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Research Article

Correlation of bacterial vaginosis with preterm labour: a case control study

Waseeqa Nigeen[1]*, Abdus Sami Bhat[2], Khushboo Gulzar[1], Shehnaz Taing[1]

[1]Department of Obstetrics & Gynaecology, Government Medical College, Srinagar, J & K, India
[2]Department of Pediatrics, Government Medical College, Srinagar, J&K, India

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***Correspondence:**
Dr. Waseeqa Nigeen,
E-mail: waseeqanigeen@gmail.com

ABSTRACT

Background: Bacterial vaginosis (BV) is thought to be an important risk factor and predictor of preterm labour. Prevention and early detection and treatment of BV can decrease the incidence of preterm labour. Primary objective of this study was to find out correlation of bacterial vaginosis with preterm labour. Secondary objectives were determination of most important criterion among the Amsels criteria and risk factors for BV among literacy, residence and parity.
Methods: A Case-control study carried out at a tertiary care hospital in north India. 100 women with preterm labour and 200 women with term labour after fulfilling inclusion criteria were enrolled as cases and controls respectively. Epidemiological and clinical details were recorded. Bacterial vaginosis was diagnosed by Amsels criteria. Prevalence in both groups was calculated. Statistical analysis was then done to find out association between bacterial vaginosis and preterm labour.
Results: Among all women enrolled 94 had bacterial vaginosis. The overall prevalence of bacterial vaginosis in both groups combined was 31.33%. It was 42% in cases and 26% in controls. The difference was statistically significant (p=0.007). Whiff test emerged as the strongest criterion if used alone with a sensitivity of 98% and specificity of 81% when compared to whole Amsels criteria. Bacterial vaginosis was found more in illiterate women and those who had given birth previously.
Conclusions: Bacterial vaginosis is significantly more prevalent in women with preterm labour. Whiff test can be used alone in centres where the patient load is too high. Illiteracy is a risk factor that can be modified to bring down incidence of bacterial vaginosis.

Keywords: Preterm labour, Bacterial vaginosis, Amsels criteria

INTRODUCTION

Preterm labour refers to the onset of uterine contractions of sufficient strength and frequency to effect progressive dilatation and effacement of cervix between 20 and 37 weeks of gestation.[1] Although all births before 37 weeks of gestation are considered premature, births before 32 weeks of gestation (2% of all births) account for most neonatal deaths and disorders. Overall incidence of preterm labour is reported to be 6-15% and 40-50% of these occur spontaneously whereas 25% occur following preterm, pre-labour rupture of membranes. Preterm birth is a leading cause of perinatal morbidity and mortality worldwide and is associated with 60-80% of deaths of infants without congenital abnormalities.[2] Almost 40% cases of preterm labour are due to infections. Ascending infections have been identified as the most important preventable cause of preterm labour. Amongst the ascending infections, bacterial vaginosis (BV) is a major cause of preterm labour. In BV the normal vaginal flora is replaced by anaerobic organisms. These organisms are Gardnerella vaginalis, Bacteriodes, Mobiluncus, Mycoplasma hominis, Peptostreptococcus, Fusobacterium and Prevotella. Bacterial vaginosis is the

most common lower genital tract syndrome among women of reproductive age group. Current studies have found the prevalence of BV among non-pregnant women to range from 15-30% and up to 50% of pregnant women have been found to have bacterial vaginosis.[3-6] Bacterial vaginosis is diagnosed by various methods. These methods are Amsels criteria, Gram stain (Nugent score/Hay Ison grading), Bacterial vaginosis blue test which measures vaginal fluid sialidase activity.[7-9] The diagnostic criteria commonly used for bacterial vaginosis is Amsels Criteria. It involves assessing four clinical parameters with the existence of three or more parameters corresponding to a diagnosis of bacterial vaginosis. At least three of the four criteria should be present for a confirmed diagnosis of bacterial vaginosis. Bacterial vaginosis can be cured by antibiotics, systemic and topical with spontaneous relapse occurring more commonly among women treated with topical compared with systemic antibiotics.[10]

Keeping in view the importance of BV in predicting the outcome of pregnancy and its overall impact on maternal and child health and the need for its prompt and early diagnosis, this study was designed to look at the correlation between bacterial vaginosis and preterm labour in our population. This study also tried to find out the most significant of the Amsels criteria, which could be used alone for the diagnosis of bacterial vaginosis without compromising the diagnostic power of whole Amsels criteria. This is relevant in developing countries like ours where there is scarcity of resources and time. By using a single test we can save both.

METHODS

The study was conducted in the Postgraduate Department of Obstetrics and Gynaecology, Lalla Ded Hospital, Government Medical College, Srinagar, India.

This was a case control study that was conducted in the labour ward of the hospital from August 2011to October 2012. Patients who fulfilled following inclusion criteria were enrolled as cases: gestational age between 28 to 36+6 weeks, singleton pregnancy, intact membranes, painful uterine contractions >2 in 10 minutes, each lasting >45 seconds, cervical dilatation between 1-4cm ,cervical effacement >25%. In addition patients having any of the following conditions were excluded: gestational age <28 weeks, history of antepartum haemorrhage (APH), urinary tract infections, medical complications of pregnancy such as moderate to severe anaemia, pregnancy induced hypertension (PIH), diabetes mellitus, history of leaking per vaginum or absent membranes, multiple pregnancy, intrauterine growth restriction (IUGR), fetal malformations intrauterine death (IUD), antibiotic therapy in the last four days, fever, polyhydramnios, cervical surgery, incompetent cervix or blood transfusion within last seven days. Controls included patients presenting in labour with gestational age more than 37 completed weeks. Rest of the inclusion and exclusion criteria were same as described above.

On the basis of prevalence of BV in preterm labour in previous studies, a sample size of 150 patients in each group was calculated. However we ended up enrolling 100 patients in cases group and 200 patients in control group as the study period was limited. However the described change in sample size is least likely to affect the validity of results

While evaluating the results of the study, relevant epidemiological and clinical data were collected from every patient. Epidemiological data included literacy status, location; urban or rural, age and parity while clinical data included history, general physical examination, and obstetric examination. Baseline investigations including obstetric ultrasound. All women were screened for bacterial vaginosis by Amsels criteria:

The tests of Amsels criteria to detect bacterial vaginosis are:

1. Vaginal pH >4.5
2. Thin greyish white homogenous discharge.
3. Whiff's test.
4. Presence of clue cells in vaginal smear.

When three out of the above four tests were positive the patients were diagnosed as having bacterial vaginosis.

The women in the study were placed in dorsal position and clean unlubricated speculum was introduced in the vagina to retract the posterior vaginal wall and the amount, colour and pH of the vaginal fluid was measured directly with pH indicator strips (with pH marking 1-14).

Sterile cotton swabs were used to obtain vaginal material from the posterior vaginal fornix for wet mount preparation and amine test (Whiff test). Whiff test was performed by adding a drop of 10% potassium hydroxide to the vaginal fluid and sniffing the mixture. The test was interpreted as positive if a fishy aroma was noted.

A swab containing vaginal fluid was obtained and immediately placed in 0.5ml of saline which was examined under microscope at 400x for presence of clue cells.

While evaluating literacy of subjects, a literate person was defined as one who could read and write with understanding in any language.

Informed consent was taken from all the participating subjects. The study was approved by the institutional ethics committee.

Statistical methods

All the data obtained was entered in the Microsoft Excel sheet. Data was checked multiple times and all errors removed. Unpaired Student't' test was used to calculate p-value for all quantitative variables, while odds ratio (OR) was calculated for qualitative variables. Chi-square test (two tailed) was also used for calculating p-values for qualitative variables. Calculations were done with the help of STATA-15 Statistical Software.

RESULTS

Table 1: Comparison of mean age of the two groups.

Age (completed years)	Cases (n=100)	Controls (n=200)	p value
Mean±SD	26.75±3.2.	26.69±2.8	0.86

100 women admitted with clinical features of preterm labour were enrolled as cases and 200 women admitted with term labour were enrolled as controls after applying inclusion and exclusion criteria. Informed consent was taken from all cases and controls before enrollment.

Table 2: Age group distribution of the two groups.

Age (years)	Cases (n=100) (%)	Controls (n=200) (%)	P value
<20	2 (2%)	4 (2%)	1.0
20-25	31 (31%)	58 (29%)	0.82
26-30	56 (56%)	121 (60.5%)	0.53
>30	11 (11%)	17 (8.5%)	0.62

Table 3: Comparison of mean gestation between the groups.

Gestation (completed weeks)	Cases (n=100)	Controls (n=200)	p value
Mean±SD	32.85±2.1	38.1±1	0.0001
Median	33	38	
IQR(1^{st} and 3^{rd})	31-35	37-39	

The mean age of the cases was 26.75±3.2 years while it was 26.69±2.8 years in controls (Table 1). There was no significant difference in the age profile of the two groups (Table 2). The mean gestation of the cases was 32.85±2.1 weeks, while it was 38.1±1 weeks in controls, as shown in Table 3. The parity distribution of the cases and controls is shown in table 4. The difference was not significant between the two groups. Fifty eight (58%) cases as compared to 112 (56%) controls were from a rural background. The difference was not significant (Table 5).

Table 4: Comparison of parity between the two groups.

Parity	Cases (n=100) (%)	Controls n=200) (%)	p value
0	36 (36%)	76 (38%)	0.46
1	29 (29%)	54 (27%)	0.82
2	25 (25%)	52 (26%)	0.96
>2	10 (10%)	18 (9%)	0.94

Among all women enrolled 94 were detected to have bacterial vaginosis on the basis of Amsels criteria. The overall prevalence of bacterial vaginosis in both groups combined was 31.33%. It was 42% in cases and 26% in controls. The difference was statistically significant (p=0.007) with an odds ratio of 2.2 (95% CI 1.2 4.4) (Table 6).

Table 5: Location distribution of the two groups.

Location	Cases (n=100) (%)	Controls (n=200) (%)	p value
Rural	58 (58%)	112 (56%)	0.37
Urban	42 (42%)	88 (44%)	

Whiff test was positive in 130 women out of 300.It missed two bacterial vaginosis cases and picked up 36 false positives. Whiff test was found to have a sensitivity of 98% and specificity of 81% which is the best as compared to other tests. Identification of clue cells had the highest specificity among all the criteria at 90%. Its positive predictive value was 81%, negative predictive value was 95%. Vaginal discharge was found to be very nonspecific with specificity of 42%. Although the sensitivity was good at 98%, it picked up 118 women as BV positive who did not actually have it. Among all the criteria low pH of more than 4.5 was the most nonspecific, although it had a high sensitivity, it had a positive predictive value of only 41%. The number of false positives was too huge to make it a test worth using alone (Table 7).

Table 6: Correlation of bacterial vaginosis and preterm delivery.

Bacterial Vaginosis	Cases (n=100) (%)	Controls (n=200) (%)	OR	CI	p value
Present	42 (42%)	52 (26%)	2	1.3-3.5	0.0073
Absent	58 (58%)	148 (74%)			

Among 94 women having bacterial vaginosis 69 did not receive any formal education (73%) as compared to 106 out of 206 (51%) among those not having BV. The difference was very significant OR 2.5 (95% CI 1.5-4) with a p value of 0.0006 .The percentage of women with

previous birth in BV group was 72% as compared to 58.5% in non BV group. The difference was significant with the odds ratio of 1.9 (95% CI 1.1-3.1) and p value of 0.027. Among the 94 BV positive women 50 were from rural background (53%) and among 206 BV negative patients 120 were from rural background (58%). The difference was not statistically significant (p 0.5) (OR 0.8 95% CI 0.5-1.3), Thus there did not seem to be any correlation between location and risk of bacterial vaginosis (Table 8).

DISCUSSION

The association between BV and adverse pregnancy outcome has now been proven beyond doubt. In almost every corner of the world across racial and ethnic differences bacterial vaginosis remains an important risk factor for adverse pregnancy outcome including preterm labour and preterm delivery. There have been multiple studies from India also which have shown similar observations in Indian population. We also tried to look at the correlation between bacterial vaginosis and preterm delivery in a cohort of women belonging to a population residing in a part of Jammu and Kashmir (North India), being taken care of by our hospital.

The odds of having bacterial vaginosis in women having adverse pregnancy outcome has ranged from 1.06 to 5.99 among various international studies.[14,15] There are however a few studies that have not been able to demonstrate any significant association between bacterial vaginosis and preterm delivery, but their number is very small and they have their own limitations.[16,17]

In the present study 42% of women admitted with preterm labour with no obvious risk factors for preterm delivery had bacterial vaginosis as compared to 26% in those admitted with term labour (OR 2, 95% CI 1.3-3.5, p value 0.0073). Our study supports other similar studies that have shown a strong association between bacterial vaginosis and preterm labour. Among Indian studies also almost similar results were shown by Kumar Aruna and Khare Jyoti, who showed that prevalence of bacterial vaginosis, was 44.5% in their cohort of women with preterm labour as compared to 23.5% in controls.[11] In another Indian study by Goyal R and Sharma P et al bacterial vaginosis was diagnosed more in women delivering prematurely than in women delivering at term (31.6% vs 15%) ($p<0.05$).[12] Other countries from the subcontinent have also reported similar prevalence and high risk of preterm labor among bacterial vaginosis positive women. A study from Pakistan by Islam and Safdar et al is one among such studies where bacterial vaginosis was found in 44% of the women who delivered preterm.[13]

Bacterial vaginosis can be diagnosed by various ways. These are based on Gram stain, bacterial culture, quantitative polymerase chain reaction of DNA and clinical criteria called as Amsels criteria. Out of these only Amsels criteria are based on clinical assessment and are widely used by physicians across the world for the diagnosis of bacterial vaginosis. There have been many studies that have compared these diagnostic tests with one another. Sha BE, Chen HY, et al, showed that, the sensitivity and specificity of Amsel criteria in their cohort were 37% and 99%, respectively as compared to Nugent score.[18] The sensitivity and specificity of bacterial cultures were limited. Schwebke et al[19] reported the sensitivity and specificity of Amsel criteria compared to Nugent score to be 70% and 94%, respectively, in a cohort of nonpregnant women. Although these studies show that Nugent score is a better diagnostic test than Amsels criteria but it is rarely used in a clinical setting because of the time involved and the expertise that is needed while interpreting the test. Amsels criteria hence remain the most widely used diagnostic criteria for bacterial vaginosis. We tried to look at the most significant test among the tests on which Amsels criteria are based. This was done in order to find out whether there is a test that can be singularly used to pick up bacterial vaginosis as far closely possible as are picked up when all the Amsels criteria are used. According to Amsels criteria a patient is labelled as having bacterial vaginosis if three out of four criteria are fulfilled. This is especially relevant in poor resource limited countries where patient load in the hospitals is very high and time constraints do not allow administration of full Amsels criteria.

Table 7: Performance of various individual criteria as compared to whole Amsels criteria.

Positive test	BV present (n=94)	BV absent (n=206)	Sensitivity	Specificity	PPV	NPV	PLR	NLR
Whiff test	92	38	98%	81.5%	70%	98%	5.3	0.03
Clue cells	85	20	90.4%	90.2%	81%	95.3%	9.3	0.1
discharge	92	118	98%	42.8%	43.8%	97.7%	1.7	0.04
pH >4.5	94	146	100%	32.1%	41%	100%	1.4	NR

BV: Bacterial vaginosis; PPV: positive predictive value; NPV: negative predictive value; PLR: Positive likelihood ratio; NLR: Negative likelihood ratio

In our study we found that whiff test is the most sensitive and specific bedside test among all the tests with a sensitivity of 98% and a specificity of 81%. Identification of clue cells also turned out to be a good test as it had a good sensitivity of around 90% and specificity of 90%. The advantage of a Whiff test over the identification of clue cells is that the former is an easy test and can be done bed side while the latter is a laboratory test. From our study we infer that if only whiff test is used we can pick up almost 98% cases of bacterial vaginosis, however, it will pick up 19% normal subjects as having bacterial vaginosis, which is not very high. A patient negative on whiff test would be almost certain not to have bacterial vaginosis (NPV 97.7%). The other two criteria including vaginal discharge and pH>4.5 were very nonspecific with specificities well below 50% although their sensitivities matched that of whiff test. So if we were to use these tests alone, the number of false positives will be high and that would lead to overtreatment of normal subjects and placing of extra financial costs of treatment on the system. The reason behind very high prevalence of discharge includes its subjective nature of assessment, and high prevalence of other infections causing discharge like trichomoniasis, vaginal candidiasis and sometimes streptococcal infections. Elevated pH can also be due to recent intercourse and contamination by cervical secretions. Similar observations were made by Chakraborty et al[20] who found that out of 39 patients presenting with vaginal discharge only 15 had bacterial vaginosis. They also found that pH > 4.5 as very nonspecific. Similar findings by Kumar Aruna and Khare Jyoti have shown whiff test to be the most significant bed side test among all the Amsels criteria and discharge and pH > 4.5 as very nonspecific.[11]

Table 8: Association of literacy, location and parity with bacterial vaginosis.

Risk factor	BV present (n=94) (%)	BV absent (n=206) (%)	OR	CI	P value
Illiterate	69 (73%)	106 (51%)	2.5	1.5-4.0	0.0006
Parity ≥1	68 (72%)	120 (58.5%)	1.9	1.1-3.1	0.027
Rural	50 (53%)	120 (58%)	0.8	0.5-1.3	0.5

BV: Bacterial vaginosis

Although our primary aim was not to look at the risk factors for bacterial vaginosis, but we did look at some of the risk factors which were available to us. Such studies are important as identification of risk factors can help in decreasing the incidence of bacterial vaginosis and thereby preventing its adverse effects including preterm labour and preterm delivery. This is also important in presence of the proven fact that treatment for bacterial vaginosis does not always and consistently lead to decrease in adverse pregnancy outcomes associated with it. Thus it becomes more important to identify the risk factors and modify them if possible to decrease the incidence of bacterial vaginosis. Among the risk factors that we looked at were educational status of the patients, urban or rural location and parity. Out of these three education is the only modifiable risk factor and we found that bacterial vaginosis is less prevalent in literate as compared to illiterate women who did not have access to education as literate women are more likely to have a good personal hygiene, are health conscious and seek early medical advice.

Among 94 women having bacterial vaginosis 69 did not receive any formal education (73%) as compared to 106 out of 206 (51%) among those not having bacterial vaginosis. The difference was very significant OR 2.5 (95% CI 1.5-4) with a p value of 0.0006. The effect of education on incidence of bacterial vaginosis is expected as it is likely to increase awareness about sexual health, lead to healthy sexual practices and early seeking of medical care.

Similar observations were made by various researchers before. T. Ashraf Ganjoei while looking at the risk factors for bacterial vaginosis found that low education level was a significant risk factor for bacterial vaginosis. OR 3.8 (1.68-8.64).[21] He also found smoking and previous preterm delivery and premature rupture of membranes as having significant association with bacterial vaginosis. In yet another study that looked at the effect of ethnicity and education on rates of bacterial vaginosis Claudia Holzman et al found that "lower education level was a significant predictor of bacterial vaginosis among both African, American and White women irrespective of ethnicity" thereby underlining the role of education in preventing bacterial vaginosis.[5] Kumar Aruna and Khare Jyoti et al also made similar observations. Thus improving the educational status of women overall among other innumerable advantages can decrease the incidence of bacterial vaginosis and thereby decreasing the incidence of adverse pregnancy outcome.[11]

Our study did not show any significant difference in the prevalence of bacterial vaginosis among rural or urban women. Although it seems rural women should have more of bacterial vaginosis, our study did not conform to this impression. Multiple previous studies also did not show rural location as a risk factor for bacterial vaginosis. Some significant Indian studies among these are as follows. Bhalla P, Chawla R studied prevalence of bacterial vaginosis in rural and urban communities of Delhi.[22] They found that overall bacterial vaginosis was diagnosed in 32.8% subjects. Highest prevalence was seen in urban slum (38.6%) followed by rural (28.8%) and urban middle class (25.4%). The difference between rural and urban middle class was not significant. Kosambiya JK, Vikas K Desai et al tried to estimate the prevalence of reproductive tract infection, sexually transmitted infection among women in urban and rural

areas of Surat.[23] They found that bacterial vaginosis was found in 24% of urban and 25% of rural women. They showed a similar prevalence in both groups. Similar observations were made by Chakraborty et al who had enrolled 50 women each of rural and urban background and found that bacterial vaginosis was present in 26% of rural women as compared to 30% among urban women.[20] The reason behind the lack of difference may be the increasing awareness about sexual health among rural women, improving educational status in rural areas. Less incidence of high risk sexual behaviour including multiple sexual partners, which is a risk factor for bacterial vaginosis, has been discussed by some. The other reason may be that only well off people reached our referral centre thereby underestimating the true prevalence among rural women. Increased risk of vaginal infections in rural women, however, should not be written off completely as there may be a selection bias in hospital based studies. In a community based study published in Lancet 1989 Bang RA and Bang AT et al enrolled 650 rural women and found that 55% had gynaecological complaints, which is high.[24] Rate of gynaecological infection was also high. Hence they have recommended that in rural areas of developing countries, gynaecological and sexual care should be a part of primary health care. Although the incidence of vaginal infections may be dropping in rural areas, continued efforts to improve sexual and gynaecological health care in these areas should be a priority.

Upon the comparison of parity we found that the percentage of women with previous birth in bacterial vaginosis group was 72% as compared to 58.5% in non-bacterial vaginosis group. The difference was significant with an odds ratio of 1.9 (95% CI 1.1-3.1) and p value of 0.027. It means the odds of having bacterial vaginosis was significantly lower in nulliparous women as compared to those with history of previous birth. Other studies also have shown that bacterial vaginosis is found more in women who had previously given birth. There have been no studies that have evaluated the effect of number of previous pregnancies with future risk of bacterial vaginosis. Smart S and Singel A et al in their study, aimed at addressing the social and sexual risk factors for preterm delivery, found that among other risk factors history of previous pregnancy was associated with increased risk for bacterial vaginosis (OR 1.5 $p<0.0006$).[25] Bhalla P and Kaushika A et al showed that bacterial vaginosis showed positive correlation with a parity of more than two.[26] The reason for increased risk in non-nulliparous women is not certain and it may not be directly related to parity. Increased risk may reflect more sexual activity in a woman who already had given birth as compared to those who have not. The fact that bacterial vaginosis increases with increased duration of married life was shown in same study by Bhalla P et al and they attributed it to increased exposure to sexual activity.[12] However, this hypothesis needs further validation through studies which focus on these specific points.

CONCLUSIONS

Bacterial Vaginosis is an important risk factor for preterm delivery and therefore requires early identification and treatment. Whiff test and identification of clue cells can be used individually in place of whole Amsels criteria without compromising the diagnostic value thereby saving the time and resources in a resource limited setting. Education of women may be instrumental in bringing down the overall incidence of bacterial vaginosis. However our study was a small study not designed to look at all predictors of preterm delivery and all risk factors for bacterial vaginosis, which need larger studies.

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