Thai Journal of Obstetrics and Gynaecology
March 2023, Vol. 31, No. 2, pp. 107-114.

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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Received: 17 September 2021, **Revised:** 29 December 2021, **Accepted:** 19 January 2022

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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