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REVIEW

Treatment of BV and Poor Pregnancy Outcomes: What Do Inconclusive Results Mean?

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Abstract: Bacterial vaginosis (BV) has been shown to double the risk of preterm delivery in pregnant women. But trials of antibiotic treatment of BV in pregnant women to prevent preterm delivery have been inconclusive. Without a good understanding of the etiology of BV, interpretation of these results has been challenging. As currently diagnosed, BV appears to be a heterogeneous condition and women with BV might have different risk profiles for preterm delivery depending on the presence of certain bacteria. Additionally, current diagnostic approaches may not adequately discriminate BV from other abnormal vaginal flora associated with mild inflammation. Finally, BV associated bacteria appear to ascend into the uterus very early in pregnancy resulting in early pregnancy losses and very early preterm delivery. In future trials efforts should be made to differentiate abnormal vaginal flora that might be associated with mild inflammation from BV and women should be screened and treated as early as possible, preferably before 20 weeks of gestation.

Keywords: bacterial vaginosis, antibiotic treatment, pregnancy outcomes

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Introduction

Bacterial vaginosis (BV) is the most common cause of abnormal vaginal discharge in women of reproductive age.[1] In the United States of America, prevalence as high as 29.2% has been reported among women aged 14–49 years. Women from minority groups are the most affected with prevalence as high as 51.4% among non-Hispanic blacks, 31.9% among Mexican Americans, and 23.2% among non-Hispanic whites.[2] BV has been shown to be associated with poor pregnancy outcomes including early[3] and late miscarriages,[4–8] preterm pre-labor rupture of membranes, low birth weight, and preterm labor and delivery.[9–11] The cause of BV is still being debated[12,13] and the mechanisms through which BV leads to poor pregnancy outcomes is not well understood. BV is a poly-microbial syndrome or an ecological disorder of the vaginal flora in which the normal *Lactobacillus*-dominant flora is replaced by a 100–1000-fold increase in the numbers of anaerobic bacteria.[14] It is believed that BV associated bacteria ascend to the uterus to cause intra-uterine infection that results in pregnancy losses.[15] However, trials of antibiotic treatment of BV in pregnant women to prevent preterm delivery have been inconclusive.[16–28] In the absence of a well established etiology for BV and of a better understanding of the patho-physiology that results from BV to preterm delivery, interpretation of trials that evaluated treatment of BV with antibiotics to prevent preterm delivery has been challenging. Do the inconclusive trial results mean that the association between BV and preterm delivery is not causal? Or do they mean that the intervention (antibiotic treatment) is not appropriate? In this paper we revisit the definition of both normal and abnormal vaginal flora, BV and preterm delivery, the results of experimental interventions to treat BV with antibiotics for prevention of preterm delivery, and suggest direction for future research.

Vaginal Flora

Normal vaginal flora

The vaginal flora, the bacteria that live inside the vagina, of a healthy asymptomatic woman or "normal vaginal flora" consists of a wide variety of anaerobic and aerobic bacterial genera and species dominated by the facultative, microaerophilic, anaerobic genus *Lactobacillus*.[29] The activity of *Lactobacillus* sp. is essential to protect women from genital infections and to maintain the natural healthy balance of the vagina. In normal conditions, *Lactobacillus* sp. utilize available glycogen to produce lactic acid, which acidifies the vaginal pH to less than 4.5,[30,31] inhibiting the growth of non-acid tolerant and potentially pathogenic microorganisms.[32,33] Moreover, some *Lactobacillus* sp. also produce hydrogen peroxide, a potent antimicrobial molecule, which has been shown to protect against acquisition of BV.[34]

In recent years, molecular techniques based on the analysis of rRNA gene sequences have been developed, providing powerful tools to reveal the phylogenetic diversity of the microorganisms found within complex ecosystems and understand community dynamics.[36–38] One major advantage of the molecular-based technique as compared to the traditional culture-base methods is the ability to detect several bacterial populations not readily culturable. Application of these culture-independent methods to the studies of vaginal flora show that healthy women had vaginal bacterial biotas which is dominated by Lactobacilli and is made of very limited bacterial species (1 to 6) whereas women with abnormal vaginal flora had greater bacterial diversity (up to 35 bacterial species for BV).[37,39]

Abnormal vaginal flora

Donders has proposed a summary of the definition and classification of abnormal vaginal flora.[40] Generally, vaginal flora is classified in three categories: normal, intermediate, and abnormal. The classification is usually based on the relative quantities of lactobacillary morphotypes and/or other microorganisms present in the vaginal fluid.[41–44] The Gram-smear scoring and the lactobacillary grading system are the two most commonly used classification systems.

The Gram-smear scoring system proposed by Nugent et al summed the weighted quantification (0, 1 to 4) of the following morphotypes to yield a score of 0 to 10 for each person: large Gram-positive rods—lactobacillus morphotypes—weighted such that absence yields the highest score, small Gram-negative to -variable rods—*Gardenerella vaginalis* and *Bacteroides* sp. morphotypes, and curved Gram-negative rods—*Mobiluncus* sp. morphotypes. A score of 7 or higher corresponds to full blown bacterial vaginosis; a score of 4 to 6 is defined as intermediate vaginal flora, and a score of 0 to 3 corresponds to normal vaginal flora.[43]

Donders et al recently proposed another scoring system called "lactobacillary grades" with four categories in which abnormal vaginal dominated by aerobic microorganisms is clearly differentiated from BV.[41,42] In this new scoring system, the normal—grade I—flora corresponds to predominantly lactobacillary morphotypes, with very few coccoid bacteria present. The intermediate—grade II—flora corresponds to a diminished lactobacillary flora, mixed with other bacteria. This group is subdivided into slightly disturbed, fairly normal: grade IIa (if lactobacillary morphotypes still outnumber the other bacteria) and moderately disturbed, rather abnormal: grade IIb (if lactobacillary morphotypes are less abundant than the other morphotypes). Finally, the grossly abnormal—grade III—flora consists of numerous other bacteria, with no *Lactobacilli* present.[41,42] Lactobacillary grade III indicates complete disruption of the normal vaginal flora: other bacteria have completely overgrown the *Lactobacilli*, which are no longer visible. Donders et al separate this group into "aerobic vaginitis" and bacterial vaginosis: a typical characteristic of BV being the absence of inflammatory reaction while in aerobic vaginitis, the grade III flora are dominated by coarse, coccid bacteria and are often accompanied by numerous parabasal epithelial cells and increase leukocytosis. Additionally, culture of this flora often grows aerobic microorganism such as Escherichia coli, group B streptococci, enterococci.[41,42]

Bacteria vaginosis

BV is a disruption of the normal vaginal flora—shifting from the predominance of *Lactobacillus* species to an overgrowth of Gram-negative and anaerobic bacteria (*Gardenerella vaginalis, Bactroides sp, Provetella sp, Mobiluncus sp*, and *Peptostreptococcus sp*) and genital mycoplasmas (*U. urealyticum* and *M. hominis*).[45] However, these microorganisms, including *Mobiluncus sp.* are naturally found in the vaginas of healthy women[45–47] and the threshold at which they may become pathogenic is not clear. To add to the confusion, the disruption can resolve spontaneously without any intervention,[16] while treatment with either of the two antibiotics used in the recommended first-line therapy (metronidazole or clindamycin) does not always result in full resolution of BV[48] and recurrence rates above 20% at 1 month after treatment have been reported.[49]

BV is often asymptomatic. Clinically, it is characterized by the presence of a thin, homogeneous discharge which adheres to the vaginal walls, vaginal pH above 4.5, release of fishy odor upon alkalinization with 10% potassium hydroxide and clue cells on a saline wet mount. If three of these four Amsel criteria are met, the patient is diagnosed with BV.[50] The laboratory diagnosis of BV is based on Gram-stained vaginal smear scoring. The Nugent score has been reported as reliable and replicable for the diagnosis of BV.[43] As described above, a score of 7 or higher corresponds to full blown bacteria vaginosis while a score of 4 to 6 is defined as intermediate vaginal flora.[43]

Preterm Delivery and Role of Infection

Preterm deliveries, ie, those that occur at less than 37 weeks gestation usually occur as a result of medical decision for either maternal or fetal indication in 33% of cases that occur before 30 weeks, 26% of those that occur between 31–34 weeks, and 22% of deliveries between 35–36 weeks of gestation.[51] Spontaneous preterm births occur as a consequence of spontaneous preterm labor or preterm pre-labor rupture of fetal membranes before the onset of labor. Four different pathways lead to spontaneous preterm birth: excessive myometrial and fetal membrane overdistention, decidual hemorrhage, precocious fetal endocrine activation, and intrauterine infection or inflammation.[52,53] Infections play a major role in the genesis of spontaneous preterm birth and may account for 25%–40% of these events.[54,55] The frequency of infection in preterm birth is inversely related to the gestational age. Infection is rare in late preterm deliveries (at 34 to 36 weeks) but is present in most cases in which birth occurs at less than 30 weeks.[15] Women with preterm pre-labor rupture of membranes at very early gestational age—between 24 to 28 weeks for instance—are at increased risk of having underlying intrauterine infection.[53] Microorganisms may gain access to the amniotic cavity and fetus using any of the following pathways: 1) ascending from the vagina/cervix (in the case of BV), 2) haematogenous dissemination through the placenta, 3) retrograde seeding from the peritoneal cavity, and 4) accidental introduction during invasive procedures.[54,55] In women in spontaneous preterm labor with intact membranes, the most commonly identified bacteria are *Ureaplasma urealyticum, Mycoplasma*

hominis, Gardnerella vaginalis, peptostreptococci, and bacteroides species.[15]

BV and Risk of Preterm Delivery

Three meta-analyses in English from two independent groups are available on MEDLINE on the association of BV and poor pregnancy outcomes.[10,11,56]

The first meta-analysis by Flynn et al includes eighteen studies published in 1996 or earlier.[56] Only two[57,58] of these 18 studies reported a non-statistically significant association between BV and preterm delivery. However, analyses showed substantial and significant evidence of publication bias in favor of large studies showing an increased risk of preterm delivery among women with BV (negative association). In addition, there was substantial and highly significant heterogeneity among studies.[56] Women with BV were found to be more likely to deliver a preterm infant (summary random Odds ratio (OR) 2.05; 95% Confidence Interval (CI), 1.67–2.50) or an infant weighing less than 2500 grams (1.73; 1.11–2.69). Seven[9,59–64] of these eighteen studies reported adjusted estimates for the association between BV and poor pregnancy outcomes. The summary estimates of the adjusted ORs were lower than that obtained from unadjusted data (1.60; 1.44–1.74).[56] In the largest study included in this meta-analysis, the adjusted effect size was the lowest (1.4; 1.1–1.8)—after adjustment for smoking, race, any previous delivery of a low-birth-weight infant, any loss of an earlier pregnancy, gravidity, maternal age, marital status, any use of antibiotics, and any colonization with *C. trachomatis, N. gonorrhoeae, T. vaginalis,* or group B streptococci.[65]

The second and third meta-analyses are from Leitich and collaborators.[10,11] Their initial analysis included eighteen studies published prior to July 2001. Of these eighteen studies, eight[5,6,63–68] were published prior to 1996 and had been included in the Flynn analyses. No formal assessment for publication bias was reported. Considerable and highly significant heterogeneity was also found among these studies. The summary random OR for the association between BV and delivery before 37 weeks was roughly identical to that found by Flynn et al (2.19; 1.54–3.12; thirteen studies involving 14740 women). However, the strength of the association between BV and delivery before 37 weeks was strongly correlated with the timing of BV diagnostic in pregnancy. The summary OR for the association between BV and delivery before 37 weeks of gestation was: 7.55 (95% CI, 1.80–31.65; three studies involving 1400 women) when screening for BV was done before 16 weeks of gestation, 4.20 (95% CI, 2.11–8.39; five studies involving 2763 women) when it was done before 20 weeks of gestation, and 1.53 (95% CI, 1.29–1.82; nine studies involving 12459 women) when screening was done at 20 weeks of gestation or after. However, when the authors updated their analyses in 2005, while the summary OR for the association between BV and delivery before 37 weeks did not change: (2.16, 1.56–3.00; 24 studies involving 24190), screening earlier in pregnancy was no longer associated with a risk for preterm delivery that was distinctively different from the result of the main analysis: 2.97 (95% CI, 1.48–5.98; seven studies involving 3292 women) when screened before 16 weeks, 2.15 (95% CI, 1.34–3.43; eleven studies involving 9688 women) when screened before 20 weeks, and 1.89 (95% CI, 1.27–2.83; fifteen studies involving 15415 women) when screened at 20 weeks or later. BV was strongly associated with late miscarriages (6.32; 3.65–10.94; five studies and 2010 women) and there was no evidence of heterogeneity among the five studies in this sub-analysis.[11]

Overall, BV appears to double the risk of delivery before 37 weeks. However, the considerable and highly significant heterogeneity among studies call into question the validity of this association. This heterogeneity among studies can be attributed to at least 3 sources: 1) the heterogeneity of the cut-off for the definition of preterm delivery and the varying role of infection depending on the cut-off, 2) the timing of BV screening in pregnancy and potential for left censoring bias, 3) the heterogeneous nature of BV as a condition itself.

As discussed above, the role of infection in preterm birth is inversely related to the gestational age: rare in late preterm deliveries (34 to 36 weeks) but present in most cases in which birth occurs before 30 weeks of gestation.[15] The strong association between BV and late miscarriage and the lack of heterogeneity among studies included is indicative of a potential causal relation. It appears that BV associated bacteria ascend to the uterus very early in pregnancy and induce miscarriages or very early preterm deliveries throughout the second trimester. The high heterogeneity and the

weakening of the strength of association observed with increasing gestational age correlate well with the known decreasing role of infection in preterm delivery as the gestational age increases.

There is a potential for left censoring bias in studies of the association between BV and preterm birth that arises from the fact that when the screening is done late (at 20 weeks or after), some women who were at very high risk of early pregnancy loss because of BV or BV-associated bacteria might have already experienced the negative outcome and are not included in these studies. Thus depending on the timing of BV screening, women included in studies might have different risk profile for preterm delivery.

As currently diagnosed, BV is a confusing and heterogeneous condition. For instance, depending on co-infection with *M. hominis, Bacteroides* sp., or both, women with BV may have a completely different risk profile during pregnancy.[65,69] In a recent study, Vogel et al[70] found evidence to suggest that *U. urealyticum* might be a necessary cofactor to induce poor pregnancy outcomes. Moreover, though a typical characteristic of BV is the lack of inflammatory reaction, in a study to determine which clinical signs have an important contribution in BV diagnosed by Nugent's criteria, Hapsari et al reported that women with BV more often have yellowish color discharge compared to those without BV.[1] It has been shown that women with five or more neutrophils per high power oil field are statistically at increased risk for early preterm delivery (<32 weeks) and if the inflammation was associated with elevated vaginal PH > 5, the OR for early preterm delivery was 2.9 (95% CI, 2.0–4.3).[71] These observations have been corroborated by results of a study of the predictive value for preterm birth, of abnormal vaginal flora, bacterial vaginosis and aerobic vaginitis during the first trimester of pregnancy which demonstrated that the absence of *Lactobacilli*, partial BV (defined as patchy streaks of BV flora or sporadic clue cells mixed with other flora), and *M. hominis*, but not full BV, were associated with an increased risk of preterm delivery after 24 weeks.[72] The suggestion that *Mycoplasma* and *Ureaplasma* are important cofactors for BV to induce preterm delivery raises the question of whether antibiotics used in trials of treatment of BV to prevent preterm are appropriate.[73]

Antibiotic Treatment to Prevent Preterm Delivery

Antibiotics traditionally used to treat BV include the 5-nitro-imidaloes (metronidazole), or lincosamide (clindamycin).[74] Metronidazole inhibit anaerobes that support *Gardeneralla vaginalis* but do not affect *Lactobacilli*.[75] However, metronidazole is not active against aerobes and has no activity against *Mobiluncus, Ureplasma urealyticum and Mycoplasma hominis*.[73] Clindamycin on the other hand, exhibits a broad range of antimicrobial activity. *Streptococci*, *Gardenerella vaginalis, Neisseria gonorrhoea,* and *Mobiluncus* are sensitive to clindamycin.[76] But *Ureplama urealyticum* is only partially sensitive to clindamycin.[77] In addition, clindamycin is also active against the protective *Lactobacillus* species, and as a consequence of treatment, the vagina could become colonized by potentially pathologic Gram-negative organisms against which clindamycin has poor activity such as *E coli*.[73]

Both metronidazole and clindamycin have been shown to be effective in the eradication of symptoms of BV, achieving clinical cure in about 91% of cases after two weeks,[75] though relapse rates above 20% at 1 month after treatment have been reported.[49] The recent Cochrane review on antibiotics for treating bacterial vaginosis in pregnancy shows that antibiotic treatment of BV in pregnancy does not reduce the risk of delivery before 37 weeks of gestation. However, treatment before 20 weeks' gestation was associated with a reduction of risk of delivery before 37 weeks (OR, 0.63; 95% CI, 0.48–0.84; five trials, 2387 women). Treatment was also found to be associated with a reduction of risk of delivery before 37 weeks and the risk of late miscarriages in women with abnormal vaginal flora (intermediate flora or bacterial vaginosis) (OR, 0.51; 95% CI, 0.32–0.81; two trials, 894 women, and 0.25; 95% CI, 0.08–0.79; one trial, 485 women).[78]

The fact that early treatment (before 20 weeks of gestation) appears to reduce the risk of preterm birth and that of late miscarriages adds weight to the above observation that the association between BV and late miscarriages and very early preterm birth might be causal. Additionally, the observation that antibiotic treatment of abnormal vaginal flora rather than treatment of BV alone might reduce the risk of preterm is consistent with the report by Donders et al that abnormal vaginal flora and *M. hominis*, but not full BV in

the first trimester, are associated with an increased risk of preterm delivery.[72]

Conclusion

This review sought to further the understanding of the association between BV and preterm delivery and why trials of antibiotics treatment of BV in pregnancy to prevent preterm delivery have not yielded a conclusive result. As currently diagnosed, BV is a very heterogeneous group in terms of risk profiles for preterm delivery. There is increasing evidence that Mycoplasmas and Ureaplasmas might be important co-factors for BV to induce preterm delivery.[69,72] In addition aerobic vaginitis and mixed flora which correspond to Nugent score between 4 and 8 might equally be important.[71,79] It appears that bacteria associated with BV and other aerobic vaginitis ascend to the uterus very early in pregnancy and induce early pregnancy losses through the second trimester and by ≥20 weeks of gestation when women are screened for most trials, that women at very high risk for late miscarriage or very early preterm have already experienced the negative outcome.[79] Future trials of treatment with antibiotics to prevent preterm delivery in pregnant women with BV or abnormal vaginal flora should screen women for BV as early as possible. With the increasing availability of molecular techniques, it will also be helpful to examine the specific role of BV-associated bacteria in preterm delivery and their clearance by antibiotics use in the intervention treatment to prevent poor pregnancy outcomes. The role of inflammation in the pathogenesis of preterm is also increasingly being recognized.[55,71] Future studies of BV and preterm delivery could reduce heterogeneity by combining the lactobacillary grading of vaginal flora with the Nugent score to clearly differentiate mild vaginitis from vaginosis.[42,43]

Finally, it is important to note that though *Mycoplasma* are resistant to metronidazole, this drug is often effective in treating BV and in eliminating *M. hominis*,[77] suggesting that *M. hominis* prospers in the milieu created by the other bacteria. If treatment is to be administered early in pregnancy, it will be important to pay special attention to the high recurrence rate observed after treatment and identify means to reduce this.[49] Although there is increasing evidence that BV is sexually transmitted,[12] we have recently demonstrated that reported consistent condom use was not effective in preventing this recurrence though it statistically reduced new acquisition,[13] pointing to actual failure of treatment to eradicate BV associated bacteria.

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Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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