An observational study of bacterial vaginosis in preterm and term labour at a tertiary care centre in South India

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Abstract
Background: Bacterial vaginosis (BV) is a clinical condition caused by replacement of the normal hydrogen peroxide producing Lactobacillus species with high concentrations of aerobic and anaerobic bacteria. Studies have shown that spontaneous abortion, preterm labour (PTL), premature birth, preterm premature rupture of membranes, amniotic fluid infection, postpartum endometritis are increased because of infection with BV. In India not many studies have been done to estimate the association of BV with peripartum and perinatal complications, hence this study was taken up to know the association of BV in preterm and term patients.

Objectives: To study the prevalence of BV in women presenting with preterm and term labour and to analyze its association as the causative factor of PTL. To analyze the maternal and fetal complications associated with BV.

Methods: An observational study involving 100 patients with preterm and term labour (50 patients in each group) was conducted at a tertiary care hospital in South India. BV was determined to be present or absent on the basis of Amsel’s criteria. Pearson’s chi-square test was used to demonstrate the difference between both groups with respect to various categorical data. Independent t-test was used to compare the mean maternal age and mean gestational age at admission in both the groups.

Results: The proportion of patients who fulfilled Amsel’s criteria for the diagnosis of BV was significantly more in PTL group as compared to term labour group, and the difference was statistically significant. In PTL group more number of neonates born to women who had BV had low birth weight as well as neonatal complications as compared to those born to women without BV. Maternal peripartum complications observed were also more in women with BV as compared to women without BV in PTL group.

Conclusion: BV is major risk factor for PTL. Therefore the testing for BV and its prompt treatment may reduce the risk of PTL. This will also go a long way in the prevention of neonatal complications due to prematurity.

Keywords: Bacterial vaginosis, Preterm labour, Term labour

Introduction
“The etiology of preterm labour (PTL) remains unknown, prediction lacks specificity, prophylaxis is unhelpful, diagnosis is difficult and the benefits and risks of tocolytic therapy still being debated”. The above quote testifies to the complexity of PTL, a process that ultimately results in considerable neonatal morbidity and mortality. PTL and delivery are among the most challenging obstetric complications encountered. It complicates about 5-10% of all pregnancies and in about 30% it is due to deliberate medical intervention and in the remainder due to spontaneous PTL which is associated with 75% of all perinatal deaths[1]. Prevention of morbidity lies by identifying the high risk patients.

Risk factors fail to predict as many as 70% of PTL. No single intervention has been thoroughly studied as much as transvaginal scan (TVS) and vaginal smear examination in screening for PTL. Bacterial vaginosis (BV) affects 6–32% of pregnant women [2]. BV may be symptomless or it may be accompanied by increased vaginal discharge which is a risk factor for preterm delivery, as well as being associated with peripartum complications such as preterm premature rupture of membranes (PPROM), chorioamnionitis, and postpartum endometritis. Western studies have revealed significant association of BV with preterm delivery, miscarriage and maternal infection [3]. In India not many studies have been done, hence this study was taken up to analyze its association as the causative factor of PTL.

Material & Methods
Detailed clinical history from the subjects was taken and recorded after obtaining informed consent. Clinical examination was done. Routine hematological, Urine and biochemical test were performed. Vaginal swab was collected from lower one-third of the vaginal wall which was subjected to Gram staining, wet mount and KOH test. The pH of vaginal discharge was tested using litmus paper. BV was diagnosed if 3 or more of the following criteria were present (Amsel’s criteria):
elevated vaginal pH > 4.5; thin, homogeneous gray-white discharge; amine odor upon the addition of 10% KOH to vaginal fluid on a glass slide (whiff test); and the presence of ‘clue cells’ on microscopic examination of vaginal fluid. A score of 0 to 10 was assigned depending on the Gram staining findings, on the basis of the relative proportions of easily distinguished bacterial morphologic types (i.e. large gram-positive rods, small gram-negative or variable rods, and curved rods). A score of 0 was assigned to the most lactobacillus-predominant vaginal flora, and a score of 10 was assigned to a flora in which Lactobacillus were largely replaced by Gardnerella, Bacteroides, and Mobiluncus (Nugent’s scoring)[4]. Women fulfilling the Amsel’s criteria and/or a score of 7 or more on Gram’s staining of the vaginal smear (Nugent’s score) were considered to have BV. The results were tabulated and analyzed using SPSS version 17.0. Pearson’s Chi-square test was used to find out the significance of differences in the various categorical data in both the groups. Independent t-test was performed to compare the mean maternal age and mean gestational age at admission in both the groups. The number of patients studied in both preterm and term labour groups was 50 each.

Inclusion Criteria:
Preterm Labour (Group 1): Gestational age ≤ 37 weeks with regular uterine contractions, Cervical dilatation ≥ 1 cm but < 4 cm and effacement ≥80% with intact fetal membranes.

Term labour (Group 2): Gestational age > 37 completed weeks, Spontaneous onset of labour with regular uterine contractions. Cervical dilatation ≥ 1 cm but < 4 cm with intact fetal membranes.

Exclusion Criteria: Pregnancies with Rh isoimmunization, Multiple gestation, Cervical cerclage, Structural uterine abnormalities, established fetal anomalies, complicated with medical disorders like hypertension, diabetes, chronic renal disorders, thyroid disorders, gastrointestinal disorders, severe cardiac disorders. Use of antibiotics in the preceding two weeks, use of tocolytic agents and corticosteroids during the current pregnancy were excluded from the study.

Results
The mean maternal age in both the groups was comparable 25.6 yrs and 25.3 yrs in PTL group and term labour group respectively with p value of 0.792. The mean gestational age at the time of admission in PTL was 33.5 weeks whereas in the term labour was 39 weeks. Previous history suggestive of sexually transmitted infection was present in 6(12%) of preterm and 1(2%) of term labour subjects with a p value of 0.050. The PTL group had more number of patients with the different grades of discharge as compared to term labour group as shown in Table [1]. The difference was statistically significant with p value of 0.005. The proportion of patients with vaginal discharge suggestive of BV was significantly more in PTL group 14(28%) as compared to term labour group 2(4%) with a p value of 0.001 as shown in Table [2]. Basic Vaginal pH was significantly more in preterm 22(44%) compared with term labour group 7(14%) with p value of 0.001 Table [3].

Whiff test was positive in 22(44%) in PTL and 9(18%) in term labour group. The proportion of patients who were diagnosed to have BV according to Amsel’s criteria was significantly more in PTL group than in term labour group, with a p value of 0.001 as shown in Table [4]. Significantly more number of patients in PTL group tested positive on vaginal swab culture sensitivity as compared to term labour group (p = 0.048) as shown in Table [5]. The mean CRP value recorded was significantly more in PTL group(1.287 ± 1.058) than in term labour group(0.748 ± 0.553), and the difference was statistically significant(p=0.002). In PTL group 53.3% of neonates born to BV positive mothers had low birth weight (LBW) as compared to 82.8% of neonates born to BV negative mothers. This difference was not significant statistically (p=0.230). In term group 50% of neonates born to BV positive mothers had LBW as compared to 18% of neonates born to BV negative mothers. This difference was not significant statistically (p=0.180).

In PTL group 3(20%) of neonates born to BV positive mothers had neonatal complications as compared to 4(11.4%) of neonates born to BV negative mothers. This difference was not significant statistically. In term labour group no neonates of BV positive mothers had neonatal complications, while 2(4.1%) of neonates of BV negative mothers had neonatal complications. The difference was not significant statistically. In PTL group 4(26.6%) of patients who were BV positive had postpartum complications as compared to 8(22.8%) of patients who were BV negative. This difference was not significant statistically (p=0.333). In term labour group none of the patients who were BV positive had post-partum complications, while 6(12.5%) of patients who were BV negative had post partum complications (p=0.270).

<table>
<thead>
<tr>
<th>Type of discharge</th>
<th>Preterm labour (n=50) (%)</th>
<th>Term labour (n=50) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No discharge</td>
<td>14 (28.0)</td>
<td>25 (50)</td>
</tr>
<tr>
<td>White mucoid discharge</td>
<td>12 (24.0)</td>
<td>19 (8.0)</td>
</tr>
<tr>
<td>White curdy discharge</td>
<td>10 (20.0)</td>
<td>4 (8.0)</td>
</tr>
<tr>
<td>Greyish white discharge</td>
<td>8 (16.0)</td>
<td>2 (4.0)</td>
</tr>
<tr>
<td>Grey frothy discharge</td>
<td>4 (8.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Greenish frothy discharge</td>
<td>2 (4.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>
Table 2: Discharge suggestive of Bacterial Vaginosis in preterm and term labour group

<table>
<thead>
<tr>
<th>Discharge</th>
<th>Suggestive of BV N=16</th>
<th>Not suggestive of Bv N=84</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PretermLabour (N=50)</td>
<td>14 (28.0)</td>
<td>36 (72)</td>
<td>Highly significant (p&lt;0.001)</td>
</tr>
<tr>
<td>TermLabour (N=50)</td>
<td>2 (4)</td>
<td>48 (96.0)</td>
<td>Highly significant (p&lt;0.001)</td>
</tr>
</tbody>
</table>

p < 0.001 (highly significant)

Table 3: Vaginal pH in preterm and term labour group

<table>
<thead>
<tr>
<th>Ph</th>
<th>Basic n (%)</th>
<th>Acidic n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm labour (n=50)</td>
<td>22 (44)</td>
<td>28 (56)</td>
<td>50 (100)</td>
</tr>
<tr>
<td>Term labour (n=50)</td>
<td>7 (14)</td>
<td>43 (86)</td>
<td>50 (100)</td>
</tr>
<tr>
<td>Total (n=100)</td>
<td>29 (29)</td>
<td>71 (71)</td>
<td>100 (100)</td>
</tr>
</tbody>
</table>

p = 0.001 (highly significant)

Table 4: Diagnosis of Bacterial vaginosis according to Amsel’s criteria in preterm and term labour group

<table>
<thead>
<tr>
<th>Amsel’s Criteria</th>
<th>≥ 3 Criteria n(%)</th>
<th>≤ 3 Criteria n(%)</th>
<th>Total (N=100) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm Labour (N=50)</td>
<td>15 (30.0)</td>
<td>35 (70)</td>
<td>50 (100)</td>
</tr>
<tr>
<td>Term Labour (N=50)</td>
<td>2 (4)</td>
<td>48 (96.0)</td>
<td>50 (100)</td>
</tr>
<tr>
<td>Total (N=100)</td>
<td>17 (17)</td>
<td>83 (83)</td>
<td>100 (100)</td>
</tr>
</tbody>
</table>

p = 0.001 (highly significant)

Table 5: Results of High vaginal swab culture in preterm and term labour group

<table>
<thead>
<tr>
<th>HVS C/S</th>
<th>Preterm Labour (N=50)</th>
<th>Term Labour (N=50)</th>
<th>Total (N=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Flora</td>
<td>39 (78.0)</td>
<td>47 (94.0)</td>
<td>86 (86.0)</td>
</tr>
<tr>
<td>Candida</td>
<td>4 (8.0)</td>
<td>3 (6.0)</td>
<td>7 (7.0)</td>
</tr>
<tr>
<td>Group B Streptococcus</td>
<td>1 (2.0)</td>
<td>0 (0.0)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>6 (12.0)</td>
<td>0 (0.0)</td>
<td>6 (6.0)</td>
</tr>
</tbody>
</table>

p = 0.048 (significant)

Discussion

A similar study done by Chawanpaiboon S et al. had a mean maternal age of 26.7 yrs and 26.6 yrs respectively. Both the groups had equal number of primigravida and multigravida. The PTL group had 60% primigravida and 40% multigravida while the term group had 51.8% primigravida and 48.2% multigravida[2]. The mean gestational age at admission was 33.6 weeks and 38.6 weeks in preterm and term groups respectively. In our study the mean gestational age at admission was 33.5 weeks in PTL group and 39.0 weeks in term group.

In PTL group 12% of patients had previous history of sexually transmitted infections as compared to 2% in term labour group. In the present study the most common sexually transmitted infection reported was Trichomoniasis. According to the results of the largest prospective study in the USA, T. vaginalis was significantly associated with LBW and preterm delivery[5]. Azargoon and Darvishzadeh[6] showed that there was no significant correlation between T. vaginalis with PTL birth.

The PTL group had significantly more number of patients with different grades of discharge as compared to term group (72% vs. 50% respectively). Study done by Paulo. CG et al have proved that lower genital tract infections are very common among apparently healthy looking pregnant women with an overall prevalence of 40-54%[7].

The number of patients who fulfilled Amsel’s criteria for diagnosis of BV were more in PTL group (30%) as compared to term group (4%). This observation correlated with other studies where it was concluded that BV is one of the important risk factors for PTL. Study by Hillier and coworkers showed that patients with BV were 40% more likely to have preterm delivery[8]. The study by Subtil et al also confirmed the association of BV with increased risk of preterm delivery[9]. In the present study BV was diagnosed in 30% of the patients who presented with PTL. Study by Mittal et al also showed 30% prevalence of BV in preterm group[10]. Another study done by Svare et al. showed a prevalence of 16% in PTL patients[11].

The number of patients having vaginal discharge suggestive of BV in PTL group were significantly more than in term labour group (28% vs. 4% respectively). In the study conducted by Chawanpaiboon S et al. discharge suggestive of BV was present in 24% and 25% of patients with PTL and term labour respectively[2].

Preterm group had more number of patients with basic vaginal pH as compared to term group (44% vs.14%). The number of patients having a positive Whiff test in preterm group was significantly more as compared to term labour group (44% vs.18%).

In the present study clue cells were not detected in either of the two groups. Absence of clue cells can be explained by the possibility of them having chronic
infection in which clue cells were absent due to local immune response to IgA antibodies. According to Easmon et al. it is not always necessary to see clue cells to make a diagnosis of BV and it is not included in the scoring system by Nugent which is more systematic and has a specificity of 95%[12].

The number of patients who had other genital tract infections were more in PTL group as compared to term group (22% vs. 6%). The commonest infections found in this study were Enterococcus (12%) followed by Candida (8%) and Group B Streptococcus (2%) in PTL group and Candida (7%), Enterococcus (6%) and Group B Streptococcus (1%) in term labour group.

Study conducted by Benchetrit et al. showed the presence of GBS colonization in 26% pregnant women evaluated[13]. In the present study 3% of the patients in PTL group were positive for GBS (2% in preterm group and 1% in term group).

Simoes et al. studied Candida albicans and found a prevalence of 19.3% for vaginal candidiasis in normal pregnant women in the third trimester[14]. In the present study, Candida albicans was identified in 8% of PTL group and 6% of term labour group. In the previous studies it has been shown that this association was influenced by increased levels of circulating oestrogens and deposition of glycogen and other substrates in the vagina during pregnancy[15]. Microbiota that inhabit the vagina play an important role in the spread of illnesses and the maintenance of a healthy genital tract.

The mean C-Reactive Protein value recorded in PTL group was 1.287 and in term labour group it was 0.748. In the study done by Halder A et.al out of 250 patients, 78 (31.2%) were CRP positive and 172 (68.8%) were CRP negative. CRP positivity showed positive association with PTL with odds ratio 2.384 (95% CI: 1.153–4.928 & p value 0.01)[16].

Study done by Hillier et al also showed the relation of BV with a significantly reduced mean birth weight[8]. Study by Svar et al also showed lower mean birth weight in BV [11]. In the present study however more number of neonates born to preterm BV negative group (82.8%) had LBW as compared to neonates born to preterm BV negative mothers (53.3%). This can be explained by the probability of factors other than BV responsible for LBW like anaemia and malnutrition which was present in 22 and 16 patients respectively in the present study.

In PTL group 20% of neonates born to BV positive mothers had congenital pneumonia, as compared to 11.4% of neonates born to BV negative mothers who had respiratory distress. In term labour group none of the neonates of BV positive mothers had neonatal complications, while 4.1% of neonates of BV negative mothers had respiratory distress, whereas none of the neonates of BV positive mothers had neonatal complications. This can be explained by the probable chances of other factors giving rise to such a complication in the term group such as birth asphyxia. This observation probably indicated that BV had no role in the causation of neonatal complications in case of term labour in this study.

In PTL group 26.6% of patients who were BV positive had postpartum complications as compared to 22.8% of patients who were BV negative; the most commonly seen complication was puerperal pyrexia. In term labour group none of the patients who were BV positive had post partum complications, while 12.5% of patients who were BV negative had post-partum complications. Out of the patients who had complications 5 patients had puerperal pyrexia and 1 patient had atomic PPH. This finding probably suggests the possibility of factors other than BV giving rise to post-partum complications in term women.

Eschenbach and co-workers were among the first researchers who studied the relationship between BV and PTL. In their study, 49% in preterm group and 24% in full term group had BV. Later they showed the correlation between BV and chorioamnionitis and PTL[16]. Prematurity Prediction Study carried out on 3000 women in the United States has shown the relationship between BV and PTL[17]. Vida Modares Nejad and co-workers, carried out a study to establish the association of BV and PTL, on 160 patients in Iran in 2008, reporting 25% prevalence of BV in patients with PTL and 11.3% prevalence in term patients[19]. In the present study BV was diagnosed in 30% of patients with PTL and 4% of patients in term labour.

**Conclusion**

From the present study it can be concluded that BV is a significant risk factor for PTL. Therefore the testing for BV and its prompt treatment may reduce the risk of PTL. This will also go a long way in the prevention of neonatal complications due to prematurity.

**Conflict of Interest: None**

**Source of Support: Nil**

**References:**


