Comparison Between Modified Amsel's Criteria and The Old Standard Methods in Diagnosis of Bacterial Viginosis

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مقارنة طريقة أمسل ألمحوره مع الطرق القديمة في تشخيص التهاب المهبل الجرثومي

الخلاصة

أجريت دراسة مقطعيه(Cross sectional study لمقارنة طريقة أمسل المحورة مع الطرق التشخيصية القديمة والمعتمدة في تشخيص التهاب المهبل الجرثومي(Nugent's system & Amsel's method) .

جمعت المسحات المهبلية من ٢٣٣ امرأة تشتكي من احد العوارض المهبلية (كالافرازات،الرائحة الغير محببه،الحكه ،والحرقة) وذلك خلال زيارتهن للعيادة الاستشارية للنسائية و التوليد في مستشفى الزهراء للولادة والأطفال للفترة الزمنية من تشرين الأول/٢٠٠٦ ولغاية نهاية أيار/٢٠٠٧.

إن طريقة أمسل المحورة (Modified Amsel's method) تعتمد في التشخيص على صفتين بدلا من ثلاث من اصل أربع صفات سريريه (Amsel's criteria) معتمده في تشخيص التهاب المهبل الجرثومي.

وقد وجد أن هذه الطريقة كفوئه ودقيقه وذات حساسية عالية، وخصوصية، و قيم تنبؤيه جيده بالمقارنة مع الطرق القديمة ألمعتمده ويمكن تطبيقها سريريا في تشخيص التهاب المهبل الجرثومي بدون فقدان في قيم الحساسية والخصوصية وفي القيم التنبؤيه.

Abstract

A cross sectional study was conducted to compare modified Amsel's method with the old standard methods(Nugent's system & Amsel's method) in the diagnosis of bacterial vaginosis.

Vaginal swabs were collected from 233 women who were complaining from one or more of vaginal symptoms including discharge, malodor, itching, burning, and dyspareunia, and attending Obstetric and Gynecologic Outpatient Clinic in Al-Zahra Maternity and Pediatric Teaching Hospital in Al-Najaf Governorate, during the period from November/2006 to the end of May/2007.

modified Amsel's method is depending on two out of four instead of three out of four Amsel's criteria for diagnosing bacterial vaginosis and it was found that it is efficient and significantly applicable, without losing of sensitivity, specificity, and predictive values.

Introduction

Bacterial vaginosis (BV) is a polymicrobial disorder characterized by an increase in the vaginal pH over 4.5, a reduction in or absence of Lactobacillus colonization and overgrowth of several facultative and obligatory anaerobic bacteria(1), including Gardenerella vaginalis, anaerobic gram

negative rods (Bacteriodes spp., Prevotella spp., and Porphyromonas), Mobiluncus spp., and others(2,3,4)

It is a common cause of vaginitis in women who are sexually active during childbearing age(5).

Bacterial vaginosis is associated with adverse pregnancy outcomes, upper genital tract infections such as pelvic inflammatory disease, endometeritis, post-gynaecological surgery infections, cervicitis, urinary tract infections, cervical intraepithelial neoplasia, and increased risk of sexual acquisition of human immune deficiency virus infection(6,7,8).

The two most common symptoms associated with BV are vaginal discharge and malodor (9). However bacterial vaginosis can not be adequately diagnosed on the basis of symptoms since nearly half of the women who meet the clinical criteria for bacterial vaginosis report no noticeable symptoms(10,11) .

The diagnosis of bacterial vaginosis was made by using Amsel's criteria, the presence of three of the following four criteria is considered to be consistent with the presence of BV: (i) a characteristic thin homogenous discharge, (ii) vaginal pH more than 4.5, (iii) clue cell on saline wet mount, and (iv) release of a fishy amine odor on addition of 10% KOH(12). However, this clinical criteria can be simplified by using 2 of 4 rather than 3 of 4 (Amsel's criteria), without loss of sensitivity and specificity(13).

Nugent et al(14). described a Gram stain scoring system from 0 to 10 based on the number of Lactobacilli, Gram-negative to Gram-variable bacilli, and Gram-negative curved bacilli, a score from 7-10 indicates BV infection. The Gram stain is believed by many to be the gold standard for diagnosing bacterial vaginosis(15).

However, interpreting the Gram stain requires experience, and it's often difficult to get timely results for the clinical diagnosis of BV(13).

Materials and Methods

A cross sectional study of 233 women recruited from various locations at Al-Zahra Maternity and Pediatric Teaching Hospital in Al-Najaf Governorate over a six month period beginning in November, 2006.

Any woman had one or more of vaginal symptoms (discharge, malodor, itching, burning, and dyspareunia), and undergoing a speculum examination at the Outpatient Clinic was eligible to participate in the study.

Because all the specimens were taken from patients who were already undergoing pelvic examination, the study was approved by the College of Medicine and the Department of Obstetrics and Gynecology of the same college. Thus; a separate, specific informed consent was not obtained, and information from the study was not used for the clinical care or the management of the patient.

Women were excluded if they were taking antibiotic or topical vaginal medication within the last three days, or if there was vaginal bleeding or any signs of cervicitis on examination, and/or if the patient was at high risk pregnancy.

According to a prepared case sheet, descriptive variables of the patients obtained during evaluation includes age, address and social class, marital status, pregnancy status, parity, history of abortion, symptoms of the patients, menstrual cycle history, contraceptive choice, presence of BV associated factors like vaginal douching or recent antibiotic therapy, and past history of genital tract infection.

Unlabricated vaginal speculum was inserted, and vaginal fluids were collected from posterior vaginal fornix with three disposable cotton swabs.

One swab was used for vaginal pH determination; another swab was used to test release of fishy odor "whiff test".

Third swab was used for wet film and Gram stain to score then according to Nugent's criteria(13).

The diagnosis of BV was based on Nugent's scoring system of the Gram stained smear which is regarded as the gold standard for diagnosis of BV(14).

Each Gram-stained smear was evaluated for the following morphotypes under oil immersion (1000 magnification).

1-Gram-variable rods (G. vaginalis morphotypes) and small Gram-negative rods (Bacteroides spp. Morphotypes).

2-Curved Gram-variable rods (Mobiluncus morphotypes).

3-Large Gram-positive rod (Lactobacillus morphotypes).

Each morphotypes was quantitated from 1+ to 4+ with regard to the number of morphotypes per oil immersion field which is ranged from (0-no morphotypes to 4+: 30 or more morphotypes, table1).

Table(1): The Nugent's scoring system.

Points	*Lactobacillus* morphotypes	*Gardenerella* &*Bacteroides* morphotypes	*Mobiluncus* morphotypes
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

All points are added together to obtain the final score; score (0-3) indicate normal Lactobacillus flora, score (4-6) intermediate flora, and score (7-10) indicate BV.

Gram stain reagents were prepared according to Collee et al. (1996).

Modified Amsel's criteria

Original Amsel's method was established in 1983 (Amsel et al., 1983), where a positive diagnosis requires that the patient satisfies three of the following criteria:

a) Discharge: a thin, homogenous, milky, adherent discharge as it characterized at the time of swabs collection.

b) PH >4.5: vaginal pH determined by means of pH indicator strip (Whatman) which range from 4-6.

c) Whiff test: when some drops of 10% potassium hydroxide solution was added to vaginal secretion from one vaginal swab, a release of "fishy" amine odor signified a positive whiff test.

d) Clue cell: vaginal secretions on third swab were combined with 2-3 drops of normal saline and used for the preparation of two slides.

One slide was covered with a coverslip for wet film, which then examined under 400 magnification for presence of clue cells.

The second swab was allowed to be air dried and heat fix, then Gram stained for the purpose of Nugent's scoring.

The modified Amsel's criteria (2 out of 4) was compared with the classical Amsel's criteria (3 out 4) by testing it against the Nugent's scoring system which is regarded the gold standard for the diagnosis of BV.
3.3.3.2

Statistical analysis

- The sensitivity, specificity, positive, and negative predictive values, and their 95% confidence intervals (C.I) have been calculated for each of the individual criterion, different combinations of criteria, Amsel's criteria, and for Quick Vue Advance *G. vaginalis* test.

Data were entered and stored in Microsoft Access software and analyzed by SPSS version 14, the statistically significant differences were assessed with chi-square test at two level of probability(p≤ 0.05, p≤ 0.01).

Results

Evaluation of each Amsel's criterion

Vaginal pH was the criterion of the highest sensitivity, at 91%, the sensitivity of the remaining Amsel's criteria ranged from 60-75% table (2)and(3).

Demonstration of clue cell on microscopical examination was the criterion of the highest specificity, at 93%, thin homogenous discharge and vaginal pH have a low specificity, 51%, 63%, respectively, while positive amine odor "whiff test" have a high specificity, at 88%.

Table (2): Laboratory finding of each individual criterion(Amsel's criteria) among patients with and without BV.

Type of Amsel's criterion		Bacterial vaginosis		Total
		Positive(%)	Negative(%)	
1. Thin discharge	Positive	111(69.4)	36(49.3)	147
	Negative	49(30.6)	37(50.7)	86
2. Vaginal pH> 4.5	Positive	145(90.6)	27(37.0)	172
	Negative	15(9.4)	46(63.0)	61
3."whiff test"	Positive	97(60.6)	9(12.3)	106
	Negative	63(39.4)	64(87.7)	127
4. Clue cell	Positive	121(75.6)	5(6.8)	126
	Negative	39(24.4)	68(93.2)	107
	Total	160(100)	73(100)	233

Table (3): Evaluation of *sensitivity, **specificity,* PPV, **** NPV, and 95% confidence intervals(C.I) of each Amsel's criterion among the studied patients.**

Amsel's criterion	Sensitivity (C.I)	Specificity (C.I)	PPV (C.I)	NPV (C.I)
1.discharge	0.69 (0.62-0.76)	0.51 (0.39-0.62)	0.75 (0.68-0.82)	0.43 (0.33-0.54)
2.vaginal pH	0.91 (0.85-0.94)	0.63 (0.51-0.74)	0.84 (0.78-0.89)	0.75 (0.62-0.85)
3."whiff test"	0.61 (0.53-0.68)	0.88 (0.77-0.94)	0.92 (0.84-0.96)	0.50 (0.41-0.59)
4. clue cell	0.76 (0.68-0.82)	0.93 (0.84-0.97)	0.96 (0.91-0.99)	0.64 (0.54-0.72)

*Sensitivity=true positive/total positive(true positive + false negative).
** Specificity= true negative/total negative(true negative+ false positive).
*** Positive predictive value PPV= true positive/true positive+ false positive.
**** Negative predictive value NPV= true negative/true negative+ false negative.

Evaluation of different combinations of any two Amsel's criteria in comparison to the traditional Amsel's method.

Different combination of any two Amsel's criteria and the traditional Amsel's method were evaluated by calculating their sensitivity, specificity, negative, and positive predictive values, and their corresponding 95% C.I as summarized in table (4), (5).

The sensitivity of any combination of 2 Amsel's criteria ranged from 60-66%, while the sensitivity of 3 or more Amsel's criteria equal to 62%.

The specificity of most combinations of any 2 Amsel's criteria was nearly equal or more than the specificity of the traditional Amsel's method, at 93%, except that the specificity of (pH+ discharge) combination recorded the lowest value of specificity than other combinations, at 66%.

Table (4): Laboratory finding of different combinations of two Amsel's criteria among patients with and without BV:

Type of Amsel's criteria		Bacterial vaginosis		Total
		Positive(%)	Negative(%)	
1.pH+discharge	Positive	104(65)	25(34.2)	129
	Negative	56(35)	48(65.8)	104
2.pH+"whiff test"	Positive	102(63.8)	12(16.4)	114
	Negative	58(36.2)	61(83.6)	119
3.pH+clue cell	Positive	105(65.6)	5(6.8)	110
	Negative	55(34.4)	68(93.2)	123
4.clue cell +"whiff test"	Positive	96(60)	1(1.4)	97
	Negative	64(40)	72(98.6)	136
5.clue cell +discharge	Positive	105(65.5)	3(4.1)	108
	Negative	55(34.4)	70(95.9)	125
6."whiff test"+ discharge	Positive	98(61.3)	6(8.2)	104
	Negative	62(38.7)	67(91.8)	129
7.traditional Amsel's method (≥3 criteria)	Positive	99(61.9)	5(6.8)	104
	Negative	61(38.1)	68(93.2)	129
	Total	160(100)	73(100)	233

Table (5): Evaluation of sensitivity, specificity, NPV, PPV, and 95% C.I for different combinations of 2 Amsel's criteria in comparison to traditional Amsel's method.

Type of combined Amsel's criteria	Sensitivity (C.I)	Specificity (C.I)	PPV (C.I)	NPV (C.I)
1. pH+ discharge	0.65 (0.57-0.72)	0.66 (0.54-0.76)	0.81 (0.73-0.87)	0.46 (0.36-0.56)
2. pH+ "whiff test"	0.64 (0.58-0.71)	0.84 (0.73-0.91)	0.89 (0.82-0.94)	0.51 (0.42-0.60)
3. pH+ clue cell	0.66 (0.58-0.73)	0.93 (0.84-0.97)	0.95 (0.89-0.98)	0.55 (0.46-0.64)
4. Clue cell +"whiff test"	0.60 (0.52-0.68)	0.99 (0.92-1.00)	0.99 (0.94-1.00)	0.53 (0.44-0.61)
5. Clue cell+ discharge	0.66 (0.58-0.73)	0.96 (0.88-0.99)	0.97 (0.91-0.99)	0.56 (0.47-0.65)
6."whiff test"+ discharge	0.61 (0.53-0.69)	0.92 (0.82-0.97)	0.94 (0.87-0.98)	0.52 (0.43-0.61)
7. Traditional Amsel's ≥ 3criteria	0.62 (0.54-0.69)	0.93 (0.84-0.97)	0.95 (0.89-0.98)	0.53 (0.44-0.61)

Discussion

depending on Nugent's scoring system as standard diagnostic test for BV, it was found that elevated vaginal pH >4.5 was the criterion of the highest sensitivity at 91%, but of low specificity at 63%, table (3).

This finding was the same as that reported by other studies(9,16,17,18,13), who all found that pH was the highly sensitive Amsel's criterion and this is because of enzymes and metabolic by-products of BV- associated (such as keto acids and succinates) together with absence of lactic acid leading to rises of vaginal pH.

Vaginal pH >4.5 criterion was of low specificity because it could be associated with menstruation, recent sexual intercourse, excessive cervical discharge, infection with T.vaginalis, and it is more common in postmenopausal women(19,13).

However; normal vaginal pH can exclude the diagnosis of BV (20,21)

Demonstration of clue cell on microscopical examination was the criterion of the highest specificity at 93%, followed by the release of positive amine odor "whiff test" at 88%.

A fact that was similar to what was reported by other researchers who found that demonstration of clue cell on wet film preparation is considered to be the most accurate diagnostic criterion for BV.(9,22,15,17,18)

Clue cell are formed when G. vaginalis present in high numbers, and adhere to exfoliated epithelial cells in the presence of an elevated pH.

Visualization of thin homogenous discharge on examination was the criterion of the lowest specificity at 51%, this finding was similar to that registered by other workers who found that discharge criterion is not specific for BV, seen in about half of patients with BV, and in about one third of patients without BV(23,15,13).

Discharge criterion is not specific for BV because it could be found in women with moniliasis, and in women without vaginitis(22).

When different combinations of any two Amsel's criteria and the traditional Amsel's method were compared by calculating their sensitivity, specificity, negative, and positive predictive values, it was found that the sensitivity of any combined two criteria ranged from 60% to 66%, and the sensitivity of the traditional Amsel's method was at 62%, table (5).

The specificity of any two combined criteria was nearly equal, the same , or more than that of traditional Amsel's method at 93%, an exception of that is the combination of (discharge+ pH) criteria which was of the lowest specificity at 66%.

Both positive, and negative predictive values for any combined two criteria were nearly equal, the same, or more than that of the traditional Amsel's method at 95%, 53%, respectively.

Therefore, these findings are supportive to the new modified Amsel's method for the diagnosis of BV which depend on 2 clinical criteria instead of 3 out of 4 without significant losing of sensitivity , specificity, positive, and negative predictive values.

Gutman et al(13). demonstrated that the diagnosis of BV can be simplified by finding 2 out of 4 Amsel's criteria, instead of 3 out of 4 criteria without losing of sensitivity and specificity whatever the type of the combined two criteria.

While the present study and Forsum et al(24). found that combined (pH+ discharge) criteria have lower specificity than that of traditional Amsel's method (3 criteria). Forsum et al(24) proposed that it is better to exclude the combination of (discharge and pH) criteria for the diagnosis of BV as a modified Amsel's method, and he suggested to test pH criterion (highly sensitive criterion) and combined it with one of the two specific criteria (demonstration of clue cell and positive amine odor test) for the diagnosis of BV.

References

1-Eschenbach , D. A. (1993). History and review of bacterial vaginosis. Am. J. Obstet. Gynecol. **169**: 441-445.

2-Holst, E. (1991). Bacterial vaginosis, clinical and microbiological findings. Forth edition, edited by: Horowitz, B. J.; Mardh, P. A., eds: vaginitis and vaginosis. New York, Wiley-Liss. 115-120.

3-Spiegel, C. A. (1991). Bacterial vaginosis. Clin. Microb. Rev. Vol.**4**: 485-502.

4-Hillier, S. L.; Krohn, M.; Klebanoff, S. J and Eschenbach, D. A. (1992). The relationship of hydrogen-peroxide producing Lactobacilli to bacterial vaginosis and genital microflora in pregnant women. Obstet. Gynecol. **79**: 369-373.

5-Ramonibai, J. T.; Kavita, R. and Legori, M. (1998). Prevalence of bacterial vaginosis in women attending antenatal, gynecology, and STD clinics of medical college, Thirn vaanthapuram. J. Academy. Clinical. Microbiologia. **1**: 107-111

6-Cu-Uvin, S.; Hogan, J. W.; Warren, D.; Klein, R. S.; Pieper, J.; Schumann, P.; Holmberg, S., Anderson, J.; Schoenbaum, E.; Valhor, D.; and May, K. H. (1999).
7-Harmanli, D. H.; Cheng, G. Y.; Nyirejesy, P.; Chatwani, A. and Gaughan, J. P. (2000). Urinary tract infection in women with bacterial vaginosis. Obstet. Gynecol. **95**: 710-712.
8-Mc Gregor, J. A.; Lavelline, D.; Franco-Buff, A.; Todd, J. K. and Maskowski, E. L. (2000). Protease production by micro organisms associated with reproductive tract infection. Am. J. Obstet. Gynecol. **154**: 109-114.
9-Eschenbach, D. A.; Hillier, S. L.; Critchlow, C. W.; Stevens, C.; DeRoven, T. and Holmes, K. K. (1988). Diagnosis and clinical manifestation of bacterial vaginosis. Am. J. Obstet. Gynecol. **158**: 819-828.
10-Deborah, C. M.; Paul, E. A. and Ronnie, F.L. (2003). Bacterial vaginosis: Studd ,J(ed). Progress in obstetric and gynecology. **15**: 185-211.
11-Klebanoff, M. A.; Schwebke, J. R.; Zhang, J.; Narsal, T. R. and Andrews, W. W. (2004). Vulvovaginal symptoms in women with bacterial vaginosis. Obstet. Gynecol. 104: 267-272.
12-Amsel, R.; Totten, P. A.; Spiegel, C. A. and Chen, C. K.; Eschenbach, D.; Holmes, K. K. (1983). Non specific vaginitis. Diagnostic criteria and microbial and epidemiological association. Am. J. Med. **74**: 14-22.
13-Gutman, R. E.; Peipert, J. F.; Weitzen, S. and Blume, J. (2005). Evaluation of clinical methods for diagnosing bacterial vaginosis. Obstet. And Gynecol. **105**:551-556.
14-Nugent, R. P.; Krohn, M. A. and Hillier, S. L. (1991). Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J. Clin. Microb. **29**: 297-301.
15-Myziuk, L.; Romanowski, B. and Johnson, S. C. (2003). BV Blue test for diagnosis of bacterial vaginosis. J. Clin. Microbiol. **41**: 1925-1928.
16-Al-Zubaidy, K. G. M. (2001). Epidemiological study of some sexually transmitted disease in Najaf province. MSc. Thesis. Kufa University.
17-Beverly, E. S.; Chen, H. Y.; Wang, Q. J.; Zariffard, M. R.; Cohen, M. H.; and Spear, G. T. (2005). Utility of Amsel criteria, Nugent score, and quantitative PCR for Gardenerella vaginalis, Mycoplasma hominis, and Lactobacillus species for diagnosis of bacterial vaginosis in Human Immunodeficiency virus-infected women. J. Clin. Microb. **43**(9): 4607-4612.
18-Goyal, R.; Sharma, P.; Kour, I.; Aggarwal, N. and Talwar, V. (2005). Diagnosis of bacterial vaginosis in women in labour. J. K. Science. **7** (1): 1-4.
19-Brizzolara, S.; Killeen, J.; and Severino, R. (1999). Vaginal pH and parabasal cells in postmenopausal women. Obstet. Gynecol. **94**: 700-703.
20-Caillouette, J. C.; Sharp, C. F.; Zimmerman, G. J. and Roy, S. (1997). Vaginal pH as marker for bacterial pathogens and menopausal status. Am. J. Obstet. Gynecol. **167**: 1270-1275.
21-Carr, P. L.; Felsenstein, D. and Friedman, R. H. (1998). Evaluation and management of vaginitis. J. Gen. Int. Med. **13**: 335-346.
22-Thomason, J. L.; Gelbart, S. M.; Anderson, R. J.; Walt, A. K.; Osypowski, P. J.;and Broekhuizen, F. F. (1990). Evaluation of diagnostic criteria for bacterial vaginosis. Am. J. Obstet. Gynecol. **162**: 155-160.
23-Al-Saadi, M. K. (2003). Microbiological study of bacterial vaginosis and it's correlate with the risk of urinary tract infection among pregnant women. MSc. Thesis. College of Medicine. Kufa University.