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Thai Journal of Obstetrics and Gynaecology
March 2023, Vol. 31, No. 2, pp. 83.

EDITORIAL

This second issue of Thai Journal of Obstetrics and Gynaecology 2023 contains many interesting articles. One special article is “Hormonal Replacement Therapy after Gynecologic Cancer Treatment”.

Editor in Chief and managing staff of Thai Journal of Obstetrics and Gynaecology (TJOG) already attended “System development and quality improvement of Thai journals in the Scopus database, Phase 2” on February 24, 2023 at Mandarin Hotel Bangkok, Rama 4 Road, Bang Rak, Bangkok, Thailand. Editorial Board of TJOG looks forward to continuously raising the quality of the TJOG.

RTCOG mid year Meeting 2023 will be held during 25-29 April 2023 at Centara Grand at Central Plaza Ladprao Bangkok, Thailand. The theme of this meeting is “Good Practice”. All RTCOG members are cordially invited to participate this scientific meeting.

Wish to see you at RTCOG mid year Meeting 2023 at Centara Grand at Central Plaza Ladprao Bangkok, Thailand

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

Thai Journal of Obstetrics and Gynaecology
March 2023, Vol. 31, No. 2, pp. 84-88.

SPECIAL ARTICLE

Hormonal Replacement Therapy after Gynecologic Cancer Treatment

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ABSTRACT

Patients diagnosed with gynecologic cancers are principally managed by radiation, chemotherapy, and total hysterectomy with bilateral salpingo-oophorectomy. The resultant loss of ovarian function associated with gynecologic cancer treatments poses major health concerns as patients face the long-term effects of early menopause. The health implication entails vasomotor symptoms, osteoporosis, cognitive impairment, and increased cardiovascular risks, to name a few. Patients with gynecologic cancers are likely to require intervention, as loss of ovarian function due to cancer treatments tend to produce more severe symptoms than those from natural menopause. Hormonal replacement therapy (HRT) has shown to be an excellent option for treating menopausal symptoms. However, initiating HRT remains a challenge due to the expression of hormone receptors in most gynecologic cancers. This article aims to provide current evidence regarding HRT in managing menopause after gynecologic cancer treatment.

Keywords: hormonal replacement therapy, gynecologic cancer, menopause.

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Introduction

Worldwide, an estimated annual incidence of gynecologic malignancies is approximately more than 3.6 million[1]. Gynecologic malignancies are principally managed by radiation, chemotherapy, and total hysterectomy with bilateral salpingo-oophorectomy. The resultant precipitous loss of ovarian function especially in women less than 45 years of age causes more severe menopausal symptoms than the natural course. Vasomotor symptoms, psycho-cognitive effects, risk of osteoporosis, and cardiovascular disease are all heightened[2,3]. The management of menopausal symptoms after gynecologic cancer treatment depends on the patient's age, comorbidities, and tumor type and staging. The aim of this article is to provide current evidence regarding hormone replacement therapy (HRT) in managing menopause after gynecologic cancer treatment.

Epithelial ovarian cancer/fallopian tube/ primary peritoneal cancer

The five main histologic types of epithelial ovarian carcinoma are low-grade serous, high-grade serous, endometrioid, clear cell, and mucinous. The results of two randomized trials and two meta-analyses demonstrated that postoperative HRT has no deleterious effect on survival outcome in women with ovarian cancer. Guidozzi F et al[4] studied 130 women who had been diagnosed with epithelial ovarian cancer (EOC). They were randomized to continuous oral conjugated equine estrogen (ERT) or not (non-ERT). Follow-up 48 months revealed no significant difference in disease-free interval (DFI) and overall survival (OS) between the two groups. The ERT group and non-ERT group had median OS of 44 and 34 months, respectively. In another randomized study by Eeles RA et al[5], 150 premenopausal and postmenopausal women with EOC were randomly assigned to either no HRT or HRT group, and were provided various hormonal regimens according to physician preference. After a 5-year follow-up, this study showed that OS and relapsed-free survival were significantly improved in women who were receiving HRT. Based on the results of two meta-analyses[6,7], postoperative HRT used among women with ovarian cancer neither heighten tumor recurrence nor minimize OS. However, there are some limitations on how the results can be used. Small sample sizes in most studies limited available evidence to determine the use of HRT in the various histological subtypes of EOC. Additionally, there was also inconsistency in hormonal regimens among studies, which resulted in insufficient data for subgroup analysis for optimal HRT regimen.

The suitability of HRT may depend on the type of ovarian cancer. Estrogen receptor (ER) and progesterone receptor (PR) expressions vary with each type of EOC. The positivity of ER and PR are higher in low-grade serous carcinoma than that in high-grade serous carcinoma[8]. Consequently, the use of HRT in low-grade serous carcinoma is not recommended, especially in the advanced disease of this tumor type. Moreover, there is a paucity of evidence regarding high-grade serous carcinoma and HRT.(9) On the other hand, HRT does not seem to have an effect on survival for endometrioid ovarian cancer, which is an estrogen sensitive tumor. Therefore, HRT has been considered appropriate in early-stage disease. Nonetheless, HRT should be avoided in cases of advanced stage disease with residual disease after surgery[10]. Meanwhile, for all patients treated for clear cell and mucinous cancer, evidence showed that HRT can be implemented[3].

Ovarian germ cell tumors

Currently available data supports the use of HRT in patients with germ cell tumors if indicated[3].

Granulosa cell tumors

Since granulosa cells are estrogen dependent, a history of this type of ovarian cancer may be thought of as a contraindication for HRT. However, research has yet to show any negative effect[9].

Endometrial cancer

Endometrial cancer is found primarily among women with postmenopausal status, with only 25% of cases occurring in premenopausal women[11]. The

standard treatment begins with total hysterectomy with bilateral salpingo-oophorectomy and surgical staging. Subsequent adjuvant treatments are determined by the presence or absence of risk factors and risk of recurrence. Following surgical procedures, menopausal symptoms may be implicated.

The randomized controlled trial by Barakat et al[12] recruited 1,236 endometrial cancer patients who have undergone a total hysterectomy and bilateral salpingo-oophorectomy with or without subsequent adjuvant therapy. The patients were previously evaluated to include only those who had no other invasive malignancies in the last five years. Half of the patients were given ERT for three years, although the regimen and route were not specified. Meanwhile, the control group was given a placebo. After 35.7 months of follow-up, both ERT and control group had comparable progression free survival (PFS) and OS. In regards to exogenous estrogen replacement for relieving menopausal symptoms, the study concluded that endometrial cancer recurrence was low in patients with early stages of endometrial cancer. Nonetheless, the safety and recommendation of ERT for endometrial cancer patients remained inconclusive.

The Cochrane review attempted to assess the safety of HRT among patients previously treated for endometrial cancer by analyzing one randomized trial (Barakat et al) and five observational studies[13]. This review concluded that there was insufficient evidence to consider HRT after treatment of endometrial cancer. The benefit of treatment should be weighed against the risk of recurrence. However, the limited data suggest that HRT may be a reasonable option for women with low-grade and early-stage endometrial cancer. There was no data on higher stages of endometrial cancer.

Uterine Sarcoma

Uterine sarcoma accounts for about 3% of all uterine cancer cases. Histology of uterine sarcoma ranges from low-grade endometrial stromal sarcoma (LGESS), high-grade endometrial stromal sarcoma (HGESS), undifferentiated uterine sarcoma, and leiomyosarcoma (LMS), which is the most common subtype[14-15]. Uterine sarcoma may be hormone-dependent, as some are found to express ER and PR[16]. Therefore estrogen and progesterone receptor testing should be initiated to determine suitability for HRT for menopausal symptoms after cancer treatment. Regardless, non-hormonal treatment for menopausal symptoms should be considered for these patients[17].

Cervical Cancer

Squamous cell carcinoma constitutes up to 90% of cervical cancer histology, while adenocarcinoma makes up for the remaining 10-20%[18]. Chemotherapy, radiotherapy or surgery are the main treatment modalities for cervical cancer. These treatments may induce sudden-onset of premature menopause in women due to the removal of ovaries in surgical treatments or the destruction of oocytes from radiation toxicity[19]. Cervical cancers are hormonal-independent, thus contraindications for topical and systemic HRT have not been evident[3]. The choice of unopposed or opposed estrogen would be dependent on the patient's hysterectomy status. To prevent stimulation of the endometrium, opposed estrogen is provided for those who were treated with chemo-radiation with preservation of the uterus, while unopposed estrogen is provided for patients that underwent hysterectomy[3].

In the limited studies, no significant difference in oncologic outcome was observed amongst patients diagnosed with cervical squamous cell carcinoma who received HRT[20-22]. A prospective study by Ploch et al[20] examined 120 cervical cancer patients, 80 of whom received HRT in the form of opposed estrogen. Throughout five years of regular follow-up examination, no serious adverse effects of HRT were evident. The HRT and control group had 80 and 65 percent five-year survival, respectively, which was statistically insignificant. The difference in disease recurrence between the two groups was also statistically insignificant, where there were 20 and 32 percent recurrence in the HRT and control groups, respectively. Additionally, the HRT group was found to experience a milder degree and shorter duration of post-radiological complications.

There are differences in hormone receptor

expression between squamous cell carcinomas and adenocarcinomas. One study evaluated estrogen and progesterone receptor expression in patients with cervical adenocarcinoma and their oncologic outcomes. Estrogen and progesterone receptors were expressed in 39 and 33 percent, respectively. Throughout the 24 months period of follow-up, these receptor expressions did not influence the clinicopathological parameters, which included the clinical stage, age, histology, tumor size and grading, lymphovascular space invasion, lymph node status, and disease recurrence. The overall and disease-free survival of the patients were also not correlated[23]. Therefore, the estrogen and progesterone receptors are not recommended to yield the prognostic value of cervical adenocarcinoma. These results are in coherence with a systematic review of ten articles, which found no relationship between HRT and the incidence of cervical cancer[19]. Nonetheless, one study found HRT to have a positive association with cervical adenocarcinoma, although statistical significance was not reached[24]. Although there is a paucity of data, a majority of available studies suggested an absence of significant relationships between HRT and the risks of cervical cancer.

Conclusion

The management of menopausal symptoms after gynecological cancer treatment depends on their age, tumor type and stage, and thus should be individualized. Shared decision making is necessary for initiating HRT. High quality and randomized data examining the use of HRT are still lacking. Therefore more research is needed to provide stronger evidence to guide practice.

Potential conflicts of interest

The author declares no conflicts of interest.

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OBSTETRICS

Adverse Neonatal Outcomes in Relation to Modes of Delivery in Thick Meconium-stained Amniotic Fluid with NICHD Category I Fetal Heart Rate Pattern

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ABSTRACT

Objectives: This work studied the association between adverse neonatal outcomes and modes of delivery in thick meconium-stained amniotic fluid pregnant women with normal fetal heart rate (FHR) patterns and the key factors affecting the adverse neonatal outcomes.

Materials and Methods: This research was a retrospective cohort study. The investigation was conducted by collecting the data of 271 singleton pregnant women who had 37-42 weeks of gestation, were diagnosed with thick meconium-stained amniotic fluid and a normal FHR pattern was determined, according to the National Institute of Child Health and Human Development (NICHD) classification and were admitted to Hatyai Hospital during January 2015 to December 2020. Multivariate analysis was used to determine the association between mode of delivery and neonatal outcomes.

Results: Modes of delivery were not associated with adverse neonatal outcomes. The important factor that was associated with adverse neonatal outcome was oxytocin use > 3 h (adjusted odds ratio (OR) 3.07, $p = 0.01$). Risk factors of meconium aspiration syndrome (MAS) were the patients who did not receive antenatal care (adjusted OR 31.13, $p = 0.04$) and the induction of labor (adjusted OR 3.91, $p = 0.04$). However, increasing the Apgar score could reduce the risk of MAS (adjusted OR 0.61, $p < 0.001$). A risk factor of neonatal intensive care (NICU) admission was preeclampsia (adjusted OR 6.15, $p = 0.02$) but increasing the Apgar score at 1 min could reduce the risk of NICU admission (adjusted OR 0.49, $p < 0.001$).

Conclusion: Modes of delivery were not associated with adverse neonatal outcomes. The significant factor associated with an adverse neonatal outcome was oxytocin use > 3 h.

Keywords: thick meconium-stained amniotic fluid, adverse neonatal outcome, mode of delivery.

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พรนภัส เชาวนาสัย, พักตร์ประภา ไชยภักดี

บทคัดย่อ

วัตถุประสงค์: *เพื่อหาความสัมพันธ์ระหว่างผลลัพธ์การคลอดของทารกที่ไม่พึงประสงค์กับวิธีการคลอดในภาวะขี้ เทาข้นปนในน้ำคร่ำในกลุ่มทารกที่มีหัวใจเต้นปกติและศึกษาปัจจัยที่มีผลต่อการคลอดของทารกที่ไม่พึงประสงค์*

วัสดุและวิธีการ: *เป็นการศึกษาย้อนหลังของสตรีตั้งครรภ์เดี่ยวช่วงอายุครรภ์ตั้งแต่ 37 ถึง 42 สัปดาห์ ที่ได้รับการวินิจฉัยว่ามีภาวะขี้เทาข้นปนในน้ำคร่ำและทารกหัวใจเต้นปกติตามการแบ่งกลุ่มของ NICHD จำนวน 271 คน ที่มาคลอดบุตร ณ โรงพยาบาลหาดใหญ่ ระหว่างเดือนมกราคม พ.ศ.2558 ถึงเดือนธันวาคม พ.ศ.2563 และวิเคราะห์หาความสัมพันธ์ระหว่างวิธีการคลอดกับผลลัพธ์การคลอดของทารกที่ไม่พึงประสงค์โดยใช้สถิติชนิด multivariate analysis*

ผลการศึกษา: *วิธีการคลอดไม่ได้สัมพันธ์กับผลลัพธ์การคลอดของทารกที่ไม่พึงประสงค์แต่ปัจจัยสำคัญที่มีผลต่อผลลัพธ์การคลอดของทารกที่ไม่พึงประสงค์คือ การใช้ยาเร่งคลอด oxytocin นานกว่า 3 ชั่วโมง (adjusted OR 3.07, p = 0.01) ปัจจัยที่มีผลต่อภาวะทารกสำลักขี้เทาเข้าปอด ได้แก่ สตรีที่ไม่ได้ฝากครรภ์ (adjusted OR 31.13, p = 0.04), การชักนำการคลอด (adjusted OR 3.91, p = 0.04) ส่วนทารกที่มีคะแนนประเมินแรกคลอดที่สูงขึ้นจะมีโอกาสเกิดภาวะทารกสำลักขี้เทาเข้าปอดน้อยลง (adjusted OR 0.61, p < 0.001) ปัจจัยที่มีผลต่อการเข้าหออภิบาลทารกแรกเกิดภาวะวิกฤต ได้แก่ มารดาที่มีภาวะครรภ์เป็นพิษ (adjusted OR 6.15, p = 0.02) ส่วนทารกที่มีคะแนนประเมินแรกคลอดที่ 1 นาที สูงขึ้นจะมีโอกาสเข้าหออภิบาลทารกแรกเกิดภาวะวิกฤตน้อยลง (adjusted OR 0.49, p < 0.001).*

สรุป: *วิธีการคลอดไม่ได้สัมพันธ์กับผลลัพธ์การคลอดของทารกที่ไม่พึงประสงค์แต่ปัจจัยสำคัญที่มีผลต่อผลลัพธ์การคลอดของทารกที่ไม่พึงประสงค์คือ การใช้ยาเร่งคลอด oxytocin นานกว่า 3 ชั่วโมง*

คำสำคัญ: *ภาวะขี้เทาข้นปนในน้ำคร่ำ, ผลลัพธ์การคลอดของทารกที่ไม่พึงประสงค์, วิธีการคลอด*

Introduction

Meconium comprises fetal bowel contents consisting of various products of secretion[1]. Thick meconium is a thick dark green amniotic fluid or any meconium-stained amniotic fluid that contains lumps of meconium[2].

The incidence of thick meconium-stained amniotic fluid in Hatyai Hospital is 3.02%, which is less than that in previous studies (5.61%[3] and 5.47%[4]). Meconium staining of the amniotic fluid (MSAF) has been reported more commonly in term (7-22%) and post-term deliveries (23-52%)[5]. This may be explained by three theories regarding the fetal passage of meconium. Firstly, the fetus may pass meconium in response to hypoxia causing fetal compromise. Secondly, meconium may be representative of normal gastrointestinal tract maturation under neural control. The last theory explains that meconium passage follows vagal stimulation due to umbilical cord entrapment causing bowel peristalsis to increase[1].

Meconium-stained amniotic fluid is considered to be a bad predictor of fetal outcomes. The presence of meconium in amniotic fluid is a potentially serious sign of fetal compromise and is associated with poor perinatal outcomes[6]. Meconium aspiration syndrome (MAS) is significantly associated with fetal acidemia at birth[7]. Infants born through meconium-stained amniotic fluid have a five-fold increase in perinatal mortality, compared with low-risk patients with clear amniotic fluid[8]. It is difficult to make the decision whether to continue normal vaginal delivery or to proceed with cesarean delivery for the best neonatal outcome in the thick meconium-stained amniotic fluid pregnant women with a normal fetal heart rate (FHR) pattern, according to the National Institute of Child Health and Human Development (NICHD) classification[9].

There are no previous guidelines on the proper mode of delivery and no studies about the association between neonatal outcomes and modes of delivery in thick meconium-stained amniotic fluid pregnant women who had a normal FHR pattern (NICHD category I) in Thailand. Therefore, the primary objective in this study was to investigate the association between adverse neonatal outcomes and modes of delivery in thick meconium-stained amniotic fluid pregnant women with a normal FHR pattern (NICHD category I). The secondary outcome was to find the key factors affecting the adverse neonatal outcomes in this group. We assumed that the mode of delivery may be associated with adverse neonatal outcomes.

Materials and Methods

This work was a retrospective cohort study. This study was approved by The Institutional Review Board of Hatyai Hospital. The investigation was performed by collecting the data of pregnant women who were diagnosed with thick meconium-stained amniotic fluid and were admitted to the labor ward in Hatyai Hospital during January 2015 to December 2020.

The inclusion criteria were singleton pregnant women at 37-42 weeks of gestation with a normal FHR pattern (NICHD category I) and cephalic presentation. Those pregnant women who had non-cephalic presentation, multifetal pregnancy, abnormal FHR pattern (NICHD category II, III), the need for instrumental delivery (forceps and vacuum extraction), abnormal placenta, intrauterine fetal growth restriction, or fetal anomaly were excluded.

Among the 1,200 pregnant women diagnosed with thick meconium-stained amniotic fluid, 271 patients met the inclusion criteria of this study. Among the 271 patients, 127 patients were in the cesarean section (C-section) group and the rest were in the normal vaginal delivery (NVD) group.

The sample size was calculated using the formula for descriptive study:

$n = (Z_{\alpha/2}+Z_{\beta})^{2*} (p_1 (1 - p_1) + (p_2 (1- p_2)) / (p_1- p_2)^2)$.

Based on a previous study[5], where

$Z_{\alpha/2} = 1.96$, $Z_{\beta} = 0.84$, $p1 = 0.15$ and $p2 = 0.04$. The minimum sample size needed for each group is 126 cases.

After the admission, the management of labor

and delivery were provided according to the institutional guidelines. Continuous electronic fetal monitoring was offered to all pregnant women during labor and delivery and all FHR tracing underwent interpretation by the attending obstetricians. The definition of thick meconium-stained amniotic fluid was dark green in color and containing particulate matter[10] that was diagnosed by two midwives or the attending obstetricians at the time of rupture of the membranes or initial per-vaginal examination during admission when rupture of the membranes occurred before the patient arrived in the labor room. For the patients who needed induction or augmentation of labor, the oxytocin dosage used was about 4-16 mU/min and could be adjusted by uterine contraction. The indication of induction was similar between two groups such as overt diabetes mellitus (DM), preeclampsia and oligohydramnios. In our protocol, the cesarean section was performed in thick meconium-stained amniotic fluid pregnant women with abnormal FHR patterns (NICHD category II and III), but there was no guideline for management of the mode of delivery in patients with normal FHR patterns (NICHD category I).

All data included demographic data (age, gravida, body mass index (BMI), gestational age, antenatal care), medical condition (chronic hypertension, overt DM, human immunodeficiency virus (HIV), hepatitis B, systemic lupus erythematosus (SLE), asthma, hyperthyroid, anemia), obstetric risk factors (gestational hypertension, preeclampsia, GDM, oligohydramnios, chorioamnionitis), intrapartum assessment (onset of labor, duration of oxytocin use, duration of labor, duration rupture of membranes (ROM), cervix dilation at the time of diagnosis of thick meconium), neonatal outcome (neonatal intensive unit (NICU) admission, neonatal medium care unit (NMCU) admission, MAS, respiratory distress syndrome (RDS), persistent pulmonary hypertension of the newborn (PPHN), Apgar score, intubation, cardiopulmonary resuscitation (CPR), transient tachypnea of the newborn (TTNB), sepsis, pneumonia), and maternal complications (postpartum hemorrhage (PPH), uterine atony, uterine segment tear, metritis) were collected from Hatyai Hospital medical records.

Statistical analysis was done by use of R program version 4.0.2. Descriptive statistics including frequencies and cross tabulations were performed. Multiple logistic regression fitted with an odds ratio (OR) with a 95% confidence interval (CI) was calculated. Adjusted OR were calculated by entering the proposed explanatory variables having a significant p value < 0.05. Univariate and multivariate analyses were used to determine the association between neonatal outcomes and modes of delivery.

Results

The study found that the median maternal age and birth weight in the C-section group were greater than in the NVD group ($p = 0.01$ and $p = 0.01$, respectively). The number of patients who completed antenatal care in the C-section group was higher than that in the NVD group ($p = 0.04$). The gestational age, BMI, and medical conditions between the two groups were not significantly different. For the obstetric risk factor data, the number of patients diagnosed with gestational diabetes mellitus (GDM) in the C-section group was higher than those in the NVD group ($p = 0.05$). The patients diagnosed with chorioamnionitis were found in the C-section group only ($p = 0.05$). The other obstetric risk factors such as gestational hypertension, preeclampsia, and oligohydramnios were not statistically different between the two groups.

The most common indication of neonatal admission was meconium aspiration syndrome (MAS) followed by neonatal sepsis. Jaundice was found in the NVD group to a greater extent than in the C-section group ($p = 0.04$). Indications of cesarean section delivery were cephalopelvic disproportion (55 cases, 43.31%), previous cesarean section (8 cases, 6.30%), thick meconium-stained amniotic fluid (53 cases, 41.73%), and failed induction (11 cases, 8.66%). The demographic data are shown in Table 1.

Table 1. Demographic data of the studied patients.

Characteristics	C-section (N=127) n (%)	NVD (N=144) n (%)	p value
Age (years)			0.03
≤ 20	19 (14.96%)	34 (23.61%)	
21-25	30 (23.62%)	45 (31.25%)	
26-30	38 (29.92%)	39 (27.08%)	
> 30	40 (31.50%)	26 (18.06%)	
Median (IQR)	28 (23,32)	25 (21,29)	0.01
Gravida			0.06
Primi	72 (56.7%)	64 (44.4%)	
Multi	55 (43.3 %)	80 (55.6%)	
BMI (kg/m^2)			0.34
< 25	71 (55.90%)	93 (64.58%)	
25-29.9	32 (25.20%)	30 (20.84%)	
≥ 30	24 (18.90%)	21 (14.58%)	
Gestational age			0.07
37-38	29 (22.83%)	39 (27.08%)	
39-40	78 (61.42%)	95 (65.98%)	
41-42	20 (15.75%)	10 (6.94%)	
Antenatal care			0.04
Complete	123 (96.86%)	131 (90.96%)	
Incomplete	2 (1.57%)	11 (7.64%)	
No ANC	2 (1.57%)	2 (1.40%)	
Medical condition			
Chronic hypertension	1 (0.79%)	1 (0.69%)	1.00
Overt DM	1 (0.79%)	0 (0.00%)	0.47
HIV	0 (0.00%)	0 (0.00%)	0.30
Hepatitis B	2 (1.57%)	4 (2.78%)	0.69
SLE	0 (0.00%)	0 (0.00%)	0.30
Asthma	3 (2.37%)	0 (0.00%)	0.10
Hyperthyroid	0 (0.00%)	1 (0.69%)	1.00
Anemia	11 (8.69%)	12 (8.28%)	0.48
Obstetric risk factor			
Gestational HT	2 (1.57%)	2 (1.39%)	1.00
Preeclampsia	8 (6.32%)	4 (2.78%)	0.27
GDM	8 (6.32%)	2 (1.39%)	0.05
Oligohydramnios	7 (5.53%)	3 (2.08%)	0.20
Chorioamnionitis	4 (3.16%)	0 (0.00%)	0.05
Birth weight (grams)	3280	3087	0.01
Sex			
Male	67 (52.76%)	78 (54.17%)	
Female	60 (47.24%)	66 (45.83%)	
Indication for NICU and NMCU admission			
MAS	24 (18.90%)	24 (16.67%)	0.75
Neonatal sepsis	16(12.60%)	9 (6.25%)	0.11
Hypoglycemia	2 (1.57%)	0 (0.00%)	0.22
Pneumonia	3 (2.36%)	1 (0.69%)	0.34
Jaundice	9 (7.09%)	23 (15.97%)	0.04
TTNB	7 (5.51%)	2 (1.39%)	0.09
Amphetamine positive	1 (0.79%)	0 (0.00%)	0.47

Values are given as median (interquartile range) and number (%).
C-section: cesarean section, NVD: normal vaginal delivery, IQR: interquartile range, BMI: body mass index, ANC: antenatal care, DM: diabetes mellitus, HIV: human immunodeficiency virus, SLE: systemic lupus erythematosus, HT: hypertension, GDM: gestational diabetes mellitus, NICU: neonatal intensive unit, NMCU: neonatal medium care unit, MAS: meconium aspiration syndrome

According to the study, the number of patients who received induction of labor and the duration of labor in the C-section group were both higher than in the NVD group ($p = 0.02$ and $p < 0.001$, respectively). Most patients in both groups did not receive oxytocin. However, it was observed that the number of patients who received oxytocin > 3 h in the C-section group was greater than those in the NVD group ($p < 0.001$).

The median cervical dilatation at the first instance of thick meconium diagnosis in the NVD group was greater than that in the C-section group. Moreover, most patients in the NVD group had cervical dilatation ≥ 6 cm ($p < 0.001$). Uterine atony and uterine segment tear were found in the C-section group only ($p < 0.001$) and were not found in the NVD group ($p = 0.05$). The intrapartum data are shown in Table 2.

Table 2. Intrapartum data.

Intrapartum	C-section (N=127) n (%)	NVD (N=144) n (%)	p value
Onset of labor			0.02
Spontaneity	50 (39.37%)	70 (48.61%)	0.16
Augmentation	65 (51.18%)	71 (49.31%)	0.85
Induction	12 (9.45%)	3 (2.08%)	0.02
Duration of oxytocin use (h)			< 0.001
None	64 (50.39%)	90 (62.50%)	
≤ 3	20 (15.75%)	39 (27.08%)	
> 3	43 (33.86%)	15 (10.42%)	
Median duration labor (h)	4 (2,6)	2 (1,4)	< 0.001
Median duration ROM (h)	3 (1.5,6)	2 (1,4)	0.06
Cervix dilation (centimeters)			< 0.001
< 6	96 (75.59%)	61 (42.36%)	
≥ 6	31 (24.41%)	83 (57.64%)	
Median (IQR)	3 (2,5)	6 (4,9.2)	< 0.001
Maternal complication			
(n = 26)	(n = 20)	(n = 6)	
PPH	7 (35.00%)	5 (83.33%)	0.60
Uterine atony	9 (45.00%)	0 (0.00%)	< 0.001
Uterine segment tear	4 (20.00%)	0 (0.00%)	0.05
Metritis	0 (0.00%)	1 (16.67%)	1.00

Values are given as median (interquartile range) and number (%).
C-section: cesarean section, NVD: normal vaginal delivery, ROM: rupture of membranes, IQR: interquartile range, PPH: postpartum hemorrhage

The number of patients who had adverse neonatal outcomes including MAS, RDS, intubation, PPHN, CPR, TTNB, neonatal sepsis, pneumonia, NICU admission, NMCU admission, and Apgar score < 7 at 1 and 5 min in the C-section group was greater than in the NVD group ($p = 0.01$). The median duration of hospital neonatal admission between two groups showed no difference ($p < 0.001$). Transient tachypnea of newborns (TTNB) was found in the C-section group to a greater extent than in the NVD group ($p = 0.01$). Jaundice was found in the NVD group to a greater extent than in the C-section group ($p = 0.04$). These data are shown in Table 3.

Table 4 presents the univariate analysis which found that the factors associated with adverse neonatal outcomes (MAS, RDS, intubation, PPHN, CPR, TTNB, neonatal sepsis, pneumonia, NICU admission, NMCU admission, Apgar score < 7 at 1 and 5 min) were C-section (crude OR 2.07, $p = 0.01$) and duration of oxytocin (crude OR 1.10, $p = 0.02$).

Table 3. Neonatal outcome.

Neonatal outcome	C-section (N=127) n (%)	NVD (N=144) n (%)	p value
Apgar score			
1 min			0.05
< 7	13 (10.24%)	7 (4.86%)	
≥ 7	114 (89.76%)	137 (95.14%)	
5 min			0.66
<7	4 (3.15%)	3 (2.08%)	
≥ 7	123 (96.85%)	141 (97.92%)	
Adverse neonatal outcome	4 (2,6)	2 (1,4)	< 0.001
Admission NICU	3 (1.5,6)	2 (1,4)	0.06
Admission NMCU			< 0.001
Duration of admission (day) (Median (IQR))			
MAS	24 (18.90%)	25 (17.36%)	0.51
RDS	1 (0.79%)	1 (0.69%)	1.00
PPHN	4 (3.15%)	4 (2.78%)	1.00
Intubation	20 (15.75%)	15 (10.42%)	0.26
CPR	2 (1.57%)	0 (0.00%)	0.22
TTNB	11 (8.66%)	2 (1.39%)	0.01
Pneumonia	3 (2.36)	1 (0.69)	0.34
Jaundice	9 (7.09)	23 (15.97)	0.04
Hypoglycemia	2 (1.57)	0 (0.00)	0.22
Neonatal sepsis	16 (12.60)	9 (6.25)	0.11

Values are given as median (interquartile range) and number (%).
C-section: cesarean section, NVD: normal vaginal delivery, NICU: neonatal intensive unit, NMCU: neonatal medium care unit, IQR: interquartile range, MAS: meconium aspiration syndrome, RDS: respiratory distress syndrome, PPHN: persistent pulmonary hypertension of the newborn, CPR: cardiopulmonary resuscitation, TTNB: transient tachypnea of the newborn

Table 4. Univariate analysis of adverse neonatal outcomes.

Maternal factor	Crude OR (95%CI)	p value
Cesarean delivery	2.07 (1.24,3.46)	0.01
Age	1.03 (0.99,1.07)	0.17
Gravidity	1.01 (0.61,1.68)	0.97
BMI	1.03 (0.98,1.08)	0.19
Gestational age	0.95 (0.75,1.21)	0.70
ANC group	3 (2.08%)	
- Incomplete ANC	141 (97.92%)	
- No ANC	2 (1,4)	< 0.001
Hepatitis B	2.05 (0.40,10.35)	0.39
Asthma	4.09 (0.37,45.73)	0.25
Anemia	0.40 (0.13,1.20)	0.10
Gestational HT	2.03 (0.28,14.68)	0.48
Preeclampsia	2.08 (0.65,6.65)	0.22
GDM	2.07 (0.58,7.35)	0.26
Oligohydramnios	3.16 (0.87,11.50)	0.08
Onset of labor	0 (0.00%)	0.22
- Augmentation	2 (1.39%)	0.01
- Induction	1 (0.69)	0.34
Duration of oxytocin	1.10 (1.02,1.2)	0.02
Duration labor	1.02 (0.93,1.12)	0.68
Duration ROM	1.05 (0.99,1.11)	0.13
Cervix dilate	1.05 (0.97,1.14)	0.25

Values are given as odds ratios (95% confidence interval)
OR: odds ratio, CI: confidence interval, BMI: body mass index, ANC: antenatal care, HT: hypertension, GDM: gestational diabetes mellitus, ROM: rupture of membranes

Table 5 presents multivariate analysis of the factors associated with adverse neonatal outcomes (MAS, RDS, intubation, PPHN, CPR, TTNB, neonatal sepsis, pneumonia, NICU admission, NMCU admission, Apgar score < 7 at 1 and 5 min). Although univariate analysis data demonstrated that C-section was associated with adverse outcomes in Table 4, the multivariate analysis showed that C-section was not associated with adverse outcomes (adjusted OR 0.89, p = 0.80). The only factor associated with adverse neonatal outcomes was oxytocin use > 3 h, which increased the risk of adverse neonatal outcomes by 3 times (adjusted OR 3.07, p = 0.01). The cut point of 3 h was from the statistical calculation by using the median and percentile range that results in significantly different adverse neonatal outcomes between the two groups.

Table 5. Multivariate analysis of adverse neonatal outcomes.

Maternal factor	Adverse outcome (case) (n = 90) n (%)	Crude odds ratio (95%CI)	p value	Adjusted odds ratio (95%CI)	p value
Cesarean delivery	54 (60.00%)	2.06 (1.24, 3.44)	0.01	0.89 (0.38, 2.10)	0.80
Duration of oxytocin > 3 h	29 (32.22%)	2.93 (1.34, 6.40)	0.01	3.07 (1.30, 7.23)	0.01

Values are given as odds ratio (95% confidence interval) and number (%).
CI: confidence interval

The study observed that the mode of delivery was not associated with MAS and NICU admission from the univariate analysis (p = 0.42 and p= 0.10, respectively) but the patients who did not receive antenatal care had a 31-fold increase in risk of MAS (adjusted OR 31.13, p = 0.04), and a 3.91-fold increase in risk of induction of labor (adjusted OR 3.91, p = 0.04). Increasing the Apgar score to 1 minute reduced the risk of MAS (adjusted OR 0.61, p < 0.001), as shown in Table 6.

Table 6. Multivariate analysis of MAS.

Factor	Crude OR (95%CI)	Adjusted OR (95%CI)	p value
Anemia	0.18 (0.02,1.33)	0.13 (0.01,1.18)	0.07
Oligohydramnios	4.55 (1.27,16.36)	2.53 (0.56,11.55)	0.23
Augmentation	1.06 (0.56,2.01)	0.92 (0.46,1.86)	0.83
Induction	3.14 (1.01,9.78)	3.91 (0.91,12.47)	0.04
Maternal complication	3.02 (1.28,7.12)	2.37 (0.90,6.26)	0.08
Apgar score at 1 min	0.59 (0.47,0.74)	0.6 (0.47,0.77)	< 0.001
No ANC	14.32 (1.46, 140.88)	31.13 (1.13, 855.94)	0.04

Values are given as odds ratio (95% confidence interval)
MAS: meconium aspiration syndrome, OR: odds ratio, CI: confidence interval, ANC: antenatal care

Moreover, the study revealed that preeclampsia increased the risk of NICU admission by 6 times (adjusted OR 6.15, p = 0.02. Every 1-point increase in the Apgar score at 1 min reduced the risk of NICU admission by 0.49 times (adjusted OR 0.49, p < 0.001) as shown in Table 7.

Table 7. Multivariate analysis of NICU admission.

Factor	Crude OR (95%CI)	Adjusted OR (95%CI)	p value
BMI (kg/m2)	1.05 (0.99,1.12)	1.04 (0. 96,1.12)	0.35
Preeclampsia	7.35 (2.23,24.23)	6.15 (1.39,27.15)	0.02
Duration of oxytocin use	1.10 (1.00,1.21)	1.10 (0.98,1.23)	0.11
Apgar score at 1 min	0.45 (0.35,0.60)	0.49 (0.35,0.67)	< 0.001
Apgar score at 5 min	0.35 (0.20,0.59)	0.73 (0.47,1.14)	0.17

Values are given as odds ratio (95% confidence interval)
NICU: neonatal intensive unit, OR: odds ratio, CI: confidence interval, BMI: body mass index

Discussion

This study observed that the adverse neonatal outcomes (MAS, RDS, intubation, PPHN, CPR, TTNB, neonatal sepsis, pneumonia, NICU admission, NMCU admission, and Apgar score < 7 at 1 and 5 min) in the C-section and NVD groups were not significantly different. This finding was slightly different from those in previous studies which found that intubation, RDS and NICU admission were higher in NVD groups; however, MAS and asphyxia were not different between groups[5]. The difference in outcomes may be due to this study had smaller sample size than the previous study.

Although univariate analysis of this study showed that C-section was associated with adverse neonatal outcomes ($p = 0.01$), multivariate analysis observed that C-section was not associated with adverse neonatal outcomes ($p = 0.80$). Furthermore, oxytocin use > 3 h was a significant confounding factor because the number of patients who received induction of labor and oxytocin use > 3 h in the C-section group was higher than in the NVD group. Therefore, the significant factor affecting adverse neonatal outcomes was oxytocin use > 3 h, not C-section. This finding correlated with a previous study[11]. This could be explained by oxytocin transiently compromising fetal circulation by increasing the duration, frequency, and strength of uterine contractions. The uterine and umbilical artery flow resistance increased significantly during uterine contractions reflecting a rapid and exaggerated increase of vascular resistance in both arteries which affects neonatal outcomes[12].

Overall, many significantly serious outcomes are associated with thick meconium-stained amniotic fluid, such as MAS and NICU admission. This study demonstrated that significant risk factors of MAS were patients not receiving antenatal care, receiving induction of labor, and having low Apgar scores for the newborn. This corresponded with previous studies describing the risk of MAS as significantly greater in the presence of fetal distress and a low Apgar score[13-16]. The induction of labor was a risk factor of MAS which could be explained by the tendency of induction to take a long time for labor, and prolonged labor caused an increase in the production of fetal cortisol which increased colonic contractions leading to passage meconium[17]. Additionally, a lower Apgar score of 1 min and preeclampsia significantly increased the risk of NICU admission, which agreed with the study of Duhan et al[18].

Additionally, this study showed that modes of delivery are not associated with adverse neonatal outcomes. Hence, the pregnant women who had thick meconium-stained amniotic fluid with normal FHR patterns (NICHD category I) did not necessarily require cesarean delivery. The results of the study can be applied in counseling the patients and guiding proper management about the mode of delivery in this patient group. For the patients who need oxytocin for induction should avoid long durations of oxytocin use and closed fetal heart rate monitoring, especially in oxytocin use > 3 h. Because these factors were associated with adverse neonatal outcomes.

The strength of our study was that there were no previous studies about mode of delivery and neonatal outcomes in pregnant women with thick meconium-stained amniotic fluid with NICHD category I FHR patterns in Thailand. This research was presented as the first investigation in Southern Thailand. However, there were some limitations of this study such as incomplete clinical data due to the retrospective study, and the selective bias cannot totally be excluded because this study population (thick meconium-stained amniotic fluid with NICHD category I FHR pattern) may have benefitted from the creation of the new institutional protocol. This study cannot totally exclude fetal growth restriction (FGR) due to non-available data such as prenatal fetal Doppler, amount of atrial fibrillation (AF), and Ballard score in some cases. The duration after the diagnosis of thick meconium-stained AF until delivery may affect the outcomes of this study. However, this data was not available in our center, so should be

interpreted with caution when assessing the correlation between modes of delivery and neonatal outcomes. We suggest that future study should include this factor for data analysis.

Conclusion

Modes of delivery were not associated with adverse neonatal outcomes in pregnant women who had thick meconium stained amniotic fluid with anormal FHR pattern (NICHD category I). The significant factor affecting the adverse neonatal outcomes was oxytocin use > 3 h.

Potential conflicts of interest

The authors declare no conflicts of interest.

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OBSTETRICS

Asymptomatic Bacteriuria in Thai Pregnant Women with Preterm Delivery: Prevalence, Pathogens and Pregnancy Outcomes

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ABSTRACT

Objectives: The primary aim was to determine the prevalence of asymptomatic bacteriuria (ASB) in Thai pregnant women with a preterm delivery. The secondary aims were to identify common causative organisms and their antibiotic susceptibilities, and to compare the pregnancy outcomes between ASB-positive and ASB-negative patients.

Materials and Methods: The medical records of low-risk pregnant women with a preterm delivery at Siriraj Hospital from January 2014 to May 2020 were reviewed. Patient characteristics, urine culture results, and pregnancy outcome data were recorded. ASB-positive was defined as the growth of at least 10^5 colony-forming units per milliliter (cfu/ml) isolated from a midstream, clean-catch urine specimen.

Results: A total of 826 eligible women were included. The prevalence of ASB was 3% (25/826). The predominant organism was Escherichia coli (E. coli) (48%). All E. coli were susceptible to nitrofurantoin and only 25% were susceptible to ampicillin. No significant adverse outcomes were detected in the ASB-positive group.

Conclusion: The prevalence of ASB in Thai pregnant women with a preterm delivery was low. Antibiotic treatment should be based on the common organisms and local antibiotic susceptibility patterns.

Keywords: asymptomatic bacteriuria, preterm delivery, pregnancy outcome.

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ความชุกและเชื้อก่อโรคของภาวะการมีเชื้อแบคทีเรียในปัสสาวะแบบไม่มีอาการและผลการตั้งครรภ์ในสตรีไทยที่มีการคลอดก่อนกำหนด

วศินี สุขเฉลิมชัย, นลัท สมภักดี, ภัทรวลัย ตลึงจิตร, พรพิมล เรืองวุฒิเลิศ, บูรยา พัฒนจินดากุล

บทคัดย่อ

วัตถุประสงค์: *วัตถุประสงค์หลัก คือ เพื่อหาความชุกของภาวะการมีเชื้อแบคทีเรียในปัสสาวะแบบไม่มีอาการ (asymptomatic bacteriuria; ASB) ในสตรีไทยที่มีการคลอดก่อนกำหนด วัตถุประสงค์รอง คือ เพื่อหาชนิดของเชื้อก่อโรคและแบบแผนของความไวต่อยาปฏิชีวนะ และเปรียบเทียบผลการตั้งครรภ์ระหว่างกลุ่มที่มีและไม่มีภาวะ ASB*

วัสดุและวิธีการ: *ทำการศึกษาย้อนหลังในสตรีไทยที่มีความเสี่ยงต่ำ และมีการคลอดก่อนกำหนดระหว่าง มกราคม พ.ศ. 2557 ถึง พฤษภาคม พ.ศ. 2563 โดยเก็บข้อมูลพื้นฐาน ผลการเพาะเชื้อจากปัสสาวะ และผลการตั้งครรภ์ ภาวะ ASB วินิจฉัยเมื่อตรวจพบเชื้อแบคทีเรียในปริมาณตั้งแต่ 10^5 colony-forming units (cfu)/mL ขึ้นไปจากการเก็บปัสสาวะเพื่อเพาะเชื้อด้วยวิธี clean-catch midstream urine*

ผลการศึกษา: *ความชุกของภาวะ ASB ในสตรีไทยที่มีการคลอดก่อนกำหนดเท่ากับร้อยละ 3 (25 จาก 826 ราย) โดยเชื้อก่อโรคที่เป็นสาเหตุหลัก คือ Escherichia coli (ร้อยละ 48) ซึ่งทุกรายมีความไวต่อยา nitrofurantoin และมีเพียงร้อยละ 25 ที่มีความไวต่อยา ampicillin เมื่อเปรียบเทียบผลการตั้งครรภ์ไม่พบว่ากลุ่มที่มีภาวะ ASB มีผลการตั้งครรภ์ที่ไม่พึงประสงค์มากกว่ากลุ่มที่ไม่มีภาวะ ASB อย่างมีนัยสำคัญ*

สรุป: *ความชุกของภาวะ ASB ในสตรีไทยที่มีการคลอดก่อนกำหนดค่อนข้างต่ำ การให้การรักษาด้วยยาปฏิชีวนะควรพิจารณาถึงเชื้อก่อโรคที่พบได้บ่อย รวมถึงแบบแผนของความไวต่อยาปฏิชีวนะ*

คำสำคัญ: *ภาวะการมีเชื้อแบคทีเรียในปัสสาวะแบบไม่มีอาการ, การคลอดก่อนกำหนด, ผลการตั้งครรภ์*

Introduction

Asymptomatic bacteriuria (ASB) is the presence of a significant number of bacteria in a urine specimen collected properly from a person without symptoms of urinary tract infection (UTI), such as dysuria, frequent voiding, and incomplete voiding[1-3]. Significant bacteriuria is defined as the growth of at least 10^5 colony-forming units per milliliter (cfu/ml) isolated from a voided midstream, clean-catch urine specimen. Various causative organisms have been identified with the most commonly reported isolate being Escherichia coli (E. coli). Other organisms include gram-negative bacteria, such as Klebsiella pneumoniae (K. pneumoniae), Proteus mirabilis (P. mirabilis), and group B streptococci[1, 4-5]. ASB is common in pregnant women and, if left untreated, could lead to acute pyelonephritis in up to 25%-50% of cases[5, 6]. In addition, ASB has been associated with several adverse outcomes such as hypertensive disorder, low birth weight, preterm delivery, and increased fetal mortality[6-9]. However, the relationship between ASB and preterm delivery remains controversial[10].

International organizations including the American Academy of Pediatrics (AAP), the American College of Obstetricians and Gynecologists (ACOG), the National Institute for Health and Care Excellence (NICE), the World Health Organization (WHO), and the US Preventive Services Task Force, recommend screening for bacteriuria at the first prenatal visit. A positive urine culture result should be promptly treated[11-15]. In Thailand, ASB screening in pregnant women is still not routinely and widely practiced[16]. However, at Siriraj Hospital, urinalysis and urine culture are carried out in pregnant women who present with preterm labor and antibiotic treatment is provided if there is evidence of UTI, such as pyuria or significant bacteriuria.

Depending on the population sampled, the reported prevalence of ASB varies from 2% to 12%[6, 9, 17]. In 2018, Kamel et al[18] reported a 5% prevalence of ASB in Egyptian pregnant women with preterm labor. The prevalence of ASB in Thai pregnant women has been reported to be approximately 10%[16, 19]. However, data on the prevalence of ASB in Thai pregnant women with a preterm delivery has not been published. Common organisms and antibiotic susceptibility patterns vary across populations and over time, requiring ongoing surveillance to inform medical practice.

Therefore, this study was conducted to determine the prevalence of ASB in Thai pregnant women with a preterm delivery. The secondary objectives were to evaluate the common causative organisms and their antibiotic susceptibilities, and to compare pregnancy outcomes between the ASB-positive and ASB-negative patients.

Materials and Methods

Study design and participants

This retrospective study was conducted after the approval of Siriraj Institutional Review Board (COA no. Si381/2020). The medical records of low-risk, Thai pregnant women who had a spontaneous, preterm delivery at the Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand from January 2014 to May 2020 were reviewed. Data were collected on patient characteristics, complete blood count, urine culture on admission, and pregnancy outcomes.

Urine culture was performed on a midstream, clean-catch specimen. Significant bacteriuria, defined as the growth of at least 10^5 colony-forming units per milliliter (cfu/ml) isolated, was designated ASB-positive whereas ASB-negative denoted insignificant bacteriuria (less than 10^5 cfu/ml isolated), or no growth. Maternal outcomes included gestational age at delivery, gestational diabetes mellitus, pregnancy-induced hypertension, postpartum hemorrhage, chorioamnionitis, maternal sepsis, postpartum endometritis, and wound complication. Neonatal outcomes included birth weight, intraventricular hemorrhage, retinopathy of prematurity, respiratory distress syndrome, apnea of prematurity, necrotizing enterocolitis, hyperbilirubinemia, neonatal sepsis, ventilator requirement, duration of hospital stay, and admission to the neonatal intensive care unit.

The subjects were pregnant women aged 18 years or older with an uncomplicated singleton pregnancy who had a spontaneous preterm delivery at 24 to 36 weeks' gestation. Gestational age determination

was based on the last menstrual period with a confirmed ultrasound examination during the first or second trimester. Patients with urinary symptoms at the time of specimen collection, a history of a renal disease or any abnormality of the genitourinary tract that impairs voiding, and those with medical or obstetric complications were excluded.

Sample size calculation and statistical analysis

Sample size calculation was based on the primary objective. A study in 2018 by Kamel et al[18] reported the prevalence of ASB in patients with preterm labor to be 5%. Based on a prevalence of 5% and a confidence level of 0.95 with an allowable error of 0.015, the sample size was calculated to be 811 women.

All data analyses were performed using PASW Statistics version 18.0 for Windows (SPSS, Inc., Chicago, IL, USA). Descriptive data are presented as number and percentage, mean ± standard deviation, or median and range (P25-P75), as appropriate. Chi-square or Fisher's exact test was used to compare qualitative characteristics between groups. Student's t-test or Mann-Whitney U test was used to compare continuous data between groups. A p value of less than 0.05 was considered statistically significant.

Results

Eight-hundred and twenty-six women with a mean maternal age of 29.8 ± 6.2 years were enrolled. The mean gestational age at delivery was 33.6 ± 2.4 weeks. Twenty-five (3%) women had significant bacteriuria. Baseline demographic and clinical characteristics were comparable between the ASB-positive and ASB-negative groups (Table 1).

Table 1. Comparison of demographic and clinical characteristics (n = 826, unless otherwise specified).

Characteristics	Reported values*		
	ASB-positive n = 25	ASB-negative n = 801	p value
Age (years)			0.134[a]
18-34	16 (64%)	616 (76.9%)	
≥ 35	9 (36%)	185 (23.1%)	
Gravidity			0.572[a]
1	9 (36%)	398 (49.7%)	
2	11 (44%)	264 (33%)	
3	3 (12%)	92 (11.5%)	
≥ 4	2 (8%)	47 (5.9%)	
Pre-pregnancy body mass index (kg/m^2)	26.4 ± 4.9	25.8 ± 4.5	0.492[b]
Hemoglobin on admission (g/dl) (n = 813)**			0.716[a]
< 11	6 (24%)	215 (27.3%)	
≥ 11	19 (76%)	573 (72.7%)	
Income (Baht/month)			0.782[a]
< 10,000	3 (12%)	54 (6.7%)	
10,000-20,000	5 (20%)	170 (21.2%)	
20,001-30,000	5 (20%)	188 (23.5%)	
30,001-40,000	5 (20%)	118 (14.7%)	
40,001-50,000	3 (12%)	75 (9.4%)	
> 50,000	4 (16%)	196 (24.5%)	
Education (n = 823)***			0.099[a]
Low education (≤ primary school)	17 (68%)	409 (51.3%)	
High education (≥ secondary school)	8 (32%)	389 (48.7%)	
Gestational age at urine collection (weeks)			0.676[c]
< 28	2 (8%)	52 (6.5%)	
≥ 28	23 (92%)	749 (93.5%)	

* Data are reported as number and percentage or mean ± standard deviation. ** Results were available from only 788 women in ASB-negative group. *** Results were available from only 798 women in ASB-negative group. [a] Chi- quare test, [b] t-test, [c] Fisher's exact test. ASB: asymptomatic bacteriuria, g/dl: gram per deciliter, kg/m^2: kilogram per square meter

Among the ASB-positive women, E. coli was the most common causative organism (48%), followed by Enterococcus faecalis (E. faecalis) (16%), and K. pneumoniae (8%). Antibiotic susceptibility patterns of the common organisms are shown in Table 2. All E. coli were susceptible to nitrofurantoin, imipenem, meropenem, piperacillin/tazobactam, amikacin, and cefepime. Ninety-two percent were susceptible to ceftriaxone, 83% were susceptible to amoxicillin/clavulanate, and 25% were susceptible to ampicillin. E. faecalis, the second most common isolate, was 100% susceptible to ampicillin, gentamicin, penicillin, and vancomycin. Seventy-five percent were susceptible to fosfomycin and 25% were susceptible to ciprofloxacin.

All 826 pregnancies resulted in live births. Maternal outcomes for ASB-positive and ASB-negative patients are shown in Table 3. No significant differences in adverse maternal outcomes were detected. Ten newborns (one from the ASB-positive group and nine from the ASB-negative group) were referred to other hospitals after delivery and so only one available outcome, neonatal birth weight was included.

Table 2. Antibiotic susceptibility pattern of common isolated organisms.

Antibiotics	Reported values*		
	E. coli (n = 12)	E. faecalis (n = 4)	K. pneumoniae (n = 2)
Ampicillin	3 (25%)	4 (100%)	ND
Amoxicillin/clavulanate	10 (83%)	ND	2 (100%)
Amikacin	12 (100%)	ND	2 (100%)
Cefepime	12 (100%)	ND	2 (100%)
Ceftriaxone	11 (92%)	ND	2 (100%)
Ciprofloxacin	10 (83%)	1 (25%)	2 (100%)
Cefuroxime	11 (92%)	ND	ND
Gentamicin	8 (67%)	4 (100%)	2 (100%)
Fosfomycin	ND	3 (75%)	ND
Imipenem	12 (100%)	ND	2 (100%)
Meropenem	12 (100%)	ND	2 (100%)
Norfloxacin	4 (33%)	ND	ND
Nitrofurantoin	12 (100%)	ND	ND
Piperacillin/tazobactam	12 (100%)	ND	2 (100%)
Penicillin	ND	4 (100%)	ND
Trimethoprim/sulfamethoxazole	5 (42%)	ND	2 (100%)
Vancomycin	ND	4 (100%)	1 (50%)

* Data are reported as number and percentage. ND: not done

Table 3. Comparison of maternal outcomes among the 2 study groups. (n = 826)

	Reported values*		
	ASB-positive (n = 25)	ASB-negative (n = 801)	p value
Gestational age at delivery (weeks)			0.651[a]
< 34	10 (40%)	357 (44.6%)	
≥ 34	15 (60%)	444 (55.4%)	
Gestational diabetes mellitus	7 (28%)	120 (15%)	0.089[b]
Pregnancy-induced hypertension	3 (12%)	71 (8.9%)	0.484[b]
Postpartum hemorrhage	4 (16%)	55 (6.9%)	0.096[b]
Chorioamnionitis	2 (8%)	44 (5.5%)	0.645[b]
Maternal sepsis	0 (0%)	4 (0.5%)	1.000[b]
Postpartum endometritis	0 (0%)	6 (0.7%)	1.000[b]
Wound infection/dehiscence	1 (4%)	13 (1.6%)	0.352[b]

* Data are reported as number and percentage. [a] Chi-square test, [b] Fisher's exact test. ASB: asymptomatic bacteriuria

All other outcomes of interest were obtainable from the remaining 816 newborns (24 ASB-positive and 792 ASB-negative). Neonatal outcomes in the ASB-positive and ASB-negative groups are compared in Table 4. No significant differences in adverse neonatal outcomes were detected.

Table 4. Comparison of neonatal outcomes among the 2 study groups (n = 816, unless otherwise specified).

	Reported values*		
	ASB-positive (n = 24)	**ASB-negative (n = 792)**	**p value**
Neonatal birth weight (grams) (n = 826)**	2216.4 ± 636	2123.6 ± 538.5	0.399[a]
Intraventricular hemorrhage	2 (8.3%)	43 (5.4%)	0.386[b]
Retinopathy of prematurity	1 (4.2%)	14 (1.8%)	0.363[b]
Respiratory distress syndrome	8 (33.3%)	286 (36.1%)	0.833[b]
Apnea of prematurity	1 (4.2%)	144 (18.2%)	0.101[b]
Necrotizing enterocolitis	0 (0%)	37 (4.7%)	0.621[b]
Hyperbilirubinemia	18 (75%)	602 (76%)	1.000[c]
Neonatal sepsis	6 (25%)	111 (14%)	0.138[b]
Ventilator requirement			0.873[c]
CPAP	4 (16.7%)	159 (20.1%)	
Endotracheal tube	3 (12.5%)	89 (11.2%)	
Median hospital stay (days)	10.5 (5-19)	8 (5-24)	0.867[d]
Admission to NICU			
Admission	7 (29.2%)	190 (24%)	0.559[c]
Median stay (days) (n = 197)	13 (2-33)	8 (3-22)	0.725[d]

* Data are reported as number and percentage, mean ± standard deviation, or median (P25-P75)
** Results were from 25 ASB-positive cases and 801 ASB-negative cases
[a] T-test, [b] Fisher's exact test, [c] Chi-square test, [d] Mann-Whitney U test
ASB: asymptomatic bacteriuria, NICU: neonatal intensive care unit

Discussion

ASB has been associated with adverse perinatal outcomes that can be prevented by screening and treatment. Previous studies in several countries have reported a prevalence among pregnant women of 2% to 12%[6, 9, 17]. The prevalence of ASB in Thai pregnant women across all trimesters was reported to be approximately 10%[16, 19]. However, we measured a 3% prevalence of ASB in women with a preterm delivery, closer to the 5% prevalence in Egypt reported by Kamel et al[18]. The different result we observed may be explained by the fact that we included only pregnant women who had a preterm delivery from a single hospital setting, and differences in the socio-economic status, social behaviors, and education of the studied population might contribute to the outcome[16, 19-20]. In this study, E. coli was found to be the most common causative organism (48%), followed by E. faecalis (16%), and K. pneumonia (8%). These results were similar to other studies that also reported E. coli as the most common isolate[6, 9, 21]. Diverse antibiotic susceptibility patterns are observed across geographical areas due to the use of different antibiotics. Therefore, antibiotic treatment should be based on the common organisms and local antibiotic susceptibility patterns.Our study has some important strengths. First, this is the first survey of ASB prevalence, causative organisms and antibiotic susceptibility patterns, and outcomes in Thai pregnant women with a preterm delivery. Second, our sample size was large and sufficient to test the main objective of prevalence. Our study also has some limitations. The number of patients in the ASB-positive group was small, due to an unexpectedly low prevalence rate. Therefore, a larger study is warranted to evaluate between-group outcome comparisons. Moreover, due to the retrospective study design, there were some potential confounding factors that could affect the adverse pregnancy outcomes such as differences in intrapartum management and route of delivery.

Conclusion

In summary, the prevalence of ASB in Thai pregnant women with preterm delivery was low. E. coli was the dominant causative organism and was very susceptible to nitrofurantoin. We recommend nitrofurantoin for ASB in pregnant women due to its high susceptibility and safety.

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Potential conflicts of interest

The authors declare no conflicts of interest.

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OBSTETRICS

Depletion of Vaginal Lactobacilli and Risk of Preterm Birth in Pregnant Women Delivered at Prapokklao Hospital

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ABSTRACT

Objectives: To study the effect of depleted vaginal lactobacilli and risk of preterm birth in pregnant women at Prapokklao Hospital.

Materials and Methods: Ambispective case-control study has been conducted on pregnant women, who delivered at the Department of Obstetrics and Gynaecology, Prapokklao Hospital between January 2020 and May 2021. The data from preterm group (study case) were compiled retrospectively. The term group (study control) was a prospective study. The smear of vaginal secretions was collected, Gram stained examined by microscopic examination for morphotypes and number of lactobacilli and assigned according to Hay's criteria (grade I: normal, grade II, III: abnormal)

Results: A total of 455 pregnant women aged between 14 and 46 years were included: 112 (25%) with preterm pregnancy and 343 (75%) with term pregnancy. The proportions of pregnant women in the preterm groups who having depleted vaginal Lactobacilli was greater than term groups ($p < 0.001$). After adjusted for age, pre-pregnancy body mass index, incomplete antenatal care and grades of Lactobacilli ($p < 0.05$) demonstrated grades II, grade III Lactobacilli were all significantly associated with the incidence of preterm birth. (grades II Lactobacilli odds ratio (OR) 3.6, 95% confidence interval (CI) 2.06-6.5) (grade III Lactobacilli OR 17.8, 95%CI 7.4-43.1).

Conclusion: Pregnant women with preterm birth had a higher proportion of having depletion of vaginal Lactobacilli than control.

Keywords: lactobacilli, term, preterm, ambispective study.

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เชื้อ Lactobacilli ในช่องคลอดที่ลดลงกับความเสี่ยงของการการคลอดก่อนกำหนดในหญิงตั้งครรภ์โรงพยาบาลพระปกเกล้า จังหวัดจันทบุรี

สุรีพร จันเข็ม, วัชรินทร์ เฉิดฉิ้ม

บทคัดย่อ

วัตถุประสงค์: *ผลของจำนวนเชื้อ lactobacilli ในช่องคลอดที่ลดลงกับการคลอดก่อนกำหนดในหญิงตั้งครรภ์*

วัสดุและวิธีการ: *การศึกษาแบบ Ambispective case control study ที่โรงพยาบาลพระปกเกล้า จังหวัดจันทบุรี ระหว่างเดือนมกราคม พ.ศ. 2563 ถึงเดือนพฤษภาคม พ.ศ. 2564 ในหญิงตั้งครรภ์ที่คลอดก่อนกำหนด (กลุ่ม case เก็บข้อมูลย้อนหลังจากเวชระเบียน) เปรียบเทียบกับหญิงตั้งครรภ์ที่คลอดครบกำหนด (กลุ่ม control เก็บข้อมูลไปข้างหน้า) เก็บสิ่งคัดหลั่งจากช่องคลอดเพื่อดูจำนวนและลักษณะเชื้อ lactobacilli ตาม Hay's criteria (grade I: normal, grade II, III: abnormal)*

ผลการศึกษา: *หญิงตั้งครรภ์จำนวน 455 ราย อายุ 14 ถึง 46 ปี คลอดก่อนกำหนด 122 ราย คลอดครบกำหนด 343 ราย กลุ่มที่คลอดก่อนกำหนดพบว่า หญิงตั้งครรภ์ที่มีปริมาณเชื้อ lactobacilli ในช่องคลอดลดลง มีสัดส่วนมากกว่ากลุ่มที่คลอดครบกำหนดอย่างมีนัยสำคัญ ($p < 0.001$) หญิงตั้งครรภ์ grade II, III lactobacilli มีโอกาสคลอดก่อนกำหนดเป็น 3.6 และ 17.8 เท่า ($p < 0.001$) และเมื่อทำการวิเคราะห์ multivariate analysis โดยใช้ตัวแปรอายุ, ดัชนีมวลกายก่อนตั้งครรภ์, การดูแลฝากครรภ์ที่ไม่สมบูรณ์ และระดับแลคโตบาซิลลัส ปรับค่าใน logistic regression model พบว่าหญิงตั้งครรภ์ grade II, III lactobacilli สัมพันธ์กับอุบัติการณ์การคลอดก่อนกำหนดอย่างมีนัยสำคัญทางสถิติ (grade II Lactobacilli OR 3.6, 95%CI 2.06-6.5) (grade III Lactobacilli OR 17.8, 95%CI 7.4-43.1).*

สรุป: *หญิงตั้งครรภ์ที่คลอดก่อนกำหนดมีสัดส่วนของปริมาณเชื้อ lactobacilli ในช่องคลอดลดลง มากกว่าหญิงตั้งครรภ์ที่คลอดครบกำหนดอย่างมีนัยสำคัญทางสถิติ*

คำสำคัญ: *เชื้อ Lactobacilli, คลอดก่อนกำหนด, Ambispective study*

Introduction

Preterm birth is described as birth between age of viability and the 37 week of pregnancy[1]. Preterm birth is the leading cause of perinatal morbidity and mortality[2]. According to the World Health Organization, around 15 billion babies were born preterm[3]. Preterm birth is related for 75% of neonatal deaths and 50% of long-term morbidity, such as respiratory disease and neurodevelopmental impairment[4].

During pregnancy, the vagina of women becomes dominated by lactobacilli, resulting in an extremely acidic environment that minimizes the likelihood of aerobic pathogens invading[5, 6]. This pathogen has been associated with preterm labor, premature rupture of membranes, postpartum endometritis, and neonatal sepsis, among other obstetric complications[7]. Therefore, preterm birth may be related to the loss of lactobacilli[8]. Moreover, a recent study reported the abnormal depletion of lactobacilli during pregnancy has been associated with preterm birth[9].

Lactobacilli morphotypes in Gram stained smears were defined as gram positive bacilli[10]. Even though a recent study compared patient characteristics and pregnancy related outcomes between pregnant women with a lactobacillus dominated vagina and those with lactobacillus loss, it is possible to use Hay's criteria to determine if the presence of lactobacilli is normal or reduced by using Gram staining to classify women[11]. Hence, preterm labor could be predicted by detecting aberrant bacterial colonization of the genital tract[12].

The objective of this study was to analyze the effect of depleted vaginal lactobacilli and risk of preterm birth in pregnant women at Prapokklao Hospital, Chanthaburi. The hypothesis of this study is pregnant women with vaginal lactobacilli depletion have a higher risk of preterm birth.

Materials and Methods

This ambispective (retrospective and prospective) case control study has been conducted on pregnant women, who delivered at the Department of Obstetrics and Gynaecology, Prapokklao Hospital between January 2020 and May 2021. Pregnant women's medical records were divided into two groups. The preterm group's data (study case) was compiled retrospectively. The term group (study control) was a prospective study. The study protocol was approved by the Ethics committee for Research on Humans in Chanthaburi. (CTIREC 029/64). Before enrolling, eligible study subjects were given information about the study and gave their written informed consent to participate.

Sample size calculation

The results of vaginal swab Gram stain were reviewed in the medical records of preterm pregnant women who delivered at Prapokklao Hospital's Department of Obstetrics and Gynecology between January and June 2020. We found a 40% prevalence of depleted lactobacilli in the vagina (Gram-stained smears morphotype grade II, III)

Tabatabaei et al[13] and Bahareh[14] conducted a research of premature labor on a 1:2 and 1:3 ratio of cases to controls in the previous studies. Thus, the researchers employed a 1:3 ratio of cases to controls in this study.

Stata software was used to compute sample size using two sample proportions with a power of 80% and a type I error of 5% (alpha 0.05). The required sample size was predicted to be 392 people, with 98 preterm births and 294 term deliveries (controls).

Inclusion criteria for the study cases (preterm births) were pregnant women who delivered before 37 weeks and after 28 weeks of gestational age. The controls group were pregnant women who gave birth between 37 and 42 weeks of gestation. On the other side, exclusion criteria were pregnant women with a history of preterm labor, pregestational diabetes mellitus, hypertension, heart disease, preeclampsia, Rh-negative, multifetal pregnancy, cervical cerclage, structural uterine anomaly, structural cervical anomaly, fetal anomaly, anemia, intrauterine growth

restriction, placenta previa, abruptio placenta, intrauterine fetal death, polyhydramnios, urinary tract infection, alcohol, drinking, smoking, drug user, and no antenatal care.

Vaginal specimen collection

The medical records were used to acquire vaginal swab Gram stain results from the preterm birth group, and Gram stain was obtained from a vaginal swab taken during an examination to acquire a sample of vaginal secretions. Also, this is a method of examination that is comparable to routine practice. Age, gravida, gestational age, pre-pregnancy body mass index (BMI), previous history of abortion, and prenatal care were reviewed from the delivery/labor record (FM-OBS-05) (04), which was the retrospective data collection, of the preterm group for this study. For the term pregnant group, demographic data such as age, gravida, gestational age, pre-pregnancy BMI, previous history of abortion, and antenatal care were collected from delivery/labor record (FM-OBS-05) (04) as same as the preterm group but start collecting data after they came to admit in the hospital. When they arrived at admission, they gathered vaginal swab Gram stain from vaginal secretions in addition to usual practice, which has no negative effects on the body or impairs performance in routine practice. It is the prospective data collection.

Per vaginal examination by the resident, a smear of vaginal secretions was collected by inserting a sterile cotton-tipped wooden swab in the vaginal canal. The swab was rolled round through the vaginal canal. Swabs were then smeared on a plain glass slide and air-dried at room temperature. The slides were Gram stained, examined under oil immersion at a magnification of 1,000 by medical laboratory technologist, and assigned according to Hay's criteria[11] by the resident. Gram-stained vaginal smears were categorized as grade I: normal predominantly Lactobacilli morphotypes (bacteria > 30 cells/ oil field), grade II: intermediate or reduced lactobacilli morphotypes (bacteria 1-30 cells/ oil field), grade III: abnormal few or absent Lactobacilli morphotypes (bacteria < 1 cell/ oil field).

Statistical analysis

For statistical analysis, baseline characteristics of the participants were presented as frequency, percentage, and mean ± standard deviation. Aside from that, the Chi-squared test was used to compare category data between groups, while the student's t-test was used to compare continuous data. The relationships between grades of Lactobacilli and preterm birth were investigated using both univariate and multivariate analysis. For multivariate analysis, variables with p value less than 0.05 in univariate analysis were entered in the logistic regression model. All statistical analyses were performed in Stata software version 16.0 and the statistical significance was p value less than 0.05.

Results

A total of 455 pregnant women were included: 112 pregnant women or 25% were preterm pregnant and 343 pregnant women or 75% were term pregnancy. The mean age of preterm pregnant women was 28.7 ± 6.76 years, 38.3% in gravida 2, 10.7% were underweight, 26.7% were overweight/ obesity, 22.3% of preterm pregnant women had a previous history of abortion, 24.1% of incomplete antenatal care, 45.5% of late antenatal care. There was no statistically significant difference between the two groups regarding gravida ($p = 0.124$), previous history of abortion ($p = 0.173$) and early antenatal care ($p = 0.059$) (Table 1).

The pregnant women in the preterm groups were found grade I Lactobacilli to be lower than term groups (37.5% vs 79.0%). Grade II, grade III Lactobacilli was greater than term groups (32.1% vs 18.0%) (30.3% vs 2.9%) ($p < 0.001$) (Table 2).

Table 1. Demographic data.

Characteristics	Preterm group n (%) 112 (25)	Term group n (%) 343 (75)	p value
Age, mean (± SD) (years)	28.7 (± 6.7)	26.6 (± 6.3)	0.003
Gravida			0.124
1	29 (25.8)	139 (40.5)	
2	43 (38.3)	109 (31.7)	
3	22 (19.6)	53 (15.4)	
4	12 (10.7)	30 (8.7)	
5	5 (4.4)	8 (2.3)	
6	1 (0.8)	4 (1.1)	
Gestational age, mean (± SD) (weeks)	34.8 (± 2.1)	38.9 (± 1.0)	< 0.001
Pre-pregnancy BMI groups*			< 0.001
Underweight	12 (10.7)	8 (2.3)	
Normal weight	70 (62.5)	115 (33.5)	
Overweight/obesity	30 (26.7)	220 (64.1)	
Previous history of abortion			0.173
Yes	25 (22.3)	57 (16.6)	
No	87 (77.6)	286 (83.3)	
Complete antenatal care (≥ 5 times)			< 0.001
Yes	85 (75.8)	317 (92.4)	
No	27 (24.1)	26 (7.5)	
Early antenatal care (≤ 12 weeks)			0.059
Yes	61 (54.4)	221 (64.4)	
No	51 (45.5)	122 (35.5)	

* Pre-pregnancy BMI groups (kg/m^2): underweight < 18.5, normal weight 18.5-24.9, overweight/obesity ≥ 25
SD: standard deviation, BMI: body mass index

Table 2. Lactobacilli analysis.

Lactobacilli	Preterm group n (%) 112 (25)	Term group n (%) 343 (75)	p value
Gram-stained smears morphotype, GPR			< 0.001
Grade I	42 (37.5)	271 (79.0)	
Grade II	36 (32.1)	62 (18.0)	
Grade III	34 (30.3)	10 (2.9)	

GPR: gram-positive rods

Factors associated with preterm birth from the univariate analysis included age, pre-pregnancy BMI, incomplete antenatal care and grades of Lactobacilli. Given the following string variables; age > 34 years (odds ratio (OR) 1.9, 95%CI 1.0-3.4, p = 0.024) overweight/obesity (OR 0.2, 95%CI 0.1-0.3, p < 0.001), incomplete antenatal care (OR 3.8, 95%CI 2.1-6.9, p < 0.001), grades II Lactobacilli (OR 3.7, 95%CI 2.2-6.3, p < 0.001) and grades III Lactobacilli (OR 21.9, 95%CI 10.09-47.6, p < 0.001); univariate analysis showed statistically significant relation with preterm birth.

Based on multivariate analysis; age < 20 years (OR 0.2, 95%CI 0.08-0.6, p = 0.006), age > 34 years (OR 4.4, 95%CI 2.0-9.9, p < 0.001), underweight (OR 3.0, 95% CI 1.0-9.3, p = 0.044), overweight/obesity (OR 0.1, 95% CI 0.1-0.3, p < 0.001), incomplete antenatal care (OR 5.2, 95% CI 2.2-12.3, p < 0.001), grades II Lactobacilli (OR 3.6, 95%CI 2.06-6.5, p < 0.001) and grades III Lactobacilli (OR 17.8, 95%CI 7.4-43.1, p < 0.001); were all significantly associated with the incidence of preterm birth (Table 3).

Table 3. Factors associated with preterm birth.

Factors	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p value	OR (95% CI)	p value
Age, years				
< 20	0.7 (0.3-1.5)	0.363	0.2 (0.08-0.6)	0.006
20-34	1 (reference)		1 (reference)	
> 34	1.9 (1.0-3.4)	0.024	4.4 (2.0-9.9)	< 0.001
Pre-pregnancy BMI groups				
Underweight	2.4 (0.9-6.3)	0.061	3.0 (1.0-9.3)	0.044
Normal weight	1 (reference)		1 (reference)	
Overweight/obesity	0.2 (0.1-0.3)	< 0.001	0.1 (0.1-0.3)	< 0.001
Complete antenatal care (≥ 5 times)				
Complete ANC	1 (reference)		1 (reference)	
Incomplete ANC	3.8 (2.1-6.9)	< 0.001	5.2 (2.2-12.3)	< 0.001
Gram-stained smears, GPR				
Grade I	1 (reference)		1 (reference)	
Grade II	3.7 (2.2-6.3)	< 0.001	3.6 (2.06-6.5)	< 0.001
Grade III	21.9 (10.09-47.6)	< 0.001	17.8 (7.4-43.1)	< 0.001

OR: odds ratio, CI: confidence interval, BMI: body mass index, ANC: antenatal care, GPR: gram-positive rods

Discussion

Many factors contribute to preterm birth, including an inappropriate vaginal environment that allows pathogens to invade[5,6], infection, and the activation of inflammatory mediators[15,16]. Abnormal vaginal microflora or abnormal depletion of lactobacilli has been associated with preterm birth[8]. This study has shown that the vaginal lactobacilli of the preterm pregnant women were lower than term pregnant women with statistically significant. This result concurred with the study of Drew[9]. Those study has shown that pregnant women who have a loss of lactobacilli, with no evidence of bacterial vaginosis, have a higher risk than controls of preterm labor and preterm premature rupture of membranes. Similarly, Tabatabaei[13] documented vaginal lactobacilli may be associated with decreased risk of preterm birth. Similar to the study of Donders[17], the results found that women without abnormalities of

the vaginal flora in the first trimester had a 75% lower risk of delivery before 35 weeks compared with women with abnormal vaginal flora (OR 0.26, 95% CI 0.12-0.56) and the absence of lactobacilli was associated with increased risks of preterm birth (OR 2.4, 95% CI 1.2-4.8). In the study conducted by Verstraelen[18] has been demonstrated that normal microflora was associated with a 4-fold decreased risk of spontaneous preterm birth (95%CI 0.1-0.6, $p < 0.001$)

Based on the Gram staining method and microscopic examination, which is characterized by gram-positive bacilli, our study design was interested in morphotypes and number of lactobacilli. As know that, depletion of Lactobacilli is a part of bacterial vaginosis of which Nugent's scoring system is the gold standard diagnostic method. However, Gram staining method and microscopic examination is simple and convenient in clinical practice. It can be used to make an initial diagnosis in the reduced or absent vaginal lactobacilli. If this condition can be treated, it may help to prevent the invasion of pathogens and preventing preterm birth.

For the strength of this study, the preterm group was selected according to the specific criteria, to reduce the impact of other factors that result in preterm birth such as previous preterm labor, pregestational diabetes mellitus, hypertension, heart disease, preeclampsia, multifetal pregnancy, cervical cerclage, structural uterine anomaly, structural cervical anomaly, fetal anomaly. The limitation of this study was the difference time interval for data collection between case and control group. For preterm birth group was retrospective data collection. However, term group was prospective data collection. The exposure for preterm birth group has already occurred, before the study, and the outcomes were still ahead of the study. So, the time sequence may effect on the occurrence of the disease. In our opinion, prevention of preterm birth by screening and diagnosis for abnormal vaginal lactobacilli should be implicated as a necessary step. Moreover, the use of lactobacilli supplementation for these women may be a therapeutic option to prevent preterm birth.

Conclusion

Pregnant women with preterm birth had a higher proportion of having depletion of vaginal Lactobacilli than control.

Potential conflicts of interest

The authors declare no conflicts of interest.

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GYNAECOLOGY

Effects of Crocin on Human Sperm Viability, Motility, Morphology, DNA Fragmentation and Reactive Oxygen Species Levels after Freezing and Thawing

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ABSTRACT

Objectives: To investigate the effects of crocin supplementation during solid surface vitrification (SSV) and liquid nitrogen vapor freezing (LNF) of human spermatozoa on post-thaw sperm parameters.

Materials and Methods: Thirty-six normozoospermic semen samples were used in the study. Post prepared semen samples were divided into five aliquots: one served as non-cryopreserved control; two were vitrified, with or without crocin supplementation (SSV ± 10 µg/ml crocin); and the last two aliquots were frozen in liquid nitrogen vapor, with or without crocin supplementation (LNF ± 10 µg/ml crocin).

Results: After cryopreservation, sperm motility (93.54 ± 3.55%, 70.10 ± 10.94% and 53.58 ± 12.06% in controls, SSV and LNF groups, respectively, $p < 0.001$) and sperm viability (79.82 ± 18.86%, 57.74 ± 21.99% and 49.69 ± 22.25%, $p < 0.001$) decreased significantly in both methods. However, the SSV groups had a significantly higher sperm motility (70.10 ± 10.94% and 53.58 ± 12.06%, $p < 0.001$) and viability (57.74 ± 21.99% and 49.69 ± 22.25%, $p < 0.001$) than the LNF groups. Supplementation with crocin 10 µg/ml in cryoprotective agent did not improve sperm motility and viability in both cryopreservation methods. Also, no effect was noted on sperm morphology, sperm deoxyribonucleic acid (DNA) integrity, and both the intracellular and extracellular reactive oxygen species (ROS) levels.

Conclusion: Crocin supplementation during vitrification and liquid nitrogen vapor freezing did not improve the outcome of post-thaw sperm.

Keywords: crocin supplementation, sperm cryopreservation, vitrification, vapor freezing .

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ผลของสารโครซินต่ออัตราการรอดชีวิตของตัวอสุจิมนุษย์, การเคลื่อนไหว, ลักษณะรูปร่าง, การแตกหักของดีเอ็นเอและระดับสารอนุมูลอิสระ หลังขบวนการแช่แข็งและการละลาย

วรัญญา เรืองชัยนิคม, อุบล แสงอนันต์, ธีระพร วุฒยวนิช, วราภรณ์ ภิรมย์เลิศอมร, อุษณีย์ แสนหมี่

บทคัดย่อ

วัตถุประสงค์: *เพื่อศึกษาผลของการเติมสารโครซินในขบวนการแช่แข็งและละลายอสุจิมนุษย์ ทั้งวิธีการแช่แข็งแบบเนื้อแก้วและการแช่แข็งในไอของไนโตรเจนเหลว โดยดูอัตราการรอดชีวิต, การเคลื่อนไหว, ลักษณะรูปร่าง, การแตกหักของดีเอ็นเอและระดับสารอนุมูลอิสระของตัวอสุจิ*

วัสดุและวิธีการ: *การศึกษานี้ใช้น้ำอสุจิที่ผ่านเกณฑ์มาตรฐาน จำนวน 36 ตัวอย่าง นำไปผ่านกระบวนการปั่นแยกตัวอสุจิตามวิธีมาตรฐาน แล้วนำอสุจิที่ได้แบ่งเป็น 5 ส่วน ส่วนที่ 1 (กลุ่มควบคุม) ไม่ผ่านขบวนการแช่แข็ง อีก 2 ส่วนทำการแช่แข็งแบบเนื้อแก้ว (SSV) โดยเติม/ไม่เติมสารโครซิน (10 ไมโครกรัม/มิลลิลิตร) และ 2 ส่วนสุดท้ายทำการแช่แข็งในไอไนโตรเจนเหลว (LNF) โดยเติม/ไม่เติมสารโครซิน (10 ไมโครกรัม/มิลลิลิตร)*

ผลการศึกษา: *อสุจิหลังผ่านขบวนการแช่แข็ง พบว่าอัตราการเคลื่อนไหว (ร้อยละ 93.54 ± 3.55, 70.10 ± 10.94 และ 53.58 ± 12.06 ในกลุ่มควบคุม, SSV และ LNF ตามลำดับ, $p < 0.001$) และอัตราการรอดชีวิต (ร้อยละ 79.82 ± 18.86, 57.74 ± 21.99 และ 49.69 ± 22.25, $p < 0.001$) ลดลงในทั้งสองวิธีอย่างมีนัยสำคัญทางสถิติ แต่อย่างไรก็ตามการแช่แข็งแบบเนื้อแก้วมีอัตราการเคลื่อนไหว (ร้อยละ 70.10 ± 10.94 และ 53.58 ± 12.06, $p < 0.001$) และอัตราการรอดชีวิต (ร้อยละ 57.74 ± 21.99 และ 49.69 ± 22.25, $p < 0.001$) มากกว่าวิธีการแช่แข็งในไอไนโตรเจนเหลวอย่างมีนัยสำคัญทางสถิติ การเติมสารโครซินในขบวนการแช่แข็งไม่ได้เพิ่มอัตราการเคลื่อนไหวและการรอดชีวิตของตัวอสุจิ ในการแช่แข็งทั้งสองวิธี และไม่มีผลต่อลักษณะรูปร่าง, การแตกหักของดีเอ็นเอและระดับสารอนุมูลอิสระทั้งในและนอกตัวอสุจิ*

สรุป: *การเติมสารโครซินในขบวนการแช่แข็งและละลาย ไม่ว่าจะด้วยวิธีแช่แข็งแบบเนื้อแก้วหรือการแช่แข็งในไอไนโตรเจนเหลวไม่ได้ช่วยเพิ่มผลลัพธ์ที่ดีต่อตัวอสุจิ*

คำสำคัญ: *การเติมสารโครซิน, การแช่แข็งอสุจิ, การแช่แข็งแบบเนื้อแก้ว, การแช่แข็งในไอของไนโตรเจนเหลว*

Introduction

The biotechnological applications of cryopreservation are evolving and advancing at a rapid pace. Sperm cryopreservation has now become an important part of assisted reproductive technology, and it is routinely practiced worldwide. Male fertility preservation is performed for various reasons.

Recent researches on sperm physiology and cryobiology have contributed to the improvement in sperm cryopreservation techniques. Cryopreservation can irreversibly damage the sperm structure and affect deoxyribonucleic acid (DNA) integrity through dehydration, osmotic shock, formation of intra- and extracellular ice crystals, and the generation of reactive oxygen species (ROS)[1]. Mature spermatozoa are very susceptible to damage from ROS because their plasma and mitochondrial membranes contain polyunsaturated fatty acids, and they possess no antioxidant defense mechanism in their cytoplasm[2]. Male patients, with high levels of ROS in seminal fluid, are known to have abnormal sperm morphology, decreased sperm viability, and lower fertilizing potential[3]. During cryopreservation, an excessive amount of ROS associated with increased production and reduced antioxidant activity causes oxidative stress and apoptosis that affect sperm motility and DNA integrity resulting in decreased fertility potential of the spermatozoa[4-7]. The separation of motile spermatozoa from the seminal plasma, during the preparation step before cryopreservation is possibly another contributory factor, as it removes the anti-oxidant defense system in the seminal plasma[8]. Although cryoprotective agents may help prevent ice crystallization, they have intrinsic toxicity and may cause osmotic stress, resulting in sperm membrane destabilization and protein denaturation[9].

Our previous study showed that rapid freezing of spermatozoa gave significantly better outcomes than the standard slow programmable freezing, even after repeated freezing and thawing[10]. Despite this dramatic success, we seek to further improve the method such that cryopreserved sperm would have a survival rate approaching 90%. Our literature search indicated that supplementation with antioxidants, such as vitamin C[11], vitamin E[12], glutathione, steroids, leptin[13], and crocin[14] might improve the outcome of sperm cryopreservation.

Crocin is a carotenoid antioxidant that helps eliminate ROS, especially superoxide anion. Crocin has been used as an antioxidant in medical treatment, as well as in assisted reproductive technology (ART). As cryopreservation generates free radicals that decrease sperm quality, researchers are interested in whether crocin would improve the outcomes of sperm freezing. Sapanidou et al[14] added crocin to the bovine sperm cryopreservation medium. They found a significant reduction in ROS formation and lipid peroxidation. The sperm motility, sperm viability, and acrosomal integrity increased significantly. The fertilization rate and the number of embryos were also increased. Crocin, exert an antioxidant effect on apoptosis signaling pathways and prevent DNA fragmentation and morphological changes of apoptosis that are induced by tumor necrosis factor-α and serum-glucose deprivation[15]. A study in red deer sperm by Dominguez-Robolledo et al[16] found that the addition of crocin 1 mM to sperm cryopreservation media significantly reduced ROS and increased sperm motility. However, a dose greater than 2 mM had a negative effect and significantly increased lipid peroxidation. Unfortunately, previous studies in experimental animals were conflicting, and there has been no study in humans. In this study, we investigated the effects of crocin supplementation during solid surface vitrification (SSV) and liquid nitrogen vapor freezing (LNF) of human spermatozoa on post-thaw sperm parameters.

Materials and Methods

Participant selection criteria

Semen samples were collected from male partners of infertile couples, who visited our infertility clinic at Maharaj Nakorn Chiang Mai University Hospital. They collected sperm samples into sterile containers by masturbation after abstinence of two to seven days. Only semen with normal parameters, according to the World Health Organization reference values (WHO

2010) were included in the study. All participants gave their written informed consents for the use of their semen for research. The exclusion criteria were: 1) medical diseases or used medication that could affect spermatogenesis, 2) positive serology for human immune-deficiency viruses (HIV),3) a history of exposure to radiotherapy/chemotherapy, 4) varicocele, genital infection or leukocytospermia, and 5) did not understand Thai language or did not want to participate in the study.

Semen preparation

Semen samples were allowed to liquify at 37°C for 30-60 minutes. Semen samples were layered on top of 80% and 40% discontinuous Sil-Select Plus gradients (Fertipro NV, Beernem, Belgium), then centrifuged at 350 g for ten minutes. The sperm pellet was washed twice with 4 ml of Earle's Balanced Salt Solution (EBSS; Biological Industries, Kibbutz Beit Haemek, Israel), supplemented with 0.3% human serum albumin (HSA; Life Global, Guilford, CT, 0.03M sodium pyruvate (Cat. No. H0887; Sigma) and centrifuged at 200 g for five minutes. The final pellet was suspended in 500 µl of the same medium and divided into five aliquots. The first aliquot (100 µl) served as a non-frozen control and was immediately assessed for sperm motility, sperm morphology, kinematics of sperm movement, sperm viability, DNA integrity, extracellular and intracellular ROS. The flow chart of the study is shown in Fig. 1.

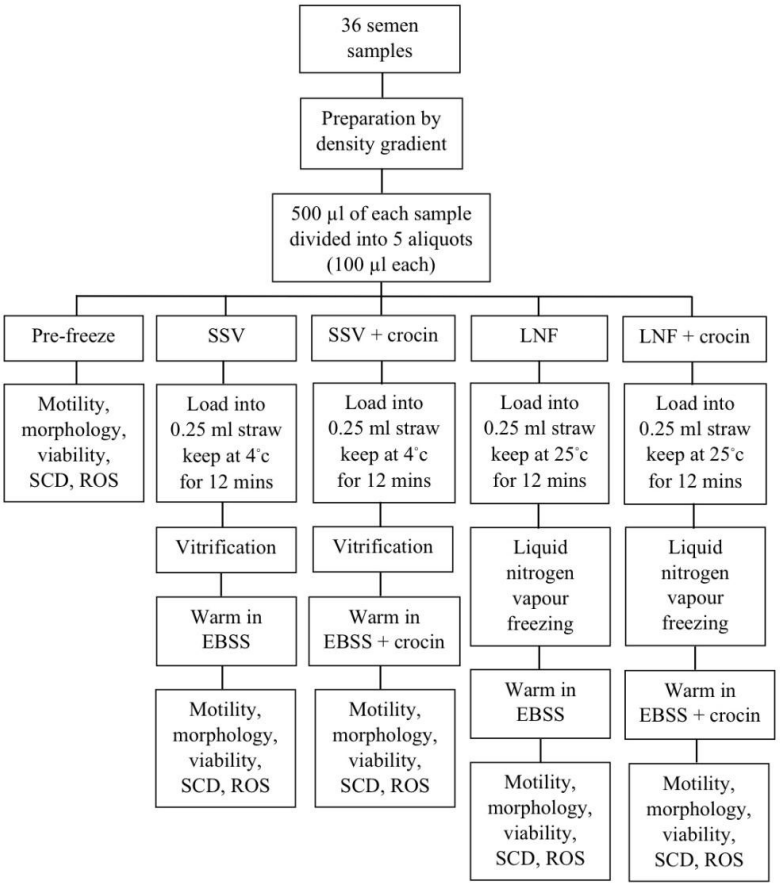

Fig. 1. Flow diagram of the study.

SSV: solid surface vitrification, LNF: liquid nitrogen vapor freezing, SCD: sperm chromatin dispersion, ROS: reactive oxygen species, EBSS: Earle's balanced salt solution

Cryopreservation methods

Vitrification was performed on the second and third aliquots. Each aliquot containing 100 µl of prepared sperm was mixed dropwise with an equal volume of the cryoprotective medium. The second aliquot was mixed with plain cryoprotectant, while the third aliquot was mixed with the same cryoprotectant supplemented with crocin (Sigma Chemical, St. Louis, MO, USA) 10 µg/ml. The in-house medium contained 10% glycerol, 10% human serum albumin (HAS), 133 mM glycine, 5.5 mM glucose, 100 mM Trehalose, 12.2 mM sodium pyruvate, and 20 mM hydroxyethyl piperazineethanesulfonic acid (HEPES). The mixtures were loaded into 0.25 ml straws and left to incubate at 4˚C for 12 minutes, then inserted into a pre-cooled in-house aluminium block, previously submerged in liquid nitrogen.

The fourth and fifth aliquots were cryopreserved by the liquid nitrogen vapor technique. The aliquots were mixed dropwise with an equal volume of warm (37°C) cryoprotective media (SpermFreeze, Lifeglobal, USA), the fourth aliquot without crocin supplementation, and fifth aliquot with crocin 10 µg/ml. The mixtures were loaded into straws and incubated at room temperature for 12 minutes, then placed in a horizontal position ten centimeters above liquid nitrogen for 15 minutes, before plunging into liquid nitrogen.

After at least one week of storage in liquid nitrogen, the straws were warmed in water at room temperature (25-28 °C). Warmed samples were washed in Earle's Balanced Salt Solution (EBSS) (second, fourth aliquots without crocin supplementation and third, fifth aliquots with crocin 10 µg/ml) and centrifuged at 200 g for five minutes to remove the cryoprotectants. Post-warmed samples were immediately assessed for sperm motility and kinematics, sperm morphology, viability, extracellular and intracellular ROS level and DNA integrity.

Sperm assessment

Sperm motility and kinetics were assessed using an HTM IVOS II computer-assisted semen analyzer (CASA; Hamilton Thorne Biosciences, Beverly, MA), equipped with a Clinical Human Motility II software. The kinematic parameters measured included: the velocity of smooth average cell path (VAP), mean curvilinear velocity (VCL), mean straight-line velocity (VSL), the amplitude of lateral head displacement (ALH), percent linearity (LIN = VSL/VCL x100) and percent straightness (STR = VSL/VAPx100).

The washed samples were smeared on glass slides and labeled accordingly. They were stained with Diff-Quick, and then assessed with HTM IVOS II computer-assisted semen analyzer (CASA; Hamilton Thorne Biosciences, Beverly, MA). For every slide, at least 200 spermatozoa were read in duplicates.

Sperm viability assessment, 10 µl from each aliquot was mixed with 10 µl of 0.5% Eosin-Y (Sigma Chemical) on a glass microscopic slide. Viable sperm appeared unstained, whereas the stained ones (red) were dead. At least 200 spermatozoa were counted in duplicates.

The level of extracellular ROS was assessed by a chemiluminescence technique, using a Glomax 20/20 luminometer (Turner Biosystems Inc., Sunnyvale, CA, USA). This method was used because it is currently the most sensitive and most used method to measure ROS levels. In principle, ROS and specific reagents will react and emit photons that pass through the photomultiplier tubes (PMT) of the luminometer. The results are measured as relative light units (RLU) of counted photons per minute (CPM) or as millivolts per second (mV/s). An earlier study determined a cut-off value of ROS for normal semen as < 20 RLU/sec/10^6. In our study, the intra-assay coefficient of variation (CV) of this method was 9.7% and the inter-assay CV was 9.8%.

The level of intracellular ROS (hydrogen peroxide radicals) was assessed by an imaging flow cytometry technique, using Amnis® FlowSight (Merck, Darmstadt, Germany)[17]. The machine combines the speed, sensitivity, and phenotyping abilities of flow cytometry with the detailed imagery (x20 magnification) and functional insight of microscopy. At least 5,000 live spermatozoa were assessed per sample.

We employed a sperm chromatin dispersion (SCD) test to assess sperm DNA fragmentation. The

principle of the technique involves sperm embedded in an agarose matrix and lysed to deproteinize the nuclei. Spermatozoa with intact DNA will show extended halos of DNA dispersion. The halos represent relaxed DNA loops, while non-dispersed chromatin displays DNA fragmentation.

Statistical analysis

The sample size was calculated based on a post-cryopreservation sperm motility. We regarded 10% absolute increase in sperm motility to be of clinical significance if the crocin supplementation was effective, with 90% power with an alpha error of 0.05, 25 subjects were required.

Data expressed as mean were compared by repeated measure analysis of variance (ANOVA) if they were normally distributed. Otherwise, they were transformed into normal distribution before ANOVA tests. If there were significant differences, Tukey HSD post-hoc test was done. A p value < 0.05 was statistically significant.

Results

Thirty-six normozoospermic semen samples were included in this study. Participants' age and pre-processing sperm parameters are shown in Table 1.

Table 1. Mean age (± SD) and sperm parameters (± SD) of participants.

Parameters	Mean ± SD
Age (years)	34.8 ± 5.0
Volume (ml)	3.3 ± 1.6
Sperm concentration (millions/ml)	62.7 ± 41.5
Total motility (%)	71.1 ± 16.0
Progressive motility (%)	65.6 ± 16.3
Normal morphology (%)	10.8 ± 4.6

SD: standard deviation

Sperm motility (93.54 ± 3.55%, 70.10 ± 10.94% and 53.58 ± 12.06% in controls, SSV and LNF groups, respectively, p < 0.001) and sperm viability (79.82 ± 18.86%, 57.74 ± 21.99% and 49.69 ± 22.25%, p < 0.001) decreased significantly after cryopreservation in both methods (Table 2). The solid surface vitrification (SSV) group had a higher post-thawed sperm motility (70.10 ± 10.94% vs 53.58 ± 12.06%, p < 0.001) and viability (57.74 ± 21.99% vs 49.69 ± 22.25%, p < 0.001) than LNF groups (Table 2). Supplementation with crocin 10 µg/ml in the cryoprotective agents did not improve sperm motility and sperm viability in both methods.

Table 2. Sperm motility, viability, morphology, ROS levels, and DNA integrity in controls and post-cryopreserved samples, with or without crocin supplementation in the cryoprotective media.

Parameters (n = 36)	Control	Vitrification		Liquid nitrogen vapor	
		SSV	SSV + crocin	LNF	LNF + crocin
Total motility (%)	93.54 ± 3.55	70.10 ± 10.94 [a,b,c]	71.96 ± 12.23 [a,b,c]	53.58 ± 12.06 [a]	55.16 ± 17.79 [a]
Progression (%)	89.34 ± 5.24	63.61 ± 11.52 [a,b,c]	65.33 ± 12.20 [a,b,c]	47.20 ± 12.57 [a]	48.90 ± 17.00 [a]
Viability (%)	79.82 ± 18.86	57.74 ± 21.99 [a,b,c]	59.92 ± 22.91 [a,b,c]	49.69 ± 22.25 [a]	49.90 ± 21.34 [a]
Morphology (%)	16.22 ± 7.98	14.47 ± 9.67	14.15 ± 7.18	14.04 ± 8.89	14.46 ± 9.00
ROS (RLU/sec/106)					
Extracellular	1.01 ± 1.33	3.22 ± 7.10	2.66 ± 6.54	4.66 ± 9.48	3.69 ± 8.96
Intracellular	315.83 ± 250.61	275.07 ± 229.61	334.25 ± 313.91	282.13 ± 258.69	347.7 ± 420.11
DNA fragmentation (%)	44.69 ± 10.16	39.64 ± 12.19	40.53 ± 14.13	41.67 ± 9.57	43.97 ± 12.36

ROS: reactive oxygen species, DNA: deoxyribonucleic acid, SSV: solid surface vitrification, LNF: liquid nitrogen vapor freezing

Repeated measure ANOVA, [a] p < 0.001 significant differences vs. control, [b] p < 0.001 significant differences vs. LNF, [c] p < 0.001 significant differences vs. LNF + crocin

VAP, VCL, and ALH significantly decreased after cryopreservation by both methods when compared to controls. STR significantly increased in the SSV group (Table 3). Sperm DNA fragmentation and sperm morphology were not different between controls and cryopreserved groups (Table 2). Crocin supplementation did not show any change in the percentage of sperm DNA fragmentation and sperm morphology in both cryopreservation groups. Both cryopreservation methods increased extracellular ROS when compared with controls, but the increase was not significantly different (Table 2, $p > 0.05$). Supplementation with crocin in cryoprotective agents decreased ROS level, but this decrease did not reach statistical significance in both groups ($p > 0.05$). Both cryopreservation methods non-significantly decreased intracellular ROS when compared with non-cryopreserved controls. Crocin supplementation slightly increased intracellular ROS levels in both methods, but this increase did not reach statistical significance in both groups ($p > 0.05$).

Table 3. Sperm kinematics in controls and post-cryopreserved samples, with or without crocin supplementation in the cryoprotective media.

Parameters (n = 36)	Control	Vitrification		Liquid nitrogen vapor	
		SSV	SSV + crocin	LNF	LNF + crocin
VAP	64.61 ± 12.05	46.91 ± 10.82 [a]	51.34 ± 11.34 [a]	50.19 ± 12.02 [a]	49.83 ± 10.93 [a]
VSL	43.81 ± 12.71	35.67 ± 10.64 [b]	39.98 ± 11.21	36.29 ± 11.21 [c]	37.65 ± 10.62
VCL	127.84 ± 25.28	95.20 ± 17.72 [a]	101.88 ± 20.23 [a]	105.98 ± 24.04 [a]	105.19 ± 20.62 [a]
ALH	7.12 ± 1.37	5.30 ± 0.88 [a]	5.41 ± 1.07 [a]	5.80 ± 1.24 [a]	5.70 ± 1.03 [a]
STR	67.72 ± 9.93	73.45 ± 8.27 [d]	74.90 ± 7.75 [e]	70.33 ± 8.73	70.66 ± 8.84
LIN	36.92 ± 9.06	38.61 ± 7.48	40.25 ± 7.60	35.79 ± 7.47	35.95 ± 7.30

SSV: solid surface vitrification, LNF: liquid nitrogen vapor freezing

Repeated measure ANOVA [a] $p < 0.001$ significant differences vs. control, [b] $p = 0.022$ significant differences vs. control, [c] $p = 0.042$ significant differences vs. control, [d] $p = 0.046$ significant differences vs. control, [e] $p = 0.005$ significant differences vs. control

Discussion

Crocin was chosen as a supplement in this study because previous in vitro experimental and clinical studies have shown its beneficial effects on sperm parameters and embryo quality[14, 15], and there has been no study on its use in human sperm cryopreservation. Sperm viability, DNA fragmentation, and morphology did not change significantly after cryopreservation, which was in agreement with our previous studies[10]. Post-cryopreserved sperm had a significant decrease in total motility, progressive motility, and many sperm kinematic parameters. However, the importance of CASA sperm kinematics is not completely understood and still under debate. Current data indicates that VSL and VCL are important kinetic parameters that are related to the speed of sperm motion. They are better correlated with fertility in humans than the percentage of motile sperm[18]. A study by Donnelly et al[19] found that the percentage of morphologically normal sperm correlated most strongly with IVF fertilization rate, and VAP correlated most significantly with pregnancy rate. In our study, it was reassuring that cryopreservation did not result in a significant change in sperm morphology, but it decreased total and progressive motility, VAP, VSL, VCL, and ALH. The decrease in these sperm kinematics could be due to cryoinjury itself or because of ROS, or both. LIN (= VSL/VCL x 100) did not change significantly in both cryopreservation groups, because there was a proportionate reduction in both VSL and VCL. On the other hand, the percent straightness (STR = VSL/VAP x 100) significantly increased in the vitrification group, compared with a non-significant increase in the liquid nitrogen vapor group. Clinically, post-cryopreserved sperm in the vitrification group would cover more distance in a shorter period of time than those in the liquid nitrogen vapor group, implying a better sperm kinematic. A previous study found that samples from which IVF pregnancy resulted had significantly higher LIN and STR than those from which pregnancy was not achieved[19].

We evaluated both the intra- and extracellular ROS levels in the non-frozen controls and both cryopreserved groups. We hypothesized that cryoinjury would increase the levels of ROS both inside and outside the cells. The findings that extracellular ROS levels were increased after both cryopreservation methods, and decreased, although not significantly, by the addition of the antioxidant crocin, was compatible with our assumption. However, the non-significant decrease in intracellular ROS after both cryopreservation methods was unexpected. Our results were in agreement with similar studies that demonstrated a non-significant decrease in intracellular ROS after programmable freezing of boar sperm[20], and liquid nitrogen vapor freezing of fish sperm[21]. In another study on human sperm cryopreservation, using the liquid nitrogen vapor techniques, there was a significant reduction in the percentage of ROS-positive sperm by transferase-mediated dUTP nick end labeling (TUNEL) assay after freezing compared to that before freezing[22]. The reasons behind the reduction in the amount of intracellular ROS were not known, but these authors postulated that it was probably due to cryoinjury to mitochondria, which resulted in both the reduction in intracellular ROS production and the energy required for sperm motility. Unfortunately, they did not measure extracellular ROS to support their assumption. The increased levels of extracellular ROS in our study suggested that ROS production was increased and then transported to the outside. This increase should come from the spermatozoa rather than exogenous sources, as leukocytospermia was one of our exclusion criteria, and we processed the sperm through density gradient centrifugation to clean up dead spermatozoa and white cells before cryopreservation. The measured levels of intracellular ROS might have been falsely low, probably due to the inactivation of esterase enzymes. This process could occur through protein oxidation by ROS itself or through cryoinjury. With a decrease in the activity of esterase enzymes, less amount of dichlorodihydrofluorescein diacetate (DCFH-DA) would be converted to dichlorodihydrofluorescein (DCFH), resulting in a lower level of the fluorescent signals from 2' , 7' - dichlorofluorescein (DCF). We postulated that crocin supplementation counteracted the detrimental effect of ROS on the esterase enzyme, thereby; increase the amount of the fluorescent signals. We observed from the imaging flow cytometer that the presence of intracellular ROS localized in the sperm head and proximal sperm tail. This was compatible with current knowledge that intracellular ROS were mainly produced from two sources: 1) the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system at the plasma membrane of the sperm head, and 2) the NADH-dependent oxidoreductase system in the mitochondria[6]. The intensity of fluorescent signals from the sperm head was observed to be more intense and more obvious than those from the sperm tail. We had no information on the amount of ROS that was contributed by each compartment. However, the lower intensity of intracellular ROS signals in the tail compartment might have been due to its smaller size, rather than a lower production rate. ROS are known to diffuse readily through specialized aquaporin pores in the cell membranes. ROS produced from mitochondria could, therefore, exit the tail compartment more readily than those from the head compartment due to its higher surface to volume ratio. Our finding that crocin supplementation in the cryopreservation media did not significantly reduce the amount of extra-cellular ROS could be due to many factors. Pharmacokinetic studies indicate that crocin ($C_{44}H_{64}O_{24}$; MW 976.972 Daltons) is a very polar substance and is not absorbed into the blood circulation as an intact molecule after oral ingestion. There is no information on its diffusion across the cell membrane[23]. In general, a polar molecule with > 5 hydrogen bond donors, > 10 hydrogen bond acceptors, and a molecular size > 500 Daltons are usually not membrane permeable[24]. We, therefore, assumed that crocin might remain largely outside the cells to combat ROS that were released from the spermatozoa. In this regard, crocin might act passively to scavenge ROS after they had exerted their detrimental effects inside the spermatozoa. The other reason for its ineffectiveness was that the amount of crocin required for optimal antioxidant activity could vary

among different individuals, making it difficult to see a beneficial effect when the same dose was used in all subjects. The third possible reason was that crocin was added to the cryoprotective media and sperm washing solution, but it was absent in sperm preparation. As ROS were known to be released after osmotic change and centrifugation[25], this could limit the beneficial effect of crocin in our study. Despite an adequate sample size in this study, we encountered many limitations that should be addressed in future studies. First, we should incorporate both intra- and extracellular antioxidants to see if the combination could significantly improve post-cryopreserved sperm survival. The inclusion of an agent that directly inhibited ROS production might also be beneficial. This cocktail of antioxidants and ROS inhibitors should be present both in the cryopreservation and washing media throughout the entire process of sperm processing, freezing, and washing. It was also advisable to use a direct method of intracellular ROS measurement, instead of an indirect one that was employed in this study.

Conclusion

In conclusion, the supplementation of crocin during vitrification and liquid nitrogen vapor freezing did not improve the outcome of post-thaw sperm.

Potential conflicts of interest

The authors declare no conflicts of interest.

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OBSTETRICS

Intrapartum Maternal Capillary Blood Glucose in Diabetic Pregnancy and Risk Factors Associated with Neonatal Hypoglycemia

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ABSTRACT

Objectives: To find an association between intrapartum maternal capillary blood glucose in diabetic pregnancy and neonatal hypoglycemia, and find the factors affected by this condition.

Materials and Methods: The study was a retrospective cohort study of 677 cases of diabetic pregnancies, delivered at Hatyai Hospital from October 2016 to September 2019. The primary outcome was to find an association between intrapartum maternal capillary blood glucose in diabetic pregnancy and neonatal hypoglycemia. The secondary outcome was to find factors that may be associated with neonatal hypoglycemia. Multiple logistic regression was used for analysis which quantifies the magnitude of association. Adjusting for covariates was done. The association was expressed as odd ratio and was interpreted as significant at p value < 0.05.

Results: From 677 cases reviewed, pregestational diabetes mellitus was 67 cases (9.90%) and gestational diabetes mellitus was 610 cases (90.10%). Neonatal hypoglycemia was recorded at 64 cases (9.45%). Following analysis, we found that a high level of capillary blood glucose of more than 110 mg/dL during intrapartum periods in diabetic pregnancy was associated with neonatal hypoglycemia (adjusted odds ratio (aOR) 2.46, 95%CI 1.40-4.32, p = 0.002). Cesarean delivery was also associated with this condition (aOR 4.04, 95% CI 2.15-7.55, p < 0.001).

Conclusion: Intrapartum capillary blood glucose levels exceeding 110 mg/dL and cesarean delivery in diabetic pregnancy were associated with neonatal hypoglycemia.

Keywords: blood glucose, diabetes mellitus, hypoglycemia, intrapartum, neonate, pregnancy.

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ความสัมพันธ์ของระดับน้ำตาลปลายนิ้วระยะคลอดในสตรีตั้งครรภ์ที่เป็นเบาหวานและปัจจัยที่เกี่ยวข้องกับการเกิดภาวะน้ำตาลต่ำในทารกแรกเกิด

วรรณิดา นิปกะกุล, รัตน์พร เบญจมานนท์

บทคัดย่อ

วัตถุประสงค์: *ศึกษาความสัมพันธ์ของระดับน้ำตาลปลายนิ้วระยะคลอดในสตรีตั้งครรภ์ที่เป็นเบาหวานกับการเกิดภาวะน้ำตาลต่ำในทารกแรกเกิด และหาปัจจัยที่มีผลต่อภาวะน้ำตาลต่ำในทารกแรกเกิด*

วัสดุและวิธีการ: *เป็นการศึกษาจากเหตุไปผลแบบย้อนหลัง (Retrospective cohort study) ในสตรีตั้งครรภ์ที่มีภาวะเบาหวานที่คลอด ณ โรงพยาบาลหาดใหญ่ ตั้งแต่เดือนตุลาคม พ.ศ.2559 ถึงเดือนกันยายน พ.ศ.2562 จำนวน 677 คน วัตถุประสงค์หลักคือ ความสัมพันธ์ของระดับน้ำตาลปลายนิ้วระยะคลอดในสตรีตั้งครรภ์ที่เป็นเบาหวานกับการเกิดภาวะน้ำตาลต่ำในทารกแรกเกิด วัตถุประสงค์รอง คือ หาปัจจัยที่มีผลต่อภาวะน้ำตาลต่ำในทารกแรกเกิด โดยใช้ multiple logistic regression ประเมินระดับความสัมพันธ์ วิเคราะห์ปรับค่าตัวแปรร่วม แสดงค่าความสัมพันธ์เป็น odd ratio และค่านัยสำคัญกับที่ $p < 0.05$*

ผลการศึกษา: *จากการศึกษาผู้ป่วย 677 คน พบภาวะเบาหวานก่อนการตั้งครรภ์ 67 คน (ร้อยละ 9.90) และเบาหวานที่เกิดจากการตั้งครรภ์ 610 คน (ร้อยละ 90.10) พบภาวะน้ำตาลต่ำในทารกแรกเกิด 64 คน (ร้อยละ 9.45) จากการวิเคราะห์พบว่าภาวะน้ำตาลสูงเกินกว่า 110 มก/ดล ในระยะคลอดในสตรีตั้งครรภ์ที่เป็นเบาหวานมีความสัมพันธ์กับการเกิดภาวะน้ำตาลต่ำในทารกแรกเกิด (adjusted odds ratio (aOR) 2.46, 95%CI 1.40-4.32, $p = 0.002$] และพบว่าการคลอดโดยการผ่าคลอดมีความสัมพันธ์กับการเกิดภาวะน้ำตาลต่ำในทารกแรกเกิด (aOR 4.04, 95% CI 2.15-7.55, $p < 0.001$)*

สรุป: *ระดับน้ำตาลปลายนิ้วระยะคลอดในสตรีตั้งครรภ์ที่เป็นเบาหวานที่ระดับน้ำตาลสูงเกินกว่า 110 มก/ดล และการคลอดโดยการผ่าคลอดสัมพันธ์กับการเกิดภาวะน้ำตาลต่ำในทารกแรกเกิด*

คำสำคัญ: *ระดับน้ำตาล, เบาหวาน, ภาวะน้ำตาลต่ำ, ระยะคลอด, ทารกแรกเกิด, สตรีตั้งครรภ์*

Introduction

Diabetes in pregnancy is divided into pregestational and gestational diabetes. As in the Clinical Practice Guideline for Diabetes 2017[1], treatment of this patient group is aimed to decrease adverse events in pregnant women and deliver healthy newborn without complications.

According to Hatyai Hospital's records, diabetes in pregnancy was found in 3% of all pregnancies delivered from 2017-2018, and increased to 5.1% in 2019. This trend will continue to increase in the future, so it is important for obstetricians to have knowledge of this practice to decrease the number of complications as much as it possible.

Hypoglycemia in the newborn can cause dyspnea, lethargy and seizure. The causes can be due to low newborn birthweight, prematurity, infection, diabetic mother and other reasons[2, 3, 4, 5]. The poor control of blood sugar level in diabetic pregnancy in the intrapartum period is thought to affect hypoglycemia in newborns. As in the American College of Obstetricians and Gynecologists (ACOG) practice bulletin guidelines, controlling blood sugar in the range of 70-110 mg/dL was suggested in pregestational diabetes pregnancy[6]. No suggestion regarding gestational diabetes pregnancy was mentioned[7]. However, there is still limited evidence to support this practice. Many recent studies have produced different results and have stated that controlling of blood sugar level in the intrapartum period did not decrease the hypoglycemia in newborns[8, 9]. Moreover, in some systemic reviews, the association was still inconclusive[10].

The researcher is interested in this subject and aims to discover the association between this condition and assist in the adjustment of the practice inpatient care, prevent complications, and reduce the number of unnecessary operations.

We set the primary objective to find an association between intrapartum maternal capillary blood glucose in diabetic pregnancy and neonatal hypoglycemia. The secondary objective was to find factors that may be associated with neonatal hypoglycemia.

Materials and Methods

A retrospective cohort study was performed by reviewing the electronic medical records of a diabetic pregnancy group between October 2016 and September 2019. This study was approved by the Research Ethics Committee of Hatyai Hospital (REC-HY) (Protocol number 31/2564).

The sample size was calculated using the formula for cohort studies. The total sample size of 641 cases was the minimum requirement. ICD10 of O240-O244 + O80, O81, O82 was used to search patients included in the medical records, with singleton and term pregnancy cases. A Total number of 730 case records were reviewed, while 53 cases (7.26%) of patients with incomplete records and death fetus in utero were excluded. The remaining 677 patient records, including 530 cases in tight control blood sugar group (< 110 mg/dL) and 147 cases in a nontight control blood sugar group (≥ 110 mg/dL) were reviewed intensively and data subsequently extracted. The value of blood sugar ≥ 110 mg/dL, stated as non-tight control blood sugar, was used to compare results with those of the previous study which used the same range of blood sugar[8]. With patients who had several values of blood sugar levels, the final level was used to show the manner of tight control blood sugar, i.e.: if the patient had hyperglycemia and then received insulin therapy or a change of intravenous fluid, and then the blood sugar dropped to < 110 mg/dL, the patient was categorized in the tight control blood sugar group. Maternal hypoglycemia (< 70 mg/dL) was found in a small number of cases, and all case received therapy such as intravenous glucose loading or a changed of fluid solution to increase the amount of glucose form. All cases showed improvement.

Categorization of this patient group also depended on the final value of blood sugar.

Neonatal hypoglycemia was diagnosed using 2011 American Academy of Pediatrics (AAP) guidelines[11]. Neonates with plasma glucose < 40 mg/dL and any symptoms including tachypnea, jitteriness, cyanosis, seizure, apneic episode, weak and highpitched cry, floppiness or lethargy, poor feeding or eyerolling neonates with plasma glucose < 25 mg/dL after birth - 4 hours postdelivery, and neonates with plasma glucose < 35 mg/dL at 4 hours - 24 hours postdelivery were defined as neonatal hypoglycemia. Data on presenting symptoms and specific treatment for these neonates were also collected.

From the data collected, overt diabetes mellitus was the same as pregestational diabetes, which was the diabetes that found before pregnancy or laboratory suspected (HbA1C ≥ 6.5%, fasting blood sugar (FBS) ≥ 126 mg/dL, random plasma glucose ≥ 200 mg/dL). Gestational diabetes mellitus (GDM) was diabetes that arises during pregnancy and does not meet the above criteria. GDMA1 required only dietary or lifestyle adjustments to control the level of blood sugar, while the GDMA2 required medication such as insulin to control blood sugar levels within the targeted range. Pre-pregnancy body mass index (BMI) was classified as Asian population range. Underweight is BMI < 18.5 kg/m^2, normal range BMI is 18.5-22.9 kg/m^2, overweight is BMI 23-24.9 kg/m^2, obese is BMI 25 - 29.9 kg/m^2, and morbid obese is BMI ≥ 30 kg/m^2. Antepartum blood sugar control level was classified as well control patient if ≥ 80% of blood sugar collected was within the targeted range. And classified as partial and poor control if 50-79% and < 50% blood sugar collected was in the targeted range, respectively. Neonatal birthweights were classified as low birthweight if weight < 2,500 grams, normal birthweight if weight 2,500 - 4,000 grams, and fetal macrosomia if weight more than 4,000 grams.

Statistical analyses were performed using STATA (Statacorp, USA) software, version 16SE. Descriptive statistics were used to demonstrate demographic data. Continuous data was presented with mean ± standard deviation (SD). The correlation of two discrete data were analyzed with Chi-squared test and Fisher's exact test as appropriate. The correlation of linear data was analyzed using t-test. Multiple logistic regression was used for analysis, which quantified the magnitude of association between intrapartum maternal capillary blood glucose and neonatal hypoglycemia. Other factors that may be associated with these conditions were also analyzed. For all analyzed results, a p value < 0.05 was considered statistically significant.

Results

Between October 2016 - September 2019, total 25,664 pregnancies delivered at Hatyai Hospital. Of the 677 reviewed cases, pregestational diabetes mellitus was 67 cases (9.90%) and gestational diabetes mellitus was 610 cases (90.10%). Maternal demographic, antenatal and intrapartum characteristics of pregnant women in the study are presented in Table 1.

Non-tight control capillary blood glucose levels in the intrapartum period, which was defined as capillary blood glucose levels at 110 mg% or higher, were found in 147 (21.71%) cases. The remaining 530 cases were in a tight control capillary blood glucose level and calculated as 78.29% of all cases.

Neonatal characteristics were reviewed (Table 2). Hypoglycemia was found in 64 (9.45%) newborns of all births from maternal diabetes. Non-hypoglycemia was found in 613 (90.55%) newborns of all births.

Table 1. Antenatal and intrapartum maternal characteristics (n = 677).

Maternal Characteristics	n (%)
Age (years), mean (SD)	32.98 (5.84)
Age range (years)	
< 20	11 (1.62)
20-24	48 (7.09)
25-29	118 (17.43)
30-34	206 (30.43)
≥ 35	294 (43.43)
Gravidity, Median (IQR)	2 (2-3)
Gravidity	
< 4	519 (76.66)
≥ 4	158 (23.34)
Parity, Median (IQR)	1 (0-2)
Parity	
< 4	642 (94.83)
≥ 4	35 (5.17)
Gestational age at delivery (days), mean (SD)	270.21 (7.84)
Diabetes mellitus	
Pregestational diabetes	67 (9.90)
GDMA1	391 (57.75)
GDMA2	219 (32.35)
Pre-pregnancy BMI (kg/m^2), mean (SD)	28.06 (5.95)
Pregestational BMI classification (kg/m^2)	
Underweight (< 18.5)	21 (3.10)
Normal (18.5 - 22.9)	114 (16.84)
Overweight (23 - 24.9)	84 (12.41)
Obesity (25 -29.9)	230 (33.97)
Morbid obesity (≥ 30)	228 (33.68)
Antepartum insulin use	
No	394 (58.20)
Yes	283 (41.80)
Antepartum blood sugar control level	
Well	372 (54.95)
Partial	187 (27.62)
Poor	118 (17.43)
Intrapartum CBG level (mg%)	
< 110	530 (78.29)
110-140	106 (15.66)
> 140	41 (6.06)
Mean capillary blood glucose (mg%), mean (SD)	95.15 (20.23)
Frequency of intrapartum CBG monitoring	
Baseline determination alone	327 (48.30)
Every hour	105 (15.51)
Every 2 hours	223 (32.94)
Every 4 hours	20 (2.95)
Every 6 hours	2 (0.30)
Intrapartum CBG level	
Tight control	530 (78.29)
Non- tight control	147 (21.71)
Intrapartum insulin use	
No	658 (97.19)
Yes	19 (2.81)
Mode of delivery	
Normal spontaneous delivery	320 (47.27)
Cesarean section	348 (51.40)
Operative vaginal delivery	9 (1.33)

GDMA1: gestational diabetes mellitus class A1, GDMA2: gestational diabetes mellitus class A2, BMI: body mass in-dex, CBG: capillary blood glucose, SD: standard deviation, IQR: interquartile range

Table 2. Neonatal characteristics and outcomes (n = 677).

Neonatal characteristics	n (%)
Sex	
Male	357 (52.73)
Female	320 (47.27)
Birthweight (grams), mean (SD)	3332.26 (498.15)
Birthweight classification	
Low birthweight	22 (3.25)
Normal birthweight	589 (87.00)
Fetal macrosomia	66 (9.75)
CBG, mean (SD)	63.24 (18.73)
Time for CBG sampling	
30 minutes	16 (2.36)
1 hour	643 (94.98)
2 hours	18 (2.66)
Hypoglycemia	
No	613 (90.55)
Yes	64 (9.45)
Presence of hypoglycemic symptoms (n = 64)	
No	19 (29.69)
Yes	45 (70.31)
Specific treatment for hypoglycemic neo-nates (n = 64)	
Feed	43 (67.19)
D10W	15 (23.44)
Increase IV fluid rate	6 (9.37)

CBG: capillary blood glucose, D10W: dextrose 10% in water, SD: standard deviation

Table 3 shows the univariate analysis of factors that may affect intrapartum blood glucose levels in diabetic mothers. There were six factors that significantly affected with $p < 0.05$, types of diabetes, mode of delivery, antepartum insulin used, antepartum blood sugar control level, frequency of intrapartum capillary blood glucose monitoring and intrapartum insulin used.

Table 4 shows the univariate analysis of factors that may affect neonatal hypoglycemia. There were 11 factors that significantly affected this conditions at $p < 0.05$, gravida exceeding 4, mean gestational age, types of diabetes, maternal pre-pregnancy BMI classification, mode of delivery, maternal antepartum insulin used, maternal antepartum blood sugar control level, frequency of intrapartum maternal capillary blood glucose monitoring, maternal intrapartum non-tight control capillary blood glucose level, newborn birthweight classification, and time of newborn capillary blood glucose sampling.

The multiple logistic regression analysis of the association between intrapartum maternal capillary blood glucose levels and neonatal hypoglycemia is shown in Table 5. We found that high levels of capillary blood glucose during intrapartum period exceeding 110 mg/dL in diabetes pregnancy is associated with neonatal hypoglycemia (adjusted odds ratio (aOR) 2.46, 95%CI 1.40-4.32, $p = 0.002$). Mode of delivery was also associated with this condition (aOR 3.05, 95% CI 1.80-5.15, $p < 0.001$). After subgroup analysis was performed, the result showed that cesarean delivery route increases neonatal hypoglycemia compared with spontaneous vaginal delivery (aOR 4.04, 95%CI 2.15-7.55, $p < 0.001$). Other factors were not significantly associated with this condition after using multiple logistic regression.

Table 3. Univariate analysis of factors associated with intrapartum maternal capillary blood glucose level.

Factors	Tight control CBG group n = 530 (%)	Non-tight control CBG group n = 147 (%)	p value
Age range (in years)			0.391†
< 20	7 (1.32)	4 (2.72)	
20 - 24	35 (6.60)	13 (8.84)	
25 - 29	90 (16.98)	28 (19.05)	
30 - 34	168 (31.70)	38 (25.85)	
≥ 35	230 (43.40)	64 (43.54)	
Gravidity			0.242*
< 4	401 (75.66)	118 (80.27)	
≥ 4	129 (24.34)	29 (19.73)	
Parity			0.501*
< 4	501 (94.53)	141 (95.92)	
≥ 4	29 (5.47)	6 (4.08)	
Gestation age at delivery (days), mean (SD)	270.20 (8.12)	270.25 (6.80)	0.938‡
Diabetes mellitus			0.002†
Pregestational diabetes	48 (9.06)	19 (12.93)	
GDMA1	325 (61.32)	66 (44.90)	
GDMA2	157 (29.62)	62 (42.18)	
Pregestational BMI classification (kg/m^2)			0.356†
Underweight (< 18.5)	15 (2.83)	6 (4.08)	
Normal (18.5 - 22.9)	87 (16.42)	27 (18.37)	
Overweight (23 - 24.9)	71 (13.40)	13 (8.84)	
Obesity (25 - 29.9)	174 (32.83)	56 (38.10)	
Morbid obesity (≥ 30)	183 (34.53)	45 (30.61)	
Antepartum insulin use			0.016*
No	327 (61.70)	67 (45.58)	
Yes	203 (38.30)	80 (54.42)	
Antepartum blood sugar control level			0.016*
Well	302 (56.98)	70 (47.62)	
Partial	147 (27.74)	40 (27.21)	
Poor	81 (15.28)	37 (25.17)	
Frequency of intrapartum CBG monitoring			< 0.001†
Baseline determination alone	280 (52.83)	47 (31.97)	
Every hour	68 (12.83)	37 (25.17)	
Every 2 hours	163 (30.75)	60 (40.82)	
Every 4 hours	18 (3.40)	2 (1.36)	
Every 6 hours	1 (0.19)	1 (0.68)	
Intrapartum insulin use			< 0.001†
No	528 (99.62)	130 (88.44)	
Yes	2 (0.38)	17 (11.56)	
Mode of delivery			0.008†
Normal spontaneous delivery	238 (44.91)	82 (55.78)	
Cesarean section	287 (54.15)	61 (41.50)	
Operative vaginal delivery	5 (0.94)	4 (2.72)	
Sex			0.643*
Male	277 (52.26)	80 (54.42)	
Female	253 (47.74)	67 (45.58)	
Birthweight classification			0.519†
Low birthweight	16 (3.02)	6 (4.08)	
Normal birthweight	465 (87.74)	124 (84.35)	
Fetal macrosomia	49 (9.24)	17 (11.56)	

GDMA1: gestational diabetes mellitus class A1, GDMA2: gestational diabetes mellitus class A2, BMI: body mass in-dex, CBG : capillary blood glucose, SD: standard deviation. *Chi-square test, †Fisher's exact test, ‡ t-test

Table 4. Univariate analysis of factors associated with neonatal hypoglycemia.

Factors	Without neonatal hypoglycemia n = 613 (%)	With neonatal hypoglycemia n = 64 (%)	p value
Age range (years)			0.067†
< 20	10 (1.63)	1 (1.56)	
20 - 24	43 (7.01)	5 (7.81)	
25 - 29	104 (16.97)	14 (21.88)	
30 - 34	196 (31.97)	10 (15.63)	
≥ 35	260 (42.41)	34 (53.13)	
Gravidity			0.014*
< 4	462 (75.37)	57 (89.06)	
≥ 4	151 (24.63)	7 (10.94)	
Parity			0.239†
< 4	579 (94.45)	63 (98.44)	
≥ 4	34 (5.55)	1 (1.56)	
Gestational age at delivery (days), mean (SD)	270.45 (8.03)	267.92 (5.27)	0.014‡
Diabetes mellitus			0.002*
Pregestational diabetes	55 (8.97)	12 (18.75)	
GDMA1	366 (59.71)	25 (39.06)	
GDMA2	192 (31.32)	27 (42.19)	
Pregestational BMI classification (kg/m2)			0.005†
Underweight (<18.5)	18 (2.94)	3 (4.69)	
Normal (18.5-22.9)	105 (17.13)	9 (14.06)	
Overweight (23-24.9)	81 (13.21)	3 (4.69)	
Obesity (25-29.9)	215 (35.07)	15 (23.44)	
Morbid obesity (≥30)	194 (31.65)	34 (53.13)	
Antepartum insulin use			0.006*
No	367 (59.87)	27 (42.19)	
Yes	246 (40.13)	37 (57.81)	
Antepartum blood sugar control level			0.004*
Well	349 (56.93)	23 (35.94)	
Partial	160 (26.10)	27 (42.19)	
Poor	104 (16.97)	14 (21.88)	
Frequency of intrapartum CBG monitoring			0.024†
Baseline determination alone	293 (47.80)	34 (53.13)	
Every hour	90 (14.68)	15 (23.44)	
Every 2 hours	209 (34.09)	14 (21.88)	
Every 4 hours	20 (3.26)	0 (0.00)	
Every 6 hours	1 (0.16)	1 (1.56)	
Intrapartum insulin use			0.095†
No	598 (97.55)	60 (93.75)	
Yes	15 (2.45)	4 (6.25)	
Intrapartum CBG level			0.004*
Tight control	489 (79.77)	41 (64.06)	
Non-tight control	124 (20.23)	23 (35.94)	
Mode of delivery			< 0.001†
Normal spontaneous delivery	306 (49.92)	14 (21.88)	
Cesarean section	299 (48.78)	49 (76.56)	
Operative vaginal delivery	8 (1.31)	1 (1.56)	
Sex			0.100*
Male	317 (51.71)	40 (62.50)	
Female	296 (48.29)	24 (37.50)	
Birthweight classification			< 0.001†
Low birthweight	21 (3.43)	1 (1.56)	
Normal birthweight	543 (88.58)	46 (71.88)	
Fetal macrosomia	49 (7.99)	17 (26.56)	
Time for CBG sampling			0.005†
30 minutes	12 (1.96)	4 (6.25)	
1 hour	588 (95.92)	55 (85.94)	
2 hours	13 (2.12)	5 (7.81)	

GDMA1: gestational diabetes mellitus class A1, GDMA2: gestational diabetes mellitus class A2, BMI: body mass in-dex, CBG: capillary blood glucose, SD: standard deviation. * Chi-square test, † Fisher's exact test, ‡ t-test

Table 5. Crude and adjusted logistic regression models of neonatal hypoglycemia and intrapartum maternal capillary blood glucose level.

	Neonatal hypoglycemian					
	Crude			Adjusted		
	OR	95%CI	p value	OR	95%CI	p value
Intrapartum maternal CBG in non-tight control level	2.21	1.28-3.82	0.004	2.46	1.40-4.32	0.002
Mode of delivery	2.91	1.72-4.93	< 0.001	3.05	1.80-5.15	< 0.001
- Normal spontaneous delivery	Reference			Reference		
- Cesarean section	3.58	1.93-6.63	< 0.001	4.04	2.15-7.55	< 0.001
- Operative vaginal delivery	2.73	0.32-23.38	0.359	2.27	0.25-19.96	0.459

CBG: capillary blood glucose, OR: odds ratio, CI: confidence interval

Discussion

The pregnancies delivered at Hatyai Hospital were 25,664 cases between October 2016 - September 2019. To estimate the proportion of diabetic cases, diabetic pregnancies was found 960 cases or 3.74%. Which was lower than that of the United States at 6.5%[12] but higher than the previous study in Thailand which found diabetes in 2.32% of all pregnancies[13]. After dividing the groups of diabetes, pregestational diabetes mellitus was found 82 cases (0.32%) of all pregnancies and gestational diabetes mellitus in 806 cases (3.41%) of all pregnancies. This was lower than that in the USA, which was found 1.5% and 5%, respectively.

Our study population was predominantly gestational diabetes mellitus, especially type A1 which was managed with dietary control. The majority of parturients were obese and morbidly obese. The incidence of intrapartum maternal blood glucose in non-tight control level was 21.71%, similar to that found in other studies[8]. Our study determined the last blood glucose prior to delivery as the controlled level and assumed that high blood sugar level controlled with intravenous fluid solution changed and insulin administration and dropped to lower than 110 mg/dL in the tight control group. Some studies found that duration of hyperglycemic levels, or mean blood sugar during labor, demonstrated the better results[14,15], but our study did not intensively detail this.

The ACOG practice bulletin number 201 recommends monitoring blood glucose levels every hour during labor in pregestational diabetic pregnancy[6]. However, the frequency of glucose monitoring in this institution is no consensual and baseline determination is mostly conducted alone, depending on the individual physician's decision.

Insulin administration was used in the form of regular insulin and continuous intravenous infusion, but with high capillary blood glucose levels exceeding 110 mg/dL, not every case received insulin. This may be related to the differences in individual practices of the physicians. However, in the liberal high level of blood sugar (> 140 mg/dL), every case received intravenous insulin.

The majority of infants were born to diabetic mothers in this institution appear to have weights appropriate for gestational age. Fetal macrosomia was found at 9.75%. This was related to suboptimal antepartum glycemic control and some studies have found this leads to neonatal hypoglycemia[16,17]. However, our study found no association.

The incidence of neonatal hypoglycemia among diabetic parturient in this study was found to be 9.45%, similar to the previous study[2, 8]. We did not find any maternal antepartum characteristics or managements that associated with neonatal hypoglycemia, but in the intrapartum period, we found that intrapartum maternal capillary blood glucose at the non-tight control level and mode of delivery as a cesarean route, were significantly associated with neonatal hypoglycemia.

In the context of maternal hyperglycemia levels, neonatal hypoglycemia is thought to be due to the increase in fetal glucose levels that stimulate the fetal pancreas to synthesize excessive insulin causing fetal

hyperinsulinemia. This can cause diminished hepatic glucose production in neonates. After birth, a fall in plasma glucose concentration, while the insulin level is still high, finally results in neonatal hypoglycemia. Some studies have stated that chronic hyperglycemia or antepartum suboptimal control of blood sugar results in this condition[14,18]. Hyperglycemia in the intrapartum period is considered in the same way, but the results were still inconclusive[10]. The cut-off level to determine hyperglycemia was different in practice. We used a capillary blood glucose level of 110 mg/dL or more to determine high, or as in non-tight control level. Some studies have shown that the percentage of time spent in the hyperglycemic level may affect this[14,15]. Our results supported the association between intrapartum high capillary blood glucose levels of more than 110 mg/dL and neonatal hypoglycemia, but we lack the detail about the time spent. Further studies may be beneficial.

Some studies explored the relationship between cesarean deliveries and the neonatal hypoglycemia[19, 20, 21], and stated that this route of delivery was also found in the higher incidence of neonatal hypoglycemia. Factors were fasting time before surgery and high glycemic load. However, these factors were not detailed in our studies, so further research is recommended.

Compared to the previous study, our study had a larger number of cases and includes more details about the antepartum blood glucose control level[8]. However, with the retrospective study design, our study had limitations, as possible inclusion of confounding factors, selection bias or misclassification bias from data collection. It also did not detail some data, such as indication for cesarean delivery, incidence of shoulder dystocia, fasting time, intravenous fluid during intrapartum period and dosage of insulin. These limitations may be reduced in further studies which include a greater amount of detailed data.

Hypoglycemia in neonate is also influenced by factors such as premature birth, infection, low birth weight, antepartum glyburide used, etc.[2, 3, 4, 5] Some cases had many factors that augment the affected to this condition. Awareness of the potential results and control of intrapartum level of maternal glucose to normal levels is highly recommended.

Conclusion

Intrapartum capillary blood glucose exceeding 110 mg/dL in diabetic pregnancies was associated with neonatal hypoglycemia. Another factor affected this condition was cesarean delivery.

Acknowledgments

This study was approved by the Institutional Review, Hatyai Hospital, Songkla, Thailand.

Potential conflicts of interest

The authors declare no conflicts of interest.

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OBSTETRICS

Postpartum Blues and Late Postpartum Depressive Symptoms at Her Royal Highness Princess Mahachakri Sirindhorn Medical Center

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ABSTRACT

Objectives: To identify association between postpartum blue and late postpartum depressive symptoms (LPDS) and incidence of both conditions with other risk factors of LPDS at Her Royal Highness Princess Mahachakri Sirindhorn Medical Center.

Materials and Methods: Case-control study where 128 mothers were assigned to complete Thai Edinburgh Postnatal Depressive Scale (Thai EPDS) at 2 days and 6 weeks postpartum to detect postpartum blues and LPDS, respectively. Risk factors of LPDS were assessed using bivariate analysis. Multiple logistic regression was used to identify the association between postpartum blues and LPDS along with other risk factors of LPDS.

Results: Majority of participants in this study were aged between 26 - 35 years old (52.4%), and multiparous women (67.2%) accounting more than nulliparous women (32.8%). Postpartum blues was significantly associated with LPDS with adjusted odds ratio (OR) 7.16 (95% confidence interval (CI) 1.87-27.39). The incidence of postpartum blues was 24.2% while the incidence of LPDS was 15.6%. Other significant risk factors were lack of support in newborn care with aOR of 4.48 (95% CI 1.31-15.33), breastmilk inadequacy with aOR of 8.62 (95% CI 2.33-31.84) and Bangkok and vicinity habitation with aOR 4.99 (95% CI 1.29-19.28).

Conclusion: Postpartum blues are highly associated with late postpartum depressive symptoms and being an early prediction of postpartum depression.

Keywords: postpartum blues, postpartum depression, late postpartum depressive symptoms.

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อารมณ์เศร้าหลังคลอดกับอาการซึมเศร้าหลังคลอดตอนปลาย ณ ศูนย์การแพทย์สมเด็จพระเทพรัตนราชสุดาฯ สยามบรมราชกุมารี

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บทคัดย่อ

วัตถุประสงค์: *หาความสัมพันธ์ระหว่างอารมณ์เศร้าหลังคลอด (Postpartum blues) กับอาการซึมเศร้าหลังคลอดตอนปลาย (Late postpartum depressive symptoms) ณ ศูนย์การแพทย์สมเด็จพระเทพรัตนราชสุดาฯ สยามบรมราชกุมารี และศึกษาอุบัติการณ์ของภาวะทั้งสอง รวมถึงปัจจัยเสี่ยงต่างๆ ของอาการซึมเศร้าหลังคลอดตอนปลาย*

วัสดุและวิธีการ: *การศึกษาแบบมีกลุ่มควบคุม (Case-control study) เพื่อหาความสัมพันธ์ระหว่างอารมณ์เศร้าหลังคลอดกับอาการซึมเศร้าหลังคลอดตอนปลาย โดยมารดากลุ่มตัวอย่าง 128 คน ได้ทำแบบสอบถาม Thai Edinburgh Postnatal Depressive Scale (Thai EPDS) ที่สองวันและหกสัปดาห์หลังคลอดเพื่อหามารดาที่มีอารมณ์เศร้าหลังคลอดและมารดาที่มีอาการซึมเศร้าหลังคลอดตอนปลาย และใช้ multiple logistic regression เพื่อเพื่อหาความสัมพันธ์ระหว่างอารมณ์เศร้าหลังคลอดกับอาการซึมเศร้าหลังคลอดตอนปลาย และปัจจัยเสี่ยงอื่นๆ*

ผลการศึกษา: *มารดาส่วนใหญ่ที่เข้าร่วมในการศึกษามีอายุในช่วง 26-35 ปี คิดเป็นร้อยละ 52.3 และพบเป็นมารดาที่ตั้งครรภ์ท้องหลังมากกว่าท้องแรก คิดเป็น ร้อยละ 67.2 และ 32.8 ตามลำดับ จากการศึกษาพบว่าอารมณ์เศร้าหลังคลอดและอาการซึมเศร้าหลังคลอดมีความสัมพันธ์กันอย่างมีนัยสำคัญทางสถิติ (adjusted odds ratio (OR) 7.16, 95% confidence interval (CI) 1.87-27.39) โดยพบอุบัติการณ์ของอารมณ์เศร้าหลังคลอด ร้อยละ 24.2 และอุบัติการณ์ของอาการซึมเศร้าหลังคลอดตอนปลาย ร้อยละ 15.6 ปัจจัยเสี่ยงต่ออาการซึมเศร้าหลังคลอดตอนปลายได้แก่ การขาดความช่วยเหลือในการดูแลทารก aOR 4.48 (95% CI 1.31-15.33), ปัญหาน้ำนมแม่ไม่เพียงพอ (aOR 8.62, 95% CI 2.33-31.84) และการอาศัยในกรุงเทพฯ และปริมณฑล (aOR 4.99, 95% CI 1.29-19.28)*

สรุป: *อารมณ์เศร้าหลังคลอดและอาการซึมเศร้าหลังคลอดมีความสัมพันธ์กันอย่างยิ่ง โดยสามารถใช้อารมณ์เศร้าหลังคลอดทำนายภาวะซึมเศร้าหลังคลอด (Postpartum depression) ได้*

คำสำคัญ: *อารมณ์เศร้าหลังคลอด, ภาวะซึมเศร้าหลังคลอด, อาการซึมเศร้าหลังคลอดตอนปลาย*

Introduction

Postpartum is a stressful moment for women. It increases the risk of several mental illnesses. Spectrum of postpartum mood disorders ranging from postpartum blues to postpartum depression. Postpartum blues or so-called maternity blues is a time-limited mood or emotional instability occurring within 1st week postpartum, and usually resolves within ten days as the process of mental adaptation. Mothers with postpartum blues still have happiness as predominant mood but tend to be more emotionally labile and experience various depressive symptoms such as insomnia, anxiety, inability to concentrate and irritability. On the other hand, postpartum depression shares the same symptoms to postpartum blues but with more of its severity. The symptoms mimic major depressive disorder according to diagnostic and statistical manual of mental disorders (DSM-V) criteria such as depressed mood, sleep disturbance, unexplained guilt, loss of energy, diminished ability to concentrate or even suicidal thought or attempts. Postpartum depression usually lasts longer than 2 weeks and may continue up to 12 months. Postpartum blues are spontaneously resolved by six weeks postpartum. Women whose symptoms persist beyond six weeks are considered to experience late postpartum depressive symptoms (LPDS). LPDS poses risk for postpartum depression[1, 2].

ACOG committee opinion No.757 recommended that Obstetricians and Gynecologists should perform mental health assessment using validated screening tools to identified mothers with postpartum depression at comprehensive postpartum visit, and provide referral program together with close monitoring care after detection of high risk mothers[3]. Since postpartum depression associated with maternal and neonatal morbidity, one of the most severe consequences is maternal suicide and infanticide, thus being able to diagnose patients with LPDS helps them to receive timely intervention and may prevent clinical progression to postpartum depression[3, 4].

Several risk factors have been associated with postpartum depression in Thailand. They are including extreme maternal age, divorced status, low family income, unplanned pregnancy, newborn sickness, non-breastfeeding mother, unemployed, inadequate support from family and domestic violence[5]. Furthermore, recent studies showed that postpartum blues was one of the important risk factors of postpartum depression[6, 7]. However studies in Thailand are yet to show such association.

The purpose of this study was to identify association between postpartum blues and LPDS at Her Royal Highness Princess Mahachakri Sirindhorn Medical Center. Secondary objectives were to find the incidence of postpartum blues and LPDS using Thai EPDS questionaire, and to identify other significant risk factors of LPDS.

Materials and Methods

Participants

Thai mothers who gave birth at the HRH Princess Maha Chakri Sirindhorn Medical Center between 1st May 2020 - 31st Dec 2020 who are more than 18 years old with single livebirth newborn and have given consent were enrolled in the study. Mothers who were diagnosed with psychological disease e.g., bipolar disorder and depressive disorder in past history or in current treatment process, mothers who undergone first questionnaire at 2 days postpartum with the result that highly suggestive of underlying depressive symptoms, and mothers who were unable to follow-up at postpartum clinic were excluded from the study.

Methods

This was a case-control study conducted at the HRH Princess Maha Chakri Sirindhorn Medical Center between 1st May 2020 - 31st December 2020. It was approved by Ethical Committee of Srinakharinwirot University.

In sample size calculation, a case-control study formula was used with alpha at 0.05 and beta at 0.2. We used postpartum blues as an exposure and late postpartum depressive symptoms group (LPPD group) as a case group. From previous study in Thailand, the incidence of late postpartum depressive symptoms was about 20% at 6 weeks postpartum[8], result in ratio of late postpartum depression group (LPPD) and non-late postpartum depressive group (non-LPPD group) at 0.2. Proportion of postpartum blues mother in LPPD group

from previous study in Japan was 50% and proportion of postpartum blues mother in non-LPPD group was 10%. After calculation, a total of 72 participants were required to identify association between postpartum blues and LPDS[6].

All eligible mothers who gave birth to single livebirth newborn between 1st May 2020 - 31st Dec 2020 at the HRH Princess Maha Chakri Sirindhorn Medical Center were given informed consent before participating in this study. At the time of the first questionnaire, evaluation of underlying psychological disorder, e.g., bipolar disorder and depressive disorder were carried out by history taking. Patients with psychological disorder were withdrawn from the study and were assigned for psychiatrist referral. Then the first self-administered questionnaire was offered at 2 days postpartum during hospitalization. The questionnaire inquired demographic data of participants including age, habitation (Bangkok and vicinity habitation or country habitation), religious, education, occupation, personal income, family income, dysmenorrhea, unplanned pregnancy(pregnancy at the time when no children were desired), family history of psychological disorders, length of hospital stay, birth body weight of newborn and newborn status after birth. In this study, we defined sick newborn status as neonatal intensive unit (NICU) admission. Then postpartum blues was evaluated by using Thai Edinburgh Postnatal Depressive Scale (Thai EPDS) with the cut-off score of 10 and more.

The second self-administered questionnaire was then completed at 6 weeks postpartum. Mothers were alone in an isolated room at the postpartum clinic, apart from family, baby and doctors. The questionnaire included postpartum and parenting information, e.g., support in newborn care, sleep deprivation (defined by total sleep time less than 6 hours of sleep per night as postpartum mother's average nighttime total sleep time was 6 hours during first 2 months[9]), breastmilk adequacy (subjectively by mothers) and evaluation of LPDS using Thai EPDS score of 10 or more.

The Edinburgh Postnatal Depressive Scale was developed in 1987 to detect postpartum depression because the standard screening tools for general population may not detected postpartum depression due to maternal adaptation and less concerned of symptoms. EPDS is 10-item self-reporting screening tools containing questions about many depressive symptoms. At cut-off point 12/13, it has 86% sensitivity, 78% specificity and a positive predictive value of 73% for postpartum depression[10]. EPDS is used worldwide and available in many languages such as French, Spanish, Dutch, Swedish, Chinese, Turkish, Arabic and Thai[11].

Thai Edinburgh Postnatal Depressive Scale (Thai EPDS) was developed compared with DSM-IV criteria in diagnosed postpartum depression. It has a degree of agreement at 0.38, accuracy at 0.93 and Cronbach's alpha coefficient of 0.80. At the cut-off score of 10, Thai EPDS has 88-90% specificity and 60-100% sensitivity in detection minor and/or major depression[12, 13].

Statistical analysis

Descriptive statistics were used to analyze dermographic data and characteristic of participants presented in percentage, mean ± standard deviation (SD), median and interquartile range.

Univariate analysis using chi-square test and independence t-test were performed to depict the differences between late postpartum depressive symptoms and non-depressed group for the following factors: age, parity, occupation, dysmenorrhea, unplanned pregnancy, habitation, route of delivery, second stage duration, length of hospital stay, low birth weight status of newborn, family income, family type, support in newborn care, sleep deprivation, breastfeeding status, breastmilk adequacy and postpartum blues. Factors with significant level at 0.3 after univariate analysis was then undergone colinearity tested then multiple logistic regression was performed. All statistical analysis was performed using SPSS vesion 26.

Results

Total participants of 171 mothers were enrolled. Forty-three were unable to follow-up at postpartum period and were excluded. No participant has ongoing psychological disorder. Consequently 128 mothers participated in this study. Demographic data of late postpartum depressive symptoms group and non-late postpartum depressive symptoms group are shown in

Table 1. Categorical data were demonstrated in frequency and percentages. All continuous data which had non-normal distribution were demonstrated in median and interquartile range.

The only risk factor which was significantly different between LPPD and non-LPPD group was postpartum blues. However, the proportion of women who had young age, unplanned pregnancy, low family income, low birth weight and long second stage of labor duration tended to have more late postpartum depressive symptoms.

Among 128 participants, thirty-one had postpartum blues at 2 days postpartum. Incidence of postpartum blues in our study was 24.2%. Incidence of late postpartum depressive symptoms was 15.6%.

Table 1. Participants characteristics and camparison between postpartum depression and non-postpartum depression group.

Variables	Total (n = 128)	LPPD group (n = 20)	Non-LPPD group (n = 108)	p value
Age (years)				0.467
18 - 25	43 (33.6%)	5 (25%)	38 (35.2%)	
26 - 35	67 (52.3%)	13 (65%)	54 (50%)	
More than 35	18 (14.1%)	2 (10%)	16 (14.8%)	
Habitation				0.130
Urban and suburban	83 (64.8%)	10 (50%)	73 (57%)	
country	45 (35.2%)	10 (50%)	35 (43%)	
Religious				0.525
Buddhist	107 (83.6%)	18 (90%)	89 (82.4%)	
Muslim	21 (16.4%)	2 (10%)	19 (7.6%)	
Education				0.348
Less than high school	19 (14.8%)	5 (25%)	14 (13%)	
High school to junior college	67 (52.3%)	10 (50%)	57 (52.8%)	
Bachelor or more	42 (32.8%)	5 (25%)	37 (34.2%)	
Planned pregnancy				0.094
Yes	79 (61.7%)	9 (45%)	38 (35.2%)	
No	49 (38.3%)	11 (55%)	70 (64.8%)	
Income (baht)				0.793
Less than 10,000	48 (61.7%)	8 (40%)	40 (37%)	
10,000 - 30,000	71 (55.3%)	10 (50%)	61 (56.5%)	
More than 30,000	9 (7%)	2 (10%)	7 (6.5%)	
Family Income (baht)				0.298
Less than 20,000	52 (40.6%)	5 (25%)	47 (43.6%)	
20,000 - 50,000	65 (50.8%)	13 (65%)	52 (48.1%)	
More than 50,000	11 (8.6%)	2 (10%)	9 (8.3%)	
Dysmenorrhea				0.647
Yes	70 (54.7%)	11 (55%)	48 (44.4%)	
No	58 (45.3%)	9 (45%)	60 (55.6%)	
Family history/ past history of psychological disorder				1.000
Yes	0	0	0	
No	128 (100%)	20 (100%)	108 (100%)	
Parity				0.456
Nulliparous	42 (32.8%)	8 (40%)	34 (31.5%)	
Multiparous	86 (67.2%)	12 (60%)	74 (68.5%)	
Second stage of labor duration (minutes)	0 (0,10)	0 (0,5)	1 (0,10.5)	0.181
Route of delivery				0.380
Normal vg delivery	54 (42.2%)	6 (30%)	48 (44.4%)	
Operative vg delivery	3 (2.3%)	1 (5%)	2 (1.9%)	
Cesarean section	71 (55.5%)	13 (65%)	58 (53.7%)	
Birth weight (grams)	3070 (2810, 3370)	3040 (2690, 3260)	3070 (2850, 3385)	0.322
Estimated blood loss after delivery (ml)	400 (200, 600)	375 (200, 500)	400 (200, 600)	0.690
Newborn status after birth				0.742
Well baby	107 (83.6%)	16 (80%)	91 (84.3%)	
Sick NB	21 (16.4%)	4 (20%)	17 (15.7%)	
Length of hospital stay (days)	4 (3,5)	4 (3.5,5)	4 (3,5)	0.158
Postpartum blues				< 0.01
Yes	31 (24.2%)	12 (60%)	19 (17.6%)	
No	97 (75.8%)	8 (40%)	89 (82.4%)	

LPPD: late postpartum depressive symptoms, NB: newborn

Risk factors for LPDS after univariate analysis are shown in Table 2. Risk factors of LPDS with p value less than 0.3 from univariate analysis were enrolled in multiple logistic regression. Those factors were sleep deprivation (odds ratio (OR) 1.71, 95% confidence interval CI 0.64-4.60), unplanned pregnancy (OR 2.25, 95%CI 0.86-5.91), isolated family type (OR 1.92, 95%CI 0.73-5.03), second stage of labor duration (OR 3.00, 95%CI 0.65-

13.78), length of hospital stay (OR 2.89, 95%CI 0.49-16.95), low birth weight newborn (OR 3.64, 95%CI 0.79-16.63), lack of support in newborn care (OR 6.86, 95%CI 2.46-19.18), breastmilk inadequacy (OR 7.13, 95%CI 2.39-21.26), operative delivery including operative vaginal delivery and cesarean section (OR 1.87, 95%CI 0.67-5.22) and Bangkok and vicinity habitation (OR 2.09, 95%CI 0.79-5.47).

Table 2. Possible risk factors of late postpartum depressive symptoms after univariate analysis.

Factors	Crude OR	95%CI	p value
Age (years)			
18 - 25	1.05	0.19 - 6.00	0.954
26 - 35	1.93	0.40 - 9.44	0.419
More than 35	1		
Habitation			
Bangkok and vicinity	2.09	0.79 - 5.47	0.135
Country	1		
Religious			
Buddhist	1		
Muslim	0.52	0.11 – 2.43	0.407
Family type			
Isolated	1.92	0.734 – 5.027	0.184
Extended	1		
Education			
Less than high school	2.64	0.66 – 10.55	0.169
High school to junior college	1.30	0.41 – 4.10	0.657
Bachelor or more	1		
Employment			
Unemployed	1.11	0.37 – 3.35	0.858
Employed	1		
Income (baht)			
Less than 10,000	0.70	0.12 – 4.01	0.689
10,000 – 30,000	0.57	0.10 – 3.17	0.524
More than 30,000	1		
Family Income (baht)			
Less than 20,000	0.48	0.08 – 2.86	0.419
20,000 – 50,000	1.13	0.22 – 5.85	0.889
More than 50,000	1		
Planned pregnancy			
Yes	1		
No	2.25	0.86 – 5.91	0.099
Marital status			
Married	1		
Divorced	1.37	0.15 – 12.92	0.784
Parity			
Nulliparous	1.45	0.54 – 3.88	0.458
Multiparous	1		
Second stage of labor duration			
Less than 10 min	1		
10 min and more	3.00	0.65 – 13.78	0.158
Route of delivery			
Normal vaginal delivery	1		
Operative vaginal delivery and Cesarean section	1.87	0.67 - 5.22	0.234
Birth body weight			
Less than 2500 g	3.64	0.79 – 16.63	0.096
2500 g and more	1		
Estimated blood loss			
Less than 500 ml	1		
500 - 1000 ml	0.75	0.26 – 2.16	0.594
More than 1000 ml	1.43	0.26 – 7.74	0.679
Breastmilk adequacy			
Yes	1		
No	7.13	2.39 - 21.26	< 0.01
Adequate support in newborn care			
Yes	1		
No	6.86	2.46 – 19.18	< 0.01
Sleep deprivation (less than 6 hours of sleep per night)			
Yes	1.71	0.64 – 4.60	0.287
No	1		
Length of hospital stay			
≤ 5 days	1		
> 5 days	2.89	0.49 – 16.95	0.240
Postpartum blues			
Yes	7.03	2.53 - 19.54	< 0.01
No	1		

OR: odds ratio, CI: confidence interval

Table 3 shows risk factors of late postpartum depressive symptoms after multiple logistic regression. Significant risk factors of late postpartum depressive symptoms, after adjusted by sleep deprivation, family income, unplanned pregnancy, isolated family type, second stage duration, length of hospital stay, and low birth weight newborn, were postpartum blues (adjusted odds ratio (aOR) 7.156, 95%CI 1.87-27.39), lack of support in newborn care (aOR 4.479, 95%CI 1.31 -15.33), breastmilk inadequacy (aOR 8.620, 95%CI 2.33-31.84) and Bangkok and vicinity habitation (aOR 4.989, 95%CI 0.05-0.775).

Table 3. Risk factors of late postpartum depressive symptoms after multiple logistic regression.

Factors	Crude OR	95%CI	p value	Adjusted OR	95%CI	p value
Postpartum blues	7.03	2.53 - 19.54	< 0.01	7.156	1.87-27.39	< 0.01
Lack of support in newborn care	6.86	2.46 – 19.18	< 0.01	4.479	1.31–15.33	0.017
Breastmilk inadequacy	7.13	2.39 - 21.26	< 0.01	8.620	2.33-31.84	< 0.01
Operative delivery (including operative vaginal delivery and cesarean section)	1.87	0.67 - 5.22	0.234	3.244	0.84-12.54	0.088
Bangkok and vicinity habitation	2.09	0.79-5.47	0.135	4.99	1.29-19.28	0.02

* Adjusted Odds ratio by sleep deprivation, family income, unplanned pregnancy, isolated family type, second stage duration, length of hospital stay and low birth weight newborn. OR: odds ratio, CI: confidence interval

Discussion

The incidence of LPDS in our study was 15.6% and many possible factors of late postpartum depressive symptoms were studied. After multiple logistic regression, significant risk factors of late postpartum depressive symptoms were postpartum blues (OR 7.156, 95%CI 1.87-27.39), lack of support in newborn care (OR 4.479, 95%CI 1.31-15.33), breastmilk inadequacy (OR 8.620, 95%CI 2.33-31.84) and urban habitation (OR 4.989, 95%CI 0.05-0.775).

The incidence of postpartum blues and late postpartum depressive symptoms are usually underestimated due to lack of detection, unawareness and self-limited nature. Evidences from recent studies have supported that postpartum blues is associated with postpartum depression. Maternal depression has consequences that may affect the whole family including impaired maternal-child interactions, unhealthy interaction between couples and disrupt relationship of whole family members[6, 7].

In this study, the incidence of postpartum blues was 24.2%, while the incidence of LPDS was 15.6%. As mentioned earlier that postpartum blues was a significant risk factor of LPDS, participants who experienced postpartum blues early during hospitalization were prone to develop depressive symptoms up to 7 folds greater than those without postpartum blues. Other significant risk factors identified in this study were lack of support in newborn care, breastmilk inadequacy and habitation. Inadequate support in newborn care from other family members resulted in maternal exhaustion and led to late postpartum depressive symptoms. Breastmilk inadequacy, feeling of dissatisfaction in amount of breastmilk, posed a higher risk for late postpartum depressive symptoms. Participants who live in Bangkok and vicinity, had higher risk in developing late postpartum depressive symptoms. On the other hand, operative delivery doesn't posed a higher risk for late postpartum depressive symptoms.

Postpartum blues incidences are different among countries, reaching as high as 50% in many countries[10, 11]. In Thailand postpartum blues are around 45.7 - 51.8%[12, 13], which is much higher than the incidence in this study. One study included only postpartum mother with newborn admission in NICU care which might be the causative effect of higher blues incidence[13]. Another was a multicentered study conducted in 4 hospitals located in Bangkok and nearby province which may have different setting of socioeconimic background of participants from our study[12].

Incidence of LPDS is lower than postpartum blue, ranging from 3.5-63.3%, depending on characteristics of the population. Some population produce higher risk of postpartum depression e.g. socioeconomic, cultural

influences and social construction[4, 14]. However, studies in Thailand showed the incidence of postpartum depression ranging from 8.4% to 16.8% in which consistent with our study[8, 13].

This study showed that postpartum blues was associated with LPDS after adjusted with multiple logistic regression. It was consistent with a study conducted in Japan which showed odd ratios of 6.17[6]. Another significant risk factor was breastmilk inadequacy. Evidence from multiple studies demonstrated that mothers without breastfeeding or having difficulties in breastfeeding were at risk for postpartum depression[4, 5, 13]. Furthermore, lack of support in newborn care and urban habitation posed higher risk of LPDS. This was relevant with several previous studies. Bangkok and vicinity habitation may promote LPDS because each individual family might be less involved to one another and having more separated lifestyle[4]. While newborn care was a great deal of motherhood, lack of support from other family members may induced a feeling of overwhelming responsibility and guilt to many mothers[4, 5, 10, 15].

In this study, no participant were drop out due to emerging severe depression that need earlier intervention prior to 6 week postpartum visit. All women with LPDS detected in this study were refered to psychiatry clinics for proper intervention. Twenty mothers that had significant depressive symptoms by screening tools were refered to psychiatrist and four of them had major depressive disorder (all had history of postpartum blues at 2 days postpartum). Additionally, three mothers were diagnosed with adjustment disorder, six mothers were assigned to follow-up without certain diagnosis and unfortunately seven mothers were lost to follow-up.

The strength of this research was this being the first case-control study in Thailand to identify the association between postpartum blues and LPDS. Identification of postpartum blues can help in early detection of postpartum depression, promoting the development of standard screening programme, follow-up protocols and provide interventions for psychological support.

Limitation of this study was that documentation of postpartum blues and late postpartum depressive symptoms were taken place at a single point of time. Extended timing of study can provide precise magnitude ef problems. Furthermore, calculation for sample size was aimed to defined the association between postpartum blues and late postpartum depressive symptoms as a primary objective. More sample size may be needed to identify precise magnitude effect and significance of other risk factors. Also, this study was a single-centered study. This may limit the generalization, thus multicentered study is suggested.

Conclusion

Postpartum blues were highly associated with late postpartum depressive symptoms and being an early prediction for postpartum depression. Early detection of postpartum blues during postpartum hospitalization is useful to identify patient at risk of developing late postpartum depressive symptoms.

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Potential conflicts of interest

The authors declare no conflicts of interest.

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OBSTETRICS

The Incidence and Associated Factors of Perineal Wound Infection Following Vaginal Delivery in Charoenkrung Pracharak Hospital, Bangkok, Thailand

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ABSTRACT

Objectives: To measure the incidence and assess the associated factors for perineal wound infection and dehiscence following vaginal delivery.

Materials and Methods: This retrospective study was conducted between October 2018 and September 2020 in Charoenkrung Pracharak Hospital. The incidence of perineal wound infection and dehiscence was calculated. There were 4,015 delivered vaginally. A total of 2,589 pregnant women were enrolled. The data collection tools included postpartum ward daybook, electronic patient records, and clinical data of perineal wound examination at first 72 hours after delivered was reviewed. The association factors were analyzed by logistic regression.

Results: The incidence of perineal wound morbidity was 2.9% (1.7% of wound infection and 1.2% of wound dehiscence). Gestational hypertension (adjusted odds ratio (aOR) 3.77, 95% confidence interval (CI) 1.28-11.12), number of vaginal examinations > 4 (aOR 4.21, 95%CI 2.29-7.73), neonatal birth weight ≥ 3 kg (aOR 4.28, 95%CI 1.89-9.7) and registered nurse with less than 5 years of experience (cOR 3.04, 95 % CI 1.46-6.35) increased the risk of wound infection. Prophylactic antibiotic reduced the risk of perineal wound infection (aOR 0.29, 95%CI 0.1-0.82). There was no significantly associated risk factor for perineal wound dehiscence.

Conclusion: Perineal wound morbidity was found to be 2.9% with 1.7% of perineal infection and 1.2% of perineal dehiscence. The number of vaginal examinations > 4, neonatal birth weight ≥ 3,000 grams, gestational hypertension, and healthcare providers with experience < 5 years increased the risk of perineal wound infection. Prophylactic antibiotics reduced the incidence of infected perineal wounds.

Keywords: perineal wound, infection, dehiscence.

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อุบัติการณ์ และปัจจัยที่มีความสัมพันธ์กับการติดเชื้อแผลฝีเย็บหลังคลอดในหญิงตั้งครรภ์ที่คลอดบุตรทางช่องคลอดในโรงพยาบาลเจริญกรุงประชารักษ์

นันทวัฒน์ ทองทิพย์, อังสุมาลิน ศรีหล้า, จิรพร เหลืองเมตตากุล

บทคัดย่อ

วัตถุประสงค์: *เพื่อศึกษาอุบัติการณ์และปัจจัยที่มีความสัมพันธ์กับการติดเชื้อแผลฝีเย็บและการเกิดแผลฝีเย็บแยกหลังคลอดในหญิงตั้งครรภ์ที่คลอดบุตรทางช่องคลอด*

วัสดุและวิธีการ: *การศึกษาย้อนหลังในช่วงเดือนตุลาคม พ.ศ.2561 จนถึงเดือนกันยายน พ.ศ.2563 โดยศึกษาอุบัติการณ์แผลฝีเย็บติดเชื้อ และแผลแยกในหญิงตั้งครรภ์ซึ่งคลอดบุตรทางช่องคลอดที่โรงพยาบาลเจริญกรุง-ประชารักษ์ ทั้งหมด 4,015 คน โดยสุ่มกลุ่มตัวอย่างจำนวน 2,589 คน ซึ่งคลอดบุตรทางช่องคลอดและเย็บแผลฝีเย็บ โดยเก็บข้อมูลย้อนหลังจากสมุดตรวจแผลประจำวันและเวชระเบียน ซึ่งตรวจที่ 72 ชั่วโมงแรก หลังคลอด และนำผลลัพธ์มาเพื่อศึกษาอุบัติการณ์ และวิเคราะห์ปัจจัยที่มีความสัมพันธ์กับการติดเชื้อ และการแยกของแผลฝีเย็บหลังคลอด*

ผลการศึกษา: *การศึกษาพบว่าเกิดผลแทรกซ้อนของแผลฝีเย็บทั้งหมด ร้อยละ 2.9 โดยเป็นแผลติดเชื้อ ร้อยละ 1.7 และแผลแยก ร้อยละ 1.2 จากการศึกษาพบว่าภาวะความดันโลหิตสูงหลังการตั้งครรภ์ (adjusted odds ratio (aOR) 3.77, 95% confidence interval (CI) 1.28-11.12) จำนวนการตรวจภายในมากกว่า 4 ครั้ง (aOR 4.21, 95%CI 2.29-7.73) น้ำหนักทารกแรกเกิด ≥ 3 กิโลกรัม (aOR 4.28, 95%CI 1.89-9.7) และพยาบาลวิชาชีพที่มีประสบการณ์การทำคลอด < 5 ปี (cOR 3.04, 95 % CI 1.46-6.35) เป็นปัจจัยที่เพิ่มการเกิดแผลฝีเย็บติดเชื้อ และพบว่าการใช้ยาปฏิชีวนะเพื่อป้องกันการติดเชื้อเป็นปัจจัยที่ลดการเกิดแผลฝีเย็บติดเชื้อ (aOR 0.29, 95%CI 0.1-0.82) สำหรับแผลฝีเย็บแยกนั้นพบว่าไม่มีปัจจัยที่มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติ*

สรุป: *การศึกษาพบว่าอุบัติการณ์เกิดผลแทรกซ้อนของแผลฝีเย็บทั้งหมด ร้อยละ 2.9 โดยอุบัติการณ์ของแผลฝีเย็บติดเชื้อ ร้อยละ 1.7 และอุบัติการณ์ของแผลฝีเย็บแยก ร้อยละ 1.2 โดยปัจจัยที่มีความสัมพันธ์กับการเกิดแผลฝีเย็บติดเชื้ออย่างมีนัยสำคัญทางสถิติ คือ ภาวะความดันสูงจากการตั้งครรภ์ จำนวนการตรวจภายใน > 4 ครั้ง น้ำหนักทารกแรกเกิด ≥ 3 กิโลกรัม และประสบการณ์ผู้ทำคลอด < 5 ปี ส่วนการใช้ยาปฏิชีวนะสามารถป้องกันการติดเชื้อแผลฝีเย็บได้อย่างมีนัยสำคัญทางสถิติ*

คำสำคัญ: *แผลฝีเย็บ, ติดเชื้อ, แผลแยก*

Introduction

Perineal wound infection and dehiscence usually occur in the first 72 hours-14 days of delivery and are associated with physical, mental, and future sexual problems to postpartum mothers. The perineal wound also causes pain, incontinence, and dyspareunia owing to inflammation[1]. The key factors causing the infected perineal wound are: (1) maternal factors associated with diseases, diabetes, primiparity, advanced maternal age, weight, education, and ethnicity, (2) fetal factor: baby birth weight (> 4000 g), and (3) delivery factors: operative vaginal delivery, perineal incision episiotomy, and degree of perineal tear. Buppasiri et al compared spontaneous laceration, restrictive episiotomy, and routine episiotomy and found that episiotomy could reduce the severity of the perineal wound and its complications since third and fourth-degree perineal lacerations were associated with an elevated perineal wound infection risk[2]. Other factors linked to perineal wound infection include postpartum hemorrhage. In addition, the risk of infection and antibiotic exposure could be increased during second stage labor[3-5].

In obstetrics, the perineal repair is considered as a crucial surgical procedure, anapproximately 85% of pregnant women with vaginal delivery had perineal injuries, including spontaneous laceration and episiotomy[6, 7]. Perineal lacerations cause a variety of problems for postpartum mothers[8]; the most common risk of perineal wound is the infection of the perineal wound[3]. A study conducted by a systematic review revealed that perineal wound infection ranged from 0.1 to 23.6%, and perineal wound dehiscence was from 0.21% to 24.6%[9], based on the regional and ethnic differences. Perineal wound infection ranged from 0.2% to 3.96% in Thailand by some studies[10-12]. However, some studies found that wound infection was as high as 20.7% in Bangkok[3].

The Charoenkrung Pracharak Hospital is a 464-bed advanced tertiary hospital in Bangkok. In 2019, the hospital received a transfer from other hospitals to provide maternity services, and the hospital recorded a total of 3,053 births with 1,930 vaginal deliveries in the same year. The records were kept in the hospital database, comprised of incidents and related factors to avert difficulties and after-birth impacts on postpartum mothers. Typically, the delivery has an impact on mental health, customers' satisfaction, service quality, hospital revenue loss, and costs of maternity care after childbirth, especially increased hospital costs and longer hospital stays.

The present study aimed to investigate the incidence of perineal wound infection and dehiscence and to determine the factors associated with postpartum perineal wound infection.

Materials and Methods

This retrospective cross-sectional study was conducted between October 2018 and September 2020 in Charoenkrung Pracharak Hospital, Bangkok, Thailand. The study protocol was approved by the Bangkok Metropolitan Administration Human Research Ethics Committee and conducted to investigate the health status of pregnant women delivered in Charoenkrung Pracharak Hospital. The sample size was calculated based on available literature. Kiennukul et al identified a 3.96% incidence of perineal wound infection, using a 95% confidence level and 10% data loss. A total of 2,589 vaginal deliveries with perineal wound repair were included from sample size calculation. Excluded criteria were pregnant women who had vaginal deliveries with no perineal tear, those with gestational age < 28 weeks, birth weight < 1,000 grams, and those with incomplete medical records.

The evidence of perineal wound morbidity was identified in (1) the postpartum ward daybook, recording the perineal wound examination at 24, 48, and 72 hours after delivery and in (2) electronic patient records. According to Jones et al, the perineal wound infection was examined from the first 24 hours to 28 days after childbirth, with the highest infectious inflammation in the first 72 hours[9, 16-18]. Thus, our hospital follows this guideline, requiring the doctors and nurses to assess perineal wounds at 24, 48, and 72 hours after birth. Therefore, we used the perineal wound examination at the first 72 hours after delivery to identify the perineal wound morbidity.

Perineal wound infections were diagnosed in our hospital following the presence of purulent discharge and positive bacterial culture. We defined perineal wound dehiscence as a gap of > 1 cm between wound edges. In this study, the diagnosis of the perineal wound was defined following the Center for Disease Control (CDC) guidelines, as either the presence of purulent discharge or a wound abscess (pain, swelling, redness)[13]. The perineal wound dehiscence was defined as a gap of > 1 cm between wound edges[14, 15]. Perineal wound morbidity was defined as perineal wound infection and dehiscence.

We collected the demographic data of patients, potential associated factors resulting in perineal morbidity in the antepartum and intrapartum period, the degree of perineal wound tear, and the health care providers who repaired perineal wounds in labor. We also collected data related to prophylactic antibiotics used by the patients. The prophylactic antibiotic was defined as the administration of antibiotics after vaginal delivery with the perineal tear, administered to prevent infection. In our hospital, we administered prophylactic antibiotics following the third- or a fourth-degree perineal tear, after operative vaginal deliveries, severe perineal trauma, prolonged procedure (more than 1 hour), obese pregnancy, vaginally delivered by a medical student, and postpartum hemorrhage. The regimen of prophylactic antibiotic comprised of single-dose second-generation cephalosporin intravenously (cefoxitin 1 g intravenously, or clindamycin 900 mg intravenously if allergic to penicillin) for third- or fourth-degree tear, and the oral regimen: clindamycin or amoxicillin for other indications. Perineal trauma: injuries to the vagina, its management, and microbiology results were recorded in the postpartum daybook.

In this study, the primary outcome was the incidence of perineal wound infection. The secondary outcomes were the incidence of perineal wound dehiscence and the associated risk factors of perineal wound morbidity. Descriptive statistics were used to summarize the data in a manageable form in frequencies, percentages, mean, and standard deviation using IBM SPSS version 26. The regression analysis was used to analyze the associated risk factors with estimated odds ratios (OR) and 95% confidence intervals (CI). A p values < 0.05 was considered statistically significant level. Multiple logistic regression analysis was used to select the associated factors that were statistically significant and to select category variables significant level < 0.2.

Results

Between October 2018 and September 2020, a total of 6,353 pregnant women had childbirth deliveries at Charoenkrung Pracharak Hospital. Of 6,353 pregnant women, a total of 2,338 were cesarean delivery (36.8%) and 4,015 were vaginal delivery (63.2%). We excluded 133 (3.3%) pregnant women who had undergone vaginal delivery without a perineal wound. From those who had undergone vaginal delivery with perineal wound repair, we collected the pregnant women who met the inclusion and no exclusion criteria from the medical record chart consecutively until we collected 2,589 women according to sample size calculation. Of these, episiotomy was performed in 2,219 (85.7%) women. From the group of all women with perineal wound repair, 75 of 2,589 women (2.9%) had perineal wound morbidity, 44 of 2,589 women (1.7%) had perineal wound infection, and 31 of 2,589 women (1.2%) had perineal wound dehiscence.

As revealed in Table 1, the average age of pregnant women was 26.96 ± 6.37 years. We found the greatest number of perineal wound morbidity in women aged 20-34 years old. Nulliparous pregnancy accounted for 17.3% of perineal wound morbidity, to be similar to the incidence in multiparous pregnancy. Thai pregnant women made up the majority of the population in this study and comprised a higher number of women with perineal wound morbidity (77.3% vs 22.7% of non-Thai ethnicity). However, the incidence of perineal wound morbidity increased in non-Thai ethnicity. Most of the pregnant women were secondary school graduation, and the incidence of perineal wound morbidity was highest in women with under primary school education. The mean BMI was 20.94 ± 2.96 kg/m^2, and this study showed the highest number of perineal wound morbidity was reported in the women with normal BMI (82.7%). The average infant weight

was 3,054.24 ± 419.42 grams, and the greatest number of perineal wound morbidity was found in women who had a neonatal birth weight between 3,000 and 3,499 grams.

Table 1. Characteristics and incidence of perineal wound morbidity.

Characteristics (mean ± SD)	No morbidity n (%)	Morbidity n (%) n = 75 (2.9)	Incidence of morbidity	Infection n (%) n = 44 (1.7)	Dehiscence n (%) n = 31 (1.2)
Age (years)					
(26.96 ± 6.37)					
< 20	309 (12.4)	6 (8)	1.9	2 (4.5)	4 (12.9)
20 - 34	1826 (72.6)	61 (81.4)	3.3	37 (84)	24 (77.4)
≥ 35	379 (15)	8 (10.6)	2.1	5 (11.5)	3 (9.7)
Parity					
Primipara	424 (16.9)	13 (17.3)	3.0	8 (18.2)	5 (16.1)
Multipara	2090 (83.1)	62 (82.7)	2.9	36 (81.8)	26 (83.9)
Ethnicity					
Thai	2101 (83.6)	58 (77.3)	2.7	35 (79.5)	23 (74.2)
Non-Thai	413 (16.4)	17 (22.7)	4.1	9 (20.5)	8 (25.8)
Education					
Under primary	134 (5.3)	9 (12)	6.7	5 (11.4)	4 (12.9)
Primary	473 (18.8)	17 (22.7)	3.5	6 (13.6)	11 (35.5)
Secondary	1503 (59.8)	37 (49.3)	2.4	25 (56.8)	12 (38.7)
≥ Bachelor	404 (16.1)	12 (16)	2.9	8 (18.2)	4 (12.9)
BMI (kg/m^2)					
(20.94 ± 2.96)					
< 18.5	221 (8.8)	5 (6.7)	2.2	1 (2.3)	4 (12.9)
18.5 - 24.9	2037 (81)	62 (82.6)	3.0	37 (84.1)	25 (80.7)
25 - 29.9	204 (8.1)	6 (8)	2.9	5 (11.4)	1 (3.2)
≥ 30	52 (2.1)	2 (2.7)	3.8	1 (2.2)	1 (3.2)
Baby birthweight (grams)					
(3,054.24 ± 419.42)					
< 2,500	203 (8.1)	4 (5.3)	1.9	1 (2.3)	3 (9.7)
2,500 - 2,999	893 (35.5)	16 (21.3)	1.7	6 (13.6)	10 (32.3)
3,000 - 3,499	1078 (42.9)	44 (58.7)	4.0	29 (65.9)	15 (48.3)
3,500 - 3,999	311 (12.3)	9 (12)	2.8	7 (15.9)	2 (6.5)
≥ 4,000	29 (1.2)	2 (2.7)	6.8	1 (2.2)	1 (3.2)

SD: standard deviation, BMI: body mass index

Table 2 displays the univariate regression analysis for the associated factors for perineal wound infection and perineal wound dehiscence. We found significantly higher incidence of perineal wound infection in pregnant women with the number of vaginal examinations were more than 4 times (OR 3.78, 95%CI 2.08 - 6.88), the experience of provider < 5 years (OR 3.04, 95%CI 1.46 - 6.35), neonatal birthweight ≥ 3,000 gm (OR 4.08, 95%CI 1.81 - 9.19). In contrast, prophylactic antibiotics reduced the incidence of infected perineal wounds (OR 0.37, 95%CI 0.13-1.05).

Our study demonstrated significantly increased risk of perineal wound dehiscence in the experience of the provider less than 5 years (OR 5.6, 95%CI 2.25

-13.96), pregnancy who graduated primary school (OR 2.92, 95%CI 1.28-6.67), and pregnant women who not graduated primary school (OR 3.66, 95%CI 1.17-11.51).

Table 2. Univariate regression analysis for associated risk factors for perineal wound infection and perineal wound dehiscence.

	(n = 2589) n (%)	Infection n (%) n = 44	Crude OR (95%CI)	Dehiscence n (%) n = 31	Crude OR (95%CI)
Age (years)					
< 20	315 (12.2)	2 (4.5)	0.31 (0.77-1.33)	4 (12.9)	0.99 (0.34-2.89)
20 - 34	1887 (72.9)	37 (84.1)	1	24 (77.4)	1
≥ 35	387 (14.9)	5 (11.4)	0.65 (0.25-1.67)	3 (9.7)	0.60 (0.18-2.02)
Ethnicity					
Non-Thai	430 (16.6)	9 (20.5)	1.29 (0.69-2.719)	8 (26.8)	1.76 (0.78-3.96)
Education					
Under primary	143 (5.5)	5 (11.4)	2.2 (0.83-5.83)	4 (12.9)	3.66 (1.17-11.51)
primary	490 (18.9)	6 (13.6)	0.75 (0.31-1.84)	11 (35.5)	2.92 (1.28-6.67)
secondary	1540 (59.5)	25 (56.8)	1	12 (38.7)	1
≥ bachelor	416 (16.1)	8 (18.2)	1.19 (0.54-2.67)	4 (12.9)	1.24 (0.4-3.87)
Associated disease					
Diabetes mellitus	245 (9.5)	3 (6.8)	0.7 (0.21-2.27)	2 (6.5)	0.66 (0.16-2.77)
Hypertension	207 (8)	5 (11.4)	1.49 (0.58-3.82	3 (9.7)	1.24 (0.37-4.1)
Gestational HT	93 (3.6)	4 (9.1)	2.76 (0.97-7.88)	1 (3.2)	0.89 (0.12-6.62)
Pre-eclampsia	73 (2.8)	1 (2.3)	0.8 (0.11-5.88)	2 (6.5)	2.42 (0.56-10.32)
Anemia	101 (3.9)	2 (4.5)	1.18 (0.28-4.93)	-	-
Substance abuse (amphetamine)	44 (1.7)	-	-	2 (6.5)	4 (0.92-17.31)
Meconium stain	308 (11.9)	5 (11.4)	0.95 (0.37-2.43)	6 (19.4)	1.79 (0.73-4.41)
Vaginal examination > 4 times	595 (23)	23 (52.3)	3.78 (2.08-6.88)	9 (29)	1.38 (0.63-3)
Operative delivery	55 (2.1)	2 (4.5)	2.24 (0.53-9.49	-	-
Episiotomy 3rd/ 4th degree tear	2220 (85.7)	41 (90.9)	1.67 (0.6-4.71)	28 (90.3)	1.56 (0.41-5.15)
3rd/ 4th degree tear	43 (1.7)	-	-	-	-
2nd stage					
< 30min	2084 (90.4)	33 (75)	1	25 (80.6)	1
≥ 30min	505 (19.5)	11 (25)	1.38 (0.7-2.76)	6 (19.4)	0.99 (0.4-2.43)
PPH					
< 500	2404 (92.9)	39 (88.6)	1	31 (100)	-
≥ 500	185 (7.1)	5 (11.4)	1.68 (0.66-4.33)	-	-
Prophylactic antibiotic	543 (21)	4 (9.1)	0.37 (0.13-1.05)	2 (6.5)	0.26 (0.06-1.08)
Baby birthweight					
< 3,000	1116 (43.1)	7 (15.9)	1	13 (41.9)	1
≥ 3,000	1473 (56.9)	37 (84.1)	4.08 (1.81-9.19)	18 (58.1)	1.05 (0.51-2.15)
Provider					
RN < 5 yr	703 (27.1)	21 (47.7)	3.04 (1.46-6.35)	21 (67.6)	5.6 (2.25-13.96)
RN ≥ 5 yr	1098 (42.4)	11 (25)	1	6 (19.4)	1
Nurse student	366 (14.1)	9 (20.5)	2.49 (1.02-6.06)	2 (6.5)	1 (0.2-4.98)
Medical student	219 (8.5)	-	-	2 (6.5)	1.68 (0.34-8.37)
Resident	103 (4)	3 (6.8)	2.97 (0.81-10.8)	-	-
Attending OB/GYN	100 (3.9)	-	-	-	-

OR: odd ratio, CI: confidence interval, HT: hypertension, PPH: postpartum hemorrhage, RN: registered nurse.

Table 3 shows the multivariate regression analysis of the associated risk factors for perineal wound infection. We found that gestational hypertension (adjusted odds ratio (aOR) 3.77, 95%CI 1.28-11.12) and

number of vaginal examinations more than 4 (aOR 4.21, 95%CI 2.29-7.73) and neonatal birth weight ≥ 3,000 grams (aOR 4.28, 95%CI 1.89-9.7) increased the risk of perineal wound infection. Similarly, using prophylactic antibiotics significantly reduced the risk of infected perineal wounds (aOR 0.29, 95%CI 0.1-0.82).

Table 3. Multivariate regression analysis of the associated risk factors for perineal wound infection.

	(n = 2589) n (%)	Infection n (%) n = 44	Crude OR (95%CI)	Adjusted OR (95%CI)	p value
Gestational HT	93 (3.6)	4 (9.1)	2.76 (0.97-7.88)	3.77 (1.28-11.12)	0.023
Vaginal examination > 4 times	595 (23)	23 (52.3)	3.78 (2.08-6.88)	4.21 (2.29-7.73)	< 0.001
Prophylactic ATB	543 (21)	4 (9.1)	0.37 (0.13-1.05)	0.29 (0.1-0.82)	0.019
Baby Birthweight					< 0.001
< 3,000	1116 (43.1)	7 (15.9)	1	1	
≥ 3,000	1473 (56.9)	37 (84.1)	4.08 (1.81-9.19)	4.28 (1.89-9.7)	
Provider					0.097
RN < 5yr	703 (27.1)	21 (47.7)	3.04 (1.46-6.35)	2.66 (1.26-5.6)	
RN ≥ 5yr	1098 (42.4)	11 (25)	1	1	
Nurse student	366 (14.1)	9 (20.5)	2.49 (1.02-6.06)	2.45 (0.99-6.03)	
Medical student	219 (8.5)	-	-	-	
Resident	103 (4)	3 (6.8)	2.97 (0.81-10.8)	3.44 (0.8-14.82)	
Attending OB/GYN	100 (3.9)	-	-	-	

OR: odd ratio, CI: confidence interval, HT: hypertension, ATB: antibiotic, RN: registered nurse, OB: obstetric, GYN: gynecology.

Discussion

A review of the previous studies, the incidence of perineal wound morbidity depended on the ethnicity and region of the country. Worldwide, the incidence of perineal wound infection had been 0.1% to 23.6%, and perineal wound dehiscence had been 0.21% to 24.6%[9]. In Thailand, the incidence of perineal wound infection ranged from 0.2 to 3.96%[10-12]. The incidence reported in this study was consistent with the previous studies, in which incidence of perineal wound morbidity was 2.9%, 1.7% was perineal wound infection and 1.2% was perineal wound dehiscence.

Similarly, from previous studies, the number of vaginal examinations, neonatal birthweight, prophylactic antibiotics, and gestational hypertension were the risk factors associated with the high incidence of perineal wound morbidity. The study of Nell[16] found the frequent vaginal examination ≥ 5 increased the risk of the infected perineal wound, which was consistent with present study of which the number of vaginal examinations > 4 significantly increased the risk of perineal wound infection[16]. Frequent vaginal examinations may introduce infection during the time of delivery[17], we considered studying the aseptic technique and indication of vaginal examination in the future.

In this study, the risk of developing perineal wound infection to be associated with neonatal birthweight ≥ 3,000 grams, which was consistent with previous studies that also found an association between neonatal birthweight greater than 3,500 grams and perineal wound infection[18-20]. Because of the physical structure of southeast Asian women, the neonatal birth weight ≥ 3,000 grams might cause an extended perineal tear and result in perineal wound infection.

Moreover, health care providers (registered nurse, nurse student, medical student, and resident of OB-GYN) who had experience < 5 years were associated with perineal wound infection. In the same way, previous study discovered by midwives had an increased perineal wound infection risk[21]. Our study found that there was no significant difference in the incidence of perineal wound infection between registered nurses with more than 5 years of experience and attending staff. In the other way, registered nurses with less than 5 years of experience had significantly

higher incidence of perineal wound infection than registered nurses with more than 5 years' experience.

Although no statistically significant correlation factor was found for perineal wound dehiscence, the study discovered that non-Thai pregnant women who might not understand Thai or English language and pregnant women who did not graduate from secondary school had a high incidence of perineal dehiscence which was consistent with previous study conducted in Thailand[3]. Difficulties in communication, which hindered teaching of postpartum perineal wound care were associated with these problems.

In this study, we demonstrated that prophylactic antibiotics significantly reduced the risk of perineal wound morbidity. However, there is no sufficient evidence to assess the clinical benefits or harms of routine antibiotic prophylaxis for episiotomy repair after vaginal delivery. Knight et al[22] also discovered that prophylactic antibiotics reduced the incidence of perineal wound infection in operative vaginal delivery. Moreover, previous studies found that prophylactic antibiotics also reduced infection in the first-degree and second-degree perineal tear, however, no sufficient evidence to support this assertion[22-24]. The present study discovered no association between perineal wound infection and the performance of operative vaginal delivery, third or fourth-degree perineal tear, and postpartum hemorrhage, which was inconsistent with other previous studies[14, 18, 19]. In contrast, our study found that prophylactic antibiotics reduced the risk of perineal wound infection in the second-degree tear. However, there was no sufficient evidence to conclude the correlation between prophylactic antibiotics and associated risk factors.

This study faces some limitations because it was a single center and retrospective study, limited in terms of data diversity, and insufficient medical record data. Furthermore, we only explored medical record in the first 72 hours of hospitalization, so the incidence of perineal wound infection might be lower than it appeared. The strength of our study was that we used the definition of perineal wound infection from the CDC. In addition, the perineal wound examination in this study was only performed by physicians and registered nurses. The benefits of antibiotic prophylaxis for second-degree perineal tear and other risks are to be determined by the future study.

Conclusion

Perineal wound morbidity was found to be 2.9% with 1.7% perineal infection and 1.2% perineal dehiscence. The number of vaginal examinations > 4, neonatal birth weight ≥ 3,000 grams, gestational hypertension, healthcare providers with experience < 5 years increased the risk of perineal wound infection. Prophylactic antibiotics reduced the incidence of infected perineal wounds.

Potential conflicts of interest

The authors declare no conflicts of interest.

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CASE REPORT

Dermoid Cyst in an Accessory Ovary: A case report

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ABSTRACT

Accessory ovaries are rare in incidence and tumour arising from these ovaries is extremely rare. We reported incidental finding of dermoid cyst during caesarean section in a 32-year-old, gravida 4, para 3-0-0-3 for fetal distress. Resection of the cyst of accessory ovary done and histological evaluation confirmed diagnosis of mature cystic teratoma or dermoid cyst.

Keywords: accessory ovary, supernumerary ovary, dermoid cyst.

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Introduction

Accessory ovaries are rare gynaecological conditions with reported incidence of 1:29,000 - 700,000 cases[1]. Tumour arising in accessory ovaries are extremely rare. The case reported here is unique in that a benign cystic teratoma (dermoid cyst) in an accessory ovary was inadvertently discovered at the time of emergency lower segment caesarean section done for acute fetal distress.

Case Report

A 32-year-old, gravida 4, para 3-0-0-3 at 37 weeks gestation underwent emergency lower segment caesarean section (EMLSCS) for acute fetal distress, however intra-operatively we noted the presence of left ovarian cyst measured 5 cm x 5 cm which was not noticed in all ultrasonography done in antenatal period. The cyst surface was smooth with the appearance of hair and sebum which clinically suggestive of dermoid cyst. Upon thorough exploration, we revealed one normal ovary connected to the same ovarian ligament attached to the left ovarian cyst (Fig. 1).

There was a clear demarcation in between these two ovaries and the dermoid cyst derived from the left accessory ovary. Both right and left ovaries were located at the normal location and appeared to be normal (Fig. 2).

Other anomaly was not identified during exploration of the abdominal cavity. Resection of the dermoid cyst was performed over the left accessory ovary. The operation was uneventful, and she recovered well postoperatively. The histopathology from the resected cyst was benign mature cystic teratoma of the left accessory ovary (Fig. 3A, 3B)

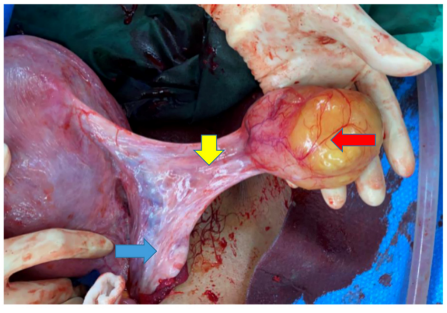

Fig. 1. Intraoperative findings showed presence of a dermoid cyst at the left accessory ovary (red arrow), where the accessory ovary located near and attached (yellow arrow) to the left normal ovary (blue arrow).

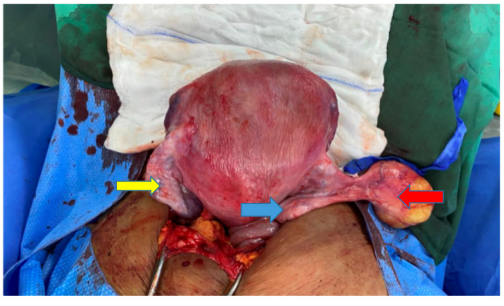

Fig. 2. Posterior view revealed a 5x5 cm dermoid cyst seen over the left accessory ovary (red arrow), both right (yellow arrow) and left (blue arrow) ovaries normally located and appeared normal.

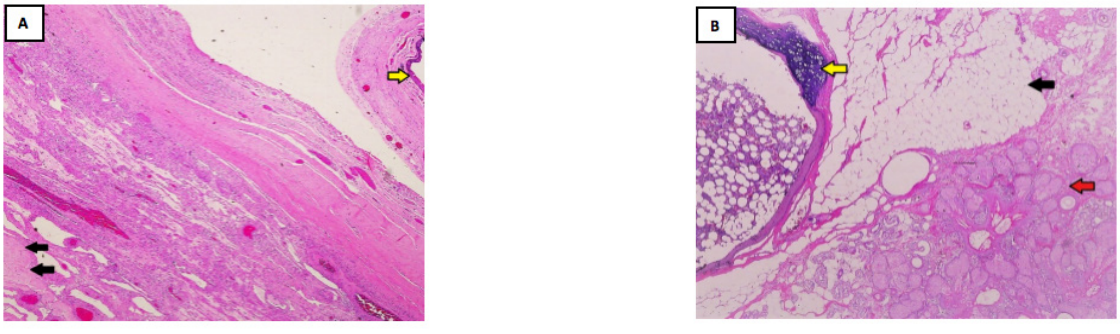

Fig. 3. (A) The cyst wall composed of ovarian stroma with corpus albican (black arrow) lined by stratified squamous epithelium (yellow arrow) (H&E, 40X). (B) In other areas, the cyst wall showed skin and its appendages (red arrow), cartilage (yellow arrow), bone with its hematopoietic cells and adipocytes (black arrow). (H&E, 40X).

Discussion

Ectopic ovary is an extremely rare gynaecological condition, whether accessory or supernumerary ovaries. The first description of an accessory ovary was reported in 1864 by Grohe and first case of supernumerary ovary was reported in 1890[2]. Only a few cases of ectopic ovary have been reported and in the most cases, including the present case, the ectopic ovary was an incidental finding. Accessory ovaries are often subcentimetric and patients are usually asymptomatic, thus preoperative diagnosis is challenging[3]. As with our case, it was an incidental finding intra-operatively, which the left accessory ovary was located near and attached to the normally placed

left ovary with presence of dermoid cyst at the left accessory ovary.

Accessory ovaries arise from a splitting of the developing ovarian primordium on the germinal ridge and are supplied by vessels continuous with those of the normally placed ovaries along with the mesoovarium. Due to its rarity, there is no studies or reviews comment on the most common site of accessory ovary, however by the definition itselft, accessory ovary is located near to the normally placed ovary, and may be connected with the ovary itself, broad ligament, ovarian ligament or infundibulopelvic ligament while supernumerary ovary, the ovarian tissue is entirely separate from the normally placed ovary, and it arises from a separate primordium[4].

There have been two theories that have been postulated with regards to the development of accessory ovary. Firstly, based on an embryological theory, they were formed as a result of abnormal separation of a small portion of the developing and migrating ovarian primordium[5]. Secondly, the formation of accessory ovaries developed in cases of acquired conditions such as inflammation and surgery, as explained by Lachman and Berman theory. This theory explains that part of the ovarian tissue detached from the ovary could implant anywhere in the pelvic cavity and continue to function in vivo.

With the presence of an accessory ovary, there is also an increased incidence of urogenital ridge defects. From 19 cases of accessory ovary reviewed by Wharton, five cases had defects such as accessory fallopian tube, bicornuate uterus and agenesis of the kidney[4]. Accessory ovary is often excised during surgery as it has both the functional and potential pathological behaviours of the normal ovaries[6].

In the literature, various cases report of tumors from the accessory or supernumerary ovaries, such as dermoid cyst, serous cystadenoma, Brenner tumor, steroid cell tumor, and sclerosing stromal tumour[7-10]. Dermoid cysts are the most common germ cell tumours as they account for up to a quarter of all ovarian tumors in reproductive age. A dermoid cyst in an accessory ectopic is an extremely rare entity, and the incidence is unknown. Only a few cases of dermoid cyst in an accessory ovary have been reported[8,9]. The patient with disease accessory ovary carries risk of cyst accident such as twisted, ruptured or haemorrhage and the risk of malignant transformation, thus the surgical excision is the treatment of choice[11].

As found in our case, the histological evaluation confirmed the finding of a mature cystic teratoma or dermoid cyst in the accessory ovary. The specimen composed of all 3 germ cell components include skin and its appendages, respiratory type epithelium, bone, cartilage and adipocytes with the absent of immature elements. The remaining of ovarian stroma as well as corpus albicans are also appreciates.

The appropriate surgical procedure is the resection of accessory ovary including the disease if any, as in our case the accessory ovary as separated from the infundibulopelvic ligament and other adjacent structure. In cases where the disease accessory located along the infundibulopelvic ligament, extra caution is needed as the resection of the accessory ovary or ovarian reconstruction poses risk of vascular injury, hematoma and can jeopardize the blood supply to main ovary. The other risk is ureteric injury as the common site of iatrogenic injury is at the infundibulopelvic ligament.

In our case, the intraoperative was uneventful and she had good recovery following this surgery.

Conclusion

The accessory ovary is asymptomatic in most cases and usually an incidental finding. Recognition of the accessory ovary is important to avoid unnecessary reconstruction of the ovary on the ovarian ligament and other structure. Complete excision of the pathological accessory ovary is recommended as it carries the risk of torsion, ruptured, infection and malignant transformation.

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Potential conflicts of interest

The authors declare no conflicts of interest.

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