Diagnostic role of Pap-smear in patients with vaginal discharge

THESIS
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List of abbreviations

Abbreviation	Meaning
ACOG	American College of Obstetricians and Gynecologists
ACS	American Cancer Society
ALTS	ASCUS/LSIL Triage study
ASC	A typical squamous cells
ASCUS	A typical squamous cells of undetermined significance
ATECC	Atypical endo-cervical columnar cells
BV	Bacterial vaginosis
CIN	Cervical intraepithelial neoplasia
CIS	Carcinoma in situ
CMV	Cytomegalovirus
DVI	Direct visual inspection
FDA	Food and Drug administration
G.V	Gardnerella vaginalis
HHV	Human herpes virus
HIV	Human immunodeficiency virus
HPV	Human Papillomavirus
HSIL	High grade Squamous intraepithelial lesion
ICC	Invasive cervical carcinoma
IUD	Intrauterine device
KOH	Potassium hydroxide
LBC	Liquid-based cytology
LCR	Long control region
LEEP	Loop electrosurgical excision procedure
LLETZ	large loop excision of the transformation zone
LSIL	Low grade Squamous intraepithelial lesion
NCI	National Cancer Institute
OCP	Oral contraceptive pills
PAP	Pap smear Papanicolaou smear
PCR	Polymerase chain reaction
PID	Pelvic inflammatory disease
RVVC	Recurrent vulvovaginal candidiasis
SCC	Squamous cell carcinoma
SCJ	Squamocolumnar junction
STDs	Sexual transmitted diseases
TMI	Time of first marriage
VVC	Vulvovaginal candidiasis

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Review of Literature

Chapter 1

Microbiology of the Vagina

Vaginal microflora

The vagina and cervix of healthy females harbor an indigenous microflora. The normal vaginal flora is fairly homogeneous and consists of aerobic and anaerobic bacteria. The aerobic components include *lactobacilli,* Group B and D *streptococci*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and gram-negative enteric rods such as *Escherichia coli* **(Larsen and Galask, 1980)**.

Anaerobic cocci were reported in 7% to 57% of the cultures, predominantly *Peptostreptococcus asaccharolyticus* and *Peptostreptococcus anaerobius*, anaerobic gram-negative bacilli were isolated from most of the cultures; their isolation rates were between 57% and 65%. The predominant strains were *Prevotella bivia*, *Prevotella disiens* ,*Bacteroides fragilis* group **(Larsen and Galask, 1980)**.

Normal variations in cervico-vaginal flora are related to the effects of age, pregnancy, and menstrual cycle **(Larsen and Galask, 1980)**.

The microflora in females before puberty, during the childbearing years, during pregnancy, and after menopause are not uniform. Colonization with lactobacilli is low in prepubertal females and postmenopausal females and high in pregnant females as well as those females in their reproductive years who are not pregnant **(Brook, 2002)**.

At the time of puberty, *lactobacilli* become the predominant inhabitant of the vagina, presumably because of the effect of estrogens on the glycogen content of vaginal epithelial cells **(Paavonen, 1983)**.

When estrogen levels falls, such as in postpartum or menopausal women, the prevalence of *lactobacilli* declines **(Redonodo-lopez, 1990)**.

A healthy vaginal ecosystem is dominated by certain species of *Lactobacillus*, which exert a significant influence on the microbiology of the vagina, some species of *Lactobacillus* suppress the growth of other endogenous bacteria in the vagina through the production of organic acids such as lactic acid, $H_2 O_2$ and bacteriocins or lactocins **(Antonio, 1999)**.

The production of organic acids maintains the vaginal pH at < 4.5, thereby creating an inhospitable environment for the growth of most endogenous pathogenic bacteria. In addition to lactic acid, the combination of H_2O_2 and lactocins further suppresses the endogenous pathogenic bacteria to maintain a healthy vaginal ecosystem **(Redondo-Lopez, 1990)**.

Ninety-six percent of *Lactobacillus* species found in a healthy vaginal ecosystem produced $H_2 O_2$, whereas only 6% of the *lactobacilli* recovered from women with bacterial vaginosis (BV) produced $H_2 O_2$ **(Eschenbach, 1989)**.

Hillier (1992), has postulated that H_2O_2 inhibits the growth of vaginal microorganisms either directly or through the enhancement of the enzyme peroxidase-halide.

H_2 O_2 produced by lactobacilli was found to be lethal to *Gardnerella vaginalis*, *Bacteroides bivia*, and *Escherichia coli* ***(*****Klebanoff ,1991)**.

Hawes et al (1996), found that vaginal colonization with H_2 O_2-producing *lactobacilli* was associated with a decrease in the occurrence of BV.

A third component to this system is the production of bacteriocin or lactocin, which are proteins that have bactericidal activity, can inhibit adverse group of bacteria, including *G vaginalis* **(Brink, 1994)**.

Bacteriocin produced by vaginal *lactobacilli* appears to have a broad spectrum of activity and inhibits a wide range of gram-positive and gram-negative bacteria **(McGroaty,1993).**

McLean and McGroarty (1996), suggested that lactic acid and low pH work synergistically with bacteriocin and may be more important than hydrogen peroxide in inhibiting the growth of *G vaginalis*.

Aroutcheva et al (2001), suggest that the number of bacteria (biocell mass) in the vaginal environment is responsible for pH changes. The **biocell mass** or numbers of bacteria are significant with regard to the quantity of organic acids produced. Thus, the mere presence of lactobacilli and their ability to produce lactic and other organic acids is not the only important factor in maintaining a healthy vaginal ecosystem—also important is the active growth and the increase in number of bacteria present.

(Newton et al, 2001) showed that several factors appear to have a major impact on the vaginal flora:

1) Hormones, especially estrogen, which support the growth of at least

Lactobacillus and *Candida* species.

2) The menses, which appears to increase the growth of non-*Lactobacillus* flora.

3) STDs influence the growth of non-STD bacteria in the vagina

4)Genetic differences, lead to variations in the attachment of bacteria to epithelial cells, which in turn influence the ability of the bacteria to compete with other microorganisms.

5) The interactions among microbial species, exogenous STD microorganisms and non-STD microorganisms in the vaginal flora.

Vaginal cycle

The transient hormonal changes which occur each month over the course of the menstrual cycle affect vaginal flora.:

Estrogen increases the vaginal epithelial activity resulting in thickened epithelium with glycogen deposited in the superficial cells, the glycogen is metabolized by *lactobacilli* to lactic acid resulting in vaginal acidity of about PH 4.5 **(Wilson, 2004)**.

Several studies have suggested that abnormal vaginal flora develop most frequently at the beginning of the menstrual cycle when the estrogen levels are lowest and the tendency for Gram- positive cocci to appear mostly at the beginning of the menstrual cycle was also noticed **(Keane, 1997)**.

Bacterial vaginosis occurs most commonly at the beginning of the menstral cycle when estradiol levels are at their lowest **(Wilson, 2007)**.

Estradiol levels increase during the follicular phase of the menstrual cycle which may account for mid- cycle spontaneous remission of bacterial

vaginosis and the high level of spontaneous remission of BV during pregnancy **(Hay, 1997)**.

A microbiological study throughout the menstrual cycle found the rate of recovery of *lactobacilli* increased over the cycle and the concentration of non lactobacilli species was higher during menstruation **(Eschenbach, 2000)**.

Perimenopausal and postmenopausal women have low estrogen levels and they have been found to have high rates of abnormal vaginal flora at 35%and 70% **(Taylor-Robinson, 2002)**.

Etiology of vaginal discharge:

1- Normal physiologic discharge

Normal vaginal secretions are composed of vulvar secretions from sebaceous, sweat, bartholin ,and skene glands; transudate from the vaginal wall; exfoliated vaginal and cervical cells; cervical mucous; endometrial and oviductal fluids and micro-organisms and their metabolic products .Normal vaginal secretions are floccular in consistency,white in colour ,and usually located in the dependent portion of the vagina (posterior fornix). The type and amount of vaginal secretions are influenced by hormonal levels, vaginal secretions may increase in the middle of the menstrual cycle because increase in the amount of cervical mucus **(Huggins and Preti, 1981)**.

2- Pathological vaginal discharge:

Causes of Vaginal discharge are many & can be summarized in this table:

Table (1): Causes of vaginal Discharge

Non-infective

-Physiological

-Cervical ectopy

-Foreign bodies, such as retained tampon

-Vulvual dermatisis

Non-sexually transmitted infection

-Bacterial vaginosis

-*Candida* infections

Sexually transmitted infections

- *Chalamydia trachomatis*

-*Neisseria gonorrhoeae*

-*Trichomonas vaginalis*

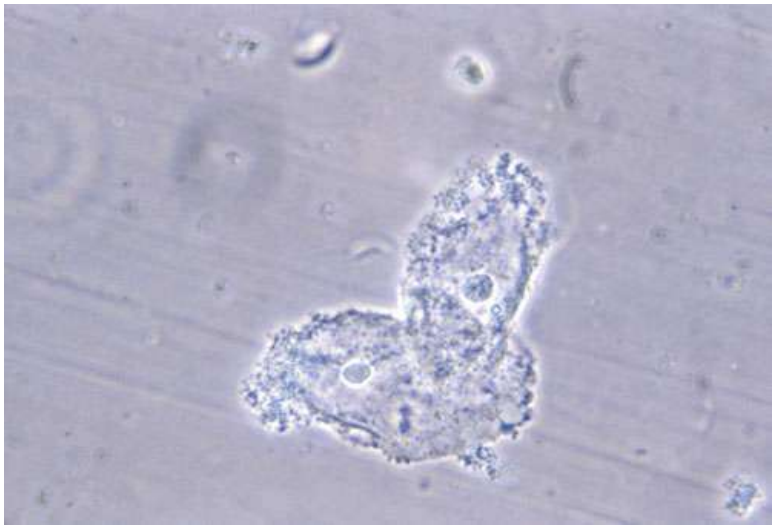
Quoted from: Spence and Melville 2007

Bacterial vaginosis:

Epidemiology

Bacterial vaginosis is the most prevalent cause of symptomatic vaginitis in sexually active women. At same time, 50% of the total number of bacterial vaginosis infections are asymptomatic **(Schwebke, 2003).** Bacterial vaginosis is characterized by shift of vaginal flora from normal lactobacilli dominant to flora with greatly reduced numbers of lactobacilli and an overgrowth of *Gardnerella vaginalis*, *Mycoplasma hominis*, and anaerobic

bacteria such as *peptostretococci*, *Prevotella* spp and *Mobiluncus* spp **(Wilson,2004)**.



Figure(1):Clue cells are epithelial cells with bacteria adhering to their surface and sometimes obscuring their borders. Clue cells indicate bacterial vaginosis ***(Library of the Centers for Disease Control and Prevention).***

Risk factors of bacterial vaginosis*:*

1- Young age **(Ralph, 1999)**.

2- Black ethnicity **(Goldenberg, 1996)**.

3- Douching **(Hawes, 1996)**.

4- IUD as contraception **(Avonts, 1990)**.

5- Recent change in sexual partner **(Hawes, 1996).**

6-Multiple partners compared with one partner **(Nillson, 1997)**.

7-Smoking **(Schwebke, 1999),**cigarette smoke contains chemicals like nicotine which demonstrated in cervical mucus of smokers and may directly alter the vaginal microflora or may act by depleting Langerhans cells in cervical epithelium leading to local immunosuppression. This may be responsible for change of cervical flora causing bacterial vaginosis.

Etiology of Bacterial vaginosis*:*

Bacterial vagionosis is not a monoetiologic infection but rather a synergistic polymicrobial disequilibrium of the vaginal ecosystem .The exact mechanism for the onset of bacterial vaginosis remains a mystery. It is associated with a reduction in hydrogen peroxide producing lactobacilli, a rise in the vaginal pH, and the overgrowth of BV associated organisms. But which of these happen first, and which is the most is still a matter of debate **(Wilson, 2004)**.

1) *Reduction in the lactobacilli and hydrogen peroxide production*

(Klebanoff et al, 1990), showed in vitro that combining myeloperoxidases with hydrogen peroxide and a halide produced a potent oxidant, which was toxic to BV associated bacteria. Myeloperoxidases activity has been found in vaginal fluid and cervical mucus, and chloride is present in cervical mucus. The toxin effect of this LB+/myeloperoxidase/chloride system was rapid, with reduction in numbers of *G vaginalis* at 15 minutes and complete loss of viability at 60 minutes.

(Pavlova et al, 1997) suggest that associated organisms are sensitive to lactic acid and hydrogen peroxide, suppression of *lactobacilli* must come before overgrowth of BV associated bacteria .

2) *Change in pH:*

The low pH of the vagina (3.2- 4.8) is attributed to production of lactic acid by *lactobacilli* metabolism, and by the conversion of glycogen to lactic acid by estrogenished vaginal epithelial cells **(Wilson, 2004)**.