"Prevalence of Bacterial Vaginosis and its associated risk factors using Gram's staining and culture in pregnant women attending obstetric OP with white discharge at Government Maternity Hospital, Tirupati."

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Introduction:

In women, Bacterial vaginosis is the commonest reproductive tract disorder of childbearing age worldwide and accounts for one-third of vaginal infections.[1] It is distinguished by foul-smelling vaginal discharge, which on addition of 10% potassium hydroxide, gives a fishy odor. Other features like presence of clue cells, a few lactobacilli and polymorphonuclear leucocytes are also seen in Bacterial vaginosis.

Bacterial vaginosis can happen to anyone, both pregnant and not pregnant women. In some cases, there will be no inflammation and only foul-smelling vaginal discharge. In general, the prevalence is higher in parts of Africa.[2] Prevalence shown to be different depending on race and ethnic group.[3]

The complex balance of the microflora of vaginal changes in bacterial vaginosis, called polymicrobial syndrome. An increase in facultative and anaerobic bacteria and decrease in lactobacilli, either separately or together, are observed. Gardnerella vaginalis, Atopobium vaginae, Peptostreptococcus, Bacteroides fragilis, Sneathia, Mobiluncus, Propionibacterium, Leptotrichia, Veillonella spp., Fusobacterium, Ureaplasma urealyticum, Mycoplasma hominis, Staphylococcus spp., B Streptococcus spp.Vagina which is healthy typically has a small amount of

these organisms present. Due to their rarity, Mobiluncus species serve as a diagnostic indicator for BV.[4]

Pregnant women frequently experience Bacterial vaginosis, and studies have shown that BV increases the risk of adverse gynecological and obstetric outcomes like Preterm labor, premature rupture of the membranes, low birth weight, spontaneous abortion, and postpartum infections like endometritis and caesarean section wound infections.[5,5,7,8]

Early detection is crucial for prompt treatment, preventing related complications, and having a healthy pregnancy because most of the cases are asymptomatic. This is true especially for pregnant women. Due to its connection to sexually transmitted infections (STIs), bacterial vaginosis is a public health problem [9]

Various studies showed that it can be an important factor in the transmission of STIs because BV disturbs the vaginal ecosystem, making it more conducive to infection and thus impacting the dynamics of STI disease. Preterm births can be prevented by screening BV. BV is detected by Gram's stain (Spiegel criteria, Nugent's criteria) and accepted gold standard criteria (Amsel's composite criteria).[10]

To reduce all these complications there is a necessity to diagnose bacterial vaginosis early and start treatment. The first step in prevention of complications is to know the magnitude of the problem. There are few studies among pregnant women in our population, hence we felt a need to undertake this study.

Aim: To assess the prevalence of Bacterial Vaginosis and its associated risk factors using Gram's staining and culture in pregnant women attending obstetric OP with white discharge at Government Maternity Hospital, Tirupati.

Materials and Methods:

This was a Cross Sectional Study in 150 pregnant women attending obstetrics OP with complaint of white discharge per vaginum Government Maternity Hospital; Tirupati during the period from may 2021 to June 2022. **Inclusion criteria:** Pregnant Women attending obstetrics OP with complaint of white discharge. **Exclusion criteria:** Patients on antibiotic treatment,

Patients with bleeding per vaginum, or with chronic illness like genital tract malignancies. All identification details of patient like name, age, gender, marital status, parity, and address were taken. Using a sterile swab a high vaginal swab was collected. Gram's staining was done by preparing a smear and culture plates with blood agar and MacConkey's agar were inoculated and incubated in the Department of Microbiology. The values which are obtained according to the grading will be scored.

Results

Table 1: Socio-demographic characteristics

Variable		Number (n=150)	Percentage
Age category	**21 to 25 years**	29	19.3%
	26 to 30 years	57	38%
	31 to 35 years	41	27.4%
	36 to 40 years	23	15.3%
Religion	**Hindu**	90	60%
	Muslim	35	23.3%
	Christian	25	16.7%
Residence	**Urban**	57	38%
	Rural	93	62%
Socio-economic status	**Upper (Class I)**	4	2.7%
	Upper middle (Class II)	15	10%
	Middle (Class III)	47	31.3%
	Lower middle (Class IV)	14	9.3%
	Lower (Class V)	70	46.7%

Table 2: Menstrual and obstetric information of the pregnant women with white discharge

Variable		Number (n=150)	Percentage
Menstrual history	**Regular**	141	94%
	Irregular	9	6%
Contraceptive history	**IUCD**	10	6.7%
	Condom	8	5.3%
	OCPs	10	6.7%
	DMPA	2	1.3%
	None	120	80%

Gravida	**One**	**49**	**32.7%**
	Two	**75**	**50%**
	Three and above	**26**	**17.3%**
Trimester	**First**	**14**	**9.3%**
	Second	**93**	**62%**
	Third	**43**	**28.7%**

Table 3: Distribution of pregnant women by symptoms other than vaginal discharge

Symptoms	No. of patients (n=150)	Percentage
Itching	68	45.3%
Foul smell	44	29.3%
Dysuria	20	13.3%
Dyspareunia	17	11.3%
Lower Abdominal pain	14	9.3%
No other symptoms	41	27.3%

Table 4: Risk factors and its association with white discharge in pregnant women

Risk factor	No. of patient (n=150)	Percentage
Douching	10	6.7%
H/O IUCD insertion	12	8%
Diabetes	6	4%
Hypertension	6	4%
HIV Reactive	1	0.6%
Nothing significant	115	76.7%

Table 5: Characteristics of vaginal discharge among the pregnant women

Type of discharge	No. of patients (n=150)	Percentage
Mucoid discharge	54	36%
Homogenous greyish white discharge	29	19.3%
Curdy white discharge	28	18.7%
Watery discharge	26	17.3%
Frothy discharge	13	8.7%

Table 6: Gram's staining findings of vaginal discharge in the pregnant women

Gram stain	Total no. of patients (n= 150)	Percentage
Gram positive rods+	52	34.7%
Gram positive cocci+	13	8.7%
Gram negative bacilli+	26	17.3%
Pseudo-hyphae+	28	18.7%
Trichomonas+	20	13.3%
Clue cells+	33	22%
Epithelial cells+	87	58%
Pus cells+	60	40%

Table 7: Prevalence of the Bacterial vaginosis (BV) and other infectious diseases in pregnant women with white discharge

Diagnosis	Number (n=150)	Percentage
Bacterial vaginosis	42	28%
Vulvo-vaginal Candidiasis	28	18.7%
Trichomonas vaginalis	27	18%
Mixed infection	10	5.6%
None	63	42%

Table 8: No. of lactobacilli identified in vaginal discharges of pregnant women

No. of lactobacilli per oil immersion field	Overall (n=150)	With Bacterial vaginosis (n=42)	Without Bacterial vaginosis (n=108)
0	36 (24%)	25 (69.4%)	11 (30.6%)
<1	0 (0%)	0 (0%)	0 (0%)
1 to 4	12 (8%)	7 (58.3%)	5 (41.7%)
5 to 30	82 (54.7%)	10 (12.2%)	72 (87.8%)
>30	20 (13.3%)	0 (0%)	20 (100%)

χ^2 value = 54.09; df = 3; p<0.001 (Significant)

Table 9: No. of gram variable bacilli (Gardnerella vaginalis) identified in vaginal discharges of the pregnant women

No. of gram variable bacilli per oil immersion field	Overall (n=150)	With Bacterial vaginosis (n=42)	Without Bacterial vaginosis (n=108)
0	59 (39.3%)	0 (0%)	59 (100%)
<1	0 (0%)	0 (0%)	0 (0%)
1 to 4	27 (18%)	0 (0%)	27 (100%)
5 to 30	45 (30%)	23 (51.1%)	22 (48.9%)
>30	19 (12.7%)	19 (100%)	0 (0%)

χ^2 value = 94.22; df = 3; p<0.001 (Significant)

Table 10: No. of Mobiluncus like species identified in vaginal discharges of the pregnant women

No. of Mobiluncus like species per oil immersion field	Overall (n=150)	With Bacterial vaginosis (n=42)	Without Bacterial vaginosis (n=108)
0	78 (52%)	3 (3.8%)	75 (96.2%)
<1 to 4	40 (26.7%)	15 (37.5%)	25 (62.5%)
≥5	32 (21.3%)	24 (75%)	8 (25%)

χ^2 **value = 59.43; df = 2; p<0.001 (Significant)**

Table 11: Distribution of pregnant women with vaginal discharge by Nugent's score

Nugent's score	Number (n=150)	Percentage
0 to 3 (Normal)	69	46%
4 to 6 (Intermediate)	42	28%
7 to 10 (Bacterial vaginosis)	39	26%

Table 12: Effectiveness of Nugent's scoring in identifying bacterial vaginosis

Based on Nugent's score		Final diagnosis as bacterial vaginosis		Total
		Present	Absent	
Bacterial vaginosis	Present	32	7	39 (26%)
	Absent	10	101	111 (74%)
Total		42 (28%)	108 (72%)	150 (100%)

Table 13: Risk factors of Bacterial vaginosis (BV) among pregnant women

Variables		With BV (n=42)	Without BV (n=108)	p-value
1. Age	≤ 30 years	29 (33.7%)	57 (66.3%)	0.070; NS
	> 30 years	13 (20.3%)	51 (79.7%)	
2. Residence	Rural	19 (20.4%)	74 (79.6%)	0.008; S
	Urban	**23 (40.4%)**	34 (59.6%)	
3. Religion	Hindu	29 (32.2%)	61 (67.8%)	0.359; NS
	Muslim	8 (22.9%)	27 (77.1%)	
	Christian	5 (20%)	20 (80%)	
4. Socio-economic status	Class I & II	2 (10.5%)	17 (89.5%)	0.041; S
	Class III	10 (21.3%)	37 (78.7%)	
	Class IV & V	**30 (35.7%)**	54 (64.3%)	
5. Menstrual history	Regular	39 (27.7%)	102 (72.3%)	0.713; NS
	Irregular	3 (33.3%)	6 (66.7%)	
6. H/O Contraceptive	Yes	**17 (56.7%)**	13 (43.3%)	<0.001; S

usage	**No**	25 (20.8%)	95 (79.2%)	
7. Gravida	**One**	14 (28.6%)	35 (71.4%)	**0.917; NS**
	Two	20 (26.7%)	55 (73.3%)	
	Three & above	8 (30.8%)	18 (69.2%)	
8. Trimester	**First**	5 (35.7%)	9 (64.3%)	**0.431; NS**
	Second	28 (30.1%)	65 (69.9%)	
	Third	9 (20.9%)	34 (79.1%)	

S = Significant; NS = Not significant

Table 14: Comparison of symptoms other than white discharge with bacterial vaginosis

Symptoms other than white discharge	Total cases	With BV	Without BV	p-value
Itching	68	20 (29.4%)	48 (70.6%)	**0.726; NS**
Foul smell	44	14 (31.8%)	30 (68.2%)	**0.502; NS**
Dyspareunia	17	11 (64.7%)	6 (35.3%)	**<0.001; S**
Dysuria	20	3 (15%)	17 (85%)	**0.164; NS**
Lower Abdominal pain	14	5 (35.7%)	9 (64.3%)	**0.499; NS**

S = Significant; NS = Not significant

Table 15: Comparison of selective risk factors with bacterial vaginosis

Risk factor	Total cases	With BV	Without BV	p-value
Douching	10	5 (50%)	5 (50%)	0.215; NS
H/O IUCD insertion	12	6 (50%)	6 (50%)	0.151; NS
Diabetes	6	2 (33.3%)	4 (66.7%)	0.867; NS
Hypertension	6	0 (0%)	6 (100%)	0.274; NS
HIV Reactive	1	0 (0%)	1 (100%)	0.623; NS

S = Significant; NS = Not significant

Table 16: Comparison of type of vaginal discharge with bacterial vaginosis

Type of discharge	Total cases	With BV	Without BV	p-value
Mucoid discharge	54	9 (16.7%)	45 (83.3%)	**0.020; S**
Homogenous greyish white discharge	29	25 (86.2%)	4 (13.8%)	**<0.001; S**
Curdy white discharge	28	2 (7.1%)	26 (92.9%)	**0.006; S**
Watery discharge	26	3 (11.5%)	23 (88.5%)	**0.040; S**
Frothy discharge	13	3 (23.1%)	10 (76.9%)	**0.929; NS**

S = Significant; NS = Not significant

Table 17: Assessing the importance of clue cells in identifying bacterial vaginosis

Clue cells in Gram's staining	With BV (n=42)	Without BV (n=108)	p-value
Present	25 (75.8%)	8 (24.2%)	**<0.001; S**
Absent	17 (14.5%)	100 (85.5%)	

Table 18: Comparison of Gram's staining findings with final diagnosis

Finding on gram's staining	Bacterial vaginosis	Trichomonas vaginalis	Vulvo-vaginal candidiasis	Normal
Gram positive cocci	5 (38.5%)	2 (15.4%)	0 (0%)	6 (46.1%)
Gram negative rods	17 (65.4%)	8 (30.8%)	0 (0%)	5 (19.2%)
Gram positive rods	1 (1.9%)	2 (3.8%)	0 (0%)	50 (96.2%)
Pseudo-hyphae	2 (7.1%)	0 (0%)	28 (100%)	0 (0%)
Trichomonas	6 (30%)	20 (100%)	0 (0%)	0 (0%)
Clue cells	25 (75.8%)	10 (30.3%)	1 (3%)	2 (6.1%)
Pus cells	25 (41.7%)	19 (31.7%)	0 (0%)	20 (33.3%)
Epithelial cells	12 (13.8%)	11 (12.6%)	16 (18.4%)	53 (60.9%)

Table 19: Findings of the culture of white discharge in pregnant women

Culture findings	Number (n=150)	Percentage
Bacterial vaginosis (BV)	39	26%
Staph. Aureus	29	19.3%
Candida	15	10%
Gram negative bacilli	11	7.3%
E. coli	5	3.3%
Klebsiella pneumonia	3	2%
Mixed organisms with BV	17	11.3%
Normal study	31	20.7%

Table 20: Correlation of culture findings with Nugent's score

Culture findings	Median Nugent's score	Inter-quartile range (IQR)
Bacterial vaginosis (BV)	5	1 – 9
Staph. Aureus	3	1 – 7
Candida	4	2 – 5
Gram negative bacilli	4	2 – 9
E. coli	4	2 – 4
Klebsiella pneumonia	1	1 – 1
Mixed cultures	4	1 – 6
Normal study	4	1 – 7

DISCUSSION

In present study out of 150 pregnant women with white discharge, majorly 38% were in 26 to 30 years, followed by 31 to 35 years (27.4%), 21 to 25 years (19.3%) and 36 to 40 years (15.3%). Mean age of the study participants were 30.3 (±4.89) years, ranges from 21 to 40 years.

Most of the pregnant women reported with white discharge belongs to Hindu religion (60%), rural community (62%) and belongs to lower class (46.7%) based on modified BG Prasad socio-economic status classification. In a study by Yeshey Dorjey et al[11] the sociodemographic characteristics, like marital status, age, and education level, varied significantly between the patients with infective causes and non infective causes of vaginal discharge. In a study by Indu Lata et al,[12] Bacterial vaginosis occurred in 41 out of 200 patients. BV was more prevalent in groups with lower socioeconomic status.

The highest prevalence of BV was recorded in women aged 18–22 years similar to Ibrahim et al[13] while, Mengistie et al[14] recorded highest prevalence in women aged 21–29 years and in a study by Cristiano L et al[15] found that the incidence of bacterial vaginosis was commonest among the group of 18-27 years age. In a study by Ajani G et al[16] it was observed that Prevalence reported in various situations varied depending on the environment, a person's behavior, their socioeconomic standing, and the stressors they were exposed to.

The study by Adane Bitew et al[17] involved 210 women in total. Bacterial vaginosis was 48.6% common overall. The prevalence of bacterial vaginosis was slightly lower (41.5%) in younger women (15–24 years old), but it ranged from 47.8% to 60.0% in women 25 years and older. However, the adjusted odds ratio showed that age was not a significant factor in the incidence of bacterial vaginosis.

In the present study, out of 150 pregnant women with white discharge, majorly 94% women had regular menstrual history. Around 80% does not use any contraceptive method, if used any, IUCD (6.7%) and OCPs (6.7%) was used by a greater number of women. Most commonly white discharge was reported in gravida two (50%) and during second trimester

(62%). In a study by Yiewou Marguerithe Kamga et al[18] Participants who were primigravidae had a higher rate of BV than multi gravidae, albeit the difference was not statistically significant. In a study by Ibrahim et al [19]reported the highest prevalence of BV in the multigravida and second trimester of pregnancy.

In Present study, out of 150 pregnant women with white discharge, 8% women had history of IUCD insertion, 6.7% women were practicing douching, 4% had Diabetes, 4% had Hypertension, 0.6% were HIV reactive and remaining 76.7% had no significant risk factors for white discharge. The high prevalence of BV reported study by Yiewou Marguerithe Kamga et al[18] was due to the high rate of vaginal douching (221/309, 71.5%) seen among study participants. In a study by Mulinganya G et al,[21] the prevalence of BV was 26.3% and approximately half of the women with BV were asymptomatic.

In a study by Spiegel CA et al,[22]A relative reduction in the number of vaginal lactobacilli is one characteristic of this syndrome. In a study by Donugama Vasundhara et al,[23] women positive for BV harboured higher α-diversity and had lower abundance of L. helveticus. According to H. Chart et al,[24] Mobiluncus spp. are isolated from 97% of women with bacterial vaginosis (non-specific vaginitis) and is rarely found in the vagina of healthy women.

In the present study out of 150 pregnant women, majorly 46% pregnant women had overall Nugent's score of 0 to 3, followed by 28% had score between 4 to 6 and 26% had overall score between 7 to 10. By Nugent's scoring, 26% pregnant women with vaginal discharge were notified to have bacterial vaginosis.

In a study by Mohammed Ahmed et al,[25] According to Nugent's scoring system, of the 248 study participants, 51/248 (20.6%) were diagnosed as bacterial vaginosis with a Nugent score of

7–10, and 37/248 (14.9%) as intermediate with a Nugent score of 4–6. In other Indian study done by Abilasha gupta et al[26] found that out of 500 antenatal women bacterial vaginosis was diagnosed in 98 women and 350 women had normal vaginal flora nugent's scoring was (0-3), 52 out of 500 were intermediate (10.4%) i.e., Nugent's score 4-6 and 98 out of 500 (19.6%) had Gram stain consistent with bacterial vaginosis (BV) nugent's score (7-10).

In the present study apart from dyspareunia, symptoms such as itching, foul smell, dysuria and lower abdominal pain were significantly not associated with bacterial vaginosis. More number of pregnant women having dyspareunia (64.7%) had bacterial vaginosis (p<0.05; Significant). In a study by Aduloju OP et al,[27] Symptoms such as vulvar itching, dyspareunia, lower abdominal pains and characteristic of vaginal discharge such as colour and consistency were significantly associated with BV, p < 0.05. In a study by Yeshey Dorjey et al[11] it was found that foul smelly vaginal discharge was commonly reported in 24.2% patients with bacterial vaginosis. Profuse vaginal discharge was seen in bacterial vaginosis among 43.7%. Dyspareunia was reported in 18.6% of patients with bacterial vaginosis. Dysuria was present in bacterial vaginosis among 12.2%.

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