دراسة بعض الجوانب المناعية لالتهاب المهبل ببكتريا الاشريشيا القولونية النوع المصلي K١ في الانسان والارنب

اطروحة مقدمة
الى مجلس كلية العلوم – جامعة بابل
وهي جزء من متطلبات نيل درجة الدكتوراه - فلسفة
في علوم الحياة /علم الاحياء المجهرية
من

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١٤٢٦ ه ٢٠٠٥ م

بِسَمْ اَللهِ الرَّحمِن الرَّحيمِ

وَمِنْ آياته أن خلقَ لَكُم مِنَ أَنفسِكُم أَزَواجاً لتِسكُنُوا إلَيَها
وجَعَلَ بَيَنكُم مَّودَّةً وَرَحمةً إنّ في ذَلِك لآياتٍ لِّقَومٍ يَتَفَكَّرُونَ

صَدَقَ اللهُ الَعِليُّ العَظيم
سورة الروم" ٢١"

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Abstract

The nature of human vaginosis has been investigated in ٣٥٢ women at Babylon province during ٢٠٠٤-٢٠٠٥ .

Four basic topics are discussed namely, the microbial profile associated with vaginosis with emphasis on the dominat *E.coli* k١ as the major vaginosis bacterial pathogen ;assessing mucosal and systemic immune responses of *E.coli* k١ vaginosis were undertaken pathogenicity of *E.coli* k١ vaginosis in rabbits as an animal model was evaluated .

E.coli k١ vaginosis were determined in fifty patients and thirty normal women were included as a control group . From each of the patients and control groups blood with and without anticoagulant as well as vaginal swabs were collected . Serum IgG, IgA ,IgM , C_3 and C_4 concentrations were higher in patients than control by using single radial immundiffusion . Phagocytosis using nitrobluetetrazolium dye reduction (NBT) on test was evaluated ,NBT mean values increased (٦١.٠٤,٤٢.١٤) in patients in comparison with control were (٤٦.٦ ,٢٠.٠٣) in systemic and local respectively . Cell mediated immunity was evaluated using LIF. LIF was significant in patients and non significant in normal. Serum *antiE.coli* k١ agglutinins and haemagglutinins were higher in patients than those in normal subjects in both local and systemic and the ratio of serum to mucosal antibody was ١٠:١ mostly.

IL-١α and IL- ٨ concentrations were determined by using enzyme linked immunoassorbent assay (ELISA) in serum of patients and normal ,the concentration of IL-١α was higher in most cases of patients than normal while IL-٨ concentrations were decreased in most cases of patients compared with normal . Female rabbits were used as animal model for study *E.coli* k١ vaginosis and the strain was chosen from acute cases .

Three groups of female rabbits were used the first group was with live bacteria, the second with capsular (test groups) antigen and third group with normal saline (control group) and after suitable period ,blood and biopsies (genital tract and intestinal tract) were collected from these animal . Specific *antiE.coli* k١ agglutinin and haemagglutinins were higher in test groups than control group . LIF was significant in test groups while no significant in control group in both local and systemic immunity . Delayed type hypersensitivity test was used in vivo and test was positive in rabbits with capsular antigen .

IL-١ α and IL-٨ concentration in test groups were higher than control group . It can be concluded that live bacteria and capsular k١ induce immune responses humoral and cellular local and systemic and also appeared that the immune responses were disseminated from vaginal to other part of mucosal immune system .

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List of abbreviations

Ab	Antibody
Ag	Antigen
AIDS	Acquired immunedeficiency syndrome
Cap	Capsular
C_3 ,C_4	Complement component ٣,٤
Cc	Chemokine subfamily has no amino acid between cysteine
Cxc	Chemokine subfamily has single amino acid between nearestpair of cystiene
CCL	Chemokine ligand
CCR	Chemokine receptor
CD	Cluster of differentiation
CMI	Cell mediated immunity
CMIS	Common mucosal immune system
CSF	Colony stimulating factor
CTL	Cytotoxic T cell
CFU	Colony forming unit
D.W	Distilled water
ELISA	Enzyme linked immunosorbent assay
g	Gram
GM-CSF	Granulocyte macrophage –colony stimulating factor
HCG	Human chorionic gonadotropin
HBD	Human B defensin
LA	Lymphoid aggregate
LFA-١	Lymphocyte function associated antigen
LIF	Leukemia inhibitory factor
LPS	Lipopolysaccharide
M cell	Membranous cell
MAdCAM-١	Mucosal addressin cell adhesion molecule ١
MHC	Major histocompatibility complex
MIP	Macrophage inflammatory protein
M.W	Molecular weight
NBT	Nitrobluetetrazolium
PAMP	Pathogen associated molecule protein
PEG	Polyethylene glycol
PIgR	Polymeric immunoglobulin receptor
PSG-١	P selectin glycoprotein ligand -١

PIgA	Polymeric IgA
ICAM-١	Intercellular adhesion molecular -١
Ig	Immunoglobulin
IFN	Interferon
IL-	Interleukin
RANTES	Regulated upon activated ,normal T cell expressed and secreted
RNA	Riboneucleic acid
SC	Secretory component
SLPI	Secretory leucocyte protease inhibitor
SP-A	Surfactant protein A
SRID	Single radial immune diffusion
STD	Sexually transmitted disease
TCR	T cell receptor
TGF-β	Trans forming growth factor β
Th	T helper
TLR	Toll like receptor
TNF	Tumor necrosis factor
VAP-١	Vascular associated protein
VCAM-١	Vascular cell adhesion molecule -١
VIg	Vaginal immunoglobulin

الخلاصة :

درست طبيعة التهاب المهبل في الانسان في ٣٥٢ امراة في محافظة بابل خلال الفترة ٢٠٠٤-٢٠٠٥ وتضمنت الدراسة اربعة محاور وهي مسح للاحياء المجهرية المرتبطة مع التهاب المهبل مع التركيز على جراثيم الاشريشيا القولونية النوع المصلي k١ . تم وضع موديل للدراسة المناعية والامراضية لهذه البكتريا باستخدام اناث الارانب .

جمعت العينات (دم ومسحة مهبلية) من خمسين امراة مصابة بالتهاب المهبل ببكتريا االاشريشيا القولونية k١ وثلاثين امراة سوية واعتبرت النساء السويات كمجموعة سيطرة وقد تم جمع العينات المتمثلة بعينات الدم(مع مانع تجلط وبدون مانع تجلط) والمسحات المهبلية من هذه النساء ودرست المناعة الخلطية الموضعية والجهازية ,كما حددت تراكيز الاضداد IgG و IgA و IgM و مكونات المتمم٣Cو٤Cباستخدام طريقة الانتشار المناعي الشعاعي المفرد في المصل وكانت التراكيز مرتفعة في المرضى مقارنة بالاسوياء.

كذلك درست كفاءة الخلايا متعددة الاشكال النووية باستعمال طريقة اختزال النايتروبلوتترازيليوم وان الفعالية البلعمية باستخدام اختزال الصبغة NBT كانت مرتفعة(٦١.٠٤,٤٢.١٤) في المرضى مقارنة بالاسوياء (٢٠.٠٣ ,٤٦.٦) في كلا من الجهازي والموضعي على التوالي .

ودرست المناعة الخلوية والمتمثلة بعامل تثبيط هجرة الخلايا اللمفاوية وان عامل تثبيط هجرة الخلايا اللمفاوية كانت معنوية في المرضى وغير معنوية في الاسوياء في كلا من الجهازية والموضعية.

وجد ان الاجسام المضادة متخصصة في كلا من التلازن والتلازن الدموي المباشر حيث ان العيارات الموضعية والجهازية اعلى في المرضى مما في الاسوياء ودرست نسبة الاضداد المصلية الى الجهازية وكانت النسبة ١:١٠ الاكثر تكرارا.

و تم تحديد تراكيز السايتوكينات في المصل باستخدام طريقة الاليزا وكان تركيز IL-١α عاليا في معظم حالات المرضى مقارنة بالاسوياء اما تركيز IL-٨ اظهر العكس. واستخدمت اناث الارانب كموديل للدراسة الامراضية والمناعية للالتهاب المهبل ببكتريا *E.coli* k١ والسلالة المستخدمة عزلت من حالة مرضية حادة وقد استخدمت في هذه الدراسة ثلاثة مجاميع من اناث الارانب ,احداها لقحت بالبكتريا الحية والثانية لقحت بمستضد المحفظة والمجموعة الثالثة بمحلول الملح الطبيعي واعتبرت كسيطرة وبعد فترة حضانة مناسبة تم جمع عينات الدم مع مانع تجلط وبدون مانع تجلط واخذت اجزاء من الجهاز التناسلي واجزاء من الامعاء حيث تم استخلاص الكلوبيولينات المناعية الافرازية لدراسة المناعة الموضعية ثم درست المناعة الخلطية باستخدام التلازن والتلازن الدموي المباشر ,اظهرت عيارات متخصصة للمستضد في مجاميع المعاملة اعلى مما في مجموعة السيطرة في كلا من المصل . ان عامل تثبيط هجرة الخلايا اللمفاوية كان معنويا في مجاميع المعاملة وغير معنوي في مجموعة السيطرة واستعمل اختبار فرط الحساسية المتاخرة كاختبار في الحي للتحري عن حدوث المناعة الخلوية واعطى نتائج موجبة في الحيوانات المعاملة بمستضدات المحفظة . وان تراكيز السايتوكينات كانت عالية في مجاميع المعاملة اكثر مما في السيطرة .

ومن هذه النتائج نستنتج ان *E.coli* K١ الحية ومستضد المحفظة عند اعطائها للارانب عن طريق المهبل تولد مناعة موضعية وجهازية كماانها تنتقل الى اجزاء اخرى من الجهاز الموضعي .

Certification

We certify that this thesis was prepared under our supervision at the Department of Biology ,College of Science ,University of Babylon as a partial of fulfillment of the requirements for the degree of Doctor Philosophy in Biology (Microbiology)and this work has never been published anywhere .

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Dedication

To my mother……

To father ,brothers ,sister and teachers with my endless love

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The conclusions:

١- *E.coli* K١ is most bacteria cause vaginitis.

٢- K١ antigen important in induce humoral and cellular immunity in human .

٣- IL-١α increased invaginitis with *E.coli* K١ while IL-٨ concentrations were decreased

٤- Lapian *E.coli* K١ vaginosis induce humoral and cellular immunity both in local and systemic .

٥- K١ antigen when inoculation in intravaginal of rabbits lead to increase Igs concentrations and C٣,C٤ concentrations .

٦- *E.coli* K١ induce immunity in local (vaginal) and disseminated to other part of common mucosal immune system (intestinal tract) .

٧- Female genital tract of rabbits is part of common mucosal immune system .

Recommendation:

١-Preparation of subunit *E.coli* intravaginal vaccine ,its field evaluations.

٢- Study interleukin level in local secretion to complete the picture of cytokine IL-١α and IL-٨ with *E.coli* K١ vaginitis .

١ -overview

The vaginal infections are frequently the cause of distress and discomfort in adult women . Vaginosis reflects a disturbance which has caused alteration in the vaginal microflora (Faro ,١٩٩٦). The vaginal microflora is normally made up of ٥-١٥ different bacterial species, including aerobes and anaerobes, and has the potential to inflict different clinical syndrome (e.g bacterial vaginosis) and diseases (e.g pelvic inflammatory disease). Gram negative facultative anaerobic *Escherichia coli* is one of common organisms in the microflora of pregnant as well as non-pregnant women (Bartlett *etal.*, ١٩٧٧) .

Vaginal colonization of *E.coli* is associated with various genitourinary, obstetric and neonatal complications and early onset neonatal septicemia and meningitis (Schiffer *etal.*, ١٩٧٦) . Vaginal *E.coli* have been reported to be sexually transmissible to male partner (Hebelka *etal.* ,١٩٩٣) . Vaginal *E.coli* is reservoir along the fecal ,vaginal ,urinary / neonatal course of transmission .The extraintestinal *E.coli* infection ,and most dominant strains of *E.coli* have the K١ antigen (Yasuoka *etal* .,٢٠٠٢ ;Watt *etal.* ,٢٠٠٣).There is relation between the K١ capsular polysaccharide of *E.coli* and invasiveness (Schiffer *etal.*,١٩٧٦; Yasuoka *etal.*,٢٠٠٢) .Sexually transmitted diseases (STD) is a major problem and

cause of morbidity and mortality in developing countries as well as industrialized societies (Wassen *etal.*,١٩٩٦).

Although the concept of the common mucosal immune system (CMIS) has been the dominant paradigm in mucosal immunology for more than two decade , it has become increasingly clear that the CMIS is not uniform ,and that the human reproductive tracts represent components of this system with unique character (Russell,٢٠٠٢;Wu *etal.* ,٢٠٠٢) .For example ,whereas locally produced secretory immunoglobulin A (IgA) is present in the genital secretions while the dominant Ig isotype is IgG much of which is derived from the circulation. Further more female genital tracts lack organized lymphoepithelial structures resembling intestinal Peyer s' patches where mucosal immune responses are induced and disseminated to remote effecter sites .The hormone dependent lymphoid aggregates consisting of $CD_{٨}^{+}$ T cells ,B cells ,and macrophages in the uterine stratum basalis are of a different composition and probably have a different function (Moscicki *etal.* ,١٩٩٥; Johansson *etal* .,١٩٩٩ ;Wormley *etal.* ,٢٠٠٠; Yeaman *etal.* ,٢٠٠١)

Numerous experiments have shown that local instillation of non replicating antigens into the vagina of experimental animals or of human volunteers can result in development of specific antibodies in the local

secretions (Johansson *etal* ,٢٠٠١; Nardelli-Haefliger *etal.*,٢٠٠٣; Mestecky *etal.* ,٢٠٠٤; Nguyen *etal.*,٢٠٠٥).

The presence of polymeric IgA secreting plasma cells in subepithelial tissue of the female genital tract , particularly in the endocervix and to a lesser extent in the fallopian tubes and uterus has been well documented and polymeric Ig receptor,the membrane precursor form of secretory component (sc) has been demonstrated in the overlying epithelium .Thus in these locations SIgA is assembled and transported into the lumen ,the mechanism whereby IgG from the circulation or produced by resistant IgG -secreting plasma cells is transferred to the lumen remain unclear .Mucosal cell mediated immune mechanism are known as operative (Russell & Mestecky ,٢٠٠٢).

The aim of the present study are follows

١- Study of the bacterial profile of human vaginosis .

٢- Study of the *E.coli* specific systemic and local immunity for both cellular and humoral immune responses in human female genital tract.

٣- - Using animal model to study the pathogenesity of *E.coli* K١

٤- Using animal model to study the immunological properties after intravaginal immunization with capsular antigen and live bacteria.

٥- Detection of certain cytokines including chemokines ,the IL-١α and IL-٨ concentration in human serum and rabbit .

٢.١: Female Genital Tract Anatomy.

The mucosa of the vagina and outer portion of the cervix (or ecto cervix) is made up of a highly vascularized sub mucosa and a superficial, non keratinized, stratified squamous epithelium. This squamous epithelium abruptly changes to simple stratified columnar epitheliums at the transitional zone, which marks the beginning of the inner portion of the cervix (or endocervix) this is the site of hormonally regulated secretion of specialized mucus that facilitates sperm transport. The endocervix ends in the uterine cavity, the lining of which is referred to as either endometrium in the non pregnant state or decidua during pregnancy. Depending on the hormonal stimulation, the endometrium varies from ١ to ٦mm in thickness and is made of several glandular layers. The innermost layer, the stratum functionale grows and thickens prior to and after ovulation (Beckerman & Dudley, ٢٠٠١) .

If pregnancy and implantation occur, it hypertrophies further to become the nutrient- rich, intensely glandular decidua; if pregnancy does not occur, this layer is shed at the time of menses.

The site of fertilization is the fallopian tube, a muscular membranous structure lined by a highly vascular mucosa (endosalpinx), consisting of ciliated and secretary cells. The endosalpinx is thrown into numerous branched, slender longitudinal folds and is ideally suited to the maintenance, and transport of the conceptus during its five day journey to the uterus. (Beckerman & Dudley, ٢٠٠١).

٢.٢: Development Of The Normal Vaginal Flora In Women

At birth, the vagina of the female infant is sterile .After only a few days, when the mother′s oestrogen has elevated the glycogen content of the vaginal epithelial cells, the infant′s vagina is colonized by lactobacilli migrating from the mother. This is in line with generally recognized, fact that the normal bacterial flora in humans originates from mother. In most instances this flora continues to dominate throughout the individual′s lifetime, and this is also the case with the vaginal flora though it is in the vagina′s physiological environment during the life cycle (Cauci *et al.*, ٢٠٠٢)

The vaginal flora of infant girl becomes interspersed with contributions of coagulase negative *Staphylococci, Streptococci, E. coli* and other intestinal bacteria.

Small quantities of lactobacilli remains, however, and the oestrogen produced at menarche will cause a thickening of the vaginal mucosa, a prerequisite for the propagation of lactobacilli, the dominant vaginal flora of the adult female (Harstall & Corabian, ١٩٩٨). The bacteria isolated from the vaginal secretion of women of childbearing age number around $١٠^{٧}$ or $١٠^{٨}$cfu/g fluid (Redondolopez *et al.*, ١٩٩٠). This microflora composition continues until the menopause, when it is replaced by a mixed flora unlike that of the infant female, but with considerable portion of Mycoplasma species and small quantities of anaerobic bacteria.(including *Gardnerella vaginalis*).

Hormone replacement therapy, when used will cause lactobacilli to continue as the dominant flora (Forsum *et al.*, ٢٠٠٥ a).

٢.٣: Vaginosis

The vaginal ecosystem is a complex biosphere composed of a great variety of microorganisms which interact in various ways to maintain balanced or healthy state.

The ecosystem is in a constant state of flux and its equilibrium is constantly challenged by both endogenous and exogenous factors. Factors that may alter the delicate equilibrium are the following: antibiotics, hormones contraceptive preparations (oral and topical), douches , sexual intercourse , presence of foreign bodies such as IUDs , alterations in immune states, sexually transmitted disease , stress and change in partners (Harstall & Corabian,١٩٩٨).

Disease of the vagina which may cause an abnormal vaginal discharge may fall into one of three categories infectious, allergic and neoplastic whereas an unbalanced ecosystem, referred to as vaginosis, reflects a disturbance which has caused an alteration in the vaginal microflora (Faro, ١٩٩٦). It is common for the terms vaginitis and vaginosis to be used- interchangeably for an inflammation of the vagina.

The most common cause of vaginitis symptoms among women of childbearing age include bacterial, followed by candidiasis and trichomoniasis (Harstall & Corabian , ١٩٩٨).

Bacterial vaginosis is observed at many types of clinics. It is reported from primary care units, gynecological clinics , sexual transmission , infected clinics and among specific patient group such as expectant mothers abortion applicant and sex workers (Mead, ١٩٩٣).

Diagnosis of bacterial vaginosis has long been based on the clinical criteria of Amesl where by three of four defined criteria must be

satisfied ,included discharge (the typical bacterial vaginosis discharge is a thin white fluid with a consistency resembling sour milk) . PH exceeding ٤.٥ ; Whiff test (production of amines when add ١٠ % KOH) and presence (Amsel *et al ;* ١٩٨٣) .Other method was developed called Spiegels was first created to diagnosis Gram stained vaginal smear . In this method the bacteria were grouped into morphotypes ;Lactobacillus morphotypes were called elongated bacteria and Gardnerella morphotyes ,were called short bacteria (Spiegl *et al* ; ١٩٨٣).Scoring method which function well in comparison i.e Nugent scoring it is not certain that they will always identify the same category of patients. Point of care methods based on various combination of microbial product presence of RNA, or more complex laboratory instrumentations such as sensor arrays, have also been introduced for the diagnosis of bacterial vaginosis. No method for diagnosing bacterial vaginosis can at present be regarded as the best. It could be that based partly on tracit knowledge on the part of the clinical investigators scoring in the clinic- various scoring systems have been chosen to fit a particular bacterial vaginosis related problem in a particular population (Forsum *et al.* , ٢٠٠٥b)

Escherichia coli is one of the common organisms in the microflora of pregnant as well as non pregnant women. Vaginal colonization with *E. coli* is associated with various genitourinary, obstetric and neonatal complication such as severe form of pelvic inflammatory disease (Heinonen & Miettinen , ١٩٩٤) urinary tract infections (Rajan *et al.*, ١٩٩٩) very low- birth weight infants and early onset neonatal septicaemia and meningitis.

Vaginal *E. coli* has also been reported to be sexually transmissble to a male partner (Hebelka *et al.*, ١٩٩٣).

The *E. coli* implicated in various human infection can be broadly classified as intestinal and extraintestinal . Among the extraintestinal *E. coli,* uropathogenic *E. coli* and neonatal meningitis *E. coli* have been characterized in some detail by diverse approaches including phylogenetic analysis, serotyping and molecular typing such as virulence factor profiling, multilocus enzyme electrophoresis, outer membrane protein profiling and plasmid profiling. Vagina- colonized remain largely uncharacterized with regard to its serotypes, clonality and phylogenetic linkage with other *E. coli*. Some studies determined vaginal *E. coli* virulence factor profiling and serotyping in comparison with other isolated *E. coli* from blood, cerebrospinal fluid and stool (Watt *etal*; ٢٠٠٣).

The vaginal *E. coli* included ٣١ serotypes ٨ of them were common serotypes ($O_{١:}K_{١}$: $H_{١}$; $O_{١}$: $K_{١}$:$H_{٧}$; $O_{٢}$:$K_{١}$:$H_{٧}$;$O_{٤}$:$H_{٥}$; $O_{٦}$:$H_{١}$; $O_{١٨}$ ac: $K_{١}$:$H_{٧}$; $O_{٢٥}$: $H_{١}$ and $O_{٧٥}$: HNM) and this study provides, in additional evidence for a link among extraintestinal *E. coli,* supporting the concept that the vaginal *E. coli* are a reservoir along the faecal-vaginal- urinary neonatal course of transmission in the extraintestinal *E. coli* infections. The virulence factors of vaginal *E. coli* also were determined in this study these include adhesins, toxins, siderophores and invasin(Watt *et al.*, ٢٠٠٣).

٢.٤ : *E. coli* K_1 *antigen*

K_1 antigen is a linear homopolymers in which the basic sugar unit contains the carboxyl group and the acetylated amino group. The poly saccharide may exist either in O- acetylated or non O- acetylated form K_1 polysaccharide which consists of linear chains of α-٢-٨ linked N-acetylneuraminic acid and its structure has been found to be identical with group B- meningococcal capsular polysaccharide and K_1 more like the V_i antigen of *Salmonella typhi* (Szewczyk & Taylor;١٩٨٣) .

E. coli K_1 is associated with invasive disease in human and in domesticated animals. K_1 isolates account for over ٨٠٪ of *E. coli* meningititis and comprise the majority of capsular types in upper urinary tract infections in infants.

Several reports suggest that the K_1 capsular polysaccharide confers invasiveness upon *E. coli* and there are indications that polysaccharide exerts an antiphagocytic effect similar to that observed with other encapsulated bacteria (Pluschke *et al.*, ١٩٨٣).

Extensive cross reaction with *Neisseria meningititis* group B poly saccharide, *E. coli* K_{92} also react with *Neisseria meningititis*, cross reactivity in these cases is often due to the complete, identity of polysaccharide or to the occurrence of a common immunodominant sugar within the antigen molecule the capsular is the most common capsular associated with *E. coli* strains that are isolated from the blood of patients in neonatal. There is high mortality rate from systemic infection with K_1 encapsulated *E. coli* even after appropriate antibiotic therapy.

All members of Enterobacteraceae are able to elaborate a layer of surface associated polysaccharide called the capsule. The composition of these capsular polysaccharide is very much strain dependent. In *E. coli* it may be one of the ٨٠ distinct polysaccharide (designated the K antigen) or polymer derived from the ١٧٠ different O antigens. In fact whereas all polysaccharide K١ antigens form a capsule structure, the invert is not always true, not all capsules are composed of K antigens (Schembri *et al.*, ٢٠٠٤).

K١ positive *E. coli* strains have been traditionally identified by seroagglutination with antisera raised in rabbit . Another research serum agar technique has been described which takes advantage of the known immunochemical cross reactivity between the K١ capsule of miningococcus, in this technique, equine antisera to group B meningococcus polysaccharide is incorporated into nutrient agar used for cultivation of *E. coli* . Precipitin reactions can then be observed surrounding individual colonies of K١ positive *E. coli* by incorporation of the appropriate antisera (Cross *et al.*, ١٩٨٤).

A third means of identifying K١ positive *E. coli* is to determine lytic sensitivity of strains to a set of five K١- specific bacteriophages and the identification of K١ capsule by bacterial agglutination with a murine monoclonal antibody directed towards the group B- meningococcal polysaccharide (Cross *et al.*, ١٩٧٧).E.coli K١ also can be detected by using Polymerase chain reaction (Johanson & Bryan ;٢٠٠٤) .

٢.٤.١: *Immunity of capsular K١ antigen*

Robbins *et al.*, (١٩٧٤) reported the frequency of *E. coli* strains having the K١ capsular polysaccharide among isolates from CSF of newborn infants with meningitis . It was of interest that the capsular polysaccharide of K١ *E. coli* was antigenically identical to that of group B meningitis in human, these similarities suggest that the capsule may play a role in the pathogenicity of these two organisms (Schiffer *et al.*, ١٩٧٦).

Many studies described the role of K١ antigen one of these studies by (Bortolussi *etal.*,١٩٧٩)found virulence of *E. coli* strains for newborn rats was related to opsonic requirements of the strains sensitivity to the bactericidal activity of serum . K١ positive cells were resistant to the bactericidal activity of sera deficient in classical complement pathway component whereas K١ negative cells were sensitive to these sera based on these result and those from complement fixation assays, the K١ sialic acid impedes the activation of complement and thus protects the bacteria against the alternation complement pathway. Another study indicated that strains that posses the K١ capsule are resistant to killing and opsonization sera deficient in classical complement pathway activity (Pluschke *et al.*, ١٩٨٣).

In human newborns, the gastrointestinal tract seems to be the source of *E. coli* which can invade the blood stream with subsequent involvement of the central nervous system. (Orskov *et al.*, ١٩٧٧) found that the bacteria detected in approximately ٢٠٪ of fecal samples were obtained from healthy babies.

Previous protection studies using antisera from immunized animals are subjects to criticism on the basis that these may contain antibodies against more than one type of cell surface antigen. It has been shown previously that the injection of monoclonal antibodies to the K١ antigen prevents bacteremia in newborn rats.

Antibodies to *Neisseria meningititis* B- capsular antigen which is identical to the *E. coli* K١ capsule protected mice challenged with lethal doses of K١ *E. coli* although very high doses where needed and also, demonstrated that newborn rats can be protected against infection with K١ *E. coli* antibodies specific for the O- antigen of LPS. The chemical compositions of both the capsule and the O antigen seem to determine the degree of direct complement activation ,in absence of specific antibodies, the virulent bacteria cause less direct activation of complement than closely related a virulent strains (Bortolussi *et al.,*١٩٧٩*)* . The K١ capsule which is structurally identical to the capsules of group B- meningococcal is a poor immunogen, and efforts towards the development of capsular – polysaccharide vaccine have not been very rewarding.

A high percentage of adults have serum antibodies to the K١ capsule, nearly all of which are of the IgM class and have relatively low avidity and poor bactericidal activity (Pluschke & Achtman , ١٩٨٥).

٢.٥: Immunity of female genital tract.

٢.٥.١: Non specific defense.

The epithelium of the endocervix, uterus and Fallopian tubes is composed of polarized epithelial cells connected by tight junction that provide an essential barrier function, However more recent studies in women indicate that uterine peristaltic waves, occurring under the influence of estradiol treatment that enhance the movement of labeled-albumin macrosphere from the vagina into the uterus toward the ovary (Kunz *et al.*, ١٩٩٧) is of particular importance that, irrespective of stage of menstrual cycle or the use of oral contraceptives, radio- opaque dye entered the uterine lumen and fallopian tubes within ٢hr of placement in the vagina of women. As part of these studies, dye placed in the vagina of post menopausal women entered the uteri as rapidly as was found in premenopausal women . All these studies demonstrated that the polarized epithelial cells of the reproductive tract were exposed to bacteria at frequency not previously appreciated.

Further more, as the uterus and fallopian tubes have a relatively low incidence of chronic infections as determined by routine histological analysis, these studies suggest that in addition to serving as an effective barrier epithelial cells as a part of the mucosal immune system, protect against the organisms present throughout the reproductive tract (Wira *et al.*, ٢٠٠٥).

Epithelial cells at a number of mucosal surfaces are known to produce defensins, particularly β- defensins, the synthesis and or release

of these natural antibiotics is induced rapidly by infectious toxins and in general act via their amphiphilic charge on the microbial membrane to create pores or other wise affect permeability, these antimicrobials are small cationic peptides, rich in arginine with cysteine disulfide bridges with proven effectiveness against gram negative and gram positive bacteria , fungi and some viruses, Human β – defensins (HBD)١ and ٢ are produced by the epithelial cells of the female reproductive tract and have a primary role in defending mucosal surfaces against microbes.

(HBD-١) is primarily found in the genitourinary tract, and is constitutively produced, whereas HBD-٢ is usually induced by infection. Human defensin ٥ and α- defensin is also produced by endometrial epithelial cells, with highest response during the secretary phase (Wira *et al*.,٢٠٠٥).

In addition to their bactericidal activity, defensins have also shown to have multiple functions in innate immunity for example, β defensin, by binding to the chemokine receptor CCR٦, are chemotactic for immature dentritic cells and memory T cells uterine epithelial also produce CCL ٢٠/ macrophage inflammatory protein (MIP)٣ α a chemokine ligand of CCR٦ which has significant homology to defensins and has been shown to have significant antimicrobial activity (Valore *et al*., ١٩٩٨).Epithelial cells express toll like receptors in the female reproductive tract .Using human reproductive tract tissues ,found that TLRs١-٦ (Wira *etal* .,٢٠٠٥) .

Secretory leucocyte protease inhibitor (SLPI) is produced by macrophage and epithelial cells of human and rodent female reproductive tract and has a broad spectrum activity against a variety of potent pathogens, including HIV-١, neutralization of bactericidal activity with antibody indicated that SLPI in uterine secretion plays a central role in the human reproductive tract protection against gram negative and gram positive bacteria (Wira & Fahey,٢٠٠٤ ; Wira *et al*., ٢٠٠٥).

Surfactant protein A(SP-A) is a member of the collectin (collagenous lectin) family of proteins that has important host defense functions, SP-A is produced in a squamous epithelium namely the vaginal mucosa and has a localization that would allow it to contribute to both the innate and adaptive immune response, this finding supports the hypothesis that in vagina as in lung, SP-A is essential component of host defense system. A corollary hypothesis is that qualitative and quantitative alterations of normal SP-A may play a role in the pathogensis of lower genital tract inflammatory conditions (MacNeill *et al*., ٢٠٠٤).

٢.٥.٢: *Mucosal immune responses in human female genital tract*

Mucosa of the female genital tract are the portal of entry for agents responsible of sexually transmitted diseases of viral, bacterial and parasitic origin.

World wide ~ ١٢٠.٠٠٠ ٠٠٠ cases of SID are reported annually, but the true incidence is probably much higher. (Hernandez *et al*.,١٩٩٨).

Studies performed on animal models and human have convinsingly demonstrated that the mucosal and systemic compartments of the immune system display a significant degree of mutual independence . Immunoglobulins (Igs) present in external secretions or systemic fluid are represented by molecules of different physicochemical and biological properties . In external secretion of human and mammalian species the dominant Ig in secretory IgA (SIgA) consists of polymeric IgA with J chain and secretory component (sc) derived from epithelial cell, However secretions of the female genital tract differ from other external secretions (e.g salivary, milk) as well as from plasm in proportion of Ig isotype and forms (Mestecky& Russell; ٢٠٠٢).

٢.٥.٣: *Immunoglobulins and specific antibodies in female genital secretion*

Highly variable information has been reported about the presence of various Ig classes in human female genital secretions. Such discrepancies probably reflect both differences in the applied sampling method and individual variables such as age and phase of menstrual cycle(Waldman *et al*., ١٩٧١)found that SIgA was the predominant (≈٤٥٪) Ig in normal cervico vaginal secretions, The remainder consisting mainly of monomeric IgA (≈٢٠٪) and IgG (≈٣٠٪).

In the same study antibodies to *Candida albicans* were found to be mainly of IgA class. *Chlamydia Trichomatis* has been reported to act particularly stimulating on the appearance of SIgA in cervico- vaginal secretions. However, IgA levels were found to decrease with age in contrast to IgG that increases (Jalanti & Isliker, ١٩٧٧).

Some studies have only in part agreed with earlier data showing a general predominance of specific IgG in cervical secretions but a clear predominance of specific IgA antibodies in secretions from female colonized with group B- streptococci in their cervix and/ or rectum

(Hordnes *et al*., ١٩٩٦). Others have reported predominance of IgG also in normal vaginal fluid . This fits with detailed sampling study showing considerable topical variations, with IgA dominating in mucus from the endocervix and so ectocervix and IgG dominating in vaginal fluid However, when a cervical washing method was applied, probably causing more epithelial irritation, IgG was shown to predominate also in cervical mucus (Kutteh, *et al*.,١٩٩٦).

IgG occurring in genital secretions was deemed to be mainly serum- derived, but a significant enrichment of IgG١ subclass suggested some local influence, probably mucosal IgG production favoring this subclass. The maximal output of both cervical IgA and IgG, together with much smaller amounts of IgM, was found to take place ٢-٣ days before ovulation (Hocini *et al*., ١٩٩٥; Kutteh *et al*., ١٩٩٦; Russell & Mestecky, ٢٠٠٢).

Quantitation of actual number of Ig- producing , immunocytes present in genital tissues is also difficult to perform because of the uneven distribution of these cells . Immunocyte densities have only been reported as number of cells low- power fields without any precise morphometric definition of the evaluated tissue compartment . The endocervix was found to contain the largest number (≈١٢٠), The ectocervix about half of that (≈٦٦) and the fallopian tube (≈٣٩) and vaginal mucosa (≈٣٧) even fewer (Kutteh & Mestecky, ١٩٩٤).

Sc expression has been detected at variable and relatively low levels in the epithelial cells lining the villi of the tubes and in the glandular epithelium of the cervix, but not in the squamous epithelium of the vagina luminal and intra cytoplasmic epithelial staining for IgA, Sc and J chain has been taken to reflect polymeric immunoglobulin receptor (PIgR), dependent external transport of PIgA to form SIgA (Brandtzaeg, ١٩٩٧)

٢.٥.٤ : *Cellular immunity in female genital tract*

In the reproductive tract of women, the endocervix has been shown to contain most concentrated numbers of dendritic cells, macrophages and $CD_{٤}^{+}$ T cell which may favour antigen presentation and immune induction at this site in particular(Anderson, ١٩٩٦).

The endocervix contains $CD_{٤}^{+}$ and $CD_{٨}^{+}$ T cells carrying αβ or γδ TCR. Some of these Tcells have cytotoxic function but a majority of the T cells appear to be regulatory cells depending on the cytokine they produced (Ahmed *et al*., ٢٠٠١).

Unlike the intestine, the genital tract is devoid of M cells and organized follicles. Accordingly it has been assumed that induction of local immune response in the genital tract is difficult to achieve through local application of antigen (Kozlowski *et al*., ٢٠٠٢). The human female genital tract contains all the essential elements for mounting an effective immune response against genital pathogen. By enzymatically degrading genital tissue and analyzing the cells it was estimated that leucocyte represent ٦-٢٠٪ of total number of cells in fallopian tubes, endometrium cervix and vaginal mucosa. The T cells accounted for about ٥٠٪ of all leucocytes with $CD_{٨}^{+}$ cell, predominantly over $CD_{٤}^{+}$ cells. (Givan *et al*., ١٩٩٧).

(Johansson *et al.*, ١٩٩٩) found that the T cells both $CD_{٤}^{+}$ and $CD_{٨}^{+}$ were concentrated in a band directly beneath the epithelium in both endocervix and vagina. In cervical area several lymphoid aggregates (LA) have been found as consisting of numerous T cells.

These aggregates had several features of inductive mucosal lymphoid tissue aggregated lymphocytes in follicle like structures, lymphocytes invasion of the over lying epithelium and presence of high endothelial venules (HEV).

Lymphoid aggregates develop in stratum basalis of the human endometrium during the proliferative phase of the menstrual cycle, but are absent in the endometrium of postmenopausal women, suggesting that their development is hormonally influenced.

(LA) usually have a B- cell core, which is surrounded by spheroidal mass of Tcells. The T cell region is in turn surrounded by a diffuse halo of macophages. A unique feature of these LA is that, in most endometria, the T cells are usually exclusively $CD_{٨}^{+}$ T cells. The function of LA $CD_{٨}^{+}$ cells is not known however, it is of interest that endometrial $CD_{٨}^{+}$ cells isolated from secretory- phase endometria when LA are fully formed are unable to mediate cytotoxic T lymphocyte (CTL) activity fallowing stimulation with IL-٢ . This lack of CTL function is tissue specific, as $CD_{٨}^{+}$ cells from secretary phase cervix vaginal mucosa are active. CTL activity of $CD_{٨}^{+}$ cells from post menopausal endometria, which lack LA, do exhibit CTL activity. Therefore, these observations suggest that development of LA is accompanied with a concomitant reduction in CTL capacity in the uterus.

It is not definitively known whether they represent a distinct regulatory CD_8^+ T cell subset. LA develop during menstrual cycle largely by the trafficking of cells to nucleation sites within endometrium rather than by division of a limited number of precursor cells (Yeaman *et al*., ٢٠٠١).

Cell mediated immunity (CMI) and the role of T cells in host defense at the vaginal mucosa however, are largely unknown. This knowledge is critical for developing strategies to control or prevent vaginal infections caused by a vast number of pathogens, including bacteria, fungi, protozoans and viruses that require CMI host defenses for eradication . Furthermore, although deaths due to acquired immune deficiency syndrome (AIDS) have generally declined in the recent past, deaths in women due to AIDS continue to rise . In fact women infected with human immunodeficiency virus (HIV) is the fastest rising group of HIV- infected individuals , stressing the urgency of elucidating host defense mechanisms at the vaginal mucosa (Wormley *et al*., ٢٠٠٠).

Candida albicans is the causative agent in the majority of mucosal fungal infections in AIDS patients and the organism is most often diagnosed in women with acute or recurrent vulvovaginitis. CMI is the predominant adaptive host defense mechanisms against *C. albicaus* at mucosal surfaces. (Fidel *et al*.,١٩٩٥;Fidel *et al*., ٢٠٠٤).

٢.٥.٥ :*Adhesion molecules in human cervix and vagina*

Mucosal addressin cellular adhesion molecule-١ (M AdcAM-١) is known vascular adhesion molecule that directs lymphocyte to mucosal

tissue by binding to some lymphocyte expressions integrin α_4 β_7 ,such expression has never been found in the genital tissue .

Intercellular cell adhesion molecule -١ (ICAM-١) is constitutively expressed on endothelia and plays a role in lymphocyte homing by binding to lymphocyte function associated antigen (LFA-١), whereas vascullar cell adhesion molecule-١ (VCAM-١)is an inducible ligand for

the α_4 integrins expressed on lympocytes E. selectin is also expressed by activated endothelial cells . In both the cervical and vaginal tissue ICAM-١ was constitutively expressed on endothelial cells. Vascular associated protein VAP-١ and p-selectin glycoprotein ligand-١ (PSG-١) were present on lymphocyte and together with E- selectin .

P- selectin has been shown to mediate the movement of T helper ١ but not T helper-٢ into inflamed tissue (Johansson *et al.*, ١٩٩٩).

There is some evidence that genital homing of lymphocyte may involve integrins α_4 β_1 or α_L β_2 (LFA-١) which recognize VCAM-١and ICAM-١ respectively, both of which occur in genital tract endothelial cells (Russell & Mestecky, ٢٠٠٢).

٢.٥.٦: *Immunization strategies in female genital tract*

Numerous experiments have been reported in recent years on female genital immune responses to different immunization strategies, with varying results dependent upon the type of antigen, and the role of administration and other factors(Albert & Edward , ٢٠٠٠) .

Local induction of IgA and IgG antibody responses in the female mouse or rat reproductive tract can be achieved by repeated application

of large doses of non viable antigens, or the use of potent mucosal adjuvant such as cholera toxin, or in evasive viruses such as herpes simplex. Even though these strategies may result in significant local antibody production however, disseminate to other mucosal effecter sites or to the systemic compartment is limited (Johansson *et al*., ١٩٩٨; Wu *et al*., ٢٠٠٠; Kozlowski *et al*., ٢٠٠٢).

Systemic immunization generally elicits weak responses in the reproductive tract, but booster immunization given in the vagina or uterine horns have often resulted in enhanced genital tract responses (Russell, ٢٠٠٢).

Comparative studies indicated that whereas vaginal immunization induces a weak vaginal response, subserous and interperitoneal immunization with same antigen result in IgG and IgA, but subcutanous injection induces only IgG antibodies (Thapar *et al*., ١٩٩٠).

A complicating factor is that immune responsiveness of female genital tract varies during the estrous cycle and that of hormone treatment is used to maintain the animals in a constant state. This effects the outcome because estrogen suppresses and progestrone enhances immune responses. Intravaginal immunization has also been reported to generate mucosal tolerance (Alberd & Edward ,٢٠٠٠) . Probably the most remarkable development has been the observation, confirmed in many laboratories that intranasal immunization is very effective for inducing mucosal IgA antibody responses in the female genital IgA antibody responses in the female genital tract (Johansson *et al*., ٢٠٠١).

Oral immunization in human has generally been a disappointing route for eliciting genital antibody responses unless combined with local

application of the antigen, but alternative routes such as rectal and intranasal have attracted considerable attention on a count of successes obtained in experimental animals thus, rectal immunization with inactivated influenza virus resulted in the development of modest IgA antibody responses in cervical secretions within ١ month and both IgA and IgG responses in cervical and vaginal secretions after ٦ months (Russell & Mestecky, ٢٠٠٢).

٢.٥.٧: *Cytokines in female genital tract*

The human $CD_{٤}^{+}$ T cells can be classified on the basis of their pattern of cytokine production .Type ١ $CD_{٤}^{+}$ T cell ($Th_{١}$) produce IL-٢, Tumor necrosis factor (TNF)-β and interferon (IFN-γ) and are the main effectors of phagocyte- mediated host defense, which is highly protective against infectious sustained by intracellular parasites.

On the other hand Type ٢ $CD_{٤}^{+}$ T cells ($Th_{٢}$) produce IL-٤, which stimulates IgE and $IgG_{١}$ antibody production, IL-٥ (promoting the growth and differentiation of eosinophils). IL-١٠ and IL-١٣ which together with IL-٤ inhibit several macrophage functions.

The $Th_{٢}$ cell is mainly responsible for phagocyte independent host defense, e.g against certain nematodes. The differentiation of Th. cells into $Th_{١}$ or $Th_{٢}$ cell can be influenced by hormone, progesterone, at concentrations comparable with those present at the maternofetal interface during pregnancy is a potent of enhancer or augmenter production of $Th_{٢}$ type cytokines (i.e IL-٤ and IL-٥) .

Whereas, relaxin, a polypeptide hormone predominantly produced by corpus luteum and decidua during pregnancy favors the development of T cell producing IFNγ without exerting any effect on the production of

IL-٤. However, ١٧β estradial and hCG have no effect on the Th. cell differentiation into Th_1 or Th_2 cells (Piccinni ,٢٠٠٢).

Luminal and glandular epithelial cells of endometrium have been shown to produce numerous cytokines, including granulocyte-macrophage colony stimulating factor (GM- CSF), CSF-١, Tumor necrosis factor α(TNF-α) IL-١, IL-٦,IL-٨, leukemia inhibitory factor (LIF) and transforming growth factor (TGF-β). Cytokines are now

recognized as principle components of complex intercellular communication among cells in the uterus, temporal release patterns during both the menstrual and estrous cycles have been shown to regulate by sex hormones including GM- CSF .TGF-β, LIF and TNF-α, release of IL-١α by uterine epithelial and stromal cells results in increased secretion of prostaglandin E_2 and F_2 .

IL-٦ which plays a role in B cell differentiation as well as GM-CSF, which regulates granulocytes and macrophage proliferation are made and released by uterine epithelial cells both in the pregnant and non pregnant uterus. Chemokines produced by epithelial cells that are responsible for recruiting immune cells into the reproduction tract include regulated upon activation, normal T cell expressed and secreted (RANTES) and IL-٨ (Rollins ,١٩٩٧) .

Recent studies in mice and rats have focused on the production by epithelial cells of cytokines/ chemokines including TNF-α, TGF-β and $MIP_2α/CCl_2$. Other studies have shown that TGF-β regulate cellular proliferation, migration, cell differentiation and protein expression, TNF- α plays a prominent role in inflammatory processes. Investigation of TGF-β in the immature mouse uterus led to the conclusion that diethylstilbestrol, a synthetic estrogen, increase TGF-β

mRNA and protein for TGF-β١,٢,٣ in the uterus. TNF- α, which plays a prominent role in inflammatory processes, has been shown to be involved in both normal pregnancies as well as under conditions of pregnancy loss. MIP٣ α / CCl٢٠ chemokines that attracts B cell, memory T cells and immature bone marrow- derived dendritic cells, is produced by uterine epithelial cells. Important when considering the response of uterine epithelial cells to the presence of commensal bacteria, production and release of MIP٣ α / CCl٢٠ by uterine epithelial cell is rapidly up-regulated in the presence of *Escherichia coli* and pathogen associated molecular patterns ($PAMP_s$) including bacterial ligands of toll like receptors(TLR_S٢ and ٤), but not by live or heat killed *lactobacillus rhamnosus.* (Wira *et al*., ٢٠٠٥).

Chemoattractants which are reported to be possibly involved in the inflammatory response of *Trichomonas vaginalis* include leucotriene β٤ and IL-٨, which are both found in the vaginal discharge of symptomatic Trichomonasis patients.

IL-٨ is the best characterized member of α - chemokine or CXC subfamily. IL-٨ acts primarily on polymorphonuclear cells but also has potent chemotactic and stimulatory effects on T cells, basophils, and eosinophils upon exposure to inflammatory stimuli, such as lipopoly saccharide IL-١, or tumor necrosis factor, IL-٨ is released by a wide variety of cell types including monocytes/ macrophages, neutrophils, T lymphocytes, fibroblasts endothelial cells and epithelial cells. Human monocytes are known to produce IL-٨ after stimulation with *T. vaginalis* (Ryu *et al.*, ٢٠٠٤), IL-٨ levels in cervical mucus were no significant differences between follicular, ovulatory, and luteal phases in women, However IL-٨ was increased significantly during pregnancy (Luo *et al.*, ٢٠٠٠).

IL-١ levels were increased after infection with *Neisseria gonorrhoeae*, gonococci to suggest that the earlier IL-٨ and IL-٦ responses were not mediated through. The IL-١ signal apathway. The ability of gonococci to stimulate distinct proinflammatory host response, in these morphologically and functionally different compartments of the lower female genital tract may contribute directly to the inflammatory signs and symptoms characteristic of disease caused by *N. gonorrhoeae* (Fichorova *et al*., ٢٠٠١).

٣.٧ : Blood Processing (Figure ٣.١)

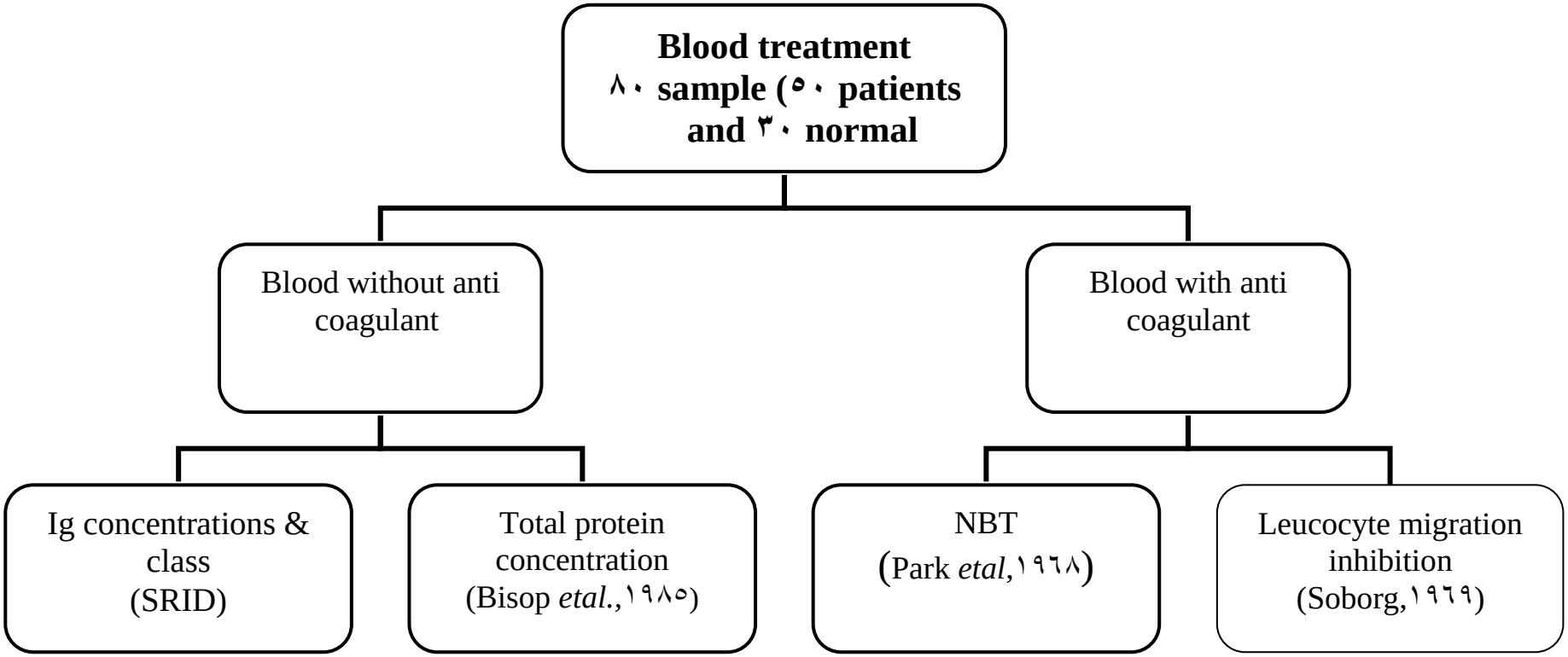


Figure (٣.١): Processing of blood sample

٣.٨: Antigen preparation

٣.٨.١: Capsular antigen

This antigen was prepared in accordance with (Kwapinsiki, ١٩٧٢)

١. The isolated bacteria *E. coli* was cultured on nutrient agar (٣٧°c) for ٢٤hr.

٢. (١٠)ml sterile D. W. were added to the culture and bacteria growth was harvested.

٣. Suspension was collected in sterile centrifuge tube and (١)ml of HCl (٠.٥)N was added to (١٠)ml of suspension.

٤. The suspension was heated in water bath at (٧٥)°C for ٣٥mins.

٥. The suspension was cooled and centrifuged to discard cells.

٦. The obtained supernatant was neutralized with NaOH (٠.٥)N to obtain (pH=٧).

٧. The neutralized solution was treated with three volume of ethyl sodium acetate (١٠ g of H_3COONa, M. W. ٨٢٠٣ BDH, in ١٠ml of ethyl alcohol).

٨. The mixture was left over night at (٤)°C the precipitate was collected and dissolved in (١)ml sterile D. W. to obtain an opalescent solution.

٩. Three ml of ethyl sodium acetate was added to obtain opalescent solution and was left for (١)hr and then centrifuged for (٥)mins at ٢٥٠٠rpm and then precipitated collected and dissolved in (١)ml sterile D. W. This antigen was used in tube agglutination with serum and VIgs and in leucocyte migration studies.

٣.٨.١.١: *E.coli* K_1 type serology

K_1 positive *E. coli* strains were identified serologically with specific antisera (poly spezi fiches ok test serum anti coli I) (Orskov *et al.*, ١٩٧٧).

٣.٨.١.٢ :Chemical identification

It was done according to (Szewczyk & Taylor ,١٩٨٣)

١. One ml of capsular antigen was added to test tube and iodate reagent was added to it .A positive result was viewed as blue granules indicating the Ag was polysaccharide .

٢. K_1 is O- acetylated. This determined as fallows

One ml NH_2OH. HCl in which ١ml KOH methanol and ١ml capsular antigen was added and left in water bath at ١٠٠°C for ١٠ mins and then cooled and ١ ml of (١٠٪) $FeCl_3$ was added . The appearance of brown color that disappeared after few mins . This indicated the presence of O- acetylated .

٣.٨.١.٣: $K1$ Temperature effects

K_1 antigen is influenced by temperature .The isolated bacteria was cultured on two eosin methylene blue plates ,one of them was incubated at ٣٧ C° and the other at ٢٢ C° for ٢٤ hr .The colonies in ٢٢ C° lost their phenotypic appearance (metallic sheen) and negative result when was mixed with K١ antisera , while the other in ٣٧ C° appeared metallic sheen and positive result with K١ antisera (Bortolussi *etal* ., ١٩٧٩).

٣.٨.٢: *Cell free culture antigen (cfc)*

١. *E. coli* K١ was cultured into tube containing ٥ ml peptone water.

٢. The tube was incubated at (٣٧)°C for (٢٤)hrs.

٣. At the end of incubation period the tube was centrifuged at ٢٥٠٠rpm for ٥mins.

٤. The culture then was filtrated by using ٠.٢٢μm (Millipore filter) and then was stored until use. According Kwapinski (١٩٧٢) and modified by (Shnawa & Thwaini, ٢٠٠٣).

٣.٩: *Detection of E. coli K١ specific antibodies*

In vaginal secretion and serum (antibody activity)

Tube agglutination (serum)and VIgs

١.Eleven clean agglutination test tubes were used and (٠.٩)ml normal saline was added to first tube and (٠.٥)ml was added to the rest tubes for sera while for VIgs ٠.٢ ml of normal saline was added to other eleven tubes .

٢. (٠.١)ml of serum was added to first tube and mixed gently by pasteur piptte and then (٠.٥)ml was transferred to the second tube until ١٠th tube. Tube number eleven was considered as a control tube containing (٠.٥)ml normal saline, while ٠.٢ml of VIgs extraction was added to tubes until ١٠th tubes .

٣. (٠.٥)ml of capsular antigen was added to each tube of serum and ٠.٢ ml of capsular antigen was added to VIgs tubes .

٤. The content was incubated at (٣٧)°C for (٢٤)hrs.

٥. The titer was recorded.

٣.١٠ :Passive hemagglutination test

It was done according to (Boyden, ١٩٥١)for both serum and VIgs and as fallows

١. Sheep blood was collected and preserved with Al sever′s solution in a proportion volume: volume The mixture was left at ٤°C for at least ٣ days

٢. (٣)ml of solution was pipette into centrifuge tube and added to (١٠)ml of saline and mixed thoroughly and centrifuged at ٢٥٠٠rpm for ٥min

٣. The supernatant was removed carefully by a Pasteur pipette. The cell was washed three times.

٤. (٢٠)ml of saline was added to (٠.٥)ml of washed red blood cells in large tube and it was mixed thoroughly. Standardized cell suspension was done by way (٢)ml of cell suspension to (١٠)ml of D. W and the optical density was read at ٥٢٠nm in spectrophotometer, the reading should fall between ٠.٤- ٠.٦

٥. (٣)ml of the cell suspension from step ٤ was pipette into centrifuge tube. Then (٣)ml of dilute tannic acid solution was added and mixed thoroughly and placed into water bath at (٣٧)°C for (١٠)mins

٦. Suspension was centrifuged for (٥)mins at ٢٥٠٠rpm and supernatant was decanted. Packed cells were resuspendend by using a Pasteur pipette. The packed cells were washed twice.

٧. To coat the tanned red blood cells with antigen (٤)ml saline, (١)ml of cell free culture and (١)ml of cell suspension was mixed thoroughly and tube was let for (١٠)min at room temperature.

٨. Tube was centrifuged for (٥)min at ٢٥٠٠rpm. The supernatant was decanted and the packed cells were washed three times and resuspended to (١)ml saline.

٩. Micro titration plate was used to dilute the serum and VIgs ٥٠ml of normal saline was added to each well of plates including ten well and eleventh well was considered as control tube for each plates

١٠. (٥٠) µl of VIgs was added to the first well and then mixed gently and pipette by micropipette to second well until ten well, while serum was diluted by adding ٠.١ml of serum to ٠.٩ ml of saline and from this tube then tranasferred ٥٠ µl to first well of plate and then mixed and pipette to second well until ten well , and (٥٠) µl of solution in ٨ step was added to each well of the eleven wells for each plates and incubated at (٣٧)°C for ٤٥mins and then titer was recorded .

٣.١١: *Interleukins*

٣.١١.١: *IL-١α ELISA*

The test was used for serum of patients and normal subjects to determine the concentration of IL-١α. This test was achieved according to the manufacturing company (Beckman coulter Tm) as fallows

١. The components of the kit were equilibrated at room temperature before use

٢. The components of the kit were prepared as the follows

Reagents	Preparation
Plate ٩٦ well	Ready to use
Standard lyophilized	Add the volume of diluent ٢ stated on the vial label
IL-١∝. Acetylcholine estrase conjugate lyophilized	Add the volume of distilled water stated on the vial label
Diluent ١	Ready to use
Diluent ٢	Ready to use
Wash solution	Dilute ٥٠ml in ٩٥٠ml of D. W
Substrate lyophilized	Add the volume of D. W on the vial label
Stop solution Tacrine	Ready to use

٣. The standard solution and the appropriate diluent were prepared to form standard curve for equation calculated. Series of dilution were as follows

Standard concentration pg/ml	IL-١α	Diluent ٢ (µl)
١٠٠٠	Reconstituted	-
٢٥٠	٣٠٠µl of ١٠٠٠	٩٠٠
٦٢.٥	٣٠٠µl of ٢٥٠	٩٠٠
١٥.٦	٣٠٠µl of ٦٢.٦	٩٠٠
٠	٣٠٠µl of ١٥.٦	٩٠٠

٤.The protocol of ELISA was included in three steps

step ١

- ١٠٠µl of standard or sample was added to wells
- ١٠٠µl of diluent ١ in standard wells or ١٠٠µl of diluent ٢ in sample wells
- The wells were incubated for ٤hrs at ٢-٨°C
- The wells were washed (manual procedure by repeating at least three cycles by turning the plate upside- down and shaking vigorously over the sink and the end cycle the micro titer plate firmly tap the inverted microtiter plate on to a clean (absorbent piper)

Step ٢

- ٢٠٠µl of conjugate was added to wells
- The plate was incubated over night at room temperature
- The wells were washed three times.

Step ٣

- ٢٠٠µl of substrate was added to wells
- The plate was incubated for ١٥min at room temperature while shaking in the dark
- ٥٠µl of stop solution was added to wells
- The plate absorbance was read at ٤٠٥nm by Beckman coulter reader.

٣.١١.٢ :IL-٨ ELISA

The test was prepared according to the manufacturing company (Beckman coulter Tm)

١. The components of the kit were equilibrated ٣٠min at room temperature before use.

٢. The components of kit were prepared as follows

Reagent	Preparation
Plate ٩٦ wells	Ready to use
Standard lyophilized	The volume of D. W was added on the vial label
Biotinylated monoclonal antibody	Ready to use
Diluent	Ready to use
Streptavidin HRP- conjugate	Ready to use
Wash solution (٢٠x)	٥٠ml of wash solution was added to ٩٥٠ml of D. W
Substrate	Ready to use
Stop solution sulfuric acid	Ready to use

٣. The standard solution (٢٠ng/ml) and appropriate diluent were prepared. The dilution series are follows.

Standard concentration	IL-٨	Diluent
٢٠٠٠pg/ml	٥٠µl of ٢٠ng/ml	٤٥٠µl
٥٠٠	١٠٠µl of ٢٠٠٠pg/ml	٣٠٠µl
١٢٥	١٠٠µl of ٥٠٠pg/ml	٣٠٠µl
٣١.٢	١٠٠µl of ١٢٥pg/ml	٣٠٠µl
٠	١٠٠µl of ٣١.٢pg/ml	٣٠٠µl

٤.The protocol of ELISA included three steps

step(١)

- ٥٠µl of standard or samples were added per well
- The plate well was incubated ٢hr at room temperature while shaking

- The wells were washed (manual procedure by repeating at least four cycles, turning plate upside down and shaking vigorously over the sink then the wells were filled with wash solution, the solution was run over the rim of wells, the plate upside down was turn while shaken vigorously over the sink and firmly tap the inverted microtiter plate onto a clean absorbent paper)

Step (٢)

- ٥٠µl of biotinylated and ١٠٠µl of streptavidin- HRP conjugate were added to wells
- The plate was incubated for ٣٠min at room temperature while shaking
- The wells were washed as pervious by mentioned

Step (٣)

- ١٠٠µl of substrate was added to each well
- The plate was incubated ٢٠mins at room temperature while shaking
- ٥٠µl of stop solution was added to each well and the absorbance was read at ٤٥٠nm by Beckman coulter Tm reader

٣.١٢: *Lapin experimental E. coli K١ vaginosis*

٣.١٢.١ :*Bacteriology*

I- Animals

Rabbits were elected as test experimental animals .They were brought from local market and of local breed (*Oryctolagus cuniculus*) and were females(١.٥-٢ Kg) . These female rabbits were checked for ecto, end haemoparasites and were found to be free and for *E. coli* K١ antibodies were also found to be seronegative . They were kept at

labium conditions during experimentation period. The overall clutch size was ١٤ (Baker, ١٩٩٨)

II- Criteria for choosing appropriate isolate

The test of bacterial isolate for infection was chosen according to the following criteria

١. The presence of clue cells in patient vagina
٢. Pus cell was of high count
٣. Phagocytic activity was high in local and systemic
٤. Isolate in heavy growth and this isolate was forming hemolysis on blood agar
٥. The titers of agglutination tubes were high in both local & systemic responses.
٦. Appropriate isolate was obtained from clinically vaginosis cases.

III- Dose determination

The doses were used following (Bortolussi *et al*., ١٩٧٩) these doses were ٠.٥×١٠^{٤}, ٠.٥×١٠^{٥}, ٠.٥×١٠^{٦} colony forming unit per ml and each dose was used for ٢ animals, so the total six animals were used, the animals were tested if the bacterial vaginosis was developed or not taking vaginal swab from each rabbit it was examined (culture, wet & stained smear as mentioned in ٣..١). Rectal temperature degree was recorded before and after inoculation . The suitable dose was determined as that inducing clinical infection as that congestion.

IV- Infection route

٠.١ml of (٠.٥×١٠^{٤}) cfu /ml was inoculated intravaginal by sterile micropipette tips.

V- Inclusion criteria for experimental clinical vaginosis

The criteria of experimental clinical vaginosis included fever, redness of vagina, pus cells clue cells, cervical secretion, *E. coli* K١ isolated.

VI- Determination of incubation period.

The period of incubation was determined by the study of experimental clinical vaginosis in animals and these signs were determined within (٤٨)hrs.

٣.١٢.٢: *Immunology*

I- Animals

Three groups of female rabbits were used

١. The first group included three animals, which were inoculated by live bacteria.
٢. The second group included three animals which were inoculated by capsular antigen .
٣. The third group included two animals which were used as negative control .

II- Antigens

١. Viable antigen (live bacteria of *E. coli* K١) dose (٠.٥×١٠^{٤})cfu/ml was used in immunization protocol.

٢. Non viable antigen (capsular antigen) was prepared as (٣.٨) The dose was ١٠ international unit was prepared and compared with WHO standard tube ١٩٧٥, and this antigen was used in immunization protocol.

III- Immunization protocol

١. The first group of animals was inoculated with live bacteria by inoculating (٠.١)ml of (٠.٥×١٠^{٤})cfu/ml of live bacteria intravaginal and this inoculation was done at intervals of ٢ days up to ١٤ days often the ١٤ days, the animals were left one week and one of them was taken after ٦ days and study the immunologic investigation . The second animal was taken after ١٢ days and the third was taken after ١٨ days.

٢. The second group of animals was inoculated with capsular antigen by inoculating ٠.١ml of (١٠Iu capsular ag) in vaginal at ٢ days intervals. For ١٤ days and the animals were left for ٣٢ days (Esposito *at el* ; ١٩٦٩).

٣. Macrophage stimulation.

Macrophages were stimulated by using casein digest (١.٢g for ١٠٠ml D. W. and was sterilized by autoclave) ٥ml of casein digest was injected in peritoneal cavity (Bloom and Bennett, ١٩٦٦).

This stimulation occurred in all groups of animals and after ٧٢hrs the peritoneal fluid was collected and used in macrophage migration inhibitory study.

٤. skin test

٠.١ ml of capsular antigen (prepared as ٣.٨.١) was injected in the skin by using sterile syringe (size ١ml) and the erythrema and induration were recorded after (٤)hrs, (٢٤)hrs,(٤٨)hrs , (٧٢)hrs. (Tompkins *et al.*, ١٩٧٣)

IV- Immunological investigation

١.Sampling from experimental animals

a. Blood samples

Blood was collected from the heart by using sterile syringe(size ٥ml).٣ml of blood was put in tube without coagulant to obtain serum and (٢)ml was put into tubes with anticoagulant and used for leucocyte migration as (٣.٦.٢No III).

b. **Autopsies of animals** These included genital tract (vagina, horns and fallopian tubes and ovary) and intestinal tract (duodenum and appendix). They were used to study mucosal immune system in these animals.

c. Peritoneal fluid

Peritoneal fluid was collected by using sterile syringe and laid in clean tubes. This fluid was used in macrophage migration inhibition study (٣.٦.٢ number III)

٢. Mucosal Ig extraction

The mucosal Igs were extracted from vagina according to (Shnawa & Abd, ٢٠٠٥) and as follows. The vagina was cut and laid in clean plates and then was opened. (١٠)ml of formal saline was added and the mucosal layer was scarbed and the suspension was collected in centrifuge tube and centrifuged for (٣٠)mins at ٣٥٠٠rpm. The supernatant was collected and pellet was used in leucocyte migration inhibition percent . Equal volumes of PEG ٦٪ were added to supernatant and left for (٣٠)mins at room temperature and centrifuged for (٣٠)mins at ٣٥٠٠rpm . The pellet was dissolved in (١)ml of formal saline and this represents MIgs. The mucosal Igs from other parts were extracted as

mentioned with vaginal mucosal Igs except in duodenum and appendix. The digest materials were removed by washing and applying the steps as in vaginal Igs.

٣. The separation of leucocytes from vagina :(Skoog & Beck, ١٩٥٦)

The pellet formed in above resuspended to (١)ml normal saline to form suspension. Equal volume of ٣٪ dextran (٠.٣g of dextran, pharmacia company were dissolved in ١٠ml of sterile saline and sterilized solution by filtration with ٠.٢٢μm Millipore filter) was added to solution and left at room temperature for (٢٠)mins then the supernatant was centrifuged at ٢٠٠٠rpm for ٥min . The pellet was collected in capillaries tubes and was used in leucocytes migration inhibition study as in (٣.٦.٢ No III).

٤. Blood samples

Blood was collected with or without anticoagulant . The blood samples were used to separate leucocytes for LIF test and other samples were used to collect serum that were used in titrating Ab in both tube and PHA test, estimation of Ig concentrations, complement $C^{٣}$, $C^{٤}$ concentration total protein concentration.

٥.The methods used for the study of mucosa were previously described when discussed rabbit mucosal lining as shown in figure ٣.٢

Three groups of animal were killed and autopsies of genital tract (vagina, horns, fallopian tubes and ovary) intestinal tract (duodenum and appendix),these biopsies were opened by clean scissor and laid in clean Petri dishes and the mucosa were scrabed with ١٠ ml formal saline and laid in clean tubes ,centrifuged at ٣٥٠٠ rpm/٣٠ minute .The solution was formed

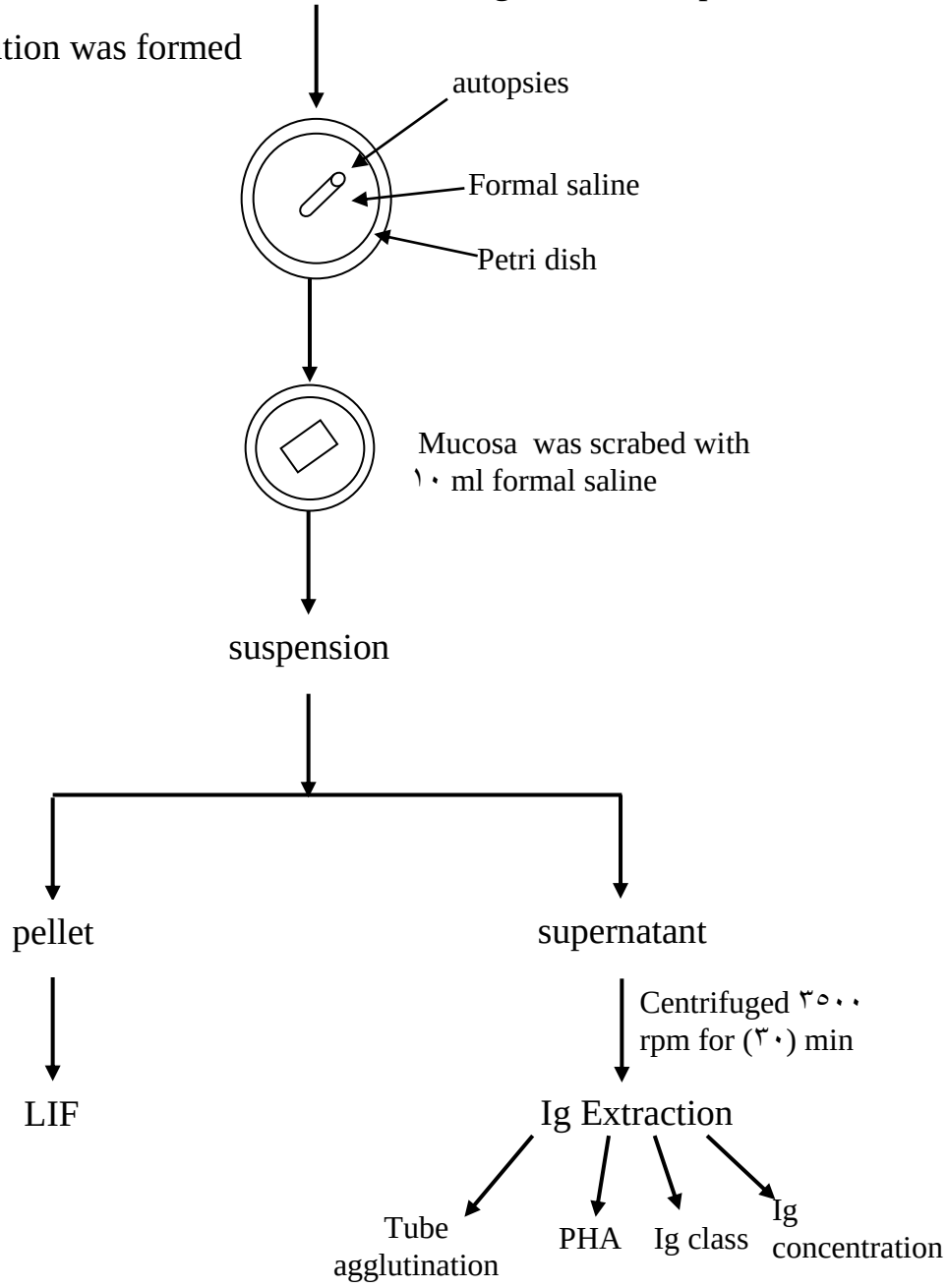


Figure (٣.٢) The study of immunological methods carried out on rabbits mucosa

٤.١. Human Vaginosis

٤.١.١: Human host clue and inflammatory cell morphology

In direct examination of vaginal swabs , clue cells were found with bacterial infection (Table ٤.١) . Mononuclear and ploymorphonuclear cells were also found in cases of bacterial vaginosis at a rate of (٢-١٠) cells/ field , likewise, this was observed with candidiasis.

Table (٤.١): Clue cells and bacterial cases

Bacteria	Presence of clue cells	Absence of clue cells
E. coli	٥٠ cases	٢ cases
Klebsiella S. P.	٨	٥
coagulase positive *Staphyloco ccus*	٤	-
Strepto coccus β- haemolyticus	٣	٢
proteus spp	٣	-
Enterobacter	٣	-

٤.١.٢: *Microbial profile.*

The study of ٣٥٢ vaginal swabs were indicated the presence of different microorganisms, most of which were non pathogenic bacteria that included *Lactobacillus spp* and diphtheroids . The pathogenic bacteria were ٨٠ and two cases belong to *Trichomonas vaginalis*. (Table ٤.٢)

Table (٤.٢): Microorganisms found in vaginal swabs

Microorganisms	Number	Percentage
Candida spp	١٠٨	٣٠.٦٨٪
Potential bacteria Pathogens	٨٠	٢٢.٧٠٪
Trichomonas vaginalis	٢	٠.٦٥٪
Non pathogenic bacteria	١٦٢	٤٦.٠٢٪
Total	٣٥٢	١٠٠٪

٤.١.٣: Bacterial profile

The bacteria that were most frequently found in cultured vaginal swabs were *E. coli* which represented ٥٢ cases out of ٨٠ cases examined ١٣ cases were *Klebsiella spp*. coagulase positive *Staphylococcus* ٤ cases ,with ٥ cases *Streptococcus* β- haemolyticus, with ٣ cases *Proteus* and ٣ cases were *Enterobacter (*Table ٤.٣)

Table (٤.٣): Bacteria contained in cultured vaginal swabs

Bacteria	Number	Percentage
E. coli	٥٢	٦٥٪
Klebsiella spp	١٣	١٦.٢٥٪
Coagulase positive *Staphylococcus*	٤	٥٪
Streptococcus β hemolytica	٥	٦.٢٥٪
Proteus spp.	٣	٣.٧٥٪
Enterobacter spp	٣	٣.٧٥٪
Total	٨٠	١٠٠٪

٤.٢: Immunological picture

٤.٢.١: protein concentration

Standard curve experiment was used, the standard protein was bovine serum albumin. The optical density was measured for each dilution using Biuret method at ٥٤٠ nm wave length where the concentration of each was considered as x_i value while the optical density

of each dilution was considered as y_i value. (Table ٤.٤) the straight line equation was obtained and found by simple linear regression analysis (Dawed & AL-Yas, ١٩٩٠).

Table (٤.٤): Statistical features for standard curve of albumin solution concentration

X_i	Y_i (g/l)	$(x_i - \bar{x})$	$(x_i - \bar{x})^2$	$(y_i - \bar{y})$	$(y_i - \bar{y})^2$	$(x_i - \bar{x})(y_i - \bar{y})$
٠.٢	٦٠	٠.١٢	٠.٠١١٤	٤٠.٣١٢	١٦٢٥.٠٥	٤.٨٣٧٥
٠.٠٧	٣٠	-٠.٠١	٠.٠٠٠١	١٠.٣١٢	١٠٦.٣٣	٠.١٠٣
٠.٠٦٥	١٥	-٠.٠١٥	٠.٠٠٠٢	-٤.٦٨٧	٢١.٩٦٧	٠.٠٧
٠.٠٦	٧.٥	-٠.٠٢	٠.٠٠٠٤	-١٢.١٨٧	١٤٨.٥٢٢	٠.٢٤٣
٠.٠٥	٣.٧٥	-٠.٠٣	٠.٠٠٠٩	-١٥.٩٣٧	٢٥٣.٩٨٧	٠.٤٧٨
٠.٠٤٥	١.٨٧٥	-٠.٠٣٥	٠.٠٠١٢	-١٧.٨١٢	٣١٧.٢٦٧	٠.٦٢٣
Σ=٠.٤٩	Σ=١١٨.١٢٥	Σ=٠.٠١٧٢٥				Σ=٦.٣٥٦٢
$\bar{x}$ =٠.٠٨	$\bar{y}$ =١٩.٦٨٧					

$$b = \frac{6.35625}{0.01725} = 368.47$$

$$a = \bar{y} - b\bar{x}$$

= ١٩.٦٨٧٥-(٣٦٨.٤٧ * ٠.٠٨)

a=-٩.٧٩

$$\hat{y} = -9.79 + 368.47 x_i$$

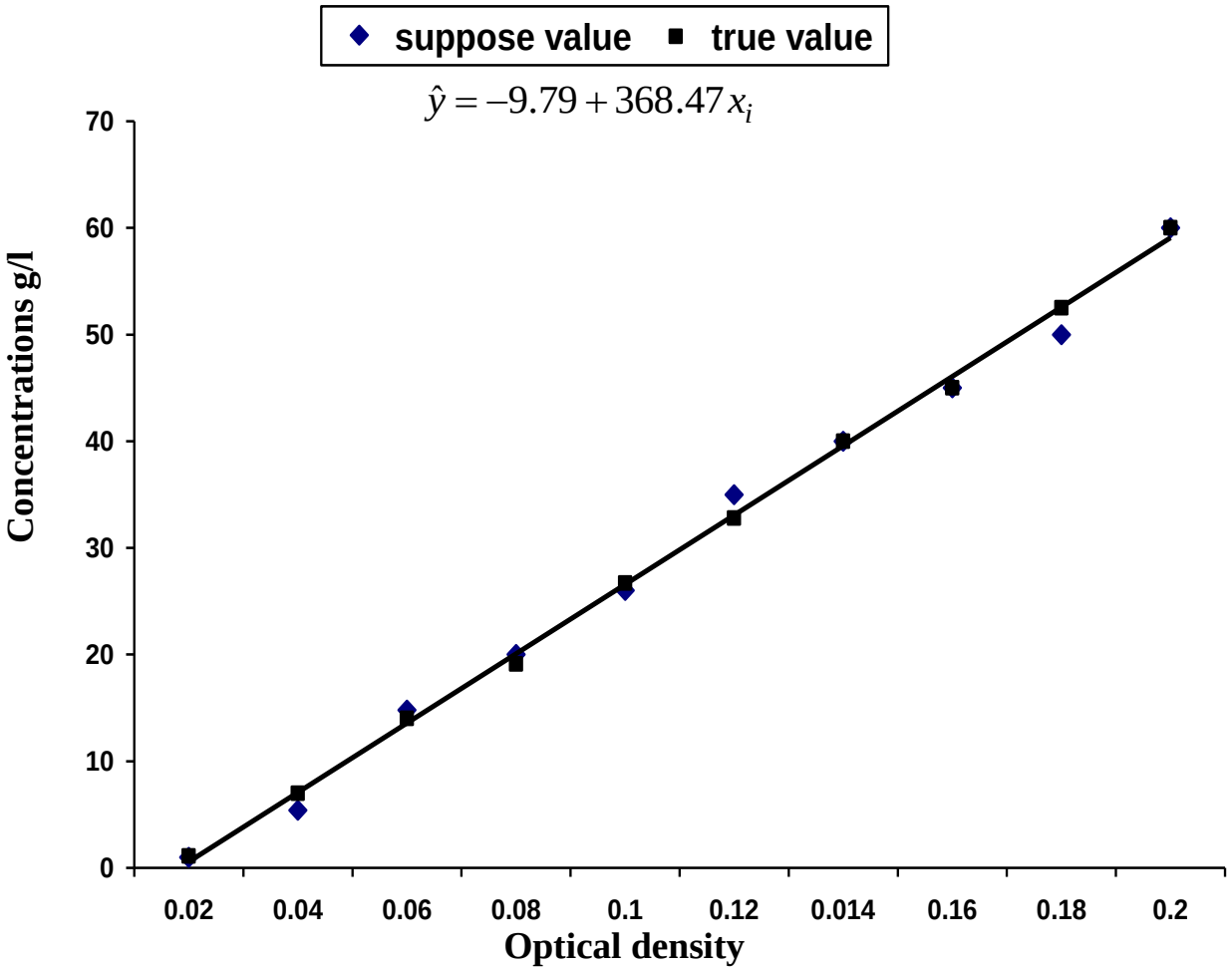


Figure (٤.١): Standard Curve for the albumin serial concentrations and their respective optical density.

٤.٢.١.١: *Total protein concentrations and vaginal immunoglobulin concentrations*

The total protein & VIg concentration were calculated from the standard curve above. Total protein concentration in patients was (٦٣.٩-٢٧٠)g/L while normal subject, was (٤٥.٤٨-١٥٦)g/L . VIgs concentration in patients was (٠.٠٧- ٢٨.٣)g/L while in normal subjects was (٠.٠٧-٢.٤٧)g/l. These results a appeared in three groups of value in patients and normal subjects table(٤.٥)

Table(٤.٥): Total protein concentrations and vaginal immunoglobulin concentrations in patients and normal subjects.

	Low concentration g/L	High concentration g/L	The Median	Mean
		Patients		
Total protein	٦٣.٩	٢٧٠	١٦٦.٩٥	١٤٢
VIgs concen.	٠.٠٧	٢٨.٣	١٤.١٨	٦.٥
		Normal subjects		
Total protein	٤٥.٤٨	١٥٦	١٠٠.٧٤	١٠٧
VIgs concen.	٠.٠٧	٢.٤٧	١.٢٧	١.١٨

٤.٢.٢: *Immunoglobulin Isotypes*

Concentration of immunoglobulin isotypes were determined by Mancini technique using single radial immunediffusion test this method was used to identify the accurate quantity of Ig and Ig class. Certain Ig concentration was detected in serum but this method was un attend to measure VIgs extraction in both patients and normal subjects.

Table(٤.٦) shows that the Ig concentrations in patients were higher than normal subject, the immunoglobulin detected were IgA, IgG and IgM . They are (٢٣.٢, ١٠٣٦.٢, ٦٤.٣) in patients while in normal were (٥٠.٤, ٢٢٣.٧, ٦٤.٣) respectively . The high concentration in patients was higher than normal concentration in all Ig isotypes.

Table (٤.٦): Immunoglobulin concentrations in sera of patients and normal subjects .

Ig class	Low mg/dl	High mg/dl	The median	Mean
		Patients		
IgA	٢٣.٢	٦٤٧	٣٣٥.١	٢٧٠
IgG	١٠٣٦.٢	١٥٦٤.٣	١٣٠٠.٢٥	١٧٢٠.٨
IgM	٦٤.٣	٣٢٢.٨	١٩٣.٥٥	١٦٣.٤
		Normal subjects		
IgA	٥٠.٤	٤٧٤.٧	٢٦٢.٥٥	٢٢٨
IgG	٢٢٣.٧	٢١٧٣.٧	١١٩٨.٧	١٣٠٠
IgM	٦٤.٣	٢٣٨.٧	١٥١.٥	١١٢.٨

٤.٢.٣: Complement Components

Certain complement components (C ٣,C ٤) were determined for both serum and VIgs. These components were detected in serum but were not present in VIgs. They were used in both patients and normal subjects (Table ٤.٧)

Table (٤.٧): Complement components in sera of patients and normal subjects.

Complement component	Low concentration mg/dl	High concentration mg/dl	median	Mean
		Patients		
C٣	١١.١	١٨١.٩	٩٦.٥	٩٩.٢
C٤	١٥.١	٥٤	٣٤.٩	٣٢.٧
		Normal subjects		
C٣	١١.١	١٨١.٩	٩٠.٩٥	٩٠
C٤	٢.٨	٥٤.١	٢٨.٤٥	٢٥

٤.٢.٤: Specific Anticapsular for K١ Antigen

Specific antibodies for capsular antigen of *E. coli* K١ were determined. The titers of specific antibody were determined in both serum and local immunoglobulins extraction (VIgs).

The titer profiles and ratio between systemic and local and their frequency are shown in table ٤.٨. The most common titer was ٦٤٠ and the ratio ١٠:١ was more predominant than other ratio in both patients and

normal subject and the ratios ٢٠:١, ٥:١ were appeared in both patients and normal subjects respectively.

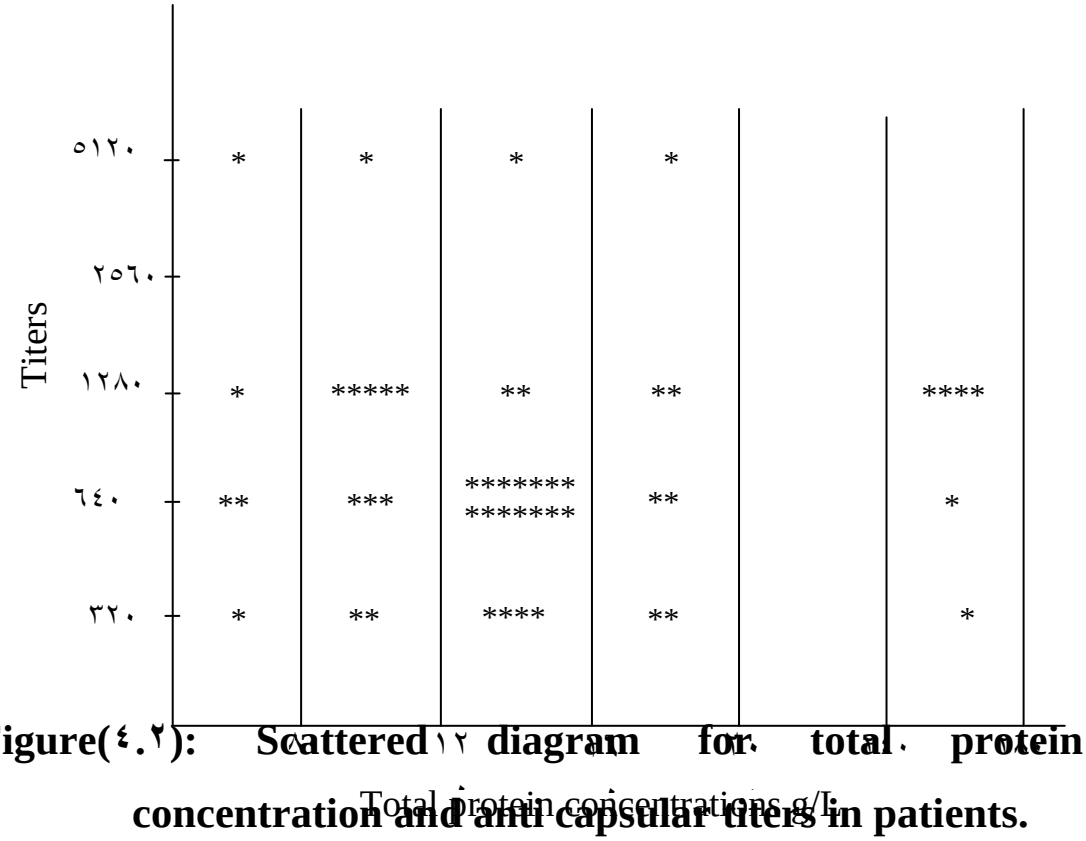
Table (٤.٨): Profile of anticapsular *E.coli* K١ antibody titer in both systemic and local in women

	Systemic	Local	Systemic/local	Frequency
Patients	٥١٢٠	٥١٢	١٠:١	٣
	٥١٢٠	١٢٨	٢٠:١	١
	١٢٨٠	١٢٨	١٠:١	١٣
	٦٤٠	٦٤	١٠:١	١٨
	٦٤٠	٥١٢	٥:١	١
	٦٤٠	٣٢	٢٠:١	٤
	٣٢٠	٣٢	١٠:١	١٠
Control	١٠	١	١٠:١	٥
	٢٠	٢	١٠:١	١٧
	٤٠	٤	١٠:١	٦
	٤٠	٢	٢٠:١	٢

٤.٢.٤.١: *Serum Protein Versus The Titer Correlates*

The relationship between the range of protein concentrations and anticapsular *E. coli* K١ titers was determined by using scattered diagram, In this diagram it can be seen that the most frequent titer was ٦٤٠, and the most cases were found in concentration range (١٢٠-١٦٠)g/L. The number of titers were decreased in high and low titer, titer ١٢٨٠ and ٣٢٠ appeared respectively, while the titers ٢٥٦٠ was not shown in most

concentrations. Figure (٤.٢). In normal subjects the titers disappeared and thus they are not found in this diagram .



Figure(٤.٢): Scattered diagram for total protein concentration and anti capsular titers in patients.

٤.٢.٤.٢: *Vaginal Ig Concentrations Versus The Titers Correlates.*

The relationship between VIgs concentrations and anticapsular *E. coli* K١ titers were determined by scattered diagram (٤.٣). Most titers were concentrated in (١-٢)g/L and (٨-١٠)g/L respectively but they decreased in higher concentration except for some cases. In normal subjects the titers were disappeared so not used this diagram in these subjects.

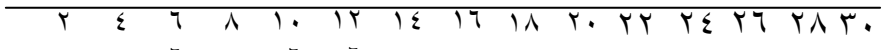


Figure.(٤.٣): Scattered diagram for the relationship between VIg concentration and anticapsular titers in patients.

٤.٢.٤.٣: *Effect of ٢ - mercaptoethanal*

The titers of agglutination in the presence of ٢- mercaptoethanol were not effected but it was effected on the prozone phenomena.

This phenomena disappeared by ٢ mercaptoethanol. This occurred in five cases.

٤.٢.٥: *Specific antibody to cell free culture antigen by using passive haemagglutintion.*

Specific haemagglutinin antibody titers are shown in Table (٤.٩). The ratio between systemic to local was higher than others and titers (٦٤) appeared as more frequent titers other and (٣٢) was the second titer. Higher titers ١٢٨, ٢٥٦ were detected in only ٣ cases and one case respectively. In normal subjects the positive result showed in the first well or second and the ratio between systemic to local was ١٠:١

Table (٤.٩): Profile of anticfc antigen in both systemic and local

	Systemic	Local	Systemic/local	Frequency
Patient	٣٢٠	٣٢	١٠:١	١٤
	٣٢٠	٦٤	٥:١	٣
	٦٤٠	٦٤	١٠:١	٢٤
	٦٤٠	٣٢	٢٠:١	٣
	٦٤٠	١٢٨	٥:١	١
	١٦٠	٣٢	٥:١	١
	١٢٨٠	١٢٨	١٠:١	١
	١٢٨٠	٦٤	٢٠:١	١
	٢٥٦٠	٢٥٦	١٠:١	١
	٢٥٦٠	١٢٨	٢٠:١	١
control	٢٠	٢	١٠:١	١٦
	٤٠	٤	١٠:١	٣
	١٠	١	١٠:١	١١

٤.٢.٥.١:Correlation between VIg concentrations and serum protein concentrations and anticfc antigen

Scattered diagram was used to determine the relationship between VIg & serum protein concentration versus titers (Fig ٤.٤, ٤.٥).

In patients the most frequent titer was ٦٤٠ then ٣٢٠ respectively and other titers decreased as ١٢٨٠ and ٢٥٦٠ VIgs concentrations were appeared in titer ٦٤ in most cases then ٣٢ and the high titers were decreased. These diagrams indicated the relationship in patient and this was not present in normal subjects.

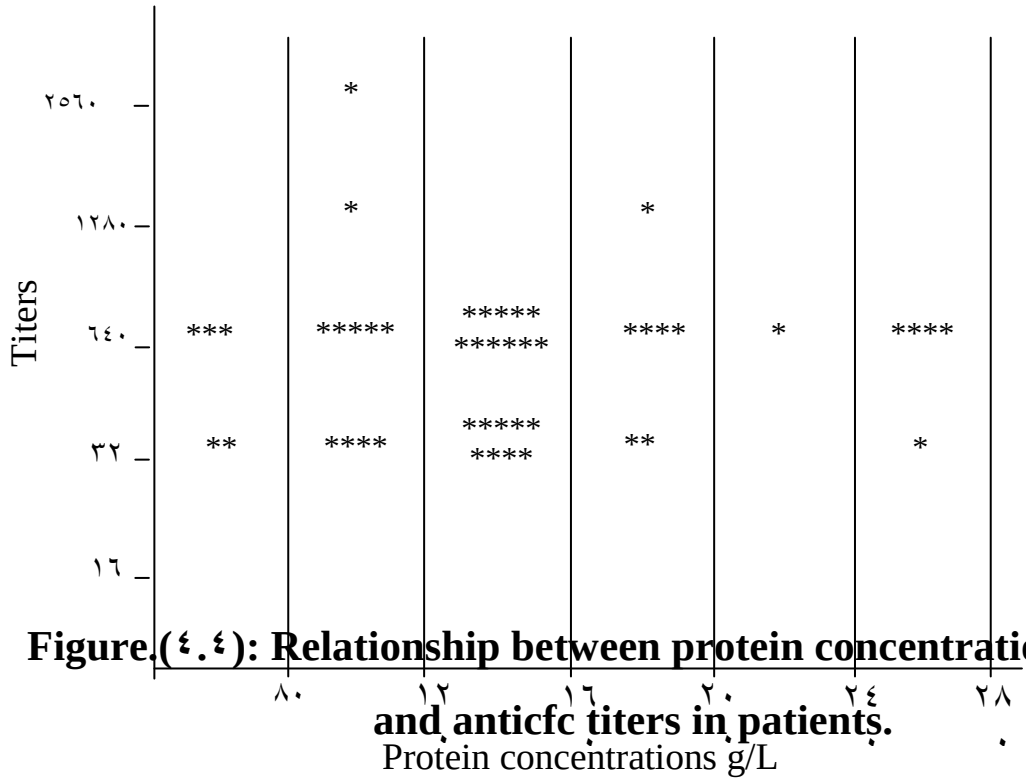
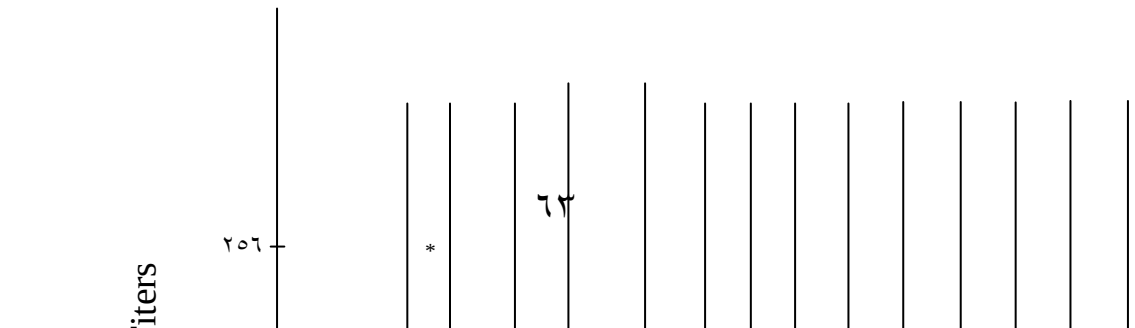


Figure (٤.٤): Relationship between protein concentrations and anticfc titers in patients.



VIgs concentrations g/L

Figure.(٤.٥):Relationship between VIg concentrations and anticfc titers in patients.

٤.٢.٦: *phagocytic activity*

Nitroblue tetrazolium (NBT) reduction was used as a marker for phagocytic activity of polymorphonuclear phagocytic cells (PMNS). The reaction was graded on the basis of the number of neutrophils with intracellular deposite of formazan per ١٠٠ neutrophits counted i.e persent positive. This test was used in peripheral blood and local vaginal secretion. The phagocytic activity in patients were more active than in normal subjects in both systemic and local(Table ٤.١٠).

Table (٤.١٠): The means and standard deviation of NBT for patients and normal subjects.

		Systemic	Local	
		NBT %	NBT %	NBT+ bacteria %
Patient	$\bar{x}$	٦١.٠٤	٤٢.١٤	٢٦.٦٤

	S	٧.٦٣	٩.٢٨	١٤.٨
Normal	$\bar{x}$	٤٦.٦	٢٠.٠٣	
	S	٩.٤	٥.٩٥	

$\bar{x}$ mean s standard deviation

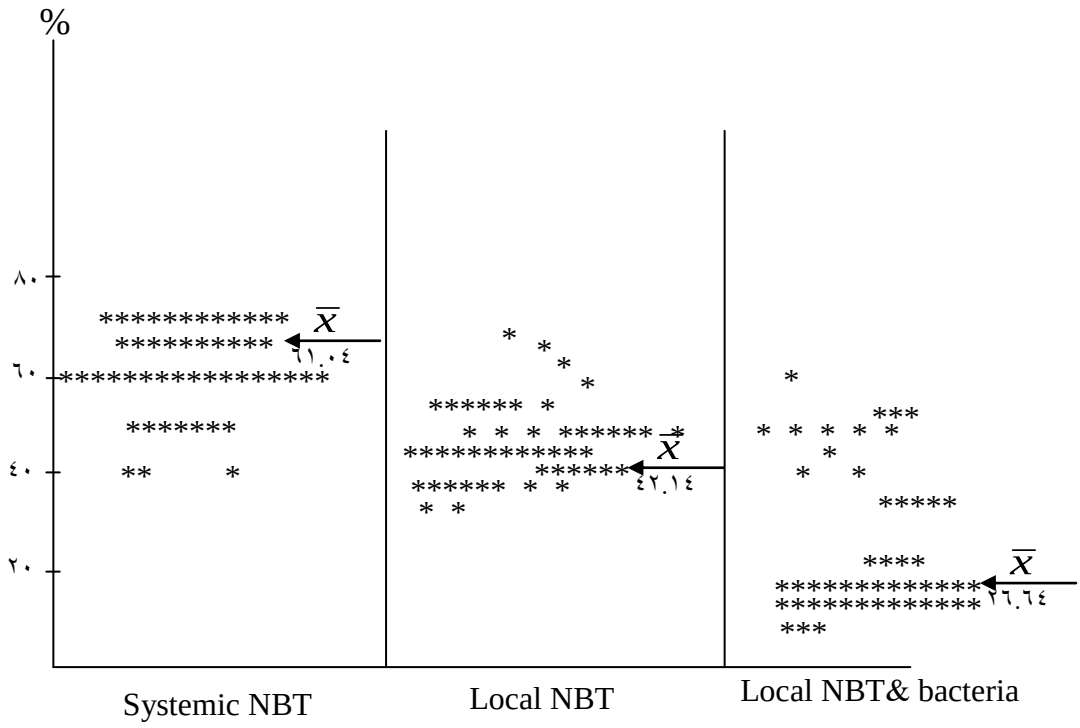
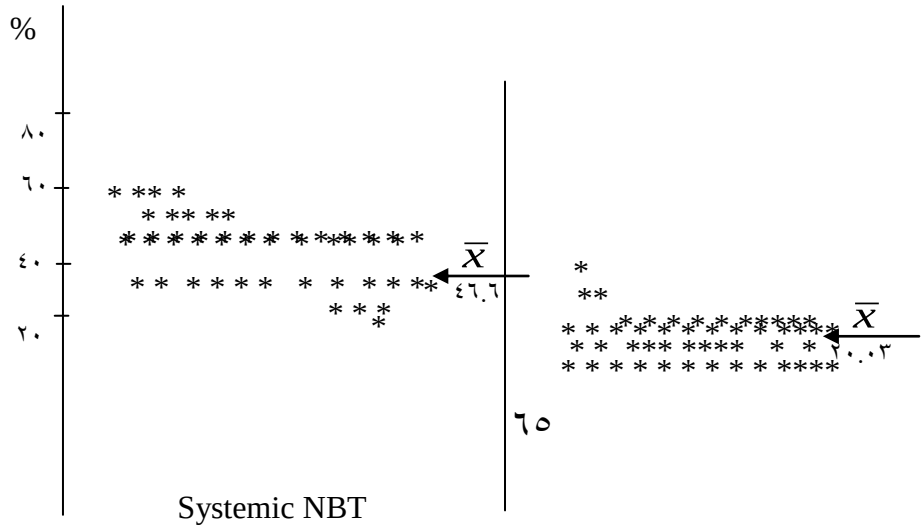


Figure.(٤.٦): Distribution of phagocytic cells in both systemic and local in patients.

]



Local NBT

Figure.(٤.٧): Distribution of phagocytic cells in both Local and systemic in normal subjects .

٤.٢.٧: *Leucocyte migration inhibition*

Leucocyte migration inhibitory factor is a ubiquitously expressed protein which is able to manifest itself as cytokine it maintains a key regulatory role in inflammation and both specific and non specific immunity. This test was carried out by using two types of antigen (capsular antigen (cap) and cell free culture antigen cfc). The percent of migration inhibition was calculated by applying the following equation

Percent inhibition=١-[(mean area of migration with antigen)/(mean area of migration without ag)]× ١٠٠

The inhibition was showed in patients in both local and systemic. Table(٤.١١) demonstrated the mean of the percent inhibition with both antigen and in both local and systemic, in normal and patients. The distribution of leucocyte migration inhibition showed in (Figures ٤.٨, ٤.٩).Significant results between ٣٠ and ٧٠ .

Table (٤.١١): The means of leucocyte migration inhibition in both local and systemic with standard deviation

	Systemic		Local	
	Cap %	Cfc %	Cap %	Cfc %

Patients				
$\bar{x}$	٥٨.٥	٤٦.٥	٦٠.٧	٤٨.٣
S	١٠.٤	١٠.٣	١٢.٣٤	١١.٩
Control				
$\bar{x}$	١٠٠	٨٩.٦٦	٨٥.٦	٨
S	٠	٨.٠٨	٣٠.١	٢٦.١

$\bar{x}$ mean

cap capsular antigen

s standard deviation cfc cell free culture antigen

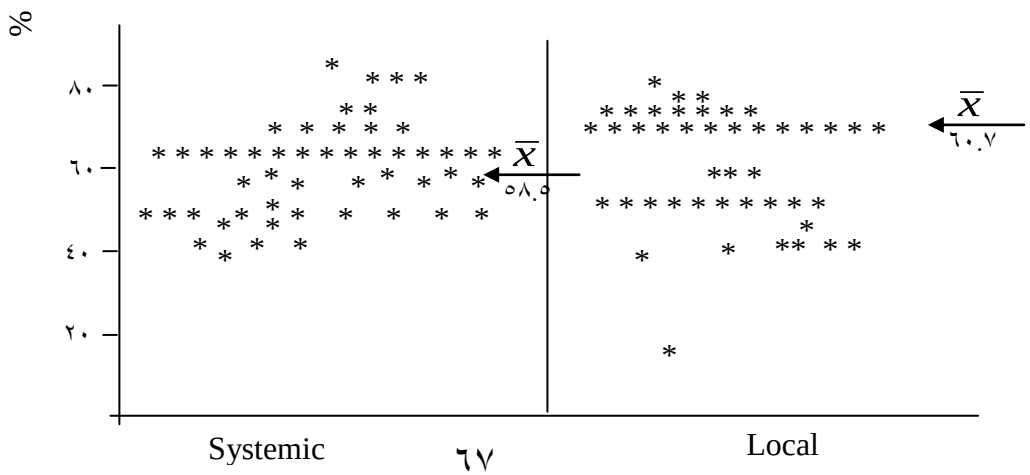


Figure.(٤.٨): Distribution of percentage of leucocyte migration inhibition in the present of capsular antigen.

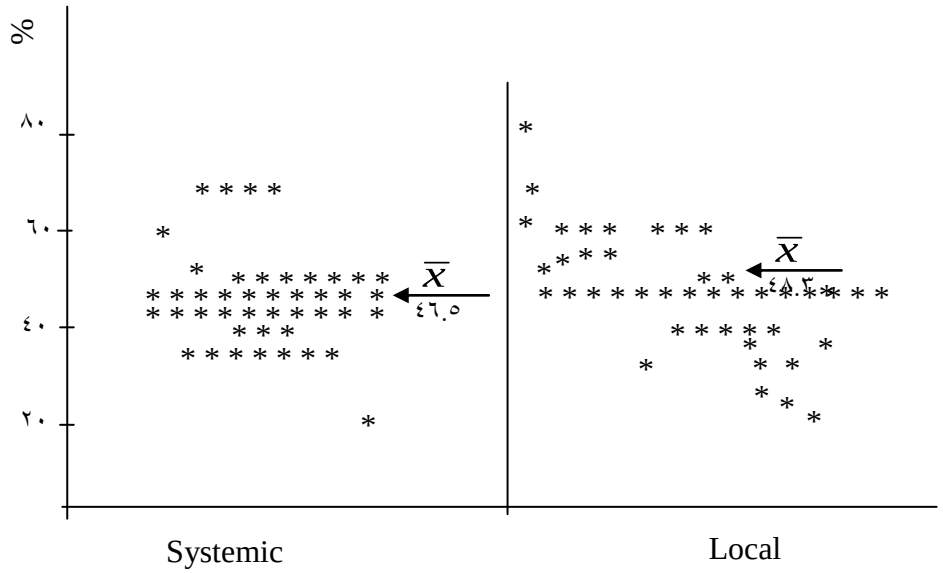


Figure.(٤.٩): Distribution of percentage of leucocyte migration inhibition in the present of cell free culture antigen .

٤.٢.٨: *IL-١ α and IL-٨*

IL-٨ and IL-١α cytokines were estimated by using enzyme linked immunosorbent assay (ELISA) were used for quantification of human IL-١α and IL-٨.The results of this test were calculated by using standard

curve fit equation . Tables (٤.١٢, ٤.١٤) show the statistical features for fit equation.

The concentration of IL-١α in sera of patients was ranged between (٢٠.٨٤٢ -١٥٥.٢٦٢) pg/ml while in normal subject it was (١٣.٢٥٣-١٥٣.٩)pg/ml(Table ٤. ١٣) .

The concentrations of chemokine IL-٨ in patients was ranged between (١٧.٩٨٦٦-٧٣٤.٤٧٦٦)while its ranged was (٥٥.٤٤٥٢-٣٥٢.٦)pg/ml in normal subjects(Table ٤. ١٥)

Table (٤.١٢): Statistical features for standard curve fit equation of IL-١α

Absorbance x_i	(y_i)pg/ml	$(x_i - \bar{x})$	$(y_i - \bar{y})$	

٠.٩٦٥	٢٥٠	٠.٦٠٨٢٥	١٦٧.٩٧٥	$\sum(x_i-\bar{x})^2=$ ٠.٥٣٩١٥٦٢٥
٠.٣٢٠	٦٢.٥	-٠.٠٣٦٧٥	-١٩.٥٢٥	
٠.١٠٧	١٥.٦	-٠.٢٤٩٧٥	-٦٦.٤٢٥	
٠.٠٣٣	٠	-٠.٣٢٤٧٥	-٨٢.٠٢٥	$\sum(y_i-\bar{y})^2=$ ٣٩٧٣٧.٢٠٧٥
∑=١.٤٢٧	∑=٣٢٨.١			$\sum(x_i-\bar{x})^2(y_i-\bar{y})^2=$ ١٤٦.١١٥٦
$\bar{x}=$٠.٣٥٦٧٥	$\bar{y}=$٨٢.٠٢٥			

$$r=\frac{\sum(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum(x_i-\bar{x})^2(y_i-\bar{y})^2}}=٠.٩٩٨$$

$a=\bar{y}-b\bar{x}=$٨٢.٠٢٥-(٢٧.٠٠٨*٠.٣٥٦٧٥)=-١٤.٦٦

$\hat{y}=a+bx_i$

$\hat{y}=$-١٤.٦٦+٢٧١.٠٠٨X_i

Table (٤.١٣): Concentration of IL-١α in sera of both patients and normal subjects

Concentration of IL-١α pg/ml (patients)	Concentration of IL-١α pg/ml in normal
٨٠.١٩٢٨	١٢٣.٨٢٥

١١٠.٠٠٣٦	١٣٠.٦٠٠٢
١٠٥.١٢٥٥	٤١.١٦٧٦
٧٥.٠٤٣٦٤٨	١٣١.١٤٢٣
١٥١.٧٦٤٤٨	١١٦.٧٧٨٨
٨٠.٤٦٣٨	١٦.٧٧٦
١٤٧.٩٧٠٣	١٣.٢٥٣
١٣٠.٠٥٨	١٧.٠٤٧٩
٢١.٣٨٤٠٦	١٢٢.٧٤١
٧٥.٠٤٣٦	١٥٣.٠٩٣٩
١٥٥.٢٦٢٠١	
١١٣.٥٢٦٧٨	
١٥٣.٣٦٤	
١٢٩.٧٨٧٢	
١٤٠..٦٢٧٥	
١٥٤.٩٩١	
٢٠.٨٤٢	
٧٨.٢٩٥٧	
٧٧.٢١١٧	
١٥٣.٠٩٣٩	
N=٢٠ $\bar{x}$ = ١٠٧.٧٠٢	N=١٠ $\bar{x}$ =٨١.٨٤٥

Table (٤.١٤): Statistical features for standard curve fit equation of IL-٨

Absorbance	Concentration	$(x_i - \bar{x})$	$(y_i - \bar{y})$	

Standard x_i	y_i pg/ml			
٢.١٧٧	٥٠٠	١.٣٢٦٣٧٥	٣٣٥.٩٥	$\sum(x_i-\bar{x})^2$ =٢.٤٧٦٥٧
٠.٦١٩	١٢٥	-٠.٢٣١	-٣٩.٠٥	
٠.٤٨٥	٣١.٢	-٠.٣٦٥	-١٣٢.٨٥	
٠.١٢١٥	٠	-٠.٧٢٨٥	-١٦٤.٠٥	$\sum(y_i-\bar{y})^2$ =١٥٨٩٤٨.٨٣
$\bar{x}$ =٠.٦٥	$\bar{y}$ =١٦٤.٠٥			$\sum(x_i-\bar{x})^2(y_i-\bar{y})^2$ =٦٢٢.٦١٦٩

$$b=\frac{622.6169}{2.47657}=٢٥١.٤٠٢٩٠٨١$$

$$a=\bar{y}-b\bar{x}=١٦٤.٠٥-(٢٥١.٤*٠.٨٥)=٤٩.٦٤$$

$$\hat{y}=a+bx_i$$

$$\hat{y}=-٤٩.٦٤+٢٥١.٤x_i$$

$$r=\frac{622.6169}{627.416625}=٠.٩٩٢$$

Table (٤.١٥) Concentration of IL-٨ in sera of both patients and normal subject

	Concentration of IL-٨ pg/ml in patients		Concentration of IL-٨ pg/ml in normal
١.	٧٣٤.٤٧٦٦	١.	٣٥٢.٦
٢.	١٠٠.٤٤٥٨	٢.	٣١١.٦٢١٨
٣.	١١٦.٧٨٦٨	٣.	٢٠٣.٠١٧
٤.	٥٦.١٩٩٤	٤.	١١٨.٥٤٦٦
٥.	٥٤.٤٣٩٦	٥.	١٢٥.٥٨٣٨
٦.	٥٠.٩٢	٦.	٦٨.١٤٠٩
٧.	٨١.٨٤٢٢	٧.	٥٥.٤٤٥٢
٨.	١٦٠.٧٨١٨	٨.	٢٧٥.٤٢٠٢
٩.	٣٢.٥٦٧٨	٩.	٢٧٩.١٩١٢
١٠.	٤٢.٨٧٥٢	١٠.	٢٥٢
١١.	٦٧.٥١٢٤		$\bar{x}$=٢٠٤.١٦٠
١٢.	٤٣.٨٨٠٨		
١٣.	٨٩.٦٣٥٩		
١٤.	٣٩.٨٥٨٤		
١٥.	١٧.٩٨٦٦		
١٦.	٥٨.٢١٠٦		
١٧.	٦٨.٢٦٦٦		
١٨.	٥٠.٩٢		
١٩.	٤٥.٦٤٠٦		
٢٠.	٣٧.٣٤٤		
	$\bar{x}$=٩٧.٥٢٩		

٤.٣: Lapin E. coli K١ Vaginosis

٤.٣.١: Infection Model

E. coli K١ experimental lapin vaginosis was produced in its mild form where, fever, vaginal congestion and or slight secretions were noticed. *E coli* K١ was isolated in ١/٣ of the infected rabbits.

Table (٤.١٦): The parameter that used to determine the infection model

Parameters	Group١	Group٢	Group٣
Dose CFU/ ml	٠.٥x١٠^{٦}	0.5×10^{5}	0.5×10^{4}
Volume/ ml	٠.١	٠.١	٠.١
Fever	+	+	+
Pus cell (٥-١٠)/field	-	+	+
Redness of mucus membrane	+	-	+
E. coli	-	-	+
secretion	+	+	+

٤.٣.٢: Immunological Model

E.coli K١ stimulate mucosal and systemic humoral immune responses as well as mucosal and systemic cellular immune responses when rabbits were immunized with lives and capsular material as specific immunologic tests were indicated.

٤.٣.٢.١: *Specific antibody titers in rabbits*

Specific antibodies appeared in serum and mucosal parts in rabbits that were inoculated with live bacteria and capsular antigen. The most frequent titers were ٦٤ in local and ٦٤٠ in systemic application of capsular antigen followed by ٣٢٠ and ٣٢ present with vaginal Igs and horns in genital tract (Table ٤.١٧) .

٣٢ was the most frequent titers by passive haemagglutination and ٦٤ appeared only in duodenum and ٦٤٠ in serum. In (Table ٤. ١٨)anticapsular Ab titers were ٦٤ and ٣٢; titers ١٢٨٠ was appeared in systemic.

Anti cfc antigen ٣٢ was most dominant than ٦٤ and in systemic was ٦٤٠. The mean serum concentration protein in rabbits received live bacteria was ١١١.٨g/L while in rabbit with capsular antigen was ١١٩.١٧g/L. The mean of mucosal immunoglobulin concentration in rabbits with live bacteria was ٢.٨٢g/L while with capsular in those treated it was ١١.١٢g/L.

The titers were not shown in normal rabbits and the mean of protein concentration was ١٢٢g/L and the mean of mucosal Ig ٣.٣٥g/L .

Table (٤.١٧): Specific antibody titers and correlate with concentration in rabbit treated live bacteria

	Anti capsular		Anti cfc		Concentration g/L	
	serum	mucosal	serum	mucosal	serum	mucosal
Rabbit ١	٦٤٠	D=٦٤	٣٢٠	D=٦٤	١٠٤.٤٣	١.٢٦
		A=٦٤		A=٣٢		٠.٥٢
		O=٦٤		O=٣٢		٨.٦
		V=٣٢		V=٣٢		٤.٩
		H=٣٢		H=٣٢		٤.٩
Rabbit ٢	٦٤٠	D=٦٤	٦٤٠	D=٦٤	١١١.٨٠٥	٠.٥٢
		A=٦٤		A=٣٢		٠.٨٩
		O=٦٤		O=٣٢		٨.٦
		V=٦٤		V=٣٢		٠.٨٩
		H=٣٢		H=٣٢		٣.٤٧
Rabbit ٣	٣٢٠	D=٦٤	٦٤٠	D=٦٤	١١٩.١٧	٠.١٥٨
		A=٦٤		A=٣٢		١.٢٦
		O=٦٤		O=٣٢		١.٢٦
		V=٦٤		V=٣٢		٠.١٥
		H=٣٢		H=٣٢		٤.٩٤
					$\bar{x}$ =١١١.٨	$\bar{x}$=٢.٨٢

D=duodenum
A=Appendix
O=Ovary and fallopian tubes
V=Vagina
H=horn
I=Immunoglobulin
$\bar{x}$ = the mean
anti cfc = anti cell free culture antigen

Table (٤.١٨): Specific antibody titers and correlate with concentration in rabbit that received capsular antigen.

	Anti capsular		Anti cfc		Concentration g/L	
	serum	mucosal	Serum	mucosal	serum	mucosal
Rabbit ١	١٢٨٠	D=٦٤	٦٤٠	D=٣٢	١١٩.١٧	١.٢٦
		A=٣٢		A=٣٢		٠.٥٢
		O=٦٤		O=٣٢		٤.٤٩
		V=٣٢		V=٣٢		٠.٥٢
		H=٣٢		H=٣٢		١٦
Rabbit ٢	٦٤٠	D=٦٤	٦٤٠	D=٣٢	١١٩.١٧	٤.٩٤
		A=٦٤		A=٣٢		١.٢٦
		O=٣٢		O=٣٢		٦.٧٩
		V=٣٢		V=٦٤		٢٣.٦
		H=٣٢		H=٣٢		١٦
Rabbit ٣	٣٢٠	D=٣٢	٦٤٠	D=٦٤	١١٩.١٧	٢٣.٦
		A=٦٤		A=٣٢		٨.٦٣
		O=٦٤		O=٣٢		٢٣.٦
		V=٦٤		V=٣٢		١٩.٦
		H=٣٢		H=٣٢		١٦
					$\bar{x}$=١١٩.١٧	$\bar{x}$=١١.١٢

D=dodenum

A=Appendix $\bar{x}$ = the mean

O=Ovary and fallopian tubes antigen

V=Vagina

H=horn

anti cfc = anti cell free culture

٤.٣.٢.٢: *Immunoglobulin isotypes in rabbits*

Immunoglobulin isotypes were determined in serum of rabbit by using single radial immunediffussion plates and also there were attempts to determine isotypes in mucosal Igs but no results were obtained

The mean of IgG concentration in rabbits with live bacteria was more than concentration in rabbits with capsular antigen while IgA concentration was similar in both, IgM concentration in rabbits with capsular antigen was more than its concentration in rabbits with live bacteria and capsular antigen more than their concentration in control .

Table (٤.١٩): Immunoglobulin isotypes in serum of rabbits

Type of treatment	IgG mg/dL	IgA mg/dL	IgM mg/dL
live bacteria	٢٩٣٧.٩	١٣٠.٨	٢٣٨.٧
	٢٨٦٤.٤	١٣٠.٨	٣٣٢.٨
	٢٩٣٧.٩	٢٢٩	٦٤.٣
	$\bar{x}$=٢٩١٣.٤	$\bar{x}$=١٦٣.٥٣	$\bar{x}$=٢٠٨.٦
capsular antigen	٢١٧٣.٧	٢٢٩	٣٢٢.٨
	٢٩٣٧.٩	١٣٠.٨	٣٢٢.٨
	٢٨٦٤.٤	١٣٠.٨	٦٤.٣
	$\bar{x}$=٢٦٥٨.٦٦	$\bar{x}$=١٦٣.٥٢	$\bar{x}$=٢٣٦.٦
Control normal saline	٢١٧٣.٧	١٣٠.٨	٦٤.٣
	٢٩٣٧.٩	٢٣.٢	٣٢٢.٨

	$\bar{x}$ =٢٥٥٥.٨	$\bar{x}$ =٧٧	$\bar{x}$ =١٩٣.٥

٤.٣.٣: *Complement components in rabbits*

Certain complement components C٣ and C٤ were determined by single radial immunediffussion test the means of C٣ and C٤ in rabbits with capsular antigen were more than their mean in rabbits with live bacteria but their means were more than the means in the control group .

Table (٤.٢٠): Complement component in serum rabbits

Type of treatment	C٣ mg/dL	C٤ mg/dL
Live bacteria	١٢٠.٦	٣٩.٤
	١٨١.٩	٢٦.٢
	١٨١.٩	٣٩.٤
	$\bar{x}$ =١٦١.٤٦	$\bar{x}$ =٣٥
capsular antigen	١٢٠.٦	٢٦.٢
	٢٥٢.٦	٥٤.٧
	٢٥٢.٦	٥٤.٧
	$\bar{x}$ =٢٠٨.٦	$\bar{x}$ =٤٥.٢
Control normal saline	١٨١.٩	٣٩.٤
	١٢٠.٦	١٥.١
	$\bar{x}$ =١٥١.٢٥	$\bar{x}$ =٢٧.٢٥

٤.٣.٤ : leucocyte migration inhibition in rabbits

The percents of leucocyte migration inhibition were studied in rabbits in three sites the peritioneal fluid, peripheral blood and vaginal mucosa. The antigens that were used in this test were capsular antigen (cap) and cell free culture antigen (cfc).

Table (٤.٢١) the percent of inhibition was calculated as the equation

$$percent\ of\ leucocyte\ migration\ inhibition\ = 1 - \frac{[(mean\ of\ migration\ area\ with\ antigen\ )]}{mean\ of\ migration\ area\ without\ antigen} \times 100$$

In table (٤.٢١) the peritoneal fluid , peripheral blood and vagina mucosa demonstrated strong migration inhibition in the presence of antigens capsular and cfc .These were showed in rabbits with capsular antigen or live bacteria while no inhibition of migration appeared in control group .

Table (٤.٢١) :Leucocyte migration inhibition study rabbits

	peritoneal		Peripheral blood		Vagina mucosa	
Type of treatment	cfc	cap	cfc	cap	cfc	cap
live bacteria	٤٠	٥٠	٦٥	٨٦	٤٣	٥٨
	٣٤	٥٩	٢٥	٥٠	٢٩	٦٥
	٤٠	٥٠	٥٩	٧٥	٤٢	٥٩
	$\bar{x}$ =٣٨ S = ٣.٥	$\bar{x}$ =٥٣ S=٥.٢	$\bar{x}$ =٤٩.٦ S=٢١.٥	$\bar{x}$ =٧٠.٣ S=١٨.٤	$\bar{x}$ =٣٨ S=٧.٨	$\bar{x}$ =٦٠.٦ S=٣.٧
capsular antigen	٤٤	٥٠	٤٤	٥٠	٢٩	٥٠
	٢٥	٥٠	٤٤	٥٠	٤٤	٥٩
	٤٠	٥٠	٤٠	٦٠	٣٩	٦٢
	$\bar{x}$ =٣٦.٣	$\bar{x}$ =٥٠	$\bar{x}$ =٤٢.٦	$\bar{x}$ =٥٣.٣	$\bar{x}$ =٣٧.٣	$\bar{x}$ =٥٧
	S=١٠.٠١	S=٠	S=٢.٣	S=٥.٧	S=٧.٦	S=٦.٢
Normal saline	١٠	٣	٥	٥	٥	٥
	١٥	٥	٩	٩	٥	٩
	$\bar{x}$ =١٢.٥	$\bar{x}$ =٤	$\bar{x}$ =٧	$\bar{x}$ =٧	$\bar{x}$ =٥	$\bar{x}$ =٧
	S=٣.٥	S=١.٤	S=٢.٨	S=٢.٨	S=٠	S=٢.٨

$\bar{x}$ = mean

s= standard deviation

٤.٣.٥:*Skin test*

Delayed type hypersensitivity reaction was studied in rabbits by using capsular antigen. Positive results were determined using various calibration double fold thickness which was measured in the rabbit after ٢٤ hr , ٤٨hr and ٧٢hr and the signs were erythrema and indurations and the indurations ١٠mm to ٢٨ mm .

٤.٣.٢ :*I L-١α and IL-٨ in rabbits*

IL-١α and IL-٨ ELISA were used to determine the concentration of

IL-١α and IL-٨ in the rabbits groups and used the same equations that used in human were used here . Table (٤.٢٢) showed that concentrations of IL-١α in test groups (live bacteria and rabbits with capsular ag) were higher than normal, and the concentrations of IL-٨ in test group had higher concentrations than normal.

Table (٤.٢٢): IL-١α and IL-٨ concentrations in rabbits

Type of treated	IL-١α concentration pg/ml	IL-٨ concentration pg/ml

live bacteria	١٦١.٧٦٦٢	١١٨.٧٩٨
	١٦٤.٢٠٥٢٨	١٢١.٣١٢
capsular antigen	٨٢.٩٠٢٨٨	٧٣.٦٨٣٢
	١٠١.٠٦٠٤١٦	١٧٣.٢١٤٦
normal saline	٣٣.٣٣٩٨	٣٤.٠٧
	٢٥.٤٤٩١٨	٦٥.٥٠١٢

٥.١ :Human vaginosis

٥.١.١ :Human host clue and inflammatory cell morphology

Vaginatis is an inflammation of vagina and vaginosis ,an imbalance in the normal flora of the vagina without inflammation .Vaginal infection are frequently the cause of distress and discomfort in adult women .The vaginal ecosystem is a complex biosphere composed of a great variety of microorganisms ,these interact in various ways to maintain a balanced of healthy state .The ecosystem is in a constantly challenged by both endogenous and exogenous factors (Harstall and Corabian ,١٩٩٨)

Results of direct examination showed that epithelial cells,clue cells and pus cells .The clue cell (asymmetrical ,mature vaginal epithelial cells with small nucleus and increase in cases where a large number of bacteria (thousands) adhere to the cell surface it is difficult to distinguish the edge of the epithelial cell ,the epithelial cell become abascure) .The clue cells were shown with bacterial infection . These cells are important to indicate bacterial vaginosis and are one of Amsel criteria and their present refer to *Gardnerella vaginalis* (Totten *etal* .,١٩٨٢ ; Catlin ١٩٩٢; Forsum (b) *etal* .,٢٠٠٥) .In the present study found that the clue cells were present with Gram negative and Gram positive bacteria and one study showed that clue cells present with vaginal *E.coli* (Rajan *etal.* ,١٩٩٩).

٥.١.٢ : Microbial profiles

The microorganisms that are found in vaginal simple swabs ,*Candida spp* (٣٠.٦٨٪) ,potential pathogenic bacteria (٢٢.٧٪) and *Trichomonas vaginalis* (٠.٦٥٪) were lower than the results found by some other researchers (Hernandez *etal* ,١٩٩٨) and in Iraq (Abas ,٢٠٠١) who found that the percentage of *Trichomonas vaginalis in vaginal swabs* was (٠.٨ %) and *candida* spp was (٤٦.١٪) .These studies were carried out in Hilla city .In Basra another study indicated that the *T. vaginali*s percentage was (١٣ %) (Mahdi *etal*. ,٢٠٠١) .In Baghdad the percentage of *T. vaginalis* was (٩.٧٪) (Al Mudhaffar ,١٩٩٥) .From these studies it can be seen that the percentage of *T.vaginalis* in vaginal swabs was low in different sites in Iraq this due to variations between individuals and method that used to obtain swabs.

In many studies on the trichomonaisis indicated that tropical countries report that ١٥-٤٠٪ of women in reproductive age are infected with *T.vaginalis* and trichomonaisis is known to be one of most common sexually transmitted diseases among women and can be a symptomatic in ٥٠٪ ٠f cases ,it is estimated that ١٨٠ million women in the world suffer from vaginitis due to *T.vaginalis* every year (Saxena and Jenkins ,١٩٩٠; Petrin *etal* . ١٩٩٨;Vanderschee *etal* ,١٩٩٩; Stary *etal* .,٢٠٠٢).

Candida albicans was the most prevalent pathogen and many studies reported that candidiasis caused primarily by *C.albicans* remain a significant problem in women of childbearing age , *C .albicans* is a commensal organism of the gastrointestinal tract and reproductive tract ,several exogenous factors predisposed menarchal women to acute vulvovaginitis candidiasis and these include hormonal modulations associated with pregnancy ,the luteal phase of menstral cycle ,high dose oral estrogen contraseptive and hormone replacement therapy and non

hormonal factor such as antibiotic usage and uncontrolled diabetes mellitus .(Sobel ,١٩٩٢; Fidel *etal* .,٢٠٠٤)and *Candida* spp has been considered as sexually transmitted disease since ١٩٦٧ (Sobel *etal.*,١٩٩٧).

Lactobacillus spp and diphtheroid present in (٤٦.٠٢٪). *Lactobacillus* considered the healthy state of vagina and have long thought to protect against vaginal infection by maintaining an acid environment or by producing metabolic such as hydrogen peroxide that inhibit other vaginal microorganisms (Skarin and Sylwan ,١٩٨٦ ; Vasquez *etal.* ,٢٠٠٢) .

The most frequently bacteria detected in the present study was *E.coli* ,it was (٦٥٪) this result was agreed with other study in which *E.coli* was one of the common organisms in the microflora of pregnancy as well as non pregnant women and has potential to inflict different clinical syndrome ,such as vaginosis and disease e.g. pelvic imflammatory disease (Bartlett *etal* .,١٩٧٧;Chow *etal* ., ١٩٨٦; Yasuoka *etal.* ,٢٠٠٢) .

Klebsiella spp. ,*Proteus spp*.and *Enterobacter spp*. are member of enterobacteriaceae and may act as potential pathogens (Hernandez *etal.* ,١٩٩٨; Todar ,٢٠٠٢) .Gram positive bacteria were appeared and these include *Staphylococcus aureus* and *Streptococcus* B-haemolytica these bacteria colonized vagina and cause disease to newborn infant (Boyer *etal* .,١٩٨٤ ; Hordens *etal.*, ١٩٩٧)

٥.٢: Immunology

٥.٢.١ : Protein and vaginal immunoglobulin (vIg) concentrations

Total protein and VIg concentrations in patients were more than their concentration in normal subjects. This result was expected because the immunoglobulins increase in both serum and local and other protein also increase in serum such as complement components ,C-reactive protein and other protein during infection (O' Reilly *etal* .,١٩٧٦; Johansson *etal.*, ٢٠٠١).

VIg concentrations in human vaginal secretion varies because of endogenous or exogenous sex hormone (Kutteh *etal* .,١٩٩٦ ; Nardelli-Haefliger *etal.* , ٢٠٠٣) .The median of VIg concentrations in normal was in a high degree similar to those found by (Wassen *etal*., ١٩٩٦; Nardelli-Haefliger *etal* . , ٢٠٠٣) The median of VIg in previous studies was nearly ٢.١ g/l while in present study was ١.٢٧ g/l. Also many studies indicated that the local response was mostly characterized by the prompt development of a concentration of IgA antibody exceeding that detected in normal (Brandtzaeg ,١٩٩٧ ; Russell and Mestecky ,٢٠٠٢) .

٥.٢.٢ : Immunoglobulins and complement components

Single radial immune diffusion plates were used to determine Ig isotypes ; complement components and their concentration .Table (٤.٦) demonstrates in general, Ig concentrations varies , this may be due to nutritional state ,hormonal state for example lower IgA concentration which was ٢٣.٢ mg/ dl in patients was while in normal , it was ٥٠.٤ mg/dl ,IgG also appeared in high concentration was ١٥٦٤.٣ mg/dl and in normal was ٢١٧٣.٧ mg/dl and IgM same concentration in both normal

and patients ,this was ٦٤.٣mg/dl .This result may be due to various factors for example Ig concentration and isotypes were variable in genital secretion due to individual variables such as age and phase of menstrual cycles and method of sampling (Brandtzaeg ,١٩٩٧).

Complement components C٣,C٤ appeared in higher concentration in patients than normal and appeared with no difference among individual .This result is usual because antigen or antigen antibody complexes stimulated the complement pathway to avoid infection (Pluschke *etal*.,١٩٨٣).

٥.٢.٣: *Specific humoral immune resposes in both systemic and local*

Specific antibodies against capsular k١ antigen in both serum and vaginal secretions are presented in table (٤.٩) of infected women. Specific secretory IgA antibodies to *Neisseria gonorrhoeae* in the genital secretion(O Reilly *etal* .,١٩٧٦ ; Mesteky *etal* ., ٢٠٠٤).Other studies were shown specific response to human papillomavirus in genital tract of women and found specific IgG,IgA to virus (Nguyen *etal*., ٢٠٠٥) .

Specific antibody to *Chlamydia* ,*Candida* ,and *Streptococcus* B haemolytica was also demonstrated in female genital tract (Brandtzaeg ,١٩٩٧ ;Russell and Mestecky ,٢٠٠٢)similar titers of antibodies were detected in both serum and local secretions in the present study .This was found also by (Mestecky *etal.* ,٢٠٠٤) ,in a study on HIV-١ IgG in serum and vaginal washes .

In contrast many studies have been reported in recent years on female genital immune responses to different immunization strategies. Variable results were noticed depending on the type of antigen and route of administration and other factor .Significant local antibody production and dissemination to other mucosal effecter sites or to the systemic

compartment is limited, probably because the genital tract lacks organized mucosal inductive sites equivalent to peyers' Patches where common mucosal immune response are generated (Wu *etal* .,٢٠٠٠).

Table (٤.٨) was appeared the high titers (٥١٢٠) and local (٥١٢) the ratio between systemic and local was ١٠:١ , ٢٠:١and ٥:١ this result were appeared titer as in (Abd and Shnawa ,٢٠٠٢) . Immunization with cholera toxin subunit B found specific IgA was increased ٤.٩ fold and other group ٢.٩ and IgG was increased ١٦ fold so present variation in level of Ig in vaginal secretion varies due to many factors(Johansson *etal* .,٢٠٠١)

The ratio between cervical secretion versus serum percentage were IgA (١٨.٥) this study to polysaccharide capsules of group B Streptococci also IgG was ٥.٤ (Hordnes *etal*.,١٩٩٨) .

In table (٤.٨) the normal subjects had low titers so the base line titer was ٨٠ and the infection titer was ٣٢٠. The titers in serum of women were similar to that appeared against *Chlamydia pneumonia* ,these titers were ٥١٢٠,٦٤٠, ٣٢٠ (Halme *etal.* ,١٩٩٨) .Figures (٤.٢ ,٤.٣) demonstrated that the distribution of titers among concentrations . Figure (٤.٢) shows that titer ٦٤٠ was higher frequency and match the concentration of(١٢٠-١٦٠) g/l and decreased in both low and high concentration of total protein ,titer (٣٢٠) also concentrated in (١٢٠-١٦٠) g/l but less frequency than ٦٤٠ , ١٢٨٠ increased in (٨٠-١٢٠)g/l and (٢٤٠-٢٨٠) g/l . This results were agreed with (Abd and Shnawa ,٢٠٠٢).

In figure (٤.٣) showed most cases present in(٠ - ٢) g/l and (٨ - ١٠) g/l and the titer ٦٤ was the most frequency titer and then ٣٢ and ١٢٨ respectively .From this it can be seen that the Ig highly variable among the individual (Brandtzaeg ,١٩٩٧ ; Kozlowski *etal.* ,٢٠٠٢ ; Russell and Mestecky , ٢٠٠٢; Mestecky *etal* . ,٢٠٠٥) .

Table (٤.٩) showed the titers of anti cell free culture antigen in both patients and normal subjects .The most frequency titer was ٦٤٠ and then ٣٢٠ ,titer ١٢٨٠ and ٢٥٦٠ ,the titer ١٦٠ was appeared . The ratios between systemic to local also demonstrated and appeared (١٠:١, ٢٠:١,٥:١) this agreed with (Abd and Shnawa ,٢٠٠٢). .

Scattered diagrams were used to show the distribution of cases among concentrations . Figure (٤.٤) was appeared that titer ٦٤٠ was distributed among various concentrations and ٣٢٠ also distributed as in titer ٦٤٠ ,other titers were appeared in few cases . In figure (٤.٥) the titer (٦٤) was concentrated in (٠-٢) g/l and (٦-٨) g/l and decreased with high concentration ,titer ٣٢ was appeared similar as (٦٤) .

These results matched the results in many studies which indicated vaginal Igs are variable among individuals (Brandtzaeg ,١٩٩٧ , Mestecky *etal* .,٢٠٠٤) .

٥.٢.٤ : *Effect of ٢ - Mercaptoethanol(٢ ME).*

In the present study it was found that the ٢ ME had no effect on antibody titers with capsular k١ antigen in both local and systemic but only some cases (five cases) showed that were effect on prozone phenomena .This phenomenon was disappeared in the present of ٢ME when compared with that in normal saline .The constant of titer in presence of ٢ME was matched with many studies on humoral systemic and local immunoglobulins ,these studies indicated that the infection in vaginal lead to produce specific IgG to the microbes and SIgA but the most dominant Igs was IgG ,for example systemic and secretory humoral immunity in the normal human vaginal tract was studied by separating Igs and found a large proportion of un cleaved IgG whereas low amount of IgA includes SIgA ,monomeric and fragments ,S

IgM is at a very low level ,while free Sc molecules are abundant.(Hocini *etal.*, ١٩٩٥ ; Wu *etal.*, ٢٠٠٠).

٥.٢.٥: *Phagocytic activity .*

when pathogenic microorganisms gain access to tissues ,the connective tissue serves at time as an intermediadiary in the host - parasite relation .Since the connective tissue responds to the presence of foreign material with a sequence of reactions leading to various forms of inflammation any consideration of interaction between host and pathogens has to take into account the components of the inflammate exudate . Three main elements in inflammation play a major role in host parasite relations ,phagocytes ,bacteriocidal and bacteriostatic mechanism of plasma and exudate fluid and the changing chemical environment at the sitesof connective tissue reaction .An increase percentage of blood neutrophils reducing nitrobluetetrazolium dye (NBT) to formazan has been utilized as an indicator of acute bacterial infection and other inflammatory state ,the present study showed that the mean of phagoctic activity in systemic and local were more in patients than that in normal subjects . (McCall *etal*,١٩٧٤ ,Laharrague *etal* .,١٩٨٤) . Figures(٤.٦, ٤.٧) showed the distribution of phagocytic cells in both local and systemic in patients .Figure (٤.٦) demonstrated that most cases distributed between (٦٠ -٨٠ %) this belong to peripheral blood .Figure (٤.٧) showed that normal cases were present in (٤٠- ٦٠٪). These results are normal because the presence of bacteria (*E.coli*) in patients lead to activate the neutrophils phagocytosis . *E.coli* has lipopolysaccharide is an endotoxin of cell wall of Gram negative bacteria which causes different pathophisiological reactions in the organism of mammals ,the effects of LPS result from interaction of endotoxin with membrane of target cells such as neutrophils, monocyte /macrophage, B

lymphocyte ,blood platelet ,fibroblasts and endothelial cells .So this lead to increase the activity of neutrophils phagocytosis as (Pawlowska and Tourowski ,١٩٩٨)

٥.٢.٦: *Leucocytes migration inhibition factor*

Leucocyte migration inhibitory factor (LIF) was originally identified as a protein secreted by activated T-lymphocytes capable of inhibiting the random migration of macrophages in vitro (Bloom and Bennett ,١٩٦٦) .Recent data have shown that LIF is produced by other types of cells including macrophage and fibroblasts and has a number of functions including regulation and differentiation of immune cell . LIF is also known to be apituitary hormone ,enzyme and proinflammatory cytokine involved in several aspects of the immune response .LIF has an essential regulatory role in T cell activation (Bacher *etal* .,١٩٩٦ ; Benigni *etal.* , ٢٠٠٠) another study report the presence of LIF in the human urothelium and LIF synthesis and secretion by human transitional cell carcinoma cells. Endogenous LIF secretion may activate autocrine and or paracrine regulatory loops that enhance or sustain cancer cell growth (Meyer - Siegler *etal* ., ٢٠٠٤) .

LIF is suggested to function as regulator of proinflammatory gene and cytokine expression ,furthermore there is strong evidence that is involved in reproduction mainly in event based on inflammatory like processes such as ovulation ,the menstrual cycle and early pregnancy (Letta *etal* .,٢٠٠٢) . In the present study LIF is calculated as in (Tompkins *etal.*,١٩٧٣). The antigens used were cell free culture antigen and capsular antigen . The mean of percentage of LIF in patients showed that antigens inhibit the migration in both systemic and local while in normal subjects these antigens did not inhibit the migration ,so the

present of antigen lead to secret LIF (Tompkins *etal* .,١٩٧٣ ; Letta *etal* ., ٢٠٠٢) .

The figures (٤.٨ ,٤.٩) were showing the distribution of LIF percentages in patients and normal subjects .These figures also used by (Soborg and Bertram ,١٩٦٨). The presence of *E.coli* antigens (both capsule and cfc) as cell sensitizers inhibit the migration of leucocytes .Such significant LIF among patients can be suggestive for an *E.coli* epitope activating Th١ which in turn initiate Tdh subsets of Tcells that involve of hypersenstivity reactions .

٥.٢.٧: Cytokines

IL - ١α and IL - ٨

Cytokines are a group of low molecular weight regulatory proteins secreted by an array of cells such as lymphoctes , monocytes ,macrophages , fibroblasts ,neutrophil, endothelial cells and mast cells .Although cytokines function on a microenvironment level ,human cytokines function are most commonly assessed at the macro- levels can be readily assessed by using commercially available enzyme immunoassays (Janson *etal* .,٢٠٠١) . In the present study standard curves fit equation were calculated for IL-١α (table ٤.١٢) .Table (٤.١٣) indicated concentrations of IL-١α in patients were higher than normal subject and the range of IL-١α concentration in patients was (٢٠.٨٤٢ - ١٥٥.٢٦٢) pg/ml while in normal subject was (١٣.٢٥٣ -١٣١.١٤٢) pg/ml ,similar were found by (O Neill , ٢٠٠٠ ; Maisey *etal*. ,٢٠٠٣) that IL- ١α increased in infection .

Chemokines are small chemoattractant peptides that are structurally very similar ,as are cognate receptors . Many of the proximal signal transduction pathways that are activated after receptor ligation are also very much a like (Olson &Ley ,٢٠٠٢) .

Chemokines may have originated from proteins with essential intracellular function through gene duplication and selected mutation at relatively recent evolutionary stage .Based on genomics efforts ,it has been estimated that there were may be as many as ٤٠ to ٥٠ human chemokines.chemokines domians are defined by the presence of four cysteines in highly conserved positions . One major chemokine subfamily is called CXC because the two amino acids nearest the N-termini of these proteins are separeted by single amino acid .this is in contrast to the other major subfamily which is called CC because these two cysteines are adjacent. Third chemokine subfamily denoted C because of the lone cysteine in the N-terminal domian . The fourth subfamily CX_3C has three domian differs from other chemokines by the presence of three amino acids intervening between the first two cysteines ,this subfamily is an integral membrane protein such as fractalkine or neurotactin while other subfamilies are secreted proteins . Chemokines play roles in processes as organogenesis ,hematopoiesis ,neuronal communication with microglia and leukocytes trafficking . (Rollins ,١٩٩٧ ; Baggiolini ,٢٠٠١). In the present study used chemokine IL-٨ and found that the concentration of IL- ٨ in patients and the range was (٣٢.٥٧٨-٧٣٤.٤٧٦) pg /ml and in normal subjects (٥٥.٤٤٥٢ - ٢٧٩)pg/ml .

In four patients cases appeared higher concentration as in normal subjects but most cases were low concentration (٥٥.٤٤ ,٦٨.١٤) pg / ml these concentration in normal while other concentration were higher concentration .Many studies indicated that level of IL - ٨ were increased in local during infection ,such as intravenous endotoxin of *E.coli* administration increased IL-٨ in peripheral blood . Local infection in amniotic cavity is observed to be associated with an elevated IL-٨ level in amniotic fluid .IL-٨ increased in urine in patients with urinary tract infection (Ko *etal..*, ١٩٩٣; Fichorova *etal.* ., ٢٠٠١) . Vaginitis caused by

Trichomonas vaginalis showed increased in IL-٨ production from monocytes . IL-٨ influenced by many factor dose of antigen and clinical state such as symptomatic patients appeared detectable level than those of symptomatic (Ryu *etal* .,٢٠٠٤) .The present study showed various results among individual .This may depend on dose of *E.coli* and clinical state of patients .Despite the large amount of researches involving serum and plasma cytokine assessments this approach does have a number of limitations .Serum and plasma cytokine level can be affected by receptor binding ,temperature degradation ,cytokine break down within reacting cells . Since cytokines are released in a paracrine manner the levels may be varied widely depending upon when the time of subjects blood sampling .

Furthermore a major function of most cytokines is to act in intercellular communication (Janson *etal* .,٢٠٠١). It appeared from study that IL-١α concentration opposed that of IL- ٨ .The analysis of cervical mucus from pregnant women tend to show a rise of IL-٨ ,though not as high as for IL-١α and levels of IL-٨ as opposed to IL-١ α/β levels this result is similar to present study so IL levels in serum demonstrated what happened in local (Forsum *etal* .,٢٠٠٥b).

٥.٣: Lapin E.coli K١ vaginosis

٥.٣.١ : Infection model

A mild form of *E.coli* k١ experimental lapin vaginosis was noticed with the formation of fever vaginal congestion and or slight secretion . It appears that ٠.٥* ١٠٤ colony forming units is the dose required for production of vaginosis by *E.coli* . Pus cells ,clue cells ,vaginal secretion and the organisms were detected .These signs of bacterial vaginosis were noticed after ٢ days from intravaginal inoculation . This is probably related to the essential barrier provided by the epithelial cells at the end of cervix uterus and fallopian tubes . This single cell layer was initially thought to reside in a sterile environment that was only infrequently exposed to bacteria constitutively present in the ectocervix and vagina . For example viable free and sperm associated bacteria are found in the uterine lumen of the mouse .

Clearance of viable bacteria occurred within approximately ٤٨ hrs following mating and was associated with phagocytosis of bacteria as well as the presence of antimicrobial product (Parr and Parr ,١٩٨٥; Thapar *etal.* , ١٩٩٠;Kozlowski *etal.* ,٢٠٠٢)

٥.٣.٢: Humoral immune responses in rabbits

E.coli k١ antigen stimulate local and systemic humoral responses. The specific antibody against capsule antigen or cell free culture antigen in both systemic and local , Inoculation of antigen intravaginally induced specific antibody .The nature of antigen play crucial role in immune response of female genital tract (Wassen *etal.* ,١٩٩٦) . Successful local immunizations have been reported with mostly

live microorganisms as vectors while in soluble antigen have been found to be poorly immunogenic (Russell, ٢٠٠٢) .

This has been explained to be a consequence of the lack of organized lymphoid tissue and M cells in the female genital tract .Its thought that replicating antigen provide better delivery of antigen because they infect local tissue and may be more easily taken up by antigen processing cells ,such as phagocytic cells as compared to soluble antigen alone ,however ,except for defined up take mechanism .The requirements for antigen presentation to T cells in female reproductive , well met by the presence of Langerhans dendritic cells ,macrophages and even epithelial cells that express MHC class I and I I and which specifically activate T cells . These mucosal antigen presenting cell can initiate a local IgA response or whether is a dependence for trafficking of APC to immunocomptent cells T and B cells in regional lymph nodes is not well understood (Wassen *etal*. ,١٩٩٦) capsular antigen of B streptococcal was not found elicit antibody in mice after intravaginal inoculation (Hordnes *etal* .,١٩٩٧). In rabbits that intravaginal inoculation *E.coli* K ١ capsular antigens was found to elicit the production of specific antibody with high titers in the present study.

٥.٣.٣ :*Immunoglobulin and complement components* $C^{٣}$,$C^{٤}$ *in rabbits*

Little variation in Ig isotypes was detected in rabbit serum immunized with live and capsular antigens .The concentration of IgG,IgA and IgM were higher in immunized than control .This result was not unusual, since infection stimulate the lymphocytes responsible for production of antibodies (Wu *etal* .,٢٠٠٠). Mice intravaginaly inoculated with protein of *Streptococcus mutans* coupled to the B subunit of Cholera toxin lead to increased the serum IgA,IgG (Wu *etal*. ,٢٠٠٠) .

Table (٤.٢٠) the C٣ concentration were equal in rabbits treated with live bacteria and control as well as rabbits with capsular antigen had the concentration (١٢٠.٦) mg /dl while second rabbit increased (٢٥٢.٦) mg / dl .C٤ concentration also appeared varies concentration among rabbits but the means were higher in immunized rabbits than control groups .So live bacteria and capsular antigen were stimulated the complement system.

٥.٣.٤: *Leucocyte migration inhibition study in rabbits .*

Study of LIF and MIF in rabbits were demonstrated in three groups of rabbits one of which considered as a control group and other as tested groups .No migration inhibition in control group . The means of MIF and LIF were (١٢.٥ , ٤, ٧ , ٧, ٥,٧) while in test groups showed inhibition of migration and the means of MIF and LIF were (٣٦ , ٥٠ ,٤٢.٦ ,٥٣.٣ ,٥٧) these results were similar to that reported by (Tompkins *etal.* ,١٩٧٣) .

٥.٣.٥ : *Skin test*

Hypersensitive skin reaction was used as an in vivo to study cell mediated immunity in rabbits against capsular antigen and the result of this test was positive and this indicate that capsular antigen stimulated cell mediated immunity in vivo as well as in vitro which is similar to (Tompkins *etal.* ,١٩٧٣).

٥.٣.٦ : *IL-١ α and IL-٨ in rabbits.*

The rabbits inoculated with live bacteria showed an IL-١α concentration were(١٦١.٧٦٦, ١٦٤.٢٠٥٢) pg/ml and those with capsular antigen were (٨٢.٩٠٢٨ and ١٠١.٠٦) pg/ml while in control group were (

٣٣.٣٣٩ and ٢٥.٤٤)pg/ml . This also demonstrated the concentration of IL-٨ in these groups of rabbits and its concentrations in test groups were found to be higher than in control groups . This is probable due to the activity of IL-٨ as chemotactic agent in inflammation (Ko *etal*. ,١٩٩٣).

Conclusions:

١- *E.coli* K١ bacteria is the most frequent cause of bacterial vaginitis in this area .

٢- K١ antigen is important in inducing humoral and cellular immunity in human .

٣- IL-١α increased in vaginitis with *E.coli* K١ while IL-٨ concentrations were decreased in human .

٤- Lapian *E.coli* K١ vaginosis induce humoral and cellular immunity both in local and systemic .

٥- K١ antigen when inoculation in intravaginal of rabbits leads to increase Igs concentrations and C٣,C٤ concentrations .

٦- *E.coli* K١ induce immunity in local (vaginal) and disseminated to other part of common mucosal immune system (intestinal tract) .

٧- Female genital tract of rabbits is part of common mucosal immune system .

Recommendations:

١-Preparation of subunit E.coli intravaginal vaccine its field evaluation .

٢- Study the cytokine level in local secretions and peripheral blood to make complete cytokine profile for human *E.coli* K١ vaginosis.

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