EFFECT OF PROBIOTICS ON MICROBIAL FLORA IN PREGNANT WOMEN WITH BACTERIAL VAGINOSIS

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female lower genital tract is an ecological niche–aerobic and anaerobic microorganisms co-exist in a dynamic balance.

Bacterial Vaginosis (BV): affects 10 to 30% of women (Geeta gupta et.al., 2013; Hemalatha et.al., 2012; Lata et.al., 2010; Neeraja et al., 2009; Madhivanan et.al., 2008)

It is a polymicrobial vaginal condition – alteration of Lactobacillus predominant normal vaginal flora to an environment dominated by anaerobes, Gardnerella vaginalis, Mobiluncus, Prevotella, Bacteroides, and Mycoplasma species.

It is the most common infection in women of child bearing age and incidence during pregnancy is difficult to determine. Up to 40% women are asymptomatic (Susan Van et al., 2008).
Bacterial Vaginosis

- Increased frequency of other Infections
- Gynecological
  - Endometritis
  - Pelvic Inflammatory Disease
- Obstetric
  - PROM
  - PTD
  - Chorioamnionitis
- Neonatal
  - Neonatal Sepsis
- Increased frequency of other Infections
  - Candidiasis
  - Trichomoniasis
  - HIV
  - STD’s

BV is mostly sub-clinical, however, it leads to serious complications in pregnancy.
Available treatment:
Metronidazole (tablet or vaginal gel) is the most common and preferred antibiotic treatment for BV
Clindamycin (tablet or cream)
Tinidazole tablet

Side effects:
Indigenous gut flora gets disturbed due to oral antibiotic intake along with nausea
Healthy bacteria is effected by local antibiotic applications
Antibiotics are not advised in first trimester of pregnancy
Failure of antibiotics to change host receptivity to pathogen recurrences

Relapse of BV is very frequent (30-50%). Replacing the pathogenic bacteria with beneficial may aid in quicker recovery & lesser relapse.
Probiotics are "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" as defined by FAO/WHO. They have been recently studied a lot in preventing and treating vaginal infections. Lactobacilli are the commonest organisms used as Probiotics.

The rationale for use of Probiotics in women is based on the role played by the vaginal healthy microbiota and the need for restoration of this microbial ecosystem after infections.

A study on effect of probiotics (vaginal tablets containing lactobacilli) on vaginal health and pro-inflammatory cytokines showed nearly 80% cure rate (Hemalatha et. al., 2012).

There are studies showing the positive effect of probiotics as vaginal passaries in pregnant women (Samuli et al., 2001; Nishijima, 2005).
Clinical trials on probiotics use for treatment of bacterial vaginosis (BV).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Size</th>
<th>Type of study/duration</th>
<th>Intervention</th>
<th>BV cure rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ono et al., 2006b</td>
<td>40</td>
<td>R, OB, AC 30 days</td>
<td>Daily vaginal capsule containing L. rhamnosus GR-1 (109 CFU) and L. reuteri RC-14 (109 CFU) or 0.75% metronidazole gel b.i.d. for 5 days</td>
<td>65% compared to 33% metronidazole (P = 0.056)</td>
</tr>
<tr>
<td>Mastromarino et al., 2009</td>
<td>34</td>
<td>R, DB, PC 3 weeks</td>
<td>Daily vaginal tablet containing ≥109 CFU of L. brevis CD2, L. salivarius FV2, and L. plantarum FV9 for 7 days</td>
<td>50% compared to 6% control (P = 0.017)</td>
</tr>
<tr>
<td>Parent et al., 1996</td>
<td>32</td>
<td>R, PC 4 weeks</td>
<td>1-2 daily vaginal tablet containing L. acidophilus ≥107 CFU and 0.03 mg estriol for 6 days</td>
<td>88% compared to 22% control (P &lt; 0.05)</td>
</tr>
<tr>
<td>Hallén et al., 1992</td>
<td>57</td>
<td>R, DB, PC 20-40 days</td>
<td>Vaginal suppository containing L. acidophilus 10^{8.9} CFU or placebo b.i.d. for 6 days</td>
<td>21% compared to 0% control (P = NS)</td>
</tr>
</tbody>
</table>

R = randomized; DB = double blind; PC = placebo controlled; OB = observer blind. AC = active controlled. CFU = colony forming units.
Probiotics when used locally are effective in BV cure. However, the effects are transient and recurs within 3 to 4 weeks. Therefore, in the current study we used oral probiotics.

**Hypothesis**

We hence supplementation of probiotics to pregnant women will treat and prevent relapse of Bacterial vaginosis. Probiotics help to attain healthy vaginal flora.

**Objective:**

To study the effect of oral probiotics supplementation on Bacterial vaginosis (BV) cure and relapse in pregnant women.

This study is first of kind where oral supplementation of probiotics was given for pregnant women with BV.
ETHICAL APPROVALS

Institutional Ethical Committee (IEC) has approved the study, **EC NIN no: CR1/I/2014**

Drugs Controller General India (DCGI) approval obtained, **DCGI no: CT-/180/2012**

Clinical Trials Registry – India (CTRI) registered on 29th January 2013.

CTRI Registration No: **CTRI/2013/01/003337**

Osmania Medical college Ethics Scientific committee approval was obtained.

SAMPLE SIZE

Assuming 88% cure from BV in the probiotic and antibiotic treated group and 40% cure in placebo and antibiotic treated group (Kingsley et.al., 2006, Microbes and Infection: 1450-54) 50 women / group required to get significance at 5% with 80% power. Expecting 25% attrition, 70 women will be recruited per group i.e. 140 women with BV will be randomized into probiotic and placebo groups (but all of them are treated with local antibiotic, Clindamycin).
Inclusion Criteria

Pregnant women in 26-28 weeks of gestation

Exclusion Criteria

Cervical incompetence (Circlage in current gestation)

HIV infected and Hbs AG

Multiple gestation

Systemic arterial hypertension under medication

Chronic asthma requiring intermittent therapy

Patient not willing to comply with the clinical study instructions

Pre existing Thyroid disease

Gestational Diabetes / preeclampsia
Study design
Randomized, double blind, placebo controlled study conducted at Government Maternity Hospital

Supplementation
Probiotic - Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 with the concentration of $2 \times 10^8$ CFU/capsule. (The strains were initially isolated from vagina of healthy women by Dr. Gregor Reid & Dr. Andrew Bruce).

Placebo
Screening of the subjects is done by using **Nugent’s score** of the Vaginal smears.

<table>
<thead>
<tr>
<th>Species</th>
<th>None (0)</th>
<th>&lt;1</th>
<th>1-4</th>
<th>5-30</th>
<th>&gt;30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus morphotypes</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>G. vaginalis</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Mobiluncus spp</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

The count of all species was taken as total score.

<table>
<thead>
<tr>
<th>Total Score</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3</td>
<td>Normal Flora</td>
</tr>
<tr>
<td>4-6</td>
<td>Intermediate</td>
</tr>
<tr>
<td>7-10</td>
<td>Indicative of BV</td>
</tr>
</tbody>
</table>

Amsel’s criteria was also followed as the diagnostic measure:

- Homogenous white discharge
- Amine or fishy odor when added KOH
- pH more than 4.5
- Presence of clue cells
Image 1- Vaginal smear examination

Normal smear

Healthy microbiota- Lactobacilli

InfECTive smear

Epithelial cell whose borders are obscured by gram variable, pleomorphic pathogenic bacteria called ‘Clue cell’
Pregnant women in their initial days of third trimester
356 women screened for BV
Nugent’s score > 3 and Amsel’s criteria positive = 140
Anthropometry and blood sample were collected, Vaginal and
fecal samples were collected for analysis and 140 pregnant women
were randomized

Visit 1

Probiotic arm: 70 women Antibiotic
for seven days + Probiotic
supplementation until delivery

Placebo arm: 70 women Antibiotic
for seven days + Placebo
supplementation until delivery

30 days: Vaginal samples for BV
screening (n=64). Vaginal samples for
DNA isolation and Real-Time PCR
analysis & Microbiome analysis

30 days: Vaginal samples for BV
screening (n=67). Vaginal samples for
DNA isolation and Real-Time PCR
analysis & Microbiome analysis

Visit 2

Within 3-5th days of delivery,
Neonates’ anthropometry

Within 3-5th days of delivery,
Neonates’ anthropometry

Visit 4

45 days after delivery vaginal
samples were collected for BV
screening (n=25)

45 days after delivery vaginal
samples were collected for BV
screening (n=31)

Visit 5

Data could not be collected
from 9 subjects due to migration

Data could not be collected
from 34 subjects due to migration
Results
Incidence of BV in the screened subjects

Based on Nugent’s score, of the 356 pregnant women, 23.3% (83) had BV, 21.3% (76) had intermediate flora and 55.3% (197) had normal vaginal flora (Figure 1). Based on Amsel’s criteria, 42.1% (150) had BV and the rest were normal 57.8% (206). Of these pregnant women, 42% (150) were positive for BV by both the methods (Nugent’s score and Amsel’s criteria).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Probiotic group</th>
<th>Placebo group</th>
<th>Total</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.50±2.34 (70)</td>
<td>22.57±2.65 (70)</td>
<td>22.54±2.49 (140)</td>
<td>0.866</td>
</tr>
<tr>
<td>Height (cms)</td>
<td>153±5.2 (70)</td>
<td>153±5.19 (70)</td>
<td>153±5.19 (140)</td>
<td>0.881</td>
</tr>
<tr>
<td>Weight (kgs)</td>
<td>54.21±9.33 (70)</td>
<td>54.53±7.97 (70)</td>
<td>54.3±8.6 (140)</td>
<td>0.853</td>
</tr>
<tr>
<td>BMI</td>
<td>23.14±3.73 (70)</td>
<td>23.31±3.23 (70)</td>
<td>23.2±3.47 (140)</td>
<td>0.918</td>
</tr>
<tr>
<td>Hemoglobin (gm/dl)</td>
<td>10.12±1.36 (70)</td>
<td>10.29±1.45 (70)</td>
<td>10.2±1.4 (140)</td>
<td>0.466</td>
</tr>
<tr>
<td>Gestational age at recruitment</td>
<td>28.6±1.27(70)</td>
<td>27.8±1.82(70)</td>
<td>28.57±1.36(140)</td>
<td>0.872</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>39.8±1.92 (68)</td>
<td>38.97±1.31 (68)</td>
<td>38.9±1.6 (136)</td>
<td>0.327</td>
</tr>
<tr>
<td>Birth weight</td>
<td>2.73±0.39 (67)</td>
<td>2.62±0.5 (69)</td>
<td>2.67±0.43 (136)</td>
<td>0.985</td>
</tr>
<tr>
<td>New born baby’s length (cms)</td>
<td>48.79±1.7 (56)</td>
<td>47.25±2.3 (60)</td>
<td>47.9±1.8 (116)</td>
<td>0.083</td>
</tr>
<tr>
<td>Chest circumference (cms)</td>
<td>31.3±1.6 (56)</td>
<td>31.1±1.7 (60)</td>
<td>31.2±1.7 (116)</td>
<td>0.410</td>
</tr>
<tr>
<td>Head circumference (cms)</td>
<td>33±1.35 (56)</td>
<td>32.8±1.36 (60)</td>
<td>32.9±1.7 (116)</td>
<td>0.335</td>
</tr>
<tr>
<td>Mid upper arm circumference (cms)</td>
<td>9.58±0.84(56)</td>
<td>9.550.74(60)</td>
<td>9.560.79(116)</td>
<td>0.828</td>
</tr>
<tr>
<td>Low birth weight</td>
<td>13%</td>
<td>15%</td>
<td>20.7%</td>
<td>0.301</td>
</tr>
<tr>
<td>Pre-term births</td>
<td>5.0%</td>
<td>6.0%</td>
<td>5.8%</td>
<td>0.633</td>
</tr>
</tbody>
</table>
The demographic details of the subjects were similar in both the groups. In the anthropometric measurements, length of the baby was observed to be higher in the probiotic group compared to placebo group but was not statistically significant. The Nugent’s scoring during the post-natal follow-up significantly decreased in probiotic group compared to placebo showing reduction of infection. Based on Amsel’s criteria, during both the follow-ups probiotic group showed a significant reduction of infection. Presence of “Clue cells” which is considered as the manifestation of the infection also reduced during post-natal follow-up in probiotic group. Real-Time PCR analysis showed the dynamics of the microbial flora by increase of lactobacilli species quantity and in particular the supplemented strains.
Results continued...

Interestingly the other healthy species like *L. gasseri* and *L. jensenii* increased in probiotic group.

As expected, *G. vaginalis* decreased in probiotic group compared to placebo group.

L. Iners remained to be in similar quantities in both the groups.
Interpretations....

- The supplemented strains L. reuteri and L. rhamnosus; has colonized successfully and L. reuteri has shown specifically higher increase in probiotic group demonstrating the benefit of supplementation.

- L. gasseri and L. jensenii were always associated with normal vaginal flora and these two strains increased in the probiotic group suggesting that the probiotics may favorably affect the overall vaginal flora.

- Therefore, it may be speculated that oral supplementation of probiotics in pregnant women can aid in restoring the normal flora of vagina and can reduce the relapse of BV infection.

- The samples were also processed for Next Generation Sequencing and results awaited.