IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

PERFORMANCE EVALUATION OF AMSEL CRITERIA, NUGENT SCORING, BV BLUE TEST AND REAL – TIME PCR FOR DIAGNOSIS OF BACTERIAL VAGINOSIS

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ABSTRACT

Background: - Bacterial vaginosis (BV) is the most common cause of vaginal discharge among women in reproductive age. BV is associated with alteration of normal vaginal flora. BV is a sexually associated condition. It is characterized by increase in vaginal pH and presence of vaginal lactobacilli, *Gardnerella vaginalis* and Mobiluncus. BV is of special public health concern in India because of the high burden of reproductive and pregnancy related morbidity. High co-infection rates with sexually transmitted infections (STI) can increase the possibility of BV. BV can also increase the risk of pelvic inflammatory disease (PID). Pregnant female with BV can also increase the risk of preterm labour, premature rupture of membranes, chorioamnionitis and postpartum endometritis.

Methods: - A total of 250 samples of vaginal fluid samples were coming to departmental laboratory of Department of microbiology of the patients attending OPD Gynaecology and Obstetrics department of Santosh Medical College & Hospital, Ghaziabad in one year. High vaginal swab samples were taken from females were whiff test, gram stained, BV Blue test & RT-PCR were done. Diagnosed on the basis of Amsel's criteria, Nugent scoring, BV Blue test & RT-PCR for bacterial vaginosis.

Results: - Prevalence of BV were 66.8%. According to various tests it was found that Amsel's criteria shows 74%, Nugent scoring showed 66.8%, BV Blue test showed 68.8% & RT-PCR showed 66.8% bacterial vaginosis positive.

Conclusion: - Bacterial vaginosis can be considered a health problem among females. As Nugent scoring was considered as the gold standard method for diagnosis of bacterial vaginosis. As concluded that RT-PCR test is most sensitive and specific.

INTRODUCTION

Bacterial vaginosis is the most prevalent reason of vaginitis in females of reproductive age group. Bacterial vaginosis can be symptomatic or asymptomatic. There were more than 90 million patients come with bacterial vaginosis in worldwide per year [1]. Bacterial vaginosis mainly led to vaginitis and can became a reason to cause fetal loss, chorioammionitis, cervicitis, endometritis, urinary tract infection, cervical intraepithelial neoplasia, pelvic inflammatory disease i.e. PID, preterm labor & can deliver low birth weighted infants [2-5]. Vaginitis is also called as inflammation of vagina, normally featured by following vaginal discharge containing many WBC, vulvar itching, vulvar irritation, vaginal odor, vaginal erythema, dyspareunia & dysuria [6,7].

Sialidase enzyme activity was identified in one of ten clinical segregations of Gardenella Vaginalis. Gardenella vaginalis is presently found in mainly in all females that are having Bacterial Vaginosis [8]. Traditionally, BV is diagnosed in accordance to the criteria of Amsel et al[9] whereas following three of the four signs are important i.e. vaginal fluid pH is greater than 4.5, homogeneous vaginal discharge on examination, detection of fishy odor by adding 10% potassium hydroxide to vaginal fluid and with the presence of clue cells (>20%). Another method that is used widely is based on grading and scoring the microbiota in Gram stained smear vaginal fluid i.e. Nugent scoring (7-10)[10]. BV Blue test is a new point of care rapid chromogenic test based on detection of elevated sialidase activity in vaginal fluid of patients suffering from bacterial vaginosis[11]. Since it is possible to develop PCR assays that are quantitative for the numbers of specific types of organisms in the genital tract, a PCR-based assay could be less subjective in diagnosing BV than some of the available tests[11].

In addition, bacterial vaginosis itself is a risk factor for pelvic inflammatory disease, HIV, STIs, and other obstetric disorders.[12]

BV is often diagnosed clinically based on the Amsel criteria, wherein three of the following four signs must be evident: vaginal fluid pH greater than 4.5; homogeneous vaginal discharge on examination; detection of a fishy odor upon addition of 10% potassium hydroxide to vaginal fluid; and the presence of significant clue cells (>20%) [13]. The BV Blue test was compared to the standard method for the diagnosis of BV by Gram stain using Nugent score as a gold standard. As BV Blue test is a new point-of-care rapid chromogenic test based on the detection of elevated sialidase activity in vaginal fluid of patients suffering from bacterial

vaginosis[10]. The BVBlue test is used to aid in the diagnosis of BV. It detects elevated activity of vaginal-fluid sialidase, an enzyme produced by bacterial pathogens, such as gardnerella, mobiluncus, bacteroides, and prevotella.[14]

The purpose of our study to identify prevalent microorganisms causing Bacterial Vaginosis. Our study also aims also focusses on the related risks, rapid, specific, sensitivity and cost-effective test for the advancement of patient care causing bacterial vaginosis.

MATERIALS & METHODS

A cross - sectional study with 250 samples was conducted in the Department of Microbiology in collaboration with Gynaecology and Obstetrics OPD, Santosh Medical College & Hospital, Ghaziabad in one year.

SAMPLE COLLECTION

Sample were collected by gynaecologist by Cusco's bivalve speculum were introduced without any lubricant in vagina to retract the vaginal wall, three high vaginal swabs were used for collection d sample from posterior vaginal Wall and lateral vaginal wall by sterile swab stick. The swabs were recapped and transported to microbiology laboratory Santosh hospital for further processing.

SAMPLE PROCESSING

1. The sample were collected in three polyurethral swabs.
2. First swab was used to identify Amsel criteria and for gram staining (Nugent scoring).
3. Second swab were used for BV Blue test.
4. Third swab were used for Real – Time PCR for the detection of bacteria causing Bacterial Vaginosis.

Whiff test: A drop of the vaginal fluid were taken on a grease free glass slide. To this one drop of 10% KOH will be added. An intense, putrid, fishy odor indicates positive reaction.

AMSEL'S CRITERIA: Amsel's composite criteria includes

(1) the presence of a homogeneous vaginal discharge
(2) pH of the vagina being > 4.5
(3) the presence of clue cells
(4) a 'fishy' amine odor of the vaginal discharge which is with a positive whiff test.

DIAGNOSIS BY NUGENT'S CRITERIA: The vaginal discharge were smeared on clean glass slides, air dried, heat fixed and stained by Gram's staining. Each bacterial morpho-type was quantitated under an oil immersion objective (l00x) by using the following scheme. Large Gram-positive rods will be taken as lactobacillus morphotypes; small Gram-negative to Gram-variable rods will be considered as G.vaginalis and Bacteroides spp. morphotypes; curved Gram variable rods were considered as Mobiluncus spp. morphotypes.

Nugent scoring of Gram stained smear for bacterial vaginosis

Organism Morpho type	**Number/oil immersion Field**	**Score**
Lactobacillus – like (parallel sided, gram positive rods)	>30	0
	5-30	1
	1-4	2
	<1	3
	0	4
Mobiluncus- like (curved, gram negative rods)	<5	2
	<1-4	1
	0	0
Gardnerella/bacteroides-like (tiny, gram variable coccobacilli and pleomorphic rods with vacuoles)	>30	4
	5-30	3
	1-4	2
	<1	1
	0	0

Note: Total score: -0-3 Normal; 4-6 Intermediate, repeat test later; 7-10 Bacterial vaginosis.

BV Blue Test: The second swab were inserted in the BV Blue testing vessel and incubated for 10 min at 37°C. Two drops of BV Blue developer solution was added, and the colour reaction was immediately read. A blue or green colour indicates the sample contain elevated levels of sialidase and was therefore positive. A yellow colour indicates a negative result and no increasing sialidase activity.

Polymerase chain reaction (PCR) assay the third swab was used for Real – Time PCR assay for detection of the major BV causative agents, such as G. vaginalis, A. vagina, and Mobiluncus spp., targeting their 16S ribosomal ribonucleic acid (rRNA) genes were performed. All Mobiluncus spp. positive samples will be additionally examined by a species-specific PCR test for the identification of M. curtisii. The DNA will be amplified using the following protocol: initial denaturation (95 °C for 5 min), followed by 30 cycles of denaturation (95 °C for 45 s), annealing (58 °C and 69 °C for 45 s), and extension (72 °C for 45 s), with a single final extension of 7 min at 72 °C. PCR products will be separated in 1% agarose gel for 50 min at 140 V, stained with ethidium bromide , and detected by UV transillumination (wavelength: 312 nm). The amplification products were identified on the basis of fragment length.

Statistical analysis: Data were entered in the statistical analysis system (SAS). The collected data were presented in the form of tables, graphs, pie charts etc. Different statistical tests like P value (Probability value), two tailed Fisher exact test, Chi – square test, t test and Confidence interval etc. will be used accordingly.

RESULTS

In the present study, 250 vaginal fluid samples that were cross-sectionally studied from April 2018 - Feb 2019, in the Department of microbiology, laboratory of Microbiology in collaboration with Department of Gynaecology and Obstetrics OPD of Santosh Medical College &Hospital, Ghaziabad. The observations of the study are detailed below:

TABLE I

PREVALENCE OF BACTERIAL VAGINOSIS POSITIVITY IN VAGINAL FLUID SAMPLE

Total no. of cases studied	Total no. of Bacterial Vaginosis positive cases	Total no. of Bacterial Vaginosis negative cases
250	167(66.8%)	83(33.2%)

TABLE II

CHARACTERSTICS OF THE WOMEN

S.NO.	CHARACTERSTICS	NUMBER OF POSITIVE FOR BV (%)
1.	**AGE GROUP**	
	18 – 25	41(16.3%)
	26 – 35	109(43.5%)
	36 – 45	100(40.2%)
2.	**EDUCATIONAL QUALIFICATION**	
	GRADUATE	48.4
	HIGH SCHOOL	.8
	INTERMEDIATE	49.6
	POST- GRADUATE	1.2
3.	**MENSTURAL HISTORY**	
	IRREGULAR	11.2
	MENOPAUSE	1.6
	REGULAR	87.2
4.	**MARITAL STATUS**	
	MARRIED	88.8
	UNMARRIED	10.8
	WIDOW	.4
5.	**PREGNANCY**	
	14 WEEKS	.8
	26 WEEKS	.4
	36 WEEKS	.8
	ABSENT	98.0
6.	**HISTORY OF ABORTION**	
	1	6.8
	2	2.8
	3	1.2
	NULL	89.2
7.	**PELVIC INFLAMMATORY DISEASE**	40.2

8.	**SEXUALLY TRANSMITTED INFECTION**	11.2
9.	**ORAL CONTRACEPTION**	38.8
10.	**ANTIBIOTICS**	14.4
11.	**HABITS**	
	ALCOHOLISM	12.0
	NO	25.6
	SMOKING	24.4
	TOBACCO CHEWING	38.0
12.	**GRAVIDA**	
	0	12.0
	1	12.8
	2	10.8
	3	1.6
	4	37.6
	5	10.8
	6	9.2
	7	3.6
	8	1.2
	9	.4

TABLE III

AMSEL'S CRITERIA FOR VAGINAL FLUID SAMPLES

S.NO.	AMSEL'S CRITERIA	PERCENT
1.	0	13.6
2.	1	4.4
3.	2	8.0
4.	3	16.8
5.	4	57.2

TABLE IV

NUGENT SCORING OF VAGINAL FLUID SAMPLES.

S.NO.	NUGENT SCORING	Frequency	Percent
1.	Negative	84	33.6
2.	Positive	166	66.4
	Total	250	100.0

TABLE V

PREVALANCE OF BV BLUE TEST POSTIVITY IN VAGINAL DISCHARGE SAMPLE

S. NO.	BV BLUE TEST	FREQUENCY	PERCENT
1.	NEGATIVE	78	31.2
2.	POSITIVE	172	68.8
	TOTAL	250	100.0

TABLE VI

PREVALANCE OF RT-PCR POSITIVITY IN VAGINAL DISCHARGE SAMPLE

S.NO.	RT-PCR	FREQUENCY	PERCENT
1.	Negative	83	33.2
2.	Positive	167	66.8
	Total	250	100.0

TABLE VI

COMPARISION OF VARIOUS DIAGNOSTIC TESTS FOR DIAGNOSIS OF BACTERIAL VAGINOSIS

S.NO.	DIAGNOSTIC TESTS	SENSTIVITY	SPECIFICITY
1.	Amsel's criteria vs Nugent scoring	89.73%	100%
2.	Amsel's criteria vs BV Blue test	91.89%	96.92%
3.	BV Blue test vs Nugent scoring	100%	92.86%
4.	RT-PCR vs Nugent scoring	100%	98.8%
5.	BV Blue test vs RT-PCR	97.09%	100%

- The rate of Bacterial Vaginosis positivity vaginal fluid samples 66.8%.Maximum patients (43.5%) were in age group 26 – 35 years and minimum (16.3%) in age group 18-25.Maximum patients were with Intermediate degree i.e. 49.6% & minimum were with high school degree i.e. 0.8%.Females with regular, irregular and menopausal menstrual history in which females with regular menstrual history were 87.2% (maximum), 1.6% were with menopause (minimum).The rate of married females included in the study were 88.8%.The rate of women maximum was not pregnant i.e., 98%.The rate of women coming with history of abortion included in our study were with null, (maximum) i.e. 89.2%.The rate of women included in the study using barrier method of contraception were 38.8%.The rate of females with pelvic organ infection included in our study were 40.2%.The rate of women included in study with sexually transmitted infection were 15.2%.Maximum females were not on antibiotic i.e. 85.6%.Rate of female patients with different habits that are tobacco chewing, smoking & alcoholism with 38%, 24.4% & 12% respectively. Maximum number of female patients with more than four gravida i.e. 37.6% & minimum with nine gravida were 0.4%. In various vaginal fluid samples we found 185/250 positve in according to Amsel's criteria.In various vaginal fluids we found 166 positive cases according to different organisms morphotypes (7-10) in oil immersion as seen nugent score pattern after gram staining. Out of 250 samples BV Blue test give 172 positive cases in which we found 6 samples were give false positive.We found RT-PCR gave 167/250 in different vaginal fluid samples. In comparison to Amsel's criteria with Nugent scoring were 89.73% sensitive & 100% specific. In comparison to Amsel's criteria with BV Blue test were 91.89% sensitive & 96.92% specific. In comparison to BV Blue test vs Nugent scoring were 100% sensitive & 92.86% specific. In comparison to RT-PCR vs Nugent scoring were 100% sensitive & 98.8% specific. In comparison to BV Blue test vs RT-PCR were 97.09% sensitive & 100% specific.

DISCUSSION

Bacterial Vaginosis is a most prevalent type of condition that are crucial type of clinical complexity but are more irregular type of makeup of different types and distinctive type of bacteria that are present [11]. Still there were some another diagnostic method that were developed, like for example polymerase chain reaction (PCR), BV Blue test, Amsel criteria & Nugent scoring. However, many of the tests are costly and that are sensitive and specific that does not give a large benefit to the classical methods. Methods like Amsel and Nugent's scoring are the most practical, applicable and efficient type for identification of bacterial vaginosis, mainly in developing countries [1].

Table I shows the prevalence of bacterial vaginosis positivity in vaginal fluid samples that were 167/250 i.e. 66.8% as compared to previous studies. A study by **Dr Rao SR et al** showed 174 (48%) bacterial vaginosis positivity rate out of 362 cases. Table II shows 26 – 35 years i.e. 43.5% cases were included in study. There were many previous studies that supports our study, the studies by **Mahajan et al, Seth AR et al, Dr Rao SR et al, Tiyyagura S et al** & **Madhivanan et al** showed the mostly affected age group were 20 – 30 years of age group. females were studied till Intermediate i.e. 124(49.6%). A study by **Madhivanan P et al** included females that were uneducated i.e. 133 (48.1%). Women with regular menstrual history i.e. 87.2% A study by **Baruah et al** showed prevulatory (1-14 days) were 48/103 i.e. 46.6% & postovulatory (14-28 days) were 55/103 i.e. 53.39% bacterial vaginosis positive cases. In our study 88.8% of the married women were positive. Similar results were found by **Morris MC et al** showed 17.2% positivity rate in married females. Females were 14 weeks (0.8%), 26 weeks (0.4%), 36 weeks (0.8%) & unpregnant women were 98%. A study by **Aduloju OP et al** showed in second trimester were 43 (71.7%) were positive & in third trimester were 15 (25%) positive. women with a history of abortion were with 1 time, 2-time, 3 time & Null i.e. 6.8%, 2.8%, 1.2% & 89.2%. A study by **Seth SR et al** showed 30.3% were coming for abortion. Our study showed 40.2% women were with pelvic organ infection. Another study by **Seth AR et al** showed 41.07% were included with pelvic organ infection. 15.2% of the women with sexually transmitted infection. A study by **Seth AR et al** 20.5% patients were with sexually transmitted infection. 39.1% female were using barrier method of contraception. A study by **Kurewa et al** & **Seth AR et al** showed 60% & 32.14% respectively were using barrier method of contraception. showed 14.4% women were with a history of antibiotics. A study by **Ijeoma CC et al** showed 9 (13%) bacterial vaginosis positive with metronidazole & 7 (10.4%) bacterial vaginosis positivity with clindamycin. Maximum number of females with tobacco chewing i.e. 38% as compared to female doing smoking (24.4%) & alcoholism (12%). A study by **Seth AR et al** showed highest number of females were tobacco chewing 25.8%, smoking 1.7% & alcoholism were 10.71% included in the study. Maximum number of females coming with more than four gravida i.e. 37.6% there were a study that supports our study by **Kurewa et al** showed 61% patients coming with multi gravidae. Table III Vaginal fluid samples showing Amsel's criteria with 0, 1, 2, 3 &4 were 13.6%, 4,4%, 8%, 16.8% & 57.2% respectively. A study by **Mahajan G et al** showed 60/200 positive cases by Amsel criteria. Table IV showed 66.4% were bacterial vaginosis positive according to Nugent scoring pattern. Another study by **Mahajan G et al** showed 68/200 positive cases by nugent scoring. Table V shows 68.8%

were positive with BV Blue test. A study by **Shujatullah F et al** showed 240 (97.6 %) samples positive by BV Blue test. Table VI shows 66.8% were positive by RT-PCR. A study by **Kuster JG et al** showed 55 (36.42%) positive out of 151 vaginal discharge samples by RT-PCR. Amsel's criteria vs Nugent scoring gives 89.73% sensitive & 100% specific. Amsel's criteria vs BV Blue test shows 91.89% sensitive & 96.92% specific. BV Blue test vs Nugent scoring were 100% sensitive & 92.8% specific. RT-PCR vs Nugent scoring shows 100% sensitivity & 98.8% specific. BV Blue test vs RT-PCR shows 97.09% sensitive & 100% specific. A study by **Shujatullah F et al** shows Amsel's criteria were 67.1% sensitive & 90.6% specific. BV Blue test 97.6 % sensitive & 97.5% specific. Another study by **Khatoon R et al** concluded that the performance of BV blue test was better in comparison with Nugent score, with a high sensitivity & specificity of 95.3% & 92.1% respectively. Similar study by **Myziuk et al** who demonstrated the sensitivity & specificity of BV blue test were 91.7% & 97.8% respectively whereas , a study by **Kampan et al** showed BV Blue test with sensitivity of 100% and specificity with 98.3% on comparision to Nugent scoring. A study by **Kusters JG et al** showed PCR wa highly sensitivity 92% & specificity 96% as compared to Nugent scoring for diagnosis of bacterial vaginosis.

CONCLUSION

According to the present study, we found RT-PCR test is most sensitive as compared to other test i.e. Amsel's criteria, Nugent score and BV Blue test. As RT-PCR test is more expensive and takes much time as compared to other tests. We also found that after RT-PCR test & Nugent scoring are more effective and it is cost effective also. As concluded that RT-PCR test is most sensitive and specific.

Conflict of interest: None.

Source of funding: Nil.

Ethical clearance: Taken.

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