Recurrent Bacterial Vaginosis

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Bacterial vaginosis (BV) is a common cause of vaginal discharge in women of childbearing age. In some individuals, it recurs frequently after treatment, frustrating both the patient and the physician. Standard BV treatment—metronidazole or clindamycin, administered either intravaginally or orally—is followed by relapse in approximately 30% of cases, within one month. Our inability to prevent relapse reflects our lack of understanding of how BV originates. BV has been associated with infectious morbidity in obstetrics and gynecology. Recent studies have found it to be a risk factor for HIV spread. These findings increase the need for us to be able to control recurrent BV and reduce its prevalence in the general population.

Introduction

Bacterial vaginosis (BV) is the most common cause of abnormal vaginal discharge in women of childbearing age. It is characterized by an overgrowth of anaerobic or facultative anaerobic organisms in the vagina. This is accompanied by a rise in pH from below 4.5 to levels as high as 7.0, and a fishy smell due to increased levels of polyamines and trimethylamine. In some women, BV relapses and remits spontaneously. Many women are asymptomatic during transient phases of BV, but some experience frequent symptomatic episodes, which cause frustration for both patients and their physicians. If we are to prevent relapse, we need a better understanding of the factors that lead to the development of BV. Only then are we likely to improve treatments, which currently have a failure rate of 30% after 1 month [1,2].

Apart from the need to improve care for patients, control of BV may have important public health implications. BV has been associated with complications in pregnancy, including miscarriage, preterm birth, and postpartum endometritis. Studies investigating the use of antibiotics during pregnancy have not always confirmed that current treatments are effective in improving pregnancy outcome [3•]. BV is associated with infectious morbidity following gynecological surgery, such as hysterectomy or termination of pregnancy. In recent studies, BV is emerging as a co-factor for sexually transmitted infections and, importantly, acquisition of HIV by women. If ways of reducing the prevalence of BV can be found, they may help reduce the incidence of sexually transmitted HIV infection [4]. This review will focus on the etiology of BV and possible approaches to recurrent BV.

Etiology and Natural History of BV

We do not know the initial step in the onset of BV. From in vitro studies, we know that, at a pH of less than 4.5, hydrogen peroxide (H_2O_2)-producing Lactobacilli effectively inhibit the growth of BV-associated organisms, but the effect wanes at higher pH levels [5]. Is a change in pH—such as the change that occurs at the time of menstruation or following unprotected intercourse—sufficient to trigger BV? If sufficient numbers of bacteria are inoculated into the vagina by a partner, will BV develop? Another possible trigger is anything that reduces the number or quality of lactobacilli. Interestingly, broad-spectrum antibiotics that inhibit lactobacilli do not seem to trigger BV [6], possibly because they also inhibit some of the BV organisms. Several investigators have used Gram-stained vaginal smears (prepared by the study subjects) to examine day-to-day changes in vaginal flora; a scoring system that classifies normal, intermediate, and BV flora facilitated these studies [7,8].

Prevalence

Epidemiological associations can generate hypotheses about the etiology of BV. The reported prevalence of BV varies widely—from 5% to 51%—between different populations. In the United States, Bump and Buesching [9] reported a prevalence of approximately 13% among adolescent girls. We reported a similar prevalence among women in a gynecology clinic [10] and an antenatal clinic [8] in the United Kingdom. The incidence of BV is reported to be higher among women receiving abortions (28%) [11], and among a group of women receiving in vitro fertilization (IVF) treatment (24.6%) [12•]. A high incidence of BV was reported in some US populations, such as pregnant inner-city women (32.5%) [13]. The highest incidence, however, was reported by the Rakai Project Study Group, in rural Uganda, where 50.9% of women had BV, and 23.8% demonstrated a prevalence for *Trichomonas vaginalis* [14]; 80% of these women were asymptomatic. Most women

with Trichomoniasis have intermediate flora or BV, so the role of *Trichomonas* in this high prevalence of BV needs to be determined.

These studies report point prevalences. Three studies of asymptomatic volunteers examined self-prepared vaginal smears by Gram stain, collected over a period of one to two months. Gram-stain changes compatible with the onset BV occurred in 36% (nine of 25) [15], 25.5% (13 of 51) [16•], and 12% (two of 17) of the study subjects [17]. It may be that, in most populations, the majority of women develop asymptomatic BV at some time. The entity that causes a BV relapse is not necessarily the same one that initiated the first episode.

Epidemiological Associations

Most studies find associations between BV and factors including being of the black race, receiving oral sex, smoking, and using an intrauterine contraceptive device (IUCD). Anecdotally, many women report the occurrence of BV after changing sex partners. There is some evidence that BV behaves as a sexually transmitted disease (STD), but the original study by Bump and Buesching found no difference in BV prevalence between virgin and nonvirgin adolescent women [9]. What do recent clinical studies tell us?

Observational Studies

Schwebke and colleagues [16•] carefully examined, by Gram stain, daily vaginal smears from 51 women at low risk for STDs. Each participant collected daily smears for as long as 6 weeks. Only 11 women had normal flora throughout the length of the study; 25 demonstrated intermediate-pattern flora at some point; and 13 developed BV, five reporting symptoms. Significant associations were made between developing BV and prior BV (44% vs 12%), mean number of lifetime partners (13.4 vs 7.15), and a greater mean number of episodes of receptive oral sex (3.6 vs 1.4). Only oral sex remained significant in the multivariate analysis. Changes in flora were also associated with menses, and use of vaginal medication or spermicide. The earlier study by Keane and colleagues [17] similarly reported that increasing abnormalities of vaginal flora occur in the first 9 days of the cycle. Another study of staff volunteers concluded that "abnormal flora" occurs in most women at some point, and that we might need to revise our concept of what "normal flora" is [15].

None of these studies reported the onset of BV following unprotected intercourse, although it is often hypothesized that the alkaline nature of semen might encourage the growth of BV organisms.

One other study examined daily smears from 18 women with recurrent BV. Again, BV usually developed early in the menstrual cycle and resolved spontaneously in the second half of the cycle. It frequently followed episodes of vaginal candidiasis and, if anything, was more likely to resolve after unprotected sex with a regular partner, than to appear. In some women, the onset and resolution of BV occurred within two to three days, usually passing through an intermediate stage [18].

The association with resolution of candidiasis is interesting, in light of an in vitro study that demonstrated inhibition of candidal growth by putrescine and cadaverine [19]. These amines are produced by *Gardnerella* and other organisms found in BV. An earlier study also reported an association between *Candida* and recurrent BV [20].

Sexual Lifestyle

A Swedish study employed midwives to conduct interviews with 996 women at family planning and youth clinics. The prevalences of BV and *Chlamydia trachomatis* were 13.7% and 8.9%, respectively [21]. The authors reported that, compared with women who have neither condition, women who have either BV or *Chlamydia* demonstrate similarly higher-risk lifestyles. Thus, women with BV are more likely than those without it to have had multiple sexual partner in the previous month, or to have taken part in group sex.

Vaginal Douching

Vaginal douching and other washing practices are frequently cited as a cause of disturbance of the vaginal flora, leading to the onset of BV. In a prospective study, douching was associated with the loss of protective H_2O_2-producing lactobacilli and the acquisition of BV [22]. A case-control study of 200 women attending a genitourinary medicine clinic in London investigated associations between vulval washing, vaginal washing, and douching and BV [23•]. BV was more common in black Caribbean women than in white women (odds ratio [OR], 2.1, 95%; confidence interval [CI], 1.1–4.1). Use of bubble bath, antiseptic solution, and douching was more common among women with BV. Prior history of BV was the strongest predictor for current BV (OR, 13.4; CI, 5.5–32.6). In the multivariate analysis, after controlling for washing practices, there was no ethnic difference in the incidence of BV. The authors commented that little is really known about the frequency of douching in the United Kingdom, but 30% of their study population reported using any preparation intravaginally.

In contrast, consider a Chapel Hill, North Carolina, study of 842 women early in the third trimester of pregnancy. After controlling for many possible confounding variables, including douching, these authors reported that race remains associated with BV, which they found in 22.3% of blacks and, in comparison, 8.5% of whites. Black women were also more likely to have the highest Nugent scores (9 or 10), weighted by the presence of high counts of *Mobiluncus* morphotypes. Although other published studies report that, in the United States, black women are

2.5 times more likely to douche than are white women, this study [24] recorded douching among only 5% of blacks and 4% of whites. The authors correctly noted that behavior may change during pregnancy. We need randomized, prospective studies of douching, to genuinely ascertain its importance.

Microbiological Studies

One possible treatment for BV is to recolonize the vagina with healthy lactobacilli. A recent study of 215 sexually active women used whole-chromosomal DNA probes to determine the commonest strains of vaginal lactobacilli. The most prevalent strain was *Lactobacillus crispatus* (32%), followed by *Lactobacillus jensenii* (23%), and a previously undescribed species, designated *Lactobacillus 1086V* (15%) [25•]. An in vitro study investigated the rate of acid production by *Lactobacillus* species [26]. They acidified their growth medium to an asymptotic pH of 3.2 to 4.8. In contrast, BV-associated organisms—such as *Gardnerella vaginalis*, *Prevotella bivia* and *Peptostreptococcus anaerobius*—reached an asymptotic level at pH 4.7 to 6.0, consistent with the pH levels found in BV. The authors calculate that 3 mL. of semen would be acidified at a rate of 0.56 to 0.75 pH units per hour. Unfortunately they do not speculate on whether a transient pH change, induced by a single episode of unprotected intercourse, is likely to favor the growth of BV organisms sufficiently to produce a change in the bacterial flora. However, repeated episodes of intercourse within 24 hours might produce a longer period of favorable growth conditions.

One group of researchers studied pregnant women, looking at the microbiological differences, on Gram stain, among BV, intermediate flora, normal flora, and smears from spontaneous reverters, who had returned to normal. Microbiologically, the intermediate flora was associated with moderate concentrations of BV organisms, such as *Microplasma hominis* and *G. vaginalis* [27]. In a study of the *Lactobacillus* populations, lactobacilli were isolated from 19 of 50 women with BV [28•]. Of the 12 strains that were characterized, 11 were H_2O_2-producing and, contrary to expectations, apparently did not prevent the onset of BV. Relatively high concentrations of "BV organisms" were also found in the women with intermediate flora and in the reverters, in the presence of lactobacilli.

Another study, from Boston, looked at the symbiotic relationship between *Gardnerella*, which is a facultative aerobe, and *P. bivia* [29]. The former produces amino acids that are used by the latter. In turn, *Prevotella* produces ammonia, which is used by *Gardnerella*, in a symbiotic fashion. The same group also demonstrated another symbiotic relationship, in vitro, with *P. bivia* making amino acids available for *Peptostreptococcus anaerobius* [30].

A new hypothesis to explain the occurrence of BV is elegantly discussed by Blackwell [31•]. In the food industry, *Lactobacillus* phages are known to affect yogurt cultures. They can remain in a temperate state, or become lytic, when as much as 99% of the *Lactobacillus* population may be killed. They have been isolated from human lactobacilli from the vagina [32•] and gut, and from lactobacilli in yogurt. The *Lactobacillus* phages from yogurt lactobacilli were shown to inhibit vaginal lactobacilli [33]. Blackwell hypothesizes that phages might be transmitted by sexual intercourse, dairy products or feco-oral spread. Moreover, carcinogens such as benzo[a]pyrene diol epoxide [34], which is present in cigarette smoke, can induce lysogeny in cultures. When a male sexual partner transmits a *Lactobacillus* phage that triggers a woman's BV, it should be no surprise that antibiotic therapy has no effect on her relapse rate. Clearly, further prospective studies are warranted.

Endometritis

One possible explanation for the sometimes-observed relationship between BV and multiple sex partners is that BV might arise secondary to other causes of inflammation, such as infection with *Chlamydia*. BV is associated with endometritis [35], and with isolation of BV-associated organisms from the endometrium. A further case-control study from the same group concludes that plasma-cell endometritis is found more commonly during the proliferative phase of the menstrual cycle (OR, 4.5, 1.6–12.4). These researchers interpret this to indicate that organisms associated with upper genital tract infection (*ie*, *Chlamydia*, *Neisseria gonorrhoeae*, and BV-associated organisms) are more likely to ascend during this phase. This contrasts with ultrasound recordings demonstrating spontaneous ascent of contrast medium (albumin) from the cervix, through the uterus and Fallopian tubes, to the peritoneal cavity at the time of ovulation, which J. A. McGregor demonstrated at the 1998 bacterial vaginosis meeting, "BV-98," in Aspen, Colorado, in September, 1998.

Hillier and colleagues [36] carefully studied 178 women with suspected salpingitis, defining endometritis as the presence of five or more neutrophils per x400 field in the endometrial surface epithelium, plus the presence of plasma cells in the endometrial stroma. They reported an association between endometritis and *N. gonorrhoeae*, *C. trachomatis*, anaerobic gram-negative rods, and being non-white, but not between endometritis and BV. The prevalence of BV was 56% in women with endometritis and 46% in those without. A reasonable conclusion is that BV organisms are frequently found in the endometrium of women with endometritis, and that there is communication between the vagina and the endometrium.

In many women with BV, the presence of subclinical endometritis is compatible with the associations between BV and infections arising after gynecological surgery, termination of pregnancy, miscarriage in the second trimester of pregnancy, chorioamnionitis, and preterm birth. Researchers at Leeds, in the United Kingdom, studying 771 women undergoing IVF, found an association between

early miscarriage (mainly chemical pregnancies) and BV [12•]. After these researchers controlled for factors such as smoking, no prior live birth, and three or more prior miscarriages, the overall risk of first-trimester loss associated with BV increased (adjusted odds ratio = 2.67, 1.26–5.63). The authors discuss the likelihood that the early-pregnancy loss might be due to endometritis interfering with early implantation. In contrast, a report from Bristol found no association between BV and early loss, in 301 women undergoing IVF treatment [37]. With conflicting results from different studies, we need further research in this area.

Treatment

Recent studies of treatment for BV have assessed the total dose of topical agent required for short-term eradication of BV. Researchers found that intravaginal metronidazole gel can be used once a day for 5 days, instead of twice a day for five days, at the same strength and dosage [38]. In an intent-to-treat analysis, cure rates after 1 month were 118 of 207 (57%) of those treated once daily and 129 of 209 (62%) of those treated twice daily. Similarly, 2% clindamycin cream has been used for 3 days rather than the standard duration of 7 days. At 28-day follow-up, 41% of the clindamycin group were classified as either "success" or "improved," versus 4% in the placebo group [39]. Oral metronidazole remains the mainstay of treatment [40].

The choice of antibiotic may be important. Clindamycin is more active against most of the organisms found in BV, but is active also against lactobacilli. In some cases, there is period of a few days following such treatment before lactobacilli re-emerge. Researchers compared the efficacy of different antibiotics and concluded that, after 1 month, there is no significant difference in *Lactobacillus* colonization rates, from one antibiotic to the next [6]. Metronidazole is less active against *Gardnerella*, *Mobiluncus* and *Mycoplasma hominis*. Nevertheless, it is usually sufficient to restore the balance of the vaginal flora to normal. One study found that women who experience rapid BV relapses are likely to have residual abnormalities, such as intermediate flora on Gram stain, or a raised pH following treatment. Therefore, standard treatment may not be adequate for all patients.

It is possible that organisms persist in the endometrium after treatment. If their eradication is important, as might be the case in pregnancy, metronidazole might be inadequate. Thus, a combination of metronidazole and a macrolide might be superior to cover, for instance, *M. hominis* and *Gardnerella*. A small study reports the selection of strains of *Gardnerella* with decreased sensitivity, following repeat treatments with metronidazole [41]. A group in Manchester, United Kingdom, has been studying the growth of vaginal organisms in biofilms, which may be a better model of the vaginal environment than standard culture is. The sensitivity of some organisms to antibiotics differs considerably, comparing biofilm activity and standard-culture activity. Thus, in the biofilm, a strain of *Gardnerella* was much less susceptible to amoxicillin and erythromycin and a strain of *Lactobacillus acidophilus* was less sensitive to clindamycin, than these same microorganisms were to these same agents in standard cultures.

No studies have specifically addressed the issue of how to manage recurrent BV. General advice—to avoid vaginal douching, washing with soaps, and using antiseptics—should be given, but applying this advice usually does not produce a cure. Recognized triggers—such as the use of spermicide and diaphragms, and IUCDs—should be discussed. There is, however, no guarantee that removing an IUCD will lead to immediate resolution of the problem. BV has been associated with nongonococcal urethritis (NGU) in male sexual partners [42]. In a cohort study, 12 (71%) of the male partners of 17 women with BV had NGU, compared with seven (33%) partners of 21 women who did not have BV (OR, 4.8; $P = 0.049$). Whether the BV was causing the NGU, or the inflammation was triggering the BV is a matter for speculation. It is reasonable, however, to examine the partners of women with recurrent BV and to treat the couple for NGU, if it is found.

One possible explanation for frequent recurrences of BV is that healthy lactobacilli, particularly those that produce H_2O_2, are absent after treatment. The concept of recolonizing the vagina with strains of protective lactobacilli is being investigated. Recolonizing the vagina with of combination of *L. crispatus* and *L. jensenii* is being studied, but clinical results are not yet available. In a small study from Belgium, 32 women with BV or intermediate (*Lactobacillus*-deficient) flora were treated, for 6 days, with vaginal tablets, each containing 50 mg of a lyophilisate of viable, H_2O_2-producing *L. acidophilus* and 0.03 mg of estriol [43]. There was a significant benefit, with a cure rate, after 6 weeks, of 88% in the treatment group and 22% in the placebo group.

We encourage patients to collect a daily vaginal smear, for 1 to 2 months, so we can assess the frequency of relapse, the time of the menstrual cycle when BV arises, and whether there is any association with *Candida* [18]. Most often, the BV arises just before or around the time of menstruation. If that is the case, I prescribe metronidazole, either 400 mg, orally, twice daily, or 0.75% gel, once daily, for 3 days, to use at the onset of menstruation. This is continued for 3 to 6 months, in the hope that it will eliminate the BV trigger. Some women require more frequent suppressive treatment. If there is a history of candidiasis, I add regular topical or systemic treatment for it. This approach is supported by one study in which 66 women were treated for BV with 2 G oral metronidazole, followed by maintenance with metronidazole-nystatin or placebo vaginal suppositories applied at bedtime for 3 days after menstruation, over 6 consecutive menstrual periods [44]. After 6 months of follow-up, the overall cumulative object-

ive cure rate in those receiving the active agent was 100%, compared with 76% in the placebo group.

In practice, with repeated treatment, BV eventually stops relapsing, but the time for such resolution is variable [18,45]. Further prospective studies of the natural history of recurrent BV and its treatment are needed.

Complications

No studies have addressed the question of whether women with frequent recurrences of BV are at increased risk for complications associated with BV, compared with women who experience occasional episodes of BV. Nor have researchers generally distinguished between symptomatic and asymptomatic subjects. A review of the efficacy of treatment to alter pregnancy outcome or other complications is outside the scope of this article. However, if we could find a way to suppress relapse after treatment for BV, we could reduce the prevalence of BV so that the complications with which it is associated should be reduced. It is likely that eradicating BV before the woman conceives would be more effective than attempting to treat it during an established pregnancy.

In the Rakai study of more than 11,000 men and women, periodic mass treatment was prescribed, every 10 months, for three treatment cycles [46•]. The treatment group received metronidazole, 2 G, as a single dose, along with azithromycin and ciprofloxacin. At 20 months, the prevalence of trichomoniasis was decreased by 9.3% in the treatment group and by 14.4% in the control group. However, prevalence of BV was decreased only in pregnant women. There was no reduction in the rate of acquisition of HIV, between the groups.

Several studies have supported a role for BV in HIV transmission. Clinical studies have reported an association from Thailand [47], and several African countries [48–50]. Putative mechanisms were reviewed extensively by Hillier [51•]. They include destruction of local antibody through cleavage by protease and sialidase enzymes [52], and production of an HIV-inducing factor, which was associated with BV and the presence of *M. hominis* [53] and an alteration in the vaginal cytokine pattern in favor of a Th2-type, rather than a protective Th1-type response [54].

Conclusions

The management of women with symptomatic recurrent BV is difficult. At present—having discussed the putative mechanisms by which relapses might be triggered, and the potential role of douching and other washing practices—we are left with intermittent treatment, in an attempt to suppress the condition. It is also worthwhile to examine male sex partners of women with BV, for asymptomatic NGU. New therapeutic approaches are needed; these might involve combinations of antibiotics, and the application of healthy H_2O_2-producing lactobacilli.

One area of interest, currently, is the relationship between BV and endometritis, which might explain many of the upper genital tract complications associated with BV. If we can learn how to prevent recurrent BV, we might improve the outcome of pregnancy for women with prior preterm birth or miscarriage who have BV; eliminating BV before conception promises to be more beneficial than treating it during pregnancy. By reducing the overall prevalence of BV we might even be able to reduce the incidence of HIV spread through heterosexual intercourse.

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