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Comparison of Vaginal pH between Bacterial Vaginosis and Non-bacterial Vaginosis in Preterm Labour

Jiratchaya Kleebsuwan, M.D.,*,
Chanya Thamrongwuttikul, M.D.,*,
Watcharin Chirdchim, M.D.*.

** Department of Obstetrics and Gynaecology, Prapokklao Hospital, Chanthaburi 22000, Thailand*

ABSTRACT

Objectives: The aim of this study was to compare vaginal discharge pH between bacterial vaginosis (BV) and non-bacterial vaginosis in preterm labour.

Materials and Methods: An analytical cross-sectional study was carried out on patients admitted to the obstetrics ward of Prapokklao Hospital from June 2016 to January 2017. The main inclusion criterion was the presence of preterm labour without premature rupture of membranes or vaginal bleeding. Vaginal discharge was collected for pH measurement and Gram stain. The Nugent criteria from the University of British Columbia were used for BV diagnosis.

Results: The final analysis was based on data from 105 participants. The prevalence of BV was 71.4%. The mean of vaginal pH was 5.97±0.40 for the BV group and 5.20±0.36 for the non-BV group, $z=6.674$, $p < 0.001$.

Conclusion: Mean vaginal pH in the BV group was higher than in the non-BV group.

Keywords: Bacterial Vaginosis, Nugent criteria, Preterm labour, Vaginal pH.

Correspondence to: *Jiratchaya Kleebsuwan, M.D., Department of Obstetrics and Gynaecology, Prapokklao Hospital, Chanthaburi 22000, Thailand, E-mail: roseapple-medicine@hotmail.com*

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เปรียบเทียบค่าความเป็นกรดด่างของช่องคลอดในหญิงตั้งครรภ์ที่มีภาวะเจ็บครรภ์คลอดก่อนกำหนดที่มีและไม่มีภาวะติดเชื้อแบคทีเรียในช่องคลอด

จิรัชญา กลีบสุวรรณ์, จรรยา ธำรงวุฒิกุล, วัชรินทร์ เฉิดฉิ้ม

บทคัดย่อ

วัตถุประสงค์: *เพื่อเปรียบเทียบความเป็นกรดด่างของช่องคลอดในหญิงตั้งครรภ์ที่มาด้วยภาวะเจ็บครรภ์คลอดก่อนกำหนดที่มีและไม่มีภาวะติดเชื้อแบคทีเรียในช่องคลอด*

วัสดุและวิธีการ: *เป็นการศึกษาวิจัยเชิงวิเคราะห์แบบ cross-sectional ที่ห้องคลอด โรงพยาบาลพระปกเกล้า จังหวัดจันทบุรี ในหญิงตั้งครรภ์ที่มาด้วยภาวะเจ็บครรภ์คลอดก่อนกำหนดที่ไม่มีภาวะน้ำเดินก่อนกำหนด หรือเลือดออกจากช่องคลอด และรับไว้รักษาในโรงพยาบาลระหว่างเดือนมิถุนายน 2559 ถึงเดือนมกราคม 2560 โดยหญิงตั้งครรภ์ที่มาด้วยภาวะเจ็บครรภ์คลอดก่อนกำหนดทุกรายที่อยู่ในเกณฑ์คัดเข้าจะถูกเก็บสิ่งคัดหลั่งจากช่องคลอดเพื่อตรวจวัดค่าความเป็นกรดด่างและย้อมตรวจ Gram stain โดยใช้ Nugent criteria ของ University of British Columbia ในการวินิจฉัยภาวะติดเชื้อแบคทีเรียในช่องคลอด*

ผลการศึกษา: *หญิงตั้งครรภ์ที่มาด้วยภาวะเจ็บครรภ์คลอดก่อนกำหนดเข้าร่วมการศึกษาจำนวน 105 ราย ได้รับการวินิจฉัยว่ามีภาวะติดเชื้อแบคทีเรียในช่องคลอด 71.4% พบมีค่าเฉลี่ยความเป็นกรดด่างของช่องคลอดในกลุ่มที่มีและไม่มีการติดเชื้อแบคทีเรียในซ่องคลอด 5.97±0.40 และ 5.20±0.36 ตามลำดับ ($z=6.674$, $p < 0.001$)*

สรุป: *การศึกษานี้พบว่าค่าความเป็นกรดด่างในกลุ่มที่มีภาวะติดเชื้อแบคทีเรียในช่องคลอดสูงกว่ากลุ่มที่ไม่มีการติดเชื้อแบคทีเรียอย่างมีนัยสำคัญทางสถิติ*

คำสำคัญ: *การติดเชื้อแบคทีเรียในช่องคลอด, เกณฑ์ Nugent, เจ็บครรภ์คลอดก่อนกำหนด, ค่าความเป็นกรดด่างของช่องคลอด*

Introduction

Preterm labour is defined as regular contractions of the uterus that start before 37 weeks of pregnancy, resulting in changes in the cervix[1]. The preterm birth rate in Thailand was 10% in 2012[2] and regarded as a leading cause of both deficient maternal health and neonatal morbidity or mortality[1,3-4]. Based on five years of data from Prapokklao Hospital, it was reported that preterm births occurred at a rate of 6.23%. There are several risk factors of preterm labour, with previous studies reporting that vaginosis and cervicitis may have significant roles[5].

Bacterial vaginosis (BV) is the most common lower genital tract infection in women of reproductive age, resulting from a change in flora bacteria to mostly anaerobic bacteria. In pregnant women, BV is associated with preterm labour, early and late miscarriag, premature rupture of membranes, chorioamnionitis and low birth weight[6,7]. The incidence rate of BV in preterm labour is about 25.8%[8].

There are many criteria for the diagnosis of BV, including Amsel's criteria, Nugent's criteria and anaerobic culture[9-11]. Amsel's criteria is a simple bedside tool. However, a vaginal pH cut-off point of 4.5 may be unreliable because it is defined in the normal range of vaginal pH for pregnant women (3.5-6.0)[12]. Several studies have investigated vaginal pH change during pregnancy without infection, which showed consistently at 4.58 for within term delivery and 5.43 for preterm delivery[13]. No study has been conducted to determine the vaginal pH change with BV in pregnant women compared with non-BV. The Nugent scoring system and culture are inconvenient due to potential delays and high costs.

Accordingly, the aim of this study was to determine differences in vaginal pH between BV and non-BV patients in preterm labour.

Materials and Methods

This analytic cross-sectional study was carried out between June 2016 and January 2017 at Prapokklao Hospital, Chanthaburi, Thailand.

Sample size calculation

A pilot study conducted in February 2016 with 30 data records showed that the mean vaginal pH values for a BV group and a non-BV group of patients were 5.47 ± 0.54 and 5.18 ± 0.34, respectively. Sample size calculation was based on the difference in vaginal pH between the two groups. A one-tail test with 80% power was utilised for this purpose. The means vaginal pH from the pilot study were used for the calculation and giving at least 30 participants for each group. The results determined the chance for BV diagnosis at 0.6, requiring at least 45 samples for the BV group and 30 samples for the non-BV group.

The inclusion criteria for this study were women with singleton pregnancies and gestational age between 24^{+1} weeks to 36^{+6} weeks. The women had to have undergone routine ultrasonography (between 18^{+0} to 22^{+0} weeks), presenting with regular uterine contractions (4 times in 20 minutes or 8 times in 60 minutes) with cervical change in effacement ≥ 80% and dilation ≥ 1 cm. Exclusion criteria comprised premature rupture of membranes, vaginal bleeding, lack of understanding in Thai and last sexual intercourse within 72 hours. Consequently, 111 pregnant women were deemed eligible for the study. Six subjects were excluded because the Gram stain slides were lost and the patients delivered before recollection. As a result, 105 pregnant women actually participated in this study (Fig. 1).

After data and specimen collection, all preterm pregnancies were admitted to the labour room and treated following clinical guidelines for preterm labour. If cervical change advanced to the active phase of labour or spontaneous rupture of the amniotic membranes occurred, antibiotics for GBS prophylaxis was used in preparation for delivery. In cases diagnosed with BV, metronidazole at 1,200 mg/day was given for 7 days.

This study was approved by the Prapokklao Ethics Committee. Prior to enrollment, all eligible participants received information regarding the study and signed an informed consent form. The baseline characteristics of participants, antenatal data, vaginal

discharge for pH and Gram stain were collected and recorded.

Vaginal specimen collection procedure

A sterile dry speculum was inserted into the patient's vagina and 2 sterile cotton swabs were placed into the posterior fornix, one for aerobic culture (routine investigation in Prapokkloa Hospital) and another for Gram stain and pH measurement. All vaginal Gram stains were assessed for number of organisms and Clue cells in the required time by an official microbiologist, reviewed by the author and then checked for accuracy by a second official microbiologist for confirmation. Vaginal pH was measured using pH indicator paper with discrimination of 0.5. Vaginal discharge from the cotton swabs was placed on the pH indicator paper for 1 minute and read by 2 clinicians at the same time. Cervical change was assessed by training residents. Any conflicting opinions for Nugent score or pH level were resolved by mutual agreement. In some cases, a third opinion was sought.

Diagnosis of BV

Nugent criteria from the University of British Columbia were used for diagnosis in this study. Nugent scores of 4-6, which consisted of clue cells or a score of 7 or more, were reported as BV infection (Table 1)[14]

Allocation

This prospective study enrolled participants then diagnosed them as BV or non-BV prior to allocation into the two groups. The early period of this cross-sectional study identified a larger proportion of preterm patients with BV compared to non-BV, as evidenced by the ratio of 2.5 to 1 for BV to non-BV, at the end of the study period.

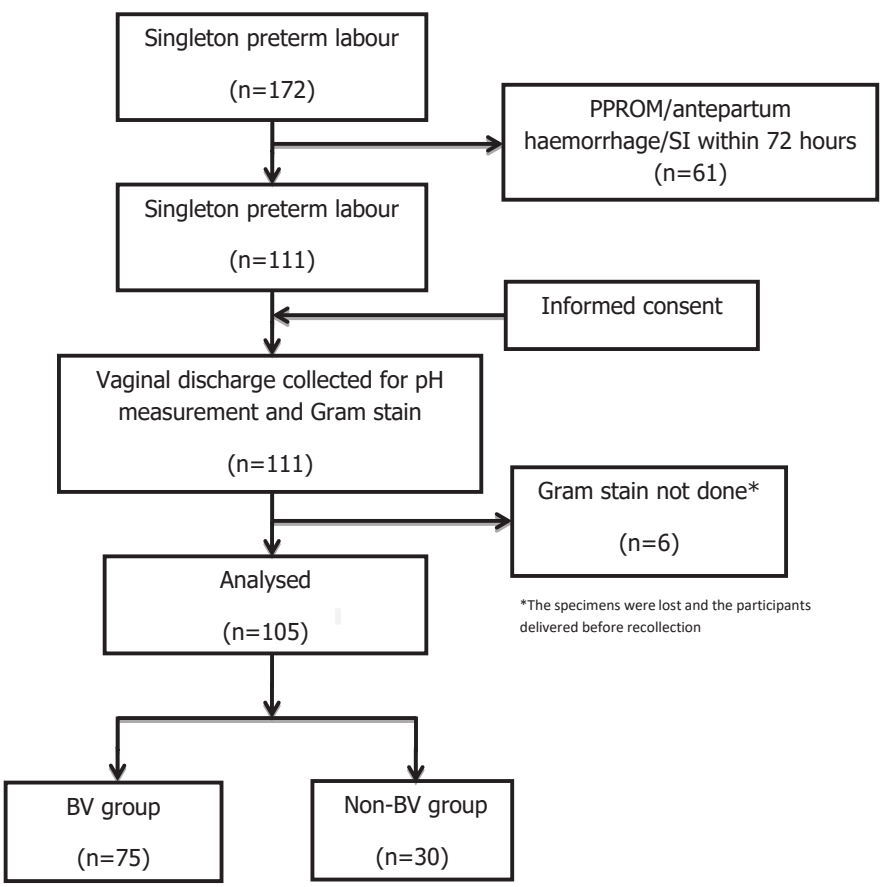

Fig. 1. The study flowchart.

Table 1. Laboratory interpretation of vaginal smears[14].

Gram positive rod*	Score	Small gram-variable CB, GNR*	Score	Curve gram-negative bacilli*	Score	Total score
≥ 30	0	0	0	0	0	
5 - 30	1	< 1	1	< 1	1	
1 - 4	2	1 - 4	2	1 - 4	1	
< 1	3	5 - 30	3	5 - 30	2	
0	4	≥ 30	4	≥ 30	2	

* number of organisms seen/100X objective, CB: curve bacilli, GNR: Gram negative rod

If N score is:	AND	Then Report
0 - 3 4 - 6	Clue cells NOT present	Smear NOT consistent with BV
4 - 6 ≥ 7	Clue cells ARE present	Smear consistent with BV

Statistical analysis

Baseline and antenatal characteristics among the two groups were analysed using percentage, mean, standard deviation (SD), exact probability test and independent t-test for maternal age and admission hematocrit. Differences in vaginal pH and gestational age between the groups were analysed using the Mann-Whitney U test because data had violation of normality assumption.

Results

In this study, 105 preterm labour pregnancies were enrolled and examined. The majority of patients had a high school level of education (59.1%), lived in Chanthaburi Province (89.5%) and were unemployed (45.7%). They had low and normal pre-gestational BMI at 32.4% and 39%, respectively. The BV and non-BV groups were similar for baseline and antenatal characteristics (Table 2).

Table 2. Baseline and antenatal characteristics.

Characteristics*	BV status		p value
	BV group (n = 75)	Non-BV group (n = 30)	
Pre-gestational overweight*	24 (32.0%)	6 (20.0%)	0.108
Nulliparous	30 (40.0%)	14 (40.7%)	0.662
Anaemia	35 (46.7%)	12 (40.0%)	0.665
Maternal age (years)**	25.6 ± 6.8	26.0 ± 7.2	0.723
Hematocrit (%)**	33.1 ± 3.9	34.2 ± 3.5	0.200
Gestational age (weeks)**	33.6 ± 3.0	32.7 ± 3.7	0.333†

* BMI ≥ 23.0 kg/m^2 (Asia), ** Mean ± SD, † Mann-Whitney U test

The prevalence of BV diagnosed utilising the Nugent criteria from University of British Columbia was 71.4%, while 29 cases (27.6%) had a Nugent score ≥7. Mean of vaginal pH in the BV group was higher than in the non-BV group (5.97±0.40 and 5.20±0.36, respectively, z=6.674, p < 0.001).

All diagnosed BV patients were orally treated with metronidazole and 48% delivered during the same visits comparable to 66.7% of non-BV patients (p = 0.129). The most common reasons for preterm deliveries included advanced cervical change, failure to inhibit following preterm labour practice guidelines, and fetal/maternal complications.

Discussion

BV during pregnancy is usually associated with a fishy-smelling discharge and is difficult to diagnose using Amsel's reproductive age standard criteria. The vaginas of pregnant women had a wider range of pH value than women who weren't pregnant. The three main causes of this phenomenon include a dramatic increase of the hormone circulated concentration from placental production[15], decreasing bacterial variety[16] and the reduction of vaginal lactobacilli producing lactate and H_2O_2[17]. In unnecessary cases, providing antibiotics can promote resistance to bacteria in the future.

The authors were interested in the changes of vaginal pH in preterm labour patients with BV. Thus, the study design invigilated confounding factors such as the premature rupture of amniotic membranes, bleeding from the genital tract and sexual intercourse within 72 hours. Our observations found that the prevalence of BV in pregnancy was higher than recorded at Siriraj Hospital (71.4% and 25.8%, respectively) because of different gestational age criteria and tools. The Siriraj Hospital study included gestational ages between 28^{+0}-36^{+6} weeks using the BVBlue test[8] (detection and measurement of microbial enzymes, sialidases: present among bacteria, viruses, mycoplasmas, fungi and protozoa)[18], whereas our study was conducted at gestational ages between 24^{+0} - 36^{+6} weeks using the Nugent criteria from University of British Columbia. However, the prevalence of BV was similar to the study by Siriraj and another study in Rio de Janeiro (27.6%, 25.8% and 28.1% respectively) if diagnosed by Nugent score ≥ 7[8,19]. Comparing the potential diagnostic tools, the BVBlue test is quick, easy and independent of personal skill, but it can be unspecified. In our opinion, standard benchmarks such as Amsel's criteria, Nugent score and bacterial culture may be more beneficial.

The mean vaginal pH of both BV and non-BV groups were higher than the cut-off point for Amsel's criteria, but within the normal range for vaginal pH in pregnancy. However, the mean vaginal pH in the BV group was still significantly higher than in the non-BV group. This implied that changes in bacterial flora could elevate vaginal pH with the decrease in lactic acid from Lactobacillus species and increase susceptibility to infections due to the impairment of the natural protective mechanisms of the vagina. In our opinion, the cut-off point for Amsel's criteria used in preterm pregnancy may be higher than non-pregnant women.

Interestingly, vaginal pH in preterm labour may be affected by nationality. Royce, et al., reported that the vaginal pH and flora differed by race/ethnicity[20]. While the mean vaginal pH of all non-BV patients in our study was lower than the vaginal pH of all preterm deliveries recorded by Gleeson et al. (5.20 and 5.43, respectively)[13]. In our opinion, the effect of nationality on vaginal pH should be investigated and confirmed by further studies.

Our study compared vaginal pH between BV and non-BV patients by controlling the most common confounding factors that interfere with the acid-base value. However, there were some limitations. We used wide-range pH test papers to measure vaginal pH. Further, the sample size was too small for a diagnostic study.

Conclusion

Our study determined that vaginal pH in the BV group was higher than in the non-BV group. Further studies should investigate the diagnostic value of a new vaginal pH cut-off point using Amsel's criteria for

uncomplicated preterm labour.

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Potential conflicts of interest

The authors declare no conflict of interest.

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