Original Article

Magnitude of Bacterial Vaginosis in Non-Pregnant Outpatient Women Presenting Vaginal Discharge in Police Service Hospital Peshawar, Khyber Pakhtunkhwa, Pakistan

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Abstract

Background and Objective: Bacterial vaginosis is the lower genital tract problem of females having profuse, whitish grey offensive smelling vaginal discharge that increases the risk for the development of various diseases. Preventive strategies are beneficial in targeting the risk factors, but unfortunately, no active control program and strategies have been focused on in research until now. Hence, the present study was aimed to study the prevalence of bacterial vaginosis in non-pregnant women presenting with vaginal discharge.

Methods: The study was an institution-based cross-sectional study conducted in the Gynecology Unit of the Outpatient Department for 24 months from January 2018 to December 2020. The sample size was 340 nonpregnant women according to the formula designed for Bacterial Vaginosis studies after verbal informed consent. Pre-designed questions and swabs from vaginal discharge were obtained from participants. Clinical diagnostic tests known as Amsel's criteria were used for the analysis of a sample. Data was analyzed by using SPSS software, and the chi-square test was used to study the relationship. P<0.05 were considered significant.

Results: A total of 167 patients' having ages ranged to (17-to 52 years) were suffering from bacterial vaginosis make prevalence of 49%. Bacterial vaginosis was highest in patients having an age group range of (13-52 years) making 67(55.5%), followed by lowest in the aged group of range (17-21) i.e.13 out of 78 (16.6%). In parity, 85 (25%) had parity ranging from 1-2, while 248 (73%) had parity of more than two. It was found that the female having a history of miscarriage and bacterial vaginosis was 102 (54.5%), while the female having no history of miscarriage was 65(42.2%). Intrauterine contraceptives used were 71(21%), while 269 (79%) were non-users. Out of 71 users, 40 (57.1%) had bacterial vaginosis 51 (30.6%). Women who had PH (8-9) were 49% (167) out of these 116 (69.4%). Nonusers of condoms suffering from bacterial vaginosis were 122 (48.6%), while users 41 (48%). Among pruritus patient 102 (61.2%) had bacterial vaginosis. The grey color of discharge was found in 24 (7%). White-colored discharge was found in 173 (51%), and yellow discharge was found in 105(31%) of the cases.

Conclusion: The prevalence of bacterial vaginosis was relatively high in patients with having age range of 13-52 years. Culturing of samples is needed to find out the microbiological flora variation in vaginal discharge. . [*Ethiop. J. Health Dev.* 2023; 37(2): 00-00]

Key Words: Bacterial Vaginosis, Vaginal Discharge, Amsel Criteria

Introduction

The second most common lower genital tract problems that affect the reproductive age of women having profuse, whitish grey offensive smelling vaginal discharge are Bacterial vaginosis (1). It is not referred to as bacterial vaginitis because of localized inflammation. Multiple studies have reported that the concentrations of proinflammatory cytokine interleukin-1beta (IL-1beta) are higher in women having bacterial vaginosis (2). This may be due to inhibition or degradation of IL-1beta by causative bacteria (2). Furthermore, it has been proved that vaginal microorganisms moved from the common lactobacilli to the total replacement with anaerobes, Gardenrella vaginalis, peptostreptococcus, e.g., Prevotella, Bacteroides, Atopobium, Leptotrichia. Besides the above, three Clostridium species are also responsible for bacterial vaginosis and are known as bacterial vaginosis-associated bacteria (3,4,5). In most cases, it may be asymptomatic but times present with a profuse, whitish grey offensive smelling vaginal discharge without any appropriate signs of inflammation. Overall, the symptom of Bacterial vaginosis is nonspecific and asymptomatic, hence clinical diagnostics tests such as Amsel's criteria, Nugents scoring rule, BV Blue Test, Fem Exam Card, DNA probe assay, Single swab multiplex, Antigen point-of-care assay and Nucleic Acid Amplification Testing (NAAT) will at best way for giving direction for the presence of vaginitis, and microscopic investigation to determine bacteria types responsible for vaginal infections (6). The fishy smells in bacterial

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vaginosis are due to the presence of amines that are released by bacterial proteolysis (7,8). Vaginal examination shows grey, thin, and homogenous discharge that covers the vaginal wall (9). Bacterial vaginosis is associated with poor pregnancy outcomes such as late miscarriages, preterm delivery, premature rupture of membranes, low birth weight infants, and postpartum endometritis along with pelvic inflammatory disease post-hysterectomy vaginal cuff infection, post abortal sepsis, laparoscopically confirmed salpingitis, infertility due to fallopian tube occlusion, cervical human papillomavirus infection (10). The main risk factors for bacterial vaginosis are smoking, failure or inaccurate use of condoms, intrauterine contraceptive devices, sexually transmitted disease, frequent vaginal intercourse, and vaginal douching (11). Use of Over-the-counter drugs and misdiagnosis of the disease can also lead to an increased risk of bacterial vaginosis (12). Different types of tests are used to diagnose bacterial vaginosis disease accurately. However, the worldwide accepted method for diagnosis of Bacterial vaginosis is microscopic criteria that was described by Nugent et al and clinical criteria that was developed by Amsel et al. Nugent rule was considered a gold standard for the diagnosis of bacterial vaginosis due to accuracy. Nugent's scoring system was discovered in the year of 1991 by RP Nugent and added furthermore specifications criteria to the previously established gold standard Spiegel criteria due to the involvement of different types of bacteria (13). By applying the Nugent scoring test, vaginal samples are mixed with oil and placed on a microscopic for Gardnerella, Lactobacillus, and curved gram rods for 10 high-power fields. Laterally, scores were added together to find the total scoring that ranges from 0 - 10. A Nugent score above 7 means a positive test for bacterial vaginosis. While a score range from 4-6 indicates intermediate, and a score less than 3 was termed as negative for Bacterial vaginosis (13). Furthermore, the Nugent scoring test is disregarded by a physician due to time-consuming, is expensive, and needs expertise and special equipment. Amsel criteria test has almost comparable specificity and sensitivity (91% both) to Nugent's rule, being costeffective, and does not need special equipment or expertise (14). Amsel criteria used for diagnosing bacterial vaginosis that was published in 1983 had only four parameters for the presence or absence of Bacterial vaginosis (15). For diagnosis of bacterial vaginitis, Amsel criteria contained simple tests in which a vaginal swab is taken from the vaginal discharge or cervical region of the vagina and placed under a microscope (15). A wet mount test sample taken from vaginal discharge is mixed with normal saline solution and then examined under a microscope for the presence of clue cells or white blood cells. A blurred border in the vaginal wall indicates the presence of bacterial vaginal infection. Furthermore, the Whiff test is used for checking the odor of the vaginal discharge by adding potassium hydroxide to the sample and observing it under microscopic. Fishylike odor revealed the presence of bacterial vaginosis in vaginal discharge. A vaginal pH test measures the acidity level of the vagina versus alkalinity by placing the sample on a pH strip. Vaginal pH higher than normal is the assigned of bacterial vaginosis (15). The presence of 3 out of 4 among below below-mentioned parameters must be considered significant for the diagnosis of bacterial vaginosis.

- 1. Increased homogenous thin grey or white vaginal discharge
- 2. PH of the secretion greater than 4
- 3. Positive whiff test on adding a drop of 20% potassium hydroxide
- 4. Presence of clue cells in wet preparations (16).

The prevalence of Bacterial vaginosis is 16.2% to 51% across the globe (13). However, unfortunately, this disease is highly underdiagnosed, and there is still no active control program in Pakistan. Therefore, the present study aimed to determine the prevalence of bacterial vaginosis and associated risk factors among the patient presenting with vaginal discharge in the Gynae Unit of Service Police Hospital, Peshawar, Khyber Pakhtunkhwa, Pakistan.

Material and Methods

Ethical Approval and Considerations

Before starting of research, test protocols were reviewed, duly addressed, and approved by the Department Research and Ethical Review Committee and the Medical Superintendent of the Hospital from where the sample data of the patients were collected. Verbal informed consent was obtained from each patient, family member, and partner before data collection. Complete detail about the nature of the data collection procedures were fully explained to all participants, and convinced them that they had the right to withdraw or refuse to take part in the study at any time during the study period. The participant was informed that their information would be treated confidentially.

Inclusion and Exclusion Criteria

Married non-pregnant women presenting with vaginal discharge were included. Pregnant women, unmarried, postmenopausal, and women in menstrual periods were excluded, along with those who used antibiotics in the last 7 days of presentation.

Study Design, Setting, and Duration

The study design was Institutional-based and cross-sectional.

The study was conducted in the Gynecology Unit of Services Hospital Police Peshawar, Khyber Pakhtunkhwa, Pakistan, for two years, starting from 1st January 2018 – to 31st December 2020.

Sample size of the study

The sample size for this study was calculated by using a simple formula that was designed for calculating the sample size of Bacterial vaginosis (17).

Sample size formula = n = $\frac{NZ^2P(1-P)}{D^2(N-1)+Z^2P(1-P)}$

Where n = Sample size, N Population size, Z Statistic for the level of confidence, P = Expected prevalence, D = Precision. In the formula mentioned above, the anticipated prevalence was considered P = 1.96, and the level of confidence was 95% (0.05), Population Size of 2600 and, Population Proportion of 50% yields 335 sample size. This means that 335 or more surveys/cases/measurements are needed to have a confidence level of 95% (0.05), that the real value is with ± 5 % of measurement. Hence, the sample size was selected as 340.

Processing of the Study

Cases were selected from the Gynecology Department of Outpatient of Services Hospital Peshawar, Khyber Pakhtunkhwa, Pakistan. A detailed history of patients, such as name, age, marital status, educational status, employment, parity, miscarriages, contraceptive, vaginal discharge, associated itching, dyspareunia, and color of the discharge, was obtained according to predesigned proforma. Parity was also recorded. A sterile speculum examination was done for the presence of discharge and color. Women were placed in a dorsal position for examination and sampling. The sterile speculum was passed after inspection of the vulva. Vagina was inspected for color, form, consistency, and color of discharge. Amsel criteria were used for the diagnosis of bacterial vaginosis (15). The pH paper was used for finding the acidity of vaginal discharge. The pH paper was placed on the lateral vaginal wall for one minute (in this way, it was kept away from the alkaline discharge of the cervix to control bias), and then the color of the paper was compared with the pH card of standard (control). Discharge was collected from the posterior fornix of the vagina with a sterile cottontipped swab that was available in Gynecology Out Patient Department. Full care was taken to avoid surface contamination. The collected sample was transferred to 2 slides already labeled and prepared. 1-2 drops of normal saline and a cover slip were added to the 1st slide and examined under a light microscope for clue cells or white blood cells. A drop of 20% potassium hydroxide (20% KOH by "Laboratory Chemicals" that was available in the hospital lab was added to the 2nd slide for examination of an odor of the vaginal discharge, which was known as a whiff test. Females who had 3 or more positive Amsel criteria parameters such as white/thin/gray/yellow discharge, Clue cells, pH above 4.5, and fishy odor discharge upon adding 20% KOH were labeled as bacterial vaginosis (16).

Data were prepared by Microsoft excel and analyzed by using SPSS version 20.0. Results were presented in graphs and tables. Proportions described the variables present in categories and continuous by average. Comparison among the variables was determined by using the chi-square and T test. The level of p<0.05was considered statistically significant.

Results

Data Analysis

A total of 340 cases of non-pregnant women having vaginal discharge that visited the OPD were examined during the study-designed period. All the participants had given complete data on their demographics and vaginal samples. After applying Amsel criteria, 167 participants were positive for Bacterial vaginosis, which makes the prevalence rate 49% of the total cases. Participants aged range from 17 - 52 years, and the mean age was 34.5 years with a standard deviation of ± 1.85 . It was found that bacterial vaginosis was low in patients having an age range of 17 to 25 years, i.e., 13 out of 78 (16.6%), moderate in participants having an age range 26-31 years, while highest in participants having an age range above 31 years that was 67 (55.5%). The prevalence of bacterial vaginosis in illiterate participants was 46 (37%), primary education was 38%, and college and above education 42%. Bacterial vaginosis among intrauterine contraceptive device users was 40 (57.1%), while in non-intrauterine contraceptive device users, bacterial vaginosis was 126 (46.8%). Regarding parity, 7(2%) of cases were nulliparous, 85 (25%) had a parity range of 1-2 and 248 (73%) had more than 2. Bacterial vaginosis among women who have a history of miscarriage was 102 (54.5%), while 65 (42.2%) that has no history of miscarriage had bacterial vaginosis. The bacterial vaginosis among oral contraceptive pills users was 48 (14%), while in non-users, 122 (48.6%) and 41 (48%) had bacterial vaginosis.

Dyspareunia was found in 221 (65%), and the rest of 119 (35%) had no dyspareunia. 109 (49.3%) of dyspareunia cases had bacterial vaginosis, while 58 (48.6%) of non-dyspareunia cases had Bacterial vaginosis. The relationship between demographic characteristics and bacterial vaginosis among non-pregnant participants is summarized in Table 1, table 2, and Figure 1.

Table 1: Relationship between Sociodemographic Characteristics and bacterial vaginosis among non-	
pregnant participants	

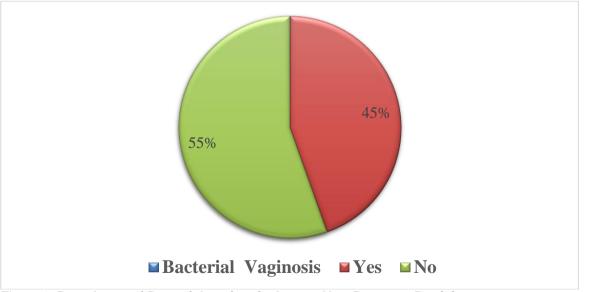
Age group (Years)	Ν	%age	Bacterial vaginosis	
			Present	Absent
≤25	78	23%	13 (16.6%)	65
26-30	140	41%	35 (28%)	105
≥31	122	36%	67 (55.5%)	55
17-52 years	340	100%		
Means age with SD 34.5±1.85	113.3±31.90	38.33±27.15	33.73±20	

Risk factors	Ν	%age	Bacter	rial Vaginosis
			Present	Absent
No education (illiterate)	122	36%	46 (37%)	76 (62.2%)
Primary education	130	38.23%	50 (38.46)	80 (61.44)
College and above	88	26.88	37 (42.04)	51(57.96)
Total	340		167	173
Distributio	n of Bacte	rial vaginosi	s as per contraceptive u	isage
Contraceptive user	71	21%	40 (57.1%)	31(43.9%)
Noncontraceptive user	269	79%	126 (46.8%)	133 (54.2%)
Condom user	85	25%	38 (44.70%)	47 (55.29%)
Non condom user	255	75%	65 (25.49)	190 (74.50%)
Distributio	on of Bacte	erial vaginosi	is as per employment st	atus
Employed	61	18%	24 (39.34%)	37 (60.4%)
Unemployed	279	82%	112 (40.14%)	167 (59.85%)
Distributio	on of Bacte	erial vaginos	is as per Miscarriage st	atus
Miscarriage	187	55%	125 (66.84%)	62 (33.1%)
No miscarriage	153	45%	78 (50.98%)	75 (49%)
Distributio	n of Bacte	rial vaginosi	s as per Dyspareunia st	tatus
Dyspareunia	221	65%	109 (49.3%)	112 (51.7%)
No Dyspareunia	119	35%	58 (48.6%)	61 (52.4%)

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Table 2: Prevalence of Bacterial vaginosis Among Non-Pregnant Participants

Bacterial Vaginosis	Number of Patients (N)	(%)
Yes	167	49%
No	173	51%
Total	340	100%





Participant Distribution Based on Abnormal Vaginal Discharge Colors

Grey color of discharge was found in 24 (7%) of the women, 17 (71%) of these had bacterial vaginosis. White-colored discharge was found in 173 (51%) of Ethiop. J. Health Dev. 2023; 37(2)

the cases, among these, 132 (76.5%) had bacterial vaginosis. Yellow discharge was found in 105 (31%) of the cases, whereas bacterial vaginosis was found in only 10 (9.7%) of women. Clear discharge was found

in 38 (11%) of cases; among these, 11 (28.94%) were positive for vaginal infection. The results are summarized in Table 3 and Figure 2.

Vaginal	Discharge Colours	Ν	% age	BV present	BV absent
	Grey	24	24 (7%)	17 (71%)	7 (29%)
	White	173	173 (51%)	132 (76.5%)	41 (24.5%)
Colors	Yellow	105	105 (31%)	10 (9.7%)	95 (93.3%)
	Clear	38	38 (11%)	11 (28.94%)	27 (72%)
		340	100	42.5±1.98	42.5±32.63



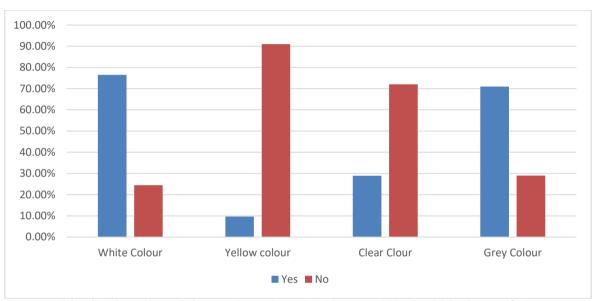


Figure 2: Distribution of participants Based on the Abnormal Vaginal Discharge Colour

Vaginal pH ranges were calculated. There were only 7 cases that had pH ranges between 4 and 5; none of these had bacterial vaginosis. pH ranges of 6-7 were recorded in 49% (167) whereas Bacterial vaginosis was found in 51 (30.6%). Women who had a pH of 8-9 were 49% (167); out of these, 116 (69.4%) had

bacterial vaginosis. Whiff test was positive in 180 (53%) of cases, 152 (84.9%) of them had bacterial vaginosis. Whiff test was found negative in 160 (47%) of cases and 14 (8.5%) out of them had BV. Clue cells were seen in 163 (48%) of women; 163 (100%) of them had bacterial vaginosis, as shown in Figure 3.

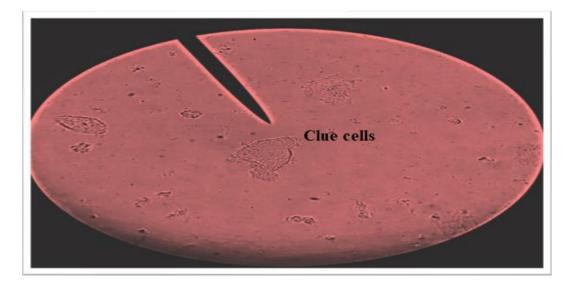


Figure 3: Clue cells on Gram- stained smears

Discussions

The present study was observational and was designed to determine the prevalence of bacterial vaginosis in non-pregnant women in the Gynecology Department of Services Hospital Peshawar, Khyber Pakhtunkhwa, Pakistan, for two years after approval. The sample size was 340 based on a simple formula. It was found that the participants suffering from bacterial vaginosis were 167, making 49% of the total sample based on Amsel criteria. While the female had no vaginal infection was 173, making 51% of the total. The same prevalence of bacterial vaginosis was reported by (Shnawa et al. 2018) in literature 48.60 % of the total sample was conducted in Soran City, Kurdistan of Iraq, confirmed present study (18). A study by Venugopal S et., reported that Bacterial vaginosis frequency was 27% in non-pregnant females (19). Another study was conducted in Nepal, the bacterial vaginosis was found to be 36.3% in non-pregnant female (20). The comparative and higher frequency of cases may be due to differences in materials and methods compared to these studies. The high rate may be probably due to the complication of miscarriage and abortion that might alter the normal flora of the vagina. Although Nugent's scoring is being claimed as the gold standard in diagnosing bacterial vaginosis , it is time-consuming, and the patient has to wait for the culture results. It is also costly for poor patients; only a few gynecologists ever have time to use this method, while the microbiology staff strength is inadequate in resourceconstraint centers like ours to operate it successfully. Amsel criteria were used in this study to provide spot diagnosis and are easy to do in an outpatient setting. Its sensitivity and specificity are comparable to Nugent's scoring because it is based on only four criteria for making the patient positive for Bacterial vaginosis (12). Moreover, as discussed above taking samples from vaginal discharge according to Amsel criteria is safe that can be collected without making the participant discomfort. Even instead of collecting a physical sample, a physician may provide that participant has a vaginal infection based on fishy odor or discharge. (12). Additionally, various studies confirmed that the prevalence of Bacterial vaginosis is Amsel criteria was more as compared to Nugent's scoring along with sensitivity.

Vaginal discharge problem was found among the participants, women having the age range 23-45 years (55.5%), while the least frequency in participants having an age range of 17 to 21 years (16.6 %). Present results are comparable with a study done by Adane Bitew in Ethiopia that showed that women having the age range 15 to 24 years had a lower prevalence rate of bacterial vaginosis, while those women whose age 25 years or above had the prevalence (47% - 60%). Hence, women having the age group above 25 years are more likely to develop of Bacterial vaginosis. A study conducted by Rajshree Bhujel (21) in Nepal found the lowest prevalence rate of bacterial vaginosis in females aged below 20 years, 16%, and highest in females aged above 30 years (60.16%. The lowest frequency was seen in age that was range 17-21 years of age, i.e., 16%, which is similar to the study as mentioned above. The highest prevalence in those above 30 years of age may be the most sexually active age group with the highest risk of pregnancies and sexually transmitted diseases, while less in the age below 20 years less exposure to sexual activity. Regarding educational status, most of the women presenting with vaginal discharge in our study having bacterial vaginosis in

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illiterate participants were 46 (37%), primary education was 38%, and college and above education 42%.

A similar report was recorded by Ibrahim S. M (22) in Nigeria that showed the prevalence rate of bacterial vaginosis in illiterate women high (54%) compared with educated women validating our result. The high cause of vaginal infection among less educated women may be due to the lack of a female consultant. Weak financial status, lack of awareness, hesitation to use medical devices, and sociocultural structure.

Multi gravida in the group showed the highest frequency of bacterial vaginosis, which was similar to a study done in Nigeria (22). This was most probably because of high coital frequency leading to a reduction in the physiological barrier in the vagina, resulting in overgrowth of abnormal pathogens (23). The present study showed a higher frequency of Bacterial vaginosis, i.e., 54.5% in a woman who had a history of miscarriages, which was almost similar to the results done in Nigeria (24). At the same time, no significant correlation was seen in another study. The present study also revealed that women using the regular intrauterine contraceptive device had a high frequency of bacterial vaginosis, i.e., 57%, which is comparable to the studies done in Nigeria (68%) (24). A study reported in India showed that females using regular intrauterine contraceptive devices have a higher chance of developing bacterial vaginosis (29.4%) compared with non-users of a device (17.20%) (Singh Hari Om (25). The contribution of an intra-uterine (2015) contraceptive device in vaginal infection may trigger vaginal flora changes that will lead to the development of bacterial vaginitis, and it increases the risk of bacterial vaginosis.

The present study revealed no significant difference in the development of disease in the participants who used condoms or non-users. However, contradictory results were recorded in the study reported by Hari Om et al. (2015) that showed that females using regular condoms have high chances of development disease 75 (59.5%) compared with non-users 51(40.5%) which may be due to incorrectly used of condom (25). No significant difference in our study group may be due to be due to the small sample size or the condom users may be very few, and even those who were using condoms were also not regular. A study reported by Ahmed S et al. (2001) in Ghana confirmed our finding that claimed that there is no proof that condom use by females protects them against bacterial vaginosis (26).

The present study revealed a direct relationship of pruritus 102 (61.2%) among participants having bacterial vaginosis making 102 (61.2%) of the total 167 (49%), which was similar to the study reported by Ibrahim et al. (2014) in Nigeria (22), showing the direct relationship of pruritis and vaginal infection. The present study revealed that the majority of women suffering from vaginal infection presented white milky discharge (76.5%), followed by grey 17 (71%) and yellow (10.9%), which is not similar to the study recorded by Ibrahim et al. (2014) in Nigeria where a majority had yellowish discharge 55(80%) followed by grey 9(13%) and white 5 (7%) (22).

Vaginal pH of 8-9 was found to be positively related to bacterial vaginosis, i.e., 69.4%, while in cases with vaginal pH of 6-7, it was 30.6%. Bacterial vaginosis was present in 84.9% of cases with positive whiff test. Clue cells were the most sensitive criteria in our study where it was seen positive in all cases (100%) of bacterial vaginosis. These are also comparable to a study recorded in South India that showed vaginal pH >5 on vagina strip in bacterial vaginosis by Hemalatha R et (2013) (27). Furthermore, the same result of the presence of clue cells and the positive whiff test was recorded by Hemalatha R et (2013) (27). In Bacterial vaginosis patients, 147 (95.5%), 71(46.1%) of the total participant.

The main limitation of our study was that it was a Hospital-Based study that did not provide enough information about the prevalence of Bacterial vaginosis in the general public community. A large sample size is needed because the small sample size did not provide information that above 30 years of age participants have a high prevalence of Bacterial vaginosis. This study only focused on non-pregnant women who ha visited the hospital within a specific duration of the study. Pregnant women, unmarried, postmenopausal ,women ,menstrual periods, and even nonpregnant women who visited after a specified period were not investigated. Similar study designs are required to be performed in a population having large populations to find out the exact prevalence of Bacterial vaginosis. To study the pattern of disease, different types of tests, such as the OSOM BV Blue test is designed for the detection vaginal sialidase activity, the Fem Exam card is designed for metabolic byproducts, Affirm VP assay is needed to study the complications of Bacterial vaginosis in women having abnormal vaginal. Only AMSEL criteria are not enough to confirm the presence of bacterial vaginosis. The clinicians are required to avoid single bacterial indicator organism assays like direct probe assays. Multiple tests, such as multiplex PCR technology, are needed that is able to detect the presence of multiple indicator organisms responsible for vaginal infection in the case of symptomatic women with recurrent vaginitis.

Conclusion

The study designed in this project suggests that the prevalence of bacterial vaginosis is relatively high in patients with having age range of 13-52 years attending the gynecological unit of Service, Police Hospital, Peshawar KPK, Pakistan. Our finding suggests that bedside of different diagnostics tests, Physicians need to adopt clinical tests such as AMSEL criteria to diagnose bacterial vaginosis rapidly along with saving time and money for the patient. AMSEL criteria will provide clues in the selection of suitable antibiotic therapy. This study was done to find out the prevalence of bacterial vaginosis in a small population; further longitudinal and follow-up studies are required to find the effects of vaginal infections on the population and associated risk factors of this problem in our country.

Declaration of Conflict of Interest

We declare that we all nodo not have any conflict of interest regarding the publication of this paper.

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