Prediction of preterm birth using a modified classification of gram-stained vaginal smears

ABSTRACT

**Objective:** The aim of this study was to identify women at risk of spontaneous preterm birth through a 4-category Gram-stained vaginal smear method and to examine the diagnostic accuracy of this classification in relation to the occurrence of preterm delivery.

**Patients & Methods:** This was a prospective study correlating first trimester Gram-stained vaginal smears with spontaneous preterm birth. Smears were categorized as normal, bacterial vaginosis-like, grade 1-like (atypical gram-positive rods) or purulent grade 1 (lactobacilli-dominated smears showing heavy leukorrhea of unknown cause).

**Results:** Abnormal Gram stain vaginal smears were associated with more than 5 fold increase in the odds of having a preterm birth when applying the modified 4 category scoring system. The presence of bacterial vaginosis (BV) like, Grade 1-like and Grade 1-PNL were associated with a significant increase in the odds of preterm labor (4, 7.8 and 6.1, respectively).

**Conclusion:** Pregnant women with abnormal Gram-stained vaginal smear have a higher risk of preterm labor. Also, the modified 4-category Gram-stained vaginal smear classification is a useful tool in predicting preterm labor.

**Key words:** preterm birth; bacterial vaginosis; Gram stain; vaginal smear

INTRODUCTION

Preterm birth is defined as delivery of an infant before 37 weeks’ gestation. Preterm birth is a serious problem occurring in 11% of all pregnancies. (1) It remains a leading cause of neonatal mortality and morbidity. (2) Bacterial vaginosis (BV) is a vaginal condition that can produce vaginal discharge and results from an overgrowth of certain types of bacteria that naturally exists in the vagina. BV, as well as other infections, had been found to be associated with an increased risk of preterm delivery in many studies. (3-5) BV had been shown to exist in almost 20% of pregnant women, however, most cases remain asymptomatic, and not all women with the condition will deliver prematurely. (6)

Various tests as Amsel’s criteria, gram staining, oligonucleotide probes and culture of organisms of bacterial vaginosis can be used for the diagnosis. (7) The Nugent scoring system of a vaginal Gram stain is the most commonly used classification of vaginal microflora status. (8) Other classifications as that by Ison and Hay had also been validated and gained popularity. (9) However, these scoring systems may not reflect the diversity and complexity of the vaginal microflora. (10, 11) Meanwhile, a number of studies had found that other nonbacterial cell types may be involved in the vaginal inflammatory response that could trigger preterm labor. (12, 13)

In 2007, Verstraalen suggested a modified 4-category Gram-stained vaginal smear classification to identify women at risk of spontaneous preterm delivery. (11) The aim of this study was to identify women at risk of spontaneous preterm birth through the modified 4-category Gram-stained vaginal smear method, proposed by Verstraalen, and to examine the diagnostic accuracy of this classification in relation to the occurrence of preterm delivery.

MATERIALS AND METHODS

This prospective study was conducted at the outpatient obstetric clinic in Mansoura University Hospital during the period from May 2008 to December 2010. It included 264 pregnant women, 5 cases were excluded due to spontaneous abortion, so the total number in this study was 259 pregnant women. A written informed consent was taken from all pregnant women in this study. Gestational age was calculated from the first day of the last menstrual period and confirmed by early ultrasound examination.

The exclusion criteria included: previous preterm labor, documented infection, multiple pregnancy, diabetic patients and women who received antibiotic or antifungal (local or systemic) treatment during pregnancy before sampling.
Each pregnant woman was subjected to Vaginal sample during her first trimester by inserting a sterile cotton-tipped wooden swab into the vagina for the purpose of vaginal microflora status assessment. The swab was rolled round through 360 degrees against the vaginal wall at the vault and carefully withdrawn to prevent contamination. Swabs were then smeared on a plain glass slide and air-dried at room temperature and heat fixed. Then, slides were Gram stained and examined under oil immersion lens at X100 magnification.

Accordingly, Gram-stained vaginal smears were categorized as normal, bacterial vaginosis-like; grade I-like (atypical gram-positive rods) or purulent grade I (lactobacilli-dominated smears showing heavy leukorhea of unknown cause).

We found that Grade I-like smears resemble grade 1 smears at first glance; however, they show predominately atypical gram-positive rods, in particular curved or irregularly shaped (bifido- or corynecocifor)gram-positive rods, that cannot be categorized as Lactobacillus (or as Bacteroides, Gardnerella or Mobiluncus) cell types and, therefore, these smears could not be assigned an Iron- and Hay score.

The purulent grade I smears (grade I-PMN) were found to have heavy leukorhea with neutrophils (PMN) in the presence of normal numbers of Lactobacillus spp. with absence of pathogens such as Candida spp. Accordingly, these smears would be considered normal according to Nugent or Iron and Hay. They show large numbers of neutrophils and hence an inflammatory response of unknown cause.

Following blinded allocation of each vaginal smear to 1 of the 4 categories, a comparison was made between patients with an 'abnormal Gram stain' (BV-like, grade I-like or grade I-PMN) and those with a 'normal Gram stain' (grade I). Other clinical data including maternal age, weight and height, obstetric history mode of conception and any special habit, as smoking, were collected in a routine manner. Also, basic investigations during antenatal care such as complete blood picture, Rh factor, blood glucose level and urine analysis were done to all women in this study.

Spontaneous preterm birth is defined as birth before 37 completed weeks of gestation (delivery between 25 and 36 weeks + 6 days) following spontaneous onset of labor or rupture of membranes. The obstetric outcome was assessed using the mean birth weight (low birth weight was defined as <2500 g) and gestational age at delivery. Outcome was recorded as early miscarriage (EM) if it was ≤13 weeks or late miscarriage (LM) if was between 14 and 24 weeks.

Statistical analysis

Continuous variables were compared with the independent samples t test. Categorical variables were compared with ordinary chi-square tests. For any reported measure, statistical significance was accepted, as the 2-tailed probability level was < 0.05. All statistical analyses were performed using the statistical software package SPSS v16.0 (SPSS, Inc, Chicago, IL).

We calculated the sensitivity, specificity, positive and negative predictive values, likelihood ratio for a positive and a negative test result and odds ratios. Calculations were performed using Meta Disc software (version 1.4). Ninety-five percent confidence intervals are also reported. Positive likelihood ratios above 10 and negative likelihood ratio below 0.1 have been noted as providing evidence of high diagnostic accuracy, whereas those above 5 and below 0.2 give evidence of moderate diagnostic accuracy.

### RESULTS

From the 259 women, 66% presented with grade I microflora on each occasion (171/259), 18.5% of the women (48/259) had BV-like microflora, 6% grade I-like microflora (16/259), while 9.2% (24/259) showed heavy vaginal leukocytosis (grade I-PMN). Seven women from the group of women with lactobacilli-dominated microflora in the absence of leukorhea had preterm birth (7/217). Preterm birth was observed in 7 ladies with BV-like microflora (7/48), 4 ladies of those with grade I-like microflora (4/16) and 5 from women who showed an episode characterized by a purulent grade I (5/24).

The sensitivity, specificity, +ve likelihood ratio (+LR), -ve likelihood ratio (-LR) and odds ratio (OR) for the classification compared to women with lactobacilli-dominated microflora (normal Gram stain) are shown in Table 2.

Table 2 shows a remarkable increase in the sensitivity of the 4 categories. Gram stain scoring method for the prediction of preterm birth from 30%, when only accounting for BV-like microflora, and up to 69% when taking into account the two other categories grade I-like and grade I-PMN. Meanwhile, the overall presence of an abnormal Gram stain was associated with more than 5 fold increase in the odds of having a preterm birth compared to women with lactobacilli-dominated microflora in the absence of leukorhea (Table 2).

The presence of grade I-like and Grade I-PMN were associated with a significant increase in the odds of preterm labor (7.8 and 6.1, respectively). The +LR and the -LR for the abnormal Gram stains were 2.5 and 0.62, respectively.

<table>
<thead>
<tr>
<th>Gram stain category</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>+LR (95% CI)</th>
<th>-LR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV-like</td>
<td>0.58 (0.35-0.77)</td>
<td>0.95 (0.91-0.98)</td>
<td>2.50 (1.35-4.61)</td>
<td>0.62 (0.16-2.46)</td>
<td>7.91 (3.33-19.27)</td>
</tr>
<tr>
<td>Grade I-like</td>
<td>0.10 (0.21-0.49)</td>
<td>0.95 (0.92-0.98)</td>
<td>1.37 (1.25-1.50)</td>
<td>0.88 (0.64-1.20)</td>
<td>7.91 (3.33-19.27)</td>
</tr>
<tr>
<td>Grade I-PMN</td>
<td>0.41 (0.28-0.56)</td>
<td>0.95 (0.92-0.98)</td>
<td>1.37 (1.25-1.50)</td>
<td>0.88 (0.64-1.20)</td>
<td>7.91 (3.33-19.27)</td>
</tr>
<tr>
<td>Abnormal Gram stain</td>
<td>0.09 (0.04-0.22)</td>
<td>0.95 (0.92-0.98)</td>
<td>1.37 (1.25-1.50)</td>
<td>0.88 (0.64-1.20)</td>
<td>7.91 (3.33-19.27)</td>
</tr>
</tbody>
</table>

* Positive likelihood ratio (+LR)
* Negative likelihood ratio (-LR)
* Odds ratio (OR)
DISCUSSION

For decades, the important objectives of reducing the preterm birth (PTB) rate have presented a challenge. In the past two decades, BV has been consistently associated with adverse outcomes of pregnancy. Specifically, BV has been shown to be associated with a two-fold increase in risk for preterm delivery. The cause of this study was to identify women at risk of spontaneous preterm birth through a 4-category Gram-stained vaginal smear method and to examine the diagnostic accuracy of this classification in relation to the occurrence of preterm delivery. Our findings had shown that extending the spectrum of vaginal Gram stain diagnosis may identify a much higher proportion of women at risk of spontaneous preterm birth than has been reported. Our results also agree with the findings from many studies. On the other hand, the results of our study are in disagreement with the findings from a recent study who denied the association between preterm labor and bacterial vaginosis. It has been suggested that abnormal vaginal microflora probably encompasses a wide range of changes in microflora (not confined to bacterial vaginosis) though few investigators seem to have addressed this issue. In our study we found that abnormal Gram stain vaginal smears were associated with more than 5 fold increase in the odds of having a preterm birth when applying the modified 4 category scoring system. The group of patients with grade 1-2 like microabcessi and those with grade 3-4 showed a significant increase in the odds of preterm labor. 

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REFERENCES