An Observational Study on Impact of Bacterial Vaginosis(BV) Infection on Female with Different Age Groups

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ABSTRACT

The most common cause of vaginal infections is bacterial vaginosis, also known as non-specific vaginitis. In our country, it is difficult to detect the organism early on and begin the appropriate treatment. This is mainly due to a lack of awareness among reproductive aged females and missed or ignored follow-up during gynecological treatment. In this disease there is vaginal discharge, which can be itchy or not. It strongly correlates with IVF implantation rates, preterm labor, premature membrane rupture, and low birth weight during pregnancy. This study sought to determine the prevalence of bacterial vaginosis among women of reproductive age. A cross sectional study wasconducted among 160 women of the reproductive age group ranging from 26 to 45 years of age at Aashakiran fertility world which is situated in the state of Punjab's GILLCO VALLEY, KHARAR. over a period of one and half years. Four groups bifurcated as 26-30, 31-35,36-40, 41-45 age included 40 patients each thus makinga total of 160. The study took over a period of one and half years for completion. The diagnosis was made on the basis of physical, biochemical and Nugent's Scoring method. Out of the total 160 women enrolled in the study 90 women showed positive signs of infection and majority of the infected females belonged to 26-30 age group.

The study shows us the high prevalence of bacterial vaginosis in age group ranging from 26 to 30 years (n=34).

The age group 41-45 had the least number of infected patients i.e., 13. Whereas the other two age groups i.e 31-35 and 36-40 had almost similar numbers of infected patients 22 and 21 respectively. The study performed below concluded that age group plays an important role in the prevalence of bacterial infection infemales undergoing IVF treatment. The result of this study suggested that the females mostly in their mid- twenties to late 30's women are more prone to developing bacterial vaginosis(BV).

Keywords: Bacterial Vaginosis, Vaginal Discharge, Preterm, Reproductive Age, Vaginal Candidiasis, Vagina

1. INTRODUCTION

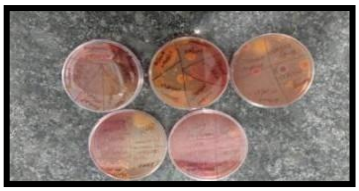
In developing nations, BV among people in their reproductive years is a serious issue. Recognizing the living being at a beginning phase and starting a legitimate treatment is undeniably challenging in ourcountry because of absence of mindfulness and appropriate development. The tough stratified squamous epithelium that covers a normal vagina isdevoid of any glands. Numerous pathogens cannot thrive in the hostile environment created by the acidity. Additionally, the normal flora in the upper third of the cervical canal, which includes Doderlein'sbacilli, diphtheroid, Group B and D streptococci, Coagulase-negative staphylococci, restricts the spreadof harmful plants. Vaginal infections are more common in females of mid-twenties (4). During menstruation, after an abortion, and during childbirth, for example, the vaginal defense mechanisms are less effective. Bacterial vaginosis, moniliasis, chlamydia, gonococcal, and trichomonad are some specific vaginal infections that occur in women of reproductive age.

The rest, with the exception of bacterial vaginosis, are mostly transmitted sexually. The most common cause of vaginal infections is bacterial vaginosis, which is alsoknown as non-specific vaginitis. The disease is associated with a low inflammatory response and is caused by Gardnerella vaginalis, the causative organism.

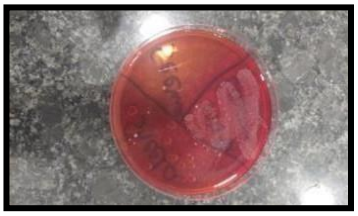
It is characterized by a shift in the vaginal flora, with an increase in Gardnerella vaginalis and resident anaerobic bacilli and a decrease in the number of Doderlein's bacilli (5). The disease causes vaginal discharge, which can be itchy or not. It is strongly linked to preterm labor, premature membrane rupture, and low birth weight during pregnancy. It may result in pelvic inflammatory disease, and antimicrobial use and chemotherapy are major risk factors for Candida infection and infertility.

Post-inflammatory changes in the fallopian tube and tube ovarian mass, which alter vaginal flora, account for the majority of infertility. Infertility can also be broughton by other organisms like Chlamydia, mycoplasmahominis.

Most common among people with low socioeconomic status, some of the factors that contribute to an imbalance in the microorganism population include vaginal discharge, immunodeficiency status, sexual activities, and antibiotic use. These vaginal diseases lead to excruciating sex, ongoing pelvic agony, tingling in the privates. The signs of an infection include changes in secretions color, smell, and quantity, as well as lesions in the vagina. The purpose of this study was to investigate the prevalence of bacterial vaginosis in women of reproductive age (6,7).



PIC1: PETRI DISHES WITH DIFFERENT BACTERIAL GROWTH



PIC2: PETRI DISH WITH BACTERIAL COLONIES

2. METHODS AND MATERIALS

Individual women of varying ages who are receiving treatment for infertility were chosen for sampling (n=160). The data was collected for each groups with 40 females of age groups lying from 25-45 age:

- Group A 40 females (26-30 years)
- Group B 40 females (31-35 years)
- Group C 40 females (36-40 years)
- Group D 40 females (41-45 years)

Preparation of Blood Agar:

Blood agar was used to isolate the suspected bacteria. Blood agar is made up of sodium chloride, sheep blood, a protein source, soybean protein digest, and agar. Blood agar was made by dissolving 11 grams of powdered agar media in 500 milliliters of distilled water in a flask. It was sterilized for 15 minutes in an autoclave at 121o C after being shaken for a while to completely dissolve the medium. The prepared media was then added to the clean plates. For the streaking, blood agar plates were used, and test plates were incubated for 24 hours in a hot air oven at 37o C. The plates showed signs of growth 24 hours later. After that, to see if bacterial vaginosis had an effect on IVF outcomes, we compared the results of IVF where bacterial growth was observed. For that in a sterile condition swab sample was proceeded from the consented female patient randomly selected as per age criteria of 4 groups from 26-30 yrs., 31-35yrs., 36-40 yrs. & 41-45yrs. of age. A non-lubricated speculum waspassed into the vagina, a Dacron-tipped swab was inserted, and a sample taken from the upper layer of the vagina. The swab was then wiped onto a pre- labelled glass slide and allowed to air dry. After that, the swab was taken out, applied to a labelled slide, and air dried properly to proceed the methods. Vaginal pH was measured by touching the collection swab to a pH indicator strip (4.0 to 7.0). The pH was recorded immediately, and the outcomes were recorded.

3. RESULTS AND DISCUSSION

The majority of the patients that were diagnosed positive for BV fell in the age group of 26-30 (34). Among this age group the greatest number of caseswere in age group 27 (16) followed by 26 (n=11), 25 (n=2), 30 (n=1), 28 (n=1), 29 (n=3). This age group hence has been found to be most vulnerable for the BV infection. The age group of 31-35 and 36- 40 had an almost similar number of infected patients i.e., 22 and 21 respectively. The maximum was of the age 32 (12), then 33 (n=5), 31 (n=2) 34 (n=1) 35 (n=3).

The age group of 36-40 had 21 patients. Maximum being in 38 (n=14), 36 (n=2), 37 (n=3), 39 (n=1), 40 (n=1).

The age group of 41-45 had the least number of patients i.e., 13 and maximum of them being of the age42 (n=6), 41 (n=1), 43 (n=2), 44 (n=1), 45 (n=3).

Below in a tabular form showing BV infected female among all the females of different age groups.

AGE GROUPS	BV+ FEMALES	PERCENTAGE
25-30 yrs.	34	37.25 %
31-35 yrs.	22	24.50 %
36-40 yrs.	21	23.05 %
41-45 yrs.	13	14.70 %

TABLE1: AGE CHARACTERSTICS OF WOMEN INFECTED WITH BV

Below mentioned in Table 2, the bacteria that cause BV and the factors that contribute to the vaginal overgrowth werefound to be significantly linked in this study. Among other things, the women's age and the Ph level of their vagina (4.5) were significantly linked to bacterial overgrowth in the vagina and BV. The vagina pH level of 4.5 was found to be significantly more correlated with women under the age of 30 had been linked to the BV causing overgrowth of bacteria in the vagina (8).

In addition to this confirm this outcome of BV correspondence to age factors in affected female the Nugent Scoring system is used to calculate and find out exact outcome. The Nugent Scoring system along with the microscopic and physical examination of females isan important part for the BV diagnosis.

CHARACTERSTICS		FREQUENCY	BV		COR (95% CI)
			YES	NO	
AGE	26-30 yrs.	40 (25%)	28	12	7 (1.57, 31.79)
	31-35 yrs.	40 (25%)	20	20	1.2 (0.48, 2.73)
	38-40 yrs.	40 (25%)	24	16	0.9 (0.45, 1.69)
	41-45 yrs.	40 (25%)	15	25	0.5 (0.30, 0.92)
DISCHARGETYPE	ODOR				
	Abnormal	120 (75%)	42	78	1
	Normal	40 (25%)	14	26	0.01 (0.0007, 0.024)
pH VALUE	<4.5	108 (67.5%)	62	48	1
	>4.5	52 (32.5%)	24	28	4.3 (2.790, 6.563)

TABLE2: FACTORS ASSOCIATED WITH BACTERIAL OVERGROWTH IN THE VAGINA

Also, as per Nugent scoring there are only few cases that falls in intermediate range and most of the females are included in BV category is listed in the table3. Thisscoring system helps in assessment of bacterial coloniespresent in vaginal flora and finding its morphotypes.

NUGENT SCORING	BV +VE	PERCENTAGE
BV	45	50%
INTERMEDIATE	08	08.82%
NORMAL	37	41.6%

NORMAL (0-3), INTERMEDIATE (4-6), BV (7-10)

TABLE3: NUGENT SCORING OF GRAM STAINING DIAGNOSIS OF BV

4. STATISTICAL ANALYSIS

Here, the effect of BV and its impact on reproductive age of female is studied. Moreover, the highest incidence of BV positive is seen in the age groups of 25-30 years of age. But the exact cause of this maybe due to the lifestyle of individuals. The treatment for this BV in women is possible if they only adhere to regular follow up and assessment during fertile period.

The total subjects considered is N=120 in which and participants of each groups is closely analyzed according to symptoms and outcome for result.

The data used in test 4.1 is from Table:1 where BV positive females are sorted in each age groups.

4.1. AGE DISTRIBUTION AND BV POSITIVE FEMALE

$\chi^2 = \Sigma [(O_i - E_i)^2 / E_i]$

Where: χ^2 = chi-square value

O_i = observed frequency for each age group

E_i = expected frequency for each age group

Using the given data, we can calculate the chi-square value as follows:

$\chi^2 = [(34 - 22.5)^2 / 22.5] + [(22 - 22.5)^2 / 22.5] + [(21 - 22.5)^2 / 22.5] + [(13 - 22.5)^2 / 22.5]$

$\chi^2 = 5.89 + 0.011 + 0.10 + 4.01$

$\chi^2 = 10.01$

Therefore, the chi-square value for the given data is 10.01. The degrees of freedom for this test would be (number of age groups - 1) = 3.

Significance level of $\alpha = 0.05$, which is a common choice in statistical hypothesis testing. We will compare the obtained chi-square value with the critical value at this significance level.

Using the degrees of freedom (df = 3) and the chosen significance level ($\alpha = 0.05$), we can look up the critical value from the chi-square distribution table. For df = 3 and $\alpha = 0.05$, the critical value is approximately 7.815.

Comparing the obtained chi-square value ($\chi^2 = 10.01$) with the critical value (7.815), we see that the obtained chi-square value is larger than the critical value.

Since the obtained chi-square value exceeds the critical value, we can reject the null hypothesis at the $\alpha = 0.05$ significance level. This indicates that there is a statistically significant association between age groups and the number of females. In other words, the observed distribution of females across age groups is significantly different from what would be expected under a uniform distribution.

The graphical distributions are correlated in bar chart for positive females and their age distribution. Patients were classified into 4 categories were positive tested females showing highest to least percentage of effected female

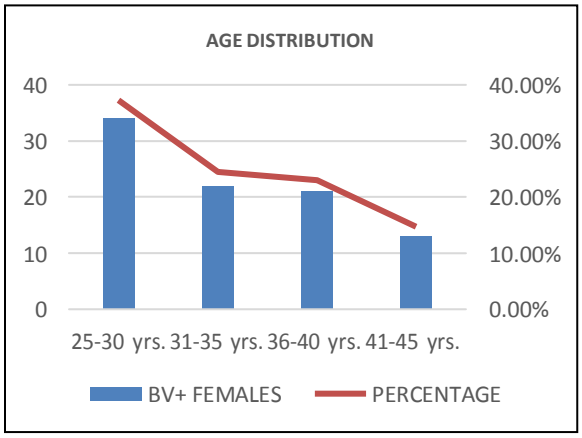


FIG1: BAR CHART FOR BV POSITIVE FEMALES AS PER AGE DISTRIBUTION

4.2. DIFFERENT FACTORS AS PER AGE DISTRIBUTION

To calculate the expected frequencies, data collected from Table:2 where we assume no association between age groups and BV. We will use the row totals, column totals, and the total sample size.

Total sample size = 160

Step 2: Calculate the chi-square value.

The chi-square value is calculated using the formula:

$\chi^2 = \Sigma [(O_i - E_i)^2 / E_i]$

Where: χ^2 = chi-square value

O_i = observed frequency for each cell

E_i = expected frequency for each cell

Using the given data and the expected frequencies, we can calculate the chi-square value:

$\chi^2 = [(28 - 7)^2 / 7] + [(12 - 3)^2 / 3] + [(20 - 5)^2 / 5] + [(20 - 5)^2 / 5] + [(24 - 6)^2 / 6] + [(16 - 4)^2 / 4] + [(15 - 3.75)^2 / 3.75] + [(25 - 6.25)^2 / 6.25]$

$\chi^2 \approx 347.96$

Therefore, the chi-square value for the AGE vs. BV table is approximately 347.96

2.1 Discharge Type vs. Odor

$\chi^2 = \Sigma [(O_i - E_i)^2 / E_i]$

Where: χ^2 = chi-square value

O_i = observed frequency for each cell

E_i = expected frequency for each cell

Using the given data and the expected frequencies, we can calculate the chi-square value:

$\chi^2 = [(42 - 31.5)^2 / 31.5] + [(78 - 58.5)^2 / 58.5] + [(14 - 10.5)^2 / 10.5] + [(26 - 19.5)^2 / 19.5]$

$\chi^2 \approx 12.36$

Therefore, the chi-square value for the Discharge Type vs. ODOR table is approximately 12.36.

Comparing the obtained chi-square value ($\chi^2 \approx 12.36$) with the critical value (3.841), we see that the obtained chi-square value is larger than the critical value.

Since the obtained chi-square value exceeds the critical value, we can reject the null hypothesis at the $\alpha = 0.05$ significance level. This indicates that there is a statistically significant association between Discharge Type and Odor.

The clustered graph showing the different physical factors that correlates with BV outcome in effected females. In addition, to this the age distribution is kept in notice to find out the significant correlation with age and BV outcome.

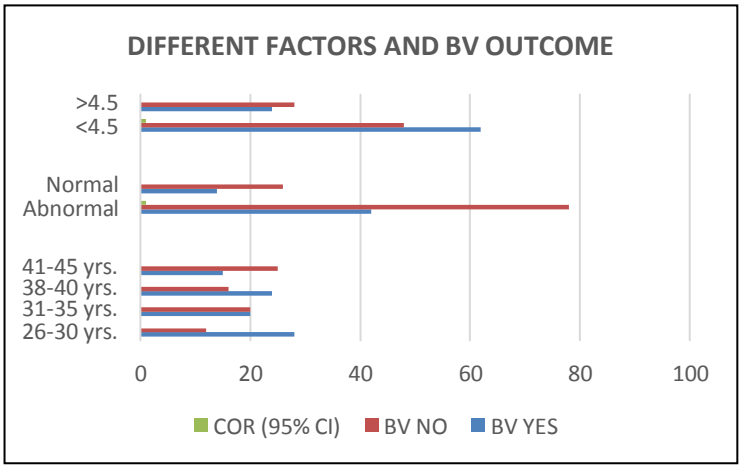


FIG2: CORRELATION OF DIFFERENT FACTORS WITH BV OUTCOME

4.3 NUGENT vs BV OUTCOME

In this test analysis the data is collected from Table:3 where the findings of Nugent score with BV is done. In which the outcome of BV positive female is done on the basis of Table:2 where different correlated factors are used to find out total outcome of BV positive females.

Here, chi-square value is calculated using the formula:

$\chi^2 = \Sigma [(O_i - E_i)^2 / E_i]$

Where: χ^2 = chi-square value

O_i = observed frequency for each cell

E_i = expected frequency for each cell

Using the given data and the expected frequencies, we can calculate the chi-square value:

$\chi^2 = [(45 - 25)^2 / 25] + [(8 - 4.41)^2 / 4.41] + [(37 - 20.8)^2 / 20.8]$

$\chi^2 \approx 27.085$

Therefore, the chi-square value for the NUGENT SCORING table is approximately 27.085.

Comparing the obtained chi-square value ($\chi^2 \approx 27.085$) with the critical value (3.841), we see that the obtained chi-square value is much larger than the critical value.

Since the obtained chi-square value exceeds the critical value, we can reject the null hypothesis at the $\alpha = 0.05$ significance level. This indicates that there is a statistically significant association between NUGENT SCORING and BV status.

Therefore, based on the chi-square test, we can conclude that there is evidence to suggest that there is a significant association between NUGENT SCORING and BV status. The NUGENT SCORING category is significantly related to the presence or absence of BV.

In this graph below displaying distribution of females according to age groups. According to Nugent Scoring criteria the outcome of the BV positive is around 50% i.e., half of the total subjects enrolled in this study. And the least percentage of female is in intermediate category.

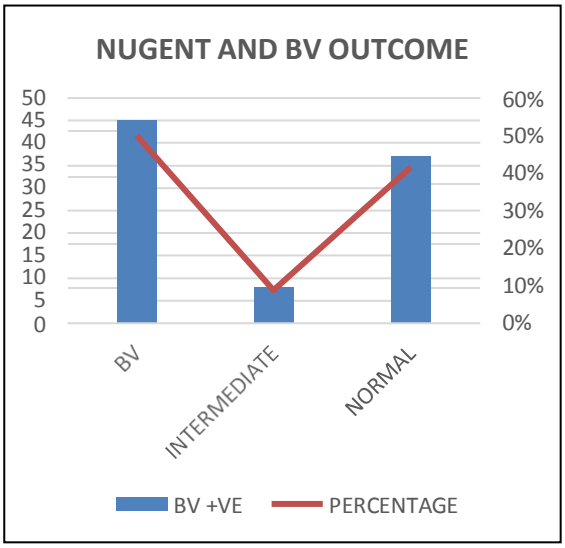


FIG3: DISTRIBUTION OF FEMALES ACCORDING TO BV POSITIVE, INTERMEDIATE & NORMAL

5. CONCLUSION

From the analysis presented above, and the described results of bar charts of all variables that is applied in thisstudy, final findings show a correlation of BV along with IVF outcome in females that is affected with infection. The prevalence of bacterial vaginosis has been the subject of numerous studies in various parts of India. Also, test was carried out using Chi- square method to establish this correlation by keeping age, and other related factors in parallel. Our study's prevalence of bacterial vaginosis was 50%, which was higher thanthe previous study by Bhalla et al. (40.66%) and in Barbados (33%) (3). Numerous other prevalence ranges from 25.4% to 38.6% have been reported in reviews of the literature. The first group i.e., 26 to 30-year-old hadthe highest prevalence. According to our study, femalesover the age of 40 had a lower incidence, which is in linewith previous research by Ako et al.

However, there was a significant and protective link between the types of discharge and BV (9). The study demonstrates the high prevalence of bacterial vaginosis and the importance of routine PAP smears for omen in reproductive age. The simple method of analysis and treatment should be focused on to forestallmorbidities.

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