# BACTERIAL VAGINOSIS IN PREGNANCY

**Thesis**

**Submitted for the Partial Fulfillment of Master Degree in Clinical and Chemical Pathology**

**By**

**Ghada Abdel Wahed Ismail**
**(M.B., B.Ch.)**



**Supervisors**

**PROF. DR. IBRAHIM KHALIL ALY**
**Professor of Clinical Pathology**
**Faculty of Medicine, Ain Shams University**

**PROF. DR. ALAA EL-DIN EL-ETRIBY**
**Professor of Gynecology and Obstetrics**
**Faculty of Medicine, Ain Shams University**

**DR. NEVINE NABIL KASSEM**
**Lecturer of Clinical Pathology**
**Faculty of Medicine, Ain Shams University**

**FACULTY OF MEDICINE**
**AIN SHAMS UNIVERSITY**
**1993**

بسم الله الرحمن الرحيم

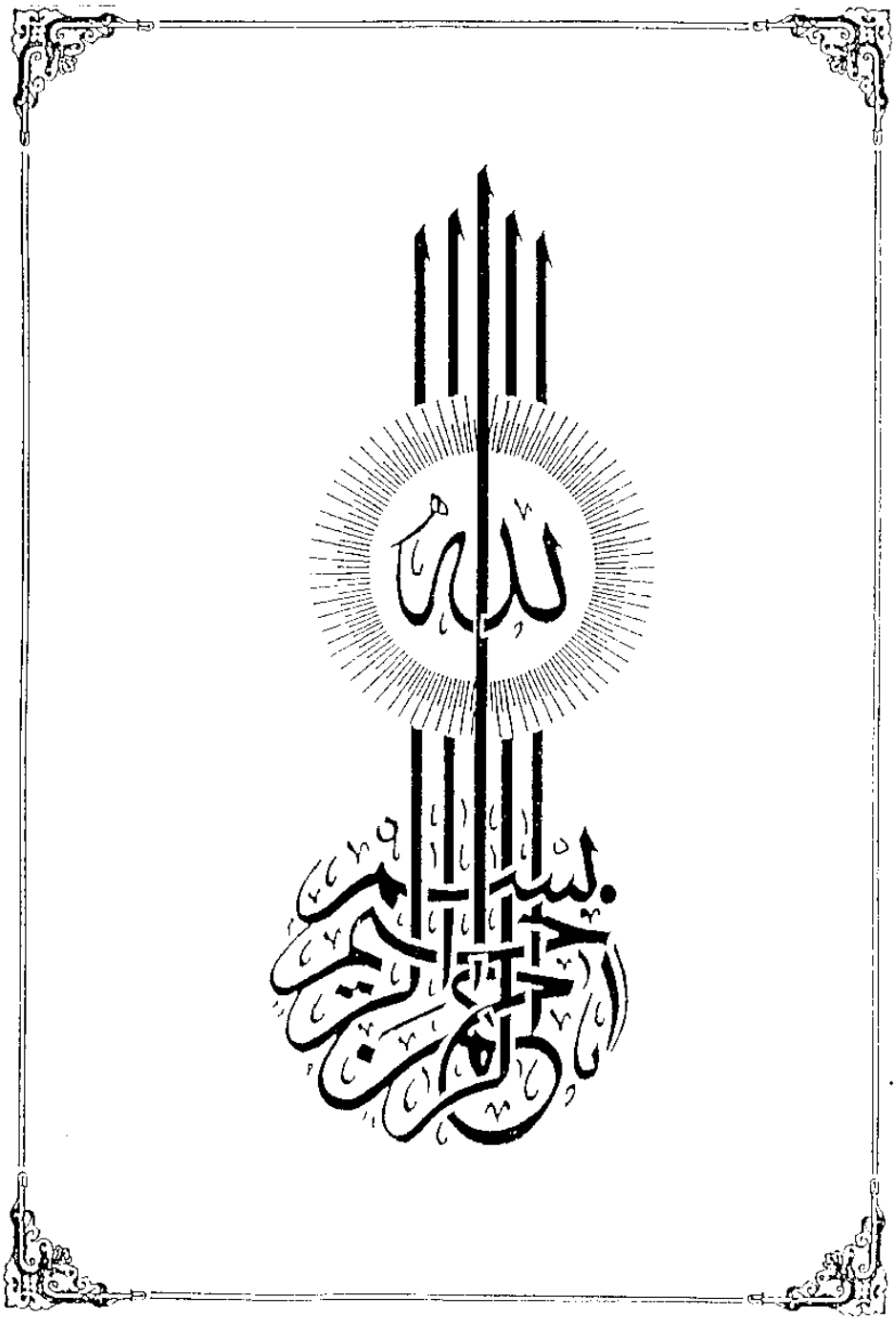
اقرأ باسم ربك الذي خلق

خلق الإنسان من علق

اقرأ وربك الأكرم الذي علم بالقلم

علم الإنسان ما لم يعلم

العلق ١-٥



# CONTENTS

# INTRODUCTION AND AIM OF THE WORK

# INTRODUCTION

Bacterial vaginosis is a syndrome which is diagnosed clinically by homogenous grey vaginal discharge, vaginal pH >4.5, positive amine odour test and the presence of clue cells. Microbiologically, bacterial vaginosis is characterised by a shift in the vaginal flora from the dominant flora of Lactobacillus species to a mixed vaginal flora that includes Gardnerella vaginalis (Nugent et al., 1991).

Gardnerella vaginalis was the commonest organism constantly isolated from the vaginal secretions of patients with non-specific vaginitis (Yong and Thompson, 1982). Gardnerella vaginalis produces larger amount of phospholipase A2 than other bacteria, an enzyme capable of initiating prostaglandin synthesis by cleavage of arachidonic acid, the prostaglandin precursor which is thought to be the central hormone that stimulate labour (Gravett et al., 1986). Gardnerella vaginalis can occur as an endogenous organism in the vaginal flora in a population of healthy women but are numerically increased in women with bacterial vaginosis in whom they are present in most cases.

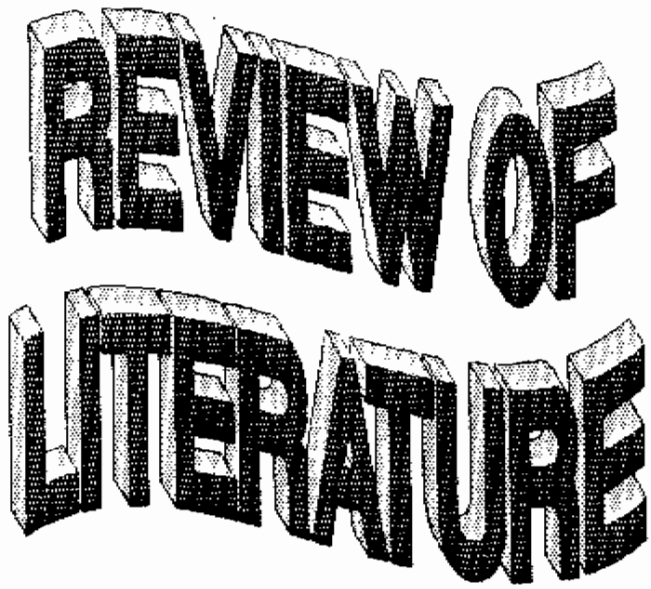
Although pregnant women with and without bacterial vaginosis had similar demographic and obstetric factors, neonates born to women with bacterial vaginosis had lower

mean birth weight that did neonates born to women without bacterial vaginosis. Bacterial vaginosis is significantly associated with premature rupture of membrane, premature labour and amniotic fluid infection (Gravett et al., 1986).

The importance of an accurate reproducible inexpensive laboratory method to diagnose bacterial vaginosis has increased with the recent association of vaginosis with placental infection and premature delivery. A reliable standardized method of gram stain interpretation provides graduation of the disturbance of vaginal flora. A new scoring system that uses the most reliable morphotypes from the vaginal smears was proposed for diagnosing bacterial vaginosis. This standardized score also facilitates further research concenring bacterial vaginosis because it provides graduations of the disturbance of vaginal flora which may be associated with different levels of risk for pregnancy complications (Nugent et al., 1991).

## AIM OF THE WORK

The aim of this work is to establish an inexpensive and reproducible laboratory methods to diagnose bacterial vaginosis by using the new scoring criteria based on gram stain.



# THE NORMAL VAGINAL ECOSYSTEM

**Normal microflora:**

The healthy human body harbour an extensive number of microorganisms that inhabit surfaces and cavities exposed or connected to the external environment. Each area of the body has a unique collection of microorganisms. The genera and species of bacteria detected are dependent on the physiologic conditions present in that particular area. These microbes are collectively called "normal microflora" (Finegold, 1977).

Only those microbes with genetic and biochemical properties that allow the best use of existing environmental conditions will survive and replicate and thus become part of the normal microflora, thus the microflora is not a static population, but in a dynamic state where types and population levels detected continually fluctuate with the changing environment (Lopez et al., 1990).

It is important to understand the composition of the normal microflora for two reasons. First, some of these microbes have a physiologic function, second, their disruption can sometimes result in or predispose to infection (Drason and Hill, 1974).

Although most of the micro-organisms are not pathogenic where they are normally found, their presence can occasionally result in local disease or in disease at locations distant from the site of colonization under circumstances that enhance their virulence expression, e.g., immunosuppressive, damage to the mucosal barrier and the presence of foreign bodies (Lopez et al., 1990).

**The vaginal microflora:**

The vaginal microflora and its unique microflora form a finely balanced ecosystem, with the vaginal environment controlling the microbial types present and the microflora in turn control the vaginal environment (Stahl and Hill, 1986). The ecosystem changes in response to external stimuli such as hormones level variations (Lopez et al., 1990).

Since the end of the 19th century the normal vaginal flora of women in the fertile age has been considered to be dominated by Lactobacilli, the Doderlein bacteria properly consisting of several Lactobacillus species. Lactobacillus is the most prevelant organism in the normal vaginal flora, although quantitative and qualitative studies of such flora have demonstrated that many other facultative and obligate anaerobic organisms are present in high concentrations (Larsen and Galask, 1980). Diphtheroid bacilli comprise most of the remaining facultative gram-positive rods in the

female genital tract, these organisms are considered to be of low virulence. Since the early report of Doderlein, 1894. Diphtheroids have been considered to play a protective role in the female genital tract preventing colonization by more virulent species (Lopez et al., 1990).

The normal vaginal flora often include group B Hemolytic Streptococci, anaerobic Streptococci. Bacteroides species, Clostridia, Gardnerella vaginalis, Ureaplasma urealyticum and sometimes Listeria or Mobiluncus species. In some women the introitus contains a heavy flora resembling that of the perineum (Jawetz et al., 1991).

**Factors controlling the normal vaginal microflora:**

**A) Age:**

The vagina of the newly born child is sterile, organisms make their appearance in 12 - 14 hours after birth. At first, they consist of Staphylococci, Enterococci and Diphtheroids, but these are often replaced in 2 or 3 days by a practically pure culture of Lactobacilli. At this time, glycogen is demonstrable in the vaginal epithelium, and the vaginal secretion itself is acidic. The occurrence of glycogen appears to be due to the presence of oestrogen derived from the maternal circulation. Soon, this is excreted in urine, glycogen is no longer demonstrable in the epithelium, the Lactobacilli disappears and the vaginal

secretion reverts towards alkalinity. From now on till puberty, the vaginal secretion remains alkaline, and there is varied microbiota, the most common organisms being S.epidermidis and Diphtheroid bacilli, followed by alpha-hemolytic Streptococci and Lactobacilli, and in smaller numbers still by non-hemolytic Streptococci and E.coli. (Hammerschlag et al., 1978).

At puberty, glycogen is again deposited in the vaginal wall, the secretion becomes acidic, thereby preventing the establishment of potentially harmful microorganisms and the Lactobacillus establishes itself as the predominant anaerobic organism together with Streptococci, Diphtheroid, Fungi and yeasts (Linton, 1990).

After menopause, Lactobacilli again diminish in number and a mixed flora returns (Linton, 1990).

**B) Lactobacilli:**

Lactobacilli are the most prevelant and often the most numerous organisms in the normal vagina. The predominant species isolated are Lactobacillus acidophilus and Lactobacillus fermentum with Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus jensenii, Lactobacillus casei, Lactobacillus delbrueckü and Lactobacillus salivarius

isolated less frequently (Rogosa et al., 1960 and Wylie et al., 1969).

In contrast, Giorgi and his colleagues in 1987 using DNA-DNA homology, suggested that the identification of Lactobacillus acidiphilus was erroneous and the Lactobacillus crispatus, Lactobacillus jensenii, Lactobacillus fermentum and Lactobacillus gassari were the predominant vaginal species.

Since the first description of Lactobacilli by Doderlein it has been widely assumed that Lactobacilli normally present in the vagina against the overgrowth of potentially pathogenic endogenous flora and exogenous pathogens. The pathogenesis of bacterial vaginosis (BV) is thought to be due to the elimination or reduction of antibacterial activity expressed by endogenous vaginal Lactobacilli (Skarin and Sylwan, 1986). Lactobacilli inhibit the in vivo growth or occurrence of BV by G.vaginalis, Mobiluncus, Peptostreptococcus and Bacteroides. Similarly, Candida vaginalis following systemic antibiotic therapy has been attributed to loss of the protective vaginal population of Lactobacilli. Several mechanisms have been suggested for how Lactobacilli control the vaginal ecosystem, but each remains unproven (Lopoz et al., 1990).

**Possible control mechanisms by Lactobacilli:**

1) Low vaginal pH:

Low vaginal pH is believed to be a primary mechanism controlling the composition of the vaginal microflora. Although lactic acid produced during lactobacilli metabolism may contribute to vaginal acidity it is not necessarily the primary flora controlling source of low vaginal pH. Fatty acids, including lactic acid produced by vaginal epithelial cells and released into the secretions may be a more important source (Preti and Higgins, 1975). However, Skarin and Sylwan in 1986 attributed most in vitro growth inhibition of Gardnerella and other anaerobic bacteria to acid production of Lactobacilli.

Thus the contribution of Lactobacilli to the acidic pH of the vagina remains unknown. Nevertheless, Lactobacilli are acidophilic and whether or not they contribute to low pH, they do thrive at an acidic pH of 3.5 - 4.5; such pH values are found in the normal vagina throughout the menstrual cycle (Lopez et al., 1990).

2) Competition for adherence:

Long-term bacterial colonization of the vagina is thought to require adherence of the bacteria to the vaginal mucosa. Initial localization of bacteria at the epithelial surface is due to lost cell/bacterial surface charge

interactions (Smith, 1977). Estrogen level fluctuations, with accompanying alternations in vaginal cell charge, could alter bacterial adherence properties and hence affect the composition of the vaginal microflora. Lactobacillus cell wall fragments block adherence of uropathogens to uroepithelial cells. This finding suggests that steric hindrance of uropathogen is a primary protective factor (Chan et al., 1985).

The Lactobacillus casei rhamnosus isolated by Reid and his associates exhibited the greatest ability to prevent the adhesion of uropathogen to uroepithelial cells. In addition, this organism was considered physically as being the largest bacterium which adhered sufficiently well to cells to cover the largest epithelial surface area (Ried et al., 1987). In a study of in vitro adherence to vaginal epithelial cells Mardh and Westrom (1976) reported that Gardnerella cells were more able to adhere to exfoliated vaginal epithelial cells than were Lactobacilli. In contrast, Sobel and coworkers (1981) demonstrated that Lactobacilli and Gardnerella adhered equally well to vaginal epithelial cells at pH 7.- but not at pH 4.5 (normal vaginal pH).

3) Hydrogen peroxide production:

Production of $H_2O_2$ is a well recognized method of bacterial antagonism (Dahiya and Speck, 1968).