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SCREENING OF BACTERIAL VAGINOSIS IN PRETERM LABOR

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ABSTRACT

Bacterial vaginosis is the most common lower genital tract infection among women of reproductive age, and has been associated with a number of significant obstetric and gynecologic complications. Treatment regimens recommended by the Centers for Disease Control and Prevention in pregnant women include metronidazole 250 mg orally three times daily for 7 days or clindamycin 300 mg orally twice a day for 7 days. Cure rates vary in published studies, and this syndrome tends to recur after treatment in both pregnant and nonpregnant women. There is currently no consensus as to whether to screen for and treat bacterial vaginosis in pregnancy. Treatment has not been shown to decrease adverse obstetric outcomes in the general population at low risk for prematurity, although oral treatment for at least 7 days may be effective in decreasing preterm birth rates in women who have a history of a prior preterm delivery. Further study is required in order to advance our knowledge and understanding of the effects of this syndrome in pregnant women, and to make definite conclusions regarding the role of treatment in pregnancy.

KEYWORDS: Bacterial vaginosis, labor.

INTRODUCTION

There are great numbers of microorganisms living in balance with each other and their host making the "vaginal flora". This vaginal flora consists of lactobacilli that named after their function of producing lactic acid. The presence of certain number of micrococci is acceptable in a healthy vaginal environment. It contains also multiple aerobic or facultative species and obligates anaerobic organisms.[1]

As normal pregnancy progresses the total number of commensal organisms in the vagina increase, by term, the vagina is colonized by organisms of low virulence, which pose no significant threat to the fetus passing through the birth canal.

The natural protection of the vagina:

• The natural protection of the vagina is determined by several factors; the acidity of the vagina (pH), its cell layer (stratified squamous epithelium), the balance between the microorganisms forming the vaginal flora, and the vagina secretes leukocyte protease inhibitor protein to protect tissues against toxic inflammatory products and infection.[2]

• The vaginal pH ranges between 4 and 4.5. It is believed to result from lactobacillus species' production of lactic acid, fatty acids, and other organic acids. This acidic environment inhibits the growth of yeast and other unwanted organisms.[3]

• Normal vaginal flora protects female and makes her less susceptible to Human Immune-Deficiency Virus (HIV) and other sexually transmitted infections (STD).

Lactobacilli produce hydrogen peroxide (H2O2) which is toxic to bacteria by producing toxic hydroxyl radicals.[4] Altered vaginal flora: Several events may alter lower reproductive tract flora as over use of broad-spectrum antibiotics, sexual intercourse, vaginal douches, and bleeding in pregnancy (blood serve as a source of nutrient to bacteria and make the vaginal environment more alkaline as blood PH= 7.4[5], When lactobacilli are absent or reduced the vaginal environment becomes alkaline (as opposed to acid). This environment promotes overgrowth of certain anaerobic organisms such as Gardenerella vaginalis, Mycoplasma hominis, and Mobiluncus species, and often results in an infection called bacterial vaginosis (BV).[6]

Physiological vaginal discharge: Normal vaginal discharge (leucorrhea) is white, becomes yellow on contact with air due to oxidation. It consists of desquamated epithelial cells of the vagina and cervix, mucous originated from cervical glands, and fluid comes from vaginal transudes.[7]

. Normal leucorrhea is increased during pregnancy because of increased estrogen production and increased vascularity of the vagina.[8] Increase amount of a discharge, a change in its color or smell, bloody vaginal discharge, irritation, itchiness, burning sensation in the vagina, burning with urination and discomfort or pain during intercourse; are signs of a pathology called vaginitis.[9]

Back ground

Bacterial vaginosis was first recognized in the late nineteenth century. Descriptive microbiology for vaginal infections began in 1894, when Doderlein[10] described the presence of lactobacilli in normal vaginal flora. In 1914 Curtis[11] reported an association between abnormal anaerobic vaginal flora and vaginal discharge, and Gardner and Dukes[12] first described the organism Gardnerella vaginalis in 1955. The syndrome of bacterial vaginosis was initially referred to as nonspecific vaginitis, which was well-characterized in many studies.[13]

Several changes in nomenclature occurred over the years. In 1984, the term bacterial vaginosis was proposed and became widely accepted. The normal vaginal flora consists of both aerobic and anaerobic bacteria. The predominant microorganisms are Lactobacillus spp, accounting for greater than 95% of all bacteria present.[14]

These organisms are believed to provide defense against infection by maintaining an acidic pH in the vagina. The lactobacilli of normal women tend to contain more hydrogen peroxide-producing species, which damage organisms lacking free radical scavengers such as many of the bacterial vaginosis-associated organisms, thereby inhibiting vaginal colonization by these organisms.[15]

Both pregnant and nonpregnant women colonized with adequate numbers of hydrogen peroxide-producing lactobacilli have decreased acquisition of bacterial vaginosis compared with women not having these bacteria.[16] In contrast, bacterial vaginosis is a polymicrobial syndrome resulting in a decreased concentration of lactobacilli and an increase in pathogenic bacteria. These organisms include G vaginalis, Mobiluncus spp, Bacteroides and Prevotella spp, and Mycoplasma spp.[17]

Prevalence and epidemiology

As already noted, bacterial vaginosis is very common. Studies have revealed that the prevalence varies widely depending on the patient population. In private offices, the prevalence has ranged from 4% to 17%, whereas in gynecology clinic populations, the prevalence has been higher, at 23%.[18]

In college students, the prevalence has ranged from 4% to 25%, and it has been as high as 61% in women attending sexually transmitted disease clinics.[19] Studies of pregnant women

have documented similar prevalence rates to those seen in nonpregnant populations, ranging from 6% to 32%.[20] Epidemiologic studies have identified several risk factors for the acquisition of bacterial vaginosis. It has been associated with racial origin, smoking, sexual activity, contraceptive practice, and vaginal douching. Bacterial vaginosis is more common in women of the black race, women who smoke[21], women who are sexually active (although it has been isolated in virginal women as well), and women who use vaginal douches.[22]

Diagnosis of Bacterial vaginosis is a syndrome that can be diagnosed both clinically and microbiologically. In 1983, Amsel and colleagues[23] published a paper outlining clinical diagnostic criteria, and these are still in use today. The clinical diagnosis of bacterial vaginosis is made if three of the four following signs are present.[24]:

1) An adherent and homogenous vaginal discharge.

2) Vaginal pH is greater than 4.5 618 yudin.

3) Detection of clue cells (vaginal epithelial cells with such a heavy coating of bacteria that the peripheral borders are obscured) on saline wet mount.[25]

4) An amine odor after the addition of potassium hydroxide (positive whiff test) Although these criteria are widely used, they have been criticized because of inherent difficulties with such a diagnostic scheme. With the exception of pH, the remainder of the criteria are either subjective (appearance of discharge, whiff test) or potentially technically difficult (appearance of clue cells in the saline wet mount examined by microscopy).[26]

Four laboratory methods have been used to diagnose bacterial vaginosis:

(1) culture of vaginal fluid for G vaginalis.

(2) biochemical tests for metabolic byproducts of vaginal bacteria (gas-liquid chromatography).

(3) assays for proline aminopeptidase.

and (4) direct Gram's stain of vaginal secretions.[27]

Gvaginalis is found in high concentrations in almost all women who have bacterial vaginosis, but is also often found in the vaginal flora of normal women.[28]

Also, the isolation of any one specific organism on culture does not reliably predict bacterial vaginosis, so culture is not felt to be valuable in the diagnosis.[29] Chromatography equipment is not readily available in many laboratories. Therefore, Gram's stain of vaginal fluid is the most widely used and evaluated diagnostic method for bacterial vaginosis. To perform a

Gram's stain, vaginal discharge is collected on a glass slide, allowed to air-dry, stained in the laboratory, and examined under oil immersion for the presence of bacteria. This diagnostic method has several advantages, including a permanent record, a high frequency of interpretable results, low cost, and ease of transport and storage.[30]

Over the past 2 decades, there have been two main Gram's stain diagnostic schemes used for the evaluation of vaginal infections. In 1983, Spiegel and colleagues[31] published a paper describing an objective way to diagnose bacterial vaginosis by Gram's stain.

Bacterial vaginosis was present by the Spiegel criteria if Lactobacillus morphotypes were fewer than five per oil immersion field, and if there were five or more other morphotypes (gram-positive cocci, small gramnegative rods, curved gram-variable rods, or fusiforms) per oil immersion field. If five or more lactobacilli and fewer than five other morphotypes were present per oil immersion field, the Gram's stain was considered to be normal. Although used for many years, the Spiegel system was criticized because there was no account for the spectrum of severity.[32]

In 1991, Nugent and colleagues addressed this problem and developed a scoring system. This system is presented in Table 1. Each morphotype on the stain is quantitated from 1+ to 4+ with regard to the number of morphotypes per oil immersion field, and a corresponding score is assigned. The scoring system allows for gradations in severity. The criterion for bacterial vaginosis is a score of 7 or higher.

A score of 4 to 6 is considered intermediate, and a score of 0 to 3 is considered normal. Bacterial vaginosis has consistently been shown to be a risk factor for adverse obstetric outcomes such as preterm labor and delivery, preterm premature rupture of membranes, spontaneous abortion, chorioamnionitis, and postpartum infections such as endometritis and cesarean section wound infections.[33]

Despite these associations, it is still not clear whether screening for and treatment of bacterial vaginosis in pregnancy can reliably reduce the incidence of these complications. Further, for clinicians who elect to screen pregnant women, the op620 yudin timal time to screen, a screening test to use, and treatment to administer are all uncertain. The United States Preventive Services Task Force (USPSTF) published a statement in 2001[34] concluding that the available evidence was insufficient to recommend for or against routinely screening

women at high risk for preterm birth for bacterial vaginosis, and recommending against screening average-risk asymptomatic pregnant women. If one chooses to treat this syndrome in pregnancy, the 2002 Centers for Disease Control and Prevention sexually transmitted diseases treatment guidelines recommend using either metronidazole 250 mg orally three times daily for 7 days or clindamycin 300 mg orally twice a day for 7 days.

There is no evidence that metronidazole is teratogenic or mutagenic, and it is considered safe for use in pregnancy.[35] Topical agents are not recommended. There have been many trials over the past 2 decades exploring the treatment of bacterial vaginosis in pregnant women. These trials have evaluated the efficacy of various treatment regimens in achieving and maintaining cure.

Oral and vaginal metronidazole and clindamycin have been used in various treatment trials. The studies have also investigated whether the treatment of abnormal vaginal flora can reduce the incidence of prematurity and other bacterial vaginosis-associated adverse pregnancy outcomes. The varying results of these trials can be difficult to interpret, and the aim of this section is to summarize and consolidate this body of literature for the reader.[36]

Cure rates following treatment Definitions of cure vary widely among published trials on treatment of bacterial vaginosis, which may account for variation in reported treatment efficacy rates. As well, studies of the natural history of this syndrome have shown that it gradually recurs with longer follow-up in pregnant and nonpregnant women, and rates of cure depend on the timing of follow-up evaluations.[37]

In oral treatment trials, cure rates have consistently been greater than 70%. Several trials have used oral metronidazole. Hauth and colleagues[38] demonstrated resolution of bacterial vaginosis (defined as less than three of four clinical signs and normal flora on Gram's stain) in 70% of women 2 to 4 weeks after treatment with oral metronidazole and erythromycin; McDonald and coworkers[39] showed cure rates (by Gram's stain or culture of G vaginalis) of 76% 4 weeks following two 2-day courses of metronidazole 400 mg twice daily; Carey and colleagues[40] reported normalization of vaginal flora on Gram's stain in 78% of women after two 2 gram doses of oral metronidazole; and Klebanoff and coworkers[41] found cure (defined as a Nugent score of b7 on Gram's stain) in 78% of patients following two 2-gram doses of oral metronidazole. In studies employing oral clindamycin, McGregor and colleagues[42] published cure rates of 92.5% 2 to 4 weeks after treatment, and a recent study completed by

Ugwumadu and coworkers[43] using oral clindamycin 300 mg taken twice daily for 5 days resulted in cure rates (defined as a Gram's stain Nugent score of 0 to 3) of 90%.

In addition to oral treatment trials, there have been many studies using vaginal preparations, most commonly clindamycin cream, with cure rates ranging from 33% to 86%. In randomized controlled trials of clindamycin cream versus placebo, Joesef and coworkers[44] demonstrated a cure rate (defined as a Nugent Gram's stain score of less than 7 with a normal pH) of 85.5% 2 weeks after treatment in 340 pregnant women; Kekki and colleagues[45] reported normalization of vaginal flora (defined by Spiegel criteria) in 66% of 187 patients 1 week following treatment; Kurkinen and coworkers[46] found cure rates (on Gram's stain) of 33% in 51 women 2 weeks after treatment; and Lamont and colleagues[47] demonstrated a range of cure rates (71% to 78%) using several different criteria for cure in over 200 pregnant women 3 and 6 weeks post-treatment. A study by McGregor and coworkers[48] clearly showed that cure depends on the timing of follow-up, with rates of 90% at 1 week and 60% to 70% at 4 weeks post-treatment.

There are very few studies including both oral and vaginal treatment. In a study by Yudin and colleagues[49], pregnant women who had bacterial vaginosis were randomized to receive either oral metronidazole for 7 days or vaginal metronidazole gel for 5 days.

Cure rates were defined in three ways:
(1) microbiologic cure—Gram's stain score of 0 to 3,
(2) clinical cure—the absence of all four clinical signs, and
(3) therapeutic cure—a combination of both microbiologic and clinical cure. The results demonstrated that at 4 weeks after treatment, cure rates were greater than 70% for any of the three criteria, and were equivalent for oral and vaginal therapy.[50]

There have been many trials designed to determine whether treatment of bacterial vaginosis in pregnancy can impact on the frequency with which adverse outcomes, especially premature delivery, are encountered. Despite the consistent association between bacterial vaginosis and preterm birth, the results of these treatment trials have not been consistent. The reason for this lack of clarity in the literature may be that studies have used mixed populations (women at both low and high risk for preterm birth) and different treatment modalities (systemic and local therapy).[51]

In trials enrolling women from the general population who are at average risk for preterm birth, there does not seem to be any benefit to screening for and treating bacterial vaginosis. Studies involving this category of women have used both oral and vaginal treatment regimens. McGregor and colleagues[52] randomized women who had bacterial vaginosis from 16 to 27 weeks' gestation to receive intravaginal clindamycin or placebo. There were no significant differences in adverse outcomes such as preterm birth, preterm labor, or low birthweight between the two groups, despite adequate treatment and eradication of bacterial vaginosis. Similarly, Joesef and coworkers[53] found no difference in preterm delivery rates between women who had bacterial vaginosis at 14 to 26 weeks randomized to topical clindamycin or placebo. A study from Finland[54] found no difference in rates of preterm birth or puerperal infections among women enrolled at 12 weeks' gestation and receiving vaginal clindamycin versus placebo; and an Italian group[55] reported no difference in the frequencies of preterm delivery, low birthweight, or gestational age at birth in women enrolled between 14- and 25-weeks' gestation and randomized to topical clindamycin or placebo. Oral treatment trials in women at low risk for preterm birth have had similar results. In two large trials, McDonald and colleagues[56] found no difference in preterm delivery rates in 879 women randomized to oral metronidazole or placebo at 24 and 29 weeks' gestation; and Carey and coworkers[57] reported no differences in rates of preterm birth, low birthweight, or preterm premature rupture of membranes among 1953 pregnant women randomized to oral metronidazole or placebo from 8 to 22 weeks' gestation.s

Methods

This case-control study was done in the alkhaek hospital with the aim of investigating the relationship between bacterial vaginosis and preterm labor.

Considering previous studies and the expected Odds ratio of 2.5, the prevalence rate of 10% for BV in our society, a =0.05 and ß = 20%, a number of subjects in each group were calculated 66, but in order to increase the accuracy, 80 subjects were studied in each group. With the consequent sampling method, 160 patients were enrolled in the study. Preterm labour was the main inclusion criteria for selecting the patients. We define preterm labour as follow:

A. at least four contractions in 20 minutes or 8 contractions in 40 minutes causing.

B. cervical dilatation (> 1 cm) and

C. effacement (>80%). Those with cervical deficiency, placenta previa, abruptio placenta, Premature rupture of membrane (PROM), uterus abnormality and multiple pregnancies were excluded from the study. Subjects of the control group were selected from full term pregnant women referred to the Delivery Ward because of labour pain.

Vaginal discharge was taken with an ayre spatale from the posterior culdesac and spread on two lamellae. The first lamella was used for the Whiff test. That is after adding KOH in the case of amine odor, the diagnosis of BV was confirmed. The second lamella was fixed in alcohol for PapSmear test and was studied by an expert for the presence of clue cells. The diagnosis of BV was established when three of four Amsell's criteria were positive:

1. Vaginal fluid pH > 4.6
2. Gray homogeneous vaginal discharge
3. Positive Whiff test
4. The presence of clue cells in Pap Smear or wet mounts

The collected data were analyzed by SPSS and adjusted OR was calculated for various factors.

RESULTS AND DISCUSSION

Bacterial vaginosis is the most common cause of vaginal discharge in reproductive age with the prevalence rate of 10-15%.7 Bacterial vaginosis is often asymptomatic and is found in upto 20% women during pregnancy**.**

Depending on how often the population is screened.15 In surveying the relationship between bacterial vaginosis and educational level, no significant difference was observed. Newton's study reported that bacterial vaginosis had a direct relationship with a low cultural level. Eschenbach and coworkers were among the first researchers who studied the relationship between bacterial vaginosis and preterm labour. In their study 49% in preterm group and 24% in full term group had bacterial vaginosis. Later they showed the correlation between bacterial vaginosis and chorioaminiotis and preterm labor.18 Prematurity Prediction Study carried out on 3000 women in the United States has shown the relationship between bacterial vaginosis and preterm labor.[58]

In the present study, 20 women (25%) in the preterm group had bacterial vaginosis while in the full-term group only 9 women (11.3%) had BV that shows a significant difference

between the two groups and proves the association of BV with preterm labor. The prevalence rate of BV during pregnancy has been reported to be 10-30% in various studies. In a study done in Isfahan, the prevalence rate of BV in preterm subjects was 27.7%.

The results of the present study are similar to other studies. In our study, 20% of preterm subjects had a previous history of preterm labour, while in the full-term group only 6.3% had a history of preterm labour which is a significant difference between the two groups. In a study done in 1996 in England, 500 cases of repeated abortion were surveyed and a higher rate of BV was found in those with a history of abortion in the second trimester comparing to those with early abortion.[59]

In our study, no significant difference in the prevalence rate of BV was found between preterm cases with a different history of abortion. In a study done on 2000 subjects in Indonesia, the sensitivity and specificity of Whiff Test for the detection of BV were respectively 6.58% and 73.2% and the sensitivity and specificity of clue cells were respectively 43.1% and 99.6%.6 A Brazilian study has found 20% prevalence of bacterial vaginosis among asymptomatic pregnant women.19 In addition, a significantly increased risk of neonatal complications was found among this population.20 This study was conducted to evaluate the impact of treatment for bacterial vaginosis among a population of Brazilian pregnant women. Our study compares well with the Brazilian study.

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