Association of intermediate Nugent Score and bacterial vaginosis with sexually transmitted infections and vulvovaginal candidiasis

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Abstract

Background: Bacterial vaginosis is a common vaginal syndrome among females, which leads to significant morbidity and complications, if left untreated. The association of bacterial vaginosis with various sexually transmitted infections has been mentioned in previous literature. However, studies on the intermediate Nugent Score are lacking. This study was planned to examine the association of sexually transmitted infections with the intermediate Nugent Score.

Materials and Methods: The study included was conducted to include females presenting with vaginal discharge, burning micturition, itching, lower abdominal pain and infertility. The Nugent scoring was used to categorize patients into those having normal flora, intermediate or bacterial vaginosis. Conventional and molecular techniques targeting *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, Syphilis, *Neisseria gonorrhoeae* and vulvovaginal candidiasis were performed.

Results: A total of 3,531 clinical samples were collected from females with a median age of 28.0 years. The number of patients with bacterial vaginosis and intermediate Nugent Score and positive cases were significantly higher in the 21–35 years age group ($P < 0.0001$). We observed that the likelihood of test results being positive for *Trichomonas vaginalis* was higher ($P < 0.05$), as the abnormality of the vaginal flora increased. *Mycoplasma hominis* was observed to be significantly higher in the intermediate Nugent Score group than the BV-positive patients (0.6 vs 0.2, $P = 0.002$). The number of vulvovaginal candidiasis cases in both the bacterial vaginosis-negative and bacterial vaginosis-positive groups were nearly the same (9.3 vs 9.8%).

Limitations: Individual follow-up couldn't be performed on the patients.

Conclusions: We observed that the dysbiosis in vaginal microbiota, with an increase in Nugent scoring, was significantly associated with an increased risk for the acquisition of sexually transmitted infections and vulvovaginal candidiasis.

Key words: Bacterial vaginosis, Intermediate nugent score, *Mycoplasma hominis*, *Trichomonas vaginalis*, Vulvovaginal candidiasis.

Plain Language Summary

This clinical study evaluated the association of bacterial vaginosis and intermediate stage, as per Nugent's Score, with various sexually transmitted infections and vulvovaginal candidiasis. We observed an incremental increase in the likelihood of acquisition of sexually transmitted infections and vulvovaginal candidiasis with the increase in the abnormality of vaginal flora.

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Introduction

Bacterial vaginosis (BV) is a common vaginal syndrome due to the alteration of normal vaginal flora. The vaginal lactobacilli are replaced and dominated by the growth of anaerobes and gram-negative bacteria *(Gardnerella vaginalis*).[1] This leads to disruption of vaginal microflora resulting in grey or white, thin, and malodorous discharge. Worldwide, the prevalence of bacterial vaginosis varies substantially, ranging from 15 to 49%.[2–5] It has been associated with complications related to women's reproductive health and contributes to morbidity among females in developing countries. Traditionally, Nugent scoring is used to categorise vaginal flora as bacterial vaginosis normal, intermediate and positive.[6] Previously, many studies have observed association and increased susceptibility of various sexually transmitted infections (STIs) in BV positive and negative patients.[7] In a recent systematic review and meta-analysis, a nearly 2-fold higher risk for acquiring *Trichomonas vaginalis* was observed in bacterial vaginosis-positive patients than in those without bacterial vaginosis.[8] Also, concurrent vaginal *Candida* colonization was independently associated with approximately two-fold increased odds for bacterial vaginosis.[9]

Studies on intermediate Nugent Score (BV intermediate) are lacking. There is a growing body of literature that vaginal bacterial communities, like lactobacillus, play a pivotal role in preventing colonization and infection by pathogens causing STIs and vulvovaginal candidiasis. Only a few prospective studies have been assessed to see the association of vaginal microbiota with the incidence of STIs and vulvovaginal candidiasis. However, these studies were conducted among high-risk group females, that is, female sex workers or women attending STIs clinics.[10,11] Most of these studies have observed the relationship between various STIs and vulvovaginal candidiasis between BV positive and negative patients. As known, the increase in the Nugent Score corresponds to the progression in vaginal dysbiosis and decrease in the *lactobacillus* population.[6] Bacterial vaginosis intermediate has the propensity to progress towards the BV-positive stage. Thus, it is important to study the association of STIs in the BV positive and BV intermediate categories individually. Therefore, this study was conducted to evaluate the relationship between STIs and vulvovaginal candidiasis among female subjects with negative, intermediate and positive bacterial vaginosis categories individually.

Material and Methods

Study design and recruitment

The study was conducted from 1st January 2017 to 31st December 2021 at the regional STIs reference, research and training center of the Postgraduate Institute of Medical Education and Research, Chandigarh, which caters 14 peripheral centers in Chandigarh. A total of 3,531 clinical samples were collected from females (16–65 years of age) with vaginitis. Females presenting with any of the following chief complaints such as vaginal discharge, burning micturition, itching, lower abdominal pain, or infertility were enrolled. Two vaginal swabs were collected by the trained healthcare professional using FLOQSwabsTM (Copan, Italy) during routine pelvic examinations. Females were excluded if they had received antibiotics or antifungals in the preceding week or were menstruating or pregnant. Females with genital tuberculosis and malignancies were also excluded. The study was approved by the Institutional Ethics Committees, Postgraduate Institute of Medical Education and Research, Chandigarh (Ethics approval no. INT/IEC/2019/002222).

Microbiologic Analysis

Bacterial vaginosis: The vaginal fluid was smeared on a glass slide and stained with gram stain and reported for the presence of bacterial vaginosis, in accordance with Nugent scoring criteria.[6] Bacterial vaginosis was diagnosed if the score was 7–10; a score of 4–6 indicated intermediate vaginal flora; and a score of 0–3 indicated normal vaginal flora.

Test for *Trichomonas vaginalis*: For *Trichomonas vaginalis* culture, vaginal swabs were inoculated in Diamond's media and incubated at 37°C. The media were examined every second day for 1 week for the presence of motile trichomonads. The tvk3 and tvk7 primers were used as their targets, specifically amplifying a 261 bp sequence of the 18S SS-rRNA gene segments of *Trichomonas vaginalis*. The conventional polymerase chain reaction was performed as per the protocol.[12]

Tests for *Neisseria gonorrhoeae*: Endocervical samples were streaked on a chocolate medium. Identification was confirmed by gram stain examination, superoxol (10% hydrogen peroxide), rapid carbohydrate utilization test and a polymerase chain reaction of the *porA* and *opa* gene.[13]

Test for *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum*: The quantitative polymerase chain reaction for the detection of *C. trachomatis*, *Ureaplasma urealyticum* and *Mycoplasma hominis*, was performed using the primers from previous literature.[14]

Test for syphilis: The Venereal Disease Research Laboratory (VDRL) test was performed as per the World Health Organization guidelines.[15] The treponema pallidum hemagglutination (TPHA) test was performed by Plasmatec kit (Novacyt Group, United Kingdom) as per the manufacturer's guideline.[16]

Yeast identification: It was done by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (Bruker Daltonics MALDI Biotyper, Germany).

Data analysis and statistics

The data was entered in Statistical Package for the Social Sciences 26.0 for Mac OS (SPSS, Inc., Chicago, IL). A $P < 0.05$ was used to analyse the data.

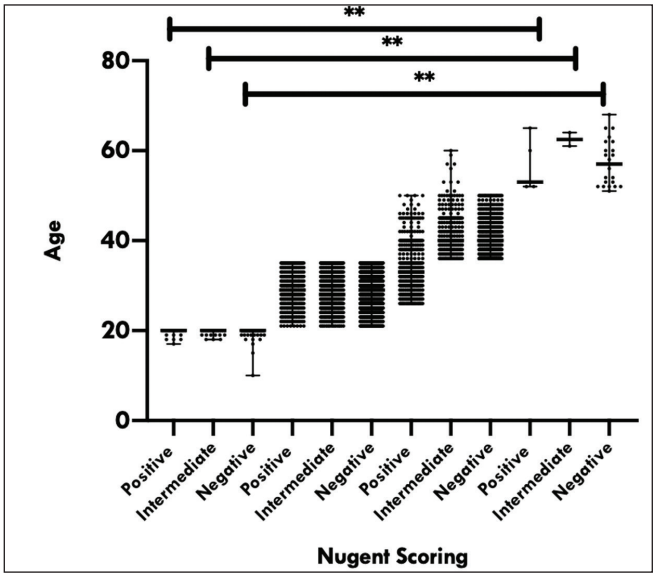

Figure 1: Relation of various categories of Nugent scoring with different age groups

Results

Characteristics of the study population

The age of females in the present study ranged from 15 to 50 years, with a median age of 28.0 years (interquartile range: 10) [Figure 1]. Based on the Nugent scoring, number of patients with positive, intermediate, and negative scores for BV were 479 (13.6%), 837 (23.7%) and 2215 (62.7%), respectively. Among the subjects with BV, 7.7% (37 of 479) were infected with one of the two sexually transmitted organisms, compared with an infection rate of 6.2% (52 of 837) in patients with BV intermediate and 2.8% (64 of 2215) for subjects without BV.

Association of age groups with bacterial vaginosis stages

The BV-positive females were mostly from 21–35 age group, followed by the age group of 36–50 years [Figure 1]. The number of positive BV cases was significantly higher in 21–35 years age group ($P < 0.0001$). Similarly, the number of BV intermediate patients was significantly higher in 21–35 years age group ($P < 0.0001$) than in other age groups.

Association of bacterial vaginosis stages with clinical symptoms

The frequency of cervicitis and discharge was significantly more in patients with BV intermediate category than in negative and positive BV groups (10.9 vs 7.1 vs 7.7%, $P = 0.003$).

Association of bacterial vaginosis stages with sexually transmitted infections

Among the subjects with BV, 25 (31.3%) were infected with *Trichomonas vaginalis* [Table 1]. We found a significant difference in patients with and without BV for *Trichomonas vaginalis* acquisition ($P < 0.05$). Patients with BV were three times more likely to be infected with TV than those without BV (95% confidence interval, 1.8–4.8) [Table 2]. Likewise, patients with BV were significantly more positive for *Candida* species than patients without BV (odds ratio, 1.2; 95% confidence interval, 0.85–1.630) [Table 2]. The likelihood of the test results being positive for *Trichomonas vaginalis* was significantly higher ($P < 0.05$) as the abnormality of the vaginal flora increased. *Mycoplasma hominis* was observed to be significantly higher in BV intermediate than BV positive patients (0.6 vs 0.2, $P = 0.002$) [Table 1]. Also, concurrent *Trichomonas vaginalis* and *Mycoplasma hominis* were found in 5 of 6 of the patients. Although not significant, an increasing trend was observed in *Ureaplasma urealyticum* infection in BV patients, than in BV normal and BV intermediate categories ($P = 0.064$) [Table 1]. *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were present in similar frequency among all the categories.

Table 1: Characteristic of bacterial vaginosis based on Nugent's scoring

	Negative (2215) *n* (%)	Intermediate (837) *n* (%)	Bacterial vaginosis positive (479) *n* (%)	*P*-value
Symptoms				
Cervicitis	159 (7.1)	92 (10.9)	37 (7.7)	0.003
Presence of discharge	1278 (57.7)	552 (65.9)	300 (62.6)	0.000
Infertility	354 (15.9)	130 (15.5)	70 (14.6)	0.749
Burning micturition	29 (1.3)	10 (1.2)	4 (0.84)	0.690
Itching	81 (3.6)	29 (3.4)	20 (4.2)	0.801
Abdominal pain	68 (3.0)	15 (1.8)	13 (2.7)	0.153
Infections				
Trichomonas vaginalis	23 (1)	32 (3.8)	25 (5.2)	0.000
Chlamydia trachomatis	3 (0.1)	1 (0.11)	0	0.725
Ureaplasma urealyticum	4 (0.2)	3 (0.3)	4 (0.8)	0.064
Mycoplasma hominis	0	5 (0. 6)	1 (0.2)	0.002
Syphilis	28 (1.3)	11 (1.3)	6 (1.3)	0.993
Neisseria gonorrhoeae	6 (0.3)	2 (0.2)	1 (0.2)	0.965
Vulvovaginal candidiasis	180 (8.2)	78 (9.3)	47 (9.8)	0.356

BV: bacterial vaginosis

Association of bacterial vaginosis with vulvovaginal candidiasis

Of all the subjects with BV, 14 (15.4%) women had vulvovaginal candidiasis. The BV patients were 1.2 times more likely to be infected with yeast infection than were subjects without BV (95% confidence interval, 0.850–1.634). The number of vulvovaginal candidiasis cases in both BV intermediate and BV positive was nearly the same (9.3 vs 9.8%). However, the difference in vulvovaginal candidiasis between BV positive and negative groups was not significant ($P = 0.336$). Figure 2 depicts the distribution of various yeast species among patients with different Nugent scores. *Chlamydia trachomatis* was the

Table 2: Relation of bacterial vaginosis with the presence of symptoms and infections

Characteristics	Bacterial vaginosis		Odd's ratio (95% confidence interval)	*P*
	Present *n* (%)	Absent *n* (%)		
Presence of discharge	300 (14.1)	1830 (85.9)	1.12 (0.92–1.36)	0.270
Infertility	70 (12.6)	484 (87.4)	0.91 (0.69–1.19)	0.543
Burning micturition	4 (9.3)	39 (90.7)	0.65 (0.23–1.83)	0.288
Itching	20 (15.4)	110 (84.6)	1.16 (0.72–1.89)	0.305
Abdominal pain	13 (13.5)	83 (86.5)	0.99 (0.55–1.81)	0.571
Cervicitis	37 (12.8)	251 (87.2)	0.94 (0.65–1.34)	0.788
Trichomonas vaginalis	25 (31.3)	55 (68.8)	3.01 (1.85–4.86)	0.000
Chlamydia trachomati	0 (0.0)	4 (100)	0.99 (0.99–1.00)	1.000
Ureaplasma urealyticum	4 (36.4)	7 (63.6)	3.66 (1.07–12.56)	0.50
Mycoplasma hominis	1 (16.7)	5 (83.3)	1.27 (0.15–10.94)	0.583
Syphilis	6 (13.3)	39 (86.7)	0.98 (0.43–2.33)	1.00
Neisseria gonorrhoeae	1 (11.1)	8 (88.9)	0.79 (0.09–6.38)	1.00
Vulvovaginal candidiasis	47 (15.4)	258 (84.6)	1.17 (0.85–1.64)	0.336

most common yeast isolated overall, followed by *Candida glabrata* (195 vs 85) [Figure 2]. Other less common yeasts were *Chlamydia trachomatis*, *Candida krusei*, *Candida kefyr, Candida parapsilosis* and *Candida lambica*.

Discussion

This study represents a large study population that provides evidence of the association of BV positive and BV intermediate with sexually transmitted infections and vulvovaginal candidiasis. The prevalence of BV in the current study was 13.6%, which is nearly similar to another study from the South India where the prevalence was 15.4%.[2] However, a higher rate of BV has been reported in various other parts of the world, ranging from 29 to 49%.[3–5]

We observed an incremental increase (3.8–5.2%) in the likelihood of acquisition of *Trichomonas vaginalis* infection with an increase in the abnormality of vaginal flora. Similar findings were also observed by Brotman *et al*., where the intermediate state was associated with a 1.5–2-fold increased risk for *Trichomonas vaginalis* infection.[17] These above findings are suggestive that females without BV but with an altered vaginal flora may pose a risk for STIs.[24] Although epidemiological data suggest a strong association of *Trichomonas vaginalis* infection with BV, the prevalence of these infections is probably underestimated since both can be asymptomatic or clinically present with common symptoms of vaginal discharge.[18] Rathod *et al*. observed a four to nine-fold increased risk of *Trichomonas vaginalis* infection among sexually active

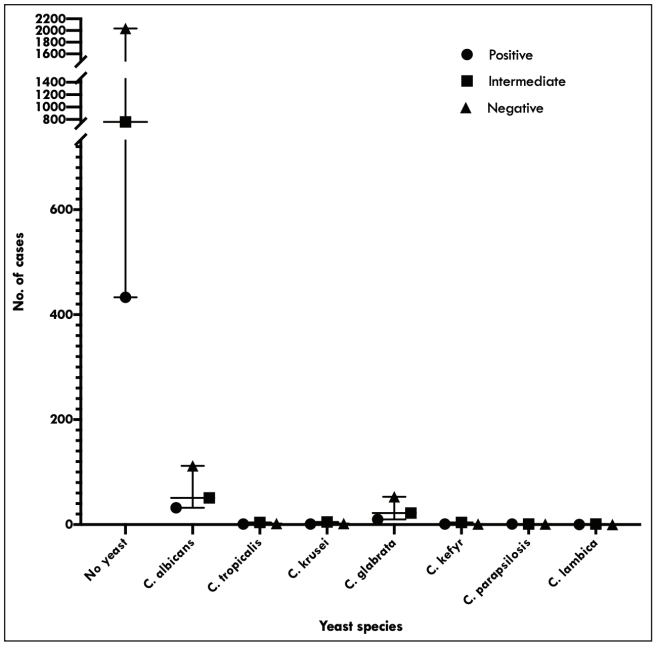

Figure 2: The distribution of various yeast species among patients with different Nugent Scores *viz*; normal, intermediate and bacterial vaginosis

females with abnormal vaginal flora, suggesting a causal role of altered microbiota on trichomonad infection.[19] *In in vitro* experiments, Hinderfeld and Simoes-Barbosa observed that biofilm produced by dysbiotic bacterias increased the adhesion of the protozoa to host cells. Thus, it is plausible that BV intermediate alters the vaginal ecology and facilitates the acquisition of STIs.

Secondly, we observed a higher number of *Mycoplasma hominis* cases in the BV intermediate category than BV positive cases ($P = 0.002$). On the contrary, Cox *et al*., observed a significantly higher presence of *Mycoplasma hominis* in BV positive (60.7%) patients compared to BV intermediate and nonbacterial vaginosis (36.4 and 8.8 %, $P < 0.001$) stages.[20] Both the BV positive and BV intermediate stages are characterized by decreased protective *lactobacilli*.[21] These findings suggest BV intermediate has unique epidemiology and represents unique taxa of the bacterial community, which needs to be defined by further studies.[21]

Mycoplasma hominis has been found in symptomatic women as well as healthy females. Various studies have observed its association with altered vaginal flora, including BV positive and *Trichomonas vaginalis* infection. Belkum *et al*. observed the highest prevalence of *Mycoplasma hominis* prevalence in the groups of patients infected with *Trichomonas vaginalis* or those who were BV positive (71 and 38%, respectively).[22] This was also observed in the present study, where the maximum number of cases (5 of 6) had concurrent *Trichomonas vaginalis* and *Mycoplasma hominis* infection. We did not observe an increase in symptoms once *Mycoplasma hominis* infection was detected along with *Trichomonas vaginalis*. However, the question remains elusive, whether in BV, the *Trichomonas vaginalis* predisposes to *Mycoplasma*

hominis infection or if this is merely an association. In one study, a symbiotic association between *Trichomonas vaginalis* and *Mycoplasma hominis* was observed to influence the host-microbes interactions, which is detrimental to their host but beneficial to both microbial partners during infections.[23] However, further research is required to study the association between these STIs.

In the current study, concurrent vaginal *Candida* colonisation was associated with approximately one-fold increased odds of BV. Although not significant, the higher trend of vulvovaginal candidiasis was present in both BV intermediate and BV positive stages [Table 1]. In India, Rathod and co-workers found a positive association between women clinically diagnosed with BV positive and prevalence of vulvovaginal candidiasis (prevalence bacterial vaginosis: bacterial vaginosis normal 12%:6.5%).[24] As both BV positive and vulvovaginal candidiasis have overlapping clinical presentation of vaginal discharge, the misclassification between vulvovaginal candidiasis and clinically defined BV is most likely possible.[24] The high prevalence of yeast infection/colonisation in BV intermediate may predispose these females to symptomatic vulvovaginal candidiasis, thus leading to repeated visits to hospitals. Also, a significant number of females who are BV positive have yeast colonisation in the vaginal ecosystem. Hence the failure of symptom resolution in patients with targeted therapy to treat BV can be due to the development of vulvovaginal candidiasis from antibiotic exposure or intrinsic failure of the therapy.[25] Thus, the identification of patients with concurrent BV and vulvovaginal candidiasis is important due to a major concern of recurrence of symptoms in patients. Lopez *et al*., observed concurrent BV and vulvovaginal candidiasis in 34% of cases and 29% of patients had a history of recurrent BV.[26] The lack of specificity of clinical signs and symptoms of vaginitis and high rate of recurrence mandate clinical and microbiological examination to determine a specific diagnosis. Failure to appreciate the vulvovaginal candidiasis in BV-positive patients leads to inappropriate therapy and increases the rate of recurrence. In accordance with previous studies, *Candida albicans* was the most frequently isolated species; however, we also observed a high prevalence rate of nonalbicans *Candida* species.[27,28] Thus, yeast identification, antifungal susceptibility, and continuous epidemiological surveys to measure changes in species distribution from *Candida albicans* to non-albicans *Candida* species are important.

A healthy vaginal microbiome plays a pivotal role in protecting the female genital tract against various STIs and vulvovaginal candidiasis. The alteration of host defence against infection due to a decrease in the protective *lactobacillus*-deficient environment leads to increased susceptibility to STIs due to the presence of BV associated organisms and their metabolites. Further release of microbial products alters the vaginal microenvironment.

The major strength of the present study is simultaneous reporting of Nugent Score, cultures, and detection of STIs. While most of the previous literature has been majorly defined in high-risk population, our study assessed females presenting for routine health care, thus providing a panoramic view of the STIs in BV intermediate.

Taking this into consideration, future research should focus on whether intervention should be recommended for females who present with BV intermediate stage. The metagenomics and transcriptomics of vaginal flora in healthy and BV females can facilitate our understanding of the interaction of microbial communities. The knowledge of these communities will assist in the development of interventions that can drive the vaginal microbiota toward a healthier state.

Ethical approval

The study was approved by the Institutional Ethics Committees, Postgraduate Institute of Medical Education and Research, Chandigarh (Ethics approval no. INT/IEC/2019/002222)

Declaration of patient consent

Institutional Review Board (IRB) permission was obtained for the study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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