Full Length Research Paper

Bacteriological study of vaginal discharge of pregnant women using Gram stain smear and culture

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Accepted 30 June, 2011

Bacterial vaginosis is caused by an imbalance of the organisms that naturally exist in the vagina. The importance of bacterial vaginosis with respect to pregnant women’s health is emphasized by the association between bacterial vaginosis and adverse outcome of pregnancy. The aim of present study was to evaluate the direct smear microscopy and culture for determination of bacteria from vaginal discharge of pregnant women. In total, 240 vaginal swabs were collected from 120 pregnant women and were screened for bacterial population. For each patient one swab was used for smear preparation and Gram staining and the second swab was used for cultivation. The prepared Gram-stained smears were observed for various morphotypes. Each morphotype was quantified on a scale from 0 to 4 and weighed to yield a score of 0 to 10, as per Nugent’s system. The bacteria grown in preliminary culture media were identified using standard identification tests. The majority of isolated bacteria in culture were Diphtheroid, Lactobacillus spp., Coagulase-negative Staphylococci and yeast. In Gram-stained smears, 78 (65%) Gram positive rods and 54 (45%) Gram positive cocci were detected. According to Nugent’s criteria, 64 cases (53.33%) were classified as having normal vaginal flora, 45 (37.5%) intermediate flora and 11 cases (9.2%) having bacterial vaginosis. The prevalence of bacterial vaginosis is not very high. However we recommend the regular screening of women with Gram stain method using Nugent’s criteria which is reliable, easy to perform and well suited for the routine clinical laboratory.

Key words: Vaginal discharge, bacterial vaginosis, Nugent’s criteria, Gram stain, culture.

INTRODUCTION

The vaginal microflora constitutes a complicated environment, composed of varying microbiological species in variable quantities and proportions and their concentrations are indicative of the vaginal health of the individual (Donders et al., 2005). The microbial ecology subject to remarkable changes over the course of lifetime induced by developmental and hormonal changes (Pybus et al., 1999). In childhood, the vaginal flora contains skin commensals and bowel organisms. At menarche, the pH falls from neutral to approximately 4, and the flora becomes dominated by lactobacilli. Many other organisms may be present in lower concentrations, including anaerobic and facultative anaerobic bacteria and Candida spp. (Donders, 1999). In women of childbearing age this system is also dominated by Lactobacillus spp., a defining characteristic of which is the ability to grow in acid media and tolerate acid conditions at pH around 4.5; lactobacilli also ferment carbohydrates to produce lactic acid (Sobel, 2000). The normal vaginal bacterial flora of healthy pre-menopausal women continues to consist predominantly of Lactobacillus spp. These are believed to play a protective role in guarding the urogenital tract against infection by
Bacterial vaginosis (BV) is a very common condition characterized by alterations of the vaginal flora with acquisition of diverse communities of anaerobic and facultative bacteria and depletion of the usually dominant Lactobacillus flora (Sobel, 2005). Accurate diagnosis of BV is important as it is associated with adverse pregnancy outcome (Myziuk et al., 2003). In a recent study, the investigators tried to evaluate the possible association of BV with serious reproductive complications in women and they reported a significance association of BV with infertility (Mania-Pramanik et al., 2009).

Currently the criteria as defined by Nugent et al. (1991) are considered as the standard procedure to score vaginal smears by Gram stain (Nugent et al., 1991). This method scores the smears in a standardized manner by quantification of some of the cell types present (Forsum et al., 2002). The present work confirms the findings from
Figure 1. Frequency of age groups in pregnant women in this study.

Table 1. The relative frequency of organisms seen in Gram-stained smears and isolated from vaginal discharge cultures.

<table>
<thead>
<tr>
<th>Isolated organisms</th>
<th>Smear Gram stain No. (%)</th>
<th>Culture No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive rods ((Lactobacillus) spp.)</td>
<td>78 (65)</td>
<td>46 (38.33)</td>
</tr>
<tr>
<td>Curved gram variable rods ((Mobilincus))</td>
<td>22 (18.33)</td>
<td>-</td>
</tr>
<tr>
<td>Gram negative rods ((Gardnerella))</td>
<td>42 (35)</td>
<td>-</td>
</tr>
<tr>
<td>Gram positive rods ((Gardnerella))</td>
<td>16 (13.33)</td>
<td>48 (40)</td>
</tr>
<tr>
<td>Gram negative rods ((Neisseria sp.))</td>
<td>12 (10)</td>
<td>8 (6.66)</td>
</tr>
<tr>
<td>Enteric Gram negative rods</td>
<td>-</td>
<td>4 (3.33)</td>
</tr>
<tr>
<td>(Staphylococcus aureus)</td>
<td>-</td>
<td>18 (15)</td>
</tr>
<tr>
<td>Coagulase negative (Staphylococcus)</td>
<td>-</td>
<td>43 (35.83)</td>
</tr>
<tr>
<td>Streptococci</td>
<td>36 (30)</td>
<td>11 (9.16)</td>
</tr>
<tr>
<td>Yeast</td>
<td>36 (30)</td>
<td>44 (36.66)</td>
</tr>
<tr>
<td>Clue cells</td>
<td>18 (15)</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2. Score of vaginal discharge in present study as per Nugent criteria system.

<table>
<thead>
<tr>
<th>Score</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4⁺</td>
<td>0</td>
<td>0</td>
<td>48 (40)</td>
</tr>
<tr>
<td>2</td>
<td>3⁺</td>
<td>1⁺</td>
<td>0</td>
<td>16 (13.3)</td>
</tr>
<tr>
<td>4</td>
<td>3⁺</td>
<td>3⁺</td>
<td>0</td>
<td>30 (13.3)</td>
</tr>
<tr>
<td>6</td>
<td>2⁺</td>
<td>4⁺</td>
<td>4⁺</td>
<td>15 (12.5)</td>
</tr>
<tr>
<td>8</td>
<td>1⁺</td>
<td>4⁺</td>
<td>4⁺</td>
<td>5 (4.2)</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>4⁺</td>
<td>4⁺</td>
<td>6 (5)</td>
</tr>
</tbody>
</table>

A: large Gram-positive rods \((Lactobacillus morphotypes)\).
B: Small Gram-variable rods \((G. vaginalis morphotypes)\).
C: curved Gram-variable rods \((Mobilincus spp. morphotypes)\).

previous studies demonstrating the usefulness of Nugent’s criteria for determination of BV in women (Delaney and Onderdonk, 2001; Tamrakar et al., 2007; Sha et al., 2005).

We examined the vaginal flora by the objective and reproducible evaluation of Gram-stained smears and culture. To determine the prevalence of BV, we applied the strict definition given by Nugent et al. (1991). We found that in pregnant women under investigation the prevalence of BV was 9.2% with scores of 8 and 10 (Table 2). In a recent work from Iran, the prevalence of BV based on the presence of clue cells and Amsel’s criteria (Amsel et al., 1983), was reported as
11.3% among 160 labouring women (Modares and Shafaie, 2008). Dadhwal et al. (2010) found a prevalence of 8.6% among 502 asymptomatic pregnant women. This was reported as 4.5% among 472 studied pregnant women by Gratacos et al. (1999). While their findings were close to ours, however in some other studies the reported prevalence was higher than this study. The reported BV prevalence in the study of Rizvi and Luby (2004) was 25%, in Abu Shaqra (2001) was 29.7%, and in Rouse et al. (2009) was 16.6%. The Nugent’s criteria was used by these investigators as an easy and reliable diagnostic tool to determine the prevalence of BV, since the clinical criteria alone may not enough for a definite diagnosis especially in pregnant women with asymptomatic BV. In a study on 492 women, BV was diagnosed in 1.6% of women on the basis of clinical criteria, while this was 4.5% according to Gram stain (Rizvi and Luby, 2004). Lactobacilli were the most prevalent organisms in Gram-stained smears in normal women with scores of 0 and 2 and were the least or none in women with BV comprising scores 8 and 10. This finding suggests that the decrease in Lactobacillus colonization could be a leading cause of the BV as previously concluded (Chaudry et al., 2004; Sobel, 2005). The cultural method and data interpretation have established that the normal flora was very diverse in tested specimens, reflecting a dynamic, polymicrobial ecosystem. While the number of some morphotypes such as Gardnerella and Mobiluncus was significant in stained smears, in culture we could not detect these morphotypes, probably due to sensitivity of these morphotypes and lack of special requirements in our culture media needed for their growth. The majority of grown bacteria in culture were Gram positive cocci and rods (Lactobacilli) with the least belonged to Gram negative entric rods. Since for diagnosis of BV, the culture is time-consuming compared to direct Gram stain and providing the special requirements for growth of fastidious morphotypes makes the process costly, we believe the Gram-stained smear alone, without culture, can be used to evaluate vaginal swab specimens for BV.

In conclusion, the overall results of this study which was conducted for the first time in our setting indicated that the prevalence of BV is not very high. However we recommend the regular screening of women with Gram stain method using Nugent’s criteria which is reliable, easy to perform and well suited for the routine clinical laboratory. This method could be used for rapid diagnosis of bacterial BV for clinicians with minimum need for confirmation by culture to prevent treatment delay.

ACKNOWLEDGEMENT

This work was supported by a grant (No. S-71-88) from Research Affairs, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

REFERENCES


