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Original Article

Abnormal Vaginal Discharge: Comparison of Clinical and Microbiological Criteria for the Diagnosis of Bacterial Vaginosis in Western Rajasthan, India

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ABSTRACT

Bacterial vaginosis (BV) is the commonest cause of abnormal vaginal discharge in women of child-bearing age with prevalence of 20-30%. BV is associated with increased susceptibility to HIV- AIDS, other sexually transmitted diseases and various obstetrics complications like preterm labour and miscarriage etc. BV is diagnosed by using Amsel's, Hay/Ison and Nugent Criteria in which Nugent's criterion is considered as the gold standard.Aim-Comparison of Amsel's, Hay/Ison& Nugent criteria for diagnosis of bacterial vaginosis and to correlate with anaemia as an associated risk factor.Method- This prospective cross-sectional study involved 150 females of reproductive age group complaining of excessive vaginal discharge attending tertiary health centre at Dr. S.N. Medical College, Jodhpur,Rajasthan , India.Vaginal swabs were collected and the diagnosis of BV was done by using Amsel's , Hay/Ison and Nugent Criteria on Gram stain.Results: Bacterial vaginosis was found in 30.66% women byAmsel's Criteria,28% by Hay/Ison Criteria and 26.66% by Nugent's scoring .According to Hay's Criteria:50% women having grade (1); 22% women lies in grade (2) & 28% in grade (3).Nugent Criteria: Show score >7 in 26.67% women, score 4-7 in 25.33%; score 0-3 in 48% women.It was also observed that bacterial vaginosis diagnosed by Nugent's score showed 50% women with Hb 5-8 gm/dl; 30% with Hb 9-12 gm/dl and 20% women with Hb>12gm/dl. Conclusion Although Nugent's criterion is considered as the gold standard for the diagnosis of bacterial vaginosis,Amsel's and Hay/Ison method showed good agreement with the gold standard method of Nugent et al. and can be used as an alternative to Nugent's criteria in busy tertiary care centre. This study shows almost similar result for all three criteria used for diagnosis of Bacterial Vaginosis. This study also proves anemia as a risk factor.

Introduction

Vaginal discharge is a common and distressful condition for a woman, which can result from a variety of physiological as well as pathological states [1]. The world over, bacterial vaginosis (BV) has been reported to be the most common cause of abnormal vaginal discharge among women in the reproductive age group [2–4]. BV is characterized by a change in the complex vaginal ecology, wherein the Lactobacillus,dominant normal flora of the vagina is replaced by a mixed microbial flora consisting of anaerobes and Gardnerellavaginalis[5–7]. It is imperative to diagnose BV especially in pregnant females and institute treatment as early as possible to prevent complications such as low birth weight infants, preterm births [8], pelvic inflammatory disease [9], postpartum endometritis [10], and infertility [11]. Several epidemiologic studies indicate that BV is associated with increased susceptibility to HIV infection [12] and also several other sexually transmitted infections (STIs), including herpes simplex virus, gonorrhoea, trichomoniasis , and chlamydia trachomatis infection [13–15].

A variety of methods were used for diagnosis until consensus was reached to define the diagnosis of BV using the composite criteria described by Amsel's et al. [16]. An alternative method of diagnosis that has been used extensively is the grading or scoring of the microbial flora in the Gram stained smears of vaginal fluid first described by Spiegel et al. in 1983 and later modified by Nugent et al. [17]. Nugent's score method smear examined by quantification of the different vaginal morphotypes, making the evaluation of smears very subjective that requires an experienced slide reader and also considerable time and skill. Therefore, a simpler version was described by Hay/Ison in 2002 [18], in which vaginal flora is divided into the following three different categories: normal, intermediate, and BV depending on the relative amount of Lactobacillus morphotypes as compared to the Gardnerella morphotypes.

We can use the method of Nugent et al. for diagnosis of Bacterial vaginosis but have had to spend considerable time in training laboratory personnel before they could independently

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and accurately read the smears. The aim of the study was to evaluate the simple reading scheme of Amsel's, Hay/Ison against the scoring method of Nugent and to assess the utility of the former in terms of ease of training and subsequently ability in arriving at a correct diagnosis.

2. MATERIAL AND METHODS:

Study design: We performed a prospective study from April 2014 to August 2014 (5 months) including women attending STD clinic at Umaid hospital, Dr. S.N. Medical College, Jodhpur,(western Rajasthan), India.

Inclusion criteria:150 non pregnant women aged between 15 and 49 years, presenting with abnormal excessive vaginal discharge were included in the study. Informed consent was taken from all women.

Exclusion criteria: Women who were menstruating, pregnant, or had received antibiotics in the past four weeks were excluded from the study.

Methodology

Sample Collection: Routine pelvic examination was done with a clean, unlubricated (or using non bacteriostatic lubricant like normal saline) speculum to inspect vaginal tissues and the presence or absence of vaginal discharge was noted. Women underwent genital examination during which 3 vaginal swabs were collected by using sterile cotton swabs from posterior vaginal fornix. Smears were prepared immediately on clean glass slides by "Roll the swab" technique (19-21) air-dried, heat fixed and labelled with patients name, date of collection and the patient's study number. Gram staining was done and examined under oil immersion (1000X magnification) for the diagnosis of bacterial vaginosis on the basis of Nugent's score and for Hay/Ison criteria.

A second vaginal sample was taken to measure pH by using pH paper graded from 3-7 and then placed on a slide for the addition of 10% potassium hydroxide (KOH) to detect an amine odour (whiff test).That slide was then evaluated for budding yeast, hyphae, and pseudo hyphae under bright-field microscopy at x400 magnification. A third vaginal discharge sample was mixed with a drop of normal saline and examined immediately under bright-field microscopy for the presence of clue cell and motile trichomonas. Those sample showing budding yeast and motile trichomonas were excluded from my study. Examination under high-powered microscopy identified the percentage of clue cells. In case of delay of more than two hours, the specimen was transported from O.P.D to Microbiology laboratory in Stuart's transport Media.

Criteria for diagnosis:

Each patient was subjected to evaluation by using clinical and microbiological parameters. The following diagnostic criteria were used in the study:

(A) Diagnosis of bacterial vaginosis based on clinical signs - Amsel's criterion (16)

Clinical diagnosis of bacterial vaginosis was considered positive on the basis of Amsel's criteria if three of the following four criteria were met (1) Discharge with fishy Odour (2) vaginal pH exceeded 4.5 (3)whiff test was positive (4) more than 20% clue cells were present on wet smear preparation. The examination was considered normal when three of these criteria were not met and neither fungi nor trichomonas were detected microscopically.

B) Gram Stain-Hay/Ison(17) & Nugent's criteria(18)-Hay/Ison Criteria: In Grade 1 Lactobacillus morphotype Predominant; Grade 2Mixed Flora Lactobacillus + G.V or Mobiluncus, Grade 3Predominantly G.V or Mobiluncus, few or absent Lactobacilli

Nugent's criteria: the amount of three morphotypes-Lactobacillus, Mobiluncus, and Gardenella were noted, quantified and scored according to table 1 of Nugent's criteria.

Score 0-3 is normal, 4-7 is intermediate and >7 is bacterial vaginosis.

After all the Gram stained smears had been evaluated and the Gram stain diagnoses were made, the results were compared with those of the clinical examination.

Results

The results were analysed by comparing all the three methods. According toAmsel's criteria,thin homogeneous vaginal discharge was present in all 150 women (100%), vaginal pH >4.5 in 36(24%), positive whiff (KOH) test in 30(20%), clue cell on wet mount 34(22.66%) and three criteria out of above four were seen in 46(30.66%) women positive for Bacterial vaginosis as shown in Table2.

On the basis of Hay/Ison criteria women with Grade 1: Lactobacillus Morphotype Predominant in 75 women (50%), Grade 2 Mixed Flora Lactobacillus + Gardenellavaginalis or Mobiluncus in 33 (22%) and Grade 3 Predominantly gardenellavaginalis or Mobiluncus, few or absent Lactobacilli (bacterial vagonosis) in 42(28%) women as shown in Table 2 / Pie Chart-2.

Nugent's scoring, BV was diagnosed in 40 (26.67%) women while 72 (48%) women had normal vaginal flora and 38 (25.33) had an intermediate (mixed) flora as shown in Table 2 / Pie Chart-1

Anaemia proved as a risk factor for bacterial vaginosis as 80% cases diagnosed with BV (by Nugent's method) were anaemic (Hb<12).Among 80% women with BV, 50% of the women had Hb 5-8 gm/dl, 30% had Hb 9-12% while among non-anaemic women (Hb>12gm/dl) BV diagnosed in (20%) an shown in Table 3.

Table 1: Nugent critaria for diagnosis of BV

Lactobacilli morphotypes average no. per HPF(1000*Ols)		Gardenella Bacteroids morphotpes average no.per HPF(1000*ol)		Curved Gram Negative/Variable rods average no. per HPF(1000*ol)	
>30	0	0	0	0	0
15-30	1	<1	1	<5	1
1-14	2	1-4	2	5+	2
<1	3	5-30	3		
0	4	>30	4		

Table 2. ComparisionAmsel'scritaria, Hays/Ison Criteria and Nugent scoring

Amsel's Criteria		**Hay/Ison Criteria**		**Nugent's Scoring**	
Criteria	No. of women	Criteria- Grade	No. of women	Score	No. of women
1. Presence of thin vaginal discharge	150 (100%)	Grade1.Lactobacillus Morphotype Predominant	75 (50%)	Score 0-3 Normal	72 (48%)
2. Vaginal PH>4.5	36 (24%)	Grade2.Mixed Flora Lacto + G.V or Mobiluncus	33 (22%)	Score 4-7 Intermediate	38(25.3%)
3. Positive whiff (KOH) Test	30 (20%)	Grade3.Predominantly G.V or Mobiluncus	42 (28%)	Score >7 Bacterial Vaginosis	40 (26.7)
4. Presence of Clue cell on Wet Mount	34 (22.7%)	Few or absent Lactobacilli			

Table 3.Distribution of cases according of Hemoglobin status

No. of Women in study	Hb level (gm/dl)	Women with BV (By Nugent criteria)
38	5-8	20 (50%)
70	9-12	12 (30%)
42	>12	8 (20%)
Total: 150		Total: 40

Figure 1 : Gram stain of vaginal smear showing clue cell (vaginal epithelial cell studded with gram negative coccobacilli)

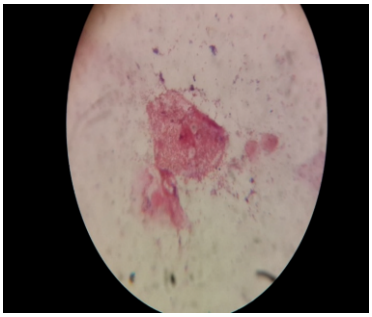
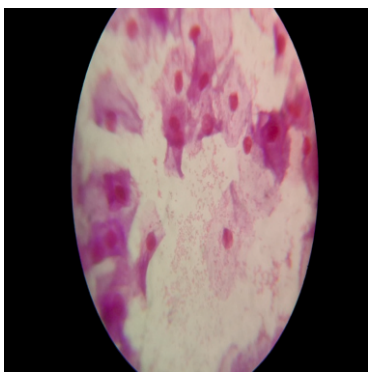
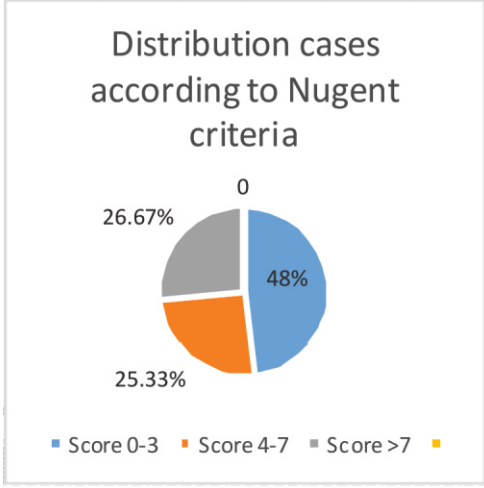


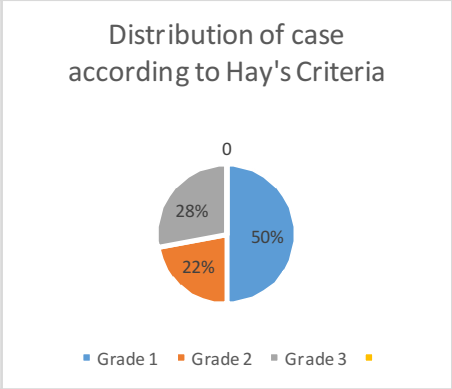
Figure 2 : Gram stain of vaginal smear showing gram negative curved rods



Pie Chart-1



Pie Chart-2



4. Discussion

Nugent scoring as the gold standard test for diagnosis of BV because the Nugent scoring system is an excellent, method for laboratory evaluation of cases of bacterial vaginosis and it is more reliable than Amsel criteria and Hay/Isoncriteia.The Nugent scoring test requires health care experts, laboratory support, and access to high-power microscopy to obtain timely results for the diagnosis of BV. Since these necessities are not always available in developing countries, it is important to have simple and reliable clinical criteria that clinicians can use in practice.

In our study, among the individual Amsel's criteria, the presence of clue cell was found to be the most reliable predicator of BV, but this test requires technically experienced hands and laboratory support. A second criterion is vaginal discharge, which is nonspecific and non-sensitive as thin homogenous vaginal discharge is not accurately interpreted. The third criteria is high pH > 4.5 have higher sensitivity but less specific. A fourth criterion is the Amine odor test (KOH test). Gutmanet al. suggested in their study that the KOH test was a highly sensitive and specific method [19].

In our study, there was a strong association of a normal Nugent's score with grade I flora and a Nugent's score of ≥7 with grade III Hay's classification, with only 2 % of women with a Nugent's score of ≤3 falling in grade II by Hay's method.

Controversy occurred in women with intermediate scores in Nugent's method (more % being placed in grade III of Hay's method). Therefore, more women were diagnosed with BV by the Hay's method. Our study indicatingthat the two methods are very alike. These results indicate that when there is a lack of time or expertise, this method of assessment of microbial flora can be used as an alternative method of diagnosis. Similar results have been reported by Larsson et al. from Sweden [22].

However, among the 40 women diagnosed BV by Nugent's method, 34 were clue cell positive (85%) and among 42 women with grade III by Hay's method, 34 women (80%) were clue cell positive signifying the importance of clue cells. Another investigator from Kolkata, India, has reported that the presence of clue cells correlates best with a positive diagnosis by Nugent's score [1]. This result indicates that by adding a score for the presence/absence of clue cells to each of these 2 methods, one can enhance the diagnosis of BV, which can be superior to the methods of Nugent et al. and Hay et al.

In our study,although the maximum no. of women (70) were mild anaemic(Hb9-12) but BV present in 12 (30%) out of 40 women while in severe anaemic women (Hb 5-8) BV present in 20(50%) women. In non-anaemic women (Hb>12) BV present in 8 (20%) women. This proves that anaemia is associated with increased risk for bacterial vaginosis.

Conclusion

Although Nugent's criterion is considered as the gold standard for the diagnosis of bacterial vaginosis (BV), the method requires an experienced slide reader and considerable time and skill. Using Nugent score for the intermediate group is most difficult. Amsel's and Hay/Ison method shows good agreement with the gold standard method of Nugent et al. and can be used as an alternative to Nugent's criteria in busy tertiary care This study shows almost similar results for all the three criteria used for diagnosis of Bacterial Vaginosis with anemia as a risk factor for it .Early diagnosis of BV can help prevent further complications, especially in pregnant women, by commencing appropriate treatment.

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