Bacterial vaginosis and genital pathogens in pregnant women

Le Roux MC, Ditsele RMM, Matebane KH, Nchabeleng M, Sethale KC
Main Topics

• Introduction
• Infection in pregnancy
• Bacterial vaginosis
• Genital pathogens
• SMU study 1: Laboratory diagnosis of BV
• SMU Study 2: BV and genital pathogens in pregnant women
• Discussion
• Conclusion
Introduction

- Intrauterine infection = one of the most important risk factors for complications in pregnancy
- Potentially preventable infections may account for up to 15% of early adverse pregnancy events and up to 66% of late adverse pregnancy events (Baud et al, 2008)
- Estimated 14.9 million preterm births in 2010, accounting for up to 18% of births in some African countries (Blencowe et al, 2012)
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Infection in pregnancy 1

• Presence of genital infection early in pregnancy increases the risk of PTB, premature rupture of membranes, low birth weight, small for gestational age, and placental abruption.

• Abnormal vaginal flora may occur:
  – because of a STI
  – colonization by an organism that is not part of the normal vaginal community, eg *Streptococcus pneumoniae*
  – by overgrowth or increased virulence of an organism that is a part of normal vaginal flora, eg. *Escherichia coli*
  – by bacterial vaginosis (BV)
Infection in pregnancy 2

- Some organisms have been associated with preterm delivery.
  - Bacterial vaginosis (BV)
  - *Neisseria gonorrhoeae*
  - *Chlamydia trachomatis*
  - *Ureaplasma urealyticum*
  - *Bacteroides* spp.
  - *Treponema pallidum*
  - Group B streptococcus (GBS)
  - *Trichomonas vaginalis*
  - *Mycoplasmia hominis*
  - Peptostreptococci.

- Ascending genital tract infections may contribute to up to 50% of premature deliveries.
Access to the amniotic cavity and the foetus gained:

- by organisms ascending from the vagina and the cervix
- hematogenous dissemination through the placenta
- retrograde seeding from the peritoneal cavity through the fallopian tubes
- accidental introduction at the time of invasive procedures such as amniocentesis.
Infection in pregnancy

• Difficult to assess the extent of the causal relationship between infection and PTB
• Colonization rates by microorganisms differ according to race, gestational age, geographical variation, and investigators.
• Socioeconomic variables, maternal smoking, genital infections and short cervix = risk factors for PTB.
• Not all contributory causes of PTB have been identified, → healthcare systems unable to target and manage relevant risk factors appropriately.
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Bacterial vaginosis 1

• Bacterial vaginosis = common cause of abnormal vaginal discharge
• Aetiology and pathogenesis remains unknown.
• Defined as:
  – ↓ vaginal *Lactobacillus* populations and ↑ diverse communities of anaerobic and facultative anaerobic bacteria species such as *Gardnerella vaginalis*, *Atopobium* spp., *Mobiluncus* spp. in the vagina leading to ↑ in vaginal pH.
Bacterial vaginosis 2

• Broad-range 16S rRNA gene PCR revealed a number of novel, fastidious, or uncultivated bacterial species including the *Clostridia*-like bacteria, bacterial vaginosis-associated bacteria (BVAB) 1, 2 and 3, and the uncultivated *Megasphaera*-like phylotype 1 (Fredricks et al, 2007)

• Although BV does not involve the cervix, it may be associated with:
  – acute cervicitis
  – post hysterectomy vaginal cuff infection
  – post abortion endometritis
  – increased risk of acquiring STIs, especially genital herpes and HIV
  – increased risk of spontaneous miscarriage ranging from 13 to 24 gestational weeks
  – preterm birth.
Bacterial vaginosis 3

• Diagnosis of BV difficult = no single responsible microbial etiologic agent

• Diagnosis can be clinical (Amsel’s criteria), by microscopy (Nugent’s score; Ison-Hay’s criteria) or molecular methods (targeting different organisms).

• With the Nugent scoring, interpretation of intermediate results is controversial.
  – Intermediate flora has been described as a less distinct transitional pattern between normal flora and BV.
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Genital pathogens

- **Neisseria gonorrhoeae:**
  - In late pregnancy is associated with preterm rupture of the membranes, preterm birth and low birth-weight, the association between infection in early pregnancy and these adverse outcomes is less clear *(RCOG, 2013)*

- **Chlamydia trachomatis:**
  - Contrasting results concerning the relation between chlamydia in pregnancy and preterm birth *(Rours et al, 2011)*

- **Trichomonas vaginalis:**
  - Estimated 1.2 fold increased risk of preterm birth *(Johnson et al, 2011)*
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SMU Study 1: Laboratory diagnosis of BV 1  
(Matebane et al, 2014)

• To compare Nugent score, standard multiplex PCR and real-time PCR for detection of BV

• Vaginal swab specimens collected from 200 pregnant women at DGMAH (TOP and antenatal clinics)
SMU Study 1: Laboratory diagnosis of BV

• Nugent scoring:
  – 0-3 = normal flora
  – 4-6 = intermediate
  – 7-10 = bacterial vaginosis flora

• Multiplex PCR:
  – BVAB 2 and Megasphaera 1

• Real-time PCR:
  – Gardnerella vaginalis, Atopobium vaginae
SMU Study 1: Laboratory diagnosis of BV

Nugent Score

- 0 (0.0%)
- 2 (1.0%)
- 3 (1.5%)
- 74 (37.0%)
- 31* (15.5%)
- 12 (6.0%)

* Includes all BV intermediate results

Nugent: 79 (39.5%)
M-PCR: 115 (57.5%)
Q-PCR: 120 (60.0%)
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• To determine the prevalence of bacterial vaginosis and genital pathogens in pregnant women, including those with a risk of adverse pregnancy outcomes

(Ditsele et al, 2014)
Patients:

- Swab specimens collected from 137 consenting pregnant women attending the ante-natal clinic of the Dr George Mukhari Academic Hospital in Pretoria, South Africa.
- 67/137 presented with a history of adverse pregnancy outcomes.

- Previous pre-term labour, miscarriage, still born foetus, PROM, IUFD, and abruptio placentae
SMU Study 2: BV and genital pathogens in pregnant women

- BV detected by Nugent scoring of Gram stained smears, and PCR targeting BV associated bacterium-2 (BVAB2) and Megasphaera-phyloptype1.
- *Neisseria gonorrhoeae* and *Chlamydia trachomatis* detected by real-time PCR (Sacace, Italy)
- *Trichomonas vaginalis* by standard PCR (Crucitti et al, 2003)
### SMU Study 2: BV and genital pathogens in pregnant women

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Age</th>
<th>Gestational Age</th>
<th>Parity</th>
<th>Gravidity</th>
<th>HIV pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without poor obstetric history (PNPOH) (n=70)</td>
<td>28 years (18-41)</td>
<td>30 weeks (21-36)</td>
<td>1.3</td>
<td>2.3</td>
<td>33% (22/66)</td>
</tr>
<tr>
<td>With poor obstetric history (PPOH)(n=67)</td>
<td>28 years (18-40)</td>
<td>30 weeks (16-36)</td>
<td>1.7</td>
<td>3.4</td>
<td>44% (19/43) (P=0.9)</td>
</tr>
<tr>
<td>TOTAL: 137</td>
<td></td>
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</tbody>
</table>
SMU Study 2: BV and genital pathogens in pregnant women

<table>
<thead>
<tr>
<th>Patient group</th>
<th>BV</th>
<th>NG</th>
<th>CT</th>
<th>TV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nugent Scoring</td>
<td>M-PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PNPOH (n=70)</td>
<td>26 (37.1%)</td>
<td>38 (54.3%)</td>
<td>2 (2.9%)</td>
<td>6 (8.6%)</td>
</tr>
<tr>
<td>PPOH (n=67)</td>
<td>27 (40.3%)</td>
<td>40 (59.7%)</td>
<td>1 (1.5%)</td>
<td>4 (6.0%)</td>
</tr>
<tr>
<td>TOTAL: 137</td>
<td>53 (38.7%)</td>
<td>78 (56.9%)</td>
<td>3 (2.2%)</td>
<td>10 (7.3%)</td>
</tr>
</tbody>
</table>
SMU Study 2: BV and genital pathogens in pregnant women

- BV (Nugent) P=0.73
- BV (PCR) P=0.61
- NG P=1.00
- CT P=0.76
- TV P=0.04
• All specimens positive for BV using the Nugent scoring were also positive by PCR.
• The 12 BV intermediate specimens were positive using PCR (100%).
• BV was detected in 37% (15/41) HIV pos patients, compared to 22% (15/53) HIV neg patients (p=0.12).

• Of the HIV pos patients with BV, 27.3% (6/22) and 47.4% (9/19) were in PNPOH and PPOH respectively (p=0.31).
SMU Study 2: BV and genital pathogens in pregnant women

• BV frequently detected, both with Nugent scoring (38.7%) and with PCR (56.9%).

• A higher prevalence of BV among patients with a poor obstetric history, difference was not significant.
  – Previous history may not have been due to the presence of infectious disease or the microbes identified in this study
  – The inclusion of women with a high risk of adverse pregnancy outcomes instead of women experiencing poor outcomes

• Limitation: Should have followed the pregnancy outcomes of all the pregnant women with BV enrolled in our study.
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Discussion 1

• Issue with intermediate Nugent scores
  – PCR has a higher discriminatory power, and it can be used to resolve the issue of ‘intermediate’ flora.
  – Women with intermediate flora may be in transition between normal flora and BV, which may indicate low bacterial loads of bacteria associated with BV.
  – The Ison-Hay’s or Claeys’ criteria may be a more accurate scoring system
Neisseria gonorrhoeae has been implicated in poor outcomes in late pregnancy

Chlamydia trachomatis: Role in PTB not defined

Trichomonas vaginalis:
- Review of 11 studies on the association of T. vaginalis with pregnancy outcomes, T. vaginalis in pregnancy was associated with an 1.2x increased risk of premature rupture of membranes; preterm birth and small for gestational age infants (Silver et al, 2014)
Discussion 3

• Debate: Screening and treatment of BV and trichomonas in pregnancy to reduce preterm birth rates?
• The high prevalence of BV, (symptomatic or asymptomatic) may indicate that pregnant women should be screened for BV, and treated to prevent adverse pregnancy outcomes.
• Prophylactic BV treatment to high risk pregnant women without confirmation by laboratory diagnosis?
  — Meta-analysis of 21 trials assessing the effects of antibiotic treatment of BV in pregnancy = little evidence that screening and treating all pregnant women with BV will prevent preterm labour and its consequences.
  — Treatment of BV during pregnancy does not improve preterm birth rates, and may in fact increase them. (Broklehurst et al, 2013)
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Conclusion

• Studies to be expanded and pregnancy outcomes evaluated in order to determine the value of screening and treating pregnant women for these pathogens.

• Given the existing infrastructure for antenatal screening and availability of effective treatment in many countries, it is essential to better understand the potential impact of antenatal screening for genitourinary pathogens with respect to the burden of preterm birth.
Acknowledgements

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