Preterm Labour and its Relation To Bacterial Vaginosis and Fetal Fibronectin

M. A. B. Gamal 1               Tammaly M. 2            Elsharif M.3

Abstract

Preterm delivery (PTD) is the leading cause of infant morbidity and mortality. Epidemiological association between PTD and various bacteria that are part of the vaginal microflora has been reported. Bacterial vaginosis is believed to be a risk factor for preterm delivery. The aim of the present study is to investigate the association between bacterial vaginosis, cervicovaginal fetal fibronectin level and the preterm delivery of infant with low birth weight. In this study a total of 288 pregnant women were enrolled during the period between May 2008 and June 2009 at Alkoms Hospital, Libya. The present study revealed that the presence of bacterial vaginosis was related to preterm delivery of a low birth weight infants. The highest risk of preterm delivery of low birth weight infants was found among those with vaginal Gardnerella vaginalis, Mycoplasma hominis and Bacteroides spp. (6.3%), as well as women with vaginal Mycoplasma hominis, Bacteroides spp and Escherichia coli (5.9%). However Lactobacillus spp. had no effect on poor pregnancy outcome and play no role with preterm delivery of infants with low birth weight. There was also a strong relation between cervicovaginal fetal fibronectin and isolation of Mycoplasma hominis, Bacteroides spp. and Escherichia coli. In conclusion, that the effect of the abnormal vaginal flora was an independent predictor of preterm delivery. Cervicovaginal measurement of fetal fibronectin in combination with detection of bacterial vaginosis may be useful for evaluation of pregnant women with threatened premature delivery of low birth weight infants.

1  Microbiology Department Faculty of Pharmacy, Al-Mergib University, Alkomas, Libya.
2  Microbiology Department Faculty of Science, Al-Mergib University, Alkomas, Libya.
3  Faculty of Marine Resources, Al-Mergib University, Zliten, Libya.
Introduction

Preterm delivery is the leading cause of infant morbidity and mortality over the world. There are many causes for preterm delivery and it remains incompletely understood (Onderdonk et al., 2003).

Scoring systems designed to identify those women at risk of preterm delivery by incorporating recognized risk factors have lacked sufficient specificity to be clinically useful. Even if a group of women at risk of preterm delivery could be identified accurately, there is no proof that prophylactic measures would be helpful, since bed rest, cervical cerclage and long term obstetric treatment have failed to provide any benefit (Gibbs et al., 1992).

There is increasing evidence that ascending infection from the lower genital tract is an important cause of preterm labour. Moreover, evidence indicates that intrauterine infection may also be involved in the genesis of significant neonatal and infant complications such as cerebral palsy and bronchopulmonary dysplasia (Cassell et al., 1993).

Extrauterine infections such as pneumonia, malaria, pyelonephritis and typhoid fever have been associated with preterm labour and delivery (Madinger et al., 1989).

Intrauterine infection has been recognized as a major factor associated with preterm labour and the most common microbial isolate, from the amniotic cavity from women with preterm labour and intact membrane, are Ureaplasma urealyticum, Fusobacterium species and Mycoplasma hominis (Abdel-Hamid and Fakr, 1993; Romero and Mazor, 1989; Tekesin et al., 2005; Kataoka et al., 2006).

Fetal fibronectin, has primarily been identified in amniotic fluid, trophoblastic tissue and the extracellular matrix of decidual basal (Langer et al., 1997). This glycoprotein is detectable in cervico-vaginal fluid during normal pregnancy at less than 21 weeks of gestation and at full term (Feinbery et al., 1991; Balu et al., 2003). However, the presence of fetal fibronectin in the cervixes or vagina after twenty weeks is abnormal (Langer et al., 1997) and may indicate mechanical or inflammatory mediated disruption of the choriodecidual interface and provides a possible marker for risk of preterm labor (Ness et al., 1998; Roman et al., 2005).
The aim of this study was to investigate the relation between bacterial vaginosis, the detection of this biochemical marker (fetal fibronectin) in the cervico vaginal secretions and preterm delivery.

**Subjects, Materials and Methods**

**I- Subjects:**

This study was conducted at Alkoms hospital, Libya between May 2008 and June 2009, 288 pregnant women between 25-28 weeks of gestation were recruited to a prospective observational study. Women were enrolled in the study during routine prenatal visits after 25-28 weeks of gestation had been completed.

For each woman, a medical, obstetrical, sexual and social history were taken and cultures of the vagina and cervix were obtained.

Exclusion criteria: Age less than 20 years, use of antibiotics in the preceding two weeks, cervical cerclage, hypertension requiring treatment with medication, insulin dependent diabetes, current use of corticosteroids and chronic renal diseases. Estimation of gestational age were based on the date of the last menstrual period and were adjusted by the obstetrical provider as needed on the basis of the results of pregnancy tests and the first pelvic examination, the fetal heart beat, and available results of ultrasonography. Preterm delivery was defined as delivery at less than 37 weeks gestation.

**II- Sampling:**

**Swabs for bacterial identification:** A clean sterile, unlubricated speculum was placed in the vagina and a sterile cotton wool swabs were used through the speculum to obtain specimen from the endocervix and posterior vaginal fornix. The sample was taken with a sterile polyester swab from the commercially available collection Kits. The swab was rolled gently in the posterior vaginal fornix and the cervix for 5-10 seconds, avoiding bloody areas when possible and extracted in 750 μl of the provided buffer solution.

**III- Bacterial identification:**

Swabs were streaked onto the primary isolation media (MacConkey agar, mannitol salt agar, cetrimide agar and blood agar plates). All cultured plates were incubated aerobically and anaerobically at 37oC for 24-48 hours.
After the incubation time, the plates were examined for any growth and the isolates were subjected to Gram stain and biochemical tests for full bacterial identification according to Colle et al., 1996. For Mycoplasma, the swabs were streaked on Columbia agar and incubated anaerobically for 72-96 hours (Koneman et al., 1997).

IV- Biochemical assay of Fetal Fibronectin:

Fetal fibronectin was determined quantitatively with an enzyme linked immunosorbent assay (Fetal Fibronectin Enzyme Immunoassay; Adeza Biomedical, Sunnyvale, California, USA) using monoclonal antibody FDC-6. The absorbency of the samples and standards was then determined in duplicate by use of an automated microtiter plate reader. Absolute fetal fibronectin concentrations were obtained and recorded in micrograms per milliliter and a value of > 0.05 µg/ml was considered positive (Lockwood et al., 1991).

V- Statistical analysis:

Analysis was done by SPSS program, Six edition, 2001. A two-tailed P value less than 0.05 was considered to indicate statistical significance.

Results

A total of 288 pregnant women were enrolled in this study. They were divided according to their age into four groups (Table 1), where the highest age group was those between 25-27 years (30.6%).

Table (1): Prevalence of pregnant women according to their age

<table>
<thead>
<tr>
<th>Group</th>
<th>Age</th>
<th>Number</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>22 – 24</td>
<td>45</td>
<td>15.6</td>
</tr>
<tr>
<td>II</td>
<td>25 – 27</td>
<td>88</td>
<td>30.6</td>
</tr>
<tr>
<td>III</td>
<td>28 – 30</td>
<td>75</td>
<td>26.0</td>
</tr>
<tr>
<td>IV</td>
<td>31 – 35</td>
<td>80</td>
<td>27.8</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>288</td>
<td>100</td>
</tr>
</tbody>
</table>

* Prevalence of age was related to total number of pregnant women.

Giving births according to gestational age is shown in Table (2), where preterm delivery was recorded in 13.9%.
Preterm Labour and its Relation To Bacterial Vaginosis and Fetal Fibronectin

Table (2): Gestational age at birth.

<table>
<thead>
<tr>
<th>Gestational age in week</th>
<th>Number of births observed</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-32</td>
<td>7</td>
<td>2.5</td>
</tr>
<tr>
<td>33-36</td>
<td>33</td>
<td>11.6</td>
</tr>
<tr>
<td>37-40</td>
<td>219</td>
<td>75.9</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>29</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>288</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table (3) and Figure (1) show the types and prevalence of the isolated microorganisms and the rate of single and mixed infections isolated from pregnant women under investigation. It was noticed that lactobacillus spp. were the most prevalent microorganism, isolated alone at 42% and mixed with Gardnerella vaginalis at 28%, while Gardnerella vaginal together with Bacteriod spp. and Mycoplasma hominis were isolated rate of 19%. However, the lowest rate of isolation (11%) was observed with Mycoplasma hominis together with Bacteroides spp. and Escherichia coli. Also, it was clear that colonization with lactobacilli was inversely associated with bacterial vaginosis (table 3 and figure 1).

Table (3): Types and prevalence of isolated organisms from the studied pregnant women.

<table>
<thead>
<tr>
<th>Isolated microorganisms</th>
<th>No. of positive cases</th>
<th>% Isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus spp.</td>
<td>120</td>
<td>42</td>
</tr>
<tr>
<td>Lactobacillus spp. + Gardnerella vaginalis</td>
<td>82</td>
<td>28</td>
</tr>
<tr>
<td>Gardnerella vaginalis + Bacteroides spp. + Mycoplasma hominis.</td>
<td>55</td>
<td>19</td>
</tr>
<tr>
<td>Mycoplasma hominis + Bacteroid spp. + E. coli.</td>
<td>31</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>288</td>
<td>100%</td>
</tr>
</tbody>
</table>
% correlated to the total number of cases under study (288).

![Figure 3: Prevalence of organisms isolated from women](image)

**Table (4): Association of bacterial vaginosis with outcomes of pregnancy among the studied group**

<table>
<thead>
<tr>
<th>Isolated microorganisms</th>
<th>Frequency of isolation</th>
<th>Women with preterm delivery</th>
<th>Women with full term delivery</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>120</td>
<td>3</td>
<td>1</td>
<td>117</td>
</tr>
<tr>
<td>Lactobacillus spp. + Gardnerella vaginalis</td>
<td>82</td>
<td>4</td>
<td>1.4</td>
<td>78</td>
</tr>
<tr>
<td>Gardnerella vaginalis + Bacteroides spp. + Mycoplasma hominis.</td>
<td>55</td>
<td>18</td>
<td>6.3</td>
<td>37</td>
</tr>
<tr>
<td>Mycoplasma hominis + Bacteroides spp. + E. coli.</td>
<td>31</td>
<td>17</td>
<td>5.9</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>288</td>
<td>42</td>
<td>14.6</td>
<td>246</td>
</tr>
</tbody>
</table>

N.B. % correlated to the total number of cases under study (288).

The results shown in Table 4 indicated that bacterial vaginosis was associated with an increased risk of preterm delivery.
Figure (2): Association between preterm and fullterm delivery & bacterial vaginosis

Gardnerella vaginalis, Bacteriodes spp., Mycoplasma hominis and E. coli were the microorganisms most strongly associated with presence of bacterial vaginosis. Also Table 4 & Figure (4) illustrate the role of individual species in the association between bacterial vaginosis and the preterm delivery of infant with low birth weight. The recovery of Gardnerella vaginalis had no effect on incidence of preterm delivery of low-birth weight infant independently of the presence of bacterial vaginosis and in the absence of Bacteroids spp., Mycoplasma hominis and E. coli there was no greater risk of premature delivery. In contrast presence of Mycoplasma hominis, Bacteroides spp. and E. coli was associated with preterm delivery of a low-birth weight infant, even in the absence of Gardnerella vaginalis.

Fetal fibronectin value was higher in pregnant women with bacterial vaginosis than in those without bacterial vaginosis (table 5 and figure 3).
Table (5): Cervicovaginal fetal fibronectin level relation to types and frequency of isolated microorganisms.

<table>
<thead>
<tr>
<th>Group</th>
<th>Isolated microorganism</th>
<th>Frequency of isolation</th>
<th>Fetal fibronectin conc.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.05 ug/ml</td>
<td>&gt; 0.05 ug/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>I</td>
<td><em>Lactobacillus spp.</em></td>
<td>120</td>
<td>103</td>
<td>86</td>
</tr>
<tr>
<td>II</td>
<td><em>Lactobacillus spp.</em> + <em>Gardnerella vaginalis</em></td>
<td>82</td>
<td>71</td>
<td>86.6</td>
</tr>
<tr>
<td>III</td>
<td><em>Gardnerella vaginalis</em> + <em>Bacteroides spp.</em> + <em>Mycoplasma hominis.</em></td>
<td>55</td>
<td>34</td>
<td>62</td>
</tr>
<tr>
<td>IV</td>
<td><em>Mycoplasma hominis</em> + <em>Bacteroides spp.</em> + <em>E. coli.</em></td>
<td>31</td>
<td>14</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>288</td>
<td>222</td>
<td>66</td>
</tr>
</tbody>
</table>

% Were correlated to the number of cases in each group.

Figure (3): Cervicovaginal fetal fibronectin level relation to types and frequency of isolated microorganisms.
Discussion

Bacterial vaginosis is one of the most common genital infections in pregnancy. From 12 to 22 percent of pregnant women have this syndrome (Hay et al., 1994). Currently, pregnant women with asymptomatic bacterial vaginosis are not routinely screened or treated for this syndrome. Given the high frequency of bacterial vaginosis, its treatment could have a substantial effect on the incidence of preterm delivery of infants with low-birth weight. Among the organisms most commonly present as vaginal microflora are members of the genus lactobacillus, which play beneficial role with the production of lactic acid and H2O2. Lactic acid being responsible in part, for the low pH of the vagina which is inhibitory for many potentially pathogenic organism that colonize the vaginal environment in low concentration. H2O2 inhibitory effect is also known for organisms not producing catalase (Antonio et al., 1999).

In this study lactobacillus spp. were the most prevalent microorganism isolated alone in 42% and accompanied with Gardnerella vaginalis in 28%. However there was an over growth of other bacterial spp.; Bacteroides spp., Mycoplasma homins and E. coli in the vagina with a corresponding disappearance of lactobacillus spp. and this explain the protective beneficial role of presence of lactobacillus spp., which creates unfavorable acidic condition that is inhibitory to other potentially pathogenic organisms (Antonio et al., 1999). The results of the present study revealed that a significant high percentage of women were harboring lactobacillus spp. delivered at term (more than 37 weeks gestation) (40.6%) and only 1% with preterm delivery (less than 37 week gestation). This means that colonization of the genital tract with lactobacillus spp., had no effect on poor pregnancy outcome and do not play any role in preterm delivery of low birth weight infants. It is noteworthy that the presence of vaginal lactobacilli appeared to protect against preterm delivery in the present study. This is in according to Krohn et al., 1999, who reported similar results.
On the other hand there was strong evidence that other bacteria (Gardnerella vaginalis, Bacteroid spp., Mycoplasma hominis and E. coli) play an important role in preterm delivery of low birth weight infants.

In absence of Mycoplasma hominis, bacteroid spp. and E. coli; Gardnerella vaginalis had no effect on incidence of preterm delivery of low birth weight infants. However presence of Mycoplasma hominis, bacteroid spp. and E. coli had the highest incidence of preterm delivery of low birth weight infants independently of the presence of Gardnerella vaginalis.

Many other studies have reported an increased risk of preterm delivery among women with bacterial vaginosis (Kurki et al., 1992, Riduan et al., 1993 and Holst et al., 1994). Also the results of this study agree with that reported by Krohn et al., 1999 and McDonald et al., 1999; Kataoka et al., 2006, they reported the association between bacterial vaginosis, including anaerobic gram-negative rods, Gardnerella vaginalis and Mycoplasma hominis and preterm delivery.

Hay et al., 1994, concluded that late miscarriage and preterm delivery are associated with the presence of bacterial vaginosis and this is independent of recognized risk factors such as previous preterm delivery.

The mechanism by which bacterial vaginosis causes the preterm birth of an infant with low birth weight is not known, but there is evidence that it cause infection of the upper genital tract, which in turn causes premature birth (Pippa et al., 2002). In other studies, bacterial vaginosis has been associated with two to threefold increases in infection of amniotic fluids, infection of the chorion and amnion and histologic chorioamnionitis (Hillier et al., 1995).

Platz-Christensen, 1993, mentioned that pregnant women with bacterial vaginosis have elevated vaginal or cervical levels of endotoxin, mucinase and interleukin 1-α, suggesting that microorganisms that cause bacterial vaginosis stimulate the production of cytokines.

However the results of this study disagree with that obtained by Onderdonk et al., 2003, who reported that there was no significant differences in frequencies of isolation and mean counts for the major
Preterm Labour and its Relation To Bacterial Vaginosis and Fetal Fibronectin

microbiologic categories between the preterm delivery group and the term delivery group.

In women with abnormal bacterial vaginosis, monitoring of cervicovaginal fetal fibronectin concentration (which is a protein of the placental membranes) was found to be higher in pregnant women suffering from bacterial infection with *Gardnerella vaginalis*, *Bacteroids spp.*, *Mycoplasma hominis* and *E. coli*.

It is believed that ascending infection may lead to intrauterine infection which may disrupts the extracellular choriodecidual basement membrane, causing release of this protein into the cervix and vagina (Newton et al., 1997 Kataoka et al., 2006).

References


Preterm Labour and its Relation To Bacterial Vaginosis and Fetal Fibronectin


