

ANALYSIS OF NUGENT AND AMSEL CRITERIA FOR THE DETECTION OF FEMALE GENITAL TRACT INFECTIONS IN A TERTIARY CARE HOSPITAL, COASTAL KARNATAKA

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ABSTRACT

BACKGROUND

Female genital tract infections are polymicrobial in nature. Among the genital infections, bacterial vaginosis is the most common infection caused by anaerobic and microaerophilic organisms such as *Bacteroides fragilis* group, *Mobiluncus* species, *Porphyromonas* species, *Prevotella* group and *Gardnerella vaginalis*.

Objective- Comparative evaluation of Nugent and Amsel criteria in diagnosis of various types of female genital tract infections.

MATERIALS AND METHODS

The present descriptive study was conducted in a tertiary care hospital in coastal Karnataka, South India during the period from July 2013 to June 2016. Of the 1217 women under study, 1054 cases were taken with complaints of vaginal discharge and 163 were included as control, which belongs to normal vaginal flora, confirmed by microscopy in the Dept. of Microbiology, Father Muller Medical College, Mangalore. High vaginal swabs from women of reproductive age group (15 - 45 years) and 163 age matched control group were examined by preliminary examination.

RESULTS

By employing Amsel and Nugent criteria, out of 1054 vaginal samples 415 (39.4%) were Normal vaginal flora, Vaginal Lactobacillosis 117 (9.3%), Bacterial vaginosis 315 (29.9%), Intermediate Bacterial vaginosis 11 (1.0%), Vulvovaginitis 6 (0.6%), Cervicitis 4 (0.4%), HIV 2 (0.2%), Candidiasis 106 (10.1%), Group B *Streptococcus* with Antenatal cases 73 (6.9%) and Trichomoniasis 5 (0.5%). No vaginal infections were detected from the control group.

CONCLUSION

BV, Candidiasis, Trichomoniasis and Vulvovaginitis are the most common conditions present among female genital tract infections. Nugent and Amsel criteria are the most cost effective and less time-consuming tests for the diagnosis of vaginal infections.

KEYWORDS

Nugent's Criteria, Amsel's Criteria, Bacterial Vaginosis, Vaginal Discharge Candidiasis, Trichomoniasis.

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BACKGROUND

The normal vaginal flora of women includes abundant prevalence of Lactobacilli, which maintain vaginal pH and acidic environment of vagina.¹ Lactobacilli are considered to play a pivotal role in preventing the overgrowth of other bacteria by production of lactic acid. The Lactobacilli maintain their dominance through combination of acidity, hydrogen peroxide, lactocins and other bacteriocin which inhibit the growth of other bacteria.² Female genital tract infections characterised by the reduction in the prevalence number of hydrogen peroxide producing Lactobacilli and overgrowth of vaginal microbiota include viruses, bacteria, parasites and fungi.³

Female genital tract infection is polymicrobial in nature and microorganisms are classified as in three forms such as-

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1. Sexually transmitted disease (STD) organisms which include *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, Herpes simplex and *Trichomonas vaginalis*,
2. Mixed aerobes and anaerobes,
3. *Mycoplasma hominis* and *Ureaplasma urealyticum*.⁴

In genital infection, mixed aerobes and anaerobes cause bacterial vaginosis (BV), vulvovaginitis, tubo-ovarian abscess, cervicitis, postsurgical and post-partum infections. Of these infections BV is the most common infection found in vaginal tract which causes vaginal discharge and thus leading to vaginal disorder in women of reproductive age.^{5,6}

The present study was intended to perform the comparative evaluation of Nugent and Amsel criteria in diagnosis of various types of female genital tract infections in a tertiary care hospital, Karnataka.

MATERIALS AND METHODS

The present descriptive study was conducted in a tertiary care hospital in Coastal Karnataka, South India, during the period from July 2013 to June 2016. Ethical clearance was obtained for the study (Ref. No. FMCC/ FMIEC/ 1298/ 2013) and written informed consent was duly collected from the patients or attendants.

Source and Collection of Data

High vaginal swabs from 1054 women of the reproductive age group in the (15 - 45) were suspected to have BV and other female genital tract infections such as itching, irritation or burning, discomfort urination, douching, using contraceptive device, infertility cases, pre-term birth and pelvic inflammatory disease constitute the test. Control group number 163 included from healthy women in the reproductive age group without any white discharge, attending for family planning consultation were collected from the Department of Gynaecology and Obstetrics. Non-random, convenient sampling technique was used.

A detailed clinical history for each woman was noted which included age, sex, severity of problem and other illnesses etc. were taken from medical records. Women with menstrual period and patients who were on medication and clinically diagnosed for any bacterial, fungal, parasitic or viral infections for upto one month prior to the specimen collection and women with diabetes mellitus were excluded from the study.

Three high vaginal swabs were collected per patient aseptically. Vaginal secretions or discharge were collected from the posterior and lateral fornices cervix area. Cervical or high vaginal swabs were collected with the help of Cusco's speculum and inoculated into sterile tube containing 0.5 mL normal saline and immediately transported to the Department of Microbiology for the further processing.

Diagnosis of Female Genital Tract Infections

Diagnosis was done based on Amsel and Nugent criteria.

Amsel's Criteria

The Vaginal Discharge was subjected to the following Tests-

- Appearance:** Appearance of the discharge was clear to white and homogeneous.
- pH Test:** pH of the discharge was tested by using pH paper, which showed a wide pH range of 1 - 14 (HiMedia Laboratories, Pvt. Ltd., Mumbai, India).
- Whiff Test:** Microorganisms produced amines, which is present in the vaginal discharge. The organisms which involved in BV volatilise in the presence of alkaline pH giving a fishy smell.
- Clue Cells:** Presence of clue cells were confirmed by screening the vaginal discharge by gram stain. Clue cells are the vaginal squamous epithelial cells studded with large number gram variable coccobacillary forms.
- According to Amsel's Criteria:** According to Amsel's Criteria: If three of the above four criteria were positive, the patient was considered to be suffering from female genital tract infections.⁷

Procedures:

pH Determination

A swab of vaginal discharge was put onto litmus paper to check its acidity. A colour reaction developed on the paper was compared to a colour comparison chart to determine the pH of the sample. pH > 4.5 indicated BV.

Amine Odour Test (Whiff Test)

Amines present in the vaginal discharge produced by the microorganisms were involved in BV. A drop of 10% potassium hydroxide (KOH) was added to some vaginal discharge put in a clean glass slide. A characteristic fishy odour was considered as positive amine odour test and was suggested of BV.

Wet Mount

A drop of vaginal discharge suspension was placed on a slide and covered with coverslip. Slide was examined microscopically using a 40x objective. Test was analysed for determination of epithelial clue cells and pus cells, *T. vaginalis* (TV) and *Candida specie*.

Nugent Score and Interpretation Criteria

Gram Stain

High vaginal discharge smeared on clean glass slides, air dried, heat fixed and stained by Gram's method using acetone alcohol (1: 1) mixture as decolouriser and dilute carbol fuchsin as the counter stain. The numbers of "clue" cells, various morphotypes of bacteria and normal epithelial cells were estimated.⁸

Nugent's Criteria

Nugent criteria is a gold standard method for detection of female genital tract infections, which is based on interpretation of Gram stain of vaginal discharge. Nugent criteria summed the weighted quantitation (0, 1 to 4+) of the following morphotypes to yield a score of 0 to 10 for each case, large gram-positive rods (Lactobacillus morphotypes weighted such that absence yielded the highest score), small gram-negative to gram-variable rods (*G. vaginalis* and *Bacteroides spp.* morphotypes) and curved gram-variable rods (*Mobiluncus spp.* morphotypes). The criterion for bacterial vaginosis was a score of 7 or higher, a score of 4 to 6 was considered intermediate and a score of 0 to 3 was considered normal.⁹ Table 1 and 2 shows the Nugent's criteria methods. In the present study, Nugent's criteria method was considered as gold standard method compared to Amsel's criteria.

Statistical Analysis

Statistical analysis was done using SPSS version 23. Fisher's exact test was applied for data analysis and Chi-square test used for comparative analysis between Amsel and Nugent criteria. The sensitivity, specificity, positive predictive value and negative predictive value were calculated by Chi-square test.

Nugent Score an Interpretation Criteria

Morphotype	Number of Organism per Oil Immersion Field (Average 10 Field)				
	None	< 1.0	1 - 4	5 - 30	>30
Lactobacillus species	4	3	2	1	0
<i>Gardnerella</i> and Anaerobic GNB	0	1	2	3	4
Curved GNB <i>Mobiluncus Species</i>	0	1	1	2	2

Table 1

Nugent's Score	Cells Present or Absent	Interpretation
0 – 3	No clue cells	Normal vaginal flora
4 – 6	No clue cells	Intermediate or not consistent with BV
4 – 6	Clue cell present	Indication of BV
≥ 7	Clue cell present or absent	Indication of BV

Table 2

RESULTS

Of the 1217 women under study, 1054 cases were taken with complaints of vaginal discharge and 163 were included as control which belongs from normal vaginal flora. High vaginal swabs were examined by Amsel and Nugent criteria.

Table 3 shows details of age-wise distribution of cases and controls. Table 4 indicates preliminary examination of 1054 vaginal samples such as *Trichomonas vaginalis* 5 (0.5%), *G. vaginalis* 223 (21.2%) and *Candida spp.* 106 (10.1%). Table 5 shows results of female genital tract infections prevalent in 1054 cases with 163 age matched control group.

Table 6 shows comparison results of Amsel and Nugent criteria in female genital tract infection cases. The sensitivity, specificity, positive predictive value and negative predictive value were calculated which indicates Nugent's criteria is the best method for diagnosis of female genital tract infections compared to Amsel's criteria.

Age Group in Year	Groups				Total	(%)
	Cases	%	Control	%		
16 to 20	56	5.3	6	3.7	62	5.1
21 - 30	413	39.2	72	44.7	485	39.9
31 - 40	283	26.9	47	28.8	330	27.1
Above 40	302	28.7	38	23.3	340	27.91
Total	1054	100.0	163	100.0	1217	100.0

X²= 3.265, p= .353, not significant

Table 3. Details of Age-Wise distribution of Cases and Controls

Groups		Positive		Negative		Fisher Exact Test p value
		No.	%	No.	%	
Trichomonas Vaginalis (TV)	Cases	5	0.5	1049	99.5	.487
	Control	0	0.00	163	100.0	
G. vaginalis (GV)	Cases	223	21.2	831	78.8	.000
	Control	0	0.00	163	100.0	
Gonococci (G)	Cases	0	0.00	1054	100.0	-
	Control	0	0.00	163	100.0	
Candida spp. (C)	Cases	106	10.1	948	89.9	.000
	Control	0	0.00	163	100.0	

Table 4. Preliminary Examination (TV, GV, G, C) of Vaginal Swabs

Groups	Clinical Condition	Groups				Total
		Cases	%	Control	%	
A.	Normal vaginal flora	415	39.4	163	100.0	578 (47.5%)
B.	Vaginal Lactobacillosis	117	11.1	0	00.0	117 (9.6%)
★C.	Bacterial vaginosis	315	29.9	0	00.0	315 (25.9%)
★D.	Intermediate Bacterial vaginosis	11	1.0	0	00.0	11 (0.9%)
★E.	Vulvovaginitis	6	0.6	0	00.0	6 (0.5%)
★F.	Cervicitis	4	0.4	0	00.0	4 (0.3%)
★G.	HIV	2	0.2	0	00.0	2 (0.2%)
H.	Candidiasis	106	10.1	0	00.0	106 (8.7%)
I.	Antenatal cases with Group B Streptococcus	73	6.9	0	00.0	73 (6.0%)
J.	Trichomoniasis	5	0.5	0	00.0	5 (0.4%)
	Total	1054	00.0	163	00.0	1217 100.0%

Fisher's exact test p value= 000 < 0.001, highly significant

Table 5. Details of the Clinical Conditions of Cases and Controls

Methods	Sensitivity %	Specificity %	Positive Predictive Value %	Negative Predictive Value %
Amsel's criteria	57.72	98.79	78.61	96.81
Nugent's criteria	96.81	78.61	57.72	98.79

Table 6. Comparison of Amsel and Nugent Methods from Female Genital Tract Infections

DISCUSSION

Female genital tract infections include a spectrum of clinical conditions such as Bacterial vaginosis, Pelvic inflammatory disease, Trichomoniasis, Vulvovaginitis, Candidiasis etc. BV is found to be the most common vaginal disorder in women of reproductive age. BV can predispose to obstetrical infections, post aortal, pelvic inflammatory disease, post-partum endometritis after caesarean, prevalence of BV obtained in patients with infertility, preterm labour.¹⁰

Initial screening of these infections was done by direct microscopy. As implied in the literature, Amsel's criteria is less sensitive than gram stain interpretation in cases of BV.^{7,11} Amsel's criteria requires a minimum of 3 to 5 vaginal swabs from each patient.^{12,13} It has been observed that routinely only a single swab is sent to the laboratory to rule out BV in the hospitals. This might be the reason why Amsel's criterion is unpopular. Ever though culture is the gold standard, the organisms present in the specimen do not survive the delay in processing. Hence, gram stain interpretation is the most popular method for routine diagnosis of vaginal infections.

Previous studies reported that Nugent's criteria is the best method for diagnosis of BV.¹⁴⁻¹⁶ Based on direct smear examination, it can classify gram vaginal smears into normal vaginal flora, intermediate BV and BV.^{9,17} Few studies have tried to formulate that gram stain scoring system was better, but are not popular as Nugent's criteria for diagnosis of BV.^{18,19}

Kusters and their colleagues performed a comparative study by using a multiplex real-time PCR assay and Nugent's score diagnosis of BV.²⁰ They reported that multiplex qPCR was a better method for diagnosis of BV compared to the Nugent's score.

In the present study, Amsel criteria and Nugent criteria were compared for the diagnosis of female genital tract infections. In comparison with Amsel's method, Nugent's Criteria was found to be 96.81% sensitive, 78.61% specific with positive predictive value of 57.72 and negative predictive value of 98.79%. In the present study, Nugent's criteria were found to be gold standard method compared to Amsel's criteria p .000, highly sensitive.

In a study by Udayalakshmi J et al, Amsel's criteria, Spiegel's and Nugent's criteria are reported equally effective methods for the diagnosis of BV,²¹ whereas another study reported Amsel's criteria is less sensitive to Nugent's criteria.^{7,11}

Indian studies conducted on general population have shown the prevalence of BV as 19% by Nugent's scoring system.²¹⁻²³ In a previous Indian study the prevalence of BV was observed as the highest in Urban slum 38.6%, Rural 28.8% and Urban middle class community 25.4%.^{24,25} However, the present study conducted on the Rural and Urban middle class community has shown the prevalence of BV 29.9% and Intermediate BV 1% by Nugent's scoring system.

Vaginal Lactobacillosis is a condition mostly found in the reproductive age group of women with odourless, white vaginal discharge and vulvovaginal itching. In a study reported by Ventolini G 15% prevalence of Vaginal Lactobacillosis in women,¹ whereas the present study was observed a prevalence of 11.1% Vaginal Lactobacillosis.

A study reported by Doyle C and their colleagues, *Trichomonas vaginalis* was significantly associated from premenstrual syndrome with headache.²⁶ However, some other studies have reported 4%²¹ and 1.5%²⁷ Trichomoniasis from infectious women. In the present study, 5 (0.5%) Trichomoniasis was observed.

Dagli and Demir had reported *Candida* spp. (7.3%),²⁸ whereas in other studies Candidiasis was obtained in 20%²¹ and 16.5%²⁷ respectively. In present study, 10.1% *Candida* species was analysed from the genital tract infections. Powell and Nyirjesy reported some of the commonly encountered problems in women like Vulvovaginitis, Vulvovaginal Candidiasis, BV and Trichomoniasis.²⁹

The present study evaluates the value of direct and preliminary examinations of vaginal discharge from infectious women by using Nugent and Amsel criteria. The time required to diagnose female genital tract infections by culture and other sophisticated newer methods will require longer time. Therefore, our study employs cost effective and less time-consuming methods to analyse these infections by Gram stain, pH test, whiff test and wet mount.

CONCLUSION

Female genital tract infections namely BV, Candidiasis, Trichomoniasis and Vulvovaginitis affect the socio-economic conditions of the women in India. Nugent and Amsel criteria are the most cost effective and less time-consuming methods for the diagnosis of such vaginal infections as compared to the traditional culture technique.

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