Original Research

A Cross-sectional Study to Investigate the Association Between Obesity and Bacterial Vaginosis Using Nugent Score

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ABSTRACT
Bacterial vaginosis is one of the very important vaginal infections with which women report in OPD. Women with BV are at an increased risk for sexually transmitted infections such as gonorrhea, chlamydia, HIV, trichomoniasis, urinary tract infection, pelvic inflammatory disease, and adverse pregnancy outcomes that include preterm birth. Thus, the study wasconductedinatertiarycarehospitaltounderstandthecorrelationbetween bacterial vaginosis and obesity and to prevent future gynecological complications in obese women. A total of 120 women of reproductive age group between 18-45 years and sexually active were enrolled in the study. Their mean age in completed years was 35.95 ± 7.17 years with the youngest woman being 20 years old and the eldest being 48 years old. It was observed thattheindividualwhodoes not have bacterial vaginosis has 3.22timestheodds to be not obese. This finding was statistically significant(p=0.002). It was found that the women who had some complications were at 7 times higher odds of being obese as compared to being not obese. A total of 81 study subjects presented with negative Amsel's criteria, out of which 29 showed the presence of bacterial vaginosis while the other 52 did not. It was concluded that bacterial vaginosis is associated with the oddsof beingfrom the Obesegroupofwomenas compared tothe non-obese women. Moreover, the Nugent score which is a gram-stain-basedcriterion for diagnosis can be preferred over Amsel's criteria for BacterialVaginosis.

INTRODUCTION
Bacterial vaginosis is one of the very important vaginal infections with which women report in OPD. Bacterial vaginosis (BV), is a common cause of vaginal symptoms among reproductive-aged women and is present in approximately 1 of every 3 women [1]. Women with BV are at an increased risk for sexually transmitted infections such as gonorrhea, chlamydia, HIV, and trichomoniasis, urinary tract infection, pelvic inflammatory disease, and adverse pregnancy outcomes that include preterm birth [2,3,4]. It is characterized by thin homogenous vaginal discharge, lower abdominal pain, fishy odor of discharge, burning micturition, itching, etc. [5,6,7] The lactic acid formed by the natural flora ofvagina under the acidic PH of the vagina provides a local mechanism of protection by inhibiting growth of otherorganisms. But changeinlocalPHleadsto the growthofanaerobicbacteria which is due to a reduction in the number of H_2O_2-producing lactobacilli making it more susceptible tothe growth of pathogenic organisms.[8,9] Treatment is recommended for symptomatic women, but 1 month after therapy only 80% of women have regained normal flora; recurrences after 1 month are also common. Little is known about howthedysbioticBV microbiomedevelopsorhowindividualbacteriainteract withthehosttoproduce disease. A relationship between increased body mass index (BMI) and gut dysbiosis has been studied widely, however, very little information is there about the relationship between BMI and BV prevalence. Thus,thestudy was conducted in a tertiary care hospital to understand the correlation between bacterial bacterialvaginosisandobesityand obesity andpreventive gynecologicalcomplications in obesewomen.

Primary Objective of the study:
- AssociationbetweenObesity(highbodymassindex)

and prevalence of Bacterial
- Vaginosis(BV)in reproductiveagegroup as assessed byNugentscore

Secondary objectives
- To Study demographic and characteristics features of bacterial vaginosis
- To study various complications associated with BV in women of reproductiveage group
- TostudytherelationshipofobesitywithBVcomplications

MATERIALS AND METHODS

Thisstudywasa cross-sectionalstudywhichwasconductedin a cross-sectionaltertiarycarematernal andchildhealthwingofa ruraltertiaryhospitalforaperiodofJanuary2020toJune 2021. A total of 120 women of reproductive age group between 18-45 years and sexually are actively reporting to the hospital with complaints of BV such as white discharge, lower abdominal pain, vulval soreness and itching, frequency & burning in micturition and dyspareunia who have high body mass index were enrolled for the study. Participants with a history of tubal ligation &hysterectomy, Pregnantfemales, sexually transmitted diseases, and Douching were excluded from the study. Thensentwastaken before theirclinical history includingthe complaints, menstrual and obstetric history, and past and specifically personal history were taken in detail. In the personal history, sexual history was also asked in detail. The symptoms too were asked in detail and co-morbidities were also noted. The woman was examined as per the Gynaecological examination protocol of the department by doing general, local, speculum, and bimanual vaginal examinations. In the general examination the BMI was calculated using height and weight and women were classified in 2 groups of Lean with normal weight women as the first group & overweight with obese women as the second group. For the women in whom there was suspicion of bacterial vaginosis,pH was checked by using simple litmus paper, characteristics of discharge were also noted and the odour of amine was checked by putting KOH on discharge.The wet mount and dry slide were prepared and sent to the Department of Microbiology. The specimen of the vaginal and cervical swab was taken and sent for microscopy and culture sensitivity too in these women.The reports were followed and all the specimens where the report was bacterialvaginosis positivewereincludedineachgroupasstudysubjects.Asth edetailedhistoryandexamination were already done and BMI was already calculated, the data was entered in the Excel sheet along with the report of AMSEL and NUGENTSCORING.Data was analyzed on MSExcel spreadsheet usingRversion4.1.2.

RESULTS

A total of 120 women participated in the study. Table 1 below shows the socio-demographics. Their mean age in completed years was 35.95 ± 7.17 years with theyoungest woman being 20 years old and the eldest being 48 years old.

Table 1: Background characteristics of study participants

Sr.no	Variable	Mean ± SD	Range (min-max)
1	Age (in completed years)	35.95 ± 7.17	28 (20-48)
2	Height (m)	1.57 ± 0.05	0.20 (1.46-1.66)
3	Weight (Kg)	65.43 ± 8.54	35 (52-87)
4	BMI (Kg/m^2)	26.37 ± 2.90	11.44 (22.55-33.98)

Table 2: Clinical history and related details

Sr.no	Aspect	n (%)
1	**Primary complaints**	
	White discharge PV	7 (5.83)
	Itching	13 (10.83)
	Burning micturition	13 (10.83)
	Lower abdominal pain	17 (14.17)
	Foul-smelling discharge PV	21 (17.50)
	Difficulty in coitus	49 (40.83)
2.	**Vaginal Discharge**	
	Present	68 (56.67)
	Absent	52 (43.33)
3.	**pH**	
	Alkaline	70 (58.33)
	Acidic	50 (41.67)
4.	**Whiff's test**	
	Negative	83 (69.17)
	Positive	37 (30.83)

5.	**Wet mount**	
	Absent	85 (70.83)
	Present	35 (29.17)
6.	**Amsel's criteria**	
	Negative	**81 (67.50)**
	Positive	39 (32.50)

Table 3 -: Nugent score

| Sr.no	Variable	Mean ± SD	Range (min-max)
1	Nugent score	5.81 ± 2.16	8 (1-9)
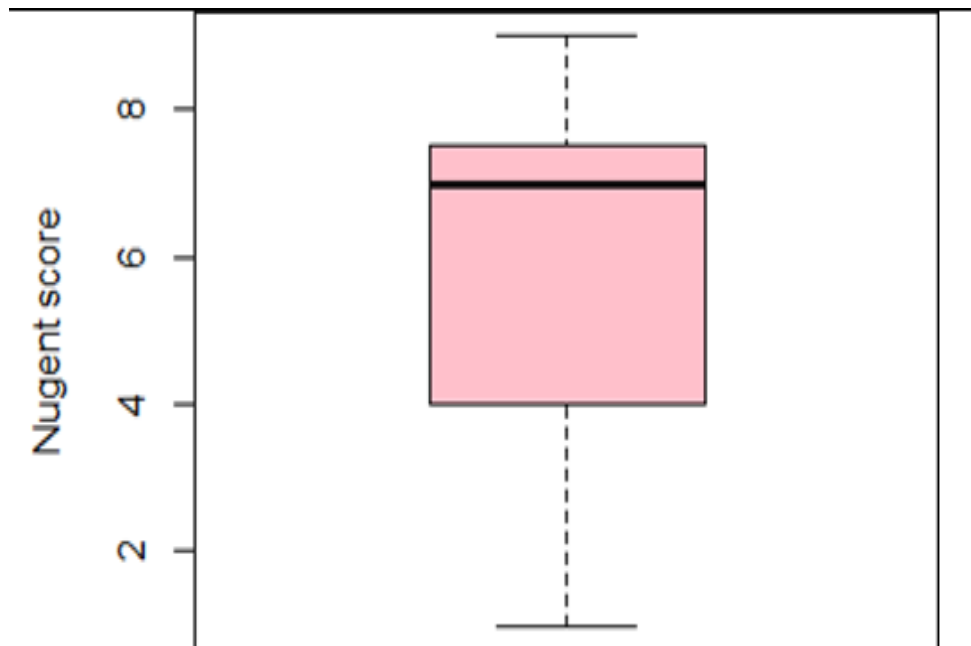
The boxplot above shows the distribution of the Nugent score across our study participants. The lower whisker marks the minimum value of the Nugent score i.e. 1 while the upper whisker marks the maximum value of the Nugent score i.e. 9. The horizontal bold line in the box marks the 50th percentile value of the Nugent Score i.e.The lower margin of the box marks the 25th percentilevalueoftheNugentscorei.e. 4 andtheuppercentageofthebox marks the 75th percentile value of the Nugentscore.
Table 4: Shows thattheindividualwhodoesnothave bacterial vaginosisareat3.22timestheodds to be not obese. This finding was statistically significant(p=0.002).

Table 4: Association of Obesity and Bacterial Vaginosis

	Bacterial Vaginosis absent	Bacterial Vaginosis present	Total	Odds ratio (95% CI)
Not obese	38	28	66	
Obese	16	38	54	3.22 (1.51 – 6.90)
Total	54	66	120	

Association of Obesity with Bacterial Vaginosis
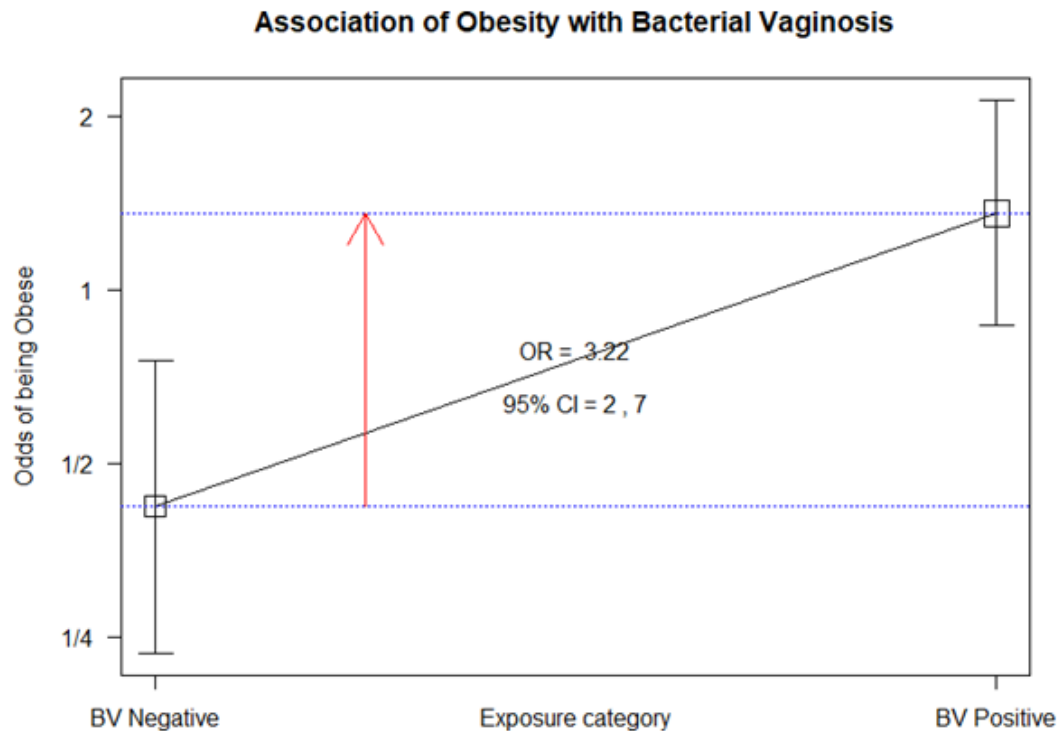
Figure: Association of Bacterial vaginosis and Obesity

Table 5a: Association of Obesity and Complications

	Cervicitis	Abortion	Endometritis	PID	Vaginitis	Total
Obese	4	2	1	5	5	17
Not Obese	1	0	0	1	2	4
Total	5	2	1	6	7	21

Table 5a shows the distribution of complications according to being obese or not. It was observed that the association of obesity and complications was seen in our study participants. It was found that the women who had some complications were at 7 times higher odds of being obese as compared to being not obese.

Table 5b: Association of Obesity and Complications

	Complication's present	complications absent	Total	Odds ratio (95% CI)
Obese	17	37	54	7.01 (2.30 – 25.90)
Not Obese	4	62	66	
Total	21	99	120	

Table 6: Independent predictors of Bacterial Vaginosis

Coefficients	Estimate	Standard error	t value	p value
(Intercept)	-2.565	1.388	-1.848	0.067
BMI	0.285	0.052	5.497	<0.001
Amsel's criteria	2.631	0.320	8.277	<0.001
Residuals: Min: -5.5450 1st quartile: -1.0614 Median: 0.0101 3rd quartile: 1.2284 Max: 3.0866				
Residual standard error: 1.635 on 117 degrees of freedom Multiple R-squared:0.4378,Adjusted R-squared:0.4282F-statistic: 45.55 on 2 and 117 DF, p-value: 2.345e-15				

Multiple linear regression was performed to derive the final estimate to explain the effect of independent determinants on the Nugent score. All the factors were considered for multiple linear regression. The final model retained body mass index and Amsel's criteria as independent predictors of Bacterial Vaginosis. It is implied that for every unit increase in BMI, the Nugent score increasesby0.285andthatifAmsel's

criteria arepositivethentheNugent'scoreincreasesby2.631. Analysis of residuals suggested that there is no significant heteroscedasticity. Adjusted R2 for the finalmodelwas0.4282implyingthatourmodelexplains42.82% variation inBacterialvaginosisas an outcome. Table 6 shows the final model obtained after multiple linearregression.
Table

7ashowsthedistributionofthestudypopulationwith37studysubjectshavingpositive Amsel's criteria and the presence of bacterial vaginosis as per Nugent score, while only 2 showed positiveAmsel'scriteriabutabsenceofbacterialvaginosis asperNugentscore.A total of 81 study subjects presented with negative Amsel's criteria, out of which 29 showed the presence of bacterial vaginosis while the other 52 did not.

Table 7a: Diagnostic accuracy of Nugent score against Amsel's criteria to diagnoseBacterial Vaginosis

		Amsel's criteria positive	Amsel's criteria negative	Total
Nugent score	Bacterial Vaginosis present	37	29	66
	Bacterial Vaginosis absent	2	52	54
	Total	39	81	120
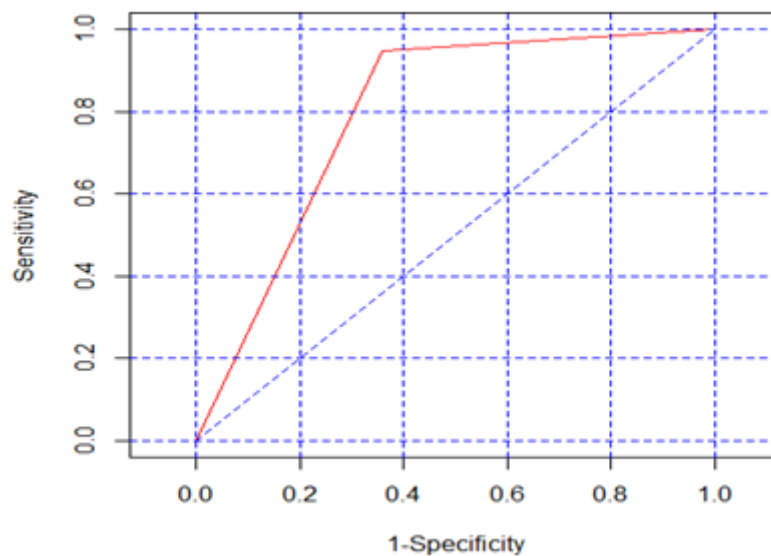
Figure: ROC curve for diagnostic accuracy of Nugent score against Amsel's criteria The table below summarizes the diagnostic accuracy of Nugent score showing the

Table 7b: Sensitivity of 94.87%, specificity of 64.20%, positive likelihood ratio of 2.64, negative likelihood ratio of 0.08, disease prevalence of 32.5%, the positive predictive value of 56.06%, negative predictive value of 96.30% and accuracy of 74.17%.

Table 7b: shows the sensitivity and specificity

Statistic	Value	95% CI
Sensitivity	94.87%	82.68% to 99.37%
Specificity	64.20%	52.77% to 74.55%
Positive Likelihood Ratio	2.65	1.96 to 3.58
Negative Likelihood Ratio	0.08	0.02 to 0.31
Disease prevalence (*)	32.50%	24.23% to 41.65%
Positive Predictive Value (*)	56.06%	48.58% to 63.28%
Negative Predictive Value (*)	96.30%	86.97% to 99.02%
Accuracy (*)	74.17%	65.38% to 81.72%

It was very evident that Amsel's criteria can miss a number of cases of Bacterial vaginosis whereasNugent's criteria identify most of them with better sensitivity and specificity.

DISCUSSION

The purpose of this cross-sectional study was to study the association between obesity and bacterial vaginosis as assessed by the Nugent score. Further, this thesis wanted to explore the characteristic features of bacterial vaginosis.Themeanageofwomenhavingbacterialvaginosiswas35.95±7.17yearswiththeyoungest womanbeing20yearsoldandtheeldest48yearsold.Multiplestudieshaveshownastrong association between the

presence of bacterial vaginosis and age >25 years (132-134). This is similar to the findings of thisstudy. Theanalysisofprimarycomplaintsinthepresentstudyrevealedthatthehighest percentage i.e., 40.83% was of difficulty in coitus followed by foul-smelling discharge PV (17.50%), lower abdomenpain(14.17%),burningmicturition(10.83%),itching(10.83%),andlowestpercentagei.e., 5.83%reportedwhitedischargePV.Itisknownfrompreviousstudiesthatmanyformso BVremain asymptomaticorpresentonlywithamalodorousvaginaldischargewithoutinflammatorycomplaints [10]. Amongst the women participating in the present study, 56.67% reported the presence of vaginal discharge while the rest said it was absent. A previous study found that increased vaginal discharge was found in 73% of the symptomatic patients whileirritativesymptoms (itching, burning, pain) were identified by 45 percent of the patients in this study, which may have been associated with other causes of vaginitis if symptoms alone had been used to direct diagnosis and care [11]. Also, Bacterial vaginosis is known to be the most common cause of vaginal discharge among women of reproductiveage which further supports our findings. However, Klebanand colleagues [12]observedthatthecomplaintssoodoranddischargewere presentin58percentof patientswithBVand57percentofthosewithoutBVduring theintervening6months,demonstrating the unreliability of symptoms fordiagnosis.ThepH of 58.33% of subjects was found to be alkaline, and others were acidic. The Whiffs test tested positive only for 30.83% of the study subjects, while that for others was negative. Evidence suggests that Bacterial Vaginosis is clinically distinguished by a thin, gray/off-white, homogeneous, malodorous vaginal adherent discharge that is more visible during intercourse and menstruation,withapH>4.5[13]A pioneering studybyGardnerandDukeshasalsoshowntherise in pH is associated with Bacterial Vaginosis [14]. This further supports the findings of the present study.

BV is usually diagnosed using Amsel's clinical criteria or Gram stain. Using Amsel's criteria, the clinical diagnosis is made by fulfilling three out of four criteria: (1) vaginal pH > 4.7. The presence of clue cells on wet mount, Thin homogeneous discharge, and Amine "fishy odor" whenpotassiumhydroxideisaddedtothedischarge.However, in the presentstudy,the wet mountis absent in 70.83% population and present only in 29.17%. Amsel's criteria were found to be negativein67.50%ofthestudypopulation,andonly32.50%werepositive.Thisindicatestheneed foramoreintensiveinvestigationworkup todiagnoseBV.TwodifferentGramstainscoringsystems, Nugent's [15] and Spiegel's [16] have been developed and compared with Amsel's clinical criteria. Inthepresentstudy,themeanNugentscoreofthewomenwas5.81±2.16,withaminimumscoreofandamaximumscor

eof9.Further,itwasfoundthatforeveryunitincreaseinBMI,theNugent score increases by 0.285 and that if Amsel's criteria are positive then the Nugent score increasesby 2.631.The diagnostic accuracy of the Nugent score showsa sensitivity of 94.87%, specificity of 64.20%, positive likelihood ratio of 2.64, negative likelihood ratio of 0.08, disease prevalence of 32.5%, a positive predictive value of 56.06%, negative predictive value of 96.30% and accuracy of 74.17%.Gram stain of vaginal discharge may be a more reliable means of diagnosing BV, and thereisevidencethatitofferstheaddedabilitytoquantifyandclassify bacterialload.[90]Forthese reasons, Gram stain has been the primary means used to diagnose BV in epidemiologic and treatment studies, with Nugent's criteria accepted as the preferredmethod.For the women who participated in this study, the mean height was 1.57 ± 0.05m and the mean weight of the study participants was 65.43 ± 8.Obesity has been highlighted as a potentially modifiable risk factor for BV in some studies (17), but not in others (18).The mean body mass index (BMI) of the women participating in the study was 26.37 ± 2.90. In one study of 2,906 U.S. women, of which 26.2% were black, 36% of obese women were BV positive; however, after adjusting for confounders, they found no relationship between BMI and BV. [19]The Present study implies that the individuals who do not have bacterial vaginosis are at 3.22 times the odds to be not obese. This finding was statistically significant (p=0.002).In this study, it was found that out of the total 120 study participants, 21 (17.50%) had some complicationslikecervicitis,abortion,endometritis,pelvicinflammatorydisease,orvaginitis.Outof the 21 participants who had complications, 17 (80.95%) were obese and 4 (19.05%) were not obese. It was found that the women who had some complications were at 7 times higher odds of being obese as compared to being not obese. Previous evidence suggests that vaginal infections can result in various gynecological and obstetric complications [20]

which is concurrent with the findings of the presentstudy.Multiple linear regression was performed to derive the final estimate to explain the effect of independent determinants on the Nugent score. All the factors were considered for multiple linear regression. The final model retained body mass index and Amsel's criteria as independent predictors of Bacterial Vaginosis. It is implied that for every unit increase in BMI, the Nugent score increasesby0.285andthatifAmsel'scriteriaarepositivethentheNugentscoreincreasesby2.631.The Nugent score thus was found to be a better test for diagnosing bacterial vaginosis as comparedtoAmsel'scriteria.Thefindingsstatethatthe highertheBMI,the morechancesofBVbeing diagnosed by Nugent'scriteria.The diagnostic accuracy of the Nugent score showsa sensitivity of 94.87%, specificity of 64.20%, positive likelihood ratio of 2.64, negative likelihood ratio of 0.08, disease

prevalence of 32.5%, a positive predictive value of 56.06%, negative predictive value of 96.30% and accuracy of 74.17%.It was very evident that Amsel's criteria can miss several cases of Bacterial vaginosis whereas Nugent's criteria identifies most of them with better sensitivity and specificity.

CONCLUSION

ThepresentstudyoverallconcludesthathavingBacterial Vaginosisisassociatedwithhigher oddsof beingfromthe Obesegroupofwomenascomparedtothe non-obese women.Moreover, the Nugent score which is a gram-stain-basedcriterion for diagnosis can be preferred over Amsel's criteria for BacterialVaginosis.

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