

## Bacterial Vaginosis

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### Intruduction

Among the causes of vaginal discharge, bacterial vaginosis (BV) is the commonest in most communities, with variation in the prevalence from one place to another and according to the method used and the group of patients studied. Evidences are available that, the disease is associated with preterm labour, pre mature rupture of the membranes, post-induced abortion pelvic inflammatory disease, post-hysterectomy vaginal cuff cellulitis and plasma cell endometritis<sup>1-4</sup>. Moreover in pregnant women bacterial vaginosis may be associated with amniotic fluid infection and post-partum endometritis<sup>4</sup>. In Sudan, the disease was first reported in 2000 by Kafi and his co-workers who found bacterial vaginosis to be the commonest cause of vaginal discharge (17.2%) in a suburban Sudanese community. It's occurrence rate was almost equal to that of trichomonas and gonorrhea (collectively)<sup>5</sup>.

Despite these facts to date, bacterial vaginosis is almost neglected as a cause of vaginal discharge, evidenced by lack of practical advices to the laboratory personnel on how to diagnose it. Moreover, no clinical attention is paid to the disease and its possible clinical outcome.

The aim of this article is to throw light on this important subject particularly history, epidemiology, clinical features, diagnosis and management. It is hoped that, this will draw the attention of the gynecologists and laboratory personnel to this subject.

**Keywords:** trichomonas, endometritis, post-hysterectomy.

### Epidemiology:

Whether bacterial vaginosis is sexually transmitted or not is controversial. In support of sexual transmission is its association with sexual activity, and recovery of *Gardnerella vaginalis* from male sexual partners<sup>6</sup>. In addition Gardner and dukes supported the possibility of sexual transmission by inducing symptomatic bacterial vaginosis in eleven out of fifteen women inoculated with vaginal secretions from other infected women<sup>7,8</sup>.

Against sexual transmission is the demonstration of bacterial vaginosis in virgins indicating that it is not exclusively sexually transmitted<sup>9</sup>.

The prevalence of the disease varies according to the population studied and the method used for the diagnosis. Reported studies from USA showed prevalences of 10-32% in obstetrical population<sup>2,7,10</sup>.

In Britain figures ranging between 11% and 28% were reported<sup>11</sup>. Previous records from Sudan revealed prevalence of (17.2%)<sup>(5)</sup>. In Pakistan Shazia and his coworkers reported infection rate of ( 60% )<sup>12</sup>.

### History:

Vaginitis caused by *Candida albicans*, *T.vaginalis* , *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, is the commonest cause of vaginal discharge. Vaginitis wherein none of these is detected used to be called non-specific vaginitis (NSV) and it was the commonest single cause of vaginal discharge. In 1914 Curtis noticed an association between abnormal vaginal flora and non-specific vaginitis. In 1955 Gardner and Dukes described a new organism (*Corynebacterium vaginalis*, *Haemophilus vaginalis* and finally *Gardenerella vaginalis*), thought to be the sole cause of non specific vaginitis, but it was gradually become evident that the disease is absent in a substantial proportion of women with *G.vaginalis*<sup>7</sup>.

With advances in micro-biological techniques, it was possible to demaonstrate in

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non-specific vaginitis, a change of vaginal micro-flora of Lactobacilli to a mixed flora consisting of Mobiluncus species, Bacteroides species, Peptostreptococci and Mycoplasma. Non-specific vaginitis was therefore preferably named bacterial vaginosis.

#### Clinical features:

Remarkable numbers of cases are asymptomatic. The main symptoms are vaginal discharge and vaginal malodor. The discharge is grey, thick, homogeneous, frothy and is often adherent to the vaginal wall which is otherwise normal. The odor is similar to that of a rotten fish. It is caused by release of volatile amines by the anaerobic bacteria. The odor is intensified by alkalization of the discharge by 5-10% potassium hydroxide and also after sexual intercourse. The frothy character of the discharge which used to be considered pathognomonic of *Trichomonas vaginalis* infection is no longer so, as about 10-15% of the cases of bacterial vaginosis give frothy discharge. Pruritis vulva is a presenting symptom in 12% of cases.

#### Diagnosis:

Several methods are used for the diagnosis of bacterial vaginosis. These include:

##### A. The composite clinical criteria:

Although commonly used, it is complicated, time consuming and more over requires special setting. This method denotes that the clinical diagnosis of bacterial vaginosis is made if 3 out of the followings are present.

1. Homogeneous grey or white vaginal discharge.
2. Vaginal pH greater than 4.5.
3. Presence of clue cells.
4. Positive amine test<sup>13</sup>.

To be more precise, David and his co-worker increased the cut-off pH to 4.7 and stated that clue cells must form 20% of the epithelial cells to be considered as a positive criterion<sup>14</sup>. Other workers used 5 criteria instead of four. The fifth criterion being absence of lactobacilli or they are outnumbered by non-lactobacilli<sup>15,16</sup>.

Clue cells are vaginal epithelial cells covered with numerous adherent bacteria (fig.1). They

can be shown by examination of wet saline preparation of vaginal discharge where they show characteristic appearance. The cells are granular and their margins are entirely obscured by bacteria. Microscopic examination of a gram stained smear will show that these adherent bacteria are gram negative rods or gram variable coccobacilli (fig.1).



Fig.(1): Gram stained vaginal smear showing a Clue cell(center).

This is of great help in differentiating clue cells from false positive clue cells which are difficult to differentiate from each other on wet preparation. However, the margins of cells are free in false positive clue cells and the bacteria adhering to them are gram positive.

Microscopic examination may reveal a false negative result for clue cells. In this case the saline preparation of vaginal discharge shows no clue cells but gram stained smear shows masses of gram negative or variable coccobacilli not adherent to epithelial cells. This can be explained by the fact that some women produce IgA which prevent adherence of bacteria to the cell surface. On examination of vaginal smear it must be born in mind that pus cells and lactobacilli are rarely found in the discharge of patients with bacterial vaginosis unless there is associated concurrent infection. Absence of pus in the discharge is due to the fact that the anaerobic bacteria involved in pathogenesis of bacterial vaginosis produce amines which inhibit chemotaxis.

Amine test performed by adding 10% potassium hydroxide solution to smear of vaginal discharge on a glass slide. The

mixture is then immediately sniffed for presence of fishy odor indicating a positive test<sup>17</sup>.

The odor is very transient therefore mixing must be done directly under the nose. The test is not specific and can be falsely positive in patients with trichomoniasis and by the presence of sperms in vagina of healthy female.

Vaginal pH can be measured by pH paper. It is usually less than 4.5. In bacterial vaginosis is between 5 - 5.5. Menstrual fluid and cervical secretion both increase vaginal pH. Therefore, both need to be excluded before considering vaginal pH as a positive criterion.

#### B. Gram stain:

Gram stain is gaining acceptance as the diagnostic test of choice because it is simple, highly sensitive and specific<sup>10, 18</sup>. Spiegel and his colleagues considered gram stain positive for bacterial vaginosis if lactobacilli morphotype were fewer than 5 per oil immersion and there are 5 or more *G. vaginalis* morphotype together with 5 or more other morphotypes such as (gram negative rod (Bacteroides), gram positive cocci (peptostreptococci) curved gram variable rods (Mobiluncus) or fusiform (fusibacteria)<sup>19</sup>. Thomason and his co-workers interpreted the gram stain as positive for B.V if *G.vaginalis* and other morphologic type exceeded lactobacilli (Figure2).

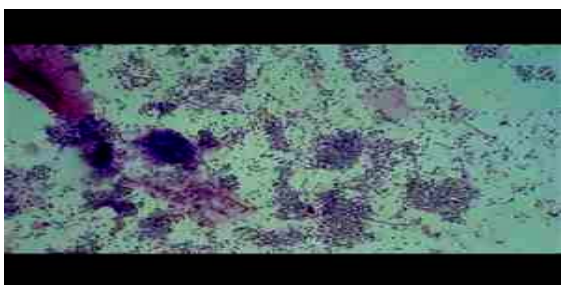


Fig.(2): Gram stained smear of vaginal discharge showing bacterial vaginosis morphotype.

The gram stain is read equivocal (indeterminate) if lactobacilli and other morphotypes are present at equal numbers<sup>20</sup>.

Gram stain criteria (bacterial morphologic types) were less accurate predictors of the disease (sensitivity 97.0%, specificity 66.2%, positive value 57.2%, negative predictive value 97.9%). Even when the bacterial morphologic type criteria were combined with presence of clue cells, predictive accuracy did not exceed that of clue cells on wet mount examination alone (sensitivity 93.9%, specificity 84.7%, positive predictive value 74.2%, negative predictive value 96.8%)<sup>20</sup>.

#### C. Culture:

The organism is facultatively anaerobic and slowly growing. It requires media enriched with blood, serum or starch. Media used for isolation are blood agar, chocolate agar and peptone starch dextrose blood agar. Selective media are available for the isolation of the organism such as those suggested by Ison and Mickelsen<sup>21, 22</sup>.

#### D. Gas liquid chromatographic (GLC) analysis:

Used for analysis of vaginal fluid for short chain fatty acids thought to be products of anaerobic bacteria. GLC is considered consistent with bacterial vaginosis if:

- The peak ratio of succinate to lactate is  $>0.4$ .
- The acetate peak is  $>3$ mm.
- The propionate isobutyrate or isovalerate peak is  $>1$  mm.

#### E. Other methods:

These include : assay for proline aminopeptidase in vaginal fluid and enumeration of clue cells in air-dried vaginal wet smear dehydrated after more than one month, which is more effective, highly sensitive (96%) and specific (98%)<sup>23</sup>.

#### Management:

Treatment is indicated in symptomatic patients and the drugs used are:

- Metronidazole: as a single oral dose of 2 grams or a 7 days course of 500mg twice per day.
- Clindamycin: orally or locally. However the possibility of pseudomembranous colitis as a

side effect have prevented the wide spread use of the oral route. Therefore clindamycin cream(2%)given as 5 grams intra vaginally once daily for 7days is preferred .Treatment of the male partner or the use of condom was found to reduce the recurrence rate in the treated women.

3. Nimorazole: In a dose of one gram orally daily for 7 days was found to be effective.

### References:

1. Gravett MG, Nelson HP, De Rouen T et al. Independent association of bacterial vaginosis and Chlamydia trachomatis infections with pregnancy outcome. JAMA 1986; 256:1899-1903.
2. Minkoff H, Grunebaum AN, Schwarz RU et al. Risk factors for prematurity and premature rupture of membranes : A prospective study of the vaginal flora. Am J Obstet Gynaecol 1984; 150:965-972.
3. Hay PE, Lamont RF, Taylor-Robinson D, et al. Abnormal bacterial colonization of the genital tract and subsequent preterm delivery and late miscarriage. BMJ 1994; 308:295-298.
4. Barbara A and Majeroni MD. Bacterial vaginosis: an update, Am Fam physician 1998; 57(6):1285 – 1289.
5. Kafi SK, Mohamed AO, Musa HA. Prevalence of sexually transmitted diseases(STD) among women in a suburban Sudanese community. Upsala J Med Sci 2000, 105: 249 -254
6. Mead PB. Epidemiology of bacterial vaginosis. Am J Obstet gynaecol 1993; 169:446-449.
7. Gardener HL, Damper TK, Dukes CD. The prevalence of vaginitis. Am Obstet Gynaecol 1957; 73:1080-1087.
8. Gardner H and Dukes CD. *G.vaginalis* vaginitis: a newly defined specific infection, previously classified non-specific vaginitis. Am J Obstet Gynaecol 1955:69; 962-976.
9. Bump RC, Buesching III WJ. Bacterial vaginosis in virginal and sexually active adolescent females: Evidence against exclusive sexual transmission Am J obstet Gynaecol 1988; 158(4): 935-939.
10. Hay PE, Taylor-Robinson, lamont RF. Diagnosis of bacterial vaginosis in a gynaecologic clinic. Birt J obstet And Gynaecol 1992; 99:63-66.
11. Blackwell Al, Philip D Thomas, Kathie Wareham et al. Health gains from screening for infection of the lower genital tract in women attending for termination of pregnancy. Lancet, 1993; 342: 306-210.
12. Shazia A, Fauzia A, Shagufta A et al. Evaluation of common organisms causing discharge. J Ayub Med Coll. Abbottabad 2009; (2):21.
13. Amsel R, Toten PA, Spiegel CA et al. Non-specific vaginitis: diagnostic criteria and microbial and epidemiologic association. Am J Med 1993; 74- 22.
14. Eschenbach D A, Hillier S, Cathy Critchlow MS et al. Diagnosis and clinical manifestation of bacterial vaginosis. Am J obstet. Gynaecol. 1988; 158:819-858.
15. Thomason JL, SM Gilbert, LM Wikosk et al. Proline Aminopeptidase activity as a rapid diagnostic test to confirm bacterial vaginosis. Obstet Gyaecol 1988; 71:607-611.
16. Majeroni BA. New concept in bacterial vaginosis. Am Fam.physician 1992; 44: 1216-1218.
17. Collee LG, Dugiud JP, Fraser AG et al. Mackie and McCartney Practical Medical Microbiology. Edition (13), 1989; churchil, Livingstone.
18. Robert PN, Marjane AK, Hillier S L. Reliability of diagnosing bacterial vaginosis is improved by standardized method of a gram stain interpretation. Journal of clin Microbiol 1991;29(2): 297-301.
19. Spiegel CA, Eschenbach DA, Amsel R et al. Curved anaerobic bacteria in bacterial vaginosis and their response to antimicrobial therapy. J of infect diseases, 1983; 148:817-822.
20. Thomason JL, Gelbart S M, Robert J et al. Statistical evaluation of diagnostic criteria for bacterial vaginosis. Am J Obstet Gynaecol 1990; 162:155-160.
21. Ison CA, Dawson SG, Hilton J et al. Comparison of culture and microscopy in the diagnosis of Gardnerella vaginalis infection. J of clin pathology, 1983; 35: 550-554.
22. Mickelsen PA, Laurence R, Mc Cathy et al. New differential medium for isolation of *Corynebacterium vaginale*. J of clin Microbiol 1977; 5;488-189.
23. Larsoon PG, Jens Jorgen, Pattz Christensen. Enumeration of clue cell in rehydrated air-dried vaginal wet smear for the diagnosis of bacterial vaginosis. Obstet and Gynaecol 1990; 76(4): 727-730.