BVBLUE Test for Diagnosis of Bacterial Vaginosis in Pregnant Women Attending Antenatal Care at Phramongkutklao Hospital

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Background: *Bacterial vaginosis (BV) is a disorder of the vaginal ecosystem characterized by a shift in the vaginal flora form the normally predominated lactobacillus to one dominated by sialidase enzyme-producing mixed flora.*

Objectives: *To compare the sensitivity of BVBLUE test for diagnosis of bacterial vaginosis with Gram stain by using Nugent score as a gold standard.*

Material and Method: *From April to June, 2004, a total of 173 pregnant women who received antenatal care at Phramongkutklao Hospital had reached the study criteria. The speculum for this exam, used in the process of collecting vaginal secretions, must not be lubricated with any lubricants. The vaginal discharge was collected from the lower 1/3 of the vaginal wall. Gram stain score and BVBLUE test were conducted and compared.*

Results: *173 patients were enrolled in the present study. BVBLUE test was compared to the standard method for the diagnosis of BV by Gram stain using Nugent score as a gold standard. The sensitivity, specificity, accuracy, positive and negative predictive value of BVBLUE test versus the Gram stain score for diagnosis of bacterial vaginosis were 94%, 96%, 96% 86%, and 98%, respectively.*

Conclusion: *BVBLUE test for diagnosis of bacterial vaginosis had high sensitivity, specificity, accuracy, positive and negative predictive value (94%, 96%, 96%, 86%, and 98%, respectively).*

Keywords: *Bacterial vaginosis, Gram stain, BVBLUE test*

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Bacterial vaginosis (BV) is a disorder of the vaginal ecosystem characterized by a shift in the vaginal flora from the normally predominant Lactobacillus to one dominated by sialidase enzyme-producing mixed flora including[1]: Gardnerella vaginalis, Bacteroides spp., Provetella spp., Mobiruncus spp. and Mycoplasma hominis. During

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pregnancy and delivery, these bacteria cause many complications[1-3] such as premature labor, premature rupture of membrane and chorioamnionitis per se. Premature labor is the most serious cause of perinatal death in all newborn babies. Recently, more and more evidence has shown that bacterial vaginosis is related to premature labor and low birth weight[1,4]. These phenomena lead to other complications in both mother and child.

Current conventional diagnostic methods are gram stain with Nugent method or Amsel's criteria. Both methods consume much time and need a microscope including a laboratory expert to interpret the result. BVBLUE Test method is the diagnostic test for bacterial vaginosis by examining the sialidase activity without using any microscope. Its result can be illustrated within 10 minutes.

This cross-sectional study was aimed to analyze the sensitivity of an instrument used for testing the bacterial vaginosis by comparing with the gold standard method and to find out the suitable diagnosis method for screening bacterial vaginosis in the institute including the preventive plan and treatment for the impacts of this disease on pregnancy and delivery.

Material and Method

The data were collected from all pregnant women, who received antenatal care (ANC) and initial physical examination at Phramongkutklao Hospital from March to June, 2004. Having the history of treatment for leucorrhea and vaginal bleeding within 72 hours before obtaining this antenatal care are exclusion criteria. One hundred and seventy-three pregnant women, who reached the study criteria, were informed about this research by the research staff. This research followed the principle of the patient's right with the approval from the Ethical Review Committee Royal Thai Army Medical Department. Each woman was asked to fill out a questionnaire at the time of screening. The questionnaires required information about demographic data, last menstrual period, past obstetric history, the history of treatment for leucorrhea and the history of vaginal bleeding within 72 hours before obtaining this ANC.

After the ANC process, each participant received pelvic examination. The speculum for this exam, used in the process of collecting vaginal secretion, must not be lubricated with any lubricants. Vaginal discharge was collected at the lower 1/3 of the vaginal wall, not the site that was close to the cervix with two cotton swabs. One swab was used for Gram's staining. The other was inserted in the BVBLUE testing vessel. Stir to mix it with the solution and left for 10 minutes at room temperature. After that, put 1-2 drops of BVBLUE test developer solution were put into that tube, mixed together and the result was interpreted within 3 minutes[5]. This procedure was performed by the research staffs that had been trained and tested for skill and correctness of such a procedure.

Principles of BVBLUE[5]

The BVBLUE system is a chromogenic diagnostic test for the detection of sialidase activity in a vaginal fluid specimen.

Evaluation of Vaginal Smears

The vaginal smears were air dried, subjected to Gram's staining, and evaluated under magnification (x 1000) by a microbiologist. The microbiologist was kept blind as to the patients. A score of 0 to 10 was assigned on the basis of the relative proportions of easily distinguished bacterial morphologic types (i.e., large gram-positive rods, small gram-negative or variable rods, and curved rods). A score of 0 was assigned to the most lactobacillus-predominant vaginal flora, and a score of 10 was assigned to a flora in which Lactobacilli were largely replaced by Gardnerella vaginalis, Bacteroides spp., Provetella spp., Mobiluncus and

Mycoplasma hominis. Scores for Gram's staining were calculated by the method of Nugent et al in Table 1[6,7].

The diagnosis for bacterial vaginosis would be indicated when the following characteristics were found.

Table 1. Standardized scoring method for evaluation of gram-stained smears for diagnosis of bacterial vaginosis[6,7]

Bacterial morphotype	Points* Scored per morphotype**				
	None	1^+	2^+	3^+	4^+
Large Gram-positive rod	4	3	2	1	0
Small Gram-negative/variable rod	0	1	2	3	4
Curved Gram-negative/variable rod	0	1	1	2	2

* Score of 0-3 points, normal; 4-6 points, intermediate; 7-10 points, bacterial vaginosis

** 1^+ < 1/1000x oil immersion field; 2+ 1-5;3+ 6-30; 4+ >30

Table 2. Demographic data

General information	Pregnant women (N = 173)				*p- Value*
	positive BV(n=36)		negative BV(n=137)		
	N	%	N	%	
1. Age					
Less than 19 years old	6	16.7	16	11.7	0.612
20 - 29 years old	18	50.0	81	59.1	
30 - 39 years old	12	33.3	39	28.5	
more than 40 years old	0	0.0	1	0.7	
2. Habitat					
Bangkok	26	72.2	93	67.9	0.617
Urban	10	27.8	44	32.1	
3. Occupation					
Government employees	1	2.8	2	1.5	0.834
Employee	22	61.1	75	54.7	
Own business	2	5.6	12	8.8	
Housewife	11	30.5	46	33.5	
Others	0.0	0.0	2	1.5	
4. Income					
1-5,000 baht	5	13.9	14	10.2	0.840
5,001-10,000 baht	14	38.8	60	43.8	
10,001-15,000 baht	6	16.7	27	19.7	
more than 15,000 baht	11	30.6	36	26.3	
5. Educational background					
Illiterate	0	0.0	1	0.7	0.431
Primary school	6	16.7	40	29.2	
High school	20	55.6	60	43.8	
Bachelor degree	10	27.7	36	26.3	
6. Gestation age (weeks)					
0-12	16	44.4	78	56.9	0.315
13-28	19	52.8	55	40.1	
29-37	1	2.8	4	2.9	
7. Parity					
1	16	44.4	83	60.6	0.219
2	14	38.9	38	27.7	
≥3	6	16.7	16	11.7	

Table 3. The relationship between Gram stain score and BVBLUE test method

BVBLUE test	Gram stain		Total
	Positive	Negative	
Positive	31	5	36
Negative	2	135	137
Total	33	140	173

$$\text{Sensitivity} = \frac{31}{33} \times 100 = 93.9\%$$

$$\text{Specificity} = \frac{135}{140} \times 100 = 96.4\%$$

$$\text{Accuracy} = \frac{31 + 135}{173} \times 100 = 95.9\%$$

$$\text{Positive predictive value} = \frac{31}{36} \times 100 = 86.1\%$$

$$\text{Negative predictive value} = \frac{135}{137} \times 100 = 98.5\%$$

1. The Gram stain score showed the value from 7 to 10 according to Nugent score (gold standard)[6,7].
2. The BVBLUE test showed the following results[5]:
   Positive result: The color of solution had changed to blue or green.
   Negative result: The color of solution had changed to yellow.

If a pregnant woman had been diagnosed for bacterial vaginosis, the guideline for the treatment was as follows[8]: Metronidazole 400 mg 1 tablet was administered twice a day (in the morning and evening), lasting for 7 days after the first trimester. This patient was followed up and was checked again the following week.

The data from the present study such as questionnaires, BVBLUE test and Gram stain score result were analyzed by SPSS 11 software. Probability values of <0.05 were considered to be statistically significant. The comparison between statistical calculation of BVBLUE test and Gram stain score was sensitivity, specificity, accuracy, positive predictive value, negative predictive value. Chisquare test was used to compare the demographic data between the positive BV group and negative BV group.

Results

From April to June, 2004, a total of 173 pregnant women who received ANC at Phramongkutklao Hospital had reached the study criteria. It was found that 33 cases had bacterial vaginosis based on the Nugent score (Table 1). The prevalent rate was 19%. About half of them (50.0%) were aged between 20-29 years. From the cases that were diagnosed for bacterial vaginosis, 72.2% of them had their habitat in Bangkok. Their occupations were employee, housewife, having their own business, and government employees (61.1% 30.5%, 5.6%, and 2.8% respectively). More than half of them (55.6%) had finished high school, 27.7% held a bachelor degree, 16.7% had finished primary school, and 0% were illiterate. Most of their income (38.8%) was between 5,001-10,000 baht. When compared to the group in whom no bacterial vaginosis was found, the general characteristics of both groups were similar ($p>0.05$) (Table 2).

The test for bacterial vaginosis by using Gram stain score, interpreted by using Nugent score, showed that 33 had a positive result. For the BVBLUE test, 36 cases were positive. Table 3 shows the relationship between Gram stain score and BVBLUE test which was applied to diagnosed bacterial vaginosis. It was found that 31 cases showed a positive result in both BVBLUE test and Gram stain score, and 135 cases had a negative result for both tests

Five cases had BVBLUE test-positive and Gram stain score-negative, whereas 2 cases had BVBLUE test-negative and Gram stain score-positive. The comparison between statistical calculation of BVBLUE test and Gram stain score with a sensitivity, specificity, accuracy, positive

and negative predictive value was 94, 96, 96, 86 and 98%, respectively.

Discussion

The present study was focused on the sensitivity of BVBLUE test so that sialidase activity was checked in all pregnant women who reached the study criteria.

BV is a disorder of the vaginal ecosystem characterized by a shift in the vaginal flora from the normally predominant Lactobacillus to one dominated by sialidase enzyme-producing mixed flora[9,10] including Gardnerella vaginalis, Prevotella spp, Bacteroides spp., and Mobiluncus spp. BVBLUE test is used to detect sialidase activity and indicated for use in women suspected of having BV.

From the studies of McGregor et al[11], Myziuk et al[12] and Bradshaw et al[13], it was found that the sensitivity and specificity of BVBLUE test was better, compared to the diagnosis for bacterial vaginosis by using Amsel criteria and having a Gram stain score as a gold standard. In addition, the study of McGregor et al[11] showed that the group of patients who were diagnosed for bacterial vaginosis had sialidase activity 4.6 times higher than the control group. However, this the present study did not touch on the quantity of sialidase activity. The research results indicated that the prevalence of pregnant women with bacterial vaginosis in Phramongkutklao Hospital was 19%. And when compared to the previous studies, it showed that the prevalence was lower than in the US reported by Minkoff[14] (31.8%) in 1984, but higher than in an English report by Hay[15] (12.1%) in 1994 and in Thailand studied by Puapermpoonsiri[16] (15.9%) in 1996. However, it was almost the same as the prevalence in Scandinavia reported by Gratacos[17] (19.6%) in 1998. Since the results concerning the prevalence of bacterial vaginosis in each research were varied, it may result from the difference of diagnostic test which was used as a gold standard. Therefore, the differences in gold standard led to the variation of sensitivity and specificity in each research results. In this the present study, the authors used vaginal Gram smears for the diagnosis of BV. Clinical signs characteristic of BV were subject to variation due to factors unrelated to infection and Gram stain of vaginal fluid is gaining acceptance as the diagnostic method of choice[6].

BVBLUE test is the diagnostic test for bacterial vaginosis by examining the sialidase activity without using any microscope. It costs 100 baht per case and its result can be illustrated within 10 minutes so treatment can be started earlier to reduce feasible complications. Whereas, the other diagnostic tests such as Amsel's criteria or Gram stain with Nugent method consume much time and need a microscope including a laboratory expert to interpret the result and cost of each method is 150 and 100 baht per case, respectively. The BVBLUE test method is rather quick for diagnosis and treatment; however, it still has limitation. For example, the BVBLUE test method cannot be the diagnostic test for other infections such as yeast infection, trichomonas vaginalis or other diseases.

The BVBLUE test should not be applied to those who have recently douched, engaged in vaginal sexual intercourse, or used spermicides, vaginal lubricants or feminine deodorant sprays within 72 hours before being tested.

Conclusion

In summary, the use of BVBLUE test makes it easy, rapid and convenient for bacterial vaginosis diagnosis. The criteria for this test is based on sialidase activity with the sensitivity 94%, specificity 96%, accuracy 96% PPV 86%, and NPV 98%. Conducting this test with other health assessment will make the diagnosis for bacteria vaginosis more reliable.

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การวินิจฉัยแบคทีเรียลวาจิโนสิส โดยวิธี BVBLUE test ในสตรีตั้งครรภ์ที่มาฝากครรภ์ที่โรงพยาบาลพระมงกุฎเกล้า

เพิ่มศักดิ์ สุเมฆศรี, ชนานันท์ ขอประเสริฐ, สุธี พานิชกุล

วัตถุประสงค์: *เพื่อศึกษาหาความไวของ BVBLUE test เพื่อการตรวจวินิจฉัย bacterial vaginosis เปรียบเทียบกับการย้อมสีแกรมจากสารคัดหลั่งในช่องคลอดโดยใช้ Nugent score เป็นวิธีมาตรฐาน*

วัสดุและวิธีการ: *ได้ทำการศึกษาแบบภาคตัดขวางในผู้ป่วย จำนวน 173 ราย ที่มาฝากครรภ์ที่โรงพยาบาลพระมงกุฎเกล้า ช่วงเดือนเมษายน พ.ศ. 2547 ถึงเดือนมิถุนายน พ.ศ. 2547 ซึ่งจะได้รับการตรวจภายในโดยใส่เครื่องมือถ่างช่องคลอด ไม่ใช้สารหล่อลื่น แล้วเก็บสารคัดหลั่งในช่องคลอดบริเวณ 1/3 ส่วนล่างของผนังช่องคลอดไม่ใกล้ปากมดลูก นำมาป้ายบนสไลด์สะอาดทิ้งไว้ให้แห้ง เพื่อนำไปย้อมสีแกรมเก็บไว้อ่านผล และนำไปทดสอบ BVBLUE test*

ผลการศึกษา: *ความสัมพันธ์ระหว่างการย้อมสีแกรมกับ BVBLUE test ในการวินิจฉัยแบคทีเรียลวาจิโนสิส พบว่า sensitivity, specificity, accuracy, positive และ negative predictive value ร้อยละ 94, 96, 96, 86 และ 98 ตามลำดับ*

สรุป: *การวินิจฉัยแบคทีเรียลวาจิโนสิส โดยการทดสอบด้วย BVBLUE นั้น มี sensitivity, specificity, accuracy, positive และ negative predictive value ร้อยละ 94, 96, 96, 86 และ 98 ตามลำดับ ค่อนข้างสูง*