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ORIGINAL ARTICLE

Bacterial Vaginosis and its Relation with Sociodemographic Determinants in Married Reproductive Age Females

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ABSTRACT

Aim: To determine the frequency of Bacterial vaginosis by pap smear cytology and find its association with its sociodemographic determinants.

Study design: Cross sectional analytical study

Place and duration of study: Department of Histopathology, Shaikh Zayed Hospital, Lahore from 1st January 2016 to 31st October 2016.

Methodology: Two hundred and sixty pap smears of married women of reproductive age group with vaginal discharge who attended Gynecology OPD were included. Proper history regarding age, socioeconomic status, age of marriage, literacy level, smoking, and nutritional status was taken.

Results: Disease prevalence was found to be 19.2%(50)in this sample of 260 while 9.1% had Candidiasis, 47%, 17.2% ,3.7%, 4.1% had Mild nonspecific inflammation, Moderate nonspecific inflammation, Moderate to severe nonspecific inflammation, and negative smears respectively. Bacterial vaginosis was associated with age, monthly income, illiteracy, early age at marriage, history of abortion and nutritional status with significant p-value ≤ 0.05. However no association was found between the disease and marital status, smoking and pallor.

Conclusion: Low socioeconomic status, literacy, age >35, nutritional status is strongly related to occurrence of bacterial vaginosis in women of reproductive age group with vaginal discharge.

Key Words: Bacterial vaginosis, Pap smear, Sociodemographic determinants

INTRODUCTION

Bacterial vaginosis (BV) is a perplexing disorder because of its unidentified cause and course of disease, despite fifty years of medical research on it. Infections of cervix and vagina are a major issue faced in the developing world. *Gardnerellavaginalis* and *Trichomonas vaginalis* cause 90% of vaginal infections. *Trichomonas vaginalis* is transmitted sexually and makes an anaerobic environment that produces a fertile soil for Bacterial vaginosis and presents most commonly as per vaginal discharge.[1]

In reproductive age group women, bacterial vaginosis is one of the common causes of vaginal discharge.[1-5] It is caused by poly microbiological agents[4] characterized by abnormalities of vaginal secretions and disturbance in vaginal ecology. The *Gardnerellavaginalis, Mobiluncus, Mycoplasma hominis, Ureaplasmurealyticum and Prevotella* are the common microorganisms involved in this[6].

The worldwide prevalence ranges from 11-48% among female population of child bearing age and varies from population to population[7]. Half of the women with Bacterial vaginosis are asymptomatic[8].

Symptomatic patients usually present with history of homogenous milky white discharge, mild itching, fishy odor[9] which becomes more evident after sexual contact[5].

Many risk factors for bacterial vaginosis including social, behavioral, cultural, ethnic, biological and sexual factors are identified[10]. These include multiple sexual partners, sexual activity at young age[4], homosexuality[2,4] douching[3,11], cigarette smoking[11], use of intra-uterine devices[3,11] nutritional status and hygienic practices.[4,5]

Cytological study by pap smear is an effective tool in early diagnosis of many treatable infections including Bacterial vaginosis[12-15]. Presence of Clue cells on cervical smear cytology (pap smear) which are desquamated vaginal epithelial cells densely layered by adherent bacteria so that their bodies are indistinct, has proved to be an important criteria for diagnosis of bacterial vaginosis.[11,16]

Pap smear is very helpful in early diagnosis and treatment in many pre-malignant conditions and cervical intraepithelial neoplasm (CIN)[12-15]. It should therefore be available to all patients coming with history of vaginal discharge and should be used as a routine screening test in women above 25 years of age[11-15].

Bacterial vaginosis is a common condition leading to serious complications in women presenting with vaginal discharge both in developed and developing countries. Earlier studies have analyzed frequency of BV in various socio economic settings but have inadequately addressed various factors causing BV and their correlation to occurrence of BV.

Secondly two studies conducted in Pakistan one in Karachi[6] and other in Rawalpindi[17] found out only the frequency of BV in the two cities with results of 55% prevalence in Karachi and 16-30% in Rawalpindi indicating a huge gap.

Considering these studies and the worst complications, BV can cause, due to late diagnosis and

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treatment, limitations of laboratory methods to diagnose it and lack of any research in Lahore city, which is the second most populous city of Pakistan and 16th largest city of the world, it is important to add some valuable information to literature, in order to identify the important modifiable factors and their relation to occurrence of BV, diagnosed by a simple noninvasive technique of Pap smear.

PATIENTS AND METHODS

This cross sectional analytical study was conducted at Department of Histopathology, Shaikh Zayed Hospital Lahore from 1st January 2016 to 31st October 2016. Two hundred and sixty pap smears of married women of reproductive age group with vaginal discharge who attended Gynecology OPD were included. Research was approved by our Ethical Review Board. Family having income of US $2 (210Rs) per day per person or less was considered Low Socioeconomic Group. Sociodemographic factors included Age, education level, income level, age at marriage. Literacy was defined as the "ability to identify, understand, interpret, create, communicate and compute, using printed and written materials associated with varying contexts**.** Persons unable to read and write were taken as illiterate and those literate were further categorized as primary, middle, metric, inter and above. Age of marriage less than 18 years was considered as early age of marriage. Slides were fixed with 85% alcohol. Alcohol, fixed slides were stained with Papanicolaou stained. These slides were mounted with DPX[15] and examined under microscope for presence of the Clue cells which are Desquamated vaginal epithelial cells densely layered by adherent bacteria so that their bodies are indistinct[18].

Pap smears of reproductive age women (15-49) either pregnant or non-pregnant, belonging to all socio economic groups, with recent history of abortion or threatened abortion with vaginal discharge having relevant clinical information with appropriate labeling and identifying information on each slide were included. Inadequate and blood stained smear were excluded.

Staining procedure and technique: Examination was scheduled 2 weeks after the 1st day of LMP, more specifically not during menses for non-pregnant women. It was made sure that vaginal medicines, vaginal contraceptives or douching were not used 48 hours before appointment and no intercourse was made a night before appointment. Samples were taken from transitional zone i.e. squamo-columnar junction that is a home for cervical dysplasia and cancers. Non-lubricated (only moistened with water) Ayre's Spatula was inserted and rotated at least 360 degrees. One half of the slide was covered to avoid coating it with fixative before the endocervical sample was applied. Brush was inserted in endocervical canal to get adequate sample. When brush was inserted some bristles were still visible. Brush was rotated getting one quarter turn only. Brush sample was then rolled over the slide and immediate fixation done. Immediate fixation is important to avoid air drying artifact which distorts the cell and impede the results[14-16].

The specimen was immediately fixed by dipping the slide in 95% ethanol for 30 minutes to prevent air artifact and cellular distortion caused by drying out and shrinkage of cells. Nuclear staining was done by dipping the slides for 2 minutes in Haematoxylin solution, after rehydrating the slides through descending grades of Alcohol and finally to water as haematoxylin stain is an aquous solution. After staining with Haematoxylin excess stain was removed by placing the slides under tap water for approximately 5 minutes. The slides were again dehydrated by passing through ascending grades of ethanol to absolute ethanol before using the alcohol based acidic cytoplasmic counter stains namely OG-6 and EA-50. The slides were dipped in OG-6 for 2 minutes and EA-50 for 5 minutes. The slides were then dipped in absolute alcohol for 15-30 seconds before dipping them in the clearing agent Xylene. The slides were then dipped in xylene for 5 minutes which has the same refractive index as glass that provides further transparency. Mounting medium (DPX) was then applied to slides and cover slip placed carefully to avoid air bubbles artifacts. Slides were then viewed under microscope[19].

The slides after staining were then scanned first at 40X, then 100X and those with the disease at 400x to look for clue cells (Fig.1). The reports were then written and the slides were then examined by the supervisor to confirm the diagnosis and check the written reports. For quality control positive cases were reassessed interdepartmentally by other consultants before issuing the final reports.

Data were entered and analyzed by using SPSS-20.0. Data for age, income class, education, Smoking and presence of BV were reported by using frequencies and percentages.

Association of all above given factors with Bacterial vaginosis was defined by using Chi-Square analysis, Binary Logistic Regression Analysis was used to see the risk of bacterial vaginosis on multiple variable (risk factors) and was described by using adjusted odd ratios with 95% confidence interval. P-value ≤ 0.05 was considered statically significant. Binary logistic regression is a statistical technique which is used to predict the odds of being a case based on the values of the independent variables (predictors). The odds are defined as the probability that a particular outcome is a case divided by the probability that it is a non-case.

Fig. 1: Slide at 400 x presenting clue cell

RESULTS

The average age of these 260 women was 32.8±6.5 years, of these 83 (31.9%) were of age above 35 years, 135 (51.9%) were illiterate or just had primary education, 64 (24.6%) had income <Rs.20000/- and 77 (29.6%) were those who got married before reaching 18th birthday. There were 42 (16.2%) pregnant, only 3 (1.2%) were smokers while 136(52.3%) had poor nutritional status. When Pap smear samples were analyzed, 50 (19.2%) were diagnosed to have bacterial vaginosis. Majority (68.1%) had NSI the differential diagnosis is given in Figure 2.

The accuracy of multivariate binary logistic model, fitted to see the association of sociodemographic determinants with BV, was 89.6% with 32(64%) of BV diagnosed correctly and 201(95.7%) of none-BV diagnosed correctly. The Nagelkerke R-square was 0.697 (Table 1).

When bacterial vaginosis was compared between groups by different factors in isolation, it was noted that the Age above 35 years, Being illiterate or having just primary education, Income ≤20000/-, Age at marriage <18 years, and poor nutritional status were all significant contributors towards bacterial vaginosis. The age at marriage had the highest odds ratio of 13.89 (6.65–29.00), none of the 42 pregnant women had BV as well as none of the 3 smokers. When binary logistic regression was applied the income ≤20000 and poor nutritional status were the two clearly significant indicators with adjusted odds ratios of 3.2 (1.2-8.5) and 7.4 (2.4-22.4) respectively. The age <35 years and age at marriage had very high adjusted odds ratio but the intervals were not measureable (Table 2).

Fig. 2: Distribution of diagnosis made for Pap smear samples

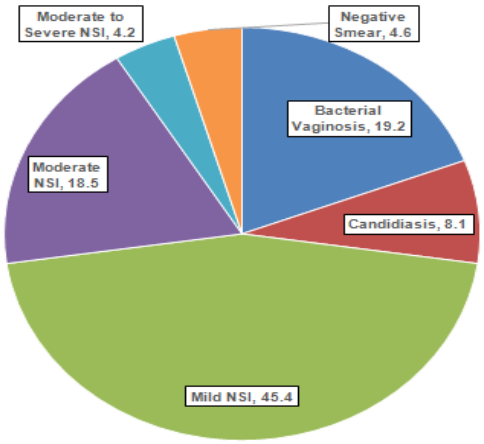

Table 1: Classification table of binary logistic multivariate regression model (with Nagelkerke R-square=0.697)

Observed	Predicted		
	Bacterial vaginosis		% correct
	Yes	No	
Bacterial vaginosis			
Yes	32	18	64.0
No	9	201	95.7
Overall Percentage			89.6

DISCUSSION

A high burden of reproductive morbidity and BV has been documented as a risk factor for both adverse birth outcomes, HIV, STD's and according to some studies for cervical cancer. This study investigated potentially modifiable socio-demographic risk factors for BV. The prevalence of BV in this sample of 260 is 19.2%, which is in accordance to study conducted by Muvunyi et al[20] 17.8% and Barouti et al[21] 17%. There were other studies with higher prevalence of 24%[22], 33%[23], 27%[24] and 44% (97 0ut of 219)[25].

However correct data regarding sexual practices and number of sexual partners is difficult to obtain in our setting considering our social norms. But it clearly concludes that its prevalence and frequency differs moderately with ethnicity and geographical distribution contributing to various personal and religious practices.[20,22,23,26]

Table 2: Frequency of bacterial vaginosis among various sociodemographic factors and its association with determinants (univariate and multivariate analysis)

Variable		Bacterial Vaginosis				Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)
		Yes		No			
		No.	%	No.	%		
Age	>35	27	32.5	56	67.5	3.23(1.71 – 6.09)	553486951.6 (0.0–c)
	≤ 35	23	13.0	154	87.0	Ref	Ref
Education	Illiterate/primary	35	25.9	100	74.1	2.57(1.32 – 4.98)	0.8(0.3 – 2.3)
	Middle/higher	15	12.0	110	88.0	Ref	Ref
Income	≤ 20000	22	34.4	42	65.6	3.14(1.64 – 6.04)	3.2(1.2 – 8.5)
	> 20000	28	14.3	168	85.7	Ref	Ref
Age at marriage	< 18	38	49.4	39	50.6	13.89(6.65–29.00)	2055978558.7(0.0 – c)
	≥ 18	12	6.6	171	93.4	Ref	Ref
Pregnancy	Yes	0	0.0	42	100.0	---	0.0(0.0 – c)
	No	50	22.9	168	77.1	Ref	Ref
H/O abortion	Yes	6	50.0	6	50.0	4.64(1.43 – 15.05)	3.8(0.7 – 22.5)
	No	44	17.7	204	82.3	Ref	Ref
Smoking	Yes	0	0.0	3	100.0	---	2.1(0.0 – c)
	No	50	19.5	207	80.5	Ref	Ref
Nutritional status	Poor	38	27.9	98	72.1	3.62(1.79 – 7.31)	7.4(2.4 – 22.4)
	Satisfactory / Good	12	9.7	112	90.3	Ref	Ref

c: The value cannot be determined

Furthermore the results of Binary Logistic Regression showed that prevalence of BV is very high in low socio economic group owing to poor nutritional status, unsatisfactory living conditions, and lack of awareness, education and access to better health facilities. Lowest prevalence was found in middle income group which is again consistent with previous studies, one carried out in Nepal[27] on 160 non pregnant women with symptomatic vaginal discharge, highest prevalence was seen among farmers 38.9% (low socio economic group) and low in those involved in business 14.7%

(high socioeconomic group), whereas in our study 32% prevalence was found in low socio economic group and 20% in high socio economic group and least in middle income group 13.5% and one in Peru by Jones[24] on 779 Peruvian women from low socioeconomic group who were tested for BV had a high prevalence of 27% (207 out of 779) as compared to previous studies done on general population**.**

Similarly low education level also has an impact on prevalence of BV. These results are in line with earlier studies conducted in various countries especially in developing countries. Literacy was found to be a major factor with high prevalence of disease among illiterate 25% and primary 26% and low incidence in inter and above 8.5% which is consistent with a previous studies conducted[27] on symptomatic vaginal discharge with 29% of total respondents illiterate and primary 25% and on pregnant women attending antenatal clinics with vaginal discharge prevalence of disease was found to be 54% in primary level education and 10% and 11% in secondary and tertiary level of education respectively[28].

Other two determinants of the disease more commonly seen in low socioeconomic class were poor nutritional status and pallor (anaemia) as this under privileged group does not have access to healthy and balanced diet due to lack of resources . In our study nutritional status was strongly linked to the disease with significant p value, however pallor was not found statistically significant as a determinant of BV with insignificant p-value. This is in line with the previous studies one carried out by Holzman et al[29] with 32% prevalence among those not taking nutritional supplements 21% among those taking regularly.

Another determinant of the disease studied, that is more common in low socio economic group is early age at marriage. It was found to be strongly associated with occurrence of BV with significant p value of ($p< 0.001$) Maximum prevalence was found in females who were married below the age of 18 years. This is consistent with a study conducted by Gergova et al[30], where 48% study population was found to have BV and was strongly linked to early onset of sexual activity with 75% of BV associated subjects starting their sexual activity by the age 15-18 years.

CONCLUSION

Low socioeconomic status, literacy, age >35, nutritional status is strongly related to occurrence of bacterial vaginosis in married women of reproductive age group with vaginal discharge.

Conflict of interest: None

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