Study of humoral Mucosal Immunity Of Urinary Tract During Infection In Menopausal Women

A Thesis

Submitted to the Council of the College of Science

Babylon University

In Partial Fulfillment of the Requirements for the Master degree

in

Biology /Microbiology

By

Hiba Jassim

دراسة المناعة الموضعيةالخلطية للجهاز البولي اثناء الخمج في النساء اليائسات

رسالة مقدمة إلى

مجلس كلية العلوم –جامعة بابل

و هي جزء من متطلبات نيل درجة الماجستير

علوم الحياة /أحياء مجهريه

من

هبه جاسم حمزة

Dedication

To my loving parents and grandmother…

To my dearest brothers and sisters…

To my respectable supervisor Prof. Dr. Ibrahim M.S. Shnawa...

And to whom I am concerned…

With respect…

Hiba J. Hamza

بِـــسْمِ آللهِ آلرحَّمنِ آلرحّيمِ

(إنَّ فِي آلسَّموَاتِ وَ آلاَرْضِ لآيَاتٍ لِلْمُؤمِنينَ)

صَدَقَ آللهُ آلعَظِيمْ

سورة الجاثية الآلاية -٣-

Acknowledgments

I want to express my sincere thanks to prof. Dr. Ibrahim M.S. Shnawa for providing me with the opportunity to carry out this scientific work. His logic way of thinking, encouragement and guidance made it possible to complete this work.

I would like to thank the head of the department of biology and the dean of the college of sciences and presidency of Babylon University for their supports.

Thanks a lot to urologist in Hilla educational hospital and Al-Hashemia hospital.

Thanks a lot to all my colleagues in research laboratory of microbiology in biology department with my wishes…. Thank to miss Evan who participated in typing my thesis.

Hiba J. Hamza

List of Abbreviations

Abbreviations	Means
٢ME	٢ mercapto ethanol
Ab	Antibody
ACTH	Adrenocorticotropic Hormone
ASC	Antibody-Secretory cell
C٣b	Component ٣b of complement
CD	Clusters of designation or cluster of differentiation
CD١٤	Clusters differentiation ١٤
CD٢	Clusters differentiation ٢
CD٣	Clusters differentiation ٣
CD٤	Clusters differentiation ٤
CD٥	Clusters differentiation ٥
CNs	Central nervous system
CRH	Corticotropin-releasing Hormone
CSF-١	Colony Stimulating factor-١
CSF-١R	Colony Stimulating factor-١ receptor
FSH	Follicle stimulating hormone
HPA	Hypothalamic-Pituitary-Adrenal
HPG	Hypothalamic-Pituitary-Gonadal
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-١	Interleukin-١
IL-١٠	Interleukin-١٠
IL-١β	Interleukin-١β
IL-٢	Interleukin-٢
IL-٤	Interleukin-٤
IL-٦	Interleukin-٦
IL-٨	Interleukin-٨
IU	International unit
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
LNs	Lymph nodes
LPS	Lipopolysaccharide
LUTI	Lower urinary tract infection

MIg	Mucosal immunoglobulin
mRNA	Messenger Ribonucleic acid
mRNACDR٣	Messenger Ribonucleic acid CD receptor ٣.
NG	No growth
NK	Natural Killer cells
PAI	Pathogenicity Islands
PEG	Polyethylenglycol
PGE٢	Prostaglandin E-٢
PIg	Polymeric immunoglobulin
PIgR	Polymeric immunoglobulin receptor
Sc	Secretory component
SCD١٤	Soluble clusters differentiation ١٤
SIgA	Secretory Immunoglobulin A
SIL-٦R	Soluble Interleukin-٦-Receptor
Th٢	T-helper ٢
THP	Tamm-Horsfall mucoprotein
TLR٤	Toll-like receptor ٤
TNF-α	Tumor necrosis factor-α
UMIg	Urinary Mucosal immunoglobulin
UTI	Urinary tract infection

List of contents

	specific mucosal humoral immune response among menopausal and adolescent during UTI.	
٥-١٢	*Streptococcus pyogenes* specific urinary mucosal humoral immune response among menopausal patients.	٨٦
٥-١٣	*Neisseria gonorrhoeae* specific urinary mucosal humoral immune response among menopausal women with UTI.	٨٦
٥-١٤	*Proteus mirabilis* specific urinary mucosal humoral immune response among adolescent UTI.	٨٦
٥-١٥	Nil urinary mucosal humoral immune responses in menopausal women.	٨٧
٥-١٦	Culture negative UTI	٨٨

List of tables

Conclusions

١. No specific bacteria can be detected only in the menopausal patients. However, the bacterial profile was alike except in incidence rate.
٢. Single as well as mixed infection was noted.
٣. Mucosal urinary immunoglobulin concentration is some what un related to specificity. Since it was noted that the low concentration with high titers, and in other, were high concentration with low titers.
٤. Menopause showed lowered bacterial specific antibody titers than adolescents.
٥. Cases producing mucosal Ig without apparent infected bacteria. Meantime, other cases produce infectious agent without detectable MIg.

Recommendations

Based on the results obtained in this study, one may recommend the investigation of mucosal cellular immune responses following same approach together with cytokine studies.

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 Master in Biology (Microbiology)

Signature

Prof .Dr.Ibrahim M.S.Shnawa

College of Science

Babylon University

Date :

In view of available recommendations, we forward this thesis for debate by the examining committee.

Signature :

Prof .Dr. Karrim H. Rasheed

Acting of head of department of biology

College of science

Babylon University

Date :

Summary:

The present study was aimed at evaluating the effect of menopause on urinary mucosal immunoglobulin (UMIgs) in urinary tract patients (UTP). Such evaluation was made by using: (١) Single urine sample, (٢) Polyethylenglycol concentration of urinary globulin and (٣) Bacterial agglutination test, the samples were included; seventy seven urinary tract infected patients their ages were ٤٦-٦٠ and thirty five from adolescent patients their ages were (١٨-٣٠) years.

UMIgs were separated by ٦٪ PEG (٦٠٠٠ MW). The concentration immune responses as evident in those menopause UTI patients. and titer were determined in menopausal patients and adolescent as control groups. The significant differences were observed in cases of *Klebsiella terrigena*, *Klebsiella oxytoca*, *Staphylococcus. aureus* & *Staphylococcus saprophyticus*. During the reaction of bacterins of the isolated organisms with UMIgs, ٢ mercaptoethanol sensitive, and resistant component were noted. The sensitive component was guessed as of serum origin. The study proved that the titers of antibodies in adolescent patient were rather higher than menopausal patients of ٣-٧ folds.

Thus, UMIgs could be used as an infection probe in menopause as well as in adolescent, while it is worth mentioning that menopause Stab may induce an immuno suppresser on humoral mucosal

الخلاصة

هدفت هذه الدراسة إلى تقييم تأثير سن اليأس على الكلوبيولين المناعي البولي لمرضى التهاب المجرى البولي. وتحقق مثل هذا التق

ويم عن طريق استخدام:

١. عينة بول واحدة. ٢. تركيز الكلوبيولين البولي باستخدام الكلايكول متعدد الاثيلين. ٣. التلازن البكتيري لكل من ٧٧ مصاب بالتهاب المجرى البولي من النساء في سن اليأس و٣٥ مصابة من الشباب.

هذا وقد فصل الكلوبيولين المناعي البولي باستخدام ٦% اثيلين كلايكول متعدد التركيز والعيار حدد من كل النساء في سن اليأس والشباب كمجموعة سيطرة اظهرت الدراسة اختلافات معنوية في حالات الاصابة بكل من *Klebsiella. terrigena* و*Klebsiella. oxytoca* و *Staphylococcus. aureus* و*Staphylococcus. saprophyticus* وتم الكشف عن مؤلفه حساسة لثاني مركبتو ايثانول واخرى مقاومة لهذه المادة أثناء التفاعل المصلي بين محاليل الكلوبيولين المناعي ومستضدات المسببات المشاركة ويتوقع ان تكون هذه المؤلفة الحساسة راجعة لجزء مصلي متناضج من المصل الى السبيل البولي.. أثبتت الدراسة إن عيار الضد عند الشباب اكبر من العيار لدى النساء في سن اليأس بحدود ٣-٧ اضعاف.

لهذا يمكن استخدام الكلوبيولين المناعي كدليل يؤشر لأثر اليأس في النساء على المناعة الموضعية في السبيل البولي. ومن الجدير بالذكر ان الشيخوخة من المحتمل ان تحث الى حد ما فعل تثبيطي في الاستجابة الخلطية المخاطية كما في فعلها لدى مرضى التهاب المجرى البولي في النساء في سن اليأس.

Chapter One

Introduction

١-١: Overview

Menopause is an important passage for all women, whether they view it as a welcome end to fertility and menstrual cycles or as a unwanted symbol of aging of the many negative symptoms associated with menopause, only vasomotor symptoms (hot flashes and night sweats) and urogenital atrophy are attributed directly to the decline in ovarian function. Other changes attributed to menopause may be related to aging itself or to psychosocial pressures that women face at midlife experience, but some are likely logical sequelae to genuine menopausal symptoms (Mc kinlay *et al*., ١٩٩٢; Prior, ١٩٩٨ Dennerstein *et al*., ٢٠٠٠; Rice, ٢٠٠٥).

١-٢: Menopause

Menopause is the cessation of menstruation for at least ١٢ consecutive months. It occurs naturally when the ovaries stop producing estrogen. The average age for menopause is ٥١(Greendale & Sowers, ١٩٩٧;Smith & Contestabile, ٢٠٠٢) However, it can occur anytime between the ages ٤٥-٥٦ years. (Paoletti & Wenger, ٢٠٠٣; Labdenperä *et al*., ٢٠٠٤).

Menopause is a normal, natural part of aging and not a medical disease or illness.(Smith & Contestabile, ٢٠٠٢)

Natural menopause,The permanent cessation of menstruation results from the loss of ovarian follicular activity and ultimate cessation of child-bearing potential (Pavelka & Fedigan, ١٩٩١).

Perimenopause,The transitional period is immediately prior to menopause and the first year after menopause (Contestabil & Derzko, ٢٠٠١).

Induced menopause,Immediate menopause is caused by medical or surgical intervention that removes or seriously damages both ovaries. (Luborsky *et al*., ٢٠٠٣).

Premature menopause,Menopause that occurs before the age of ٤٠ (Luborsky *et al*., ٢٠٠٣).

Post menopause,The period of time is after the final menstrual period (Smith & Contestabile, ٢٠٠٢; The North American Menopause Society, ٢٠٠٥).

The vasomotor symptoms associated with menopausal changes are commonly termed "hot flashes". Which are recurrent-transient episodes of flushing, perspiration and sensation ranging from warmth to intense heat of the upper body and face. When they occur with perspiration during sleep, they are called night sweats (Santoro., ٢٠٠٤).

Menopause women may have difficulty falling in a sleep, waking often during the night, or waking early. Insomnia is commonly caused by night sweat but can occur in women whether they have night sweats or not (Shaver, ٢٠٠٠).

Change in hormone levels may result in modiness, anxiety, irritability, nervousness or mild depression. However current evidence dose not support an association between estrogen levels during the menopausal transition and

the onset of clinical depression (Contestabil & Derzko, ٢٠٠٢; The North American Menopause Soceity, ٢٠٠٥).

Vaginal symptoms usually do not become problem until several years after the onset of menopause. The decrease in the amount of estrogen causes the lining of the vagina to become thin, dry and less elastica a condition known as atrophy. Vaginal secretions also diminish, which means that there is less vaginal lubrication (Greendale *et al*., ١٩٩٩ a). Also there are another symptoms such as: Urinary tract changes**,** Osteoporosis**,** Headaches, fatigue, a breast tenderness and memory loss (Contestabile & Derzko, ٢٠٠٢).

١-٣: Aims of study.

The aims of this study are to investigate the possible effect of menopausal on humoral mucosal immunology of urinary tract infection.

To verify this, a number of steps have been followed

١. Urine sample collection from menopause and control patients for isolation the pathogens and preparation the antigen from causative organisms.
٢. Separation the urinary mucosal immunoglobulin.
٣. Studying the serology examination between immunoglobulin and antigen of causative agents.

Chapter Two

Review of Literatures

٢-١: Biology of female reproductive system:

The production of germ cells is essential for the continuation of life of the species. This function in female is accomplished by the ovaries which secrete steroidal and non steroidal hormones. That not only regulate the secretion of anterior pituitary hormones but also acts on various target organs including the ovaries, mammary gland and bone (Ojeda, & Griffin, ٢٠٠٠).

٢-١-١: The ovaries (Paired structures):

The ovary has three regions: the cortex that contains the oocytes and represents most of the mass of the ovary, the inner medulla formed by stromal cells and cells with steroid-producing characteristics, and the hilum, which in addition to serving as the point of entry of the nerves and blood vessels, represents the attachment region of the gland of the mesovarium. The ovary is the major female reproductive organ, which have two function, as exo. And endocrine functions (Ojeda, & Griffin, ٢٠٠٠).

٢-١-١-١: Exocrine function (Oogenesis)

Production and release the female gametes, the ovary produces a single ovum every ٢٨ days (on average). The mature ova is derived from precursor cells-oogonia, which are present only when the female is still in the embryonic stage (by ٧ months gestation), before birth. (Ojeda, & Griffin, ٢٠٠٠).

These oogonia differentiated into primary oocytes meiosis begin but cease in prophase by birth, each primary oocyte is now surrounded by epithelial cell to form a primordial follicle, each follicle is contained in one or

more layer of granulose cells (which may provide nourishment to follicle) at birth all primordial follicles are formed, more than ١/٢ million per ovary by puberty, fewer than ٤٠٠٠٠ primordial follicles per ovary still exist. These primordial follicle become primary follicles, each month beginning at puberty, some primary follicles develop into secondary follicles, only one of which then become a mature follicle (Graafian follicle), the mature follicle releases one ova (egg) per month. Throughout the reproductive years, a female will produce an average of ٤٠٠ mature ova and the rest will degenerate of a process called follicular atresia, at menopause. Each female has no remaining ova (Rutishauser, ٢٠٠١).

٢-١-١-٢: Endocrine function:

The female reproductive system produces two main hormones and several lesser hormones.

-**Estrogen**: There are three forms of estrogen, B-estradiol (major estrogen-most potent), estrone (less potent) and estriol (least potent).

This hormone stimulates the development of secondary sex characters, stimulate breast development (estrogen alone are not sufficient to stimulate milk production).

Also estrogen stimulates uterine development, stimulate maturation of fallopian tubes, stimulate maturation of skeleton and increased fat deposition.

-**Progesterone**: Small amount secreted by ovaries, increased synthesis and release during the ٢nd half of monthly cycle.

Progesterone helps to prepare for implantation of fertilized ova in uterus, and prepares breast for milk production and release.

-**Other Hormones (relaxin):** It released at the end of pregnancy and may aid in labor (Ojeda, & Griffin, ٢٠٠٠).

٢-١-٢: Fallopian tube (ovi duct):

Ovi duct the next organ of female, reproductive tract; it is a tubular organ of relatively small diameter. The oviduct has extensively compound mucosal folds that obliterate most of lumen's volume, and the luminal epithelium is generally simple columnar epithelium.

Oviduct or fallopian tube receive single ovum from the ovary & via a structure called the fimbrial. The oviduct extend to the uterus, but fertilization generally occurs near ovary (Rutishauser, ٢٠٠١).

٢-١-٣: Uterus:

The site of implantation of fertilized ovum (conceptus),and development the concepus into the full term fetus (over ٣٨-٤٠ weeks) occur here. The uterus has three layers: endometerium, mymeterium and perimeterium. Uterus consist of three region: Body, Fundus and cervix the neek of uterus which projects into vagina (Rutishauser, ٢٠٠١).

٢-١-٤: Vagina:

Receiving the semen from the male, allows menstrual flow out of the body and act as birth canal.

The vagina walls contain vestibular gland so that are ribbed and moist.

The vagina consists of three layers:-

Mucosa-Stratified squamous epithelium and thick lamina propria, smooth muscle layer and adventitial layer (Rutishauser, ٢٠٠١).

٢-١-٥: External genitalia:

The external female genitals are collectively termed as the vulva. At the outer sides there is a pair of folds called the labia majora, which enclose two smaller folds or labia minora, which in turn enclose the clitoris and opening of the urethra and the vagina (Rutishauser, ٢٠٠١).

٢-٢: Aging change in female reproductive system:

For women, menopause or the cessation of menses is an obvious sign of aging. It is by no means the only change in female reproductive system. There is transition period called climacteric that extend for many years before and after the last meanstrual period. (Burger, ١٩٩٩).

For women, aging change involves hormone levels, physical changes in woman's entire reproductive tract, and physiological changes. Changes occur

in the intricate relationship between the ovarian hormones and these produced by the pituitary gland (in the brian). (Ojeda, & Griffin, ٢٠٠٠).

The ovaries stop releasing eggs (ova) and menstrual period stop during menopause as a result of aging female reproductive system.

In menopausal women, circulating estradiol is decreased by more than ٩٠٪ and LH levels are increased ٤-٥ fold compared with those in younger reproductive aged women (Burger, ١٩٩٩). The menopausal elevated LH levels are maintained by continued secretion of LH in pulsatile manner, with high amplitude and LH pulse frequency of approximately every ١-٢ hr (Yen *et al*., ١٩٧٢). In women, estrogen levels decline of menopause as a result of the loss of ovarian follicles (Gosden & Faddy, ١٩٩٤; Judd & Fournet, ١٩٩٤; Wich & Carnes, ١٩٩٥).

Sexual problems are relatively common in women, occurring in up to ٤٣٪ of younger women (Laumann *et al*, ١٩٩٩) and increasing with age (Berman & Bassuk, ٢٠٠٢). These result from interaction of biological, psychological and social factors (Barton & Joubert, ٢٠٠٠).

Changes in sexual function occur in many women during the immediate post menopausal years, secondary to diminished sexual response and vaginal dryness (Bachmann *et al*., ١٩٨٤).

Table (I): Symptoms related to urogenital aging may precede physical finding

Vulvovaginal	Urinary

١. Dryness	Dysuria
٢. Pruritus	Frequency nocturia
٣.Discharge	Stress incontinence
٤.Dyspareunia	Urgency/urge incontinence
٥.Post-coital bleeding	Coital incontinence

(Johnston, ٢٠٠١).

٢-٣: Biology of Menopause

٢-٣-١: Physiological changes during menopause

Two key physiological changes are associated with menopause:- The loss of primary ovarian follicules and the resulting decrease in serum and tissue estradiol. The primary estrogen in premenopausal women is ١٧ beta-estradiol, which is produced in the ovary from the aromatization testosterone (Gruber *et al*., ٢٠٠٢). Commercial estradiol products are often referred to as containing "biodentical" estrogen for this reason. Other sites, such as muscle and adipose tissue, produce smaller amount of estrogen through the metabolism of androgens. After menopause, these extragonadal sites.

The primary source of estrogen, in the form of estron and to a lesser extent, estradiol(Gruber *et al*., ٢٠٠٢).

The physiological changes that eventually result in cessation of menses and the development of menopause-related symptoms begin long befor

menopause. From menarche on, the number of primary ovarian follicles decreases, with especially marked reduction after age (Stenchver *et al*., ٢٠٠١). The loss of primary ovarian follicles appears to be the key event that triggers perimenopause (Dodson & Steiner, ٢٠٠٢).

In the past, researchers believe that menopause was caused solely by a lack of estrogen by the ovary, resulting in higher levels of follicle-stimulating hormone FSH and cessation of menses-more recent evidence suggests that inhibit B, a glycoprotein synthesized by granulose cells in the ovary, plays a major role in triggering the menopause transition (Stenchever *et al*., ٢٠٠١). FSH normally stimulates inhibin B synthesis, which then suppresses FSH via a negative feedback loop. After about age ٤٥, however, inhibin B levels fall, perhaps due to the decrease number of ovarian follicles causing arise in FSH. The increase in FSH levels can stimulate increased estradiol release from remaining follicles and can also prompt them to release estradiol more rapidly, resulting in shorter cycle. About six to twelve months before menopause, the number of follicles in even lower, and higher FSH levels fail to increase estradiol production. At this point, the reduced estradiol levels can result in menopause-related symptoms, such as hot flashed and vaginal dryness. During perimenopause, estrogen production by the ovary is erratic, such that estradiol levels are unpredictable and can fluctuate between normal, high and low. For this reason, measurement of FSH and estradiol is not helpful for diagnosis during perimenopause symptoms are a better marker of perimenopause status. (Stenchever *et al*., ٢٠٠١).

٢-٤: Immunological changes during menopause

٢-٤-١: Evidence for increase in IL-١, TNF-α and IL-٦ after natural or surgical menopause:

Spontaneous increases in the expression and secretion of the proinflammatory IL-١, IL-٦ and TNF-α with estrogen deficiency were first noted several years ago in vivo cultures of circulating monocytes (Pacifici *et al*., ١٩٨٩; Pacifici *et al*., ١٩٩١), and in bone marrow macrophages (Jilka *et al*., ١٩٩٢, Kimble *et al*., ١٩٩٤; Kitazawa *et al*., ١٩٩٤; Bismar *et al*.,١٩٩٥). Increases of these cytokines with estrogen deficiency are, however, subtle compared with the increase observed as a host reaction to infection or major tissue injury (Cohen & Cohen, ١٩٩٦). Effect or of to directly demonstrated cytokine increases with estrogen deficiency in tissue samples in vivo (Vargas *et al*., ١٩٩٦, Van Bezooijen *et al*., ١٩٩٨) or circulation (Mckane *et al*., ١٩٩٤, Girasole *et al*., ١٩٩٥, Gregory *et al*., ٢٠٠٠; Martinetti *et al*., ٢٠٠٠) Several authors have noted increases in circulating IL-٦ & TNF-α after natural or surgical estrogen deficiency has been shown to enhance the responsiveness of cells toward some of these cytokines by up-regulating cytokines receptor, numbers and cofactors of cytokine action, thus amplifying of the effects of the cytokine increases. In human, elevated soluble IL-٦ receptor (SIL-٦R) concentrations incirculation have been observed after surgical and natural menopause (Aantatore *et al*., ١٩٩٥;Girasole *et al*., ١٩٩٩; Woodward *et al*., ١٩٩٩ Keller *et al*., ٢٠٠١; Deswal *et al*., ٢٠٠١). SIL-٦R is derived from the extracellular domain of the ٨٠-KDa a receptor and is capable of presenting IL-٦ to the signal-transducer gp١٣٠, Thus enhancing cell responsiveness to IL-٦.IL-٦ is still nuclear whether these changes in cell responsiveness are direct consequence of estrogen deficiency or whether they are secondary to the increases in IL-٦ concentration because the IL-٦ is known to stimulate gp ١٣٠ gen transcription

(O'Brien & Manolagas, ١٩٩٧). There is preliminary evidence that estrogen deficiency may also enhance the responsiveness to IL-٦ through modulating IL-٦ signaling pathway (Yamamoto *et al*., ٢٠٠٠). For example, in multiple myloma cells, ١٧-β-$E_٢$ completely abolished IL-٦ inducible cell proliferation by inducing mRNA expression of the protein inhibitor of activated signal transducer and activator of transcription (Wang *et al*., ٢٠٠١).

٢-٤-٢: Toll-like receptor ٤ expression and cytokine responses in human urinary tract mucosa.

Mucosal pathogen uses divers and highly sophisticated mechanisms to gain access to the tissues at their preferred site of infection (Eden *et al*., ١٩٧٦; Bleves & Cornelis, ٢٠٠٠; Cossart & Bierne, ٢٠٠١; Sansonetti, ٢٠٠١). Adherence is a crucial first step to establish tissue contact and to break the inerta of the mucosal barrier, but in addition, the molecular interaction between bacterial and host alert the host to the danger, and the host response is activated (Agace *et al*., ١٩٩٣; Godaly *et al*., ٢٠٠١). In the urinary tract epithelial cell lines, cell activation by fimbriated *E. coli* requires primary recognition receptors for fimbrial adhesions and Toll-like receptor ٤ (TL $R_٤$) for transmembrane signaling (Frendeus *et al*., ٢٠٠١). Human urinary tract epithelial cell expresses both glycosphingolipid and mannosylated surface glycoprotein receptors, which recognize the B fimbrial adhesins (Leffler & Svanborg-Eden, ١٩٨٠) and the type ١ fimbria respectively (Ofek *et al*., ١٩٧٧). $TLR_٤$ is also expressed in immune urinary tract epithelium and the $TLR_٤$ genotype was found to regulate the in

vivo response to experimental urinary tract infection caused by p- or type١-fimbriated *E. coli* (Shahin *et al*., ١٩٨٧;Hagberg *et al*., ١٩٨٤; Schilling *et al*., ٢٠٠٣ b).

However.the extent to which $TLR_٤$ is expressed by the human urinary tract epithelium remains controversial, In addition there are contradictory reports concerning the lipopolysaccharide (LPS) responsiveness of uroepithelial cells and their expression of $CD_{١٤}$. It is well established that cells of myeloid origin express $CD_{١٤}$ and $CD_٢$ and recruit $TLR_٤$ for transmembrane signaling when exposed to (LPS) (Beutler & Rietschel, ٢٠٠٣) but the uroepithelial cell lines respond poorly to soluble LPS even though they express $TLR_٤$ (Hedges *et al*., ١٩٩٢). This has been attributed to the lack of $CD_{١٤}$ expression by these cells (Hedlund *et al*., ١٩٩٩; Bachhed *et al*., ٢٠٠٢). Other studies of $TLR_٤$ and $CD_{١٤}$ expression in the urinary epithelial cell lines either have failed to demonstrate $TLR_٤$ or $CD_{١٤}$ on the epithelium (Bachhed *et al*., ٢٠٠١; Bossolati *et al*., ٢٠٠٢) or have detected $CD_{١٤}$ and proposed that LPS responsiveness is important in urinary bladder (Schilling *et al*., ٢٠٠٣ a). There is a report that urine contains soluble $CD_{١٤}$ ($SCD_{١٤}$), but whether the concentration in uninfected urine is high enough to support $CD_{١٤}$-mediated recognition of LPS needs further study.

Cytokines are early markers of epithelial response to infection and play a key role in the innate defence (Svanborg *et al*., ١٩٩٩). Interleukin-٦ may cause fever and trigger the acute-phase response, while chemokines such as CXCL٨ (IL-٨) recruit inflammatory cells to the site of infection. The urine cytokine levels are elevated in patients with UTT (Hedges *et al*., ١٩٩٢; Oho *et al*., ١٩٩٩ ; Olszyna *et al*., ٢٠٠٠') and epithelial cells have been identified as early producers of cytokines in murine UTT model (Haraoka *et al*., ١٩٩٩; Schilling *et*

al., ٢٠٠٣ a), but the epithelial cytokine response of the human mucosa in situ has not been investigated.

٢-٥: Mucosal Immune System:

The mucosal immune system is composed of the lymphoid tissues that are associated with the mucosal surfaces of gastro-intestinal, respiratory, and urogenital tracts. It has evolved within an antigenic environment quite distinct from that in the interior of the body and so has a number of features that differentiate it from the systemic lymphoid system. These include the production of mucosa-related immunoglobulin IgA; a population of T cells with mucosa-specific regulatory properties or effecter capabilities and a mucosa-oriented cell-homing that allows lymphocytes initially activated in the mucosal follicles to migrate selectively to the diffuse mucosal lymphoid tissues underlying the epithelium. This last feature leads to the partial segregation of mucosal cells from systemic cells and thus qualifies the mucosal immune system as a somewhat separate immunologic entity (Kelsall & Strober, ١٩٩٦; McGhee, ١٩٩٩; Strober & Fuss, ٢٠٠١).

The mucosal immune system is the first line defense against pathogens encountered after ingestion and inhalation and the decline of the mucosal immune responses with age is particularly significant (Arranz *et al*., ١٩٩٢). The mucosal and systemic immune systems appear to be separate with cell activated in mucosal site homing back to that site or to another site within the common mucosal immune system (Butcher *et al*., ١٩٩٩). It has been shown that the mucosal antibody response in older people is composed of antibody with lower affinity for the immunizing antigen compared with those produce in

younger person. In addition the number of antibodies produced during an immune response is greatly increased in the elderly (Dunn-Walters *et al*., ٢٠٠٣).

The barrier function of mucosa membrane is exerted through unspecific defense mechanisms like mechanic washing & by specific immunity. Antibodies in secretion do not mainly act through bactericidal mechanisms, but rather by binding to antigen, neutralizing, agglutinating and immobilizing them. The net result will be decrease chance for bacteria to reach and bind to host epithelial cell antibodies directed against bacterial adhesions will specifically interfere with the adhesion process (Svanborg-Eden *et al*., ١٩٨٢). Mucosal immune mechanisms are believed to be important in host defense against urinary tract infection. The human immune response in urinary tract peripheral blood antibody-secreting cell (ASC), believed to originate from the mucosal surface, were investigated with the enzyme-liked immunosorbent assay ELISA. The ASC assay offers a new means for assessing the human immune response UTI and may be useful to localizing the infection (Kantele *et al*., ١٩٩٤).

The humoral mucosal immune response of the kidney involves the transport of secretory IgA (SIgA) through renal epithelial cell by the polymeric immunoglobulin receptor (pIgR) (Rice *et al*., ١٩٩٩). Some researchers, results provide the first evidence that the development of age-associated alterations possibly occurs earlier in the mucosal immune system than in the systemic immune compartment (Koga *et al*., ٢٠٠٠). Age-associated changes in the mucosal immune system have been shown that the mucosal immune system is also altered by aging because the elderly are much more susceptible to infection of the gastrointestinal tract (Schmucker *et al*., ١٩٩٦). Furthermore, marked increases in the severity and mortality caused by respiratory

pathogens such as influenza virus and bacterial pathogen *Streptococcus pneumoniae* are seen in the elderly (Mufson, ١٩٩٩; Webster, ٢٠٠٠).

Additionally, it was reported that the number of lymphocytes in peyer's patches and mesenteric lymph nodes (LNs) decreased in aged rats (Kawanishi & Kiely, ١٩٨٩).

Mucosal humoral immunology of human during persistent pyuria has been investigated in this area (Shnawa & Mehdy, ٢٠٠٤). Comparative mucosal and systemic immune response both at humoral and cellular levels in persistent pyuria patients have also been done in this area (Shnawa & Al-Amidi, ٢٠٠٥).

٢-٦: Biology of mucosal antibody

The principle antibody involved in the mucosal immunity is secretory IgA (Underdown & Mestecky, ١٩٩٤).

٢-٦-١-The structure of Immunoglobulin A:-

Immunoglobulin A (IgA) is the predominant immunoglobulin produced by plasma cells in Peyer's patches, tonsils, and other submucosal lymphoid tissues. Thus, although IgA accounts for only ١٠-١٥٪ of serum immunoglobulins, it is by far the most abundant antibody class found in saliva, tears, intestinal mucus, bronchial secretions, milk, urine and other secretions. On B-cell surfaces or in the blood, IgA exists as a monomere (M.wt=١٦٠٠٠٠)

comprising only one four-chain unit in secretion, it multimerizes to form disulfide-linked polymers of up to five such units that are associated with one molecule each of J chain and secretory component. The predominant secreted form of IgA are dimmers and trimers (Parslow *et al*., ٢٠٠١).

٢-٦-٢: The function of Immunoglobulin A:

Pathogens adapted to infect mucosa express virulence factors that allow them to adhere, colonize or invade epithelium. Secretory IgA (SIgA) prevents absorption of these bacteria and toxins by blocking their adhesion while they are still on the external side of the epithelial barrier. This activity is opposite to that antibodies associated with peripheral immunity. By preventing cellular attachment of antigen, IgA enables it to be flushed away in the stream of secreted fluids and mucous washing over the epithelial membranes. (Mazanec *et al*., ١٩٩٣).

IgA may also facilitate transport pathogens and toxins out of the body by causing them to be conveyed into bile and other exocrine secretions (Mazanec *et al*., ١٩٩٣).

Antigen specific IgA has recently been shown to neutralize viral pathogens during transport across Membranous cells "M" cells of Peyer's patches, where non degradation endosomal transport might otherwise deliver a pathogen into the host (Neutral & Kraehenbuhl, ١٩٩٤;Anonymous, ٢٠٠٤).

٢-٦-٣: Manufactured of Immunoglobulin A:-

IgA is the preponderant antibody manufactured by the body. This escaped appreciation for many years because the blood contains a relatively low concentration of IgA compared to other immunoglobulins. However ٧٥ percent of the antibody-producing cell in the body make IgA, and most of this IgA is released continuously into gastrointestinal fluid, saliva, tears, urine and other secretion up to ٤٠ mg/Kg body weight of IgA is manufactured and secreted daily in humans, which is many orders of magnitude freater than that of all other immunoglobulin isotypes (Brandtzaeg, ١٩٩٤).

٢-٦-٤: Transport of IgA:

Mucosal epithelial surfaces contain an array of host defense factors, including polymeric immunoglobulins (pIg), of which IgA is the major class. These secretory immunoglobulins (SIgA) are transported from basolateral to the apical surface of mucosal epithelial cells by polymeric immunoglobulin receptor (pIgR).(Mostov *et al*., ١٩٨٤)

In the human, the pIgR is a ١٨٨ to ١٢٠ KD a glycoprotein composed of an amino-terminal immunoglobulin-binding portion, termed secretory component (Sc), a membrane spanning element and a carboxyl-terminal cytoplasmic domain (Mostov *et al*., ١٩٨٤; Sztul *et al*., ١٩٨٥). After transcytosis of the PIgR to the apical region, both PIgR bound to immunoglobulin and unbound PIgR accumulate in endosomal vesicles called "apical recycling endosomes" (Apodaca *et al*., ١٩٩٤). The pIgR-contianing endosomal vesicles fuse with the apical membrane were the most of the ectoplasmic segment of pIgR is proteolytically cleaved and either secreted as a free Sc (FSc) or bound to polymeric immunoglobulin A as Secretory IgA (SIgA). (Rice *et al*., ١٩٩٨).

The pIgR is expressed in renal tubule epithelial cells of humans (Abranmowsky & Swinehart, ١٩٨٦) both with or without IgA (Rice *et al*., ١٩٩٨).

During urinary tract infection (UTI), secretion of SC is increased. This includes SC that is attached to IgA (Svanborg-Eden & Svennerholm, ١٩٧٨; Greenwell *et al*., ١٩٩٥) where to prevent degredation of secretory IgA (Lindh, ١٩٧٥), and FSc (Greenwell *et al*., ١٩٩٥), which may inhibit adhesion of some bacteria to cell membrane (Giuglianol *et al*., ١٩٩٥). Thus, the concentration of SIgA and FSc in the urine may influence susceptibility to UTI.

٢-٦-٥: Secretory IgA and UTI:

Expression of SIgA has been shown to be elevated in children with a symptomatic bacteriuria, acute UTI, and acute pyelonephritis, whereas it remains low in healthy controls (Hanson *et al*., ١٩٩٧). In addition ,the highest levels of SIgA are seen in children with the most severe infection and anatomic anomalies. Bacteria grow less well in the urine of children with UTI when that urine contains increased levels of IgA and IgG, suggesting a protective effect (Uehling & Balish, ١٩٧٧). In adults, many studies have found SIgA to be detectable in patient with UTI but present only in small amount in normal controls (Floege *et al*., ١٩٩٠).

Women with recurrent UTI and demonstrable urinary tract abnormality have shown increased SIgA values (Uehling, ١٩٧٣). Patients with urosepsis have higher urinary SIgA levels than do patients with lower incidence of UTI (Uehling *et al*., ١٩٩٤). Urinary SIgA expression is higher in patients with upper tract UTI than in those with uncomplicated lower tract UTI (Trinchieri *et al*., ١٩٩٠). Urinary IgA levels increased in elderly patients with a symptomatic

bacteriuria and are even higher in patients with symptomatic UTI (Nicolle & Brunka, ١٩٩٠).

How urinary SIgA play a protective role remains controversial. Studies show that basal level of locally synthesized SIgA are low in the urine of children with recurrent UTI. It has been suggested that low basal urinary levels of SIgA may represent one factor predisposing to recurrent UTI. Although the lack of SIgA seems to predispose individual to infection at respiratory and gastrointestinal mucosal sites, even a complete failure of the SIgA system does not lead to an increased UTI rate (Floege *et al*., ١٩٩٠).

Urinary immunoglobulins probably exert their protective role by interfering with bacterial adhesion to uroepithial cells (Trinchieri *et al*., ١٩٩٠). Human milk rich in SIgA antibodies to *E coli* prevents adhesion of bacteria to uroepithelial cells (Svandorg-Eden & Svennerholm, ١٩٧٨). The same effect can be seen when bacteria and uroepithial cell are incubated in urine obtained at the onset of acute pyelonephritis and containing antibody against to strain of bacteria tested (Eden *et al*., ١٩٧٦).

٢-٧: Mucosal immune system during Menopause.

Immune dysfunction occurs with aging. There is a progressive decrease in thymic mass and production of thymic hormones resulting in a decrease in naive lymphocytes and corresponding increase in memory cells. Lymphocytic proliferation responses decline, perhaps related to decreased $IL_{٢}$ production. On the other hand, certain cytokines such as IL-٦, IL-١β, TGFβ increase with age. Specific antibody response to a challenge decrease, but nonspecific immunoglobulin levels may be elevated. Monoclonal immunoglobulin protein

prevalence increases progressively with age, perhaps related to T-cell regulatory abnormalities and/or the influence of IL-٦.(Miller, ١٩٩٦)

It has been shown that aging is associated with several dysfunctional stages in Lymphocyte activation, particularly with progression of lymphocytes to state of immune unresponsiveness to ages and to an increased incidence of autoimmune disease (Hodes, ١٩٩٧; LeMaoult *et al*, ١٩٩٧ a) especially effected are T cell responses including IL-٢ production, IL-٢ receptor expression, signal transduction, and programmed cell death all of which reported in the elderly (Nordin & Collins, ١٩٨٣; Negoro *et al*., ١٩٨٦; Thoman *et al*., ١٩٩٣; Knight, ١٩٩٥; Zhou *et al*., ١٩٩٥; Miller, ١٩٩٦; Haynes *et al*., ١٩٩٧).

Because the result showed that $CD_{٤}^{+}$ T cells from aged mice exhibited lower proliferatine responses than those taken from young adult mice (Koga *et al*., ٢٠٠٠). It is possible that the age-associated reductions in antigen-specific antibody and T cell proliferation response could involve an alteration in responsiveness to T cell growth factors such as IL-٢.Indeed, a reduce frequency of IL-٢ producing $CD_{٤}^{+}$ T cells and a low IL-٢ receptor expression by this T-cell population (Nordin & Collins, ١٩٨٣; Negoro *et al*., ١٩٨٦) are one result of aging recent work showed that the effects of aging on IL-٢ production can be abrogated by exogenous IL-٢ delivery (Haynes *et al*., ١٩٩٩). The T-cells which occur in the menopause are often characterized by altered phenotypes, reduced responses to mitogens and impaired cytokine production (Flurkey *et al*., ١٩٩٢; Okumura *et al*., ١٩٩٣; Ernst *et al*., ١٩٩٣). Furthermore, these changes have been compared to that of senescent T cells, which were of memory types, showed decrease intracellular phosphorylation of $CD_{٣}$ (Miller *et al*., ١٩٩٧). Thus it is likely that these age-related T cell responses exhibit

altered help for B-cell and Ab response. Indeed, it was shown that T cells from aged mice down-regulate B cell responsiveness (Koga *et al*., ٢٠٠٠). Increased number of spleenic $CD_{٥}^{+}$ B cell producing IL-١٠ were found in aged mice (Zharhary, ١٩٨٦).

Additionally, recent studies showed that development of stable clonal B cell population In aged mice is detected by Ig heavy chain mRNA $CDR_{٣}$ size analysis (LeMaoult *et al*., ١٩٩٧ b ; LeMaoult *et al*., ١٩٩٩).

Despite, these well known age-associated change as well as recent observations made possible advances in cellular and molecular analysis of the induction and regulation of mucosal immune response which occur in elderly remain poorly defined. (Koga *et al*., ٢٠٠٠).

٢-٨:UTI and menopause immunity:

Urinary tract infection UTI represents one of most common infectious disease encountered in the practice of medicine. UTI are common especially among women. The prevalence of bacteriuria in school girls six to ١٨ years of age is ١.٢٪ among fertile women, the risk for UTI is higher and increases further after the menopause, the prevalence is ٧.٤٪ in women aged over ٦٥ years (Kunin & McCormarck, ١٩٦٨).

As is true of urinary tract infection in younger women, the vast majority of urinary tract infection in older women occur by the ascending route- colonization of the periurethral area with potentially uropathogenic bacteria is the initial event in the development of urinary tract infection in older as well as younger women. It is probable, however, that the increased prevalence of infection in older women with non-*Escherichia coli* strains of bacteria, most of which are not intrinsically uropathogenic, is a reflection of important differences in the pathogenesis of infection in different age groups. (Raz & Stamm, ١٩٩٣).

In premenopausal women, estrogens appear to encourage colonization of the vagina with lactobacilli a fastidious and generally nonuropathogenic genus of bacteria. These bacteria produce lactic acid and thereby lower vaginal pH, which inhibits the growth of many potential uropathogens. After menopause, the loss of estrogen effect on genitourinary tissues results in disappearance of *Lactobacillus* colonization and a secondary rise in vaginal pH. This allows colonization of periurethral area with potentially pathogenic specieus of bacteria such as those from the Enterobacteriaceae family, and in particular *E.coli* (Raz & Stamm, ١٩٩٣).

Loss of estrogen effect on urogenital tissues also appears to affect infection in older women, and it probably predisposes them to complicated urinary tract infection as well.

The mucosa of urethra, which orginates from the urogenital units has concentration of estrogen receptors similar to that of vaginal mucosa. Estrogen deprivation results in atrophic urethritis, vulvovaginal dryness, dyspareunia and some time urinary incontinence (Klutke & Bergman, ١٩٩٥). Observation that estrogen and progesterone affect urinary tract function include

documentation of symptomatic, cytologic, and urodynamic change during the menstrual cycle, pregnancy, and after menopause (VanGeelen *et al*., ١٩٨١; Solomon *et al*., ١٩٨٥; Tapp & Cardozom, ١٩٨٦).

Urethral closure pressure, in particular, is affected by estrogen status, and the integrity of urethral function may be important in preventing urinary tract infection as well as in maintaining continence in older women (Rud *et al*., ١٩٨٠).

After more than a half-century of research, however, there are few rigorously controlled studies of estrogen replacement in postmenopauasal women with stress incontinence, and the studies that have been published show disparate methods and results (Klutke & Bergman, ١٩٩٥).

It may be combination of larger numbers of potential uropathogens in the periurethral bacterial flora of older women plusless-effective urethral and bladder function that result in complicated urinary tract infection. The larger concentration of periurethral gram negative bacilli may simply overwhelm host defense near the urethra, and impaired bladder function make it more difficult to clear the infection (Baldassarre & Kaye, ١٩٩١).

Physiological changes occurring after menopause including increased vaginal pH and alteration in the vaginal flora to predominantly gram-negative organisms, have been reported to lead to increased susceptibility of UTI (Greendale *et al*., ١٩٩٩ b).

٢-٨-١: Pathogenesis

Urinary pathogens finds part of entry to urinary tract via ascending way from periurethral area or descending via lymph or blood flow to renal apparatus. The pathogenesis of UTIs in older women include those factors that are prominent in younger women-sexual activity and the genetically determined presence of receptors on uroepithelial cells that permits colonization by certain strains of *E. coli* (Hooton *et al*., ١٩٩٦). Women with a history of recurrent UTIs when they were young are more likely to have recurrent infections when they are older (Nicolle, ٢٠٠٠; Raz *et al*., ٢٠٠٠). Additionally, in older women, several factors assume increased importance as determinants of risk. Incontinence and dysfunctional bladder emptying due to a variety of physiologic and anatomic factors, including cystoceles and prior urologic procedures, increase the risk for infection (Raz *et al*., ٢٠٠٠).

In women residing in long-term care facilities, the use of indwelling catheters greatly increases the risk for UTI (Warren, ١٩٩٧).

٢-٨-٢: Bacterial etiology:

The most common cause of UTIs in older women living in the community is *E. coli* accounting for ٦٠-٧٠٪ of infections (Nicolle & Ronald, ١٩٨٧). Other gram negative bacilli including *Klebsiella pneumoniae* are more likely to cause infection in older women than in younger women. *Staphylococcus saprophyticus* the second most common pathogen in young women is rarely seen in older women, but enterococci, rare in young women, are associated with a small portion of infections in older women (Nicolle, ٢٠٠٠).

٢-٨-٣: Host defense mechanism to UTI in menopause:

The frequency of UTI varies with age, gender, and socio-economic background. In addition, there is individual variation in the susceptibility to UTI among persons of comparable age and socio-economic status (Svanborg, ١٩٩٣). Critically important defense mechanisms against bacterial colonization of the bladder include maturation and an efficient emptying of the bladder which continually washes out dilutes the invading organism and antimicrobial properties of the bladder mucosa, old women in the bladder correspond to a static chamber and the frequency of voiding and residual volume are crucial factor in the development of the bladder infection (Honkinen *et al.,* ١٩٩٩).

Susceptibility to UTI is increased in patients with inborn defects (for example vesico-ureteral reflux and strictures) or acquired restriction in the urine flow. these patients are often infected with a wider spectrum of bacterial species and with bacteria that are a virulent for the heathly urinary tract (deMan *et al*., ١٩٩٠; Honkinen *et al*., ١٩٩٩ a).

The low vaginal pH and the microbial diversity of the vaginal ecosystem have been found to be important in preventing vaginal colonization by *E. coli* in young women (Gupta *et al*., ١٩٩٨). Physiological changes occurring after menopause, including increased vaginal pH and an alteration in the vaginal flora to predominantly gram-negative organisms, lead to increased susceptibility to UTI (Greendale *et al*., ١٩٩٩).

One of the most important antibacterial defense mechanisms of all mucosal surfaces is the competition by indigenous microorganisms for receptors, space and substrates (Arp, ١٩٨٨).

The defense mechanisms of the ureter include urinary flow and the vesicoureteric valves which prevent reflux of urine during bladder emptying. In

older women intra-renal obstruction and vesicoureteric reflex are associated with a high risk of renal infection. The persistatic action of ureter causes turbulent flow of urine which contributes to the elimination of ascending bacteria, diminished of the persistaltic action of ureter with aging may contribute to the increased incidence of pyelonephritis.(Arp, ١٩٨٨)..

The concentration and production of urine at different times of the day and the composition of urine has significant effects on phagocytic function and complement activation (Cattel, ١٩٩٦). Age related changes in immunity involve alteration in T cell and B cell functions, that cause decay in immune function. Immuno-suppressive prostaglandin E-٢ (PGE٢) levels increase with age, even in healthy aged adults, also antibody response of old individual are characterized by lower, slower and shorter responses than those showed in younger people (Leshourd, ١٩٩٧). Increase in basal interleukin- (IL-٦) production by lymphocytes occur in aging, also specific cytokine changes that favor $Th_{٢}$ T helper responses (antibody production, including autoantibody production) while suppressing $Th_{١}$ responses (cytotoxic T cell and macrophage activation, i.e. cell- mediated immunity (High, ١٩٩٩).

Table (II): Host susceptibility factor.

Secretor status
Residual urine

Out flow obstruction
Vesico-ureteric reflex
Calculi
Structural abnormalities
Congenital
Acquired
Pregnancy
Instrumentation

(Raju & Tiwari, ٢٠٠١).

Table (III): Defense mechanism of urinary tract.

Non-specific	Normal flora of vagina
	Flushing effect of urine flow and voiding bladder glycocalyx
	Tamm-Horsfall glycoprotein
	Endotoxin induced shedding of bladder epithelial cells
	Phagocytosis
Specific	Secretory IgA
	Circulating IgM, IgG

(Raju & Tiwari, ٢٠٠١).

The predominant antibody classes in the serum were the IgG and IgM class, whereas in the urine the predominant antibodies were of the IgA and IgG classes. Antibodies against this domain would block adherence of type ١-

fimbriated *E. coli* to the bladder mucosa in situ and in vivo in an established mouse model of cystitis (Thankavel *et al*., ١٩٩٧). Host immune mechanism operating in the urinary tract includes local and systemic antibody production complement mediated killing, neutrophil phagocytosis, and cell mediated immunity. (Thankavel *et al*., ١٩٩٧)

The classical complement pathway is activated by presence of specific antibody and the alterative pathway may be activated by bacterial surface antigen. The result of complement activation is bacterial killing. However, some serotypes are relatively resistant to complement lysis and these organisms appear to predominate in cases of pyelonephritis. Phagocytosis of bacteria by neutrophils and macrophages occurs if organisms invade the bladder or kidney but is probably of little importance in most lower urinary tract infection as a composition of urine inhibit phagocyte function (Cattel, ١٩٩٦).

٢-٨-٤: Specific risk factors for complicated UTI in menopause.

After menopause, there are hormonal and pelvic floor alterations that change, the risk factors for urinary tract infection. Postmenopausal women have inherently decreased amounts of estrogen. Diminish circulating estrogen decreases the vascularity and leads to atrophy of uretheral epithelium and vaginal mucosa. Estrogen also play a role in pH balance of the vagina so that in the decrease of estrogen causes increase in the pH and that prevent or inhibit the growth of normal flora, such as Lactobacilli, and uropathogens can more easily colonize the perineum (Sotelo & Westney, ٢٠٠٤).

٢-٨-٤-١: Urinary incontinence

Older women have an even higher predisposition to develop urinary incontinence than older men, in a ratio of ٢:١. Histologically, hypertrophic changes in bladder smooth muscle, collagen, and elastic tissue occur more frequently in menopausal women. There may be degeneration and fibrosis of the bladder wall and decrease in bladder capacity, as well as diminished muscle tone in the bladder, internal and external sphincters, and pelvic floor musculature. There is also a reduction in bladder contractility.

There are many types of urinary incontinence (urge, stress, overflow, functional and mixed incontinence)urinary incontinence represent risk factors of UTI in menopausal women.

٢-٨-٤-٢: Catheters and hospitalization

About ٤٠٪ of all infections that are developed in hospitalized patients are in the urinary tract, and ٨٠٪ of those are due to catheters. The use of catheter puts older adults at risk. Symptoms of catheter-associated UTI may not be noticeable in elderly persons. Long-term catheter users face significant risks, including pyelonephritis-especially if catheterized over ٩٠ days in the last year of life. Individuals with urethral catheters are prone to damage to urethra (Wilde, ٢٠٠٤)

٢-٨-٤-٣: Renal stones

Menopause is associated with increased urinary calcium exertion, which could increase the risk for the development of calcium containing renal stones (Mattix-Kramer *et al*., ٢٠٠٣), kidney stones in some cases can cause

obstruction and cause infection, particularly pyelonephritis. Symptoms of severe urinary tract infection in people with a history of renal stones may indicate obstruction of urinary tract (Cattel, ١٩٩٦).

٢-٨-٤-٤: Diabetes Mellitus

Diabetes Mellitus puts women at significantly higher risk for a symptomatic bacteriuria. The longer of women has diabetes, the higher the risk (control of blood sugar has no effect on this condition). The risk for symptomatic complicated UTI may also be higher in women with diabetes (Cattel, ١٩٩٦).

٢-٨-٤-٥: Urinary tract abnormalities

Nearly any renal disorders increase with the risk of complicated UTI (Simon *et al.*, ٢٠٠١).

Anatomical abnormal persons who have major anatomic and functional abnormalities of urinary tract, including vesicoureteral reflux, ureteral obstruction, or foreign body (e.g. tumor) are markedly predisposed to UTI, particularly infections involving the kidney. In such persons, UTI can develop as a result of infection with bacteria, some of these abnormalities of urinary tract that cause urine to stagnate or flow back wared into the upper urinary tract such condition include the following.

-Aprolapsed bladder (cystocele) can result in incomplete urination so that urine collects create a breeding ground for bacteria.

-crevasses called diverticula are sometimes developed inside the urethral wall and can become tiny pockets for urine and debris, further increasing the risk for infection (Simon *et al*., ٢٠٠١).

٢-٩:The interaction between neroendocrine and immune system

The nervous, endocrine, and immune system interact to maintain physiological homoestasis during inflammation and stressors that induce systemic cytokine production (Bethin *et al*., ٢٠٠٠).

The hypothalamic-pituitary-adrenal axis exerts profound, multilevel inhibitory effects on the female reproductive system. Corticotrophin-releasing hormone (CRH) and CRH-induced proopiomelanocortin peptides inhibit hypothalamic gonadotropin-releasing hormone secretion, whereas glucocorticoids suppress pituitary luteinizing hormone and ovarian estrogen and progesterone secretion and rendar target tissues resistant to estradiol(Bethin *et al*., ٢٠٠٠)..

Conversely, estrogen directly stimulates the CRH gene promoter and the central noradrenergic system which may explain adult women's slight hypercortisolism; preponderance of affective, anxiety, and eating disorders; and mood cycles and vulnerability to autoimmune and inflammatory disease, both of which follow estradiol fluctuations. Several components of the hypothalamic-pituitary-adrenal axis and their receptors are present in the reproductive tissues as autacoid regulators. These include ovarian and endometrial CRH, which may participate in the inflammatory processes of the ovary (ovulation and luteolysis) and endomaterium (blastocyst implantation and menstruation), and placental CRH, which may participate in the physiology of pregnancy and the timing of labor and delivery. The hypercortisolism of the

latter half of pregnancy can be explained by the high levels of placental CRH in plasma. (Bethin *et al*., ٢٠٠٠).

This hypercortisolism causes a transient postpartum adrenal suppression that, together with esterogen with drawal, may partly explain the depressionand autoimmune phenomena of postpartum period (Chrousos *et al*., ١٩٩٨).

Gender and sex hormones exert powerful effects in the susceptibility and progression of numerous human and experimental autoimmune diseases. This has been attributed to direct immunological effect of sex hormones that impact a clear gender dimorphism on the immune system. Globally, estrogens depress T cell-dependent immune function and diseases, but inhance antibody production and aggravate B cell dependent diseases. Androgens suppress both T-cell and B-cell immune responses and vitually always result in the suppression of disease expression. Defect in the hypothalamic-pituitary-adrenal (HPA) axis have been proposed to play an important role in the pathogenesis of antoimmune diseases. Glucocorticoid response to stress, including immune challenge, is strongly inhibited by androgens and enhanced by estrogens (Dasilva, ١٩٩٩).

Signals generated by the hypothalamic-pitutary-gonadal (HPG) axis powerfully modulate immune system function. In the brain, the principal hormones of the HPG axis directly interact with astroglial cells. Thus, luteinizing hormone releasing hormone, LHRH influences hypothalamic astrocyte development and growth, and hypothalamic astrocytes direct LHRH neuron differentiation. Hormonally induced changes in neuron-glial plasticity may dictate major changes in CNS output, and thus activity participate in sex diamorphic immune responses. The impact of gendar in

neuroimmunomodulation is further underlined by the sex dimorphism in the expression of genes encoding for neuroendocrine hormones and their receptors with the thymus, and by the potent modulation exerted by circulating sex steroids during development and immunization (Marchtti *et al*., ٢٠٠٠).

Colony stimulating factor ١ (CSF-١) is homodimeric polypeptide growth factor whose primary function to regulate the survival proliferation, differentiation, and function of cell of the mononuclear phagocytic lineage. This lineage includes mononuclear phagocytic precursors, blood Monocytes, tissue macrophages, osteoclasts, and microglia of the brain, all of which possess cell surface receptor for CSF-١ (CSF-١R), the product of the C^{-fms} protooncogene, is a member of the type III tyrosine kinase receptor family. CSF-١R is also located on cells of the reproductive system including oocytes and trophoblastic cells (Arceci *et al*., ١٩٨٩; Arceci *et al*., ١٩٩٢; Cohen *et al*., ٢٠٠٢).

Chapter Three

Materials & Methods

٣-١: Solutions

٣-١-١: Normal saline

This solution was prepared by dissolving ٠.٨٥gm of sodium chloride (NaCl).BDH company (m.wt٥٨.٤٤) in small amount of distilled water and completing the final volume to the ١٠٠ ml (٠.٨٥%); this solution was sterilized by autoclave ١٢١C,١٥Ib and ١٥ min .It can be used in preparation of formal saline, and standarization secretory immunoglobulin with antigen of the gram negative bacteria (Garvey *et al* .,١٩٧٧), and preparation of antigen of the gram positive bacteria

٣-١-٢: Formal Saline

This solution was prepared by adding ٠.٠٥ ml from formaldehyde (H-CHO)BDH company (M.wt٣٠.٣) to ٩٩.٥ml (V/V) from ٠.٨٥٪ normal saline to produce ٠.٥٪ concentration; it was used to dissolved the urinary immunoglobulin and to prepare antigen of the gram negative bacteria (Lehmann *et al*., ١٩٦٨)**.**

٣-١-٣: Tris Buffer

To prepare tris buffer,١٢ gm from the Tris base ($NH_2C(CH_2OH)_3$) TAAB company (M.wt١٢١.٤)was dissolved in a small amount of distilled water and complete the volume to the ١ liter .

pH of this buffer was adjusted to ٧ adding HCl ٠.١ N. This solution was used to prepare polyethyleneglycol solution (Johnston & Thorp,١٩٨٢).

٣-١-٤: Polyethylenglycol Solution

The feasible concentration of PEG solution was ٦٪, it was prepared by dissolving ٦gm from polyethylenglycol ($HO(C_2H_4O)nH$). BDH. company (M.wt ٦٠٠٠)in small amount of Tris buffer and complete the final volume to ١٠٠ ml this solution used to separate immunoglobulin from serum and urine (Shnawa & Mehdi, ٢٠٠٤; Johnston & Thorp,١٩٨٢).

٣-١-٥: ٢-Mercapto Ethanol (٠.٠٥ M)

It was prepared by adding ٣.٩ ml from stock of ١٢.٨ molary of Collbiochem company ($HS-CH_2-CH_2-OH$) to small amount of normal saline and complete the final volume to ١liter, the solution was used to detect the effect of

this material on secretory immunoglobulin as reducing factor (Cruishshank *et at.*, ١٩٧٥).

٣-١-٦: Biuret solution

This solution was prepared by dissolving ٣gm from cupper sulfate ($CuSO_4.5H_2O$). BDH company (M. wt ١٦٦) in half liter distilled water with adding ٩ gm from sodium potassiumtartarate ($NakC_4H_4C_6.4H_2O$)-BDH company (M.wt ٢٤٩.٥)after dissolving those three components, l٠٠ml from sodium hydroxide (NaOH) ٠.٦ N was added and the final volume was complete to ١ liter by adding distilled water. This solution was used in Biuret method to measure the concentration of immunoglobulin (Ross,١٩٨٥).

٣-١-٧: Standard albumin solution

To prepared standard albumin solution, ٦٠ gm from dry–egg albumin–BDH company (M. wt ٦٥٤٠٠) was dissolved in a small amount of sodium hydroxide ٠.٦ N and the final volume was complete to١liter using NaOH.

The final concentration of albumin was ٦٠ gm /liter. Standard dilution from this solution was prepared (١:١,١:٢, ١:٤,١:٨ ,١:١٦ ,١:٣٢,١:٦٤ ,١:١٢٨ ,١;٢٥٦ ,١;٥١٢) that represents the following concentrations (٣٠,١٥ ,٧.٥,٣.٧٥,١.٨٧٥,٠.٩٣٧٥,٠.٤٦٨٧٥,٠.٢٣٤٣٧٥ ,٠.١١٧, ٠.٠٥)gm/ L respectively. The solution that was used to dilute the albumin was sodium hydroxide (٠.١N) this solution was used to prepare standard curve to detect the concentration of immunoglobulins (Ross,١٩٨٥).

٣-٢: Reagents

Kovacs (indol), methylred (MR test), Voges-proskauar (VP test) H_2O_2 solution (catalase test) and tetramethelparaphenylene_diamin mono hydrochloride (oxidase test). Reagents were prepared as in (Macfaddin, ٢٠٠٠).

٣-٣: Culture Media

To investigate menopausal women with urinary tract infections, dehydrated culture media (Table-V-) were rehydrated, prepared, sterilized dispensed and /or dispensed then sterilized in accordance with recommendation of manufacturing company.

Table (IV): The culture media used for patients investigations.

Media type	Name of producing Company
١-Blood agar base	Biolife
٢-MacConky agar	Himedia
٣-Eosin methylen blue agar	Himedia
٤-Nutrient agar	Diagnostic merck
٥- Nutrient broth	Himedia
٦-Mannitol salt phenol agar	Himedia
٧-urae agar	Himedia
٨-Simmon citrate	Himedia
٩-Pepton water	CHEM-SUPPLY
١٠-MR-VP, media	Himedia
١١-kligler iron agar	Himedia

٣-٤: Patients case history

Seventy seven menopause women of ٤٥-٦٠ years age attending Hilla Educational hospital and Al-Hashimia hospital to the period of ١٢/٢٠٠٤-٦/٢٠٠٥ suffering from urinary tract infections were the study group.

Thirty five adolescent patients with UTI ١٨-٣٠ years old were the control group.

Patients and controls were interviewed by specialist urogelists and the case history was made.

Case history was include: patients name, age, sex, menopause nature ,disease duration ,underlying disease ,past therapy ,surgical intervention.

٣-٥: Disposable tools

٣-٥-١: disposable tube type (Afma)

Disposable tubes were used to collect midstream urine sample and other biochemical tests.

٣-٥-٢: disposable plates

Disposable plates were used to cultivate the causal organisms

٣-٦: Bacteriologic Diagnosis

٣-٦-١ : Api ٢٠ E system

An Api ٢٠ E is a standardized, miniaturized version of conventional biochemical procedures used in the identification of Enterobacteriaceae and other gram negative bacteria. It was already-to-use; microtube system that performs ٢٢ standard biochemical test on pure bacterial cultures from appropriate primary isolation media.

This system consists of a strip containing ٢٠ chambers each containing of a microtube and depression called cuple.

The tube contains dehydrated substrates. The substrates are redehydrated by adding bacterial saline suspension. The strip of microtubes is then incubated for ١٨ to ٢٤ hours at ٣٥ to ٣٧ C so that the bacterium can act on the substrates.

The strip is read by noting color changes after various indicator systems which have been affected by the metabolites or added reagents. The identification of unknown bacterium is achieved by determining a seven-digit-profile index number and consulting the Api-٢٠ –E profile index booklet. Charts can also be used to determine the unknown bacteria.

٣-٥-٢: Novabiocin antibiotic disk

This antibiotic disk was produced by Bioanalyse company for invitro diagnostic use, which was resistance to diagnosis *S saprophyticus* which was resistance to novabiocin while the *S aureus* and *S epidermidis* was sensitive to novabiocin.

٣-٦-٣: Tool of serological diagnosis for group A Streptococci

It was produced by BioMerieudx company which was used for detection on serotype A of *Streptococcus pyogens* based on serological test.

According to the recommendation of supplied company, the following steps must be followed:

١-Take a loop full from culture of bacteria that grown on Brian-heart infusion agar with blood on age ١٨ hrs and added to test tube containing ٤ ml from extraction enzyme (that produce by adding ١٠ ml from distilled water to the container that contain lyophilized enzyme and mixed by the vortex).

٢-Incubate the tubes at ٣٧C in water bath for ١٥ min, to extract the charbohydrated antigen of cell wall.

٣-Put drop from extract on the slide and mixed with latex that contain the antibody associated with serotype A.

٤-Round the slide for ٢ min. and record the result and compared with positive control solution, which agglutination for ٢-٣ min in positive test

The diagnosis of uropathogens depending on (Macfaddin, ٢٠٠٠ &Holt *et al*;١٩٩٤)

(Table -V-) biochemical tests to gram positive uropathogens

Streptococcus pyogens	***S. saprophyticus***	***S. epidermidis***	***S. aureus***	**bacteria / Test**
-	-	-	-	Oxidase production
-	+	+	+	Catalase

				production
-	+	+	+	Nitrate test
+	-	+	+	Glucose fermentation
+	+	+	+	Lactose fermentation
S	R	S	S	Novabiocin ١resistant
-	-	-	+	Coagulase production
-	-	-	+	Manitol fermentation
β	γ	α	β	heamolysis
+	-	-	-	Tool of serological diagnosis for group A Streptococci

+: positive result,-: negative result; S:sensetive ;R:resistance

(Table-VI-) biochemical tests to gram negative uropathogens

E.asburiae		*P.mirabilis*		*K.terrigena*		*K.oxytoca*		*K.pneumoniae*		*E.coli*		Bacteria / test
C.B.	Api ٢٠	C.B.	Api ٢٠	C.B.	Api ٢٠	C.B.	Api ٢٠	C.B.	Api ٢٠	C.B.	Api ٢٠	
α	/	γ	/	α	/	γ	/	γ	/	α	/	heamolysis
+	+	-	-	+	+	+	+	+	+	+	+	ONPG
-	-	-	-	-	-	-	-	-	-	-	-	ADH
-	-	-	-	+	+	+	+	+	+	+	+	LDC
+	+	+	+	-	-	-	-	-	-	+	+	ODC
+	+	+	+	+	-	+	+	+	+	-	-	CIT
-	-	+	+	-	-	-	-	-	-	-	-	H٢S
-	-	+	+	-	-	+	+	-	+	-	-	URE
-	-	+	+	-	-	-	-	-	-	-	-	TDA
-	-	-	-	-	-	+	+	-	-	+	+	IND

-	-	+	-	+	+	-	+	+	+	-	-	VP
-	-	-	-	-	-	-	-	-	-	-	-	GEL
+	+	+	+	+	+	+	+	+	+	+	+	GLU
+	+	-	-	+	+	+	+	+	+	+	+	MAN
-	-	-	-	+	+	+	+	+	+	-	-	INO
+	+	-	-	+	+	+	+	+	+	+	+	SOR
-	-	-	-	+	+	+	+	+	+	+	+	RHA
+	+	-	+	+	+	+	+	+	+	-	-	SAC
-	-	-	-	+	+	+	+	+	+	+	+	MEL
+	+	-	-	+	+	+	+	+	+	-	-	AMY
-	+	-	-	+	+	+	+	+	+	+	+	ARA
-	-	-	-	-	-	-	-	-	-	-	-	OX
-	/	-	/	-	/	-	/	-	/	+	/	Metallic sheen on EMP
-	/	-	/	-	/	-	/	-	/	-	/	Oxidase production

+: positive result,-: negative result C.B.:classical biochemical test

٣-٧: Sampling

٣-٧-١: collection the specimens

Mid–stream specimen was collected and a clean catch from patients and controls after that was kept cool until arriving to the lab then examined, external genitalia were washed with soap and water and dried with sterile cotton wool. In the female, urine was passed with labia separated. The specimens was collected in a sterile container and kept cool (not more than ٢ hours) then examined.

٣-٨: Isolation of associated bacterial causal.

٣-٨-١: Methods for isolation of associated bacterial causal:-

Two methods were made to isolate the causal organisms:

٣-٨-١-١: direct method (D):-

From clean catch mid stream specimens of urine loop- full inculum

was cultured on macConky agar and blood agar at ٣٧C^{o} for ٢٤ hours under aerobic condition (Collee *et al*., ١٩٩٦).

٣-٨-١-٢:indirect method (ID):-

One ml from urine sample was mixed with ١ml from nutrient broth; this mixture was incubated on ٢٤ hours at ٣٧C^{o}, after that loop-full inoculums was subcultured into blood agar and MacConky petridishs and were incubated at ٣٧C^{o} for ٢٤ hour in aerobic condition (shnawa & Mehdi, ٢٠٠٤).

٣-٩-١: preparation of surface antigen of the gram positive bacteria.

This was prepared on (McCoy & Kenndy, ١٩٦٠, Garvey *et al*., ١٩٧٧) by using the following method:

١- Pure culture was prepared on nutrient agar. at ٣٧Cْ for ٢٤ hr

٢- Six mls from normal saline was added to the surface of growth was swept by sterile loop.

٣- The suspension was collected by sterile pasture pipette and was tubed in sterile disposable tube.

٤- Five mls from this suspension centrifuged in ٤٠٠٠ rpm at ٥ min.

٥- The deposit was washed by adding ٥ml normal saline.

٦- The supernatant was removed and added ٥ml to the deposit of phenol ٠.٥٪ (٠.٥gm from phenol crystal in ١٠٠ml normal saline) and mixed with it.

٧- One ml of this suspension was tubed in opacimeter with adding phenol solution to the opacimeter until the turbidity becomes equal to the standard tube. The final concentration of antigen is equal to the ١٠ international unit.

٨- Five international units were prepared by taking (٥ml) from the final suspension and mixed with ٥ ml from phenol solution. After that the solution was mixed well and incubated in ٣٧C^{o} in half hour.

٩- Sterility test was made by taking loop full from this suspension and streaking it on nutrient agar, then culture was incubated in ٣٧C^{o} at ٢٤ hour. The positive culture was discarded negative, however, was considered as sterile.

٣-٩-٢: Preparation of surface antigen of the gram negative bacteria.

It was prepared according to (Smith, ١٩٧٥) with some modification:

١- Twenty four hours pure culture was prepared by transport an incoulum from the growth on MacConky agar to the nutrient agar and was incubated for ٢٤ hours. at ٣٧C

٢- Six ml formal saline were added to the surface of nutrient agar and the surface growth was swept by using sterile.

٣- Loop the suspension was collected by using sterile pasture pipette.

٤- Five mls from the suspension was centrifuged at ٤٠٠٠ rpm for ٥min.

٥- Washing by adding ٥ ml of formal saline to the deposit then centrifuged at ٣٥٠٠ rpm for ٥min.

٦- Suspension was removed and the deposit was suspended by adding ٥ml from formal saline and mixed well.

٧- One ml from this suspension was tubed in opacimeter tube by adding the formal saline to the opacimeter tube until the turbidity become equal to the standard tube. Then the final concentration of antigen is equal to ١٠ international unit.

٨- Five international unit was prepared by taking ٥ml from the final suspension and with ٥ml from the formal saline.

٩- The antigen suspension was heat killed in water bath at ٦٠C^{o} for ١.٥ hour.

١٠- Sterility test was made.

٣-١٠: Urine filtration.

Before separation of immunoglobulin, part of urine sample was centrifuged at ٣٥٠٠ rpm for ٥min and filtered to separate cellular organic component and salt from urine (Bienenstock & Tomasi, ١٩٦٨; Burdon, ١٩٧٠; Kaufman *et al*., ١٩٧٠).

Filter paper (whatman no.١) was used for this purpose; the filtration paper was moisture by sterile distilled water. This method was considered the best method to filtrate urine. (Shnawa & Mehdi, ٢٠٠٤).

٣-١١: Separation of immunoglobulin from urine.

The material used for separation of immunoglobulin from urine was poly ethylene glycol PEG ٦٠٠٠ (Johnston & Thorp,١٩٨٢;Al Sa'adi,١٩٩٨;Shnawa & Alamidi, ٢٠٠٥).

١- Ten mls from urine sample was centrifuged at ٣٠٠٠ rpm for ٥ min. with removing the precipitate and collecting the supernatant.
٢- Eight mls from supernatant was filtered by using moisture filter paper (whatman no. ١).
٣- Five mls from polyethylene glycol was added to ٥ml from filtered urine and was kept in refrigerator for one hour.
٤- The mixture was centrifuged in ٦٠٠٠ rpm in ٣٠ min. after that the suspension was removed and precipitate was dissolved in ٠.٥ml of formal saline and it was transferred to the Appendrof tube.

٣-١٢: Measurement of urinary immunoglobulin. concentration.

To measure the concentration of immunoglobulin in urine we used Biuret method (Bienenstock & Tomasi, ١٩٦٨; Uehling & Steihm, ١٩٩١):-

١- Five mls of Biuret solution was tubed in spectronic tube.
٢- ٠.٢mls of immunoglobulin solution was added to the Biuret solution. This tube was represent as tube of test run.
٣- ٠.٢ml of distilled water was added to ٥ml from Biuret solution. This tube is considered as control tube.
٤- The tubes were mixed and left aside for ٣٠ min. in room temperature.
٥- The optical density was measured on wave length ٥٤٠ nanometer.

The immunoglobulin concentration was measured depending to the standard carve that has been prepared from dilutes of egg albumin solution.

٣-١٣: Serology test

Serial two fold tube dilution technique of urinary mucosal immunoglobulin was attempted against the ٥ IU density suspension of the causal organism.

Likewise, a tube dilution technique uses, ٢ME saline as a substitutant to saline as in the case of simple standard tube agglutination (Cruichshank. *et al*., ١٩٧٥ and Shnawa & Mehdi, ٢٠٠٤).

To made the agglutination test, we followed these steps

١-٨ tubes cleaned &sterilized, add ٠.٢ ml

:

normal saline (if the antigen prepare from gram positive pathogen) or ٠.٢ ml formal saline (if the antigen prepare from gram negative pathogen).

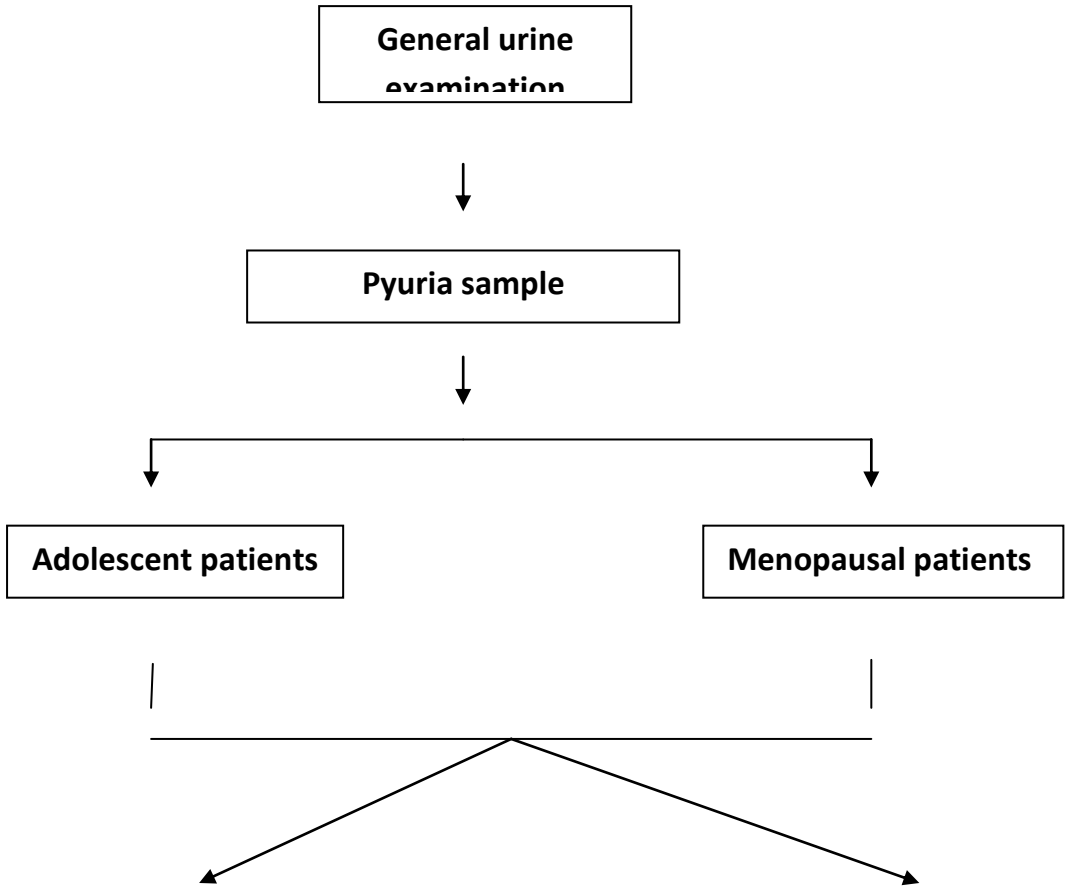
٢-Add ٠.٢ ml from UMIg to the first tube & mixed.

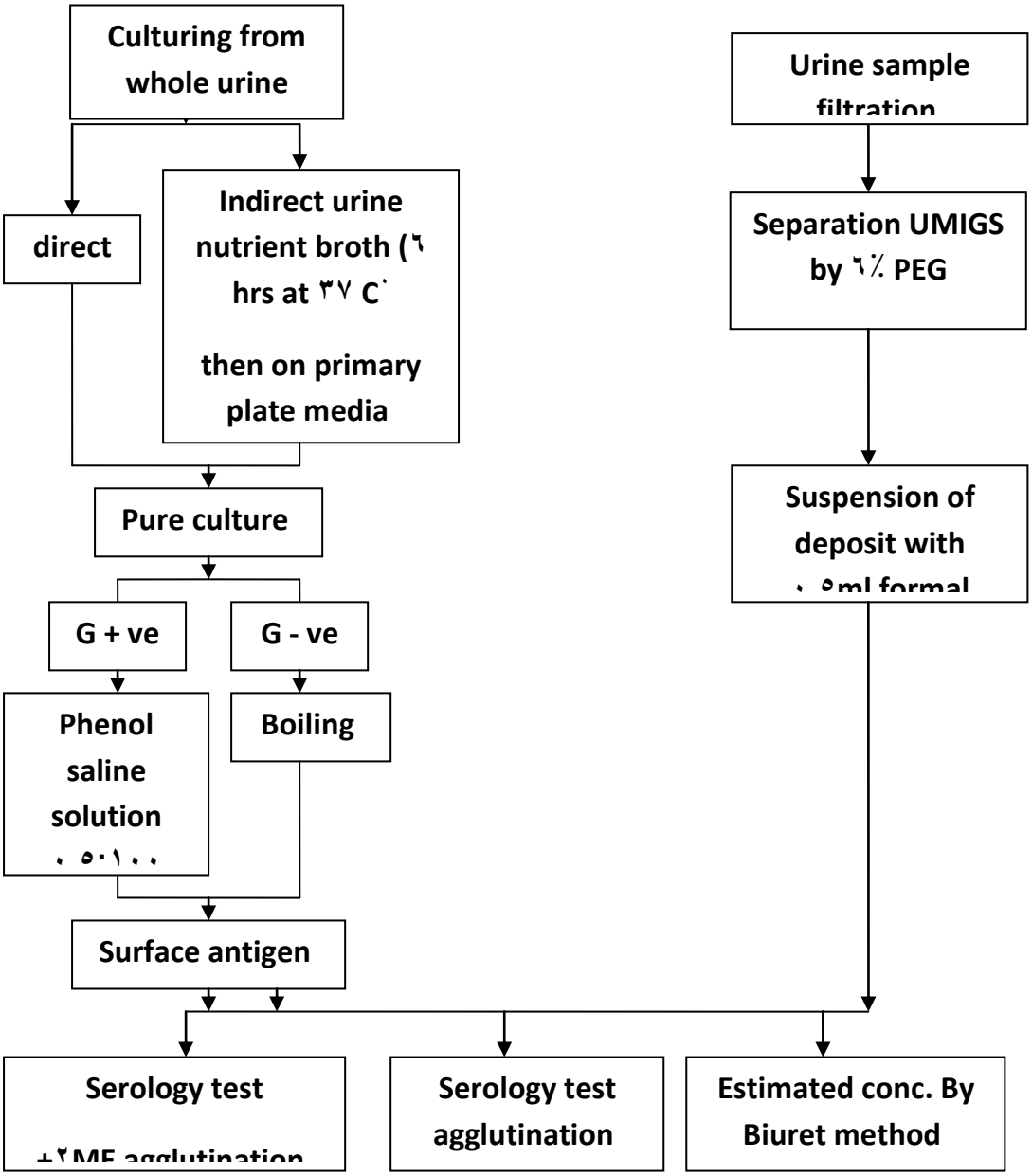
٣- Take ٠.٢ ml from the first tube to the second tube & mixed, also take ٠.٢ ml from it & added to the tube and continue to the ^{٧th} tube.

٤-The serial dilution to the UMIg become
(١:١,١:٢,١:٤,١:٨,١:١٦,١:٣٢,١:٦٤,١:١٢٨).

٥-Added ٠.٢ ml from the antigen of the causative organism to each tube (the last tube was a control tube that not contain UMIg).

٦-The tubes incubated for ٢٤ hr. at ٣٧C°and read the result. The positive result was cluster the antigen &antibody, the negative result was white cloudy.





٣-١٤: Study flow chart

٣-١٥: Statical analysis:-

There are two statistical tests were followed in this study:-

٣-١٥-١: Correlation factor

It used to study the correlation between immunoglobulin concentration and the titer of specific immunoglobulin.

$$r = \frac{\sum x : y : -(\sum x . \sum Y / N)}{\sqrt{(\sum xi2 - \frac{(\sum xi)2}{n})(\sum yi2 - \frac{(\sum yi)2}{n}}}$$

yi = titer.

yó = mean of titer.

Xi = concentration

Xó = mean of concentration.

Y^ = a+ bx.

Y^ was calculated depending on the equation of simple linear regression.

Y^ represented the value of titer depending on the study of standard curve that was referred to as the correlation between immunoglobulin concentration and antibody titer.

a =intercept of curve with y

a = y`– bX`

b =slop.

$$b = \frac{\sum (xi - x`)(yi - y`)}{\sum (xi - x`)2}$$

The equation that used to calculated the concentration of immunoglobulin

y =-٠.٦٧+١٦٨.٩xi

y =concentration of immunoglobulin

xi = optical density

١٣-١٥-٢: t- test

To determine the significant differences of the specific urinary immune globulin titer between adolescent and menopausal patients, paired observation t- statistics. Was used.

$$t=\frac{x`1-x`2}{\sqrt{\frac{s2}{n}}+\sqrt{\frac{s2}{n}}}$$

X`١= mean of titer in adolescent patients.

X`٢= variant on titer menopausal patients.

١S٢ = variant on titer adolescent patients.

n١, n٢ number of case in adolescent menopausal and menopausal patients respectively. (Al-Rawi,٢٠٠٠).

Chapter four

Results

٤-١: Score

The single infection was scored as moderate to heavy growth of one colony morphotype , while the growth of equivalent colony population indicates dimicrobic infection . Mucosal urinary immunoglobulin was characterized by the ٢ME resistance , positive biuret and immune specificity to the uropathogen .

٤-٢: Result presentation

Result will be presented in comparative manner for menopausal and adolescent patients . and evaluated by regression analysis and paired observation t-test statistics .

٤-٣: Age range

From seventy seven patients, ٤١.٥٥% were noted with in age group of (٥١-٥٥) ,while ٣٠.٩٦% of which were watched with in the age group (٥٦-٦٠) and ١٩.٤٨% were observed with in the age group (٤٦-٥٠) . [Table -١-]

٤-٤: Underlying disease and UMIg;

The concentration of urinary mucosal immunoglobulin in menopausal without underlying disease was ٠.٩٢٦٤٢ g/l, while those menopausal women with diabetes mellitus was ٢.٢٨٧١ g/l, and with urolithiasis was ١١.٨٦٠٧ g/l. [Table -٢-]

٤-٥: Nature of the uropthogen

It was evident that there was no menopausal specific uropathogen, but there was a difference in each of rate of the specific uropathogen among menopausal and adolescent patients .*Klebsiella* species were higher among menopausal than in adolescent patients.

Pseudomonas aeroginosa were not recovered among menopausal and adolescent patients.

The most common causative organisms were *E. coli* (٢٩.٨٧%, ٢٨.٥٧٪) in menopausal and adolescent patients respectively, while *K. pneumoniae* (٢٨.٥٧%, ٢٠٪) was the second among menopausal and adolescent patients accordingly. Meantime the *Enterobacter asburiae* (١٢.٩٨%, ١٤.٢٨٪) was in the third order among menopausal and adolescent patients respectively.

Staphylococcus aureus **(**١٢.٩٨%, ١١.٤٢٪) were the fourth order among menopausal and adolescent patients accordingly

Other organisms such as *Staphylococcus epidermidis***,** *Staphylococcus saprophyticus , K. terrigenae* were more common in younger than in menopausal women *K. oxytoca* were more common in menopausal than in adolescent individuals. *Streptococcus pyogens* and *Neisseria gonorrheae* were found in menopausal patients but not recovered in adolescent patients. The infection by *Proteus mirabilis* appeared among adolescent patients only. (Table -٣-)

Table -١-

Age range of menopausal UTI.

menopausal UTI ,age range		patient number
١.	٤٦_٥٠	١٥:٧٧ (١٩.٤٨٪)
٢.	٥١_٥٥	٣٢:٧٧(٤١.٥٥٪)
٣.	٥٦_٦٠	٣٠:٧٧(٣٠.٩٦٪)

Table -٢-

Menopausal UTI patient with underlying disease

seq.	underlying disease	no. of cases	mean of Mlg conc. g/l.
١.	With out underlyin disease.	٥٢	٠.٩٢٦٤٢
٢.	diabetes mellitus	١٢	٢.٢٨٧١٨
٣.	urolithosis	٨	١١.٨٦٠٧
٤.	uterus remove	٥	١.٦٧٨٩٩
.			

Table -٣-

Percent of uropathogens among menopausal

and adolescent female patients

seq.	Menopausal	the percent	Adolescent	the percent

١.	*E. coli*	٢٩.٨٧ %	*E. coli*	٢٨.٥٧ %
٢.	*K. pneumoniae*	٢٨.٥٧ %	*K. pneumoniae*	٢٠ %
٣.	*E. asburiae*	١٢.٩٨ %	*E. asburiae*	١٤.٢٨ %
٤.	*S. aureus*	١٢.٩٨ %	*S. aureus*	١١.٤٢ %
٥.	*S. epidermidis*	١٠.٣٨ %	*S. epidermidis*	١٧.١٤ %
٦.	*K. oxytoca*	١٢.٩٨ %	*K. oxytoca*	٨.٥٧ %
٧.	*K. terrigena*	٦.٤٩ %	*K. terrigena*	٨.٥٧ %
٨.	*S. pyogens*	٢.٥٩ %		
٩.			*P. mirabilis*	٥.٧١ %
١٠.	*S. saprophyticus*	٣.٨٩ %	*S. saprophyticus*	٨.٥٧ %
١١	*N. gonorrheae*	٢.٥٩ %		

٤-٦: *Escherichia coli* specific urinary mucosal humoral immune responses in menopausal and adolescent patients.

Twenty three cases of *E. coli* urinary tract infections were noted among menopausal patients. Two cases were positive for *E. coli* by preinrichment culture. (Table -٤-).

The age range of those patients were (٤٦-٥٩) years. The mean, median and range of their urinary mucosal immunoglobulin concentration were ٣.٠٥٩٧٨ g/L, ١.٥٥٥ g/L, ٠-١٨.٤٤٥ g/L respectively. While the *E. coli* mucosal specific antibody titer mean, median and range were ٦.٩٥٦٥٢, ٨ and ٢-١٦ accordingly. Statistics for the linear regression analysis showed that the correlation coefficient is significant, r =٠.٦٦٥٥ while ١٠ cases of young adult patients with *E. coli*. The age range of those patients were ١٨-٢٩ years, the means, median and range of urinary mucosal immunoglobulin concentration were ١.٢٠٨١٠٤, ٠.٩٠٢٦٦, ٠.٣٣٩٦ – ٣.٢٤٤ g/L respectively,while the urinary mucosal specific antibody titer mean, median and range were ١٨.٨, ١٦ and ٤-٣٢ accordingly. Statistics for linear regression analysis showed that r =٠.٣٧١٧٠ that mean it was statistically non significant. Thus, menopausal mucosal antibody titer mean was lower (٦.٩٥٦٥٢) than those for adolescent (١٨.٨). However paired observation analysis using t statistics showed that calculated t value was higher than the table t value which on df ٣٠ was ٣.٦٠١٨٢٣ at p =٠.٠٠٥. such finding means that it was statistically significant.

٤-٧: *Klebsiella pneumoniae* specific urinary mucosal immune responses in menopausal and adolescent patients

Twenty two cases of *K. pneumoniae* urinary tract infection were noted among menopausal women patients.Two cases were positive for *K. pneumoniae* by preinrichment culture.(table-٥-)

The age range of those patients were (٤٧-٥٩) years. The mean, median and range of their urinary mucosal immunoglobulin concentration were ١.٦٩١١٦, ٠.٨٩١٠٣, ٠-١٦.٢٢ g/L respectively. While the *K. pneumoniae* mucosal specific antibody titer mean, median and range were ٧.٤٥٤٥٤, ٨, ٤-٣٢ accordingly.

Statistics for linear regression analysis showed that r is rather significant, r =٠.٥ while ٧ case of adolescent patients with *K. pneumoniae* the age range of those patients were ١٨-٢٩ years, the means, median and range of urinary mucosal immunoglobulin concentration were ١.٤٩٤٢٢, ٠.٩٠٢٦٦, ٠.٣٣٩٦ – ٤.٣٩٢٣ g/L respectively, while urinary mucosal specific antibody titer mean, median and range were ٢٤, ١٦, ٨-٦٤ accordingly. Statistics for linear regression analysis showed that r =٠.٦٨ that mean it is significant. Thus menopausal mucosal antibody titer mean was lower ٧.٤٥٤٥٤ than those for adolescent ٢٤. The paired observation t statistics showed that t value was higher than the table t value which on df ٢٧ was ٢.٢٦١٥٤ at p =٠.٠٢٥. such finding means that it was statistically significant.

٤-٨: *K oxytoca* specific urinary mucosal humoral immune responses in menopausal and adolescent patients.

Ten cases of *K. oxytoca* urinary tract infection were noted among menopausal women patients. One case was positive for *K. oxytoca* by preinrichment culture (Table-٦-).

The age range of those patients were ٤٧-٥٩ years. The mean, median and range of their urinary immunoglobulin concentration were ٢.٣٣٣٦٨, ١.٦٧٩٢٣, ٠.٣٣٩٦–١١.١٣٥ g/l respectively. While the *K. oxytoca* mucosal specific antibody titer mean, median and range were ٧.٦, ٨, ٤-١٦ accordingly. Statistics for linear regression analysis showed that r is significant r =٠.٨٤٩٠ while ٣ cases of young female adult it patients with *K. oxytoca* the age range of those patients were ٢٢-٣٠ years, the mean, median and range of urinary mucosal

immunoglobulin concentration were ١.١١٢٣٥, ٠.٩٠٢٦٦, ٠.٨٧٩٤-١.٥٥٥ g/L respectively while the urinary mucosal specific antibody titer mean, median and range were ٣٧.٣٣٣٣٣, ٣٢, ١٦-٦٤ accordingly. Statistics for linear regression analysis showed that r was significant ٠.٧٨٧٧. Thus, menopausal mucosal antibody titer mean was lower (٧.٦) than those for adolescent (٣٧.٣٣٣٣٣). The paired observation analysis using t statistics showed that calculated t value was higher than the table t value which on df ١١ was ٢.١٠١٠٦ at p =٠.٠٥. such finding means that it was significant.

٤-٩: *K. terrigena* specific urinary mucosal humosal immune responses among menopausal and adolescent

Five case of *K. terrigena* urinary tract infection were noted among menopausal patients. (Table -٧-)

The age ranges of those patients were ٤٦-٥٨ years. The mean, median and range of their urinary mucosal immunoglobulin concentration were ١.٠٩٦٠٣٦, ٠.٩٠٢٦٦, ٠.٩٠٢٦٦ – ١.٥٥٥ g/L respectively while the *K. terringena* mucosal specific antibody titer mean, median and range were ٧.٢, ٨, ٤ – ٨ accordingly. Statistics for linear regression analysis showed that r is non significant, r = -٠.٢٣٢٦ while ٣ cases of adolescent patients with *K. terringena* the age range of those patients were ٢٥-٢٨ years the mean, median and range of urinary mucosal immunoglobulin concentration were ١.٤٤٠٣٨, ٠.١٧٤٥, ٠.١٧٤٥ – ٣.٢٤٤ g/l respectively, while the urinary mucosal specific antibody titer mean, median and range were ٣٢, ١٦, ١٦ – ٦٤ accordingly. Statistics for linear regression analysis showed that r is not significant r = -٠.٢٩٠٢٢. Thus menopausal mucosal antibody titer mean was lower (٧.٢) than those for adolescent ٣٢. The paired observation analysis using t statistics showed that

calculated t value was higher than the table t value which on df ٦ was ١.٥٤٩٠٣ at P= ٠.١. Such finding means that it was statistically non significant.

٤-١٠: *Enterobacter asburiae* specific urinary mucosal humoral immune responses in menopausal and adolescent patients,

Ten cases of *E. asburiae* urinary tract infection were noted among menopausal women patients (table-٨). The age ranges of those patients were ٤٦-٥٩ years. the means median and range of their urinary mucosal immunoglobulin concentration were ٣.٩٥١٠٣,٢.٤٢١٨,٠.٦٢١١- ١١.٩٦٨٥٧ g/L respectively while the *E.asburiae* mucosal specific antibody titer mean, median and rang were ٦.٨,٦, ٤-١٦ accordingly. Statistics of linear regression analysis showed that r is significant r = -١ while five cases of adolescent with *E. asburiae.* The age range of those patients were ١٨-٢٩ years the mean, median, and range of urinary mucosal immunoglobulin concentration were ١.٥٨٩٥٩, ٠.٩٠٢٦٦, ٠.٣٣٩٦-٥.٣١٧٨ g/L respectively. While the urinary mucosal specific antibody titer mean, median and range were ٢٨.٨, ١٦, ١٦-٦٤ accordingly.

Statistics for linear regression analysis showed that r = -٠.٥٢ that mean it is significant thus Menopausal mucosal antibody titer mean was lower (٦.٨) than those for adolescent (٢٨.٨). The paired observation analysis using t statistics showed that calculated t value was higher than the table t value which on df ١٣ was ٢.٣٥٢١٨ at P = ٠.٠٢٥ such finding means that it was statically significant.

٤-١١: *Staphylococcus aureus* specific urinary mucosal humoral immune responses in menopausal and adoleseent patients.

Ten cases of *S. aureus* urinary tract infection were noted among menopausal women patients. Only two cases were positive by indirect preinrichment culture method (Table -٩-).

The age ranges of those patients were ٤٦-٥٩ years. The mean, median and range of their urinary mucosal immunoglobulin concentration were٢.٣٦٦٠,١.٣٨٦١, ٠.٣٣٩٦ – ١٠.٦٧٥٦ g/L respectively while the *S. aureus* mucosal specific antibody titer mean, median and range were ٧.٦,٨, ٤-١٦ accordingly. Statistics for linear regression analysis showed that r is rather significant, r = ٠.٥٠٢٤ while four cases of young adult women patients with *S. aureus* the age range of those patients were ١٨-٣٠. The mean, median and

range of urinary mucosal immunoglobulin concentration were ٠.٩٢٤٩٨, ٠.٩٠٢٦٦, ٠.٣٣٩٦-١.٥٥٥ g/L respectively while the urinary mucosal specific antibody titer mean, median and range were ٣٠, ٢٤, ٨-٦٤ accordingly statistics for linear regression analysis showed that r = -٠.٥٠٣٩ that mean it is rather significant. Thus menopausal mucosal antibody titer mean was lower (٧.٦) than those for adolescent (٣٠). The paired observation for ration analysis using t statistics showed that calculated t was higher than the table t value which on df ١٢ was ١.٨٥٨٤٠ at p = ٠.٠٥. Such finding means that it was statistically significant.

٤-١٢: *Staphylococcus epidermidis* specific urinary mucosal humoral immune responses among menopausal and adolescent patients.

Eight cases of *S. epidermidis* urinary tract infection were noted among menopausal women patients (Table -١٠-)

The age ranges of those patients were ٤٦-٥٩ years. The mean, median and range of their urinary immunoglobulin concentration were ١.٥١٠٤٤, ١.١٨٤١٦, ٠.٥٠٩٥ – ٤.٩٣٣ g/L respectively while the *S. epidermidis* mucosal specific antibody titer mean, median and rang were ٥.٥, ٤, ٤-٨ accordingly statistics for linear regression analysis showed that r is not significant r = ٠.٣١٥١٨ while six cases of adolescent female patients with *S. epidermidis* the age range of those patients were ١٨-٢٨, the mean ,median and range of their urinary mucosal immunoglobulin concentrations were ١.٣٣٤٦, ٠.٩٧٥٤٨, ٠.٣٣٩٦-٢.٠٧٥٨ g/L respectively while the *S. epidermdis* urinary mucosal specific antibody titer mean, median and range were ١٦, ١٦, ٨-٣٢ accordingly. Statistics for linear regression analysis showed that r is significant r = ٠.٦٦٠٣ thus, menopausal mucosal antibody titer mean lower (٥.٥) than those for adolescent (١٦). The paired observation analysis using t statistics showed that calculated t value was

higher than table t value which on df ١٢ was ٣.٢٧٤٨٥ at p= ٠.٠٠٥. Such finding means that it was statistically significant.

٤-١٣*: Staphylococcus saprophyticus* specific urinary mucosal humoral immune responses in menopausal and adolescent patients,

Three cases of *S. saprophyticus* urinary tract infection were noted among menopausal patients (Table -١١-)

The age ranges of those patients were ٥٣-٥٦ years. The mean, median and range of their urinary mucosal immunoglobulin concentration were ١.٥٥٩٤٢, ١.٢١٧٢, and ٠.٩٥٢٦٦ – ٢.٥٥٨٤ g/L respectively. While the *S. saprophyticus* mucosal specific antibody titer mean, median and range were ٥.٣٣٣٣٣, ٤, ٤-٨ accordingly. Statistics for the linear regression analysis showed that the correlation coefficient r is non significant r = -٠.١٩٧٤ while ٣ cases of adolescent patients with *S. saprophyticus* the age range of those patients were ٢٠-٢٨ years. The means, median and range of urinary mucosal immunoglobulin concentration were ٥.٤٢٨٦٦, ٠.٨٧٩٤, ٠.٣٣٩٦ – ١٥.٠٦٩ g/L respectively while the urinary mucosal specific antibody titer mean, median and range were ٣٧.٣٣٣٣, ٣٢, ١٦-٦٤ accordingly. Statistics for the linear regression analysis showed that correlation coefficient r is non significant r = ٠.٤٧٩٩. Thus, menopausal mucosal antibody titer mean lower (٥.٣٣٣٣٣) than those for adolescent (٣٧.٣٣٣٣). The paired observation analysis using t statistics showed that calculated t value was higher than the table t value which on df ٤ was ٢.٢٥٨٢٩ at p= ٠.٠٥ such finding means that it was statistically significant.

٤-١٤: *Streptococcus pyogenes* specific urinary mucosal humoral immune responses among menopausal patients.

Three cases of *S. pyogenes* urinary tract infection were noted among menopausal women patients. (Table -١٢-)The age ranges of those patients were ٥٥-٥٩ years. The mean, median and range of their urinary mucosal immunoglobulin concentration were ٣.٨٨٧٥, ٠.٥٠٩٥, ٠-١١.١٥٣ g/L respectively. While the ***S. pyogenes*** mucosal specific antibody titer mean, median and range were ٤,٤, ٤-٨ accordingly. Statistics for the linear regression analysis showed that r is rather significant .r=٠.٥٤٠٨.

Table -١٢- *Streptoccus pyogenes* specific urinary mucosal humoral immune response among menopausal UTI.

Mucosal immune respouse/humoral menopausal				
Seq.	**Age**	**Conc. (g/l)**	**Titer Without ٢ME**	**Titer With ٢ME**
١	٥٩	٠.٥٠٩٥	٤	٤
٣	٥٥	٠	٠	
٤	٥٨	١١.١٥٣	٨	٤
Mean		٣.٨٨٧٥	٤	
Median		٠.٥٠٩٥	٤	
Range		٠-١١.١٥٣	٤-٨	
r		٠.٥٤٠٨		
t				
Equation ŷ = ٢.٩٨٩٢٧+٠.٥X				

٤-١٥: *Neisseria gonorrheae* specific urinary mucosal humoral immune responses among menopausal patients

Tow cases of *N. gonorrheae* urinary tract infection were noted among menopausal patients. (Table ١٣) .The age ranges of those patients were ٥٢-٥٣ years. The mean, and range of urinary mucosal immunoglobulin concentration were ٠.٩٤٧٣, and ٠.٣٣٩٦ – ١.٥٥٥ g/L respectively. While the *N. gonorrheae* urinary mucosal specific antibody titer mean, median and range were ٨, ٨, ٨ accordingly. Statistics for the linear regression analysis showed that the concentration coefficient r is non significant r =٠.١١٣٣.

Table -١٣-:

N gonorrheae specific urinary mucosal humoral immune responses among menopausal UTI.

Mucosal immune response/humoral				
Menopausal				
Seq.	**Age**	**Conc. (g/l)**	**Titer Without ٢ME**	**Titer With ٢ME**
١	٥٣	١.٥٥٥	٨	٤
٢	٥٢	٠.٣٣٩٦	٨	٨
Mean		٠.٩٤٧٣	٨	
Range		٠.٣٣٩٦-١.٥٥٥	٨	
r		٠.١١٣٣		
t				
Equation ŷ = ٠.٩٢٥١+٠.٦X				

٤-١٦: *Proteus mirabilis* specific urinary mucosal humoral immune responses among adolescent patients

Two cases of *P. mirabilis* urinary tract infection were noted among adolescent patients. (Table-١٤-)

The age ranges of those patients were ١٨-٢٠ years. The mean, median and range of their urinary mucosal immunoglobulin concentration were ١.٧٩١٨, ١.٧٩١٨, ٠.٣٣٩٦ – ٣.٢٤٤ g/L respectively while the *P. mirabilis* urinary specific mucosal antibody titer mean, median and range were ٤٨, ٤٨, ٣٢ – ٦٤ accordingly. Statistics for the linear regression analysis showed that the correlation coefficient r is significant, r =٠.٩٩٤٠٢.

Table -١٤-

***Proteus mirabilis* specific urinary mucosal humoral immune response among adolescent UTI.**

Mucosal immune response / humoral				
Adolescent				
Seq.	**Age**	**Conc. (g/l)**	**Titer Without ٢ME**	**Titer With ٢ME**
١	١٨	٣.٢٤٤	٦٤	٣٢
٢	٢٠	٠.٣٣٩٦	٣٢	٣٢
Mean		١.٧٩١٨	٤٨	
Range		٠.٣٣٩٦-٣.٢٤٤	٣٢-٦٤	
r		٠.٩٩٤٠٢		

Equation $\hat{y}$ = ١.٧٣١٨+١X

٤-١٧: Nil urinary mucosal humoral immune responses

. Three cases of menopausal women patients with *Streptococcus pyogens*, *Klebsiella pneumoniae* and *E. coli* UTI, their age range were ٥٣-٥٩ years, urine positive culture but they had nil urinary mucosal humoral immune responses. No mucosal urinary immunoglobulin can be obtained.(table -١٥-).

Table ١٥

Nil urinary mucosal humoral immune responses.

Case no.	Age	Culture D	Culture ID
١	٥٥	*S. pyogens +K .pneumoniae*	NG
٢	٥٣	*E.coli*	NG
٣	٥٩	*E. coli*	NG

٤-١٨: Culture negative UTI

One case of sterile pyuria was noted among menopausal women patients. The age of this patient was ٥٧ years and the concentration of mucosal immunoglobulin was ٠.٣٣٩٦ g/L

٤-١٩: Urinary mucosal humoral immune responses in dimicrobic infection

Twenty one cases of bacterial infection, their age range was ٤٥-٥٩ years. The mean, median and range of urinary mucosal immunoglobulin concentration were ١.٧٣٧٥٠ g/L, ١.٤٦٥٦٦ g/L, ٠-١١.١٥٣ g/L respectively, while the mucosal specific antibody titer mean, median and range were ٥.٨٠٩٥٢, ٤,

٠-١٦ accordingly. The titers of the bacterial specific mucosal Ig were either equal or one tube difference. (Table ١٧).

Table ١٦:Urinary mucosal humoral immune responses in dimicrobic infection in menopausal patients.

Case Number	Age	Culture Direct	Culture Indirect	Conc. (g/l)	Titer Without ٢ME	Titer Without ٢ME
١	٥٨	*E colil* *Entero. asburiae*	NG	١.٤٦٥٦٦	٤ ٨	٤ ٨
٢	٥٩	*E. coli* *K. oxytoca*	NG	١.٠١٩	٨ ٨	٨ ٨
٣	٤٦	*S. epidermidis* *K. terrigena*	NG	١.٥٥٥	٤ ٨	٤ ٤
٤	٥٩	*S. epidermidis* *S. pyogens*	NG	٠.٥٠٩٥	٤ ٤	٢ ٤
٥	٥٦	*E. coli* *S. aureus*	NG	١.٥٥٥	٨ ٤	٤ ٢
٦	٥٩	*E. coli* *Entero. asburia*	NG	٢.١٤٠٣٣	٨ ٤	٨ ٤
٧	٥٣	*S. epidermidis* *K. pneumonia*	NG	٠.٦١١٢	٤ ٤	٤ ٠
٨	٥٢	*K. pneumonia* *E. asburiae*	NG	١.٥٩٣٢	٨ ٤	٨ ٢
٩	٥٨	*S. pyogens* *K. pneumonia*	NG	٠.٣٣٩٦	٨ ٤	٤ ٤
١٠	٥٢	*E. coli*	*E. asburiae*	١.٥٥٥	١٦ ٨	١٦ ٤
١١	٥٢	*E. coli*	*S. asburiae*	٢.٣٩٩٥	١٦ ٨	١٦ ٨
١٢	٥٧	*E. asburiae* *E. coli*	NG	٣.٢٤٤	٤ ٨	٢ ٨
١٣	٥٧	*K. pneumonia*	*E. coil*	٠.٣٣٩٦	٤ ٤	٤ ٤
١٤	٤٥	*Staph. aureus* *E. asburiae*	NG	٠.٦٢١١	٤ ٨	٤ ٨
١٥	٤٩	*S. epidermidis* *K. pneumonia*	NG	٠.٣٣٩٦	٨ ٨	٨ ٨
١٦	٥٣	*S.epidermidis* *K. pneumonia*	NG	٠.٦١١٢	٤ ٤	٤ ٠
١٧	٥٩	*E. asburiae*	*E. coli*	٣.٥٢٣٥	٤ ٤	٤ ٢
١٨	٥٧	*k. oxytoca* *S.epidermidis*	NG	١.٨٩٢٨	٨ ٤	٨ ٢
١٩	٥٩	*E. coil* *K. pneumonia*	NG			
٢٠	٥٨	*K oxytoca* *S. pyogens*	NG	١١.١٥٣	١٦ ٨	١٦ ٤
٢١	٥٥	*K. pneumonia* *S. pyogens*	NG			
Mean			١.٧٣٧٥٠		٥.٨٠٩٥٢	
Median			١.٤٦٥٦٦		٤	
range			٠-١١.١٥٣		٠-١٦	

٤-٢٠: Comparative of urinary mucosal humoral immune responses for menopausal and adolescent during UTI.

There were differences between specific antibody titers for uropathogen among menopausal and adolescent female patients for the same pathogens. Paired observation t – statistics showed significant t value in case of *E. coli* ٣.٦٠١٢٣ at p =٠.٠٠٥, *S. epidermidis* ٣.٢٧٤٨٥ at p =٠.٠٠٥ also *K. pneumoniae* ٢.٢٦١٥٤ at p =٠.٠٢٥, *K. oxytoea* ٢.١٠١٠٦ at p =٠.٠٥ mean time paired observation t statistics showed significant t value in case of *E. asburiae* ٢.٣٥٢١٨ at p =٠.٠٢٥, *S. aureus* ١.٨٥٨٤٠ at p =٠.٠٥ and *S. saprophticus* ٢.٢٥٨٢٩ at ٠.٠٥. (Table -١٧-).

Table -١٧-

Comparative urinary mucosal humoral immune responses for menopausal and adolescent during UTI.

Eq.	Causal Organisms	Menopausal Titer	Adolescent Titer	T – static		significant
				Calculate	Table	
١	*E. coil*	٦.٩٥٦٥٢	١٨.٨	٣.٦٠١٢٣	٢.٧٥٠	٠.٠٠٥
٢	*K paneumonia*	٧.٤٥٤٥٤	٢٤	٢.٢٦١٥٤	٢.٠٥٢	٠.٠٢٥
٣	*K. oxytoca*	٧.٦	٣٧.٣٣٣٣	٢.١٠١٠٦	١.٧٩٦	٠.٠٥
٤	*K. terrigena*	٧.٢	٣٢	١.٥٤٩٠٣	١.٤٤٠	٠.١
٥	*E. asburiae*	٦.٨	٢٨.٨	٢.٣٥٢١٨	٢.١٢٠	٠.٠٢٥
٦	*S. aureus*	٧.٦	٣٠	١.٨٥٨٤٠	١.٧٧١	٠.٠٥
٧	*S. epidermidis*	٥.٥	١٦	٣.٢٧٤٨٥	٣.٠١٢	٠.٠٠٥
٨	*S. saprophyticus*	٥.٣٣٣٣	٣٧.٣٣٣٣	٢.٢٥٨٢٩	٢.١٣٢	٠.٠٥

Chapter Five

Discussion

٥-١: Age range of menopausal UTI:

Among menopausal UTI patients were found with dominance in the range of ٥١-٥٥ (٤١.٥٥%) followed by ٥٦-٦٠ (٣٠.٩٦%), then the age range of ٤٦-٥٠ (١٩.٤٨%) (Table-١). Other workers such as that of (Manoni *et al*., ٢٠٠٤) in a study in Thailand have found that the lower urinary tract infection (LUTI) with nocturia, urgency and stress in continence occur, particularly in post menopausal women (٥٠-٩٤) years. However, in menopause there is increase in recurrent urinary tract infection due to decrease amounts of estrogen that lead to decrease the vascularity and atrophy of urethral epithelium and vaginal mucosa (Sotelo & Westney, ٢٠٠٤). Bacteriuria is more prevalent among the elderly, with ٥٠٪ of post menopausal women having bacteriuria (Foxman, ٢٠٠٢). Thus, generally speaking the study results are of parallel results to that of (Manoni *et al*., ٢٠٠٤) and (Foxman, ٢٠٠٢) also with (Schatzl *et al*., ٢٠٠١; Vinker *et al*. ٢٠٠١; Muscatello *et al*., ٢٠٠١).

٥-٢: Underlying disease to UTI in menopause

Fifty-two women of the study patients were without underlying disease conditions, others, however, were with diabetes mellitus, urolithiasis as, common underlying diseases to UTI in menopause

(Table-٢). The rates of a symptomatic bacteriuria and UTI in diabetic women are two fold to three fold higher than those in non diabetic women; these differences were not observed in men (Stapleton, ٢٠٠٢). In hospitalized diabetic patients, particularly those with multiple organ complications, the incidence of infection and true pyelonephritis also appears to be increased. In addition, impaired cytokine secretion may contribute to a symptomatic bacteriuria in diabetic women (Geerling *et al*., ٢٠٠٠). Diabetes results in several abnormalities of the host defense system that might result in a higher risk of certain infections. These abnormalities include immunologic impairment, such as impaired migration of nutrophils, intracellular killing, phagocytosis and chemotaxis in polymorphnuclear leukocytes from diabetic patients (Valerius *et al*., ١٩٨٢) and local complication related to neuropathy, such as impaired bladder emptying (Hosking *et al*., ١٩٧٨). Also, high glucose concentration in urine may be serve as culture medium for pathogenic microorganisms .The incident of renal stones in postmenopause women was ٤٥٪ higher compared with premenopausal women (Mattix kramer *et al*., ٢٠٠٣). Menopause has been associated with an increase in urinary calcium excretion due to estrogen deficiency.

Estrogen deficiency increases the sensitivity of bone to parathyroid hormone, leading to a net increased urinary calcium excretion and possibly kidney stone formation (Riggs & Melton, ١٩٨٦).

٥-٣: Rate of uropathogens among menopausal and adolescent female patient

Largest percent was noted for *E. coli* (represented ٢٩.٨٧%) among menopausal women, and also it was the largest in adolescent (٢٨.٥٧%). The result in menopausal and adolescent patients were agreed with Nicolle & Ronald, ١٩٨٧; Hooton *et al*., ١٩٩٦; Hillier & Lau, ١٩٩٧; Raz *et al*., ٢٠٠٠; Nicolle, ٢٠٠٠; Malani, ٢٠٠٥.

K. pneumoniae & *K. oxytoca* and other gram negative bacilli were found in menopausal women higher than in adolescent women and that may be due to the lack of estrogen that lead to atrophic change in vagina, causing decrease in lactobacilli and increase in colonization with coliform bacteria (Hooton, ٢٠٠١; Pabich *et al*., ٢٠٠٣). Such results are found parallel to that of (Nicolle, ٢٠٠٠) and (Nicolle, ١٩٩٣) which showed that gram negative bacilli, including *Klebsiella pneumoniae* are more likely to cause infection in older women than in younger women. While the results of *Proteus mirabilis* which are found in adolescent patient with UTI and not found in menopausal women with UTI disagree with (Nicolle, ٢٠٠٠). Besides*, Pseudomonas aeruginosa* was not observed in any case (Malani, ٢٠٠٥).

٥-٤: *E. coli* specific urinary mucosal humoral immune response in menopausal and adolescent patients.

Twenty- three cases of *E. coli* urinary tract infection represented ٢٩.٨٧% in menopausal women while ten cases of *E. coli* represented ٢٨.٥٧% in adolescent. In menopausal patients positive relationship was found between Ig concentration and titer with r=٠.٦٦٥٥, which indicates that *E. coli* antigens induced secretory immune responses by production of SIg (Shnawa & Mahdi, ٢٠٠٤).

The uropathogenic strain of *E. coli* was belived to display a variety of virulence properties that help them colonize host mucosal surface and circumment host defense to allow invasion of the normal sterile urinary tract (Lipsky, ١٩٨٩; Sussman & Gally, ١٩٩٩;Mobley,٢٠٠٠;). A number of virulence determined have been related to the acquisition or development of UTI. Among these factor adhesions (type ١, p & s fimbriae, and a fimbrial adhesion), which bind to specific molecules in the uroepithelial, such as glycosphinglipids (Johnson, ٢٠٠٣) and another factors siderophores, toxin, capsules (Lipsky, ١٩٨٩; Yamamoto *et al*., ١٩٩٥;Andren *et al*., ١٩٩٧; Mitsumori *et al*., ١٩٩٩; Sussman & Gally, ١٩٩٩;Guyer *et al*., ٢٠٠٠; Kurazono *et al*., ٢٠٠٠; Mobley, ٢٠٠٠). Some of these virulence factor, such as necrotizing factor ١, α-hemolysin, or the satprotein a recently described autotransported protein which acts as a saproteolytic toxin, have been found to be located in pathogenicity islands (PAI) (Blum *et al*., ١٩٩٥; Hacker & Kaper, ٢٠٠٠;Guyer *et al*., ٢٠٠١).

Host specific factor associated with UTI that is caused by *E. coli* includes the production of secretory immunoglobulin A interfering with adhesion molecules, the presence of Tamm-Horsfall mucoprotein (THP) causing bacterial aggregation and wash out, bactericidal properties of the serum as well as hyrodynamic factor, ie bacterial washout (Pawelzik *et al*., ١٩٨٨; Virkola *et al*., ١٩٨٨). THP has specific receptor for several uropathogen and the bound bacteria are washed out in the urine.

Antibody responses to pilli of uropathogenic *E. coli* (have ٣٦٦٠-٣٠٤٨ pilli) were found in the serum and urine, each anti-pilli antibody totally blocked of homologous strain (Svanborg –Eden *et al*., ١٩٨٢;Kantele *et al*., ٢٠٠٣). Lower bacteria specific mucosal antibody titer was noted among menopausal patient than those reported for adolescent; this may due to decrease of B-lymphocyte

producing antibody or due to decrease synthesis and or increase in regulatory suppressor activity of T-cell finally it could be a net result of the ageing effect (Burns & Leventhal, ٢٠٠٠).

٥-٥: Urinary mucosal humoral immune response specific to *Klebsiella pneumoniae* in menopausal & adolescent patients.

Twenty-two cases of *K*. *pneumoniae* urinary tract infection representing ٢٨.٥٧% while seven cases in adolescent which represented ٢٠٪, UMIg titer were low when compared with the other infection; this due to virulence factor that present in *K*. *pneumoniae* such as capsular antigen which are essential to virulence of *K*. *pneumoniae* (Domenico *et al*., ١٩٨٢; Highsmith & Jarvis, ١٩٨٥;Clegg & Gerlach, ١٩٨٧). The capsular material form thick bundle of fibrillous structure the bacterial surface in massive layer (Amako *et al*., ١٩٨٨). This protect the bacterium from phagocytosis by polymorphnuclear granulocytes on the one hand (Podschun *et al*., ١٩٩٢; Podschun & Ulmann, ١٩٩٢) and prevent killing the bacteria by bactericidal serum factor on the other hand (William *et al*., ١٩٨٣). This result consistent with Anonymus (٢٠٠٤) and Williams & Tomas (١٩٩٠), which found that the capsules of the *K*. *pneumoniae* have high component of sialic acid which inhibiting the activation or uptake of complement component, especially C_3b and it is poorly immunogenic pilli of this bacterium associated primarily with pathogenesis of lower UTI (Iwahi *et al*., ١٩٨٣) type ١ pilli may also be involved in pathogenesis of pyelonephitis (Matsumoto *et al*., ١٩٩٠) also siderophores, serum resistance and lipopolysaccharide represent the virulence factor of *K*. *pneumoniae*.

The host response against capsules during acute pyelonephritis involves the production of serum antibodies to O antigen and occasionally to K antigen and type

١-fimbriae. These antibodies protect against haematogenous or ascending infection of UTI. While local production of immunoglobulin such as SIgA occur at an increased level in response to infection such as acute pylonephritis.

The result of this study showed that a simple positive relationship between UMIg concentration & UMIg titer this result was in accordance with Anonymus (٢٠٠٣), which proved that UMIg activity (agglutination or neutralization) was decreased in immunocompromised patients such as advanced age, diabetes and pregnancy.

٥-٦: Urinary mucosal response to *K. oxytoca* in menopausal and adolescent patients.

Ten cases of *K. oxytoca* which were represently ١٢.٩٨% of urinary tract infection among menopausal female patient while three cases of *K. oxytoca* were accounting of٨ ٥٧٪ of urinary tract infection were noted among adolescent. The result showed that strong positive linear relationship between MUIg concentration and MUIg titer r=٠.٨٤٩٠ in the menopausal women, such finding was in agreement with Shnawa & Al-Amedi,٢٠٠٥. Meantime, the result showed that there was a strong positive linear relationship between MUIg concentration and MUIg titer r=٠.٧٨٧٧ among adolescent women. This result was in agreement with Shnawa & Mehdi,٢٠٠٤; Shnawa & Al-Amedi,٢٠٠٥.

٥-٧:Urinary mucosal response to *K. Terrigena* among menopausal and adolescent patients.

Five cases of *K. terrigena* were represented by ٦.٤٩% of urinary tract infection noted among menopausal female patient while three cases represented ٨.٥٧٪ of urinary tract infection among adolescent patients.

Negative relationship between MUIg concentration and MUIg titer (r=-٠.٢٣٢٦), in the menopausal patients was noted. These result was agreed with (Dunn-Walters *et al*., ٢٠٠٣) who found that antibody response in the older people is composed of antibodies with a lower affinity for immunizing antigen compared with those produced in a younger person (the result was shown that a strong positive relationship between MUIg concentration and MUIg titer, r=١ in the adolescent patient) while the number of antibodies produced during immune response is greatly increased in the postmenopausal women (Candore *et al*., ١٩٩٧), so that (Dunn-Walter *et al*., ٢٠٠٣) found that the quality of antibodies produced in old age did not change, while the quality of antibody response differs. This may occur due to immune suppression and due to interference with immune function of B-cell, T-cell or macrophage (Todar, ٢٠٠٢). Immune suppression responses occur in bacterial infection. Since unique antigens (proteins) are the cause of this immuno suppression, the most likely explanation for this is due to:- ١-lack of co stimulatory signals (interference with cytokine secretion), ٢- Activation suppressor T-cells, ٣- Disturbances in TH_1/TH_2 activities. This leads to the production of MUIgA with low avidity, or directed against unimportant antigenic determined; they may have only week antibacterial action (Todar, ٢٠٠٢).

٥-٨:Urinary mucosal humoral immune response to *Enterobacter asburiae* among menopausal & adolescent patients.

Ten cases of *Enterobacter asburiae* urinary tract infection which comprises ٢٨.٥٧% of all menopausal cases in comparison to five cases represented ١٤.٢٨٪ in adolescent patients. This indicates that the infection with this bacteria is common in menopausal than in adolescent patient. This result agreed with other workers (Anonymous, ٢٠٠٣; Simon *et al*.,٢٠٠١). The relationship between UMIG concentration and UMIg titer among menopause was negative with r=-١, such finding was in accordance with (Dunn-Walters *et al*., ٢٠٠٣), while in the adolescent patients the relation among UMIg concentration and UMIg titer was a positive with r=٠.٥٢ and it was in agreement with that of Shnawa & Mehdi,٢٠٠٤; Shnawa & Al-Amidi,٢٠٠٥.

٥-٩: *Staphylococcus aureus* specific urinary mucosal humoral immune responses among menopausal and adolescent patients.

Ten cases of *S. aureus* urinary tract infection presenting ١٢.٩٨% in the menopausal patient and ٤ cases (١١.٤٢٪) was recorded in adolescent patients.

The presence of *S. aureus* in the urine should never be assumed to be secondary to an ascending urinary tract infection. *S. aureus* possesses resistant factors that may promote local infection by thwarting host defenses, these include coagulase that prevent neutrophil access to infection site, microcapsule (inhibit phagocytosis), protein A (inhibit IgG-mediated

opsonization) (bind Fc fragment) and (exert its antibacterial activity), clumping factor (fibrinogen receptor) inhibit opsonization (fibrin coating), catalase (interferes with intracellular killing), protease, nuclease, lipase and cytolysis (α, β, δ) (liquefaction necrosis and phagocyte dysfunction), leucocidin and gamma toxin (neutrophil cytolysis), fatty acid-metabolizing enzyme (activates bactericidal lipids) (Goldman & Bennett, ٢٠٠٠).

Also *S. aureus* exist in nature as multiple antigenic type or serotypes meaning that they are variant strains of the same pathogenic species. If the immune response is the main defense against pathogen, they will be able to shed their old antigens and present new ones to the immune system. Antigenic variation is an important mechanism used by pathogenic microorganisms for escaping the neutralizing activities of antibodies (Todar, ٢٠٠٢).

The result of menopausal and adolescent was found in accordance with (Shnawa & Mehdi,٢٠٠٤; Shnawa & Al-Amidi,٢٠٠٥).

٥-١٠: *Staphylococcus epidermidis* specific mucosal humoral immune response among menopausal and adolescent during UTI.

Eight cases of *S. epidermidis* urinary tract infection (١٠.٣٨%) while six cases of *S. epidermidis* (١٧.١٤٪) were recorded in menopausal and adolescent patients respectively.

Our result of menopausal patients were in accordance with (Shnawa *et al*, ١٩٩٩). In both menopausal and adolescent patients a positive linear correlation appeared between UMIg concentration and UMIg titer in

r=٠.٣١٥١٨ and r=١ respectively. These results also in agreement with (Shnawa *et al*, ١٩٩٩), but the titer in adolescent was approximately ٤ time more than it was in the menopausal women patients. This due to : age related changes in the immune system.
, mechanism of decline in the immune function & mechanism of disease associated with declining immune function (Shnawa *et al*., ١٩٩٩).

٥-١١: *Staphylococcus saprophyticus* specific mucosal humoral immune response among menopausal and adolescent during UTI.

Three cases of *S*. *saprophyticus* urinary tract infection which represented ٣.٨٩% were noted among menopausal patient while three cases of *S*. *saprophyticus* (٨.٥٧ %) were described in the adolescent patients. Our result of menopausal and adolescent patient were in accordance with (Malani, ٢٠٠٥). In the menopausal patient there was a negative linear correlation appeared between UMIg concentration and UMIg titer in r=-٠.١٩٧٤. This result agreement with (Dunn-Walters *et al*., ٢٠٠٣) who found that changes occur in the humoral immune response with age while in the adolescent the result showed that positive linear relationship UMIg concentration and UMIg titer (r=٠.٤٧٩٩), this result in agreement with (Shnawa & Mehdi,٢٠٠٤).

٥-١٢:*Streptococcus pyogenes* specific urinary mucosal humoral immune response among menopausal patients.

Three cases of *S. pyogens* urinary tract infection which represented (٢.٥٩%) were noted among menopausal patients, a positive linear correlation appeared between UMIg concentration and UMIg titer in r=٠.٥٤٠٨ were noted in the menopausal patient while *S. pyogens* were abscent in the adolescent patient. This result accordance with (Shnawa & Mehdi,٢٠٠٤).

٥-١٣:*Neisseria gonorrhoeae* specific urinary mucosal humoral immune response among menopausal women with UTI.

Two cases of *Neisseria gonorrhoeae* urinary tract infection which represented (٢.٥٩%) were noted among menopausal patients, while abscent in the adolescent patient. There is a positive linear relationship between UMIg concentration and UMIg titer in r=٠.١١٣٣.These results mean that *N. gonorrhoeae* not induce mucosal immune system to producing and secretion SIg.

٥-١٤:*Proteus mirabilis* specific urinary mucosal humoral immune response among adolescent UTI.

Two cases of *Proteus mirabilis* was observed are represented (٥.٧١٪) among adolescent patient. A strong positive linear relationship between UMIg concentration and UMIg titer in r=٠.٩٩٤٠٢ and these bacteria abscent in the menopausal women patients.

Proteus mirabilis is a common cause of UTI in catheterized patient and those with urinary tract abnormalities. It often infect the upper urinary tract

and it can lead to acute pyelonephritis bladder or renal stones, fewer or bacteramiae (Zunino *et al*., ٢٠٠٠). Several potential virulence factor have been suggested for *P. mirabilis* these include adhesion to uroepithelium mediated by fimbriae (Zunino *et al*., ٢٠٠٠), pore-forming hemolysins (Fraserm *et al*., ٢٠٠٢), proticine, leukocidin, endotoxin, cleavage of IgG and IgA by proteolytic enzyme (Walker *et al*., ١٩٩٩), urease production, swarming motility depend on flagella and invasion of eukaryotic cell (Zunino *et al*., ٢٠٠٠), deaminase (Svanborg-Eden & DeMan, ١٩٨٧), Polysaccharide capsules (Giuglianol *et al*., ١٩٩٥), the ability to form biofilm (Rather *et al*., ١٩٩٩; Sturgil *et al*., ٢٠٠٢). These factor enable the pathogens to overcome the various defense mechanism of the host. The result was low titer, this because *Proteus mirabilis* caused coating of bacterial surface with IgA and this possess effect on immune responses (Riedasch *et al*., ١٩٨٤).

٥-١٥:Nil urinary mucosal humoral immune responses in menopausal women.

It may be explained on the bases of one or more of the following:

١. deceasing of mucosal immunoglobulin synthesis so at the applied methodology rather unsuccessful for detection of separable detectable concentration (Koga *et al*., ٢٠٠٠).
٢. urinary mucosal immunoglobulin may be synthesized, release and catabolized by immunoglobulin splitting enzymes (Walker *et al*., ١٩٩٩).
٣. mucosal lymphocyte traffic to other effector sites of mucosal immune system (Arreaza *et al*., ١٩٩٣; Borghesi & Nicoletti, ١٩٩٤).

٤. regulatory immune response via T-suppressive subsets, that suppress B-lymphocytes from synthesis and secretion of antibodies (Miller, ١٩٩١).

٥. immunophysiological regulatory compensatory effect to depletion elsewhere in the body (Goodwin, ١٩٩٥).

٥-١٦: Culture negative UTI.

One case of sterile pyuria was noted among menopausal female patient and represented ١.٢٠٤٨١٪, these cases may be due to:- infection by *Mycoplasma hominis*, this case was dominant in older women patients and causes acute pyelonephritis with peritoneal signs, urethral syndrome due to infection by *Ureaplasma urealyticum*, *Trichomonas*, *Chlamydia* or viruses (Collee *et al*., ١٩٩٦; Simon *et al.,* ٢٠٠١), antibody coated bacteria (Al-Nasri, ٢٠٠٣), urinary tract tumor, cell wall defective bacteria, trauma, including recent instrumentation and virual infection or mycobacterial infection (Nolte & Metchock, ١٩٩٥).

٥-١٧:Urinary mucosal humoral immune responses in dimicrobic infection.

Twenty one cases were found to have been with two microorganisms. Some of these cases MUIg titer was equal in both microbes; this is consistent with (Shnawa & Mehdi, ٢٠٠٤), probably because of non antigenic competition

between both microbes and have equal immunodominant epitopes. While in many cases one of two microbes as it was shown that cause mucosal immune response higher than the other; these depend on antigenic competition or intermolecular antigenic competition.

Some bacteria such as *S. aureus* produce cell-bound Coagulase and clumping factor that cause fibrin to clot and to deposit on the cells surface. It is possible that this disguises the bacteria immunologically so that they are not readily identified as antigens targets for an immune response (Todar, ٢٠٠٢).

Other pathogens are persistent in the luminal surface of the urinary tract or the renal tubule. If there is no host cell destruction the pathogens may avoid inducing an inflammatory response, and there is no way in which sensitized lymphocytes or circulating antibodies can reach the site to eliminate the infection (Todar, ٢٠٠٢).

٥-١٨:Comparative urinary mucosal humoral immune responses for menopausal and adolescent.

T test for paired observation showed that the differences between the mucosal specific antibody titer in adolescent and menopausal during urinary tract infections were significant in case of *K. pneu*moniae, *K. oxytoca*, *K. terrigena*, *S. aureus*, *E. asburiae* and *S. saprophyticus* (Table-١٨). This means that such mucosal humoral immunosuppressive effect may be due to menopausal and aging effect (Hara *et al*., ١٩٨٧). Menopause and/or aging may induce lower mucosal antibody synthesis by mucosal B-cell system (Okumura *et al*.,١٩٩٣), or may antagonize the T-cell helper effect for B-cell system (Whisler *et al*., ١٩٩١). Finally there may be a possibility for the

presence of antagonizing peptide antigen that causes T-helper antagonists (Whisler *et al*., ١٩٩١).

Table biochemical tests to gram positive bacteria

Streptococcus pyogens	***S. saprophyticus***	***S. epidermidis***	***S. aureus***	**bacteria / Test**
-	-	-	-	Oxidase production
-	+	+	+	Catalase production
		+	+	Nitrate test
		+	+	Glucose fermentation
		+	+	Lactose fermentation
S	R	S	S	Novabiocin resistant
		-	+	Coagulase production
-	-	-	+	Manitol fermentation
β		α	*β*	heamolysis
+	-	-	-	Tool of serological diagnosis for group A Streptococci

+: positive result,-: negative result

Table biochemical tests to gram negative bacteria

N.gonorrheae		*E.asburiae*		*P.mirabilis*		*K.terrigena*		*K.oxytoca*		*K.pneumoniae*		*E.coli*		Bacteria / test
C.B.	Api ٢٠	C.B.	Api ٢٠	C.B.	Api ٢٠	C.B.	Api ٢٠	C.B.	Api ٢٠	C.B.	Api ٢٠	C.B.	Api ٢٠	
	/		/		/		/		/		/		/	heamolysis
		+	+	-	-	+	+	+	+	+	+	+	+	ONPG
		-	-	-	-	-	-	-	-	-	-	-	-	ADH
		-	-	-	-	+	+	+	+	+	+	+	+	LDC
		+	+	+	+	-	-	-	-	-	-	+	+	ODC
		+	+	+	+	+	-	+	+	+	+	-	-	CIT
		-	-	+	+	-	-	-	-	-	-	-	-	H٢S
		-	-	+	+	-	-	+	+	-	+	-	-	URE
		-	-	+	+	-	-	-	-	-	-	-	-	TDA
		-	-	-	-	-	-	+	+	-	-	+	+	IND
		-	-	+	-	+	+	-	+	+	+	-	-	VP
		-	-	-	-	-	-	-	-	-	-	-	-	GEL
		+	+	+	+	+	+	+	+	+	+	+	+	GLU
		+	+	-	-	+	+	+	+	+	+	+	+	MAN
		-	-	-	-	+	+	+	+	+	+	-	-	INO
		+	+	-	-	+	+	+	+	+	+	+	+	SOR

		-	-	-	-	+	+	+	+	+	+	+	+	RHA
		+	+	-	+	+	+	+	+	+	+	-	-	SAC
		-	-	-	-	+	+	+	+	+	+	+	+	MEL
		+	+	-	-	+	+	+	+	+	+	-	-	AMY
		-	+	-	-	+	+	+	+	+	+	+	+	ARA
		-	-	-	-	-	-	-	-	-	-	-	-	OX
-	/	-	/	-	/	-	/	-	/	-	/	+	/	Metallic sheen on EMP
+	/	-	/	-	/	-	/	-	/	-	/	-	/	Oxidase production

Mucosal immune response/humoral	Mucosal immune response/humoral
Menopausal	**Adolescent**

Seq.	Age	Conc. (g/l)	Titer Without ٢ME	Titer With ٢ME	Age	Conc. (g/l)
١	٥٨	١.٤٦٥٦٦	٤	٤	١٨	٠.٩٠٢٦٦
٢	٥٩	١.٠١٩	٨	٨	٢٧	١.٥٥٥
٣	٤٦	١.٤٦٥٦٦	٤	٢	١٨	٠.٣٣٩٦
٤	٥٠	١.٥٩٣٢	٢	٠	٢٢	٠.٨٩٧٤
٥	٥٦	١.٥٥٥	٨	٤	٢٥	٠.٩٠٢٦٦
٦	٥٩	٢.١٤٠٣٣	٨	٨	٢٥	٠.٩٠٢٦٦
٧	٥٢	٢.٣٩٩٥	١٦	١٦	٢٨	٣.٢٤٤
٨	٥٥	٢.٥٥٨٤	١٦	١٦	٢٤	٠.٨٩٧٤
٩	٥٧	٣.٢٤٤	٨	٨	٢٠	١.٥٥٥
١٠	٥٧	٠.٣٣٩٦	٤	٤	٢٩	٠.٩٠٢٦٦
١١	٥٩	٠.٣٣٩٦	٨	٨		
١٢	٥٦	١١.١٥٣	٨	٤		
١٣	٥٩	٣.٥٢٣٥	٤	٤		
١٤	٥١	١.٤٦٥٦	٨	٨		
١٥	٥٩	١١.١٥٣	٨	٤		
١٦	٥٩					
١٧	٥١	٠.٣٣٩٦	٨	٤		
١٨	٥٥	١٨.٤٤٥	١٦	٤		
١٩	٤٩	٢.١٤٠٣٣	٨	١٦		
٢٠	٥٠	٠.٣٣٩٦	٨	٤		
٢١	٥٨	٢.١٤٠٣٣	٤	٨		
٢٢	٥٣			٢		
٢٣	٥٩	١.٥٥٥	٢	٠		

٦.٩٥٦٥٢		**Mean**	١.٢٠٨١٠٤	١٨.٨
٨		**Median**	٠.٩٠٢٦٦	١٦
٢-١٦		**range**	٠.٣٣٩٦-٣.٢٤٤	٤-٣٢
		r	٠.٣٧١٧٠	
		t		
٠٠٠٠٤X		**Equation ŷ = ٠.٠٥٦٩٩=٣٢.٨٩X**		

Table ٤*:E* coli specific mucosal humoral immune response among menopausal and adolescent during UTI

Table٥: *K pneumoniae* specific mucosal humoral immune response among menopausal and adolescent during UTI

Mucosal immune response/humoral	Mucosal immune response / humoral

Seq.	Age	Conc. (g/l)	Titer Without ٢ME	Titer With ٢ME	Age	Conc. (g/l)
١	٤٧	٢.٣١٧١٤	٨	٨	٢٠	٠.٩٠٢٦٦
٢	٥٢	١.٥٩٣٢	٨	٨	١٨	٠.٨٧٩٤
٣	٥٦	١.٥٩٣٢	٣٢	١٦	٢٣	٠.٣٣٩٦
٤	٥٧	٠.٣٣٩٦	٤	٤	٢٥	٠.٩٠٢٦٦
٥	٥٩	٣.٢٤٤	٤	٢	٢٢	٢.١٤٠٣
٦	٥٣	٠.٦٢١١	٤	٠	٢١	٤.٣٩٢٣
٧	٥٣	٠.٣٣٩٦	٨	٨	٢٩	٠.٩٠٢٦٦
٨	٥٥	٠.٣٣٩٦	٨	٤		
٩	٥٣	٢.٣١٧١٤	٨	٨		
١٠	٥٥	٠.٩٠٢٦٦	٤	٤		
١١	٤٧	٠.٣٣٩٦	٨	٤		
١٢	٥٥	١.٥٥٥	٨	٨		
١٣	٤٩	٠.٣٣٩٦	٨	٨		
١٤	٥٢	٠.٣٣٩٦	٨	٤		
١٥	٣١	١٦.٢٢	٤	٤		
١٦	٥٤	٠.٨٧٩٤	١٦	٨		
١٧	٥٧	٠.٩٠٢٦٦	٤	٢		
١٨	٥٩					
١٩	٥٧	١.٤٦٥٦٦	٨	٨		
٢٠	٥٣	١.٢١٧٢	٨	٤		
٢١	٥٨	٠.٣٣٩٦	٤	٤		
٢٢	٥٥					
	Menopausal				Adolescent	

٧.٤٥٤٥٤		Mean	١.٤٩٤٢٢	٢٤
٨		Median	٠.٩٠٢٦٦	١٦
٤-٣٢		range	٠.٣٣٩٦-٤.٣٩٢٣	٨-٦٤
		r	٠.٦٨	
		t		
.٨٣٣X		Equation ŷ = ١.٤٦٥١٢+٠.٦X		

Table ٦:*K oxytoca* specific mucosal humoral immune response among menopausal and adolescent during UTI

Mucosal immune response/humoral	Mucosal immune response / humoral

Seq.	Age	Conc. (g/l)	Titer Without ٢ME	Titer With ٢ME	Age	Conc. (g/l)
١	٥٩	١.٠١٩	٨	٨	٢٢	٠.٨٧٩٤
٢	٤٧	٠.٨٧٣٥	٤	٠	٣٠	١.٥٥٥
٣	٥٥	١.٤٦٥٦٦	٨	٨	٢٨	٠.٩٠٢٦٦
٤	٥٢	٠.٣٣٩٦	٤	٢		
٥	٥٧	١.٨٩٢٨	٨	٨		
٦	٥٧	٢.٥٥٨٤	٨	٨		
٧	٥٤	٠.٣٣٩٦	٨	٤		
٨	٥٧	٢.١٤٠٣٣	٤	٢		
٩	٥٣	١.٥٥٥	٨	٨		
١٠	٥٨	١١.١٥٣	١٦	١٦		
	Menopausal				Adolescent	

٧.٦		Mean	١.١١٢٣٥	٣٧.٣٣٣٣
٨		Median	٠.٩٠٢٦٦	٣٢
٤-١٦		range	٠.٨٧٩٤-١.٥٥٥	١٦-٦٤
		r	٠.٧٨٧٧	
		t		
١٧٧٢١X		Equation ŷ = ١.٠٦٠٥٥+١.٥٧١٤X		

Table ٧:*K terrigenae* specific mucosal humoral immune response among menopausal and adolescent during UTI

Mucosal immune response/humoral	Mucosal immune response / humoral

Seq.	Age	Conc. (g/l)	Titer Without ٢ME	Titer With ٢ME	Age	Conc. (g/l)
١	٥٨	٠.٩٠٢٦٦	٨	٨	٢٥	٠.٩٠٢٦٦
٢	٥١	٠.٩٠٢٦٦	٨	٠	٢٨	٠.١٧٤٥
٣	٤٦	١.٥٥٥	٨	٤	٢٨	٣.٢٤٤
٤	٤٧	١.٢١٧٢	٤	٤		
٥	٥٥	٠.٩٠٢٦٦	٨	٨		
	Menopausal				Adolescent	

٧.٢		Mean	١.٤٤٠٣٨	٣٢
٨		Median	٠.١٧٤٥	١٦
٨ –		Range	٠.١٧٤٥-٣.٢٤٤	١٦-٦٤
		r	١	
		t		
٥٠.٥X		Equation ŷ = ١.٣٩٨٧٨+١X		

Table ٨:*E asburia* specific mucosal humoral immune response among menopausal and adolescent during UTI

Mucosal immune response/humoral			Mucosal immune response / humoral			
menopausal						
Seq.	**Age**	**Conc. (g/l)**	**Titer Without ٢ME**	**Titer With ٢ME**	**Age**	**Conc. (g/l)**
١	٥٨	١٠٤٦٥٦٦	٨	٨	٢٥	١.٠٤٨٣
٢	٥٩	١.٥٥٥	٤	٤	١٨	٠.٣٣٩٦
٣	٥٩	٢.١٤٠٣٣	١٦	٨	٢٩	٥.٣١٧٨
٤	٥٧	٣.٢٤٤	٤	٢	٢٨	٠.٩٠٢٦٦
٥	٤٦	٠.٦٢١١	٨	٨	٢٥	٠.٣٣٩٦
٦	٥٤	١.٥٥٥	٨	٤		
٧	٥٩	٣.٥٢٣٥	٤	٢		
٨	٥٩	٢.٧٠٣٣	٨	٤		
٩	٤٦	١١.٩٦٨٥٧	٤	٢		
١٩	٥٦	١١.١٥٣	٤	٢		

Adolescent

٦.٨		**Mean**	١.٥٨٩٥٩	٢٨.٨
٦		**Median**	٠.٩٠٢٦٦	١٦
٤ – ١٦		**Range**	٠.٣٣٩٦ – ٥.٣١٧٨	١٦ – ٦٤
		r	٠.٥٢	
		t		
٤٣٦٧٢X		**Equation ŷ = ٤.٠٦٤٥٩ – ١٩.٨X**		

Table ٩:*Staphylcoccus aureus* specific mucosal humoral immune response among menopausal and adolescent during UTI

Seq.	Age	Conc. (g/l)	Titer Without ٢ME	Titer With ٢ME	Age	Conc (g/l)

١	٥٩	٢.٣١٧١٤	٨	٨	١٩	٠.٩٠٢٦
٢	٥١	١.٢١٧٢	٨	٤	٣٠	١.٥٥٥
٣	٤٩	٠.٦٢١١	٨	٤	٢٩	٠.٣٣٩
٤	٥٤	٠.٨٧٣٥	٨	٨	١٨	٠.٩٠٢٦
٥	٥٦	١.٥٥٥	٤	٢		
٦	٥٢	٢.٣٩٩٥	٨	٨		
٧	٤٨	٠.٣٣٩٦	٤	٢		
٨	٤٦	٠.٦٢١١	٤	٤		
٩	٥٥	٣.٠٤١	٨	٨		
١٠	٥٨	١٠.٦٧٥٦	١٦	١٦		

Mucosal immune response/humoral	Mucosal immune response / humoral

menopausal

Adolescent

٧.٦		**Mean**	٠.٩٢٤٩٨	٣٠
٨		**Median**	٠٩.٢٤٩٨	٢٤
٤-١٦		**Range**	٠.٣٣٩٦-١.٥٥٥	٨-٦٤
		r	٠.٥٠٣٩	
		t		
.٠٨٥X		**Equation ŷ = ٠.٩٢٤٩٩-٠.٠٠٠٩٦X**		

Table ١٠ *:S epidermidis* specific mucosal humoral immune response among menopausal and adolescent during UTI

Mucosal immune response/humoral	Mucosal immune response / humoral

Seq.	Age	Conc. (g/l)	Titer Without ٢ME	Titer With ٢ME	Age	Conc. (g/l)
١	٤٦	١.٥٥٥	٤	٤	٢٥	١.٠٤٨٣
٢	٥٩	٠.٥٠٩٥	٤	٢	٢٨	٠.٣٣٩٦
٣	٥٤	١.٥٥٥	٤	٠	٢٠	٢.٠٧٥٨
٤	٥٠	٠.٩٠٢٦٦	٨	٨	٢٧	١.٥٥٥
٥	٥٧	١.٤٦٥٦٦	٤	٤	١٨	٠.٩٠٢٦٦
٦	٥٣	٠.٦٢١١	٤	٤	٢٦	٠.٨٧٩٤
٧	٤٩	٠.٥٤١٦	٨	٨		
٨	٥٢	٤.٩٣٣	٨	٨		

menopausal **Adolescent**

		Mean	١.١٣٣٤٦	١٦
		Median	٠.٩٧٥٤٨	١٦
		Range	٠.٣٣٩٦-٢.٠٧٥٨	٨-٣٢
		r	١	
		t		
.٨٣٣X		**Equation ŷ = ١.١٣٣٤٦+١x**		

Table ١١:*S saprophyticus* specific mucosal humoral immune response among menopausal and adolescent during UTI

e/humoral	Mucosal immune response / humoral

Seq.	Age	Conc. (g/l)	Titer Without ٢ME	Titer With ٢ME	Age	Conc. (g/l)
١	٥٣	٠.٩٠٢٦٦	٤	٤	٢٥	٠.٣٣٩٦
٢	٥٦	٢.٥٥٨٤	٤	٢	٢٠	١٥.٠٦٧
٣	٥٦	١.٢١٧٢	٨	٨	٢٨	٠.٨٧٩٤

menopausal **Adolescent**

٥.٣٣٣٣٣	Mean	٥.٤٢٨٦٦	٣٧.٣٣٣٣
٤	Median	٠.٨٧٩٤	٣٢
٤-٨	Range	٠.٣٣٩٦-١٥.٠٦٧	١٦-٦٤
	r	٠.٤٧٩٩	
	t		
+٠.٥X	Equation ŷ = ٥.٣٤٥٦٦+٠.٥X		

References

Abramowsky, C.R. & G.L., Swinehart. ١٩٨٦. Secretory immune responses in human kidneys. Am. J. Pathol., ١٢٥: ٥٧١-٥٧٧.

Agace, W.W.; S.R., Hedges; M., Ceska & C. Svanborg. ١٩٩٣. Interleukin-٨ & the neutrophil response to mucosal gram-negative infection. J. Clin. Investig, ٩٢:٧٨٠-٧٨٥.

Al-Rawi,K.M.,٢٠٠٠.Introduction to statistics.٢ed Agriculture &Wood College.Mousil University.

Al-Sa'adi,M.A.K.(١٩٩٨). Separation of intestinal secretory immunoglobulins,M.Sc.Thesis.College of Science .Babylon University.

Amako, K.; Meno, Y. & Takade, A. ١٩٨٨. Fine structures of the capsules of *Klebsiella pneumonia* and *Escherichia coli* K١. J. Bacteriol., ١٧٠: ٤٩٦٠-٤٩٦٢.

Anatatore, F.P.; Loverro, G.; Ingrosso, A.M.; Lacanna, R.; Sassanelli, E.; Selvaggi, L. & Carrozzo, M. ١٩٩٥. Effect of estrogen replacement on bone

metabolism and cytokine in surgical menopause. Clin Rhematol., ١٤:١٥٧-١٦٠.

Andren, A.; Stapleton, A.E.; Fennel, C.; Lockman, H.A,L Xercavins, M.; Fernandez, F. & Stamm, W.E. ١٩٩٧. Urovirulence determinants in *Escherichia coli* strain causing prostatitis. J. Infect. Dis., ١٧٦: ٤٦٤-٤٦٩.

Anonymus. ٢٠٠٣. Urinary tract infection . Infect.Immun.١١٠:١٣٨-١٥٠.

Anonymus. ٢٠٠٤. Suppression: Turning off an immune response. Infect.Immun ٦١:٨٨٤-٨٩١.

Apodaca, G.; L.A., Katz & K.E., Mostov. ١٩٩٤. Receptor-mediated trancytosis of IgA in MDCK cells is via apical recycling endosomes. J. Cell. Biol., ١٢٥: ٦٧-٨٦.

Arceci, R.J.; Shanahan, F.; Stanley, E.R.; Pollard, J.W. ١٩٨٩. Temporal expression and location of colony-stimulating factor ١ (CSF-١) and its receptor in the female reproductive tract are consistent with CSF-١-regulated placental development. Proc. Natl. Acad. Sci. USA, ٨٦: ٨٨١٨-٨٨٢٢.

Arceci, R.J.; Pampfer, S. & Pollard, J.W.١٩٩٢.Expression of CSF-١/c-fims and SF/c-kit mRNA during preimplantation mouse development. Dev. Biol., ١٥١: ١-٨.

Arp, L.H. ١٩٨٨. Bacterial infection of mucosal surfaces: An Overview of cellular and molecular mechanisms. In: Roth, J.A. (ed). Virulence Mechanisms of bacterial pathogens. American Society for Microbiology. Washington, D.C, USA: ٣-٢٣.

Arreaza, E.E.; Gibbons, J.J.Jr; Siskind, G.W. ١٩٩٣. Lower antibody response to tetanus toxoid associated with higher auto-anti-idiotypic antibody in old compared with young humans. Clin. Exp. Immunol., ٩٢: ١٦٩-١٧٣.

Bachhed, F.; M., Soderhall; P., Ekman; S., Normark & A., Richter-Dahlfors. ٢٠٠١. Induction of innate immune responses by *Escherichia coli* and purified lipopolysaccharide correlate with organ-and cell-spciefic expression of Toll-like receptors within the human urinary tract. Cell Microbiol., ١٣: ١٥٣-١٥٨.

Bachmann, G.A.; Leiblum, S.R. & Kemmann, E. ١٩٨٤. Sexual expression and its determinated in the post-menopausal women. Maturitas, ٨: ١٩-٢٩.

Backhed, F.; L., Meijer; S., Normark & A., Richter-Dahlfors. ٢٠٠٢. $TLR_{٤}$-dependent recognition of lipopolysaccharide by epithelial cells requires $sCD_{١٤}$. Cell microbial., ٤: ٤٩٣-٥٠١.

Balch, H.H. ١٩٥٥. The effect of severe battle injury and of post-traumatic renal failure on resistance to infection. Annals of Surgery, ١٤٢: ١٤٥-١٦٣.

Baldassarre, J.S. & Kaye, D. ١٩٩١. Special problems of urinary tract infection in the elderly. Med. Clin. North Am., ٧٥: ٣٧٥-٩٠.

Barton, D. & Joubert, L. ٢٠٠٠. Psychosocial aspects of sexual disorders. Aust. Fam.. Physician, ٢٤: ٥٢٧-٥٣١.

Berman, J.R. & Bassuk, J. ٢٠٠٢. Physiology and pathophysiology of female sexual function and dysfunction. World J. Urol., ٢٠: ١١١-١١٨.

Bethin, K.E.; Vogt, S.K. & Muglia, L.J. ٢٠٠٠. Interleukin-٦ is an essential, corticotrophin-releasing hormone-independent stimulator of the adrenal axis during immune system activation. PNAS, ٩٧: ٩٣١٧-٩٣٢٢.

Beutler, B. & E.T., Rietschel. ٢٠٠٣. Innate immune sensing and its roots: The story of endotoxin. Nat. Rev. Immunol., ٣: ١٦٩-١٧٦.

Bienenstock, J. & Tomasi, T.B. ١٩٦٨. Secretoey A in normal urine. J. Clin. Invest., ٤٧: ١١٦٢-١١٧١.

Bismar, H.; Diel, I.; Ziegler, R. & Pfeilschifter, J. ١٩٩٥. Increased cytokine secretion by human bone marrow cells after menopause or discontinuation of estrogen replacement. J. Clin. Endocrinol. Metab., ٨٠:٣٣٥١-٣٣٥٥.

Bleves, S. & G.R., Cornelis. ٢٠٠٠. How to survive in the host: The Yersinia lesson. Microbs. Infect., ٢: ١٤٥١-١٤٦٠.

Blum, G.; Falbo, V.; Caprioli, A.; Harcher, J. ١٩٩٥. Gene clusters encoding the cytotoxic necrotizing factor type ١, prs-fimbriae and α-heamolycin for the pathogenicity island II of uropathogenic *Escherichia coli* strain J٩٦. FEMS Microbiol. Lett., ١٢٦: ١٨٩-١٩٦.

Borghesi, C. & Nicoletti, C. ١٩٩٤. Increased of cross (auto)-reactive antibodies after immunization in aged mice: A cellular and molecular study. Int. J. Exp. Pathol., ٧٥: ١٢٣-١٣٠.

Bossolati, B.; S., David; V., Cambi; P.S., Tobias & G., Camussi. ٢٠٠٢. Urinary soluble CD١٤ mediates human proximatal tubular cell injury induced by LPS. Int. J. Mol. Med., ١٠: ٤٤١-٤٤٩.

Brandtzaeg, P. ١٩٩٤. Distribution and characteristics of mucosal immunoglobulin-producing cells. Pp. ٢٥١ in Handbook of mucosal Immunoglobulin. Academic press. Inc.

Burdon, D.W. ١٩٧٠. Quantitative studies of urinary immunoglobulins in hospital patients, including patients with urinary tract infection. Clin. Exp. Immunol., ٦: ١٨٩-١٩٦.

Burger, H.G. (١٩٩٩). The endocrinology of menopause. J. Steroid B iochem. Mol. Biol., ٦٩: ٣١-٣٥.

Burns, E.A. & Leventhal, E.A. ٢٠٠٠. Aging, Immunity & cancer. ٧: ٥١٣-٥٢٢.

Butcher, E.; Williams, M.; Youngman, K.; Rott, L. & Biriskin, M. ١٩٩٩. Adv. Immunol., ٧٢: ٢٠٩-٢٥٣.

.

Cattel, W.R. ١٩٩٦. Infection of the kidney and urinary tract. Oxford Medical Publication, pp: ١٩-٢٤٤.

Chrousos, G.P.; Torpy, D.J. & Gold, P.W. ١٩٩٨. Interaction between the hypothalamic-pituritary-adrenal axis and the female reproductive system. Annals of Internal Medicine, ١٢٩: ٢٢٩-٢٤٠.

Clegg, S. & Gerlach, G.F. ١٩٨٧. Enterobacterial fimbriae. J. Bacteriol., ١٦٩: ٩٣٤-٩٣٨.

Cohen, M.C. & Cohen, S. ١٩٩٦. Cytokine function: A study in biologic diversity. Am. J. Clin. Pathol., ١٠٥: ٥٨٩-٥٩٨.

Cohen, P.E.; Zhu, L.; Nishimura, K. & Pollard, J.W. ٢٠٠٢. Colony-stimulating factor ١ regulating of neuroendocrine pathways that control gonadal function in mice. Endocrinology, ١٤٣: ١٤١٣-١٤٢٢.

Collee, J.G.; Duguid, J.P.; Fraser, A.G.; Marmion, B.P. & Simmons, A. ١٩٩٦. Laboratory strategy in the diagnosis of infection syndrome. In Collee, J.G.; Fraser, A.G.; Marmion, B.P. & Simmons, A. (eds). Mackie & Mc Carteny practical medical microbiology.١٤th ed, pp. ٨٤-٨٨. Churchill-Livingstone, New York.

Contestabil, E.; Derzko, C. ٢٠٠٢ Canadian consensus of menopause and osteoporosis: perimenopause JOGC, ٢٤: ١٢-١٧.

Contestabile, E. & Derzko, C. ٢٠٠١. Perimenopause. J. Obstet. Gynaecol. Can., ٢٣: (١٢).

Cossart, P. & H. Bierne. ٢٠٠١. The use of host cell machinery. Immunol., ١٣: ٩٦-١٠٣.

Cruickshank, R.; Duguid, J.P.; Marmiom, B.P. & Swain , R.H.A. ١٩٧٥. Medical Microbiology. Volume ٢, ١٢th ed, pp ٤٥٩: The English language Book Society. London.

Dasilva, J.A.P. ١٩٩٩. Sex hormones and glucocorticoids: Interactions with the immune system. Annals of the New York Academy of Science, ٨٧٦: ١٠٢-١١٨.

De Man, P.; Jodal, U.; VanKooten, C.; Svanborg, C. ١٩٩٠. Bacterial adherence as a virulence factor in urinary tract infection. APMIS, ٩٨: ١٠٥٣-١٠٦٠.

Dennerstein, L.; Dudley, E.C.; Hopper, J.L.; Guthrie, J.R. & Burger, H.G. ٢٠٠٠. A prospective population-based study of menopausal symptoms. Obstet. Gynecol., ٩٦: ٣٥١-٨.

Deswal, A.; Petersen, N.J.; Feldman, A.M.; Young, J.B.; White, B.G. & Mann, D.L. ٢٠٠١. Cytokines and cytokines receptors in advanced heart failure: An analysis of the cytokine database from the vesnarinone trial (VEST). Circulation, ١٠٣:٢٠٥٥-٢٠٥٩.

Dodson, L.G. & Steiner, E. ٢٠٠٢. The perimenopausal transition. Clin. Fam.. Pract., ٤: ١٣-٢٦.

Domenico, P.; Johanson, W.G. & Straus, D.C. ١٩٨٢. Lobar pneumonia in rats produced.

Dunn-Walters, D.K.; Banerjee, M. & Mehr, R. ٢٠٠٣. Effects of age on antibody affinity maturation. Biochemcal Society Transaction, ٣١: ٤٤٧-٤٤٨.

Eden, C.S.; L.A., Hanson; U., Jodal; U. Lindberg & A.S. Akerlund. ١٩٧٦. Variable adherence to normal human urinary tract epithelial cells of Escherichia coli strains associated with various forms of urinary tract infection. Lancet ١: ٤٩٠-٤٩٢.

Ernst, D.N.; W.O., Weigle; D.J., Noonan; D.N., McQuitty & M.V., Hobbs. ١٩٩٣. The age-associated increase in IFN-γ synthesis by mouse $CD_{٨}^{+}$ T cells correlates with shifts in the frequencies of cell subsets defined by membrane $CD_{٤٤}$, $CD_{٤٥}$RB, ٣G١١ & MEL-١٤ expression. J. Immunol., ١٥١: ٥٧٥.

Floege, J.; Boddeker, M.; Stolte, H.; Koch, K.M. ١٩٩٠. Urinary IgA, urinary tract infection. Nephron., ٥٦: ٥٠-٥٥.

Flurkey, K.; R.A., Miller & D.E., Harrison. ١٩٩٢. Cellular determinants of age-related decrements in the T cell mitogen response of B٦CBAF١ mice. J. Gerontol., ٤٧: B ١١٥.

Foxman, B. ٢٠٠٢. " Epidemiology of urinary tract infections: Indence, Morbidity and economic costs". Amer. J. Med., ١١٣ (suppl. ١A): ٥S-١٣S.

Fraserm G.M.; L., Claret; R., Furness; S., Gupta & C., Hughes. ٢٠٠٢. Swarming coupled expression of the *Proteus mirabilis* hpmBA haemolysin peron. Microbiology, ١٤٨: ٢١٩١-٢٢٠١.

Frendeus, B.; C., Wachtler; M., Hedlund; H., Fischer; P., Samuelsson; M., Svensson & C., Svanborg. ٢٠٠١. Escherichia coli pfimbriae utilize the toll-like receptor ٤ pathway for cell activation. Mol. Microbiol., ٤٠: ٣٧-٥١.

Girasole, G.; Giuliani, N.; Modena, A.B.; Passeri, G. & Pedrazzoni, M. ١٩٩٩. Oestrogens prevent the increase of human serum soluble interleukin-٦ receptor induced by ovariectyomy in vivo and decrease its release in human osteoblastic cells in vitro. Clin. Endocrinol. (Oxford), ٥١: ٨٠١-٨٠٧.

Girasole, G.; Pedrazzoni, M.; Giuliani, N.; Passeri, G. & Passeri, M. ١٩٩٥. Increased serum-soluble interleukin-٦ receptor levels are induced by ovariectomy, prevented by estrogen replacement and reversed by alendronate administration. J. Bone Miner Res., ١٠: A٨٦.

Giuglianol, G.; S.T.G., Riberio; M.H., Vainstein & C.J., Ulhoa. ١٩٩٥. Free secretory component and lactoferrin of human milk inhibit the adhesion of enterotoxigenic *Escherichia coli* . J. Med. Microbiol., ٤٢: ٣-٩.

Godaly, G.; G., Bergsten, L.; Hang; H., Fischer; B., Frendeus; A.C., Lundstedt; M., Samuelsson; P., Samuelsson & C., Svanborg. ٢٠٠١. Neutrophil recruitment, chemokine receptors, and resistance to mucosal infection. J. Leukoc. Biol., ٦٩: ٨٩٩-٩٠٦.

Goldman, L.; Bennett, J.C. ٢٠٠٠. Textbook of medicine. ٢١ ed., pp ١٣-١٧١٠. A Harcourt Health Sciences Company, London.

Goodwin, J.S. ١٩٩٥. Decreased immunity and increased morbidity in the elderly.

Gosden, R.G.; Faddy, M.J. ١٩٩٤. Ovarian aging, follicular depletion & steroidogenesis. Exp. Gerontol, ٣٩: ٢٦٥-٢٧٤

Greeling, S.E.; Brouwer, E.C.; VanRessel, K.C. ٢٠٠٠. Cytokine secretion is impaired in women with diabetes mellitus. Eur. J. Clin. Invest., ٣٠: ٩٩٥.

Greendale, G.A.; Lee, N.P.; Arriola, E.R. ١٩٩٩. The menopause. Lancet, ٣٥٣: ٥٧١-٥٨٠. a

Greendale, G.A.; Sower, M.F. ١٩٩٧. The menopause transition. Endocrinol. Metab. Clin. North Am., ٢٦: ٢٦١-٧٧.

Greendale, G.A.; Zibecchi, L.; Petersen, L. ١٩٩٩. Development and validation of physical examination scale to assess vaginal atrophy and inflammation. Climacteric, ٢: ١٩٧-٢٠٤. b

Greenwell, D.; J., Petersen; A., Kulvicki; J., Harder; R., Goldblum & D.E., Neal ١٩٩٥. Urinary secretory IgA and free secretory component in pyelonephritis. Am. J. Kidney Dis., ٢٦: ٥٩٠-٥٩٤.

Gregory, M.S.; Duffner, L.A.; Faunce, D.E. & Kovacs, E.J. ٢٠٠٠. Estrogen mediates the sex difference in post-burn immunosuppression. J. Endocrinol., ١٦٤:١٢٩-١٣٨.

Gruber, C.J.; Tschugguel, W.; Schneeberger, C. & Hurber, J. ٢٠٠٢. Production and actions of estrogens. N Engl. J. Med., ٣٤٦ (٥): ٣٤٠-٥٢.

Gupta, K.; Stapleton, A.E.; Hooton, T.M.; Fennell, C.L.; Samadpour, M.; Stamm, W.E. ١٩٩٨. Inverse association of H٢O٢-producing *Lactobacilli* & vaginal *Escherichia coli* colonization in women with recurrent urinary tract infection. J. Infect. Dis., ١٧٨: ٤٤٦-٤٥٠.

Guyer, D.M.; Guther, N.W.; Mobley, H.L.T. ٢٠٠١. Secreted proteins and other features specific to uropathogenic *Escherichia coli*. J. Infect. Dis., ١٨٣ (suppl: ١): S٣٢-S٣٥.

Guyer, D.M.; Henderson, I.R.; Nataro, J.P. & Mobley, H.L.T. ٢٠٠٠. Identification of sat, an autotransporter toxin produced by uropathogenic *Escherichia coli*. Mol. Microbiol., ٣٨: ٥٣-٦٦.

Hacker, J. & Kaper, J.B. ٢٠٠٠. Pathogenicity islands and the evolution of microbes. Ann. Rev. Micrbiol., ٥٤: ٦٤١-٦٧٩.

Hagberg, L.; R., Hull; S., Hull; J.R., McGhee; S.M., Michalek & C., Svanborg-Eden. ١٩٨٤. Difference in susceptibility to gram-negative urinary tract infection between C٣H/Hej & C٣H/Hen mice. Infect. Immun., ٤٦: ٨٣٩-٨٤٤.

Hanson, L.A.; Ahlstedt, S.; Fasth, A.; Jodal, U.; Kaijser, B.; Larsson, P.; Lindberg, U.; Olling, S.; Sobl-Akerlund, A.; Svanborg-Eden, C. ١٩٩٧. Antigens of *Escherichia coli* human immune response and the pathogenesis of urinary tract infection. J. Infect Dis. [suppl] ١٣٦: S١٤٤-S١٤٩.

Hara, H.; Negoro, S. & Miyata, S. ١٩٨٧. Age-associated changes in proliferative and differentiative response of human B cells and production of T cell-derived factors regulating B cell function. Mech. Aging Dev., ٣٨: ٢٤٥-٢٥٨.

Haraoka, M.; L., Hang; B., Frendeus; G., Godaly; M., Burdick; R., Strietter & C., Svanborg. ١٩٩٩. Neutrophil recruitment and resistance to urinary tract infection. J. Infect. Dis., ١٨٠: ١٢٢٠-١٢٢٩.

Haynes, L.; P.J., Linton & S.L., Swain. ١٩٩٧. Age related changes in CD_4 T cell of T cell receptor transgenic mice. Mech. Aging Dev., ٩٣: ٩٥.

Haynes, L.; P.J., Linton; S.M., Easton; S.L., Tonkonogy & S.L., Swain. ١٩٩٩. Interleukin ٢, but not other common γ-chain binding cytokines, can reverse. The defect in generation of CD_4 effecter T cells from naïve T cells of aged mice. J. Exp. Med., ١٩٠: ١٠١٣.

Hedges, S.; M., Svensson & C., Svanborg. ١٩٩٢. Interleukin-٦ response of epithelial cell lines to bacterial stimulation in vitro. Infect. Immun., ٦٠: ١٢٩٥-١٣٠١.

Hedlund, M.; C., Wachtler; E., Johansson; L., Hang; J.E., Somerville; R.P., Darveau & C., Svanborg. ١٩٩٩. Pfimbriae-dependent, lipopolysaccharide-independent activation of epithelial cytokine responses. Mol. Microbiol., ٣٣: ٦٩٣-٧٠٣.

High, K.P. ١٩٩٩. " Micronutrient supplementation and immune function in the elderly". Clin. Infect. Dis., ٢٨: ٧١٧-٢٢.

Highsmith, A.K. & Jarvis, W. R. ١٩٨٥. *Klebsiella pneumonia*: Selected virulence factors that contribute to pathogenicity. Infect. Control, ٦: ٧٥-٧٧.

Hillier, S.L. & Lau, R.J. ١٩٩٧. Vaginal microflora in postmenopausal women who have not received estrogen replacement therapy. Clin. Infec. Dis., ٢٥ (suppl ٢): S١٢٣-S١٢٦.

Hodes, R.J. ١٩٩٧. Aging and immune system. Immunol. Rev., ١٦٠: ٥

. Holt,J.G.,Kreig,N.R.,Sneath,P.H.A.,Staley,J.T.,&Williams,S.T.١٩٩٤. Bergy

. manual of determinative bacteriology.٩th nd .Williams & Wilkins Co.

Baltimore,London.

Honkinen, O.; Lehtonen, O.P.; Ruaskanen, O.; Huovineu, P.; Mertsola, J. ١٩٩٩. Cohort study of bacterial species in children. BMJ, ٣١٨: ٧٧٠-٧٧١.

Hooton, T.M. ٢٠٠١. Recurrent urinary tract infection in women. Int. J. Antimicrob. Agent, ١٧: ٢٥٩-٢٦٨.

Hooton, T.M.; Scholes, D.; Hughes, J.P. ١٩٩٦. A prospective study of risk factors symptomatic urinary tract infection in young women. N. Engl. J. Med., ٣٣٥: ٤٦٨-٤٧٤.

Hosking, D.J.; Bennett, T.; Hampton, J.R. ١٩٧٨. Diabetic autonomic neuropathy. Diabetes Care, ٢٧: ١٠٤٣-١٠٥٤.

Iosif, C.S. & Berkassy, Z. ١٩٨٤. Prevalence of genitor-urinary symptoms in the late menopause. Acta. Obstet. Gynecol. Scand., ٦٣: ٢٥٧-٢٦٠.

Iosif, C.S. ١٩٩٢. Effect of protracted administration of estriol on lower genitourinary tract in post menopausal women. Arch. Gynecol. Obstet., ٢٥١: ١١٥-١٢٠.

Iwahi, T.; Abe, Y.; Nakao, M.; Imada, A. & Tsuchiya, K. ١٩٨٣. Role of type ١ fimbriae in the pathogenesis of ascending urinary tract infection induced by *Escherichia coli*. Infect. Immun., ٣٩: ١٣٠٧-١٣١٥.

Jilka, R.L.; Hangoc, G.; Girasole, G.; Passert, G.; Williams, D.C.; Abrams, J.S.; Boyce, B.; Broxmeyer, H.; Manolagas, S.C. ١٩٩٢. Increased osteoclast development after estrogen loss: mediation by interleukin-٦. Science, ٢٥٧: ٨٨-٩١.

Johnson, J.R. ٢٠٠٣. Microbial virulence determinant and pathogenesis of urinary tract infection. Infect. Dis. Clin. North Am., ١٧: ٢٦١-٢٧٨ VIII.

Johnstone, A. & Thorpe, R. ١٩٨٢. Immunochemistry in practice. Pp ١-٧٥. Blackwell Scientific Publication, Oxford.

Judd, H.L. & Fournet, N. ١٩٩٤. Changes in ovarian hormonal function with aging. Exp. Gerontol., ٢٩: ٢٨٥-٢٩٨.

Kantele, A.; Mottonen, T.; Ala-Kaila, K. & Arvilommi, H.S. ٢٠٠٣. Pfimbria-specific B cell responses in patients with urinary tract infection. J. Infect. Dis., ١٨٨: ١٨٨٥-٩١.

Kantele, A.; Papunen, R.; Virtaneu, E.; Mottonen, T.; Rasanen, L.; Ala-Kaila, K.; Makela, P.H. & Arvilommi, H. ١٩٩٤. Antibody-secreting cells in acute urinary

tract infection as indicators of local immune response. J. Infect. Dis., ١٦٩: ١٠٢٣-٨.

Kaufman, D.B.; Katz, R. & Mcintosh, R.M. ١٩٧٠. Secretory IgA in urinary tract infection. Brit. Med. J., ٤: ٤٦٣-٤٦٥.

Kawanishi, H. & J., Kiely. ١٩٨٩. Immune-related alteration in aged gut-associated lymphoid tissues in mice. Dig. Dis. Sci., ٣٤: ١٧٥..

Keller, E.T.; Zhang, J.; Yao, Z. & Qi, Y. ٢٠٠١. The impact of chronic estrogen deprivation on immunologic parameters in the ovariectomized rhesus monkey (*Macaca mulatto*) model of menopause. J. Repord. Immunol., ٥٠: ٤١-٥٥.

Kelsall, B.L. & Strober, W. ١٩٩٦. Host defences at mucosal surfaces. In: Clinical Immunology Principles and practice, Rich, R. ed. Mosby.

Kimble, R.B.; Vannice, J.L.; Bloedow, D.C.; Thompson, R.C.; Hopfer, W.; Kung, V.T.; Brownfield, C.; Pacifici, R. ١٩٩٤. Interleukin-١ receptor antagonist decreases bone loss and bone resorption in ovariectomized rats. J. Clin. Invest., ٩٣: ١٩٥٩-١٩٦٧.

Kitazawa, R.; Kimble, R.B.; Vannice, J.L.; Kung, V.T. & Pacifici, R. ١٩٩٤. Interleukin-١ receptor antagonist and tumor necrosis factor binding protein decrease osteoclast formation and bone resportion in ovariectomized mice. J. Clin. Invest., ٩٤: ٢٣٩٧-٢٤٠٦.

Klutke, J.J. & Bergman, A. ١٩٩٥. Hormonal influence on the urinary tract. Urol. Clin. North Am., ٢٢: ٦٢٩-٣٩.

Knight, J.A. ١٩٩٥. The process and theories of aging. Ann. Clin. Lab. Sci., ٢٥: ١.

Koga, T.; McGhee, J.R.; Kato, H.; Kato, R.; Kiyono, H. & Fujinashi, K. ٢٠٠٠. Evidence for early aging in the mucosal immune system. The Journal of Immunology, ١٦٥: ٥٣٥٢-٥٣٥٩.

Kunin, C.M. & McCormack, R.C. ١٩٦٨. An epidemiologic study of bacteriuria and blood pressure among nuns and working women. New Engl. J. Med., ٢٧٨: ٦٣٥-٦٤٢.

Kurazono, H.; Yamamoto, S.; Nakano, M.; Nair, G.B.; Terai, A.; Chaicumpa, W. & Hayashi, H. ٢٠٠٠. Characterization of a putative virulence island in the chromosome of uropathogenic *Escherichia coli* possessing agene encoding a uropathogenic-specific protein. Microb. Pathog., ٢٨: ١٨٣-١٨٩.

Labdenperä, M.; Lummaa, V. & A.F., Russell. ٢٠٠٤. Menopause: Why does fertility end before life ? Climacteric, ٧: ٣٢٧-٣٣٢.

Laumann, E.O.; Paik, A. & Rosen, R.C. ١٩٩٩. Sexual dysfunction in united states. Prevalence & Predictors. JAMA, ٢٨١: ٥٣٧-٥٤٤

Leffler, H. & C. Svanborg-Eden. ١٩٨٠. Chemical identification of a glycophingolipid receptor for *Escherichia coli* attaching to human urinary tract epithelial cells and agglutinating human erthrocytes. FEMS Microbiol. Lett., ٨: ١٢٧-١٣٤.

Lehman, J.D.; Smith, J.W.; Miller, T.E.; Barnett, J.A. & Sanford, J.P. ١٩٦٨. Local immune response in experimental pylonephritis. J. Clin. Invest., ٤٧: ٢٥٤١-٢٥٥٠.

Le Maoult, J.; J.S., Manavalan; R., Dyall; P., Szabo; J., Nikolic-Zugic & M.E., Weksler. ١٩٩٩. Cellular basis of B cell clonal population in old mice. J. Immunol., ١٦٢: ٦٣٨٤.

Le Maoult, J.; P., Szabo & M.E., Weksler. ١٩٩٧. Effect of age on humoral immunity, selection of the B-cell repertoire & B-cell development. Immunol. Rev., ١٦٠: ١١٥. a

Le Maoult, J.; S., Dlassus; R., Dyall; J., Niko-Zugic; P., Kourilsky & M.E., Weksler. ١٩٩٧. Clonal expansion of B lymphocytes in old mice. J. Immunol., ١٥٩: ٣٨٦٦. b

Leshourd, B.M. ١٩٩٧. " Nutrition and immunity in the elderly: Modification of immune responses with nutritional treatments". Am. J. Clin. Nutr., ٦٦: ٤٧٨s-٨٤s.

Lindh, E. ١٩٧٥. Increased resistance of immunoglobulin A dimmers to proteolytic degradation after binding of secretory component. J. Immunol., ١١٤: ٢٨٤-٢٨٦.

Lipsky, B.A. ١٩٨٩. Urinary tract infection in men. Epidemiology, pathophysiology, diagnosis and treatment. Ann. Intern. Med., ١١٠: ١٣٨-١٥٠.

Luborsky, J.L.; P., Meyer; M.E., Sowers; E.B., Gold & N., Santoro. ٢٠٠٣. Premature menopause in multi-ethanic population study of the menopause transition. Humen Reproduction, ١٨: ١٩٩-٢٠٦.

Macfaddin, B.A. ٢٠٠٠. Biochemical tests for identification of medical bacteria. Pp ٥٧-٤٢٤, ٣rd eed. Lippincott Williams & Wilkins. London.

Malani, P.N. ٢٠٠٥. Diagnosis and management of urinary tract infection in older women. Clinical Geriatrics, ١٣: ٤٧-٥٣.

Manonai, J.; Chiltacharoen, A.; Sarit-Apirak, S.; Vdomsubpayakul, U.; Khanacharoen, A. & Theppisai, U. ٢٠٠٤. Lower urinary tract symptoms in thai women attending the menopause clinic: Prevalence and associated factors. J. Med. Assoc. Thai., ٨٧: ١٢٦٥-٩.

Marchetti, B.; Gallo, F.; Farinella, Z.; Tirolo, C.; Testa, N.; Caniglia, S. & Morale, M.C. ٢٠٠٠. Gender, neuroendocrine-immune interaction and neuron-glial plasticity: Role of luteinizing hormone-releasing hormone (LHRH). Annals of the New York Academy of Sciences, ٩١٧: ٦٧٨-٧٠٩.

Martinetti, A.; Ferrari, L.; Celio, L.; Mariani, L.; Miceli, R.; Zilembo, N.; DiBarttolomeo, M.; Toffolatti, L.; Pozzi, P.; Seregni, E.; Bombardieri, E. & Hajetta, E. ٢٠٠٠. The luteinising hormone-releasing hormone analogue triptorelin with or without the aromatase inhibitor formestane in premenopausal breast cancer: Effects on bone metabolism markers. J. Steroid Biochem. Mol. Biol., ٧٥: ٦٥-٧٣.

Matsumoto, T.; Mizunoe, Y.; Sakamoto, N.; Tanaka, M. & Kumazawa, J. ١٩٩٠. Increased renal scarring by bacteria with mannose-sensitive pili. Urol. Res., ١٨: ٢٩٩-٣٠٣.

Mattix Kramer, H.J.; Grodstein, F.; Stampfer, M.J. & Curhan, G.C. ٢٠٠٣. Menopause and postmenopausal hormone use and risk of incident kidney stones. J. Am. Soc. Nephrol., ١٤: ١٢٧٢-١٢٧٧.

Mazanec, M.B.; J.G., Nedrud; C.S., Kaetzel & M.E., Lamm. ١٩٩٣. A three-tiered view of the role of IgA in mucosal defence. Immunol. Today, ١٤: ٤٣٠-٤٣٥.

Mc Coy, K.L. & Kennedy, E.R. ١٩٦٠. Autogenous vaccine therapy in Staphylococcic infections. J.A.M.A., ١٧٤: ١١٧-١٢٠.

Mc Ghee, J. R.١٩٩٩. Mucosal Immune Responses: An overview. In: Mucosal Immunology, Ogra PL (editor). Academic press.

Mc Kane, W.R.; Khosla, S.; Peterson, J.M.; Egan, K. & Riggs, B.L. ١٩٩٤. Circulating levels of cytokines that modulate bone resorption: Effects of age and menopause in women. J. Bone Miner Res., ٩: ١٣١٣١٣١٨.

Mc Kinlay, S.M.; Barmbilla, D.J.; Posner, J.G. ١٩٩٢. The normal menopause transition. Maturitas, ١٤: ١٠٣-١٥.

Miller, R.A. ١٩٩١. Aging and immune function. Int. Rev. Cytol.

Miller, R.A. ١٩٩٦. The aging immune system primer and prospectus. Science, ٢٧٣: ٧٠.

Miller, R.A.; G., Garcia; C.J., Kirk & J.M., Witkowski. ١٩٩٧. Early activation defects on T lymphocytes from aged mice. Immunol. Rev., ١٦٠: ٧٩.

Mitsumori, K.; Terai, A.; Yamamoto, S.; Ishitoyo, S. & Yoshida, O. ١٩٩٩. Virulence characterization of *Escherichia coli* in acute bacterial prostatitis. J. Infect. Dis., ١٨٠: ١٣٧٨-١٣٨١.

Mobley, H.L.T. ٢٠٠٠. Virulence of two primary uropathogens. ASM News, ٦٦: ٤٠٣-٤١٠.

Montgomerie, J.Z.; Kalmanson, G.M.; Guze, L.B. ١٩٦٨. Renal failure and infection. Medicine, ٤٧: ١-٣٢.

Mostov, K.E.; M., Friedlander & G., Blobel. ١٩٨٤. The receptor for transepithelial transport of IgA and IgM contains multiple immunoglobulin-like domains. Nature, ٣٠٨: ٣٧-٤٣.

Mufson, M.A. ١٩٩٩. Pheumococcal pneumonia. Curr. Infect. Dis. Rep., ١: ٥٧.

Muscatello, D.J.; Rissel, C.; Szonyi, G. ٢٠٠١. Urinary symptoms and incontinense in urban community: Prevalence and associated factors in older men and women. Inter Med. J., ٣١: ١٥١-٦٠.

Negoro, S.; S., Hara; S., Miyata; O., Saiki; T., Tanaka; K., Yoshizaki; T., Igarashi & S., Kishimoto. ١٩٨٦. Mechanisms of age-related decline in antigen-specific T cell-proliferative response: IL-٢ receptor expression and recombinant IL-٢ induced proliferative response of purified TAC-positive T cells. Mech. Aging Dev., ٣٦: ٢٢٣.

Neutral, M.R. & J.P., Kraehenbuhl. ١٩٩٤. Cellular and molecular basis for antigen transport in the intestinal epithelium. Pp. ٢٧ in Handbook of Mucosal Immunology Academic press. Inc.

Nicolle, L.E. & Brunka, J. ١٩٩٠. Urinary IgG & IgA antibodies in elderly individuals with bacteriuria. Gerontology, ٣٦: ٣٤٥-٣٥٥.

Nicolle, L.E. & Ronald, A.R. ١٩٨٧. Recurrent urinary infection in adult women: Diagnosis and treatment. Infect. Dis. Clin. North Am., ١: ٧٩٣-٨٠٦.

Nicolle, L.E. ١٩٩٣. Urinary tract infection in long-term care facillites. Infect Control Hosp. Epidomiol., ١٤: ٢٢٠-٢٢٥.

Nicolle, L.E. ٢٠٠٠. Urinary tract infection. In: Yoshikawa, T.T.; Norman, D.C. eds. Infectious disease in the aging. Towota, N.J.: Humana press, ٩٩-١١١.

Nolte, F.S. & Metchock, B. ١٩٩٥. Murray PR eds. Manual of Clinical Microbiology. ٦th ed. ASM press.

Nordin, A.A. & G.D., Collins. ١٩٨٣. Limiting dilution analysis of alloreactive cytotoxic precursor cells in aging mice. J. Immunol., ١٣١: ٢٢١٥.

Oaks, S.; Mc Cormick, A.M.; Gartland, W. ١٩٩٦. Immunological consequences of aging. Nihguide, ٢٥: PA.٩٧-٠٠٣.

O'Brien, C.A. & Manolagas, S.C. ١٩٩٧. Isolation and characterization of human gp١٣٠ promoter-regulation by STATS. J. Biol. Chem., ٢٧٢: ١٥٠٠٣-١٥٠١٠.

Ofek, I.; D., Mirelman & N., Sharon. ١٩٧٧. Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. Nature, ٢٦٥: ٦٢٣-٦٢٥.

Oho, G.; J., Braconier; A., Andreasson & C., Svanborg. ١٩٩٩. Interleukin-٦ and disease severity in patients with bacteremic and nonbacterimic febrile urinary tract infection. J. Infect. Dis., ١٧٩: ١٧٢-١٧٩.

Ojeda, S.R. & Griffin, J.E. ٢٠٠٠. Textbook of endocrine physiology. ٤th ed. Oxford university press. United State of America.

Okumura, M.; Y., Fujii; K., Takeuchi; K., Inada; K., Nakahara & H., Matsuda. ١٩٩٣. Age-related accumulation of LFA-١ high cells in $CD_{٨}^{+}CD_{٤٥}$ RA high T cell population. Eur. J. Immunol., ٢٣: ١٠٥٧.

Olszyna, D.P.; S.M., Opal; J.M., Prins; D.L., Horn; P., Speelma; S.J., Van Deventer & T., Vanderpoll. ٢٠٠٠. Chemotactic activity of CXC chemokines interleukin-٨, neutrophil-activiting protein-٧٨ in urine of patients with urosepsis. J. Infect. Dis., ١٨٢: ١٧٣١-١٧٣٧.

Pabich, W.L.; Fihn, S.D.; Stamm, W.E. ٢٠٠٣. Prevalence and determinants of vaginal flora alteration in postmenopausal women. J. Infect Dis., ١٨٨: ١٠٥٤.

Pacifici, R.; Brown, C.; Puscheck, E.; Friedrich, E.; Slatopolsky, E.; Maggio, D,; McCracken, R. & Avioli, L.V. ١٩٩١. Effect of surgical menopause and estrogen replacement on cytokine release from human blood mononuclear cells. Proc. Natl. Acad. Sci:USA, ٨٨: ٥١٣٤-٥١٣٨.

Pacifici, R.; Rifas,L.; McCracken, R.; Vered, I.; McMurtry, C.; Avioli, L.V. & Peck, W.A. ١٩٨٩. Ovarian steroid treatment blocks postmenopausal increase in blood monocyte interleukin/release. Proc. Natl. Acad. Sci. USA, ٨٦: ٢٣٩٨-٢٤٠٢.

Paoletti, R.; Wenger, N.R. ٢٠٠٣. Review of the international position paper on women's health and menopause. Circulation, ١٠٧: ١٣٣٦-١٣٣٩.

Parslow, T.G.; Stites, D.P.; Terr, A.I. & Imboden, J.B. ٢٠٠١. Immunoglobulins and immunoglobulin genes. In Medical Immunology. ١٠th ed, pp ٩٥-١١٤. Acid-free paper. New York.

Pavelka, M.S.M.; Fedigan, L.M. ١٩٩١. Menopause: A comparative life history perspecytive. Year book Phys. Anthr., ٣٤: ١٣-٣٨.

Pawelzik, M.; Heesemann, J.; Hacker, J.; Opferkuch, W. ١٩٨٨. Cloning and characterization of a new type fimbria (SIFIC-related fimbria) expressed by *Escherichia coli* $O_{٧٥}$:$K_{١}$:$H_{٧}$ blood cluster isolate. Infect. Immun., ٥٦: ٢٩١٨-٢٩٢٤.

Podschun, R. & Ullmann, U. ١٩٩٢. Isolation of *Klebsiella terrigena* from clinical specimens. Eur. J. Clin. Microbiol. Infect. Dis., ١١: ٣٤٩-٣٥٢.

Podschun, R.; Penner, I.; Ullmann, U. ١٩٩٢. Interaction of Klebsiella capsule type ٧ with human polymorphnuclear leucocytes. Microb. Pathog., ١٣: ٣٧١-٣٧٩.

Prior, J.C. ١٩٩٨. Perimenopause: The complex endocrinology of the menopausal transition. Endo. Rev., ١٩: ٣٩٨-٤٢٨.

Raju, S.B. & Tiwari, S.C. ٢٠٠١. Urinary tract infection-A suitable approach. Journal, Indian Academy of Clinical Medicine, ٢: ٣٣١-٣٣٧.

Rather, P.N.; X., Ding; R.P., Baca-DeLancey & S., Siddiqui. ١٩٩٩. Providencia stuartii genes activated by cell-to-cell signaling and identification of a gene required for production or activity of an extracellular factor. J. Bacteriol., ١٨١: ٧١٨٥-٧١٩١.

Raz, R. & Stamm, W.F. ١٩٩٣. A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infection. N. Engl. J. Med., ٣٢٩: ٧٥٣-٧٥٦.

Raz, R.; Gennesin, Y.; Wasser, J. ٢٠٠٠. Recurrent urinary tract infection in postmenopausal women. Clin. Infect. Dis., ٣٠: ١٥٢-١٥٦.

.

Rice, J.C.; J.S., Spence; J., Meggesi; R.L., Safirstein; R.M., Goldblum. ١٩٩٨. Regulation of the polymeric immunoglobulin receptor by water intake and vasopression in the rate rat kidney. Am. J. Physiol ٢٧٤ (Renal Physiol. ٤٣): F٩٦٦-F٩٧٧.

Rice, J.C.; Spence, J.S.; Megyesi, J.; Goldblum, D. M. & Safirstein, R.L. ١٩٩٩. Expression of the polymeric immunoglobulin receptor and excretion of

secretory IgA in the postischemic kidney. Am. J. Physiol., ٢٧٦ (Renel Physiol. ٤٥): F ٦٦٦-٦٧٣.

Rice, V.M. ٢٠٠٥. Post menopausal hormone therapy today. Ethnicity & Disease, ١٥ [supp١. ٢]: S٢-٣٤-S٢-٣٩.

Riedasch, G.; Ritz, E.; Rauterberg, E. ١٩٨٤. Do antibody-coated bacteria reflect local immune response in the urinary tract ?. Am. J. Nephrol., ٤: ٣٦١-٦.

Riggs, B.L.; Melton, L.J. ١٩٨٦. Medical progress series: Involutional osteoporosis. N. Engl. J. Med., ٣١٤: ١٦٧٦-١٦٨٦.

Ross, D.L. ١٩٨٥. Proteins. In Bishop, M.C.; Dhen-Von, L.J.; Fody, E.P. & Thirty three contributors (eds). Clinical chemistry principles, procedures & correlations. Pp ١٨٠-١٨١. The Murray Priniting Company, Philadelphia.

Rud, T.; Anderson, K.E,; Asmussen, M.; Hunting, A.; Ulmsten, U. ١٩٨٠. Factors maintaining the urethral pressure in women. Invest. Urol., ١٧: ٣٤٣-٧.

Rutishauser, S. ٢٠٠١. physiology and anatomy, A Basis for Nursing and Health care.: RDC Group limited C١٠٤. China.

Sansonetti, P. ٢٠٠١. Phagocytosis of bacterial pathogens: Implication in the host response semin. Immunol., ١٣: ٣٨١-٣٩٠.

Santoro, N.F. ٢٠٠٤. Treatment of menopause-associated vasomotor symptoms; Position statement of the North American Society. Menopause, ١١: ١١-٣٣.

Schatzl, G.; Temml, C.; Waldmuller, J.; Thurridl, T.; Haidinger, G.; Madersbacher, S. ٢٠٠١. A comparative cross sectional study of lower urinary tract symptoms in both sexes. Eur. Urol., ٤٠: ٢١٣-٩.

Schilling, J.D.; S.M., Martin; C.S., Hung; R.G., Lorenzo & S.H., Hultgren. ٢٠٠٣. Toll-like receptor ٤ on stromal and hematopoietic cells mediates innate resistance to uropathogenic *Escherichia coli*. Proc. Natl. Acad. Sci. USA, ١٠٠: ٤٢٠٣-٤٢٠٨. a

Schilling, J.D.; S.M., Martin; D.A., Hunsta; K.P., Patel; M.A., Mulvey; S.S., Justice; R.G., Lorenz & S.J., Hultgren. ٢٠٠٣. CD١٤ and toll-like receptor-dependent activation of bladder epithelial cells by lipopolysaccharid and type ١ piliated *Escherichia coli.* Infect. Immun., ٧١: ١٤٧٠-١٤٨٠. b

Schmucker, D.L.; M.F., Heworth; R.L., Owen & C.K., Daniels. ١٩٩٦. Impact of aging on gastrointestinal mucosal immunity. Dig. Dis. Sci., ٤١: ١١٨٣.

Shahin, R.D.; I., Engberg; L., Hagberg & C., Svanborg-Eden. ١٩٨٧. Neutrophil recruitment and bacterial clearance correlated with LPS responsiveness in local gram-negative infection. J. Immunol., ١٣٨: ٣٤٧٥-٣٤٨٠

Shnawa, I.M.S.; Abdul-Razic, M.M. & Al-Amidi, B. ١٩٩٩. The bacteriolytic complement and immuno-secnesce. Babylon University Journal, ٤: ٤٨٥-٤٨٨.

Shnawa, I.M.S.; Al-Amidi, B.H.H. ٢٠٠٥. The immune status of human persistent pyria associated *K. pneum*oniae. Kufa Med. J., ٨: ٤٢-٤٧.

Shanwa, I.M.S. & Mehdi, M.S. ٢٠٠٤. Mucosal agglutinating antibodies to the antigens of bacteria associated with urinary tract infection in human adults. Babylon University Journal, ٩: ٧٧٣-٧٨٢.

Shnawa, I.M.S. & Thwaini, Q.N.A. ٢٠٠٢. Lapin mucosal humoral versus systemic humoral and cellular immune responses post to intratesticular

administration of heat killed *C. fetus* bacterin. Babylon university Journal, ٧: ٥٣٨-٥٤٣

Shaver, J.L. ٢٠٠٠. Sleep problems II: Assessment and treatment. Menopause management, ٩: ١٤-٨.

Simon, H.; Cannistra, S.A.; Etkin, M.J.; Godine, J.E.; Huang, E.& Heller, D.; Shellito, P.C.; Stern, T.A. ٢٠٠١. Urinary tract infection. Nidus Information Services.

Smith, J.W. ١٩٧٥. Local immune response in experimental pyelonephritis in the rabbit: Lymphocyte stimulation by lipopoly saccharide in infecting organism. Immunology, ٢٩: ١٠٧٧-١٠٨٤.

Smith, T.; Contestabile, E. ٢٠٠٢. Canadian consensus on menopause and osteoporosis; Executive summary jogc, ٢٤: ٥-١١.

Solomon, C.; Panagotopoulous, P.; Oppenheim, A. ١٩٨٥. Urinary cytology studies as an aid to diagnosis. Am. J. Obstet. Gynecol., ٧٦: ٥٧-٦٢.

Sotelo, T.M. & Westney, O.L. ٢٠٠٤. Recurrent urinary tract infection in women. Business Bkiefing: Women's Health Care, ١-٦.

Stapleton, A. ٢٠٠٢. Urinary tract infection in patient with diabetes. Am. J. Med., ١١٣: ٨٠S.

Stenchever, M.A.; Droegemueller, W.; Herbst, A.L.; Mishell, D.K.; Jr, eds. ٢٠٠١. Comperhensive gynecology. ٤th ed. St. Louis: Mosby. Inc.

Strober, W. & Fuss, I.J. ٢٠٠١. The mucosal immune system. In Parslow, T.G.; Stites, D.P.; Terr, A.I. & Imboden, J.B. Medical Immunology. ١٠th ed. Pp. ٢٠٤-٢١٤. Acid-Free paper, New York.

Sturgil, GM,.; S.,Sifddiqui; X., Ding; N.C., Peccora & P.N., Rather. ٢٠٠٢. Isolation of lacZ fusions to *proteus mirabilis* genes regulated by intracellular signaling: Potential role for the sugar phosphotransferase (pts) system in regulation. FEMS. Microbiol. Lett., ٢١٧: ٤٣-٥٠.

Sussman, M.; Gally, D.L. ١٩٩٩. The biology of cystitis: Host and bacterial factor. Ann. Rev. Med., ٥٠: ١٤٩-١٥٨.

Svanborg, C. ١٩٩٣. Resistance to urinary tract infection. N. Engl. J. Med., ٣٢٩: ٨٠٢-٨٠٣.

Svanborg, C.; G., Godaly & M., Hedlund. ١٩٩٩. Cytokine responses during mucosal infection: role in disease pathogenesisand host defence. Curr. Opin. Microbiol., ٢: ٩٩-١٠٥.

Svanborg-Eden, C. & A.M., Svennerholm. ١٩٧٨. Secretory immunoglobulin A & G antibodies prevent adhesion of *Escherichia coli* to human urinary tract epithelial cell. Infect Immun., ٢٢: ٧٩٠-٨٢٧.

Svanborg-Eden, C.; Marild, S. & Korhonen, T.K. ١٩٨٢. Adhesion inhibition by antibodies. Scan J. Infect. Dis. Supp.١, ٣٣: ٧٢-٨.

Svanborg-Eden, V. & DeMan, P. ١٩٨٧. Bacterial virulence in urinary tract infection. Infect. Dis. Clin. North Am. ١٢. Gygi, D.; M.M., Rahman; H.C., Lai; R., Carlson; J., Guard-Pettier & C., Hughes. ١٩٩٥. A cell surface polysaccharide that facilitated rapid population migration by differentialed swarm cells of *proteus mirabilis*. Mol. Micrbiol., ١٧: ١١٦٧-١١٧٥.

Sztul, E.S.; K.E., Howell & G.E., Palade. ١٩٨٥. Biogenesis of the polymeric IgA receptor in rat hepatocytes. II. Lacolization of its intracellular forms by cell fractionation studies. J. Cell. Biol., ١٠٠: ١٢٤٨-١٢٥٤.

Tapp, A.J.S. & Cardozom L.D. ١٩٨٦. The postmenpausal bladder. Br. J. Hosp. Med., ٣٥: ٢٠-٣.

Thankavel, K.; Madison, B.; Ikeda, T.; Malaviya, R.; Shah, A.H.; Arumugam, P.M. & Abraham, S.N. ١٩٩٧. Localization of a Domain in the fim H adhesion of Escherichia coli type ١ fimbriae capable of receptor recognition and use of a Domain-specific antibody to confer protection against experimental urinary tract infection. The American Society for Clinical Investigation, ١٠٠: ١١٢٣-١١٣٦.

The North American Menopause Society. Menopause guidebook: Helping women make informed health care decisions through perimenopause and Beyond.Availabletathttp://www.Menopause.Org/edumaterials/guidebook/mgtoc.Htm (accessed March ٢٤, ٢٠٠٥).

Thoman, M.L.; D.N., Ernst; M.V., Hobbs & W.O., Weigle. ١٩٩٣. T-cell differentiation and functional maturation in aging mice.

Todar, K. ٢٠٠٢. Mechanisms of bacterial pathogenicity: Bacterial defense against the host immune responses. University of Wiscosin.

Trinchieri, A.; Braceschi, L.; Tiranti, D.; Dell Acqua, S.; Mandressi, A. & Pisani, E. ١٩٩٠. Secretory immunoglobulin A and inhibitory activity of bacterial adherence to epithelial cells in urine from patients with urinary tract infection. Urol. Res., ١٨: ٣٠٥-٣٠٨.

Uehling, D.T. & Balish, E. ١٩٧٧. Antibacterial activity of urinary immunoglobulins. Birth Defects, ١٣: ٤٢١-٤٢٤.

Uehling, D.T. & Steihm, E.R. ١٩٩١. Elevated urinary secretory IgA in children with urinary tract infection. Pediatrics, ٤٧: ٤٠-٤٦.

Uehling, D.T. ١٩٧٣. Immunoglobulin excretion in women with recurrent urinary tract infection. J. Urol., ١٠٩: ٣٠٢-٣٠٣.

Uehling, D.T.; Hopkins, W.J.; James, L.J.; Balish, E. ١٩٩٤. Vaginal immunization of monkeys against urinary tract infection with a multi-strain vaccine. J. Urol., ١٥١: ٢١٤-٢١٦.

Underdown, B.J. & J., Mestecky. ١٩٩٤. Mucosal immunoglobulins. Pp.٧٩ in Handbook of Mucosal Immunology, P.L. Ogra, J. Mesteck, M.E. Lamm, W. Strober, J.R.M. McGhee & J. Bienenstock, eds. Boston: Academic press, Inc.

Valerius, N.H.; Eff, C.; Hansen, N.E.; Karle, H.; Nerup, J.; Soeberg, B.; Sorenson, S.F. ١٩٨٢. Neutrophil and lymphocyte function in patients with diabetes mellitus. Acta. Med. Scand., ٢١١: ٤٦٣-٤٦٧.

Van Bezooijen, R.L.; Farih-Sips, H.C.; Papapoulos, S.E. & Lowik, C.W. ١٩٩٨. IL-١α, IL-١β, IL-٦ and TNF-α steady state mRNA levels analyzed by reverse transcription-competitive PCR in bone marrow of gonadectomized mice. J. Bone Miner Res., ١٣:١٨٥-١٩٤.

Van Geelen, J.M.; Doesburg, W.H.; Thomas, C.M.G.; Martin, C.B. ١٩٨١. Urodynamic studies in the normal menostural cycle: The relationship between hormonal changes in the menstrual cycle and urethral pressure profiles. Am. J. Obstet., ١٤١: ٣٨٤-٩٢.

Vargas, S.J.; Naprta, A.; Lee, S.K.; Kalinowski, J.; Kawaguchi, H.; Pilbeam, C.C.; Raisz, L.G. & Lorenzo, J. A.. ١٩٩٦. Lack of evidence for an increase in interleukin-٦ expression in adult murine bone, bone marrow, and marrow stromal cell cultures after ovari-ectomy. J. Bone Miner Res., ١١: ١٩٢٦-١٩٣٤.

Vinker, S.; Kaplan, B.; Naker, S.; Samuels, G.; Shapira, G.; Kitai, E. ٢٠٠١. Urinary incontinence in women: Prevalence, characteristics and effect on quality of life. A primary care clinic study. Isr. Med. Assoc. J., ٣: ٦٦٣-٦.

Virkola, R.; Westerlund, B.; Holthofer, H.; Parkkinen, J.; Kekomaki, M.; Korhonen, T.K. ١٩٨٨. Binding characteristics of *Escherichia coli* adhesion in human urinary bladder. Infect. Immun., ٥٦: ٢٦١٥-٢٦٢٢.

Walker, K.E.; S., Maghaddame-Jafari; C.V., Lockatell; D., Johnson & R., Belas. ١٩٩٩. ZAPA, the IgA-degrading loprotease of *Proteus mirabilis* is a virulence factor expressed specifically in swarmer cells. Mol. Microbiol., ٣٢: ٨٢٥-٨٣٦.

Wang, L.H.; Yang, X.Y.; Mihalic, K.; Xiao, W.; Li, D. & Farrar, W.L. ٢٠٠١. Activation of estrogen receptor blocks IL-٦ inducible cell growth of human multiple myeloma involving molecular cross-talk between ER & STAT٣ mediated by a co-regulator PIAS٣. J. Biol. Chem., ٢٧٦: ٣١٨٣٩-٣١٨٤٤.

Warren, J.W. ١٩٩٧. Catheter-associated urinary tract infection. Infect. Dis. Clin. North Am., ١١: ٦٠٩-٦٢٢.

Webster, R.G. ٢٠٠٠. Immunity to influenza in the elderly. Vaccine, ١٨: ١٦٨٦.

Whisler, R.L.; Williams, J.W.Jr.; Newhouse, Y.G. ١٩٩١. Human B cell proliferative responses during aging: Reduced RNA synthesis and DNA replication after signal transduction by surface immunoglobulins compared to B cell antigenic determinants $CD_{٢٠}$ and $CD_{٤٠}$. Mech. Aging Dev., ٦١: ٢٠٩-٢٢٢.

Wich, B.K. & Carnes, M. ١٩٩٥. Menopause and the aging female reproductive system. Endocrinol. Metab. Clin. North Am., ٢٤: ٢٧٣-٢٩٥.

Wilde, M.H. ٢٠٠٤. Urinary catheter management for the older adult patient. Clinical Geriatrics-ISSN, ١٢: ١٠٧٠-١٣٨٩.

Williams, P. & Tomas, J. M. ١٩٩٠. The pathogenicity of Klebsiella pneumonia. Rev. Med. Microbiol., ١: ١٩٦-٢٠٤.

Williams, P.; Lambert, P.A.; Brown, M.R.W. & Jones, R.J. ١٩٨٣. The role of the O & K antigens in determining the resistance of *Klebsiella pneumonia* to serum killing and phagocytosis. J. Gen. Microbiol., ١٢٩: ٢١٨١-٢١٩١.

Woodward, M.; Rumley, A.; Tunstall-Pedoe, H. & Lowe, G.D. ١٩٩٩. Associations of blood rheology and interleukin-٦ with cardiovascular risk factors and prevalent cardiovascular disease. Brit. J. Heamtol., ١٠٤: ٢٤٦-٢٥٧.

Yamamoto, S.; Terai, A.; Yuri, K.; Kurazono, H.; Takeda, Y.; Yoshida, O. ١٩٩٥. Detection of virulence factor in *Escherichia coli* by multiplex polymers chair reaction. FEMS Immunol. Med. Micrbiol., ١٢: ٨٥-٩٠.

Yamamoto, T.; Matsuda, T.; Junicho, A.; Kishi, H.; Saatcioglu, F. & Muraguchi, A. ٢٠٠٠. Cross-talk between signal transducer and activator of transcription ٣ and ER signaling FEBs Lett, ٤٨٦: ١٤٣-١٤٨.

Yen, S.S.; Tsai, C.C.; Naftolin, F.; Vandenberg, G.; Ajabor, L. ١٩٧٢. Pulsatile patterns gonadotrophin release in subject with and without ovarian function. J. Clin. Endocrinol. Metab., ٣٤: ٦٧١-٦٧٥.

Zharhary, D. ١٩٨٦. T cell involvement in the decrease of antigen-responsive B cells in aged mice. Eur. J. Immunol., ١٦: ١١٧٥.

Zhou, T.; C.K., Edwards, III & J.D., Mountz. ١٩٩٥. Preventing of age-related T cell apoptosis defect in $CD_{٧}$-fas transgenic mice. J. Exp. Med., ١٨٢: ١٢٩.

Zunino, P.; Geymonat, L.; Allen, A.G,; Legnani-Fajardo, C. & Maskell, D.J. ٢٠٠٠. Vieulence of *Proteus mirabilis* ATF isogenic mutant is not impaired in a mouse model of ascending urinary tract infection. FEMS Immunology & Medical Microbiology, ٢٩: ١٣٧-١٤٣.