

Wet Mount Microscopy A Better Diagnostic Tool for Bacterial Vaginosis Screening than Gram Staining

1. Samina Rizvi 2. Farhana Sami 3. Yasmin Hashim 4. M. Naveed uz Zafar 5. Tazeen Shah 6. Dur Muhammad

1. Assoc. Prof. of Pathology 2,3,4. Asstt. Profs. of Pathology 5. Sr. Lecturer of Pathology 6. Assoc. Prof. of Pathology Jinnah Sindh Medical University, Karachi

ABSTRACT

Objective: To compare Wet smear and Gram staining for Clue cells in screening Bacterial Vaginosis (BV) in a clinical setting where laboratory facilities are not readily available.

Study Design: Cross-Sectional

Place and Duration of Study: This study was conducted in the Department of Microbiology, Basic Medical Sciences Institute Jinnah Postgraduate Medical Center Karachi. High vaginal swabs (HVS) were collected from Gyne & Obstetric Department of Jinnah Postgraduate Medical Centre, Karachi, from Jan 2005 to Feb 2007.

Materials and Methods: One hundred and fifty high vaginal swabs (HVS) were collected from married females with or without complain of vaginal discharge. BV was diagnosed on the basis of clinical Amsel's criteria BV was diagnosed. Subsequently, clinical diagnosis was compared with laboratory based diagnosis for each woman. Two methods used were Wet Mount and Gram Staining.

Results: Out of total 150 cases, 100 females were complaining of vaginal discharge and after clinical assessment by Amsel's criteria 34 (34%) were diagnosed as cases of BV. Fifty females as (as controls) were those who were not complaining of any discharge. But among this group 8 (16%) were discovered to be suffering from BV. With the help of wet mount, the clue cells were visualized in 61 (40.7%) cases, while by Gram staining 54 cases (36.0%) were seen.

Conclusion: BV screening is important as it affects both groups of females with or without complains of discharge. For easier recognition of clue cells, which are an excellent predictor of BV, wet mount is better as compared to Grams staining method.

Key Words: Gram stain, wet mount, Clue cells, vaginal smear, bacterial vaginosis

INTRODUCTION

Bacterial vaginosis (BV) also termed as non specific vaginitis is the most common of the vaginal infections, and main cause of abnormal vaginal discharge in women of childbearing age.^{1,2,3,4} This condition predisposes women to increased susceptibility to sexually transmitted diseases^{5,4} including human immunodeficiency virus (HIV) infections and adverse pregnancy outcomes⁶. BV involves an imbalance in the vaginal bacterial ecosystem, with a decrease in hydrogen peroxide-producing Lactobacilli and an increase in *Gardnerella vaginalis*, anaerobes, and mycoplasmas.^{7,8,9} Normally, lactobacilli constitute 95% of the bacteria in the vagina. In women with BV, lactobacilli are absent or severely reduced and the concentration of other bacteria increase by $10^2 - 10^4$.¹ Studies have documented an association between bacterial vaginosis and the adverse pregnancy outcome of preterm delivery. This epidemiologic evidence has been used as a rationale for screening asymptomatic pregnant women for bacterial vaginosis.^{10,11,12} Most of the women are asymptomatic, but some women with bacterial vaginosis have a foul smelling, thin, homogeneous, frothy, vaginal discharge^{13,14}. In

addition to a nuisance infection, bacterial vaginosis can lead to a variety of obstetric and gynecological complications such as preterm birth, premature rupture of membranes, chorio amnionitis, and infection of amniotic fluid and pelvic inflammatory disease (PID)^{10,12,13,14}. As it is just an overgrowth of the normal flora of the vagina without inflammation, there is no single best method for the diagnosis of bacterial vaginosis^{13,14}. Most often, multiple criteria are used for the diagnosis of bacterial vaginosis. One of the methods of diagnosis is the Amsel's composite criteria which includes clinical diagnosis and a few simple laboratory tests^{15, 16, 17, 18}

Although Culture is the gold standard method for diagnosis of most of the bacterial diseases; it cannot become the gold standard for diagnosis of bacterial vaginosis as the organisms which are involved in BV cannot be isolated in the laboratory easily and as normal women also have this flora in their vagina in small number.^{19,20} BV represents complex changes in the vaginal flora, thus vaginal culture has no role in diagnosis. Although cultures for *G. Vaginalis* are positive in almost all women with symptomatic infection, the organism is detected in up to 50 to 60 percent of healthy asymptomatic women; thus, its

presence alone is not diagnostic of BV²⁰. Amsel's method is very popular as a means of diagnosis of bacterial vaginosis^{21, 22, 23}.

Utilization of clinical criteria which was initially described by Gardner and Dukes (1955)²⁴ and later formulated into a diagnostic tool by Amsel et al (1983)²⁵ and now accepted as "Gold Standard" for diagnosis^{26,27,28}.

When microscopy is available, the diagnosis of BV is based on the presence of at least three of the following four Amsel criteria.

- 1) Homogeneous, thin, grayish-white discharge that smoothly coats the vaginal walls
- 2) Vaginal pH greater than 4.5
- 3) Positive whiff-amine test, defined as the presence of a fishy odor when 10 percent potassium hydroxide (KOH) is added to a sample of vaginal discharge
- 4) Clue cells on saline wet mount, comprising at least 20 percent of epithelial cells

In the smear of typical homogenous discharge of bacterial vaginosis a clue cell is a specific diagnostic indicator and a BV patient typically shows "clue cells" with mixed flora consisting of large numbers of small gram-negative rods and gram-variable rods and coccobacilli^{29,30}

Clue Cells: A smear of vaginal discharge from a BV patient typically shows "clue cells" with mixed flora consisting of large numbers of small gram-negative rods and gram-variable rods and coccobacilli. Detection of the clue cell requires attention to certain characteristic features of the cell whose border appears under light microscope to be heavily stippled and granular in appearance with obscured borders resulting from adherence of gram negative to gram variable rods or coccobacilli. Lactobacilli morph types are absent or greatly reduced. Clue cell tend to occur in clumps, with the bacteria located between the squamous epithelial cells, whose cytoplasm is covered by bacteria. Secondly, it is important to note that there are very few, if any, white cells, which is in contradiction to Trichomonas infection, in which white cells are commonly present. Finally, it is important, in detecting the clue cell, to note that a normal squamous epithelial cell has a very sharp border, where as clue cell loses the sharp border because the organism adheres to the epithelial cells. The typical clue cell has the Gardnerella organism altering the border, so the sharp, distinct border is gone³¹

For significant bacterial vaginosis, more than 20% of the epithelial cells on the wet mount should be clue cells.³¹

MATERIALS AND METHODS

150 married women between the ages of 19 – 36 years, with or without complaints of vaginal discharge were

included. Women who were menstruating at the time of the specimen collection and women, who were on medication for any bacterial, fungal, parasitic or viral infections for up to one month prior to the specimen collection, were excluded. Also females who had used vaginal douches, tablets within the preceding week, and who had sexual intercourse within 24 hours were not included.

A detailed clinical history of each woman was taken and their routine pelvic examination was done with a clear, unlubricated speculum, vaginal swabs were collected. These vaginal swabs were used for gram staining, for the determination of the pH of the vagina, Wet mount and for the whiff test. Diagnosis of bacterial vaginosis was done by Amsel's criteria²⁵

Diagnosis by Amsel's criteria

Amsel's composite criteria includes the presence of a homogeneous vaginal discharge, pH of the vagina being > 4.5, the presence of clue cells in wet mount of vaginal discharge smears and a positive whiff test. Whiff test: A drop of vaginal discharge was mixed with a drop of 10% potassium hydroxide which was taken on a slide. A fishy smell indicated a positive test. According to Amsel, if 3 of the 4 criteria are positive, the patient has bacterial vaginosis.

Clue cells by Gram Staining

The vaginal discharge was smeared on clean glass slides, air dried, heat fixed and stained by Gram's method by using an acetone alcohol (1:1) mixture as a decolouriser and dilute carbol fuchsin as the counter stain. The vaginal epithelial cells which were completely covered by the gram variable coccobacilli so that their edges which normally have a sharply defined cell border became indistinct or stippled, were considered as the clue cells (Fig 1)

Clue cells by Wet Mount:

The epithelial cells were observed under microscope at 40X to determine clue cells

The following tips were utilized to determine if an epithelial cell is a clue cell or not

- 1 The number of distinguishable epithelial cells in the field of view were noted. Distinguishable epithelial cells are whole cells that are visible with a nucleus present.
- 2 To determine if any of the epithelial cells are clue cells, it is important to study only the borders of the cell. A cell is a clue cell if the borders of the cell are completely obscured with bacteria and have edges that look "grainy" or "fuzzy". If any of the border is clear, it is not a clue cell.
- 3 To determine the percentage of clue cells in the field:
 - a. Count the number of clue cells and divide that number by the total number of distinguishable epithelial cells.

For example: A field has 6 epithelial cells and 2 are clue cells. $2/6 = 33\%$. So this field is considered positive for clue cells.(Fig 2)

Subsequently, clinical diagnosis was compared with laboratory based diagnosis for each women

RESULTS

Table 1 shows the frequency of BV according to Amsel's criteria. It was observed that by use of Amsel's criteria 34% of females who were complaining of vaginal discharge had BV. While with use of this criteria BV was found only in 16% of asymptomatic females. Thus it was observed that BV is present even in females not complaining of discharge

Table No.1: Frequency of bacterial vaginosis (BV) according to amsel's criteria

Patient	No. of cases	Bacterial vaginosis (BV)
Complaining vaginal discharge	100	34(34%)
Not complaining vaginal discharge	50	08(16%)

*P <0.05 statistically significant as compared to controls (not complains of discharge)

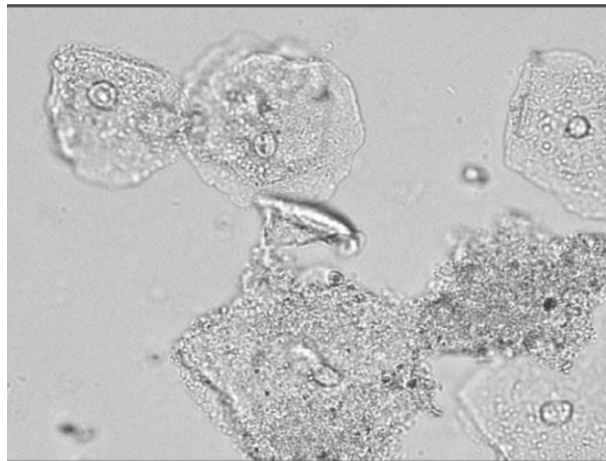


Figure No.1: Clue Cells in Wet Mount

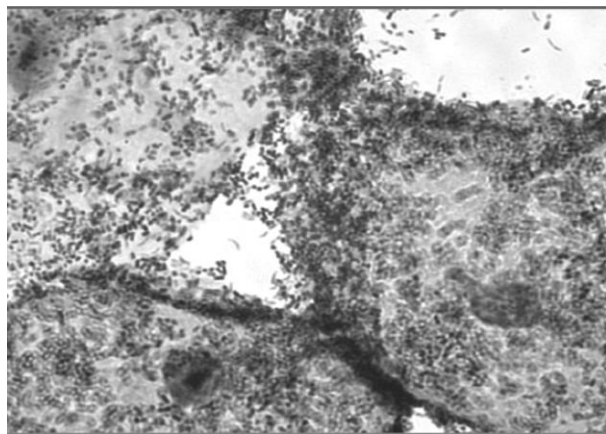


Figure No.2: Clue Cells in Gram Staining

Table No.2: Comparison of gram's staning and wet mont to detect clue cells (n= 150)

Clue Cells	Gram Method		Wet Mount	
	NO	%	NO	%
	54	36.0	61	40.7

Table 2 shows comparison between Grams staining and wet mount for detection of Clue cells .According to this wet mount is giving better results . It is present in 61 (40.7%) of females detected by wet mount while with Gram staining only 54 cases(36%) were detected.

DISCUSSION

Despite the health risks associated with Bacterial vaginosis, its high prevalence continues to be largely ignored by clinicians particularly in asymptomatic women³². Symptoms alone are poor criteria to define any type of vaginitis. Many of the patients who complain of symptoms do not have vaginitis and many patients of vaginitis have no complaints.³³ Many authorities advocate routine testing and screening in conjunction with the pap smear for an early diagnosis and better treatment of BV.^{25, 33, 34, 35}

The goal of this study was to find the frequency of BV in married females and to correlate several clinical and microbiological criteria that have been used for the screening and diagnosis of BV. We were particularly interested in the diagnostic values of simple observations and procedures that could be carried out in the physician's office, and in the correlations between each office procedures and less costly and more readily available microbiologic test for BV.

In our study we used two well described diagnostic methods for screening of BV that is by using Amsel's criteria and finding clue cells on wet mount and Gram staining.

The results of this study agree with Amsel et al^{25,36,37} that majority of women in the study remained free of any definite symptoms. In our study 100 females were complaining of discharge and only 34(34%) were diagnosed as cases of BV, while out of 50 only16 female (%) had BV in spite of the fact that they were not complaining of any discharge.

We agree with Soper³⁸ that although signs and symptoms can assist in the diagnosis, the wet mount with microscopy is the best way to confirm the diagnosis.³⁸ Studies have shown that a diagnosis of BV based on symptoms alone is often inaccurate compared to diagnosis based on laboratory criteria.^{39,40} In addition, diagnosis based solely on symptoms will miss those women who would have laboratory-confirmed diagnosis of the infection but are asymptomatic.^{40,41}

From a practical point of screening the Amsel's clinical criteria appears to satisfy the requirements. It is accurate, sensitive, specific, simple and quick to use, and relatively inexpensive technology.⁴²

Clue cells have been associated with BV by investigators.^{17,24,31} In this study also clue cells were highly associated with the presence of BV on wet mount and Gram stained specimens. We agree with^{17,21} that Wet mount as a screening test has the advantage that it can be performed immediately and therefore allows early screening, preventive diagnostic or therapeutic measures.

CONCLUSION

BV screening is important as it affects both groups of females with or without complains of discharge. For easier recognition of clue cells, which are an excellent predictor of BV, wet mount is better as compared to Grams staining method.

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Address for Corresponding Author:**Dr. Samina Rizvi**

Associate Professor of Pathology
Jinnah Sindh Medical University
Karachi

Email: saminarizvi@hotmail.com

Contact # 0300-2216831