Isolation of Gardnerella Vaginalis from Pregnant Women with Bacterial Vaginosis in Basrah, Iraq

Jihan M Al-Muk, MBChB, MSc*   Hassan J Hasony, Mphil, PhD*

Objective: To determine the prevalence of *Gardnerella vaginalis* among pregnant women and to examine the efficiency of clinical diagnosis compared to laboratory methods.

Methods: Clinical and laboratory study was designed using at least three out of four clinical signs (Homogeneous vaginal discharge, vaginal pH more than 4.5, positive amine test and the presence of clue cells) in the diagnosis of bacterial vaginosis. This was then compared to *Gardnerella vaginalis isolation.*

Setting: Basrah University Maternity Hospital.

Subjects: Pregnant women attending the maternity hospital for their periodic visits during the period from April 1995 to September 1997.

Results: Out of 413 vaginal swabs examined, *Gardnerella vaginalis* was isolated from 32 (7.7%) and by the use of clinical criteria as diagnostic procedure, the prevalence of bacterial vaginosis was found to be 7%. In cases where *Gardnerella vaginalis was* isolated, 93.1% of women had vaginal pH greater than 4.5 as compared to those with no *Gardnerella vaginalis* where only 10.2% had greater than 4.5. All women with positive cultures had positive amine test (100%). Clue cells were a constant finding in the discharge of pregnant women with positive *Gardnerella vaginalis* cultures.

Conclusion: Amine test alone or together with estimation of vaginal pH are the most suitable tests for prediction of bacterial vaginosis as it has an excellent sensitivity (100%) and specificity (97.9%). The clinical signs are simple to assess and highly related to the isolation of *Gardnerella vaginalis* (87.5%). This is in contrast to clinical symptoms which do not correlate well as more than half of *Gardnerella vaginalis* colonized pregnant women were asymptomatic.

Bacterial vaginosis has not been considered a pathogenic condition in the past, but recently it has been found to be significantly associated with obstetric infections and possess a potential threat to the fetus and newborn[1-4]. *Gardnerella vaginalis* (*G. vaginalis*) has been found to be responsible for 40-50% of all cases of vaginosis[5,6]. The presence of *G. vaginalis* with the absence of lactobacilli in vaginal secretion are characteristics of patients with bacterial vaginosis[7]. Symptomatic patients usually

----

* Department of Microbiology
   College of Medicine
   University of Basrah
   Basrah, Iraq

present with malodorous profuse vaginal discharge which may become a life-long nuisance to the women if it is not diagnosed correctly and adequately treated[8,9].

An elevated vaginal pH (5-6.5) in normal estrogenized patients is always associated with bacterial vaginosis[10-12] and the amine test odour has been shown to be related to the increase in pH[13]. However, *G. vaginalis* was most often observed adhering to the surface of exfoliated vaginal epithelial cells (clue cells) in higher number than anaerobic bacteria suggesting that *G. vaginalis* is responsible for clue cell formation which may be due to the presence of glycocalyx layer[14] as detected by wet mount or Gram's stained smears[15,16].

The objectives of this study were to estimate the prevalence of *G. vaginalis* and to compare the relation of *G. vaginalis* isolation rate to the use of various clinical and laboratory criteria in the diagnosis of bacterial vaginosis.

METHODS

Seven health centers were randomly chosen for the inclusion of pregnant women during their antenatal visits. A total of 413 pregnant women were eligible for the study. All pregnant women were interviewed following special questionnaire form and clinically examined. An unlubricated sterile Cusco's speculum was inserted and lateral and posterior vaginal fornices were swabbed with sterile cotton tipped applicators. Two vaginal samples were obtained from each pregnant women. One of the swabs was for culture to which one ml of sterile Stuart transport medium (Oxoid) was added and the other swab was for microscopic examination (Wet mount and Gram's stain). After taking the swabs, vaginal pH was measured by a pH paper placed in contact with the secretions on the speculum after it had been withdrawn. Amine test was performed by adding a drop of 10% KOH to the discharge on the used speculum and sniffed[15]. Wet mount preparation was done at the bed side within 5-10 minutes of collection of the specimen to detect the presence of *Trichomonas vaginalis*. Clue cells were diagnosed by Gram's stained smears[15]. Pregnant women were considered as a case of bacterial vaginosis when there was presence of at least three out of four clinical signs (homogenous vaginal discharge, pH greater than 4.5, positive amine test and clue cells which are epithelial cells covered with gram-negative bacilli detected on Gram's stained smears)[15,17]. The isolation and identification of different microorganisms together with various laboratory tests was based on standard methods[18]. Briefly, swabs were inoculated directly on to: chocolate, blood, MacConkey's agars and Sabouraud's dextrose agar. The inoculated culture plates were incubated at 37^0C for 48 hours. MacConkey's and Sabouraud's agar plates were incubated aerobically while chocolate and blood agar plates were incubated in candle jars to provide an increased CO_2 tension (5-10%) required for the optimal growth of *G. vaginalis*[18].

RESULTS

The characteristics of pregnant women recruited in the study is presented in Table 1. The mean age of women with *G.vaginalis* infections was 25.6 years compared to those with no *G.vaginalis* (26.9 years). Similarly, the mean age of menarche did not show any significant difference (13.4 and 12.9 years for those with or without *G.vaginalis* respectively).

Table 1. **Characteristics of pregnant women included in the study**

Character	Range	G.vaginalis culture (%)	
		Group 1 Positive:32	Group 2 Negative:381
Age (yrs)	15-43	25.6*	26.9*
Age of menarche	10-17	13.4*	12.9*
Parity	0-14	3.5	3.4
Gravidity	1-15	4.15**	4.4**
Primigravida		8 (25%)	75 (19.7%)
Multigravida		24 (75%)	306 (80.3%)
Gestational Age:			
1st trimester		4 (12.5%)	22 (5.8%)
2nd trimester		21 (65.6%)	217 (56.9%)
3rd trimester		7 (21.9%)	142 (37.3%)

Mean age   ** *Mean*

The isolated microrganisms from the study population are presented in Table 2. *G.vaginalis* was isolated from 32 cases of the recruited women, giving a prevalence rate of 7.7%. *Trichomonas vaginalis* was observed in 11 cases (2.7%) by wet mount preparations. Many pregnant women yielded more than one microorganisms, *G.vaginalis* alone was isolated from 25 (78.1%) out of the 32 positive cases. Yeast was detected in 84 (20.4%) of the pregnant women.

Table 2. Prevalence of microorganisms isolated from vaginal swabs

Microorganisms	Number (%)*
Gram's Negative:	
Gardnerella vaginalis[1]	32 (7.7%)
Escherichia coli	10 (2.4%) }
Klebsiella species	6 (1.5%) } 5.1%
Proteus species	3 (0.7%) }
Pseudomonas species	2 (0.5%) }
Neisseria gonorrhoea	3 (0.7%)
Gram's positive:	
Corynebacterium species	62 (15.0%)
Streptococcus species	25 (6.1%)
Staphylococcus species	41 (99.9%)
Others:	
Yeast: Candida sp	84 (20.4%)
Trichomonas vaginalis[2]	11 (2.7%)

Total number tested 413 [1] *G.vaginalis alone in 25 (78.1%) and mixed in 7 (21.9%).* [2] *Based on wet mount preparations*

There was no correlation between the presence of G.vaginalis and presence (40.6%) or absence (59.4%) of clinical symptoms (x^2: 0.16, p>0.05). In contrast, by the use of clinical signs in the diagnosis of bacterial vaginosis, a strong correlation was found between G.vaginalis isolation (87.5%) and bacterial vaginosis (p<0.01), Table 3. There was a good correlation between the vaginal discharge pH and G.vaginalis discharge isolation. A vaginal pH greater than 4.5 was found in 30 (93.1%) cases with positive G.vaginalis culture as compared to only 39 (10.2%) cases with no G. vaginalis. Amine test was highly related to the presence of G.vaginalis (100%) compared to the absence of G.vaginalis among women with negative amine test (only 1% were found to have Trichomonas vaginalis).

Table 3. **Occurrence of genital symptoms and clinical signs in pregnant women with *G. vaginalis* versus other aetiological agents**

Criteria	*G.vaginalis* positive culture (n:32)	Other aetiology n:381
Symptoms* 13/32 (40.6%)		
Offensive vaginal discharge	12 (37.5%)	92 (24%)
Profuse vaginal discharge	8 (25%)	37 (9.7%)
Pruritis	5 (15.6%)	93 (24.4%)
Dysuria	3 (9.4%)	54 (14.0%)
Individual clinical signs** 28/32 (87.5%)		
Homogenous discharge	23 (71.9%)	19 (5.0%)
Positive amine test	32 (100%)	4 (1.0%)
PH > 4.5	30 (93.8%)	39 (10.2%)
Clue cells	12 (37.5%)	1 (0.3%)

----

* G. vaginalis recovery in symptomatic: 8.8% and in asymptomatic 7.2%
** Prevalence of bacterial vaginosis on clinical signs was 7%

Although the presence of clue cells in women with *G. vaginalis* was not a constant finding, it was not found among *G. vaginalis* negative women except in one doubtful case. The use of clinical signs in the prediction of bacterial vaginosis (overall prevalence 7%) is presented in Table 4. Clinical signs achieve a sensitivity of 87.9% and specificity of 100%. Individual clinical signs (homogenous vaginal discharge, pH greater than 4.5, positive amine test or clue cells) were also examined as predictor for bacterial vaginosis. Of the four included signs of bacterial vaginosis (pH greater than 4.5 and positive amine test) were the most sensitive (clue cell 39.4%, pH greater than 4.5 93.9%, positive amine test 100%).

Table 4. Validity of clinical signs in the prediction of bacterial vaginosis*

Clinical signs	Sensitivity (%) (87.9%)	Specificity (%) (100%)
Homogenous discharge	82.1	95.3
PH > 4.5	100.0	89.4
Positive amine test	100.0	97.9
Clue cells	46.4	100.0

* At least 3 out of 4 clinical signs to consider bacterial vaginosis (Amsel et al 1983)

DISCUSSION

Although bacterial vaginosis is a common problem in clinical practice, only interested physician who utilize the precisely described clinical signs, can routinely diagnose bacterial vaginosis[19]. There is an urgency for the use of firm criteria for the diagnosis of bacterial vaginosis. However, this is the first attempt in southern Iraq to deal with the association of *G. vaginalis* in bacterial vaginosis from the clinical aspect.

Bacterial vaginosis is associated with pregnancy and non-pregnancy related infections of upper genital tract[3,4] and *G.vaginalis* is relatively benign in non-pregnant women. Although it's pathogenic role has been well documented in obstetrical cases[13,17], the emphasis was made on *G. vaginalis* isolation from pregnant women only. The characteristics of pregnant women (age, gestational age, parity and gravidity) have no effect on the rate of *G. vaginalis* isolation. This is in agreement with other studies[4,15,21].

The prevalence of *G. vaginalis* in this study is almost in accordance with that reported from other developing countries like South Africa (6.2%)[15]. A significantly higher prevalence of *G. vaginalis* during pregnancy was reported from developed countries; 32-41% in USA[20] which may attributed to the fact that most women recruited in these studies were single and possibly had multiple sexual partners at the same time[19] and that *G. vaginalis* colonizes uncircumcised men more frequently[10,13].

G. vaginalis was most frequently associated with yeast, a finding which was in contrast with that reported by others[22] where Trichomonas vaginalis was the most frequently associated organism with *G. vaginalis*. However, these studies were carried out on non-pregnant women and the vaginal yeast carriage is more frequent in pregnancy[17,22-24]. Therefore, we expected that there will be more chance of concomitant yeast infection than in the non-pregnant[17,24-26].

CONCLUSION

The use of vaginal pH, amine testing, wet mount preparation and Gram's stained smears of vaginal discharge are a set of criteria that have been used for the diagnosis of bacterial vaginosis[13,15]. Strong association were found between *G. vaginalis* isolation and bacterial vaginosis diagnosed by clinical criteria. These finding was similar to that reported by others[15,26]. The validity of individual clinical signs and on the basis of our results, we believe that amine sniff testing or amine test together with vaginal pH are the most suitable tests for prediction of bacterial vaginosis as it has excellent sensitivity and specificity (100% and 97.9% respectively). These results are in agreement with the results obtained by other investigator[22], who found that amine test was the most powerful indicator for bacterial vaginosis and an elevated vaginal pH in normal estrogenized patient is almost always associated with bacterial vaginosis[13,17].

REFERENCES

1.   Reynolds HD. Bacterial vaginosis and it's implication in preterm membranesis. A
        review of the literatures. J Nurse Midwifery 1991;36:289-96.
2.   McDonald HM. Prenatal microbiological risk factors associated with preterm birth. Br J Obstet Gynecol 1992;99:190-6.
3.   Gibbs RS. Chorioamnionitis and bacterial vaginosis. Am J Obstet Gynecol 1993;169:469-72.
4.   Soper DE. Bacterial vaginosis and postoperative infections. Am J Obstet Gynecol 1993;169:467-9.
5.   Gardner HL, Duckes CD. Haemophilus vaginalis vaginitis. Am J Obstet Gynecol 1955;69:962-76.
6.   Jose WE, McKensaie MJ, Lambe DW. Corynebacterium vaginalis (haemophilus vaginalis) in women with leukorrhea. Am J Obstet Gynecol 1976;126:574-8.
7.   Spiegal CA. Vaginitis/vaginosis. Clin Lab Med 1989;9:525-33.
8.   Goran L. Bacterial vaginosis. Postgraduate Doctor [special issue] 1989:38-39.
9.   Peipert JF, Moutagos AB, Cooper As, et al. Bacterial vaginosis as a risk for upper genital infections. Am J Obstet Gynecol 1997;177:1184-7.
10.   American College of Obstetrician and Gynecologist. Technical Bulletin No. 221: 1996.
11.   Association of Professors of Gynecology and Obstetrics. Diagnosis of vaginitis. Education series in women health issues. Washington, Association of Prof of Gynecology and Obstetrics: 1996.
12.   Hill GB. The microbiology of bacterial vaginosis. Am J Obstet Gynecol 1993;169:450-4.
13.   Callouette JS, Sharp CF Jr, Zummeerina CJ, et al. Vaginal pH as a marker for bacterial pathogens and menopausal status. Am J Obstet Gynecol 1997;176:1270-5.
14.   Greenwood JR. Current taxonomic status of *G.vaginalis*. Scand J infect Dis 1983;40:11-14.
15.   Amsel R, Totten PA, Spiegal CA, et al. Non specific vaginitis: Diagnostic criteria, microbial and epidemiological associations. Am J Med 1983;74:14-22.
16.   Davis JD, Conner EE, Clark P, et al. Correlation between cervical cytological results and gram's stain as diagnostic tests for bacterial vaginosis. Am J Obstet Gynecol 1997;177:532-5.
17.   Jawetz E, Melnick JL, Adelberg EA. Medical Microbiology. In: Causes of bacterial vaginosis, Chapter 23. 21st edn. Appleton & Lange: Lange Medical Books, 1998:275.
18.   Finegold SM, Baron EJ. Baile and Scott's Diagnostic microbiology. 7th edn. 1986:244.
19.   Gardner HL. Pathogenicity of *G.vaginalis* (haemophilus vaginalis). Scand J Infect Dis 1983;40:37-40.
20.   Krohn MA, Hillier SL, Eschenbach DA. Comparison of methods for diagnosing bacterial vaginosis among pregnant women. J Clin Microbiol 1989;27:1266-71.
21.   Vontner LA, Eschenbach DA. The role of *G.vaginalis* in non specific vaginitis. Clin Obstet Gynecol 1981;24:439-59.

22.   Hiller ST. Diagnostic microbiology of bacterial vaginosis. Am J Obstet Gynecol 1993;169:455-9.
23.   Sobel JD. Bacterial vaginosis. An ecologic mystery. Ann Intern Med 1989;111:551-3.
24.   Schgal SG, Nalini V. The role and prevalence of *G.vaginalis* in anaerobic vaginosis. Infection 1990;18:83-85.
25.   Spinillo A, Captazzo E, Gulminetti R, et al. Prevalence and risk for fungal vaginitis caused by non albicans species. Am J Obstet Gynecol 1997;176:138-41.
26.   Thomason JL, Schreckenberger PG, Spellacy WN. Clinical and microbial characteristic of patients with non-specific vaginosis associated with motile, curved anaerobic rods. J Infect Dis 1984;149:801-9.