)	9.25 (5.81, 14.10)	0.289
Left labia minora length (mm)	33.88 (26.60, 45.46)	24.67 (19.35, 36.01)	< 0.001*
Right labia minora length (mm)	32.20 (25.85, 45.51)	23.12 (17.25, 35.60)	< 0.001*
Protrusion of labia minora (mm)	0 (0, 6.14)	0 (0, 4.87)	0.243
	n (%)		
Color			
- Same	56 (51.85%)	25 (53.19%)	0.879
- Darker	52 (48.15%)	22 (46.81%)	
Rugorsity			
- Smooth	75 (69.44%)	36 (76.60%)	0.367
- Ridge	33 (30.56%)	11 (23.40%)	

Discussion

The high variation of female genital appearance was noted in our study. The upper limit of the labia minora width was 32.91 mm in women who were satisfied with their own appearance. Many satisfied women had the ridge of labia majora with the skin of genitalia darker than the skin of inner thigh. Widening of labia minora and its protruding out of labia majora were the most common reason for dissatisfaction of genital appearance to have cosmetic surgery[14, 15]. We found the protrusion of labia minora in 59 (38.1%) women without having any discomfort. The highest limit was 15.81 mm. Our study showed a high variation of genital appearance similar to the studies in British, Australian, and Turkish women[9-11, 16, 17]. It was also similar to Chinese women who seeking for the genital

cosmetic surgery[12]. In order to have strong evidence to counsel Thai women who seeks for genital cosmetic surgery due to the dissatisfaction of genital appearance, the data from the population of their own country is needed.

This result confirmed that the satisfaction of their own genitalia did not depend on the appearance alone but also on their expectation from the social norm. Women who decide to have the genital cosmetic surgery due to feeling difference of the external genitalia without medical indications (such as recurrent vaginal yeast infection, discomfort when wearing tight sport suites, pain when having sexual intercourse) should be reassured about the normal variation of female genitalia[2, 17].

The unnecessary genital cosmetic surgery can lead to many complications such as regret after surgery, infection, dyspareunia, scarring, distortion of external genitalia, etc[4, 18, 19]. The preoperative counselling about the real need for cosmetic surgery and understanding the normal variation of genital appearance are important to avoid those complications in unnecessary case. The complications of cosmetic surgery may lead to loss of self-esteem, distress, economic burden and legal issues [18, 19]. The reasons for genital cosmetic surgery are not only the appearances but also from many non medical reasons. In order to avoid the other unnecessary surgery, further study about non medical reasons in women who decide to have genital cosmetic surgery are advocated.

The strength of this study was that we only included the women who were satisfied with their own genital appearance with the reliable instrument (GAS questionnaire) for the strong evidence for counselling the women who decide to have cosmetic surgery with the reason of dissatisfaction of appearance alone. The age distributions of sample size in our study followed the national consensus data of Thai women. We used the standard digital caliper which was very accurate to measure the genital appearance with single operator.

The limitation of this study was that this was a hospital base. We included only the women that attend our gynecologic clinic for annual examination. Anyhow, there should be little difference of the characteristic of our population with the country data as we distributed the age group according to the data statistics. Our study did not include the women who already decided to have genital cosmetic surgery as most cases were done in plastic surgery or private surgical hospital or clinic. To have the study done in private clinic or cosmetic clinic may take more effort and longer time to conduct due to confidentiality and small numbers of cases.

Conclusion

We found the wide range of the variation of female genital appearances in Thai women who were satisfied with their own genital appearance. This information would be useful for the preoperative counseling for Thai women who are not satisfied with her own appearance and seek the genital cosmetic surgery without medical indication to avoid the regret after surgery.

Potential conflicts of interest

The authors declare no conflict of interest.

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GYNECOLOGY

Treatment of Anogenital Warts: Siriraj Hospital Experience

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ABSTRACT

Objectives: To demonstrate the characteristics, treatment modalities and outcomes in the women presenting with Anogenital Warts (AGW) at Female STI Clinic, Siriraj Hospital.

Materials and Methods: The outcomes of treatment in the patients presenting with AGW who had complete follow-up at Siriraj Female STI clinic in 2016 were reviewed. The patients with immunocompromised conditions such as systemic lupus erythematosus and human immunodeficiency virus infection were excluded from this study.

Results: Two hundred and four of 217 women with AGW were eligible for this study. The mean age was 24.6 ± 5.2 years. Most of them were married and had sexual monogamy. Education levels were similar. Most of the AGWs were located outside the vagina and with ≤ 5 lesions (range 1-20). The diameter of warts was between 1 and 5 cm. The treatment modalities were 85% trichloroacetic acid (TCA) 131 (64.3%), 5% imiquimod 57 (27.9%), cryotherapy 8 (3.9%) and surgery 8 (3.9%). The median periods of treatment were 4, 8, 5 and 1 weeks for 85% TCA, 5% Imiquimod, Cryotherapy and Surgery, respectively. Treatment modalities were changed in the groups of 85% TCA and 5% Imiquimod, for 16.0% and 1.8%, respectively. Recurrence at 3 months after being cured was highest in the groups of 85% TCA (13.0%).

Conclusion: Our results showed that 85% TCA, which is widely available, need four applications with 13% recurrence rate. Imiquimod took longer time for treatment but was associated with less recurrent. Cryotherapy and Surgery showed promising results but the data were limit.

Keywords: anogenital warts, treatment.

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การรักษาหูดหงอนไก่บริเวณอวัยวะเพศและทวารหนัก: ประสบการณ์ ณ โรงพยาบาลศิริราช

มานพชัย ธรรมคันโธ, เจนจิต ฉายะจินดา

บทคัดย่อ

วัตถุประสงค์: *เพื่อแสดงให้เห็นถึงลักษณะ, สิ่งที่เลือกใช้ในการรักษา และผลของการรักษาในสตรีที่มาขอรับการรักษาหูดหงอนไก่บริเวณอวัยวะเพศและทวารหนัก ณ คลินิกโรคติดต่อทางเพศสัมพันธ์สตรี โรงพยาบาลศิริราช*

วัสดุและวิธีการ: *การศึกษาย้อนหลังจากข้อมูลในเวชระเบียนถึงผลการรักษาหลังจากสิ่งที่เลือกใช้ในการรักษาแต่ละสิ่ง ในปี พ.ศ. 2559 ซึ่งเป็นปีที่ซึ่งกำหนดนัดตรวจติดตามครบตามกำหนดนัดหลังการรักษา ณ คลินิกโรคติดต่อทางเพศสัมพันธ์สตรี โรงพยาบาลศิริราช เกณฑ์การคัดออก คือ สตรีที่มีหูดหงอนไก่บริเวณอวัยวะเพศและทวารหนัก แต่มีภาวะภูมิต้านทานร่างกายต่ำผิดปกติ เช่น โรคลูปัส และภาวะติดเชื้อไวรัสเอชไอวี*

ผลการศึกษา: *จากสตรีที่มีหูดหงอนไก่บริเวณอวัยวะเพศทั้งสิ้น 217 ราย เข้าเกณฑ์การวิจัยทั้งสิ้น 204 ราย ค่าเฉลี่ยของอายุ คือ 26.6 ± 5.2 ปี ส่วนใหญ่มีสถานภาพสมรส และมีคู่นอนเพียงคนเดียว ระดับการศึกษาพบกระจายใกล้เคียงกันในระดับต่างๆ ลักษณะรอยโรคของหูดหงอนไก่บริเวณอวัยวะเพศและทวารหนัก (ค่าพิสัย 1-20) ส่วนใหญ่พบเฉพาะภายนอกช่องคลอด จำนวนน้อยกว่าหรือเท่ากับ 5 รอยโรค เส้นผ่าศูนย์กลางของรอยโรคหูดหงอนไก่อยู่ระหว่าง 1-5 เซนติเมตร สิ่งที่ใช้เลือกนำมารักษาหูดหงอนไก่ประกอบด้วย 85 เปอร์เซ็นต์ กรดไตรคลออะซีติก (จำนวนผู้ได้รับ = 131); 5 เปอร์เซ็นต์ อิมิควิโมด (จำนวนผู้ได้รับ = 57) การรักษาด้วยการจี้เย็น (จำนวนผู้ได้รับ = 8) และการผ่าตัดโดยจี้ไฟฟ้าหรือผ่าตัดออก (จำนวนผู้ได้รับ = 8) ระยะเวลาการหายของรอยโรคหลังการรักษาคือ 4 (2-10) สัปดาห์, 8 (4-16) สัปดาห์, 5 (3-7) สัปดาห์ และ 1 สัปดาห์ ตามลำดับ ความจำเป็นที่จะต้องเปลี่ยนวิธีการรักษาพบในกลุ่มที่ได้รับ 85 เปอร์เซ็นต์ กรดไตรคลออะซีติก และ 5 เปอร์เซ็นต์ อิมิควิโมด เป็นร้อยละ 16 และ 1.8 การเกิดรอยโรคซ้ำหลังรักษาหายแล้ว 3 เดือน พบมากที่สุดในกลุ่มที่ได้รับ 85 เปอร์เซ็นต์ กรดไตรคลออะซีติก เป็นร้อยละ 13*

สรุป: *ความสัมพันธ์ในการรักษาโรคหูดหงอนไก่บริเวณอวัยวะเพศและทวารหนัก ไม่พบความแตกต่างมากนักในระหว่างสิ่งที่เลือกใช้ในการรักษา ณ คลินิกโรคติดต่อทางเพศสัมพันธ์สตรี โรงพยาบาลศิริราช*

คำสำคัญ: *หูดหงอนไก่บริเวณอวัยวะเพศและทวารหนัก, วิธีการรักษา*

Introduction

One of the world's most common sexually transmitted infections (STIs) is anogenital warts (AGW). It is mainly caused by non-oncogenic human papilloma virus (HPV) types 6 and 11[1]. These viruses were found to be closely related to cervical cancer. AGW is the most prevalence STI among young reproductive aged[2, 3]. In Thailand, the incidence of AGW was 6.03-6.80 per 100,000[4]. However, these figures are likely to be underestimated due to psychological burden, self-image and sexual-related concern[5]. At the Siriraj Female STI Clinic, Department of Obstetrics & Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, AGW is the mostly diagnosed by either visual diagnosis or tissue biopsy[6]. AGW can occur in any parts of the anal and genital areas with or without symptoms[7]. Posterior fourchette and labia minora appear to be the most common part[8]. Visible warts can cause physical discomfort such as itching, irritation, pain, burning, inflammation and bleeding during sexual activity. Also, psychological impact in women is resulted from these AGW. Some women are worried about the transmission of the warts and its recurrence, while the others have concerns about its interruption to their sexual life and relation with their sex-partners[5]. Although these low-risk HPV viruses rarely develop malignant transformation, successful treatment provides women with a better quality of life and relationship.

There are various treatment for AGW[9] ranging from ablative techniques, surgical excision, podophyllotoxin or trichloroacetic acid (TCA) to innovative topical treatment applied by the patients such as 5% imiquimod. The traditional measures were aimed to physical destruction of the warts lesion. Severe adverse events such as burning sensation, inflammation, pain, erosion, and itching can be occurred. Other modalities such as cryotherapy, laser vaporization, electrocautery and excision are painful and expensive as well as increase risks due to the viral particles floating during the procedures. In addition, recurrence of AGW is very common[9]. 5% Imiquimod is an immunomodulator that helps to increase eradication of viruses and lesions. It is to some extent superior to the other approaches in that it does not destroy the cell tissue at the warts areas, instead it modifies immune responses and stimulates binding of several induction-specific nuclear complexes[10]. Moreover, the reduction in the disease was found in earlier week of treatment in women using the different doses of the 5% imiquimod cream[11]. Combination of use of 5% imiquimod cream followed by surgery is reported to provide lower recurrence rates than only surgical approach[12]. As data in Thai population are limited, this study aimed to demonstrate our experience in treating Thai female patients presenting with AGW at Siriraj Hospital.

Materials and Methods

This study was approved by Siriraj Ethical Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University (COA. SI375/2018). Chart-review was conducted for the patients with AGW. Information of all female patients with AGW who received treatment at the Siriraj Female STI Clinic in 2016, the year that the follow-up system was fully settled, was extracted. The patients were excluded if they had immunocompromised conditions such as human immunodeficiency virus (HIV) infection and systemic lupus erythematosus (SLE).

In 2016, the Siriraj Female STI Clinic followed the 2015 Centers for Disease Control and Prevention STI treatment guideline[13]. Size and area coverage of the lesion were the first triage of the treatment. If the lesions involved larger than 10 cm^2, surgical removal would be the first choice of treatment. Application of 85% TCA solution was the most common treatment in our clinic. The patients were then follow-up once a week. If the condition was not improved after 5 visits, combination of 85% TCA and 5% imiquimod would be started. This treatment could be used up to 16 weeks and the patients would be monthly followed-up. Cryotherapy has been started in our clinic in late 2016. The patients in this group were also followed once a weeks.

Statistical analysis was performed by STATA version 12.1. Descriptive data were presented as N(%), mean ± SD and median with range.

Results

Two hundred and four out of 217 women presenting with AGW at the clinic, 204 were eligible for our study. Nine cases of HIV-infected and 4 cases of SLE were excluded. There were 83.3% of first-time AGW. Their mean age was 24.6 ± 5.2 years. Most of them were married and had sexual monogamy. Educational levels were similarly distributed. Most AGW were outside the vagina. Most patients had ≤ 5 lesions (range 1-20). The diameter of warts were 1-5 cm. Perianal warts were found in 7 women (3.4%) but only two of them had anal intercourse. None of the participants had HPV vaccination (Table 1).

Table 1. Demographic data of women with anogenital warts (N = 204).

Category	Frequency (N = 204)	Percent
Age of women (years)		
≤ 19	21	10.3
20 - 24	96	47.1
25 - 29	61	29.9
≥ 30	26	12.7
Marital status		
Single	57	27.9
Married	129	63.2
Divorced/ separated/ widow	18	8.8
Number of lifetime partners		
1	142	69.6
2	40	19.6
3	11	5.4
≥ 4	11	5.4
Level of education		
Primary school	39	19.1
Secondary school	61	29.9
Vocational school	43	21.1
University	61	29.9
First-time diagnosis of AGW	170	83.3
Experience of anal intercourse	2	1.0
Location of warts at first visit		
External	147	72.1
External + intravaginal	25	12.3
External + perianal + intravaginal	18	8.8
Intravaginal	7	3.4
Perianal	7	3.4
Number of warts at first visit		
≤ 5	132	64.7
6 - 10	47	23.0
11 - 15	14	6.9
≥ 16	11	5.4
Diameter of warts (cm)		
≤ 0.5	111	54.4
0.6 - 1.0	67	32.8
1.1 - 1.5	7	3.4
1.6 - 2.0	2	1.0
≥ 2.1	7	3.4
Treatment modalities		
Trichloroacetic acid	131	64.3
5% Imiquimod	57	27.9
Cryotherapy	8	3.9
Surgery	8	3.9

The treatment modalities included 85% TCA 131 (64.3%), 5% imiquimod 57 (27.9%), cryotherapy 8 (3.9%) and surgery 8 (3.9%). The median duration of treatment were 4, 8, 5 and 1 weeks for 85% TCA, 5% imiquimod, cryotherapy and surgery, respectively. Treatment were changed in the groups of 85% TCA and 5% imiquimod, at 16.0% and 1.8%, respectively. Recurrence rate after 3 months of successful treatment as highest in the 85% TCA group (13.0%) (Table 2). High degree of pain within 24 hours of treatment was most common in the patients receiving TCA and surgery (Fig. 1). Overall, patients had 'high' to 'very high' satisfaction for their treatment modality (Fig. 2).

Table 2. Treatment duration, change of treatment modalities and recurrence at 3 months after being cured (N = 204).

Treatment modalities	Treatment period (weeks)	Change of treatment modalities*	Recurrence at 3 months after being cured
Trichloracetic acid (N=131)	4 (2-10)	21/131 (16.0)	17/131 (13.0)
5% Imiquimod (N=57)	8 (4-16)	1/57 (1.8)	5/57 (8.8)
Cryotherapy (N=8)	5 (3-7)	0	0
Surgery** (N=8)	1	0	1/8 (12.5)

Data presented in N(%), median (minimum-maximum)

*Change of treatment modalities for 85% trichloracetic acid refers to adding 5% imiquimod to the weekly application of 85% trichloracetic acid.

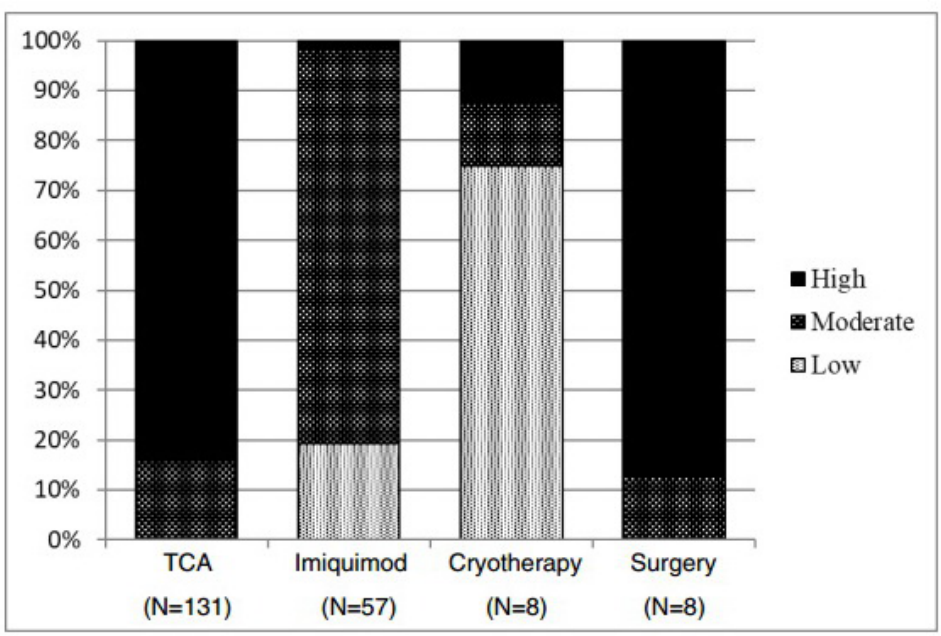

Fig. 1. Degree of pain reported by the patients 24 hours following the application of treatment (N=204).

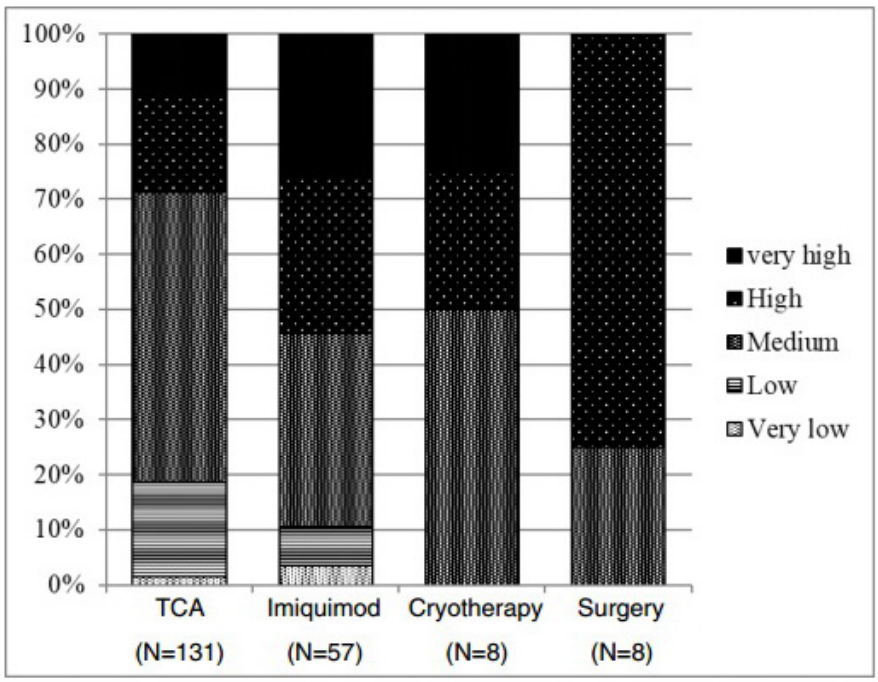

Fig. 2. Patients' satisfaction after using each treatment modality.

Discussion

Although the incidence of AGW at the Thai population is low[4], our finding suggests that the number may be underestimated. There were 170 new cases of AGW at our clinic in only one year. During the study period, our treatments were similar to the other health care units in Thailand by using 85 % TCA as the first choice. Imiquimod is currently available as an over-the-counter medicine. Contrasting to previous reviews[9], our results demonstrated high success rate of treatment in all measures. This may result from our service that include both treatment and health self-care counseling. Moreover, follow-up visits were also more frequent and regular.

These results were useful information to set up our guideline of treatments. TCA is the lowest cost, safe and widely available. Although most patients had high degree of pain within 24 hours, they were satisfied with the treatment. They concerned more about the treatment results than the side effects of medicine. The same findings were demonstrated in the surgical group.

The treatments of AGW are simple and applicable to medical students, residents and fellows. Our clinic has provided educational video on management techniques and counseling session. Some of this information can also freely accessible online. Cryotherapy provides impressive outcomes with less pain and shorter duration of treatment. This technique will be used more often for AGW in our clinic. Other novel treatments such as interferon, sinecatechins appear promising but they are not available in our clinic.

The incidence of AGW has been decreased in the countries that include quadrivalent (HPV 6, 11, 16, 18) or nonavalent (HPV 6, 11, 16, 18, 31, 33, 45, 52, 58) HPV vaccination as a national program such as Canada and Australia[14, 15]. This supports the results of the three landmark HPV studies, including FUTURE 1[16], Broad spectrum HPV[17] and V501-020[18]. The HPV vaccine against HPV type 6 and 11 do not only prevent new AGW cases but also alleviate the course of disease. Choi H compared Quadrivalent vaccine and surgical treatment in 26 Korean patients with AGW

and demonstrated that the recurrence rate was lower in the vaccine group[19]. Nonetheless, the present study can only represent information of unvaccinated population.

The well-planned follow-up schedule of patients with AGW in the specialized STI clinic, Siriraj Hospital is our the main strength. Our study is the first report on Thai female AGW patients. However, the treatment outcomes were depended on the experience and facilities of each center. Therefore, our low recurrence and high successful treatment may not represent the country data. In addition, the current study did not include HPV vaccinated population. Although AGW lesions have been cured following each treatment, the recurrence rate is still of concern.

Conclusion

Based on an experience of the Siriraj Female STI clinic, 85% TCA, which is widely available, needed for applications to treat AGW with 13% recurrence while 5% imiquimod took longer time for treatment with the side effects as immunomodulator i.e., redness, itching, swollen but was associated with lower recurrent rate. Cryotherapy and surgery appeared promising but the data were still limit.

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Potential conflicts of interest

The authors declare no conflict of interest.

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