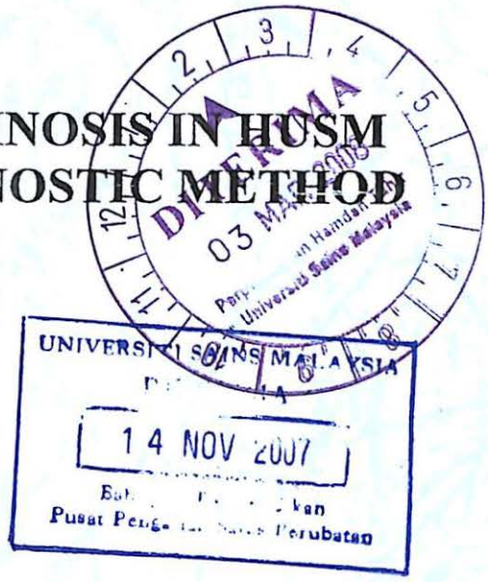

A STUDY OF BACTERIAL VAGINOSIS IN HUSM TOWARDS A STANDARD DIAGNOSTIC METHOD

Dr. AZURA BINTI HUSSIN

Dissertation Submitted In Partial Fulfillment Of The Requirement For The Degree Of Master Pathology (Microbiology)

UNIVERSITI SAINS MALAYSIA
2006

SENARAI SEMAKAN UNTUK BUKU LAPORAN AKHIR GERAN USM JANGKA PENDEK

NAMA PENYELIDIK UTAMA	:	DR. ADIBAH IBRAHIM
NAMA CO-RESEARCHER	:	DR. SITI SURAIYA MD. NOOR/ DR. FAUZIAH MOHD. IDRIS
TAJUK GERAN	:	A study of Bacterial Vaginosis in HUSM- Towards a Standard Diagnostic Method
NO.AKAUN	:	304/PPSP/6131386

SENARAI SEMAKAN SEMASA PENYERAHAN BUKU LAPORAN AKHIR
(Sila Tandakan (4) Pada Kotak Yang Berkenaan)

NO.	PERKARA	ADA	TIADA
1.	Borang Laporan Akhir Projek Penyelidikan USM Jangka Pendek	/	
2.	Borang Laporan Hasil Penyelidikan, PPSP	/	
3.	Salinan Menuskrip	/	
4.	Penyata Perbelanjaan (Financial Statement)	/	
5.	Laporan Komprehensif (termasuk kertas persidangan atau seminar dan penerbitan saintifik hasil daripada projek ini)	/	
6.	Surat pemakluman penghantaran Laporan Akhir ke Bhg. Penyelidikan	/	

Nota: * No. 1-5 - Perlu dimasukkan dalam Buku Laporan Akhir
* No.6 - Hantar terus Kepada Pn. Che Merah Ismail (RCMO) hanya salinan kepada Bhg. R&D, PPSP

My doc/checklist borang2/sue

UNIVERSITI SAINS MALAYSIA

LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA *PENDEK*

FINAL REPORT OF SHORT TERM RESEARCH PROJECT

Sila kemukakan laporan akhir ini melalui Jawatankuasa Penyelidikan di Pusat Pengajian dan Dekan/Pengarah/Ketua Jabatan kepada Pejabat Pelantar Penyelidikan

1. **Nama Ketua Penyelidik:** **DR. ADIBAH IBRAHIM**
   Name of Research Leader

   ☐ Profesor Madya/ *Assoc. Prof.* ☑ Dr./ *Dr.* ☐ Encik/Puan/Cik *Mr/Mrs/Ms*

2. **Pusat Tanggungjawab (PTJ):** **PUSAT PENGAJIAN SAINS PERUBATAN**
   School/Department

3. **Nama Penyelidik Bersama:** **DR. SITI SURAIYA MD. NOOR/ DR. FAUZIAH MOHD. IDRIS**
   Name of Co-Researcher

4. **Tajuk Projek:**
   Title of Project

   A Study of Bacterial Vaginosis in HUSM- Towards a Standard Diagnostic Method

5. **Ringkasan Penilaian/***Summary of Assessment*:	Tidak Mencukupi *Inadequate* 1	2	Boleh Diterima *Acceptable* 3	Sangat Baik *Very Good* 4	5
i) **Pencapaian objektif projek:** *Achievement of project objectives*	☐	☐	☐	☑	☐
ii) **Kualiti output:** *Quality of outputs*	☐	☐	☐	☑	☐
iii) **Kualiti impak:** *Quality of impacts*	☐	☐	☑	☐	☐
iv) **Pemindahan teknologi/potensi pengkomersialan:** *Technology transfer/commercialization potential*	☐	☐	☑	☐	☐
v) **Kualiti dan usahasama :** *Quality and intensity of collaboration*	☐	☐	☑	☐	☐
vi) **Penilaian kepentingan secara keseluruhan:** *Overall assessment of benefits*	☐	☐	☑	☐	☐

6. Abstrak Penyelidikan

(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris.** Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

Abstract of Research
(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English).
This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

ABSTRACT (ENGLISH):

Introduction: The diagnosis of Bacterial Vaginosis (BV) is commonly established by using the Amsel's criteria, which is based mainly on clinical presentation. However, BV was often under diagnosed when the clinical criteria are used because of its inherent subjectivity. A more objective method like the Nugent's criteria is more reliable especially in asymptomatic patients. This study is performed to study the prevalence of BV among pregnant women in HUSM and to evaluate its accuracy comparing to the standard Amsel's criteria. **Methodology:** A cross sectional study was randomly conducted among pregnant women, attending Obstetric and Gynaecology Clinic in HUSM for six months duration. The prevalence of BV was determined by the Amsel's and Nugent's criteria. The agreement of the two criteria was observed and the validity of the Nugent's criteria was evaluated using the Amsel's criteria as gold standard. **Results:** 270 patients were recruited into the study. The prevalence of bacterial vaginosis using the Amsel's criteria was 1.0 % (95% CI) and 2.6% (95% CI) by Nugent's criteria. The sensitivity, specificity, positive predictive value and negative predictive value of the Nugent's criteria, using the Amsel's criteria as gold standard was 100%, 98.5%, 42.9% and 100%, respectively, with moderate agreement between the two criteria (k=0.594 and p=<0.001). **Conclusion:** The prevalence of BV among pregnant women in HUSM by using the Amsel's criteria is slightly lower (1.0%) compared to the Nugent's criteria (2.6%). Both criteria could be used to diagnose BV, however Nugent's criteria is more standardized, objective and simple to perform, thus more suitable especially for research purpose.

ABSTRAK (BAHASA MALAYSIA)

Pendahuluan: Diagnosa Bakteria Vaginosis (BV) dibuat berdasarkan criteria Amsel yang berpandukan menifestasi klinikal. Insiden penyakit ini didapati jauh lebih rendah berbanding insiden sebenar kerana ketidakupayaan membuat diagnosa di kalangan mereka yang tidak mempunyai simtom. Penggunaan Nugent criteria mungkin boleh mengatasi masalah tersebut. Kajian ini dibuat untuk mendapatkan prevalen BV di kalangan ibu mengandung di HUSM dan membuat perbandingan keberkesanan diagnosa BV menggunakan criteria Nugent dan Amsel. **Kaedah:** Ia merupakan kajian cross sectional secara rawak di kalangna ibu mengandung di Klinik Ibu, HUSM, selama 6 bulan. Swab vagina kesemua pesakit diambil dan diteliti untuk diagnosa BV menggunakan kedua- dua criteria, dan perbandingan keduanya dibuat. **Keputusan:** 270 pesakit telah mengambil bahagian di dalam kajian ini. Prevalen BV di kalangna ibu mengandung di HUSM didapati sebanyak 1.0% (95% CI) menggunakan criteria Amsel dan 2.6% (95% CI) menggunakan criteria Nugent. Kriteria Nugent didapati 100% sensitive, 98.5% specific, 42.9% positive predictive value dan 100% negative predictive value (k=0.594 dan p= < 0.001). **Kesimpulan:** Kedua- dua criteria boleh digunakan untuk diagnosa BV, tetapi criteria Nugent lebih sesuai digunakan untuk kajian kerana ia lebih standard, objektif dan mudah dilakukan.

7. Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.
[Sila gunakan kertas berasingan]
Applicant are required to prepare a Comprehensive Technical Report explaning the project.
(This report must be appended separately)

Senaraikan kata kunci yang mencerminkan penyelidikan anda:
List the key words that reflects your research:

Bahasa Malaysia	**Bahasa Inggeris**
Bakteria Vaginosis	Bacterial Vaginosis
Diagnosa	Diagnosis
Kriteria Nugent	Nugent's criteria

8. Output dan Faedah Projek
Output and Benefits of Project

(a) * **Penerbitan Jurnal**
Publication of Journals
(Sila nyatakan jenis, tajuk, pengarang/editor, tahun terbitan dan di mana telah diterbit/diserahkan)
(State type, title, author/editor, publication year and where it has been published/submitted)

1. *Dissertation submitted in Partial Fulfillment of the requirements for the degree of Master of Pathology (Microbiology)*
2. *Presentation:*
   *Azura H, **Adibah I**, Fauziah I, Rosliza AR, Siti Suraiya MN. Study of demographic factors in Kelantanese Pregnant Women with Bacterial Vaginosis. 2nd National Conference on Infectious Diseases: Immunocompromised Host: Challenges in Management, on 14- 15 February 2007 at Grand Reverview Hotel, Kota Bharu, Kelantan*

   *Azura H, **Adibah I**, Fauziah I, Rosliza AR, Siti Suraiya MN. Evaluation of the Nugent's Criteria and the Amsel's Criteria in the Diagnosis of Bacterial Vaginosis. Presented at School of Medical Sciences, Kubang Kerian, Kelantan in 2006*
3. *Publication:*
   In the process for publication in Singapore Medical Journal

Dr Adibah Ibrahim

From: em.singaporemedj.0.6e3ad.157f5631@editorialmanager.com on behalf of Singapore Medical Journal [smj@sma.org.sg]
Sent: Tuesday, November 13, 2007 10:32 AM
To: dradibah@kck.usm.my
Subject: Submission Confirmation for A Study of bacterial vaginosis in HUSM towards a standard diagnostic method .

Dear Dr Ibrahim,

Your submission entitled "A Study of bacterial vaginosis in HUSM towards a standard diagnostic method ." has been received by the Singapore Medical Journal.

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author. The URL is http://singaporemedj.edmgr.com/.

Username: AIbrahim-578
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Your manuscript will be given a reference number once an Editor has been assigned.

Thank you for submitting your work to this journal.

Kind regards,

Editorial Office
Singapore Medical Journal

(b) **Faedah-faedah lain seperti perkembangan produk, pengkomersialan produk/pendaftaran paten atau impak kepada dasar dan masyarakat.**
State other benefits such as product development, product commercialisation/patent registration or impact on source and society.

NIL

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(c) **Latihan Sumber Manusia**
Training in Human Resources

i) Pelajar Sarjana:
Graduates Students
(Perincikan nama, ijazah dan status)
(Provide names, degrees and status)

Dr. Azura Husin, MD, Master of Pathology (Microbiology), completed the course and was graduated in June 2007

ii) Lain-lain:
Others

NIL

9. Peralatan yang Telah Dibeli:
Equipment that has been purchased

1. *Gloves*
2. *High vaginal swab sticks*
3. *pH paper strip*
4. *Gram stain solution*
5. *Glass slides*
6. *Pottasium hydroxide solution*

(Details of the expanses is as attached)

DR. ADIBAH IBRAHIM
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Tandatangan Penyelidik
Signature of Researcher

11 November 2007

Tarikh
Date

Komen Jawatankuasa Penyelidikan Pusat Pengajian/Pusat
Comments by the Research Committees of Schools/Centres

* Mohon dpd penyelidik name jurnal yg. telah dihantar manuskrip & apakah statusnya?.

* Period peredaran yg dibeli, jika ada.

Laporan akhir OK

ASSOC. PROF. MUSTAFFA MUSA
Chairman of Research Committee
School of Medical Sciences
Health Campus
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TANDATANGAN PENGERUSI
JAWATANKUASA PENYELIDIKAN
PUSAT PENGAJIAN/PUSAT
Signature of Chairman
[Research Committee of School/Centre]

19/2/08

Tarikh
Date

BORANG LAPORAN HASIL PENYELIDIKAN
PPSP

Tajuk geran: **A Study of Bacterial Vaginosis in HUSM- Towards a Standard Diagnostic Method**
Penyelidik: **Dr. Adibah Ibrahim, Dr. Azura Hussin, Dr. Fauziah Mohd. Idris, Dr. Suraiya Md. Noor**
Jenis geran: **Geran Jangka Pendek**
Tempoh geran: **2 tahun (1 Jun 2005 hingga 31 Mei 2007)**

Jenis laporan: Laporan Kemajuan ☐ Alatan di beli ☐ Ya:nyatakan.....

Laporan Akhir*: ☑ ☑ Tidak

OBJEKTIF SPESIFIK KAJIAN (sama spt dalam proposal asal)	**SECARA RINGKAS TERANGKAN PENCAPAIAN/HASIL**	**OBJEKTIF TERCAPAI ATAU TIDAK**
1. To study the prevalence of BV among pregnant women in HUSM using Nugent's criteria	The prevalence of BV in HUSM using Nugent's criteria was 2.6% (95% CI), comparing to its prevalence of 1.0% (95% CI) using Amsel's criteria.	Objective of the study was fully achieved
2. To evaluate the effectiveness or accuracy of gram stained method (Nugent's criteria) with currently practice method (Amsel's criteria) in diagnosing BV	The sensitivity, specificity, positive predictive value and negative predictive value using Nugent's criteria in diagnosing BV (using Amsel's criteria as the gold standard) was noted to be 100%, 98.5%, 42.9% and 100% respectively. There was a moderate agreement between the Amsel's and Nugent's criteria (k= 0.594 and p= < 0.001) in diagnosing BV. In conclusion, both Nugent's and Amsel's criteria could be used to diagnose BV. In view of its high sensitivity and specificity, together with the way the test is performed which is more standardized, objective and simple, it is recommended that Nugent's criteria to be used for the diagnosis of BV.	Objective was achieved.
3.		
4.		

- *Laporan Akhir perlu disertakan salinan manuskrip dan surat yang dihantar kepada mana-mana jurnal untuk penerbitan.*

Nama Penyelidik Utama (PI): **DR. ADIBAH IBRAHIM**
Tarikh: 11 November 2007

t.t.: Adibah Ibrahim

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SALINAN MANUSKRIP

A Study of bacterial vaginosis in HUSM towards a standard diagnostic method

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A Study of bacterial vaginosis in HUSM towards a standard diagnostic method

ABSTRACT

Introduction. Bacterial vaginosis is a common cause of vaginitis, causing many complications to the mother and foetus. The diagnosis of Bacterial Vaginosis is commonly established by using clinical presentation and microbiological tests. However, because of its inherent subjectivity it is often misdiagnosed. A more objective method like the Nugent's criteria is more reliable especially in evaluating asymptomatic population. The aims of this study are to determine the prevalence of bacterial vaginosis among pregnant women, using Amsel's and Nugent's criteria, to compare the agreement between the two criteria and to determine the validity of the Nugent's criteria to diagnose Bacterial Vaginosis.

Methodology. A randomised cross sectional study was conducted among pregnant women for six months. High vaginal swabs were performed to test for pH, Amine's test, clue cells and gram stains. The prevalence of bacterial vaginosis using both Amsell's and Nugent's criteria was determined, together with the analysis for the agreement between the two test and the sensitivity and specificity of Nugent's test.

Results: Among the 270 patients recruited, only 1.0% were noted to have bacterial vaginosis using Amsell's criteria and 2.6% using Nugent's criteria. There was a moderate agreement between the Amsel's and Nugent's criteria (k = 0.594 and p = <0.001) in diagnosing Bacterial Vaginosis. Nugent's criteria was 100% sensitive, 98.5% specific, with positive and negative predictive values of 42.9% and 100% respectively.

Conclusion: Both criteria were noted to be as effective to diagnose bacterial vaginosis. However, Nugent's criteria was found to be more objective and standardized compared to Amsell's criteria.

Keywords: Bacterial Vaginosis, Nugent's criteria, Gram staining

Introduction

Bacterial vaginosis (BV) is a syndrome of disease, characterized by a shift in the vaginal flora from the dominant flora of *Lactobacillus* spp to a mixed or polymicrobial vaginal flora that includes *Gardnerella vaginalis* (*G.vaginalis*), *Bacteroides* spp, *Mobiluncus* spp and *Mycoplasma hominis*. Among these organisms, *G.vaginalis* and *Mobiluncus* spp have been most specifically associated with disease process.

BV is increasingly recognized as a health risk with significance beyond the discomfort and annoyance of a localized vaginal infection. It is the commonest cause of vaginal discharge and is one of the leading causes for preventable preterm birth. Additionally, women with BV are more likely to present with preterm birth, pre-labour rupture of membranes (PROM) and preterm pre-labour rupture of membranes (PPROM). They are also at higher risk to develop chorioamnionitis, post partum endometritis and post-caesarean wound breakdown. Effective treatment of BV during pregnancy reduces the preterm birth rate by 30% to 50%[1,2]

These undesirable effects of BV warrant a good diagnostic tool for its diagnosis. Since 1984, the Amsel's criteria have been widely used to diagnose BV. With this criteria, the patient has to fulfill three out of four different criteria; 1) typical homogenous vaginal discharge, 2) vaginal

pH above 4.5, 3) positive Amine's test, and 4) presence of clue cells seen microscopically. These diagnostic criteria have sensitivity and specificity of 70% and 94%, respectively if gram-stain (of the Nugent's criteria) is considered as gold standard[3]. However, these criteria are not applicable to asymptomatic patients. Therefore, criteria that are more objective are introduced.

An alternative method of diagnosis BV, the Nugent's criteria has been used extensively, particularly in research studies, is the grading or scoring of microbial flora in gram-stained smear of vaginal fluid. This method reflects both the change in vaginal ecology and the microbial associations.

This criteria exclude the presence of typical homogenous vaginal discharge, the use of Amine's test and presence of clue cells microscopically which depend very much of individual experience and skills.

It was believed that by using a more objective diagnostic method to diagnose BV, we are able to pick up more cases of BV, so that early treatment could be started, thus prevent the complications of BV. If it is proven that using the Nugent's criteria is better than the conventional method, which is the Amsel's criteria, it is therefore suggested that this criteria (Nugent's criteria) to be used should screening for BV is needed.

OBJECTIVES

The objectives of this study are as follows:

1. To determine the prevalence of bacterial vaginosis among pregnant women in Hospital Universiti Sains Malaysia (HUSM) using the Amsels and Nugent's criteria

2. To compare the agreement between Amsel's and Nugent's criteria in diagnosing BV
3. To determine the validity of Nugent's criteria in diagnosing BV

METHODOLOGY

This is a cross sectional study conducted in the antenatal clinic of the Department of Obstetrics and Gynaecology of HUSM after the ethical approval from The Scientific and Research Ethical Committee, School of Medical Sciences, Universiti Sains Malaysia (USM/PPSP®/Ethics Com./2005(140.3[1]). The sample size was calculated based on single proportion formula, where the prevalence of BV was taken as 17.9% (taken from the incidence of BV in HUSM in 1998). Pregnant women, regardless of the period of amenorrhoea, whether or not they have any symptoms to suggest BV, did not have any vaginal bleeding, not receiving any antibiotic recently and not having any sexual intercourse within the past 24 hours were randomly selected using the block of 3 sampling method. Those women who were in labour were excluded. Consent was taken from each patient.

A speculum examination was performed with the patient lying in supine position with both legs flexed at the hip and knee joints. The vulva was cleaned using cotton swab soaked with normal saline solution. A sterile cuscow speculum was gently inserted into the vagina and the blades were separated to visualize the vagina and the cervix. Any evidence of vaginal discharge was further examined for its appearance and smell. Smooth and gentle swabbing done on the mid vaginal wall with two consecutive sterile swabs.

A thin smear of 2-3 cm in diameter was made immediately on the glass slide using the first swab and allowed to air-dried at room temperature. The same swab was transferred on to another glass

slide, which was submersed in sterile normal saline and covered with a cover slip. All smeared slides were arranged in a slide box prior to transportation to the Medical Microbiology Laboratory, HUSM for gram staining and clue cells examination, respectively.

The second HVS sample was used to test for pH and conducting the Amine's test. Both procedures were done immediately by the bed site.

To test for the pH, the second swab was rolled on the Universal Indicator Paper- HmbG. The reaction between the vaginal fluid was allowed to proceed for 1 minute before result was read. The change of pH paper colour was compared to the universal indicator supplied together with the pH paper as guideline.

After testing for pH, several drops of 10% potassium hydroxide (KOH) solution was then added on the swab, for detection of strong fishy odor. The presence of this odour indicates a positive Amine's test.

At the Medical Microbiology Laboratory , the smeared slides were fixed using flame for 1 to 2 seconds upon their arrival. Gram staining was later performed. The slide was read by the principal investigator and counter checked by a senior and experienced technologist under light microscope at 100X magnification (oil immersion). The identification of the bacterial was as follows. A large gram positive rods (i.e. lactobacillus morphotypes), a small gram negative to gram variable rods (i.e. *G.vaginalis* and *Bacteroides* spp. morphotypes) and a curve gram

negative rods (i.e. *Mobiluncus* spp. morphotypes). The score given to each slide was based on the scoring system mentioned in Table I.

This slide was later examined under light microscope under 40X magnification for the presence of any clue cells, which are epithelial cells studded by bacilli.

All the findings of the above procedures were documented in the study form. Based on the Amsel's criteria, BV was diagnosed if the patient fulfilled three out of the four criteria i.e. typical vaginal discharge, vaginal pH of above 4.5, positive Amine test and the presence of clue cells microscopically.

Using the Nugent's criteria, the scoring system (Table I) was used. BV was diagnosed when the score of 7 and above was obtained. Scores between 4 to 6 was considered as intermediate and score of 0 to 3 was considered as normal or negative for BV.

Patients with BV were treated with the standard regime of antibiotic for 2 weeks.

The data obtained was analysed as follows:

1. The prevalence of BV using the Nugent's and Amsel's criteria was statistically calculated by SPSS version 12.0.
2. The agreement of both criteria was calculated using kappa (k) value. The k value and level of the agreement is as shown in Table II.

3. The validity of Nugent's criteria was assessed by its sensitivity, specificity and predictive values, with the Amsel's criteria used as gold standard.

RESULTS

Demographic data

Among the 270 subjects, Malays constitute 91.9% (248 patients) of them, which was followed by the Chinese (17 patients, 6.3%), Indians (2 patients, 0.8%) and other races like Siamese and Kadazan (3 patients, 1.1%). Subject's age ranged from 23 to 73 year old, with mean age was 30.37 years (SD : 6.358). 68 out of 270 (25.2%) subjects involved were primigravida whereas 137 (50.7%) were multiparity (second to fifth pregnancy) and 65 (24.1%) were grandmultiparity (sixth and above pregnancy).

Prevalence of BV

Only three out of 270 subjects fulfilled all the Amsel's criteria (1.1%, CI = 95%) for BV. In contrast, seven subjects were positive for BV by the Nugent's criteria (2.6%, CI = 95%). Table III summarized the findings of using both criteria.

Level of agreement between the Amsel's criteria and Nugent's criteria for diagnosing BV

Smeared slides of the subjects in the normal (or negative) group by the Amsel's criteria were reevaluated with the Nugent's criteria. All the slides were also found to fulfill the Nugent's criteria.

However, four cases which were BV positive by the Nugent's criteria were found normal (or negative) by the Amsel's criteria. Thus, only moderate agreement could be shown between the Amsel's and the Nugent's criteria (Table IV).

Comparison of clinical presentation in BV cases diagnosed by Amsel's and Nugent's criteria

Presence of homogenous vaginal discharge was only found in two BV cases diagnosed by either the Amsel's or Nugent's criteria. Asymptomatic presentation (i.e. no vaginal discharge) was picked up more by using the Nugent's criteria compared to of Amsel's criteria (Table V)

Validity of the Nugent's criteria

Based on Table VI, the sensitivity of Nugent's criteria was calculated as follows:

$$\text{Sensitivity} = \frac{\text{True Positive}}{\text{True positive} + \text{False negative}} \times 100\%$$

$$= \frac{3}{3} \times 100\%$$

$$= 100\%$$

Its specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated as below:

$$\textbf{Specificity} = \frac{\text{True negative}}{\text{True negative} + \text{False positive}} \times 100\%$$

$$= \frac{263}{267} \times 100\%$$

$$= 98.5\%$$

$$\text{PPV} = \frac{\text{True positive}}{\text{True positive} + \text{False positive}} \times 100\%$$

$$= \frac{3}{7} \times 100\%$$

$$= 42.9\%$$

$$\text{NPV} = \frac{\text{True negative}}{\text{True negative} + \text{False negative}} \times 100\%$$

$$= \frac{263}{263} \times 100\%$$

$$= 100\%$$

DISCUSSION

The diagnosis of BV by using the Amsel's criteria incorporates clinical findings in its criteria as compared to the Nugent's criteria, which are based mainly on laboratory findings. Currently, the methods of choice for the diagnosis of BV are by using clinical criteria; Amsel's citeria and laboratory examination (gram stain); Nugent's criteria[4,5].

However, the individual parameters in the Amsel's criteria may be subjectively interpreted and this situation may contribute to misdiagnosis of BV cases. The accuracy of the test also depends on the expertise, the experience, the training and the education of the performer or the clinician[6] . Furthermore, clinical signs are very difficult to be standardized between clinicians and may be impossible to interpret during certain pregnancy situations, such as when the patient is having

antepartum haemorrhage and is in labour[7] .Therefore, a more standardized and widely used method i.e. the Nugent's criteria is introduced.

Besides giving a permanent record and a direct look (via light microscope) of bacterial morphotype, gram stain of the Nugent's criteria provides a shift in vaginal flora and density of bacterial involved in BV. Furthermore, the permanent record of bacterial morphotypes on smear makes it possible for it to be reassessed by other centres, which addresses the question of the reliability of this method of diagnosis[7].

The scoring system of the Nugent's criteria (refer Table I) provides a brief description of weighted combination morphology of organisms involved; lactobacilli, *G.vaginalis* or *Bacteroides* (small gram-variable rods or gram negative rods) and curved gram-variable rods. For example, the score of 0 indicates the predominance of lactobacillus and infrequence of *Gardnerella*, *Bacteroides* and *Mobiluncus* bacterial morphotypes whereas the score of 10 indicates the replacement of the lactobacilli with other morphotypes[7].

In addition, the gram stain score (by the Nugent's criteria) has been shown to correlate well with the clinical signs of BV, including raised pH, positive Amine's test and clue cells[7]. Moreover, it also provides a sensitive method for the diagnosis of BV. It has both a low false negative and a high negative predictive value too, making it an ideal diagnostic test[7].

In this study, the prevalence of BV by using the Amsel's criteria is lower (1.1%) as compared to the Nugent's criteria (2.6%). This result may be caused by the subjective nature which is

inherent in the evaluation of the Amsel's criteria. The Nugent's criteria, when compared to the Amsel's criteria as gold standard, is found to be highly sensitive (100%) and specific (98.5%)[6,7].

Schwebke *et al.* compared the Nugent's criteria for gram stain to the Amsel's criteria and they found that the former test is highly sensitive (89%)[3]. The lower specificity (83%) of the Nugent's criteria suggests that some positive gram stain results are false positive although the bacterial morphotypes are suggestive of BV. The reason is that the patient fails to fulfill the Amsel's criteria due to a spectrum of symptomatology in BV's patient, i.e. the changes of the bacterial morphotypes on the gram stain may occur without a development of the symptom.

Despite its excellent sensitivity, specificity and high negative predictive value (100%), there is a low positive predictive value (42.9%) in this study. The low PPV in this study, in turn, may be caused by the low prevalence of bacterial vaginosis cases in our hospital setting which result in false positive cases.

In this study, the Amsel's criteria (which are used as gold standard) are moderately comparable to the Nugent's criteria; with a k agreement of 0.594 and a p of less than 0.001, which is significant. When we compare the symptom presents among both criteria, it is statistically significant in the Amsel's criteria, with a p value of 0.015 (less than 0.05), using Fisher's Exact Test rather than in Nugent's criteria ($p = 0.008$).

CONCLUSIONS

Of the diagnostic methods currently available, assessment of clinical signs (the Amsel's criteria) is the "gold standard" and many clinicians continue to rely on it for the diagnosis of BV despite the inaccuracies of this approach.

With a moderate agreement between the Amsel's criteria and the Nugent's criteria, both tests are found to be acceptable methods for the diagnosis of BV. The Nugent's criteria have high sensitivity, specificity and NPV. The criteria are also standardized, simple to prepare and more objective. Therefore, the Nugent's criteria are highly recommended for the diagnosis of BV. However, its implementation still requires the usage of trained personnel to get a good microscopic evaluation.

ACKNOWLEDGEMENT

We would like to give our greatest appreciation to the Scientific and Research Ethical Committee, School of Medical Sciences, Health Campus, Kubang Kerian, Kelantan, Malaysia for approving us to proceed with this study, and the University Science of Malaysia for financing this study under its Short Term Grant (304/PPSP/6131386)

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Table I: Scoring vaginal gram stains for bacterial vaginosis using the Nugent's criteria

Organism morphotype[a]	Number of organisms/oil immersion field	Score[b]
Lactobacillus-like (parallel-sided, gram positive rods)	>30	0
	5-30	1
	1-4	2
	<1	3
	0	4
Mobiluncus-like (curved, GNB)	>5	1
	0	0
Gardnerella/Bacteroides-like (tiny, gram variable coccobacilli and rounded, pleomorphic, GNB with vacuoles)	>30	4
	5-30	3
	1-4	2
	<1	1
	0	0

Table I: Scoring vaginal gram stains for bacterial vaginosis using the Nugent's criteria

Organism morphotype[a]	Number of organisms/oil immersion field	Score[b]
Lactobacillus-like (parallel-sided, gram positive rods)	>30	0
	5-30	1
	1-4	2
	<1	3
	0	4
Mobiluncus-like (curved, GNB)	>5	1
	0	0
	>30	4
Gardnerella/Bacteroides-like (tiny, gram variable coccobacilli and rounded, pleomorphic, GNB with vacuoles)	5-30	3
	1-4	2
	<1	1
	0	0

Table II: Relationship of kappa value and level of agreement

Kappa (k) value	Level of agreement
0.00-0.2	Poor
0.21-0.4	Fair
0.41-0.6	Moderate
0.61-0.8	Good
0.81-1.0	Very good

Table III : Summary of the BV cases with their clinical presentation and laboratory findings according to Amsel's and Nugent's criteria

Number of cases	Amsel's criteria					Nugent's criteria
	Homogenous vaginal discharge		pH test	Amine test	Clue cell	Gram stain (suggestive of BV)
	Present	Not present				
1	+		+	+	+	+
2		+	+			+
3		+	+			+
4		+	+		+	+
5		+	+			+
6	+		+	+	+	+
7		+	+			+

Table IV :Level of agreement between the Amsel's and Nugent's criteria in diagnosis of BV

Patients	Nugent's criteria (BV status)	Amsel's criteria (BV status)	Statistic value
1	+	+	
2	+	+	k value = 0.594
3	+	+	$p < 0.001$
4	+	-	
5	+	-	
6	+	-	
7	+	-	

Table V : Comparison for the presence of symptom of patients between Nugent's criteria and Amsel's criteria

Variable	Patient's symptom (homogenous vaginal discharge)		Statistical analysis
	Symptomatic	Asymptomatic	
Nugent's criteria	2	5	$p = 0.088^{*}$
Amsel's criteria	2	1	$p = 0.015^{*}$,

*Presence of vaginal discharge is significant for the Amsel's criteria but not significant for the Nugent's criteria

Table VI: Showed the summary of the cases

Nugent's criteria		Amsel's criteria		Total
		Positive BV	Negative BV	
	+	3 (TP)*	4 (FP)*	7
	-	0 (FN)*	263 (TN)*	263
Total		3	267	270

*TP : true positive (Patients who were positive by Amsel and Nugent's criteria)
*TN : true negative (Patients who were negative by Amsel and Nugent's criteria)
*FP : false positive (Patients who were negative by Amsel's criteria but positive by Nugent's criteria)
*FN : false negative (Patients who were positive by Amsel's criteria but negative by Nugent's criteria)

UNIVERSITI SAINS MALAYSIA
JABATAN BENDAHARI
KUMPULAN WANG PENYELIDIKAN GERAN USM(304)
PENYATA PERBELANJAAN SEHINGGA 31 OKTOBER 2007

Jumlah Geran:	RM	Tiada Rekod	Ketua Projek:	DR. ADIBAH IBRAHIM
Peruntukan 2005 (Tahun 1)	RM	2,857 00	Tajuk Projek:	A Study of Bacterial Vaginosis in HUSM- Towards a Standard Diagnostic Method
Peruntukan 2006 (Tahun 2)	RM	2,857 00		
Peruntukan 2007 (Tahun 3)	RM	0 00	Tempoh:	01 Jun 05-30 Nov 07
			No.Akaun:	304/PPSP/6131386

Kwg	Akaun	PTJ	Projek	Donor	Peruntukan Projek	Perbelanjaan Tkumpul Hingga Tahun Lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
304	11000	PPSP	6131386		-	-	-	-	-	-	-
304	14000	PPSP	6131386		-	-	-	-	-	-	-
304	15000	PPSP	6131386		-	-	-	-	-	-	-
304	21000	PPSP	6131386		-	615.40	(615.40)	-	-	-	(615.40)
304	22000	PPSP	6131386		-	-	-	-	-	-	-
304	23000	PPSP	6131386		300.00	-	300.00	-	-	-	300.00
304	24000	PPSP	6131386		-	-	-	-	-	-	-
304	25000	PPSP	6131386		-	-	-	-	-	-	-
304	26000	PPSP	6131386		2,857.00	-	2,857.00	-	-	-	2,857.00
304	27000	PPSP	6131386		564.00	173.60	390.40	110.00	2,156.00	338.60	(1,875.60)
304	28000	PPSP	6131386		-	-	-	-	-	-	-
304	29000	PPSP	6131386		1,993.00	2,064.50	(71.50)	-	200.00	200.00	(271.50)
304	32000	PPSP	6131386		-	-	-	-	-	-	-
304	35000	PPSP	6131386		-	-	-	-	-	-	-
					5,714.00	2,853.50	2,860.50	110.00	2,356.00	538.60	394.50

LAPORAN

KOMPREHENSIVE

PROJEK

Geran projek ini yang bernilai RM 5714.00 telah diperuntukkan mulai 1 Jun 2005 sehingga 31 Mei 2007, setelah mendapat kelulusan daripada komiti etika Universiti Sains Malaysia. Agihan pertama bernilai RM2857.00 telah diperolehi pada permulaan projek. Lanjutan daripada itu, projek dimulakan dengan persediaan borang- borang persetujuan pesakit menyertai projek serta borang pengisian data. Setelah itu, pengrekrutan pesakit- pesakit telah dibuat sebagaimana protokol. Kami telah menggunakan peralatan- peralatan pakai buang seperti sarung tangan (glove), high vaginal swab sticks, kertas pH, slide kaca, larutan pottasium hydroxide dan larutan keperluan gram staining yang berada di hospital sementara menanti order dari syarikat- syarikat berkenaan. Peralatan- peralatan tersebut kemudiannya telah diganti semula apabila ia telah diperolehi daripada syarikat. Agihan kedua projek bernilai RM2857.00 telah diperolehi kemudian, dan telah digunakan untuk pembiayaan bekalan bahan mentah. Perbelanjaan peruntukan geran secara terperinci adalah sebagaimana di Jadual 1.

Pengrekrutan pesakit dan pengambilan data telah mengambil masa sehingga bulan February 2006 dan ia telah disusuli dengan penganalisaan data yang telah mengambil masa sehingga bulan Julai 2006. Keseluruhan hasil projek ini telah disiapkan di dalam bentuk disertasi pada bulan Oktober 2006.

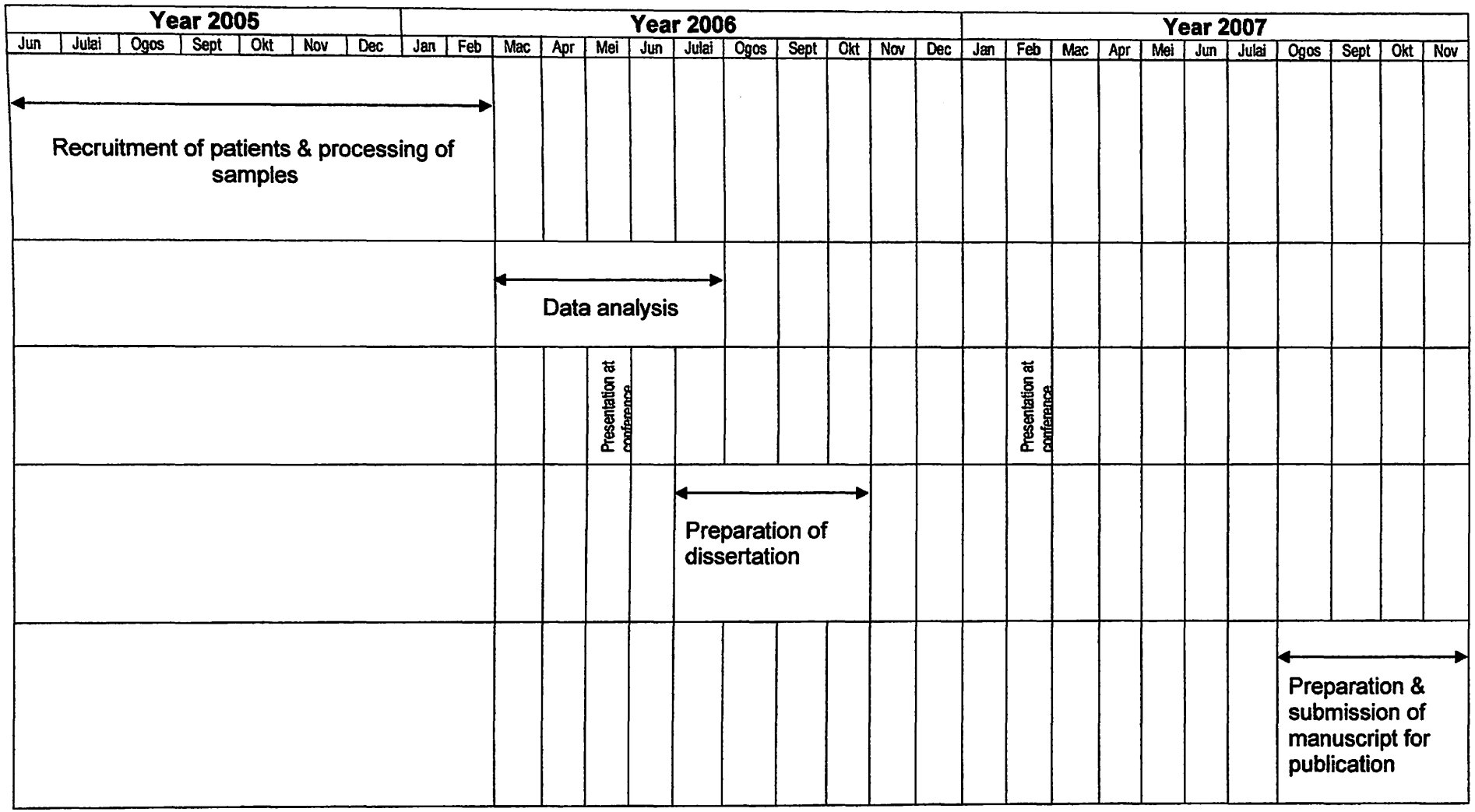
Hasil projek ini telah dibentangkan di dalam dua persidangan iaitu 'The 11th National Conference of Medical Sciences' dan '2nd National Conference on Infectious Diseases', sebagai persembahan poster. Abstrak projek ini bagi persidangan 'The 11th National Conference of Medical Sciences' telah dimuatkan di dalam The Malaysian Journal of Medical Sciences, Volume 13 Supplementary 1(2006). Selain daripada itu, projek ini juga telah dihantar ke Singapore Medical Journal untuk dipertimbangkan untuk tujuan penerbitan. Salinan daripada penerimaan journal tersebut adalah sebagaimana yang dilampirkan.

Untuk melunaskan pembayaran kepada syarikat pembekalan bahan mentah yang masih belum terlunas, tempoh tamat projek telah dilanjutkan kepada 30 November 2007.

Jadual I: Perbelanjaan projek secara terperinci

Peruntukan geran		**RM5714.00**
Akaun	**Perbelanjaan**	**Jumlah**
Vot 21000	Pendaftaran konferen dan pembiayaan percetakan poster	RM615.40
Vot 26000 dan Vot 27000	Pembiayaan bekalan bahan mentah (sarung tangan, kertas pH, slide kaca, swab sticks, larutan potasium hydroxide dan larutan untuk gram staining	RM2439.60
Vot 29000	Percetakan borang keizinan pesakit, borang data serta pemberian honororium pesakit	RM 2264.50
Jumlah perbelanjaan		RM5319.50
Baki geran		**RM394.50**

Jadual II: Milestone Projek



PB-23

EVALUATION OF NUGENT SCORE AND EACH AMSEL CRITERIA IN THE DIAGNOSIS OF BACTERIAL VAGINOSIS

Azura H*, Adibah I, Rosliza AR*, Siti Suraiya MN* Fauziah I***

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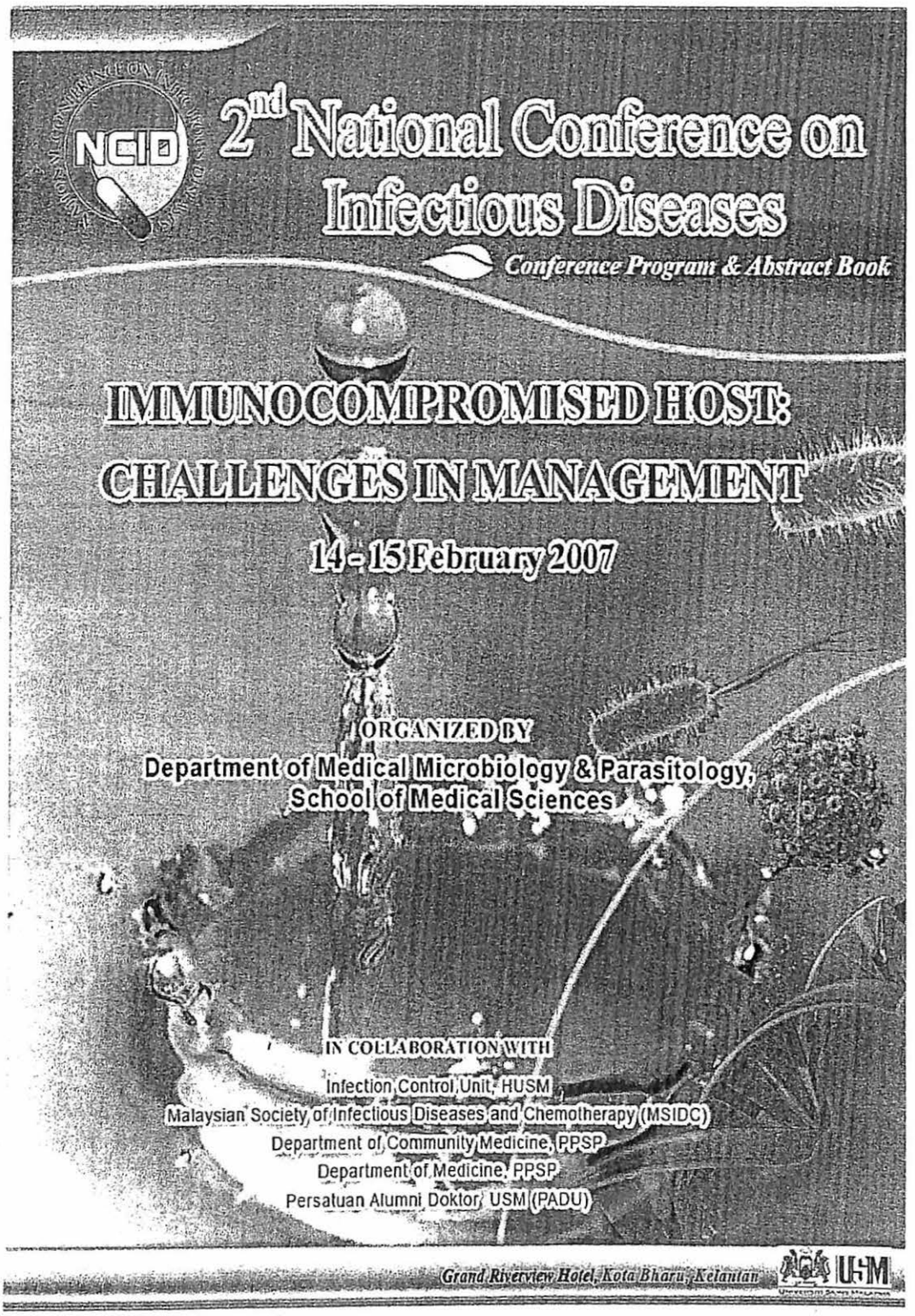
PURPOSE :
The aim of this study is to determine the prevalence of bacterial vaginosis (BV) among pregnant women using Nugent's criteria as a standard method of diagnosis. The second objective is to compare the agreement between Amsel criteria and Nugent score in the diagnosis of BV.

METHOD :
A cross sectional study was conducted among consented 270 pregnant women who attended antenatal check-up in Hospital USM, Kelantan (HUSM). Clinical data consisting of vaginal pH, "Amine test," clue cells, and appearance of the vaginal discharge (Amsel criteria) were compared with the vaginal fluid Gram stain (Nugent score) for the diagnosis of bacterial vaginosis. Using Amsel criteria as a gold standard, sensitivity, specificity, positive predictive value and negative predictive value of Nugent score and Amsel criteria were estimated.

RESULT :
The prevalence of bacterial vaginosis among anteriatal patients in HUSM was 3%. Nugent score showed a sensitivity of 100%, specificity of 98.5%, positive predictive value (PPV) of 42.9% and negative predictive value (NPV) of 100%. There was an agreement between Amsel criteria and Nugent score ($k = 0.594$ and $p = <1.0$) in diagnosing BV.

CONCLUSION :
Bacterial vaginosis is present in a significant proportion of pregnant women in this study and the use of Nugent score is recommended for its diagnosis because of its high sensitivity and specificity.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IN COLLABORATION WITH
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Department of Community Medicine, PPSP
Department of Medicine, PPSP
Persatuan Alumni Doktor USM (PADU)
Grand Riverview Hotel, Kota Bharu, Kelantan
USM

A STUDY OF DEMOGRAPHIC FACTORS IN KELANTANESE PREGNANT WOMEN WITH BACTERIAL VAGINOSIS

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Bacterial vaginosis (BV) is a syndrome of disease characterized by a shift of normal vaginal flora, to a mixed pathogenic flora resulting in symptoms of vaginal discharge and burning sensation. Ethnicity, low socio economic status and multiple sexual partners are some demographic factors that could put women at increased risk of acquiring BV. A study to observe the association of demographic factors of pregnant women in Kelantan with bacterial vaginosis was conducted prospectively from January to December 2006.

270 pregnant women attending their antenatal visit in Hospital USM were randomly selected, and questionnaires were distributed for completion to the patients. Sociodemographic profile data including ethnicity, number of sexual partner, number of pregnancy and education level was elicited. Vaginal samples from lateral vaginal wall were collected from consented patients for examinations of BV.

By using the Nugent's criteria, only seven subjects (2.6%, CI = 95%) were positive for BV. Five (71.4%) of them were Malays and two (28.6%) were from Chinese and Indian ethnic group respectively. Two of the seven patients (28.6%) with BV have only primary educational level whereas majority (5/7) has secondary and tertiary educational level. All of the patients have single sexual partner and both primigravida and multiparous women were equally affected, four (57.1%) and three (42.9%) respectively.

No significant associations were observed in this study between BV and sociodemographic data among pregnant women in Kelantan. However, further study with a more representative samples of Kelantanese women should be conducted.

A STUDY OF BACTERIAL VAGINOSIS IN HUSM TOWARDS A STANDARD DIAGNOSTIC METHOD

Dr. AZURA BINTI HUSSIN

Dissertation Submitted In Partial Fulfillment Of The Requirement For The Degree Of Master Pathology (Microbiology)

UNIVERSITI SAINS MALAYSIA
2006

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A Study of bacterial vaginosis in HUSM towards a standard diagnostic method

ABSTRACT

Introduction. Bacterial vaginosis is a common cause of vaginitis encountered in most obstetric and gynaecology clinic. It is characterized by a replacement of the *Lactobacillus* sp. in the vagina with a mixed and high concentrations of aerobic and anaerobic bacteria that comprises *G.vaginalis, Bacteroides* spp., *Mobiluncus* spp. and *Mycoplasma hominis.*

The diagnosis of BV is commonly established by using clinical presentation and or microbiological tests. However, BV is often misdiagnosed when the clinical criteria are used because of its inherent subjectivity. A more objective method like the Nugent's criteria is more reliable method of diagnosis BV especially in evaluating asymptomatic population.

A symptomatic BV cases among pregnant women, if left untreated may cause many complications, such as spontaneous abortion, preterm labor, premature birth and preterm premature rupture of the membranes. Due to the importance of reducing in many of the adverse events associated with BV, an appropriate screening method that is easy, simple to perform, reliable, sensitive and specific is required.

Objectives. The aims of this study are to determine the prevalence of bacterial vaginosis (BV) among pregnant women in HUSM, using the Amsel's and Nugent's criteria, to

compare the agreement between the Amsel's and Nugent's criteria, and to determine the validity of the Nugent's criteria in the diagnosis of BV.

Methodology. A cross sectional study was randomly conducted of pregnant women, attending Obstetric and Gynaecology Clinic in HUSM for six months duration. The prevalence of BV was determined by the Amsel's and Nugent's criteria. The agreement of the two criteria was observed and the validity of the Nugent's criteria was evaluated using the Amsel's criteria as gold standard.

Results. We enrolled 270 pregnant women whose mean age was 30.4 (SD: 6.4). In diagnosing BV, only three had bacterial vaginosis by definitive both the Amsel's and Nugent's criteria, whereas three was positive by the Amsel's criteria and seven by the Nugent's criteria.

The prevalence of bacterial vaginosis using the Amsel's criteria was 1.0 % (95% CI) and 2.6% (95% CI) by Nugent's criteria. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the Nugent's criteria for the diagnosis of bacterial vaginosis using the Amsel's criteria as gold standard was 100%, 98.5%, 42.9% and 100%, respectively. There was a moderate agreement between the Amsel's and Nugent's criteria ($k = 0.594$ and $p = <0.001$) in diagnosing BV.

Conclusions. The prevalence of BV among pregnant women in HUSM by using the Amsel's criteria is slightly lower (1.0%) compared to the Nugent's criteria (2.6%). Both the Amsel's and Nugent's criteria are acceptable methods for use in the diagnosis of BV.

However, subtle differences exist between two methods that may limit the use of the Amsel's criteria. In addition to its high sensitivity and specificity, the Nugent's criteria, are more standardized, objective and simple to perform. Thus, it is highly recommended that the Nugent's criteria be used for the diagnosis of BV.

ABSTRAK

Pengenalan. Bakteria vaginosis merupakan masalah keradangan vagina yang kerap dihadapi oleh pesakit di klinik obstetrik dan ginekologi. Bakteria vaginosis adalah disebabkan oleh penggantian normal flora di vagina iaitu *Lactobacillus* spp. kepada mikro-organisma aerobik dan anaerobik seperti *G.vaginalis*, *Bacteroides* spp., *Mobiluncus* spp. dan *Mycoplasma hominis* yang kebiasaannya tidak terdapat di vagina.

Pengdiagnosan bakteria vaginosis kebiasaannya dibuat secara klinikal atau melalui ujian mikrobiologi. Walaubagaimanapun, disebabkan subjektiviti pemeriksaan klinikal yang menyumbang ke arah ketidaktepatan pengdiagnosan kes-kes bakteria vaginosis, kriteria Nugent disarankan untuk diagnosis bakteria vaginosis kerana ia boleh digunapakai terutama untuk kes-kes ketiadaan gejala.

Kes-kes yang simptomatik bakteria vaginosis di kalangan wanita hamil, jika tidak dirawat akan menyebabkan komplikasi-komplikasi terutamanya keguguran, kelahiran pramatang, dan pemecahan selaput amnion pramatang. Kajian klinikal menunjukkan kadar penurunan komplikasi disebabkan bakteria vaginosis apabila pengesanan dan rawatan awal diberikan. Oleh itu, jenis ujian yang mudah, tepat, sensitif dan spesifik adalah perlu.

Objektif. Di antara tujuan-tujuan kajian ini adalah untuk mengetahui kadar prevalens bakteria vaginosis dikalangan wanita hamil di HUSM, menggunakan kriteria Amsel dan

Nugent disamping mendapatkan perbandingan diantara kriteria Nugent dan kriteria Amsel dan untuk mengenalpasti validiti kriteria Nugent dalam pengdiagnosan bakteria vaginosis.

Metodologi. Kajian silangan bagi tempoh enam bulan telah dijalankan dikalangan wanita-wanita hamil yang mendapat rawatan antenatal di Klinik Obsteterik dan Ginekologi, HUSM. Prevalens bakteria vaginosis diperolehi dengan menggunakan c
kriteria Amsel dan Nugent. Persetujuan antara kedua-dua kriteria telah telah diperolehi. Evaluasi validiti kriteria Nugent dibuat dengan menggunakan kriteria Amsel sebagai standard rujukan.

Keputusan. Kami telah mendapatkan persetujuan seramai dua ratus dan tujuh puluh orang wanita hamil, dalam kalangan purata umur 30.4 (SD; 6.4). Hasil daripada kajian ini, hanya tiga kes yang positif untuk kedua-dua kriteria. Melalui kriteria Amsel, hanya tiga kes yang positif, berbanding tujuh kes positif menggunakan kriteria Nugent.
Prevalens bakteria vaginosis menggunakan kriteria Nugent dan Amsel masing-masing adalah 2.6% (95% CI) dan 1.0% (95% CI).
Menggunakan kriteria Amsel sebagai standard rujukan, kiraan sensitiviti, spesifisiti, prediktif positif dan prediktif negatif kriteria Nugent adalah seperti beikut; 100%, 98.5%, 42.9% dan 100%.
Berdasarkan kajian kami, kriteria Nugent dan Amsel telah mencapai perbandingan yang sederhana dalam pengdiagnosan bakteria vaginosis ($k = 0.594$ dan $p < 0.001$).

Kesimpulan. Prevalens bakteria vaginosis dikalangan wanita-wanita hamil di HUSM adalah lebih rendah (1.1%, CI = 95%) menggunakan Kriteria Amsel berbanding Kriteria Nugent (2.6%, CI = 95%). Walaupun terdapat sedikit perbezaan di antara kriteria Amsel dan Nugent, kedua-duanya boleh digunakan dalam pengdiagnosan bakteria vaginosis. Memandangkan kriteria Nugent lebih sensitif, spesifik, standard, objektif dan mudah dikendalikan, oleh itu penggunaannya amat disarankan dalam pengdiagnosan bakteria vaginosis.

CHAPTER 1

INTRODUCTION

Bacterial vaginosis (BV) is a syndrome of disease, characterized by a shift in the vaginal flora from the dominant flora of *Lactobacillus* spp to a mixed or polymicrobial vaginal flora that includes *Gardnerella vaginalis* (*G.vaginalis*), *Bacteroides* spp, *Mobiluncus* spp and *Mycoplasma hominis*. Among these organisms, *G.vaginalis* and *Mobiluncus* spp have been most specifically associated with disease process.

BV is increasingly recognized as a health risk with significance beyond the discomfort and annoyance of a localized vaginal infection. It is the commonest cause of vaginal discharge and is one of the leading causes for preventable preterm birth (PTB). Additionally, women with BV are more likely to present with preterm birth, pre-labour rupture of membranes (PROM) and preterm pre-labour rupture of membranes (PPROM). They are also at higher risk to develop chorioamnionitis, post partum endometritis and post-caesarean wound breakdown. Effective treatment of BV during pregnancy reduces the PTB rate by 30% to 50% (Morales *et al.*, 1994 and Hauth *et al.*, 1995).

These undesirable effects of BV warrant a good diagnostic tool for its diagnosis. Since 1984, the Amsel's criteria have been widely used to diagnose BV. With this criteria, the patient has to fulfill three out of four different criteria; 1) typical homogenous vaginal discharge, 2) vaginal pH above 4.5, 3) positive Amine's test, and 4) presence of clue cells

seen microscopically. These diagnostic criteria have sensitivity and specificity of 70% and 94%, respectively if gram-stain (of the Nugent's criteria) is considered as gold standard (Schwebke *et al*, 1996). However, these criteria are not applicable to asymptomatic patients with BV. Therefore, criteria that are more objective are introduced.

An alternative method of diagnosis BV, the Nugent's criteria has been used extensively, particularly in research studies, is the grading or scoring of microbial flora in gram-stained smear of vaginal fluid. This method reflects both the change in vaginal ecology and the microbial associations.

This criteria exclude the presence of typical homogenous vaginal discharge, the use of Amine's test and presence of clue cells microscopically which depend very much of individual experience and skills.

It was believed that by using a more objective diagnostic method to diagnose BV, we are able to pick up more cases of BV, so that early treatment could be started, thus prevent the complications of BV. If it is proven that using the Nugent's criteria is better than the conventional method, which is the Amsel's criteria, it is therefore suggested that this criteria (the Nugent's criteria) to be used should screening for BV is needed.

CHAPTER 2

LITERATURE REVIEW

2.1 Definition of bacterial vaginosis (BV)

BV is a clinical entity that is characterized by a change in vaginal ecology where normal flora of Lactobacillus morphotypes is replaced by a mixed microbial flora consisting of anaerobes and *Gardnerella vaginalis* (*G.vaginalis*). This disease was previously known as a non-specific vaginitis or *G.vaginitis*, after the bacteria that were thought to cause the condition. The term "bacterial vaginosis" was agreed to replace the older term because there is no inflammatory process involved in the disease process as evidence by the absence of any polymorphonuclear cells in the vaginal discharge. Furthermore, many anaerobic or facultative anaerobic bacteria are involved in the disease process (Spiegel *et al.*, 1983, Mazzuli *et al.*, 1990 and Hillier *et al.*, 1993).

2.2 Epidemiology of bacterial vaginosis

Bacterial vaginosis, previously known as non specific vaginitis is the most common cause of abnormal vaginal discharge (Priestley *et al.*, 1996). The differences in the definition of BV lead to a wide variation of the prevalence of BV. The uncertain prevalence of BV could be because the condition is so often self-diagnosed and self-treated (Egan and Lipsky, 2000).

2.2.1 Prevalence and incidence

Vaginitis is the most common gynaecologic problem encountered by primary care physicians. It accounts for more than 10 million visits per year, with vaginal discharge being one of the top 25 reasons for which women seek medical care. BV alone accounts for approximately 50% of all cases of vaginitis (Wang, 2000).

As the most common cause of vaginitis, BV affects approximately 3 million women annually, making it as the most common cause of vaginitis (Wang, 2000). It is a disease of childbearing age and any woman is at risk of getting BV. It is more common in women with STDs and in those who have recently changed sex partner as found by Moi *et.al*, (1989) and Wang (2000). Wang (2000) had found BV cases in 10-25% of patients in general gynaecologic and obstetrics clinics and in up to 64% of patients visiting STD clinics. Saharan *et al.*, (1993) in their blinded study among patients attended an out patient department at KEM Hospital, Bombay found that fifty percent of BV cases in their study were asymptomatic. Georgijevic *et al.*, (2000), in their study also found a similar result as Saharan *et al.*,(1993).

BV is less common in prepubertal girls and postmenopausal women (Hill *et al.*, 1983) however, there were reported cases of BV among unmarried group, lesbians and smokers. A reduced rate of BV is seen among monogamous sexual relationship. Women with new or multiple sex partners, as well as women, who douche or use an intrauterine device

(IUD) for birth control, have a higher risk of bacterial vaginosis (Joesoef *et al.*, 2001 and Chiaffarino *et al.*, 2004).

The reported prevalence and incidence rates among different populations vary broadly. The prevalence in the United States, in family planning and student health clinics are 17% to 19%, in sexual transmitted diseases (STD) clinics are 24% to 37% and among pregnant women, of 10% to 29% (Egan and Lipsky, 2000). Similar variations of prevalence rate in other parts of the world are shown in table below.

Table 2.0 : Prevalence of Bacterial vaginosis by center

Center	% positive
Colombia (Bogota)	9.0
Ireland (Dublin)	5.9
Myanmar (Yangon)	15.6
Philippines (Manila)	7.5
Thailand (Bangkok)	12.5
Thailand (Khon Kaen)	11.5
USA (Philadelphia)	5.8
Zimbabwe (Harare)	24.4
Chicago	27.4
Brazil	15
Uganda	> 50
Indonesia	29.9

Adapted from CDC, by Tolosa et *al.* (2001), Reid (2004) and Krohn *et al.*, (1989).

It was suggested that host-cell receptor density, diet or other factors may play a role in the altering the vaginal ecosystem and thereby increasing the risk for BV. Naidu *et al.*, (1991) reported that severe under-nutrition women may has an increased risk of BV. Yasodhara *et*

al., (2005) who found severe under-nutrition women had degradation of immunoglobulin level and this status has a significant association with BV supported this.

It is also reported that some women develop symptoms of BV on cyclical basis for example during menses (Priestly *et al.*, 1996). These findings suggest that hormonal factors may be implicated in the etiology of the condition, most probably by changing the vaginal pH that resulted in increased risk of getting BV. However, in many cases there is no obvious factor predisposing to BV (Priestly *et al.*, 1996).

It is also estimated that, 1 in 3 women will probably get bacterial vaginosis at some time in their life and repeated infections had been reported (Hillier et *al.* 1993). Boris *et al.*, 1997 in their study of long term follow up of women who had been successfully treated, 48% remained BV free and 52% had at least recurrence once. Most relapse cases were during the first year and were significantly correlated with new sexual contacts.

The prevalence of BV in pregnant women is similar to that in the general population. It is reported that approximately 10% - 30% of pregnant women will experience BV during their pregnancy (McGregor and French, 2000). Hillier et *al.* (1993), in his multicenter population study of normal pregnant women found approximately 22% with BV, which left untreated, 66% may persist to the third trimester.

In Malaysia, there was no study done to elucidate the exact prevalence of BV among its population. However, a small prevalence study by Siw in 1998, among pregnant women in

HUSM showed a prevalence of 17.9%. However, his study was not representative BV cases in Kelantan generally and specifically in the Malaysian population.

2.2.2 The mode of transmission

In general, BV occurs more frequently among women who have early initiation of sexual activity, multiple sexual partners and among women with concurrent or prior sexually transmitted infections. However, there is sexual association between BV and STDs, treatment of the male partners does not reduce the recurrence rate following treatment. Additionally, BV can also be found in lesbian couples and even in women never sexually active and in non-abused children.

Even though the role of sexual transmission in BV is still unclear, BV cases reported to be high in sexually transmitted diseases clinics and in women with multiple sexual partners (Egan and Lipsky, 2000). McCormack (2005) also described similar findings, which BV is common in sexually active women and also common in populations with a high prevalence of sexually transmitted diseases. However, the precise contribution of heterosexual transmission to the overall epidemiology of the condition remains controversial (McCormack 2005).

Sexual transmission had been shown to be the mode of transmission especially among lesbian couples (McCormack 2005). Other factors that probably predispose to BV are, 1) sex without a condom; 2) have new sex partners or have more than one sex partner; 3) using too many perfumed soaps or bubble baths; 4) douching; 5) using intrauterine device.

However, direct contact through use of toilet seats, bedding, swimming pools, or from fomites had not been shown to be the mode of transmission in BV (Trenholm, 2002).

2.2.3 Association of BV with HIV infection

BV is more prevalent and persistent among human immunodeficiency virus (HIV) infected women, especially among whom the CD4 T-cell count is low (less than 200 cells/ml) (McCormack 2005). Besides that, BV is postulated to predispose to HIV infection, whereby BV is found to be associated with HIV-1 RNA expression in the genital tract of infected women (McCormack 2005). A low vaginal pH may inhibit CD4 lymphocyte activation and therefore decrease HIV target cells in the vagina and conversely, an elevated pH may make the vagina more conducive to HIV survival and adherence (Hill and Anderson, 1992).

A relation between BV and HIV is further supported by few studies. Martin *et al.*, (1999) in his study among commercial sex workers in Kenya found that those without lactobacilli were more likely to acquire HIV. Other study in Malawi, among 1196 pregnant women with a prevalence of BV of 24% (by the Nugent's criteria), women with BV had an increased risk of HIV seroconversion in both antenatal and postnatal periods (Hillier *et al.*, 1995).

2.2.4 Association of BV with other diseases

Controversies exist regarding the association or causal relation of BV with urinary tract infections, intrauterine device (IUD) usage, chorioamnionitis, late onset miscarriage,

preterm birth, post abortal pelvic inflammatory disease, and post caesarean section endometritis. Some authors believe that a causal relationship has not been proven and suggest that BV is merely a marker of hormonal changes, coexisting infections, or other unidentified risk factors (Egan and Lipsky, 2000). Clark et *al.*, (1994) found that patients with BV were at increased risk of developing chorioamnionitis and puerperal endometritis.

Infections of the deciduas, placenta or amniotic fluid have been consistently associated with preterm delivery. In this regard, evidence shows that BV is associated with an increased frequency of upper genital tract infections, including the chorion, amnion and amniotic fluid (Hillier *et al.*, 1995).

2.3 The pathogenesis of bacterial vaginosis.

2.3.1 Normal vaginal flora

A dynamic balance between lactobacilli and potential pathogens characterizes normal vaginal flora. After puberty, increasing levels of estrogens start to circulate, leading to a proliferation of vaginal epithelial cells and the appearance of lactobacilli. Conversion of glycogen to lactate and the formation of hydrogen peroxide (H_2O_2) from the exfoliated intermediate and superficial epithelial cells cause the pH to decrease from pH 6 to values as low as 3.5 to 4 (Caillouette *et al.*, 1997).

The H_2O_2-producing lactobacilli, which predominates the normal vaginal flora, appear to protect against exogenous infection. Any factors that lead to the change of normal vaginal

milieu; acidic condition, might permits the replacement and decrease the number of protective H_2O_2-producing lactobacilli with *G.vaginalis*, anaerobes and mycoplasmas.

2.3.2 Innate and immunological defense of the vagina

Other than that, immune mechanisms in the vagina are critically important for the prevention of reproductive tract infections (RTIs) and HIV infection (Royce *et al.*, 1997 and Witkin, 1993). The mucosal immune system contributes to the defense of the female reproductive tract. Cervical secretions contain high concentrations of complement, defensins, and immunoglobulins (Igs), which are the first line of defense against the invading pathogens (Kozlowski *et al.*, 1999). IgA secreted in vaginal fluid has been proposed as the main mucosal barrier that protects against sexually acquired HIV. Cauci *et al.*, (1998) have demonstrated an impairment of mucosal immune system in women with BV as evidenced by degradation of IgA and IgM in the vaginal washes. Sialidases produced by the pathogens (*Prevotella* and *Bacteroides* species) have been suggested to be responsible for this degradation.

A cytokines mechanism may controls both stimulation and inhibition of immune response. Local production of cytokines by various cells determines the arm of immune response to be stimulated and may also influence the magnitude of protection available in the genital tract (Kozlowski *et al.*, 1999). Recent studies indicate detectable expression of Th1 and Th2 cytokines in cervico-vaginal secretions (CVS) of normal women (Hedges *et al.*, 1998, Fidel *et al.*, 1997 and Belec *et al.*, 1995) and in those with infections. However, little