

Asymptomatic Bacterial Vaginosis in Cervical Cytology: A Preliminary Report of Incidental Finding in Routine Cervical Cancer Screening

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Abstract

Background: Bacterial vaginosis is a complex pathogenic group of bacteria which can cause a wide range of symptoms in affected women in Obstetric and Gynaecological practice. Its occurrence usually indicates alteration in the normal vaginal flora expected in majority of the women of reproductive age. Amongst the maintenance agents of the vaginal health, lactobacilli occupy a prominent place. Asymptomatic bacterial vaginosis is postulated to be linked to the development of cervical intraepithelial neoplasia by some researchers while some differ in this regard. **Objective:** To determine the rate of occurrence of asymptomatic bacterial vaginosis and associated factors in women who underwent cervical cytology for cervical cancer screening. **Study Design:** A cross sectional study involving microscopic examination of cervical smears of eligible clients presenting for routine Pap smear. **Setting:** The study was carried out in a well woman's facility established and manned by female medical doctors in Enugu state with the assistance of trained nursing staff. Various female cancers and other communicable and non-communicable disease screening are performed at the centre. The services are available to the general public including various parts of the state and other nearby states. **Methods:** This preliminary study was carried out on women presenting for cervical cancer screening between February and July 2018 in a well woman's centre in Enugu. Information on the socio-demographic and gynaecological history of the study participants were obtained and recorded in designated proformas. Smears that showed changes suggestive of bacterial vaginosis on microscopy were set aside for further evaluation. Such changes included: a conspicuous absence of normal flora of lactobacilli; filmy background of coc-

cobacilli replacing the lactobacilli, individual squamous cells covered by a layer of bacteria (clue cells); viable squamous cells showing reactive changes identified as increased nuclear size, perinuclear halo and binucleation. Data entry and analysis were done using statistical package for social sciences (SPSS) computer software version 21.0. **Results:** 285 eligible women had cervical smear done during the first six months of the 2-year project at the centre. 50.9% were above reproductive age (>45 years), 38.2% were in second half of reproductive age (31 - 45), 10.9% were in the early reproductive age (15 - 30). 13% of the women were nulliparous, 51.9% were para 1 - 4, and 35.1% were grand multiparous. Of the 285 Pap smear slides viewed, 31 had bacterial vaginosis by the above criteria. This gave a prevalence of 10.8% in the reviewed cytological smears. There was an association of bacterial vaginosis with reproductive age. There was however, no association with age at coitarche and parity. There was also, no association of bacterial vaginosis with contraceptive use. There was no association between abnormal cervical cytology and asymptomatic bacterial vaginosis with absent lactobacilli. **Conclusion:** Prevalence of bacterial vaginosis is significant in our study population who were asymptomatic. Cervical smear should be taken advantage of as a tool for both cervical pre-cancer and bacterial vaginosis screenings since same sample can simultaneously be used for both conditions during cytology. Bacterial vaginosis may be unlikely involved in pathogenesis of cervical intraepithelial neoplasia. **Recommendation:** Researchers should harmonise the standards/criteria for the diagnosis of bacterial vaginosis because there are varied criteria for the diagnosis in the literature. Given, the dual advantage of pap smear in diagnosing cervical pre cancer stages and infections, more elaborate studies are needed to determine the usefulness of treatment or otherwise of bacterial vaginosis which constitutes significant incidental findings.

Keywords

Asymptomatic Bacterial Vaginosis, Pap Smear, Cervical Cytology

1. Introduction

A woman's vagina is known to harbor loads of bacteria regarded as normal flora. There are saprophytic bacterial organisms that help in maintenance of a healthy vaginal environment and others whose presence is not beneficial and cause no obvious harm. Nature has provided for the preservation of a healthy vagina in the reproductive age by creating an acidic environment maintained by the presence of lactic acid among other factors. The lactic acid is supplied by some of the useful bacteria resident in the vagina of pre-menopausal women. Key in this liberation of lactic acid is the lactobacilli species which help not only in maintaining healthy vagina but in preventing genital infections [1]. Some groups of bacteria found in the vagina have a propensity to cause undesirable symptoms as soon as they find conducive environment to overgrow [2].

Bacterial vaginosis is a complex pathogenic group of bacteria which can cause a wide range of symptoms in affected women in obstetric and gynaecological practice. Its occurrence usually indicates alteration in the normal vaginal flora expected in majority of the women of reproductive age. Bacterial vaginosis is one of the commonest causes of vulvovaginitis in reproductive aged women [3] [4]. Although prevalence as low as 12% had been documented [5], it depends on the studied population. A recent systematic review and meta analysis estimated a high global prevalence of 23% - 29% [6]. Bacterial vaginosis can be asymptomatic in significant proportion of infected population [7] [8], yet a cause of a range of complications in pregnant and nonpregnant women. It is associated with adverse conditions such as sexually transmitted infections and human immunodeficiency (HIV); infertility, preterm birth, premature rupture of membranes etc. [9]-[14]. Clinical characteristics of bacterial vaginosis include thin homogenous, adherent vaginal discharge with fishy odour that is prominent following unprotected sex and menses; and a pH > 4.5. Though Amsel's criteria and Nugent's scoring are commonly used in diagnosis of bacterial vaginosis, Pap smear has also been found to have a comparable diagnostic specificity with these first 2 methods [2] [15] [16].

Because a significant number of women with bacterial vaginosis are asymptomatic, it may imply that the untoward effects of these organisms may get worse with further multiplication leading to the well documented adverse outcomes if undiagnosed over time. A recent study showed that asymptomatic bacterial vaginosis is associated with increased susceptibility to genital infections [17]. This study was undertaken, therefore, to determine the rate of occurrence of bacterial vaginosis in asymptomatic women, taking advantage of high specificity of Pap smear and its comparable accuracy with the Amsel's criteria and Nugent's scoring in the diagnosis of bacterial vaginosis. Also, the consideration of the numerous complications of infection by this group of organisms made it pertinent to study the rate at which they occur in asymptomatic women. Our expectation was that the findings from the study would improve the care to our gynaecological patients.

Aims

To determine the prevalence of bacterial vaginosis and its associated factors in asymptomatic women through cervical cytology smear.

2. Methods

The study was carried out in a well woman's health facility established and managed by the female medical doctors and their trained nursing staff in Enugu state of Nigeria. Various female cancers and other communicable and noncommunicable disease screening are performed at the facility. The clients come from many parts of the state including some nearby states. The project spanned for a period of 2 years but preliminary findings as presented were between February

and July 2018. Before recruitment of study subjects, they were first informed about the research and consent obtained from eligible participants before being enrolled. Eligible women were those who had no abnormal vaginal discharge, vaginal bleeding, recent use of oral or vaginal antibiotics; sexual intercourse or douching 72 hours prior to testing. Subsequently, information on their sociodemographic and gynaecological history was obtained and recorded in designated proformas. These included among others variables like current age, parity, age at coitarche, marital status, use of contraceptives presence of bacterial vaginosis. Cervical smears were then collected with cytobrush and spread on glass slides, fixed in alcohol solution and transferred to the Pathologists for pap staining and cytology. Smears that showed features of bacterial vaginosis on microscopy were set aside for further review. Changes consistent with presence of Bacterial vaginosis were rigorously searched for during the microscopic study of the slides. Such changes included a conspicuous absence of normal flora of lactobacilli; filmy background of coccobacilli replacing the lactobacilli, individual squamous cells covered by a layer of bacteria (clue cells); viable squamous cells showing reactive changes identified as increased nuclear size, perinuclear halo and binucleation. Conclusion of each report was reached after the slides were reviewed a second time by Pathologists.

Sample size was determined using the single proportion formula: $n = z^2pq/d^2$, where n = sample size; z = standard normal deviate, usually set at 1.96 which corresponds to 95% confidence level; p = the proportion in the target population estimated to have a particular characteristic *i.e.*, expected prevalence (18.4%, from a previous study [16]); $q = 1 - p$; d = precision or degree of accuracy desired, set at 0.05 for 5% precision.

Thus, for our sample size, $p = 18.4\% = 0.184$; $q = 1 - 0.184 = 0.816$

Substituting the above in the formula, we obtained a sample size of 230.7. However, 285 consenting participants were recruited to enhance the power of the study. Data entry and analysis were done using statistical package for social sciences (SPSS) computer software version 21.0. Means and standard deviations were calculated for continuous variables while categorical variables were presented as frequency and percentages. Tests for significance were performed using chi-square for categorical and students *t*-test for continuous variables respectively. Level of significance was set at *p* value <0.05. Illustration of results was done using tables and charts as appropriate.

3. Results

A total of 285 eligible women were recruited during the first six months (Feb-July 2018) of the 2-year project. As shown in **Table 1**, among other sociodemographic parameters, the mean age of the subjects was 46.17 (*SD*12.41) years. 50.9% of the study subjects were above reproductive age (>45 years), 38.2% were in second half of reproductive age (31 - 45), while 10.9% were in the first half of reproductive age (15 - 30). Majority of them were married and had

parity of ≥ 1 . They were sexually active and over 90% had their coitarche at >15 years of age. Out of the 285 Pap smear slides viewed, 31 had bacterial vaginosis by the previously outlined criteria. This gave prevalence of 10.9% among the study population. Age group 31 - 45 had the highest number of occurrence (20 out of 31 cases) as shown in **Figure 1**. The test of relationship of reproductive age and other factors with bacterial vaginosis is presented in **Table 2**. Study subjects above the reproductive age although constituted more than half of the study population had comparatively less occurrence of bacterial vaginosis than those within reproductive age. Thus, there was a significant association of bacterial vaginosis with reproductive age (p value = 0.004). However, no significant association of bacterial vaginosis was observed with age at coitarche, parity or contraceptive as illustrated in **Table 2**. There was also no association between abnormal cervical cytology and asymptomatic bacterial vaginosis.

Table 1. Socio-demographic variables.

	Frequencies	Percentage	Mean (S.D)
Age (years) by class interval grouping			
Less than 21	2	0.7	46.17 (12.41)
21 - 30	29	10.2	
31 - 40	70	24.6	
41 - 50	80	28.1	
51 - 60	69	24.2	
61 - 70	27	9.5	
70 and above	8	2.8	
Total	285	100.0	
Age (years) by reproductive age group			
15 - 30	31	10.9	46.17 (12.41)
31 - 45	109	38.2	
Above 45	145	50.9	
Total	285	100.0	
Parity			
0	37	13.0	
1 - 4	148	51.9	
Above 4	100	35.1	
Total	285	100.0	
Marital status			
Single	24	8.4	
Married	214	84.6	
Separated/divorced	2	0.7	
Widowed	17	6.0	
Missing	1	0.4	
Total	285	100.0	

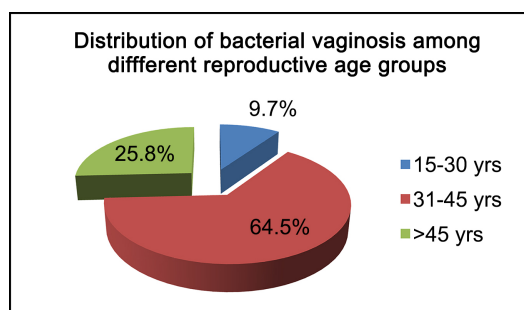


Figure 1. Distribution of bacterial vaginosis among different reproductive age groups.

Table 2. Association between bacterial vaginosis and age, parity and age at coitarche.

Variables	Bacterial vaginosis		Total	P-value
	Negative	Positive		
Age				
15 - 30	28	3	31	0.004*
	11.0%	9.7%	10.9%	
31 - 45	89	20	109	
	35.0%	64.5%	38.2%	
Above 45	137	8	145	
	53.9%	25.8%	50.9%	
Total	254	31	285	
	100.0%	100.0%	100.0%	
Parity				
0	33	4	37	0.116
	13.0%	12.9%	13.0%	
1 - 4	127	21	148	
	50.0%	67.7%	51.9%	
Above 4	94	6	100	
	37.0%	19.4%	35.1%	
Total	254	31	285	
	100.0%	100.0%	100.0%	
Age at Coitarche				
15 years and below	13	1	14	1.000
	5.1%	3.2%	4.9%	
Above 15 years	240	30	270	
	94.9%	96.8%	95.1%	
Total	253	31	284	
	100.0%	100.0%	100.0%	

*Significant $p < 0.05$.

4. Discussion

Bacterial vaginosis' prevalence of 10.9% among asymptomatic women that presented for Pap smear in our study is lower than figures obtained in the reviewed studies that used pap smear for diagnosis of bacterial vaginosis. 18.4% [16] and

48.4% [15] respectively were obtained in India at different times and settings by researchers whose investigations were on the various cervicovaginal infections diagnosed with Pap smear. Narasimha and co-workers who observed 18.4% prevalence of bacterial vaginosis did a retrospective analysis of 1252 of pap smear slides of women aged 18 - 80 years who attended gynaecology clinic for various complaints including vaginal discharge. Similar to our study, they observed that age group 31 - 40 was most commonly infected with bacterial vaginosis. Ahmad *et al.*, on the other hand, conducted a retrospective study of 220 symptomatic women aged 18 - 52 years and obtained a higher prevalence of 48.4% years later in India. These studies included women with vaginal discharge which may partly explain a higher prevalence as bacterial vaginosis is established to be a common cause of vaginal discharge. In a study of cytological finding of bacterial vaginosis in routine Pap smear, Vadana and colleagues noted that out of 100 cases of bacterial vaginosis, age groups 20 - 30 years and 31 - 40 years had the highest prevalence of 43% and 40% respectively. The age groups involved are in agreement with ours, though we noted a frequency of 64% among the most commonly affected age group, 31 - 45 years, in our study.

For studies using Gram stain to diagnose bacterial vaginosis in nonpregnant patients, the prevalence from our study was still lower than 23% (using Amsel's criteria) and 27% (with Nugent's method) observed in female students at Calabar, Nigeria whose age ranged between 16 and 35 years [18]. Unlike our finding, age group 21 - 25 years had the highest occurrence of bacterial vaginosis in the aforementioned study. This difference may be accounted for by the age limits of study participants which are not representative of all age brackets of reproductive age. Our observed prevalence is similar to 11.8% observed in an Australian community based study among females aged 16 - 25 years using Nugent's criteria [5]. This study noted association between occurrence of bacterial vaginosis and self reported symptoms of abnormal vaginal discharge and odour as well as sexual activities and lower educational level. Our study did not exclude any Pap smear-eligible -age group but focused on asymptomatic bacterial vaginosis. Also, our prevalence was lower than 24.4% recorded by Ranjit *et al.* in a study of women attending obstetrics and gynaecology clinic where symptomatic patients were used contrary to our own study population [19]. Nugent's scoring was the method used for making diagnosis in their study and they observed association of bacterial vaginosis with abnormal vaginal discharge, douching and use of anatomic-site contraceptives.

Factors associated with bacterial vaginosis have been noted to be inconsistent as observed by different researchers. Apart from the observed and well documented association of bacterial vaginosis with reproductive age [2] [15] [16] [20], we found no association with age at coitarche, parity, contraceptive or abnormal cervical cytology. In contrast to our findings, some studies demonstrated significant association of bacterial vaginosis with contraceptive use. For instance, anatomic sites contraceptives were reported as having more occurrences of bac-

terial vaginosis in some studies [19] [20] [21] while some systematic reviews and meta analysis showed that hormonal contraceptives were associated with significantly reduced prevalence, incidence and recurrence of bacterial vaginosis [22] [23]. With regards to parity, some authors, like in our study found no significant association with bacterial vaginosis [24] while some documented positive associations [13] [25]. Bacterial vaginosis is known to be more commonly seen in sexually active women. Hence, there seems to be insignificant relationship between bacterial vaginosis and age at coitarche >18 yrs [24] [26] compared to strong association which exists between it and the number of sex partners or being sexually active [3] [5] [27]. All the affected women in our study were sexually active and over 90% of them had their coitarche at age >15 years. We found no significant association between bacterial vaginosis and cervical intra epithelia neoplasms. This is at variance with previous studies including meta-analyses which reported existence of unconfirmed relationship between bacterial vaginosis and the risk of development of cervical precancerous conditions [28] [29] [30] [31]. Heterogeneity in study designs, participants, settings and lack of uniform designated gold standard method for diagnosis may account for these disparities observed among the reviewed studies as well as ours.

5. Conclusion

Prevalence of bacterial vaginosis is significant in our study population who were asymptomatic. Cervical smear should be taken advantage of as a tool for both cervical pre-cancer and bacterial vaginosis screenings since same sample can simultaneously be used to test for both conditions during cytology. Bacterial vaginosis may be unlikely involved in pathogenesis of cervical intraepithelial neoplasia.

Limitations

The fact that clients who presented for screening constituted the entire study subjects might not reflect the actual condition in the general population.

Some of the variables depended on the participants' ability to recall the required response which might not be accurately supplied.

Recommendation

Researchers should harmonise the standards/criteria for the diagnosis of Bacteria vaginosis because there are varied criteria for the diagnosis in the literature. Given, the dual advantages of pap smear in diagnosing cervical pre-cancer stages and infections, more elaborate studies should be conducted to determine usefulness of treatment or otherwise of bacterial vaginosis.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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