

**BACTERIAL VAGINOSIS IN PRETERM LABOUR AT A TERTIARY CARE CENTRE**

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**ABSTRACT: INTRODUCTION:** Bacterial Vaginosis is one of the important cause for pre-term labour which in turn can lead to infant mortality and morbidity, an easy and reliable Bacterial Vaginosis detection procedure is needed for early diagnosis and intervention to prevent adverse effects caused by bacterial vaginosis, this study provides a view on simple diagnostic techniques for early and easy detection of Bacterial Vaginosis to combat preterm labour. **METHODS AND MATERIAL:** 100 women (Study group) with preterm labour were screened for the presence of Bacterial Vaginosis by performing Whiff test, wet mount preparation, estimation of pH and Grams's staining and compared with 100 women (control group) with term pregnancy. **RESULTS:** the study group showed Bacterial Vaginosis in 42% whereas the control group had only 18%, Gram staining in the present study revealed normal flora 19%, intermediate flora 21%, BV 42% and scanty flora 16% in the study group. **CONCLUSIONS:** Preterm labour is one of the major cause for infant mortality and morbidity and Bacterial Vaginosis is one the predisposing factor for preterm labour so a simple diagnostic techniques to diagnose Bacterial Vaginosis will lead to early detection of Bacterial Vaginosis which may in turn decrease preterm labour and reduce infant mortality and morbidity.

**KEYWORDS:** Bacterial Vaginosis, Pre-Term Labour, Polymicrobial flora.

**INTRODUCTION:** Bacterial Vaginosis is of special public health concern in India because of the high burden of reproductive and pregnancy related morbidity.<sup>1</sup> BV is a very important cause of preterm labour.<sup>2</sup>

Since a long time, it has been conceived that genital tract infection during pregnancy, especially the cervix, may cause premature rupture of the membranes (PROM) and initiate preterm labor (PL) with subsequent maternal and perinatal morbidities and mortalities. Bacterial Vaginosis (BV) has been highlighted by its association with PL and PROM and other adverse pregnancy outcomes which represent a real challenge to obstetricians as they are the leading causes of high neonatal morbidity and mortality associated with prematurity.

Vaginal flora of a healthy woman is a dynamic ecosystem that can be altered easily. BV is defined as an imbalance in the normal vaginal flora with diminution in the normally-predominant lactobacilli and the proliferation of other anaerobic bacteria. A cause and effect relationship between BV and adverse pregnancy outcome can be better determined in cohort studies, in which BV is diagnosed before the onset of a number of pregnancy complications. In large percentage of cases of preterm labour (PL) the etiological factor is obscure and leads to an increased incidence of idiopathic PL.

BV is a very important cause of preterm labour (PL), hence diagnosis of BV plays a critical role in the outcome of the pregnancy.

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Global literature suggests that asymptomatic or minimally symptomatic infections are related to premature births. BV is believed to be a risk factor for preterm delivery, as well as being associated with peripartum complications such as chorioamnionitis, and postpartum endometritis.<sup>3</sup>

BV has also been linked to several puerperal morbidities including fever, and endometritis. Many studies revealed the association of BV with established preterm labor.

Bacterial Vaginosis [BV] is the most common variety of vaginal infection in which the normal predominant vaginal flora *Lactobacillus* is replaced with *Gardnerella vaginalis*, anaerobic bacteria and *Mycoplasma hominis*. The prevalence of BV is relatively high in women with poor hygiene, malnutrition, improper sanitation.

BV is polymicrobial condition with a decrease in vaginal acidity (towards alkalinity 4.5 -6.0) and decrease in *Lactobacilli*, accompanied by an increase in the number of other micro-organisms. Bacterial Vaginosis is currently thought to be a polymicrobial clinical syndrome distinguished by characteristic abnormalities of vaginal secretion and disturbances in vaginal ecology with displacement of normal lactobacillary flora by anaerobic microorganisms.<sup>4</sup> The term Vaginosis is used instead of vaginitis for this condition because there is no inflammatory response in vagina.

Routine screening for BV in higher risk group can reduce neonatal morbidity and mortality to significance extent in the developing countries.

This prospective study aims to screen for BV in patients with threatened PL and at high risk of PROM and to define a simplified screening plan applicable for the developing countries.

**MATERIALS AND METHODS:** The study was carried out in the Department of Microbiology, Rajiv Gandhi Institute of Medical Sciences (RIMS), Kadapa from March 2014 to Mar 2015, involving 100 women admitted in the Department of Obstetrics and Gynaecology, RIMS, Kadapa with idiopathic preterm labour as per selection criteria mentioned below.

### **Inclusion Criteria:**

1. Singleton pregnancy.
2. Gestational age between 28-36 wks.
3. Intact membranes.
4. Painful uterine contractions > 2 in 10 minutes each lasting > 45 seconds.
5. Cervical dilatation between 1 to 4 cm.
6. Cervical effacement > 25%.

### **Exclusion Criteria:**

1. Gestational age <28 weeks.
2. History of Antepartum hemorrhage, urinary tract infection, respiratory tract.
3. Infections, Diarrhea or any other obvious cause for preterm labour.
4. Medical complications of pregnancy such as severe Anaemia, PIH, DM.
5. History of leaking membranes or absent membranes.
6. Multiple pregnancy.
7. Intrauterine growth restriction.
8. Intrauterine foetal death.
9. Antibiotic therapy in the last 30 days.

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The study group included 100 women with preterm labour without any obvious cause for the same. Control group included 100 women carrying singleton pregnancy at term gestation. Detailed history taken, a thorough general and systemic examination was done to exclude exclusion criteria.

Patients were examined in lithotomy position. Local examination was done to exclude any local genital infections. Unlubricated cusco's speculum was introduced and per speculum examination was done after excluding leaking of the membranes.

**Evaluation of pH:** The vaginal secretion which collected on the posterior blade of Cusco's speculum was placed on a sterile glass slide for wet mount preparation. Vaginal pH measured by dipping pH paper into discharge collected on the speculum. The pH was tested by the pHydrion vivid refill papers with a pH range of 1 to 12 procured from the Himedia laboratory supplies. Color change was noted and the results were recorded.

**Whiff Test (Amine Test):** Whiff test was done by placing a drop of vaginal secretions on a sterile glass slide and few drops of 10% KOH (wt/vol) to the vaginal discharge and noting the emission of an offensive amine like odor (fishy odor) which indicated a positive amine test.

**Wet Mount Examination:** Vaginal secretions which were placed on sterile glass slide, diluted with a drop of normal saline and cover slip was placed over it and examined under Microscope (x40 magnification) for identification of clue cells, bacterial morphology and Trichomonas with special flagellated motility.

**Evaluation of Vaginal Smear:** With a sterile cotton swab the posterior vaginal wall was swept and was smeared on a clean and sterile glass slide. Smear is heat fixed and gram staining done and observed under Oil immersion objective (x1000).

A score of 0 to 10 was assigned based on Nugent's scoring system. In vaginal smear, three bacterial morphotypes are recognized. Lactobacillus morphotype (gram positive rods) Gardnerella vaginalis and bacteriodes species morphotypes (small gram negative to variable rods), Mobiluncus species morphotype (curved gram negative rods).

The amount of each morphotype detected on the smear is graded and then allocated a score as below.

Lactobacillus:	4+ = 0, 3+ = 1, 2+ = 2, 1+ = 3, 0 = 4
Gardnerella and Bacteroides	0 = 0, 1+ = 1, 2+ = 2, 3+ = 3, 4+ = 4
Mobiluncus:	0 = 0, + or 2+ = 1, 3+ or 4+ = 2

The individual score are then summed. The criterion for Bacterial Vaginosis is a total score of 7 or higher, a score of 4 to 6 considered and a score of 0 to 3 is considered normal.

### Findings of Gram Staining are classified as follows:

**Normal Flora:** Lactobacillus morphotypes predominate with few other morphotypes. Intermediate flora – Lactobacillus morphotypes reduced with other Normal flora – Lactobacillus morphotypes dominate with other morphotypes increased.

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**Bacterial Vaginosis:** Absent Lactobacillus with a great increase in other morphotypes.

**Aerobic Vaginitis:** A condition in which destructed vaginal flora has been replaced by Esch coli. Group B streptococci or enterococci.

### STATISTICS AND RESULTS:

AGE (in years)	No. of Cases	BV positive Cases	BOH History	Gram Staining	Whiff Test Positive	Clue Cells Positive	pH >4.5
< 19	02	-	-	-	-	-	-
20 - 24	80	39	10	38	38	37	45
25 - 29	14	03	03	04	05	02	04
30 - 34	02	-	01	-	-	-	-
>35	02	-	-	-	-	-	-
<b>TOTAL</b>	<b>100</b>	<b>42</b>	<b>14</b>	<b>42</b>	<b>43</b>	<b>39</b>	<b>49</b>

Table 1: Study Group (n = 100)

Age (in years)	No. of Cases	BV positive Cases	BOH History	Gram Staining	Whiff Test Positive	Clue Cells Positive	pH >4.5
< 19	08	02	-	02	02	02	02
20 - 24	55	13	03	11	15	08	17
25 - 29	27	03	06	05	01	05	08
30 - 34	07	-	01	-	-	-	-
>35	03	-	-	-	-	-	-
<b>TOTAL</b>	<b>100</b>	<b>18</b>	<b>10</b>	<b>18</b>	<b>18</b>	<b>15</b>	<b>27</b>

Control group (n = 100)

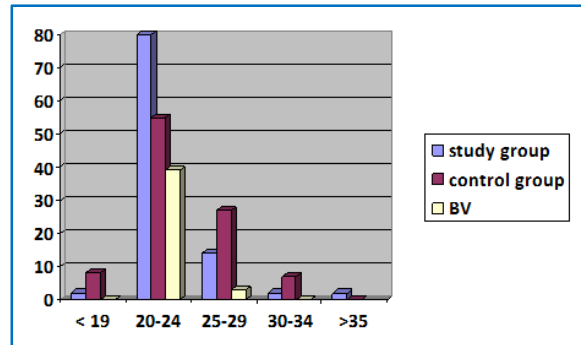
Age (in years)	Study Group	Control Group	BV Positive
<19	02	08	0
20-24	80	55	39
25-29	14	27	03
30-34	02	07	0
>35	02	03	0

Table 2: Age distribution in study and control group (n = 100)

In the study group of 100 cases, most (80) were in the age group of 20 – 24 yrs. In this group BV was seen in 39 cases. In under the 19 yrs age, there were 2 cases in the study group and 8 cases in the control group. None of these were positive for BV. In 25 to 29 yrs age group there were 14 cases in the study group and 27 in the control group. 3 in the study group were positive for BV.

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In the 30-34 yrs age group, there were 2 cases in the study group and 7 in the control group. In the age group of above 35 yrs, 2 were in the study group and 3 were in control group. There were no cases of BV in these age groups.

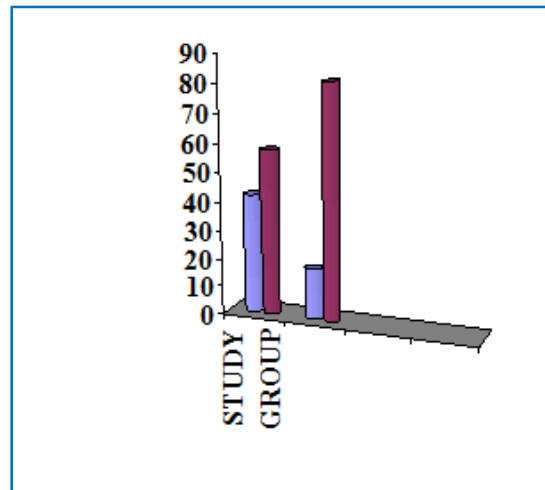


**Fig. 1**

BV	Study Group	Control Group
Women with BV	42	18
Women without BV	58	82

**Table 3: Incidence of Bacterial Vaginosis**

In the study group, 42 women had BV and in the control group 18 had BV.



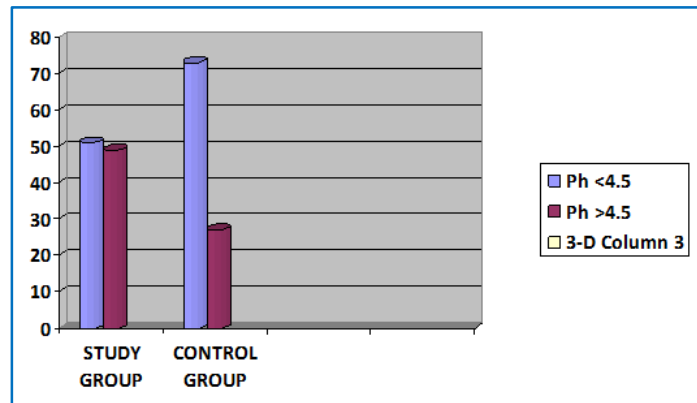
**Fig. 2**

pH	Study group	Control Group
	No. of Cases (%)	No. of Cases (%)
< 4.5	51	73
>4.5	49	27

**Table 4: Comparison of vaginal pH in both groups**

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In the study group, raised pH (more than 4.5) was observed in 49 women where as in control group this was seen in 27 women.

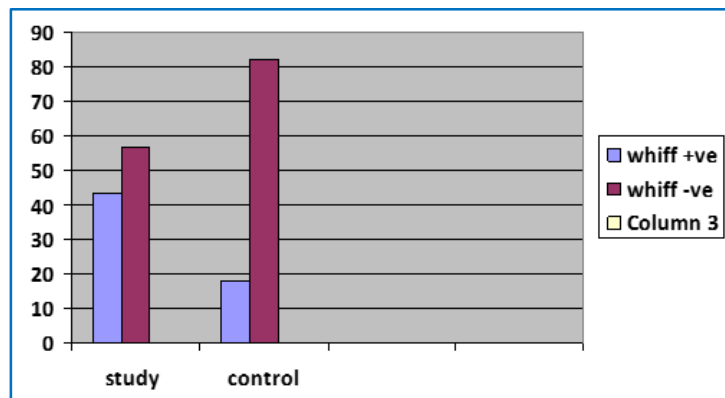


**Fig. 3**

Whiff test	Study Group No. of Cases (%)	Control Group No. of Cases (%)
Positive	43	18
Negative	57	82

**Table 5: Comparison of Whiff test among study and control groups**

Whiff test was positive in 43 women in the study group where as in the control group the test was positive in 18 women only.



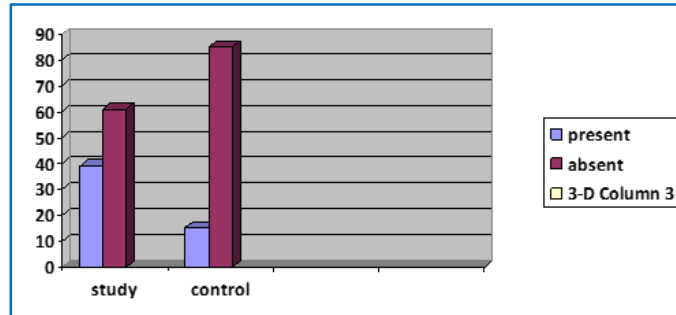
**Fig. 4**

Clue cells	Study group No. of Cases (%)	Control group No. of Cases (%)
Present	39	15
Absent	61	85

**Table 6: Comparison of Clue cells in both groups**

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Clue cells were identified and demonstrated in 39 women in the study group and in the control group only in 15 women.

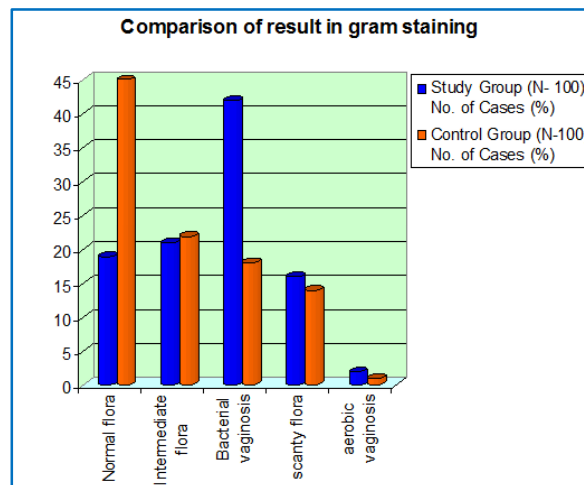


**Fig. 5**

Gram Staining Results	Study Group (n = 100)	Control Group (n = 100)	Nugent Score
	No. of Cases (%)	No. of Cases (%)	
Normal flora	19	45	0-3
Intermediate flora	21	22	4-6
Bacterial Vaginosis	42	18	7-10
Scanty flora	16	14	
Aerobic Vaginitis	02	01	-

**Table 7: Comparison of results of Gram staining**

Grams staining revealed - normal flora in 19 women, intermediate flora in 21 women, bacterial Vaginosis in 42 women and scanty flora in 16 women. Gram staining observations revealed Nugent score of 7-10 in all the cases of bacterial Vaginosis. (Table 7).



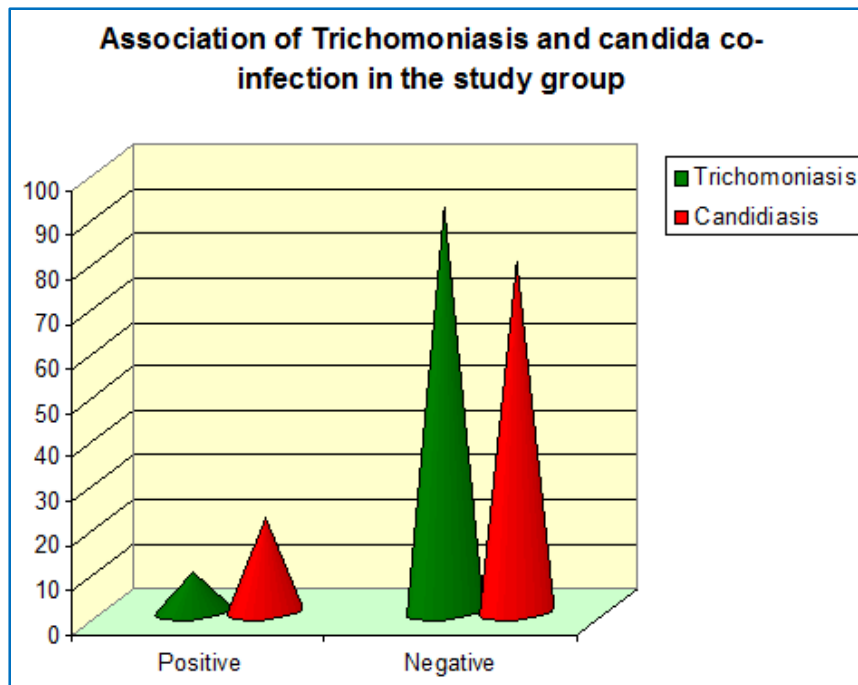
**Fig. 6**

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Name of confection	Positive	Negative	Total number of cases	BV positive
Trichomoniasis	09	91	100	6
Candidiasis	21	79	100	3

**Table 8: Association of Trichomoniasis and candida co-infection in the study group**

In the study group, 9 women showed the evidence of Trichomoniasis. Out of these, 6 women were diagnosed to be having BV. Candidiasis was seen in 21 women in the study group, out of which 3 were positive for BV. (Table 8).



**Fig. 7**

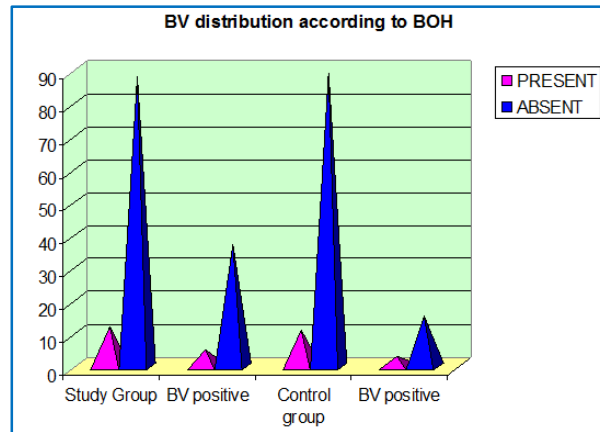
Bad Obstetrics History (BOH)	Study group	BV positive	Control group	BV positive
PRESENT	14	06	10	02
ABSENT	86	36	90	16

**Table 9: BV distribution according to BOH**

Bad obstetric history suggestive of abortions and preterm labours was recorded in 14 cases of the study group. Out of these 14 cases, 6 women were diagnosed to be having BV. In 86 women there was no suggestive bad obstetric history. In this category 36 were diagnosed to be having BV. (Table 9).



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**Fig. 8**

Gestational Age ( in weeks)	BV positive cases		BV negative cases	
	Study group	Control group	Study group	Control group
<34	32	14	40	76
>34	10	03	18	07

**Table 10: Incidence of BV according to gestational age**

In the study group, 32 cases and in the control group 14 cases were positive for BV in the gestational age of less than 34 weeks. In the gestational age of more than 34 weeks 10 cases in study group and 3 cases in control group were positive for BV (Table 10).

**DISCUSSION:** Study group of 100 women and a control group of 100 women admitted in the delivery room were tested for Bacterial Vaginosis. The diagnostic criteria in the present study were gram staining and Nugent criteria.

Sl. No.	Author	Incidence of BV
1	Madhivanan et al <sup>1</sup> 2008	19.0%
2	Awassanan Thanavath et al <sup>3</sup> 2007	19.0%
3	Azam Azargoon et al <sup>4</sup> 2006	16.0%
4	Saharan L Hillier et al <sup>5</sup> 1995	16.0%
5	Saharan SP et al <sup>6</sup> 1993	37.5 %
6	Carey JC et al <sup>7</sup> 2000	22.2%
7	Atef M Darwish et al <sup>8</sup> 2001	33.3%
8	Karat C et al <sup>9</sup> 2002	24.0 %
9	Desai veena et al <sup>10</sup> 2004	18.7 %
10	Nelofar Saleem et al <sup>11</sup> 2006	55.38%
11	Kumar Aruna et al <sup>12</sup> 2006	30.35%
12	Vineeta Gupta et al <sup>13</sup> 2009	43.6 %

**Table 11: Incidence of Bacterial Vaginosis**

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In the present study, 42 had BV (42%). This finding is almost similar to the work done by Vineeta Gupta et al (43.6%).<sup>13</sup> Similar studies by several authors yielded varying results which differ from our study. Kumar Aruna et al<sup>12</sup> reported 30.35%, Saharan SP et al<sup>6</sup> reported 37.5%, Atef M Darwish et al<sup>8</sup> reported 33.3%, Nelofar Saleem et al<sup>11</sup> reported 55.38% in their studies. The varying incidence of BV by different authors may be due to the factors like, different sample size, different geographic and socio-economic situations.

Almost similar incidence of BV by Vineeta Gupta et al<sup>13</sup> may be attributed to the similar sample size and the diagnostic criteria employed. Two diagnostic tests are commonly used for BV. Amsel criteria,<sup>14</sup> the test most commonly used in the clinical setting involves clinical conditions. There are inherent difficulties with each of the individual parameters in the Amsel criteria. The second most commonly used diagnostic test involves a Gram stain of vaginal discharge and applying Nugent criteria. The Nugent criterion<sup>15</sup> is the test most often used in epidemiologic studies in large scale, this method has several advantages that include 1) creating a permanent record that can be subsequently reviewed to confirm the diagnosis of BV and assess the reliability of the reading, 2) reporting intermediate stages of BV, which is particularly useful and 3) quantifying the amount of the three individual organisms, enabling assessment of the organism-specific risk of disease. These advantages are almost achieved in both the studies mentioned above. Thus, Nugent criteria is supposed to be ideal in diagnosing BV in the laboratory.

Gram staining in the present study revealed normal flora 19%, intermediate flora 21%, BV 42% and scanty flora 16%. A study by P Madhivanan et al<sup>1</sup> revealed normal flora 72.3%, intermediate flora 21% and BV in 68%.

In the present study, raised pH was seen in 49 cases accounting for 49%. All the BV diagnosed women showed raised pH. This is a very simple and rapid test which can be adapted to any health care setting. In a study by Atef M. Darwish et al<sup>8</sup> all the study group women with BV showed raised pH i.e., more than 4.5. Our study findings also reveal the same. Several studies in the developing countries for the screening of BV revealed similar findings. All these studies pointed out that for the diagnosis of BV the most sensitive test was pH of more than 4.5 due to its high negative predictive value coupled with the fact that it is an extremely simple and economical test to perform. Some other studies revealed a higher percentage of raised pH in BV.

Whiff test is most significant bed side test with high sensitivity, high specificity and high negative predictive value for diagnosis of BV. In our study Whiff test was positive for 43 cases which is less when compared to the study done by Atef M. Darwish et al<sup>8</sup> (75.6%) .

In present study, 32 cases were positive for BV in the gestational age of less than 34 weeks which can be compared with study done by Desai Veena A et al<sup>10</sup> (31.73%).

In a study conducted by Awassanan et al,<sup>3</sup> 15% cases were positive for BV in gestational age group less than 34 weeks and 15% in age group more than 34 weeks.

In our study group BOH was present in 14 cases out of which 6 were positive for BV; in control group BOH was present in 10 cases out of which 2 were positive for BV. This observation is in agreement with study conducted by Philip E Hay et al<sup>16</sup> who has shown that BV is associated with preterm delivery independent of recognized risk factors such as BOH.

Bacterial Vaginosis is one of the health problem accounting for the majority of cases of vaginitis and vaginal discharge in the developing countries. BV is an extremely prevalent condition in pregnancy, the true magnitude is not known because more than half of the BV cases are asymptomatic. This study focuses the importance of Nugent criteria and gram staining in the

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diagnosis of BV as a routine practice in the pregnant women. By this approach, the complications associated with BV in pregnancy can be prevented by diagnosing in early gestational age. This definitely reduces the adverse outcome of pregnancy associated with BV such as preterm labour, premature rupture of membranes, neonatal morbidity and mortality.

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