Role of Secretory Immunity in the Diagnosis of Bacterial Mastitis in Lactating Women

A Thesis
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By
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٢٠٠٥

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

”إنَّ الذينَ أمنُوا وَعمِلوا الصَالِحاتِ يَهدِيهم رَبُّهمُ
بإيمانَهِم تَجرِي من تَحتِهِمُ الأنهارُ في جناتِ
النعيم* دَعواهُم فيها سُبحانَكَ اللهُمَّ وَتَحِيتهُمُ فِيها
سَلامٌ وآخِرُ دَعواهُم أَن الحمدُ لله رَبِّ العالَمين“

صدق الله العلي العظيم
سورة يونس (٩-١٠)

دور المناعة الإفرازية في تشخيص الخمج البكتيري لالتهاب الثدي لدى النساء المرضعات

رسالة مقدمة إلى مجلس كلية العلوم جامعة بابل وهي جزء من متطلبات نيل شهادة الماجستير في علوم الحياة / أحياء مجهرية

من قبل
عروبة كطوف البيرماني

ذو القعدة ١٤٢٥هـ كانون الثاني ٢٠٠٥م

الخلاصة:

هدفت هذه الدراسة إلى التحري عن الاستجابة المناعية المخاطية الخلطية في حالات التهاب الثدي لدى النساء المرضعات.

شملت هذه الدراسة على ستين مريضة بالتهاب الثدي و ٢٠ امرأة سوية سيطرة للدراسة. إذ جرى زرع الحليب لعزل المسبب في هذه الحالات ودراسة الاستجابة الخلوية غير المتخصصة (الخلايا البيض المحببة) بالإضافة لفصل الكلوبيولين المناعي المخاطي من عينات الحليب.

من زرع الحليب ثم تثبيت التنوع البكتيري المشترك مع التهاب الثدي، إذ كان المسبب البكتيري الأكثر شيوعاً كانت المكورات العنقودية الذهبية (٢٦.٦٪). وتم التعرف الجزئي على الكلوبيولين المناعي المخاطي من خلال تفاعل بيورين ومقاومة ٢ME وتخصص الضد إلى المسبب المشترك مع الالتهاب.

كذلك تم حساب قيم المتوسط الحسابي لتركيز الكلوبيولين المناعي المخاطي اللبني للنساء المريضات هي ٠.٨ ,٠.٧٥ ,٠.٨٤ ,٠.٨٢ ,١.٠٦٥ غم/لتر على التوالي كل من الممرضات البكتيرية المشتركة في إحداث الخمج من المكورات العنقودية الذهبية وبكتريا الزائفة الزنجارية وبكتريا الكيلبسلا الرئوية وبكتريا المكورات العنقودية البشروية وأخيراً بكتريا الاشيريجيا القولونية. في حين كانت قيمة المتوسط الحسابي لتركيز الكلوبيولين المناعي المخاطي اللبني لدى النساء السويات هي ٠.٤ غم/لتر.

أشارت هذه الدراسة إلى وجود علاقة خطية بسيطة بين تركيز الكلوبيولين المناعي اللبني وبين عيار الضد المتخصص. كذلك لوحظ التنافس المستضدي ومن النوع التنافس بين الجزيئة (Intermolecular competition) في الخمج المشترك.

اعتمدت هذه الدراسة على ثلاثة مقاييس لتشخيص الخمج وهي الاستجابة الخلوية وعيار الضد المتخصص وتركيز الكلوبيولين المناعي اللبني.

توصلت هذه الدراسة إلى حقيقة مقاومة الكلوبيولينات المناعية اللبنية للعامل المختزل مثل ٢-ME ماعدا حالتين أظهرت الاختزال في الفعالية التلازينة بمقدار عيار واحد.

Abstract

The aim of the present work is the investigation of humoral mucosal immunology of women lactational mastitis. ٦٠ mastitic milk sample from lactating women are collected, Moreover ٢٠milk samples are collected from healthy women, as control subject. Four parameters are followed to define lactational mastitis as, mastitis-associated pathogens, nonspecific cellular response, bacteria specific Ab titer and MMIg concentration. MMIg is resistant to ٢-ME as reducing agent except two cases elicited reduction at one tube.

The associated mammary pathogens are characterized by direct stained preparation, culture & biochemical investigations. The age range of the patient is ١٥-٤٥ years, the high occurrence is ٦٦.٧% in ٢٥-٣٤ year. No significant difference has been observed of the occurrence between affected breast ٥٣٪ & left affected breast ٤٧٪. Direct milk stained film shows neutrophiles ٤٤.٢٪, Lymphocyte ٣١ %, monocyte ٢٣.١٪, eosinophiles ١.٣٪ & basophiles ٠.٤٪. Monomicrobic affection (٨٥.٣٪) dominate that of dimicrobic ٦.٥٪ & sterile culture is (٦.٦٪). The microbial profile of mammary affection is *Staphylococcus aureus* (٢٦.٦٪). *Pseudomonas aeruginosa* (٢١٪), *lebsiella. pneumoniae* (١٦٪) *Staphylococcus epidermidis* (١٥٪) *Eschericha coli* (٣.٣٪) *Streptococcus viridans* (١.٦٪) *protheca* spp. (١.٦٪). On the other hand, the control subject reveals that only *S. epidermidis* detected means values of MMIg concentrations of patient are ٠.٨, ٠.٧٥, ٠.٨٤, ٠.٨٢ & ١.٠٦٥g\l in respective of *S. aureus, P. aeruginosa, K. pneumoniae, S. epidermidis* & *E. coli* mastitis in comparison to ٠.٤ g\l of control subject. Bacteria specific mucosal Ab titers of the patient are higher than those of the control subject. Linear correlation is demonstrated between MMIG concentration & bacteria specific Ab titers of the patient. Antigenic competition is noted is most of the encountered dimicrobic affection.

Certification

I certify that this thesis was prepared under my supervision at Department of Biology, College of Science, Babylon University and do hereby recommended that it be accepted in partial fulfilment of the requirement for the degree of Master of Science in Biology/ Microbiology.

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In view of the available recommendation, I forward this thesis for debate by the Examining Committee.

Prof. Dr. Ali S. M. Al-Aragy
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Examining Committee Certificate

We certify that we have read this thesis entitled "***Role of Secretory Immunity in the Diagnosis of Bacterial Mastitis in lactating women***" and, as an Examining Committee, examined the student "Oruba Kuttof Al-Bermani" in its content and that, in our opinion it meets the standards of a thesis for the degree of Master of Science in Biology/Microbiology.

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Conclusions & Recommendations

A. Conclusion

١. Lactational mastitis induce cellular and humoral immune responses.

٢. Mammary mucosal immunoglobulins (MMIgs) are large locally synthesized. A though transudated component from serum are found in the mastitic milk.

٣. Mammary mucosal immunoglobulin Ab are pathogen specific.

٤. Mastitic immunoglobulin are usually present in high agglutination titers.

٥. Concentration of the immunoglobulin increased during infection.

٦. Mammary mucosal immunoglobulin can be used as an infection-diagnostic probe of mastitis.

B. Recommendation

From the results of this work one may recommend further studies on mucosal and systemic immune responses in Lactational mastitis and in comparative sense.

CHAPTER ONE

Introduction

١-١ An immunologic Overview

As the systemic immune mechanisms for natural and adaptive immunity are important against the infectious diseases, natural and adaptive mucosal immune mechanism are also important in avoiding local infections and they are suitable for mucosal Vaccination (Brandtzage *et al.*, ١٩٩٨). Mammary gland (MG) is a dual function gland. First it is a specialized gland for production of milk. An externally secreted fluid is designed to nourish the neonates, babies and youngs. This fluid provides a diverse array of bioactive substances to the developing infants during critical period of brain, immune and gut development. Second, it represents a compartment in the common mucosal immune system (Roitt *et al.*, ٢٠٠١). MG is regarded as mucosal effector sites that are responsible for production of secretory immunoglobulin during antigenic stimulation of the inductive sites such as the gut associated Lymphoid tissue (GALT), nasopharyngeal associated Lymphoreticular tissue (NALT), and the skin associated lymphoid tissue (SALT) (Brandtzaeg *et al.*, ١٩٩٨; Vancott *et al.*, ٢٠٠٠; Playfair and Chain, ٢٠٠١). Mastitis is an inflammatory condition of the breast, which is accompanied by infection. It is usually associated with lactation (WHO, ٢٠٠٠). Mastitis in Lactating women stimulates local immunity via production of an increasing amount of secretory immunoglobulin (S-IgA) during this infection processes (Tomasi *et al.*, ١٩٧٦; Sordillo *et al.*, ١٩٩٧; Groer *et al.*, ٢٠٠٤).

١-٢ Reasoning

So far it can be checked that studies concerning human mastitic MG microbiology in this visicinity seem to be sparse. However, in a previous study by Al-Gebori (٢٠٠٣) an attempt forward the profile of mammary potential pathogenic bacteria and their antibiotic resistograms was made. This work is designed to be the first step that opens the way to further mucosal immunology of women lactational mastitis.

١-٣ The Aim

is to study the mucosal humoral immune responses associated with lactational mastitis. This can be achieved by following objectives.

١. Diagnosis of MG potential bacterial pathogens.

٢. Determination of the natural of inflammatory on cells.

٣. Determination of MG mucosal secretory Igs associated with infection.

٤. Making use of MG antibodies as diagnostic probe for lactaional mastitis.

CHAPTER TWO

Literature Review

٢-١ Mucosal Immunity

The mucosal surface area is at least two hundred times that of the skin, amounting to some ٤٠٠$m^{٢}$ in adult. Epithelial barrier is comprises by numerous chemical and physical innate mechanism that co-operate with specific adaptive immunity. The mains humoral mediators of the specific mucosal immune system are S-IgA and S-IgM antibodies (Brandtzaeg *et al.*, ١٩٩٨). The mucosal immune system has mechanism that discriminates between harmless antigens, commensal microorganisms and dangerous pathogens. The innate mucosal immune system is represented by epithelial and other mucosal cells and their products. (Taskalova-Hogeenova *et al.*, ٢٠٠٢). The mammalian host has evolved organized secondary lymphoid tissue in the upper respiratory and gastrointestinal (GI) tract region that facilitate antigen uptake, processing and presentation for induction of mucosal immune responses which are collectively termed inductive sites (Brandtzaeg *et al.*, ١٩٩٨; Vaneott *et al.*, ٢٠٠٠; Brandtzaeg, ٢٠٠٢). The gut associated Lymphoreticular tissue (GALT) which also termed as (peyer's patch) comprises major inductive sites in all of the most common experimental mammalian system. The major inductive tissue for intranasal inhaled antigens in human and mice appears to be the palatine tonsils and adenoids (nasopharyngeal tonsils) which together form a physical barrier of lymphoid tissues termed "Waldeyer's ring" that is referred to as a nasopharyngeal-associated Lymphoreticular tissue (NALT). NALT and GALT in human and mice, NALT and BALT (bronchus-associated lymphoreticular tissue) in other

experimental mammalian system comprise a mucosa-associated lymphoreticular tissues (MALT) net work (Vancott *et al.*, ٢٠٠٠). Features that distinguish MALT from the other systemic lymphoid tissues are (i) the epithelium that separates the tissue from the lumen contains a specialized cell type termed M cell (membranous cell) that is closely associated with lymphoreticular cell. This epithelial cell net work is called the follicular-associated epithelium. (ii) MALT contains organized regions which include a subepithelial area (Dome), B-cell zones with germinal centers containing IgA-committed B-cell [surface IgA^+ B-cell ($SIgA^+$ B-cell)], and adjacent T-cell region with antigen-presenting cell and high endothelial venules (HEV) (Vancott *et al.*, ٢٠٠٠). Characteristic features of mucosal immunity are: strongly developed mechanisms of innate defense, the existence of characteristic population of unique types of lymphocytes, colonization of mucosal and exocrine glands by cells originating from the mucosal organized tissues (common mucosal system) and preferential induction of inhibition of the responses to nondangerous antigens (mucosal tolerance) (Tlaskalova-Hogenova *et al.*, ٢٠٠٢). M-cell exhibit thin extension around lymphoreticular cell forming an apparent pocket (Lydyard and Gross, ١٩٩٨). M-cells are adaptive at uptake and transport of luminal antigens including proteins and particulates such as viruses, bacteria, small parasites and microsphers. Others, however, suggest that M-cells may also be involved in antigen processing and presentation (Vancott *et al.*, ٢٠٠٠, Brandtzaeg, ٢٠٠٢). Dome region of the peyer's patch consists of sparse blasma cells, macrophages, as well as B and T lymphocytes. The presence of macrophages are indeed the major antigen-presenting cell in addition to the denderitic cells. T-cells in this location are approximately ٧٥٪ while exhibited a T-helper (Th) cell phenotype (Vancott *et al.*, ٢٠٠٠). Distinct follicles (B-cell zones) are located beneath the dome area of the peyer's

patch and contain germinal centers which contain the majority of S-IgA B-cell (Brandtzaeg and Farstad, ١٩٩٩). The parafollicular T cells are found in the adjacent area to follicles. T cells are mature and more than ٩٧٪ of these T-cells use the $\alpha\beta$ heterodimer form of T-cell receptor (TCR). This area contains precursors of Th cells as well as precursors of cytotoxic T Lyphocytes (Brandtzaeg, ٢٠٠٢). Naive recirculating B and T-lymphocytes enter MALT through high endothelial venules. Antigen-activated and memory B-and-T-cell populations then emigrate from the inductive sites via lymphatic drainage, circulate through the blood stream and home to mucosal effector sites (Brandtzaeg *et al.*, ١٩٩٨; Vancott *et al.*, ٢٠٠٠; Brandtzaeg, ٢٠٠٢). These effector sites include more diffuse tissues, where antigen specific T and B-lymphocyte reside perform their respective function (cell-mediated immunity and regulatory function or antibody synthesis, respectively to protect mucosal surface) (Vancott *et al.*, ٢٠٠٠). Effector sites for mucosal immunity responses include the lymphoid cells in the lamina properia region of GI, the upper respiratory, reproductive tract, and secretory glandular tissues such as mammary, salivary, and lacrimal glands (Brandtzaeg *et al.*, ١٩٩٨). Gut lumen explains that the greatest density of IgA-producing cells is seen in intestinal lamina properia (Vancott *et al.*, ٢٠٠٠). Most evidence suggest that interaepithelial lymphocyte (IEL) also serve as effector cell (Brandtzaeg, ٢٠٠٢). $\gamma\delta$ T cells constitute approximately ٤٠٪ to ٥٠٪ of the IEL and are mainly CD_8^+, CD_4^-. There is an estimated one IEL for every four to six epithelial cells (Vancott *et al.*, ٢٠٠٠). IEL are continuously exposed to mucosally encountered antigens. As such, they form a first-line defense against translocation of the microflora and against invading microbial pathogens. $\gamma\delta$ T-cells have been shown to function as regulatory cells for the regulation of mucosal immune

responses. When presented with an environmental Ag, epithelial cells endocytose it and in instances processing it. The antigen-presenting cells at the effector regions of the mucosal immune system are biased toward a $Th_{٢}$ phenotype. Effector mechanism employed to protect mucosal surface includes cytotoxic T-Lymphocyte, effector CD_4^- Th cell for cell-mediated immunity ($Th_{١}$) and for S-IgA antibodies ($Th_{٢}$) responses (Vancott *et al.*, ٢٠٠٠).

٢-١-١ Distribution of Immune Cells to Mucosal Effector Sites

After antigen-induced activation, lymphoid memory and effector cells migrate rapidly to regional nodes, from which (propably after further differentiation) they go via lymph to the peripheral blood circulation. Such primed cells express adhesion molecules (homing receptors) specific for corresponding determinantes "addressins" on endothelial cells in mucosal and glandular tissues (Butcher and Picker, ١٩٩٦). Addressins selectively expressed on the endothelial cells include the p-selectins and L- selectins, as well as glycolipid ligands, simply termed peripheral lymph node and mucosal vascular addrissins, or PNAd and MAdCAM-١, respectively (Vancott *et al.*, ٢٠٠٠). In addition, ICAM-١ and ICAM-٢ addressins function by interaction with LfA (integrin $\alpha LB_{٢}$) on lymphocyte that are also homing receptors. Lymphocytes express integrins that represent a large class of molecules characterized by a heterodimeric structure of α and β-chains pairing of $\alpha_{٤}$ with $\beta_{٧}$ represents the major integrin form present for lymphocyte homing to Mad CAM-١ expressed on high endothelial venules peyers patch $G_{١}$ tract Lamina properia (effector sites) (Brandtzaeg *et al.*, ١٩٩٨).

٢-١-٢ $CD_{٤}$, αβ T Cells Cytokines in Immunoglobulin Secretion

$CD_{٤}^+$, αβ T-cell and select cytokines are essential for induction and regulation of IgA responses. The Th-cells are required for subsequent

switching to IgG and IgA antibodies (Brandzaeg, ٢٠٠٢). Isotype switching to IgA occurs in mucosal inductive sites such as GAIT. That terminal differentiation into plasma cells producing IgA occurs in effector sites (Brandtzaeg *et al.*, ١٩٩٩; Vancott *et al.*, ٢٠٠٠). For B-cell terminal differentiation, IL-٥, IL-٦, and possibly other cytokins are important for the continued presence of plasma cell undergoing, high-rate secretion of IgA antibodies. IL-١٠ has also been shown to play an important role in the induction of IgA of synthesis in human. These findings demonstrate that $Th_{٢}$ cytokins such as IL-٥, IL-٦, and IL-١٠ all play major role in the induction of IgA responses. IL-٢ that was produced by $Th_{١}$ has been shown to enhance IgA synthesis in Lps-stimulated B-cell culture. IL-٢ is also synergistically augmented IgA synthesis in B-cell culture in the presence of Lps and TGf-B (Vancott *et al.*, ٢٠٠٠). IFN has been shown to enhance the expression of polymeric Ig receptor (PIgR) which is an essential molecule for transport S-IgA (Brandtzaeg, ١٩٩٥).

٢-١-٣ Polymeric Immunoglobulin A Transport

The molecule which is responsible for transport of PIgA into mucosal secretions is the polymeric immunoglobulin (Ig) receptor (PIgA) or secretory component (Vancott *et al.*, ٢٠٠٠). Epithelial cells produce the full-length, ١٠٠kd PIgA that is associated with nonserosal surface of epithelial cells (Brandtzaeg *et al.*, ١٩٩٨). Plasma cells in the lamina properia and acinar regions of exocrine glands produce PIgA associated with the ١٥.٦ kd peptide (J chain). Upon external secretion, the IgA with PIgR and J. Chain is termed secretory IgA (S-IgA). Most authors suggest that secretion of S-IgA follows cleavage of a ٢٠kd component of PIgR and exocytosis. Disulfide bonded portion of the PIgR stabilizes S-IgA and renders the molecule more resistant to proteolytic digestion. PIgA

and pentameric IgM can be actively transported through serous type secretory epithelia (Brandtzaeg *et al.*, ١٩٩٨; Vancott *et al.*, ٢٠٠٠).

٢-٢ Mammary Gland as Compartment of Mucosal Immune System

Lactating mammary gland is part of the mucosal immune system, and milk Abs reflects antigenic stimulation of MALT in the gut as well as in the air ways. This fact has been documented by showing that S-IgA from breast milk exhibits Abs specificities for an arry of both intestinal and respiratory common pathogens (Goldman, ١٩٩٣; May, ١٩٩٤). For example neutralizing Abs against the enterotoxin of *E. coli* and *V. cholerae* have been found in human milk (Holmgren *et al.*, ١٩٧٦; Wold and Adleberth, ٢٠٠٠). The secretory Abs are thus highly targeted against infections agent in the mother's environment, which are likely to be encountered by the infant during its first week of life. Therefore, breast feeding represents an ingenious immunelogical integration of mother and child (Brandtzaeg, ٢٠٠٢). Integration of mucosal immunity between mother and neoborn with an emphasis on migration of primed B (and probably T) cells from gut-associated lymphoid tissue (GALT) such as peyer's patch via Lymph after the Journey through mesenteric Lymph node (MN), where they divided to differentiate. The precursors are clearly committed to IgA synthesis befor leaving the MN via the thoracic duct and peripheral blood, they logged in the periglandular connective tissue of the mammary gland during lactation and late pregnancy (Roux *et al.*, ١٩٧٧; Brandtzaeg *et al.*, ١٩٩٨; Roitt and Rabson, ٢٠٠٠; Brandtzaeg, ٢٠٠٢). Homing cell to the lactating mammary gland and Lamina properia of other organs such as GALT are only IgA-bearing cell and their surface IgA is conditions and it lacks receptors for complement

(Roux *et al.*, ١٩٧٧). This distribution of precursors for IgA plasma cell beyond the gut mucosa is crucial for glandular production and subsequent occurrence in breast milk of secretory Abs (S-IgA and S-IgM). By this mechanism, the breastfed infant will receive relevant secretory Abs directed against the microflora initially colonizing his mucosae (reflecting the mother's microflora). The protection provided by this humoral defense mechanism is most readily demonstrable in populations living in poor conditions. A beneficial clinical effect is also apparent in the industrialized world, even in relation to relatively common diseases such as otitis media and acute lower respiratory tract infections. (Golding *et al.*, ١٩٩٧).

٢-٣ Human Milk Constituent in Health and Disease

Proteins in human milk are specific to human mammary production. Some human milk protein is not nutritionally (Filteau *et al.* ١٩٩٩).

Lactoferrin role is to isolate external iron not transported iron for infant metabolism. Iron transported for infant metabolism is through milk casein and lipid. Another role of lactoferrin is binding to iron making it unavailable to pathogenic bacteria. Lactoferrin is bacteriostatic by its ability to prevent growth of bacteria, such as Staphylococci and Coliform, which have iron requirement (May, ١٩٨٨; May, ١٩٩٤).

S-IgA provides local immunity by building on the walls of the intestinal tract, the oro pharynx, and the urinary tract. Thus S-IgA protects the infant from infection by preventing invasion of organism through the mycosa (Wold and Adleberth, ٢٠٠٠). Human milk contains other antimicrobial proteins such as Lysozyme, that destroys

enterobacteriaceae and gram positive bacteria by cleaving peptidoglycan from the cell wall of the gram positive bacteria and the outer membrane of gram negative bacteria (Reiter, ١٩٧٨; Semba *et al.*, ١٩٩٩). Lysozyme in combination with complement and S-IgA, exhibited significant bacteriocidal activity to *E. coli* in vitro (Gordon *et al.*, ١٩٧٩; Sordillo *et al.*, ١٩٩٧).

Sodium concentration in normal human milk is low because milk is separated from other fluid by tight junction between mammary alveolar cells. However during mastitis inflammatory cells enter the milk and the inflammation is accompanied by an opening of tight junctions which allow intercellular fluid and plasma to enter the milk (Nievelle *et al.*, ١٩٩١; WHO, ٢٠٠٠). Elevated breast milk sodium concentration is considered to be sensitive indicator to mastitis (Connor, ١٩٧٩; Filteau *et al.*, ١٩٩٩). Other immunological factors that are present in human milk as, are secretory leukocyte protease inhibitor (SLPI) that protects tissue from degradation by proteases, which are released by neutrophiles, such as elastase and cathepsin G (Semba *et al.*, ١٩٩٩) and two chemokines, interleukin-٨ (IL-٨) and regulated on activation normal T-cell expressed and secreted (RANTES) are found in relatively high concentrations in human milk (Michie *et al.*, ١٩٩٨). IL-٨ plays a role in the recruitement and activation of neutrophiles and lymphocytes (Semba *et al.*, ١٩٩٩). RANTES achemohine produced by $CD_{٨}^{+}$ Lymphocytes, natural killer cells and mammary epithelial cells, is involved in the chcmotaxis of macrophages (Semba *et al.*, ١٩٩٩). Human milk contains growth modulator such as epidermal growth factor (EGF), nerve growth factor (NGF), and insulin like growth factors (IGFs). Transforming growth factor (TGF)-alpha, TGF-beta, and granulocyte colony-stimulating factor (G-CSF) are also identified in human milk. These growth modulators are

produced either by the epithelial cells of the mammary gland or by activated macrophages, Lymphocytes (mainly T cell), or neutrophiles in the milk (Wagner *et al*., ١٩٩٥).

Human milk immune factors are associated with mother diet, as viatmin A supplementation of mother during Lactation has sevreral benefils. It can possible increase in breast milk immune factors. These component of breast milk provide passive protection to young infants and stimulate development of the infant own immune system (Goldman, ١٩٩٣; Filteau *et al.*, ١٩٩٩).

٢-٤ Mononuclear Cell System

Macrophages are part of the mononuclear phagocyte system and are derived from bone-marrow haematobiotic cells. They circulate in the blood as monocytes and after leaving the circulation through post-capillary venules. They settle in the tissue as mature macrophage and present through the loose connective tissue of the peritoneum, lymphoid tissue, pulmonary alveoli brain (microglia), in the sinusoid of the liver (Kuppffer cells), spleen sinusoids and mesangial cell in the Kidney glomerulus. Bone marrow, the lymphatic sinuses of lymph nodes. Likewise, osteoclasts of bone and interdigitation denderitic cells of the Lymphoid tissue and spleen are probably derived from monocytes (MacSween and Whaley, ١٩٩٢; Roitt and Rabson, ٢٠٠٠). Monocytes and tissue macrophage are phagocytic cells. Phagocytosis is the process of engulfment and internalization by specialized cell of particular material which includes invading microorganismn damaged cell and tissue debris, phagocytosis is enhanced if the material to be phagocytozed is coated with certain plasma protein called opsonins, a process termed opsonization. Opsonin promotes the adhesion between the material and the phagocyte cell membrane. The three major opsonins are specific Abs

of IgG class; the activated third component of complement (C_3b) together with its inactivated form of C_3bi; and plasma fibronectin. Opsonization promotes phagocytosis because opsonins on the material to be phagocytosed are ligands for specific cell membrane receptors on the phagocyte (MacSween and Whaley, ١٩٩٢; Walter *et al.*, ١٩٩٦; Kaufman and Kabelltz, ١٩٩٨; Gould and Brooker, ٢٠٠٠). Phagocytosis occurs when macrophage engulf a pathogen. The first step is opsonization, the attachment of a ligand (particle or group of molecules) to the surface of the phagocytic cell, stimulating the action of the contractile proteins myosin and action that are present within the cytoplasm. Endocytosis follows the pathogen is engulfed, entering a vacuole created by the phagocytic membrane. Lysozome in the cytoplasm fuse with the vacuol, emptying strongly acidic enzymes (catalase and myeloperoxidase) on to pathogen and destroying it (Gould and Brooker, ٢٠٠٠). Monocytes are involved in resolution of the acute inflammatory response and are then removed by Lymphatics. However, in chronic inflammation there is continued recruitment of monocyt from circulation and this depends on the increased expression of adhesion molecules on endothelial cell membrane in response to cytokine, such as interleukin-١ (IL-١) that diffuses from the site of the infection and recruits phagocytic cell and the release chemotactic factors such as C_5a and Leukotriens B_4 (MacSween and Whaley, ١٩٩٢; Levin and Antia, ٢٠٠١). The prolonged survival of macrophage also contributes to the increased number of cells; if the causative agent is toxic to cells, they may survive for only a few days, but with some agent individual cells may survive for weeks or a month. Local proliferation may also occur, but it is thought to be only minor importance in most forms of chronic infection. At sites of inflammation macrophages become "activated" and this predominating in response to the cytokines interleukin-γ. Activated macrophages are larger than resting

macrophages. They show increased protein synthesis, have increased content of lysozomal enzymes, and increased phagocytic and bacteriocidal activities. Macrophages produce secretory products which contribute to the inflammation response as hydrolytic enzymes (Lysozyme, elastase, collagenase, plasmagenass, Lysozomal acid hydrolyses); archidonic acid (prostoglanidin, and Leukotriens); complement component (many of both classical and alternative pathway); oxygen metabolities (hydrogen peroxide and hydroxyle radical and superoxide); and cytokines (IL-١, TNF-α, IL-٦) (MacSween and Whaley, ١٩٩٢). In addition to their role in early nonspecific defenses, macrophages also play a key role in antigen processing and presentation (Roitt and Rabson, ٢٠٠٠). Antigens from ingested bacteria are processed within macrophages and appear on the membrane in association with major histocompatibility complexes (MHC) class II antigen that are required by other cells (Lymphocytes) for recognition of foreign antiges. Denderitic cells are found in the splenic white pulp and lymph node paracortex, but these are accessory cell for T-cell activation, it express MHC class II, as are involved in antigen processing and presentation. Follicular denderitic cell that are found in the germinal center of the Lymph node and spleen possessed cytoplosmic processes which can retain antigen for long period time. Denderitic cells are bone-marrow derived cells. They are often called "passenger" Leucocyte and are critically important initially in the rejection of allograft. One of the best characterized of the tissue denderitic cells are langerhans cell, of the epidermis, which take up antigen applied to the skin and then migrate to the draining Lymph node via the afferent Lymphatics. In the Lymph node, the langerhans cell differentiates into an interdigitaling, denderitic cells and only then become fully capable of activating T-cells. A similar pathway is believed to operate at the gastrointestinal and respiratory

mucosal surfaces which are exposed to large amount of antigen (MacSween and Whaley, ١٩٩٢; Roitt and Rabson, ٢٠٠٠). Macrophages are the predominant cell type found in the milk and tissue of healthy Lactating mammary gland. Macrophages constitutes ٤٠-٦٠٪ of the leukocytes in the breast milk. Macrophage in the milk manufactured Lysozyme (Sordillo *et al.*, ١٩٩٧). During mastitis mechanism of the Leucocytes, or extravasation, adhesive molecule P and E-selectin appear on epithelial cell after exposure to chemoattractant. Then integrins like $CD_{١١a}$, $CD_{١٨}$, and $CD_{١١b}$, $CD_{١٨}$, on Leucocytes act to form ICAM-١. in consequence, the Leucocyte attaches firmely to the endothelium and the rolling is arrested. In the last step, Leucocyte extravasate and then migrate through tissue under the influence of chemoattractant. This step related to $CD_{٣١}$ in Leucocte and endothelial cell (Smits *et al.*, ١٩٩٨).

٢-٥ Pathogenesis

Occurrence of mammary infection depends on the interraction between of the bacterial virulence factors and defense mechanism of the host. The disease of most concern in the one produced by pathogenic microorganism. However, as a prerequisite the pathogen must not only enter the mammary gland but also be able to survive and multiply in number sufficient to produce pathogenic effect (Walter *et al.*, ١٩٩٦). The normal mammary gland is protected from such attacks by several anatomic features as nipple straight forwardness and flow mechanism of normal milk which must be overcomed by invading bacteria (Editorial, ١٩٧٦). Organism involved in causation of mastitis varies in habitat, virulence and susceptibility to host barrier (Levin and Antia, ٢٠٠١). Bacterial adhesion to epithelial surfaces occurs after nipple fissure is an important prerequisite for infection (Editorial, ١٩٧٦; Editorial, ١٩٨١). Adhesion is generally established by binding of the bacteria to the

specific receptors of the host cell surface. Most receptors are oligosaccharide residues of glycoproteins and glycolipids on the membrane (Coppa *et al.*, ١٩٩٠). Adhesion process represents a first stage in infection and followed by pathogen multiplication in the targeted site. Invasion of the epithelial cell membrane represents the second stage of infection. Bacterial densities will eventually reach a level where it will be recognized by host constitutive defenses and an inflammatory responses will be initiated by chemical signals and cytokines will be generated (Fethetston, ٢٠٠١; Levin and Antia, ٢٠٠١). Some studies referred to that pili or fimbria of the gram-negative or gram-positive bacteria are responsible on the adhesion of bacteria to the epithelial cells (Virella, ١٩٩٧) of the mammary gland. These pili were called receptor specific fimbria because they adhere with glycolipid or glycoprotein receptor. The teichoic acid and Lipotiechoic acid are also responsible on adhesion of *S. aureus* and *S. pyogenes* to the epithelial cell of the mammary gland. Adhesion was occurred by fibronectin receptors that are found on the epithelial cell (Fetherston, ٢٠٠١). Matrix proteins like collagen and fibrinogen have also important role. The net result of the mentioned processes is either to dispose the invaders or the invader succeeded in avoiding defense mechanisms and to cause mastitis (Vercellotti, ١٩٨٥).

٢-٦ Role of Mucosal Immunoglobulin in Resistance to Infection

The mucous membrane is of vital importance in protecting the host, since it is the site where potential microbial pathogens and toxic agent make their first contact with the host. The mucous membrane defenses may either prevent implementation or eliminate the agent following an initial successful colonization (Tomasi, ١٩٧٦; Brandedtzage

et al., ١٩٩٨). The major antibodies on mucous membranes are S- IgA, which function primarily by binding with microorganism by preventing their contact with the host tissue (Lei Lu and Walker., ٢٠٠١; Sait *et al.*, ٢٠٠٣). Mucosal immunogloulin has an important role in resistance of infection as through following mechanisms

١- *Viral neutralization*

Secretory Abs may protect mucous membrane against invasion by potentially pathogenic viruses (Tomasi. , ١٩٧٦; Mc ghee and Kiyono., ١٩٩٩). Secretory IgA is believed to be the major antiviral defense and it has been detected in human milk to a variety of viruses. S-IgA is shown to be active against rotavirus, Cytomigalo-virus, reovirus type ٣, rubella, herpes simplex virus, mumps virus, influenza, and respiratory syncytial viruses (May., ١٩٨٨; May., ١٩٩٤).

٢- *Agglutination activity*

S-IgA and polymeric serum IgA exhibit more efficient agglutination activity than do the corresponding serum IgA or IgG-type Ab. In fact the IgA polymers are approximately ten times more efficient than corresponding monomere and are nearly good agglutinator of IgM (Tomasi, ١٩٧٦; Walter, ١٩٩٦). S-IgA represents a multivalent molecule which makes it more active for binding to gram-negative bacteria, particularly motile bacteria. This process enhances phagocytosis by macro phage neutrophiles or prevents essential neutrient factors to reaching for bacteria (Weir, ١٩٩٢; Strober and Gaems, ١٩٩٤; Watler; ١٩٩٦). $IgG_١$, $IgG_٢$ and IgM antibodies in milk can act as bacterial opsonins that enhance phagosytosis of neutrophil and microphages (Sordillo *et al.*, ١٩٩٧).

٣- *Inhibition of bacterial adherence to mucous membranes*

For bacteria to colonize mucous surfaces, they must first adhere to the outer surface of the mucous membrane (Editorial., ١٩٨٤). S-IgA antibodies are effective in preventing adherence of bacteria to epithelial cells (Tomasi, ١٩٧٦; Lei Lu and Walker, ٢٠٠١). Human milk contains specific Abs that may prevent attachment of bacteria to the infant gasro intestinal tract (May., ١٩٩٤; Wold and Adleberth., ٢٠٠٠). S-IgA is a primary factor responsible for preventing attachment of entro pathogenic *E. coli* to gut epithelium in breast-fed infant (De Araujo and Giugliano, ٢٠٠١; Noguera-Obsenza., ٢٠٠٣). S-IgA has receptors for type-١- fimbriated *E. coli* (Wold *et al*., ١٩٩٠). The O-linked oligosaccharide chains of the IgA١ subclass are receptors for *actinomyces naeslundii* that are part of dental plaque (May, ١٩٩٤; Wold and Adleberth., ٢٠٠٠). The very complex oligosaccharide chains of secretory component interact with *Hilicobacter pylori* (Wold and Adeleberth, ٢٠٠٠).

٤- *Toxin neutralization.*

S-IgA is the major mucosal immunoglobulin that acts against many bacterial toxins (Ada and Ramsay., ١٩٩٧). S-IgA in human milk involves in resistance of bacterial toxin, for example, neutralizing Abs against the enterotoxin of *E- coli* and *Vibrio cholerae* (Holmgren *et al*., ١٩٧٦; Wold and Adleberth, ٢٠٠٠).

٥- *Other Functions*

There is enough evidence supporting S-IgA function in trafficing antigens or microorganism out of the Lamina properia back into the intestinal lumen is compelling. It has also been proposed that S-Ig may

act neutralizing ‘blanket’ to prevent the translocation of antigens derived from G١ tract across the epithelial cell layer lining the mucosal surfaces in the first place. SIg has also been suggested to model the microbiota of the G١ microbiota with the mucosal immune system and subsequent coating of the microbiota with S-Ig (Sait *et al* ., ٢٠٠٣). SIg also helps to prevent the presense of allergic reaction in gastrointestinal tract, pulmonary and genetourinary tract (Mc Ghee and Kiyono., ١٩٩٩). S-IgA lacks ordinary complement- activity properties, so they perform their role without any inflammation in the fragil mucosal surfaces.

٢-٧ Antigenic Competition

Antigenic competition describes the phenomenon that an immune responses to a particular antigen may be dimenished or enhanced in the presence of other antigens compared to when the same antigen is given alone. Several mechanisms are probably involved, but one mechanism seems to be intracellular competition among small peptide (T-cell epitopes) for biding to MHC-structures and subsequent presentation on the surface for T-cell (The European Agency For the Evaluation of Medicinal products., ١٩٩٨). Competition between Lymphocytes for stimulatory and survival signal is thought to play a pivotal role in the homeostatic control of the immune system. The steady state population sizes of naive and memory T-cell compartments, and of the resting B cell and activated IgM-secreting B cell compartment are all independently regulated by cellular competition within each compartment. Due to competition between Lymphocytes, cellular death rates and/or renewal rates are density- dependent functions of the peripheral population sizes. By such density-dependent mechanism homeostasis is established. Previous experiments have suggested $CD^{+}_{٨}$ T- cell competition was studied in vivo by reconstituting Lethally irradiated mice with mixtures of

precursors bone marrow cell from normal nontransgenic and T- cell receptor (TCR)-transgenic mice. It was shown that proliferative capacity of the TCR-transgenic cell was diminished in the presence of other T-cell indicating that competition between T-cell occured. Morever, nontransgenic cells appeared to have selective advantage over TCR-transgenic cells in seeding the peripheral Lymphoid tissue suggesting that cells were competiting for antigens (Borphans *et al*., ١٩٩٩). T-cells inhibite each other indirectly by competiting for a limiting antigenic resource. Such function can be derived from the interaction between free T-cell and free antigenic sites on Apc (Borphans *et al*., ١٩٩٩; Taussing, ١٩٧٥). Antigenic competition is immunological phenomenon is noted in the combined vaccines (more than one component). The characteristic of immune response depends on the type of cells producing the response and the antigens stimulating the process. Combined vaccine may possess a boostability of the immune response. Protein antigens induce B-Lymphocyte to produce Ab aided by T helper cells, the immune response is potentially boostable, and IgG antibody predominant. In conjncated polysaccharide antigens stimulate B cell without T-cell help producing a nonboostable response of both IgG and IgM (The European Agency For the Evaluation of Medicinal products., ١٩٩٨).

٢-٨ MMIg as Infection Probe

S-IgA is dominant Ig isotype among the others induced and synthesized at mucosal surfaces of the body and it appears selectively in the secretion, such as saliva, tears, nasal fluid, sweet, colostrum and secretions of the lung, genitourinary, and gastrointestinal tracts. (Roitt and Rabson., ٢٠٠٠). S-IgA acts to prevent uptake of antigens, thus limiting the intensity and duration and the disease (Leilu and Walker., ٢٠٠١). S-IgA is normally found in human milk, and local synthesis which

is subsequentily shown to be quantitatively important, it is the source of antigenic stimulation (Tomasi, ١٩٧٦). High concentration of MMIg occur during inflammation of the breast (Roulin and Heeb., ١٩٩٩; Groer., ٢٠٠٤). Human urinary tract recurrent bacterial infections were associated with high mucosal immunolobulin with moderate to high specific Ab titers to the antigens made from the associated causal bacterial pathogens in signal infection as compared subject. It was evident that mucosal Ab titers were concomitant with the clinical case state, ٦ titers represent base line titers, ٨-١٦ represent as a suspected titers and, ٣٢-٦٤ represent clinical titers (Shnawa and Mahdi, ٢٠٠٤). Linear simple relationship between concentration, titers in specific infections for each of the associated agent (Rossen *et al*., ١٩٧٠; Al-Amedi, ٢٠٠٣) so that the mucosal immunoglobulin could be as infection probe (Shnawa and Mahdi, ٢٠٠٤).

CHAPTER THREE

Materials and Methods

٣-١ Solutions

٣-١-١ Formal Saline

It was prepared by adding ٠.٥ml formaldehyde (H-CHO) BDH company to ٩٩.٥ml normal saline. Final concentration of the formalin solution was ٠.٥٪. This solution was used as solvent to mammary mucosal immunoglobulins and for preparation of surface antigens of gram negative bacteria (Johnston and Thorp, ١٩٨٢).

٣-١-٢ Triss Buffer Solution

It was prepared by dissolving ١٢gm triss material [$NH_2C(CH_2OH)_3$], Taab company in a small amount of Distilled water. Volume was completed to ١ liter, then pH was adjusted to ٧, that was performed by adding HCL (٠.١N). This solution was used for poly-ethylenglycole (٦٠٠٠M.W) preparation (Johnston and Thorp, ١٩٨٢).

٣-١-٣ Polyethylenglycole (PEG, ٦٠٠٠ M.W)

Solution ٦٪ concentration of PEG solution was prepared by dissolving ٦gm PEG ٦٠٠٠ ($OH-(C_2H_4O)_nH$)-BDH company in a small amount of Triss buffer solution (Item ٣-١-٢), then the volume was completed to ١٠٠ml. This solution was used for Mucosal immunoglobulins separation (Johnston and Thorp, ١٩٨٢).

٣-١-٤ ٢-Mercapoethanol Solution

It was prepared at ٠.٠٥M concentration by adding ٣.٩ml ٢-ME (٢-Mercaptothanol), ($HS-CH_2-CH_2OH$)-calbiochem company to a little

quantity normal saline, then the volume was completed to ١ titer. This solution was used to know its influence on the mucosal Igs as a reducing agent (Farrell, ١٩٩٦).

٣-١-٥ Defattination Solution

It was composed of many ingredients including abselnt ethanol ٥٢٪, Xyline ٤٤٪, and glaical acetic acid ٤٪. Final volume was ١٠٠ml. This solution was used for removing fatty material and fixation of the milk on the slide so that that it could be staining by gram stain (Francis., ١٩٨٥).

٣-١-٦ Gram Stain Solution

It was prepared according to a manufactured company (Difco, ١٩٧٥). It was used for staining bacteria and milk Leucocytes.

٣-١-٧ Biuret Solution

It was prepared by dissolving ٣gm Cuper sulfate ($CuSO_{٤}$-٥$H_{٢}O$)-BDH company in ٥٠٠ml Distilled water and ٩gm of Sodium-Potassium tartarate ($NaKCuH_{٤}C_{٦}$.٤$H_{٢}O$)-BDH company was added ٥gm potassium Iodid-Evan company and ١٠٠ml Sodium hydroxide (NaOH) were added to the Solution, then the volume was completed to ١liter by Distilled water (Ross., ١٩٨٥).

٣-١-٨ Standard Albumin Solution

٦٠ gm\l concentration of albumin solution was prepared by dissolving ٦gm bovin albumin in a little amount of sodium hydroxide (٠.٦N). Volume was completed to ١liter. Standard dilutions were prepared of this solution by using of ٠.٦N NaOH, that include, ١:١, ١:٢, ١:٤, ١:٨, ١:١٦, ١:٣٢, ١:٦٤, ١:١٢٨, ١:٢٦٥, ١:٥١٢ (g\l). Standard dilutions

were used in preparing standard curve to know mammary mucosal immunoglobulin (MMIgs) concentration (Ross., ١٩٨٥).

٣-١-٩ Benzal konium Chloride

This solution was prepared at ١:١٠٠٠ concetration, where ٠.٢ml alkyl benzal konium chloride ($C_{٢٢}H_{٤}OCIN$)-Samarra-drugs company (stolk Concentration was ٥٠٪) was added to a little quantity from Distelled water. Volume was completed to ١٠٠ml. The solution was used for preparing surface antigens of gram positive bacteria (Banker, ١٩٨٠).

٣-٢ Reagent Solution

٣-٢-١ Catalase Reagent

It was prepared by adding ٣ml hydrogen peroxide ($H_{٢}O_{٢}$) to ٩٧ml distilled water, where its concentration become ٣٪ (Baron *et al.*, ١٩٩٤). Solution was used for diagnosis of catalse-producing bacteria that are degraded to hydrogen peroxide.

٣-٢-٢ Oxidase Reagent

It was prepared immediately by dissolving ١gm tetramethyl- para phenylen diamine hydrochloride material in ١٠٠ml distilled water, then it was dispensed in a dark container (Baron *et al.*, ١٩٩٤). This solution was used for diagnosis oxidase-producing bacteria.

٣-٣ Culture Media

Culture media were prepared according to the manufacturing companies.

٣-٣-١ MacConkey Agar Medium (Mast)

The medium was used for growing gram negative bacteria and for knowing its fermentation ability of lactose sugar.

٣-٣-٢ Blood Agar Medium

It was prepared by adding ٥٪ blood to the blood agar base (Mast), that was sterilized and cooled at ٤٥C^{o}.

٣-٣-٣ Manital Salt Agar Medium (Mast)

The medium was selective for diagnosis of *S. aureus*.

٣-٣-٤ Kligler Iron Agar (Oxide)

It was used for diagnosis of gram negative bacteria, as well as for knowing their ability to produce hydrogen sulfide ($H_٢S$) gas and sugar fermentation (Glucose and Lactose Sugar).

٣-٣-٥ Sugar Fermentation Medium

Medium is composed of:

١. Basal Medium

It was prepared by dissolving ١٠gm peton, ١gm meet extract (Difco), ٥gm Sodium chloride (NaCl) and ٠.٠٨gm phenol red (BDH) in one liter distilled water, then pH was adjusted to ٧.٤. Medium was distributed on test tubes and durham tubes were added to each test tube then sterilized by autoclave (Macfaddin, ٢٠٠٠).

٢. Sugar Solutions

Sugar solutions were prepared by dissolving ١gm sugar in ١٠٠ml distilled water and sterilized by filtration, then ٠.١ml sugar solution was added to each test tube (Item-١) containing ٥ml from the basal medium. Medium was used for diagnosig pathogenic bacteria that have the ability of fermentation of sugar.

٣-٤ Biochemical Identification Method

٣-٤-١ Catalase Test

Drops of ٣٪ hydrogen peroxide ($H_٢O_٢$) solution was laid on the slide, then a few amount of the bacterial growth was mixed with the solution by wood sticks. Free oxygen on the slide surface was detected. This result refers to a positive result (MacFaddin, ٢٠٠٠).

٣-٤-٢ Coagulase Test

Bacteria were growth in the nutrient broth at ٣٧C^o for ٢٤ hour, then ٠.١ml from this culture was transported to the sterilized test tube and ٠.٥ml plasma of the human or rabbite was added to it. Moreover, ٠.١ml of the noncontaminated nutrient broth with test bacteria was laid in other tube and ٠.٥ml plasma of the human or rabbite was added to it as control tube. These tubes were incubated at ٣٧C^o for ٤ hour in the water bath. Test tubes examination was performed at each hour for detecting coagulum formation because the coagulum may be degraded after its formation, and the tube that has no coagulum was left at room temperature over night for detecting formed coagulum in late.

٣-٤-٣ Growth on The Mannitol Salt Agar

This medium was striked with bacterial culture. A colour conversion to yellow in this growing refers to positive result and mannitol fermentation. This medium was selected for diagnosis or dignosig *S. aureus* (Macfaddin, ٢٠٠٠).

٣-٤-٥ Carbohydrate Fermentation and $H_٢S$ Gas production

Slants surfaces of this medium were striked with bacterial culture, whearse. The bottom medium was inoculated by stabbing method and incubated at ٣٧C^o for ٢٤ hour. pH changes of the slant and bottom were

scored . Sugar fermentation was detected by the colour change of the phenol red reagent to yellow colour. The gas production was shown as globes or cracks at the bottom of the medium, where H_2S-producing bacteria from black precipitation in the tube bottom (Macfaddin, ٢٠٠).

٣-٤-٦ Carbohydrate Fermentation Test

Carbohydrate fermentation medium was inoculated with bacterial culture and incubated at ٣٧C^o for ٢٤-٦٩ hour. Colour change of the medium from red to yellow colour and gas formation in the durham tube indicates a positive result (Macfaddin, ٢٠٠٠).

٣-٥ Milk Sample Collection

Collection of the milk sample was achieved from December ٢٠٠٣ to April ٢٠٠٤, where ٦٠ mastitic milk were collected form patient women, diagnosed by an independent surgeon. Moreover, ٢٠ sample of milk were collected from healthy women as control subject. Milk samples were collected in sterilized plastic tubes (AFMA disposable tubes) after sterilizing the women breast with ١٪ concentration of iodine solution, then sterilized after area dryness with ٧٥٪ al-cohole (Thomsen, ١٩٨٥). First drops were neglected and a ٤ml of milk collected, then samples were transported to the labrotary in an ice box.

٣-٦ Differential Leucocyte Count

Milk sample was submitted to the microscopic direct examination, for differential Leucocyte count. The following steps has been followed:

١. Milk smear was done on the slide.

٢. It was dried by air.

٣. Slide was immered in the defattination solution (Item ١-٥) for ٦minute.

٤. This slide was stained with gram stain for Leucocyte count (Fancis., ١٩٨٥).

٣-٧ Milk Culture

full loop milk loop full inocula was cultured by quadrate striking on the blood agar and MacConkey agar, then incubated at ٣٧C^{o} for ٢٤-٤٨h. Milk culture was done, by direct or indirect.

٣-٨ Isolate Identification

Mastitic bacterial isolates were diagnosed by morphological, cultural characteristics. Biochemical tests of gram negative bacteria were also confirmed by using the Epi-٢٠ system (Biomeriex-Company). On other hand, other diagnostic tests have also been used such as:

١. Catalase test.

٢. Oxidase test.

٣. Carbohydrate fermentation and $H_{٢}S$ gas peoduction.

٤. Coagulase test.

٥. Growth on mannitol salt agar-test.

٣-٩ Mammary Mucosal Immunoglobulin Separation

Mammary mucosal Igs (MMIgs) were separated from masitic milk in according to the Johnston and Thorp (١٩٨٢) method. Some modification were made on the separation method, using the following steps:

١) ٤ml of the milk sample were mixed with ٢ml isotonic saline (normal saline ٠.٩٪) and centrifuged at ٧٠٠٠ round/minute for ١h at ٤C^{o} in cooling centrifuge tubes.

٢) Clear aqueous layer between the floating fatty layer and the pellet was removed. This solution termed 'Clarified milk'.

٣) The clarified milk solution was stired and pH brought to ٤ by hydrochloric acid and centrifuged at ٦٠٠٠r/m for ١h at ٤C^{o}.

٤) Pellet was discarded (mostly casein) and equal volume of the ٦٪ PEG was added to the supernatant volume and left at the refrigerator ٤C^{o} for ١h, then centrifuged at ١٠٠٠r/m. Pellet was suspended in ٠.٥٪ formal saline and kept in the eppendroof tube at the freezer $\cong -18C^{o}$.

٣-١٠ Mesurment of Concentration of Mammary Mucosal Immnolobulins.

Biuret method was used for measurement of mammary mucosal Igs (MMIgs) concentration. This method was also used by Mahdi (٢٠٠٠) and Al-Amedi (٢٠٠٣) in measurement of concentration of urinary Igs. Biuret method was performed according to Ross (١٩٨٥), as in the following steps.

١. ٥ml of the biuret solution were laid in the sterilized tube.

٢. ٠.٢ml of the mammary mucosal Igs was added to above tube. It was considered as test tube.

٣. ٠.٢ml of distilled water was added to ٥ml of the biuret solution in other tube. This was considered as a control tube.

٤. These tubes were mixed by vortex and left at room temperature for ٣٠ minute.

٥. Optical density was measured on the ٥٤٠nm wave length after blanking equipment by using the control tube.

٦. Concentrations of MMIgs were calculated by using of straight line equation of the stand albumin solution.

٣-١١ Antigens Preparation

١- *Surface Antigens Preparation of the Gram Positive Bacteria.*

Surface antigens were prepared according to the method that was presented by Maccoy and Kennedy (١٩٦٠).

The following steps have been used in preparing antigens

١) Pure culture isolate was done on the blood agar medium, with avoiding of the frequent culture.

٢) ٦ml of the normal saline were added to the culture and bacterial growth was swept by sterilized loop, where the suspension could be obtained.

٣) Suspension was collected and mixed by vortex for ٣ minute.

٤) ٥ml were taken from the suspension (step ٣) and centrifuged at ٤٠٠٠r/m for ٥minute.

٥) Washing of the precipitation was performed by adding ٥ml of normal saline (٠.٩٪), mixed by vortex and then centrifuged at ٣٥٠٠r/m.

٦) Supernatant was discarded and the pellet was suspended by adding ٥ml of the benzalkonium chloride (١:١٠٠٠) and mixed by vortex.

٧) ١ml was taken from the suspension (step ٦) and tubed in the opacimeter tube (W.O.H international reference). Volume was completed by adding of the benzalkonium chloride to match the

turbidity to that of standard tube. Final concentration of the antigenic suspension was ١٠ international unite.

٨) Sterility test was done on the nutrient agar medium, a loopfull of the antigenic suspension was striked on the culture medium and incubated at ٣٧C^{o} for ٢٤h. The antigenic suspension gave no bacterial growth means sterile, otherwise non sterile.

٢- *Surface antigens preparation of gram negative bacteria (Heat killed antigens)*

Surface antigens were prepared in according to the methods that was presented by Svanborg-Eden *et al.*(١٩٨٥).

The following steps have been used to prepane antigens of gram negative bacteria.

١) Pure culture of the isolate was prepared on the MacConky agar medium.

٢) ٦ml of the formal saline were added to the culture, then bacterial growth was swept by sterilized loop where the suspension could be obtained.

٣) Suspension was collected and mixed by vortex for ٣ minute.

٤) ٥ml from the suspension were taken and centrifuged at ٤٠٠٠round/mimute for ٥ minute.

٥) Washing of the pellet was performed by adding ٥ml of formal saline and mixed by vortex then centrifuged at ٣٥٠٠r/m for ٥ minutes.

٦) Supernatant was discarded and the pellet was suspended by adding ٥ml of formal saline and mixed by vortex.

٧) ١ml was taken from the suspension (step ٦) and tubed in the opacimeter tube. Volume was completed by adding formal saline to match the turbidity to that of standard tube. Final concentration of the antigenic suspension was ١٠ international unite.

٨) Antigenic suspension was heat treated by water bath at ٦٠C^o for ١.٣٠ hr.

٩) Sterility test was done on the nutrient agar medium striked with antigenic suspension and incubated at ٣٧C^o for ٢٤ hr. If antigenic suspension gave no bacterial growth this was sterilized, otherwise it was non sterilized.

٣-١٢ Tube Agglutination Test

Tube agglutination test was done between bacterial antigen (which was prepared by benzalkonium chloride method or by heat killed antigens method) and mammary mucosal Igs (MMIgs) solution to determine the titer of specific antibody (specific mammary Igs). Test was performed according to the followed method of Aloisi (١٩٧٩), and Shnawa and Mahdi (٢٠٠٤):

١. Eight agglutination tubes were used and ٠.٢ ml of normal saline was added to each tube (the test of game positive bacteria) or ٠.٢ml of formal saline (the test of gram negative bacteria).

٢. ٠.٢ml of MMIgs was added to the first agglination tube and its contents were mixed.

٣. ٠.٢ml from the content of the first tube was transferred to the second tube, then ٠.٢ml from the second tube content was transferred to the third tube and soon to the seventh tube.

٤. ٠.٢ml from the seventh tube content was transferred to out. Dilutions series of the MMIgs in the tubes were (١:٢, ١:٤, ١:٨, ١:١٦, ١:٣٢, ١:٦٤, ١:١٢٨) respectively.

٥. ٠.٢ml from the antigenic suspension was added to each agglutination tube (eight tube was regarded as control tube) because there was no Ig in its content.

٦. These tubes were incubated at ٣٧C^{o} for ١٨-٢٤h. Agglutination tubes were examinated to notice of the fine agglutination clumps. Aggregated at the tube bottom. Slight movement of the tubes, the fine agglutination clumps distributed as clumps, it would refer to a positive result, but if distributed in a homogenous fashion causing turbidity of the solution, it would refer to a negative result.

٧. Microscopic examination was attempted in case of shaky visible agglutination result.

٣-١٣ ٢-Mercaptoethanol Effect on the Specific MMIgs

Specific agglutination activity of the mammary Igs against bacterial antigens. Tube agglutination test was performed by using ٢-ME (٠.٠٥ N), it replaces normal saline or formal saline. After incubation at ٣٧C^{o} for ٢٤ hr. These tubes were examinated to determine changes in the nature of the antibody-antigen reaction. If the result still positive at the same titer, mammary Igs don't affect by ٢-ME as a reducing agent of the specific agglutination activity. Reduction of specific antibody titer means break up of the S-S bonds, otherwise no such effect on S-S bonds as in case of secretory Igs are be found (Farell, ١٩٩٦).

٣-١٤ Statistics

Standard curve experiment used standard protein such as bovin albumin (٦٠g\l) and dilutions series were prepared. Optical density was measured for each dilution using biuret method at ٥٤٠nm wave length where the concentration of each was considered as Xi value, whearse, the optical density or each dilution was considered as yi values (Table-١). Straight line equation was obtained and found by simple linear regression analysis (Dawed and Al-Yas., ١٩٩٠). Moreover, the straight line equation of this standard curve was used to measure the MMIgs concentration. Straight line equation formula was

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

$\hat{y} =$ Concentration of the mammary Igs.

a + b :Constant values

xi : Optical density of the mammary Igs solution.

Regression factor (b) was calculated by the followed equation:

$$b = \frac{SSxy}{SSx} \qquad \text{or} \qquad b = \frac{\sum xy - \left(\sum x \frac{\sum y}{n} \right)}{\sum x^2 - \left(\sum x \frac{\left(\sum x \right)^2}{n} \right)}$$

where the Sxy represents summation of the deviation squares.

a. was calculated by the followed equation

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

where :

$\bar{y}$: mean of the concentration.

$\bar{x}$: mean of the optical density.

Inaddtion, correlation factor was calculated by the followed equation.

$$r = \frac{\sum xy - \left(\sum x \frac{\sum y}{n} \right)}{\sqrt{\sum x^2 - \left[\frac{(\sum x)^2}{n} \right] \sum y^2 - \left[\frac{(\sum y)^2}{n} \right]}}$$

Table-١ Statistical Features for Standard Curve of Albumine Solution Concentration and Their Standard Titrers

x_i	y_i	$x_i y_i$	x^2	y^2
٠.٣٣٩	٦٠	٢٠.٣٤	٠.١١٤٩	٣٦٠٠
٠.٢٢	٣٠	٦.٦	٠.٠٤٨٤	٩٠٠
٠.٠٦٥	١٥	٠.٩٧٥	٠.٠٠٤٢٢٥	٢٢٥
٠.٠٤٣	٧.٥	٠.٣٢٢٥	٠.٠٠١٨٤٩	٥٦.٢٥
٠.٠٣٢	٣.٧٥	٠.١٢	٠.٠٠١٠٢٤	١٤.٠٦
٠.٠٢٥	١.٨٧٥	٠.٠٤٦٨٧٥	٠.٠٠٠٦٢٥	٣.٥١٦
٠.٠١	٠.٩٣٧	٠.٠٠٩٣٧	٠.٠٠٠١	٠.٨٧٧
٠.٠٠٨	٠.٤٦٨	٠.٠٠٣٧٤٤	٠.٠٠٠٠٦٤	٠.٢١٩
٠.٠٠٥	٠.٢٣٤	٠.٠٠١١٧	٠.٠٠٠٠٢٥	٠.٠٥٤٧
٠.٠٠٣	٠.١١٧	٠.٠٠٠٣٥	٠.٠٠٠٠٠٩	٠.٠١٣٦
٠.٧٥	١١٩.٨٨١	٢٨.٤١٩	٠.١٧١٢٢١	٤٧٩٩.٩٨٩٦

$$b = \frac{\sum xy - \left(\sum x \frac{\sum y}{n} \right)}{\sum x^2 - \left[\frac{\left(\sum x \right)^2}{n} \right]} = \frac{19.427925}{0.114971}$$

b = ١٦٨.٩

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

$$\bar{a} = 11.9881 - 12.6675 = -0.67$$

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

$$\hat{y} = -0.67 + 168.9xi$$

$$r = \frac{\sum xy - \left(\sum x \frac{\sum y}{n} \right)}{\sqrt{\sum x^2 - \left[\frac{\left(\sum x \right)^2}{n} \right] \sum y^2 - \left[\frac{\left(\sum y \right)^2}{n} \right]}}$$

$$r = \frac{19.427925}{\sqrt{(0.114971)(3362.8442}} = \frac{19.427925}{386.62956}$$

r = ٠.٩٨٨

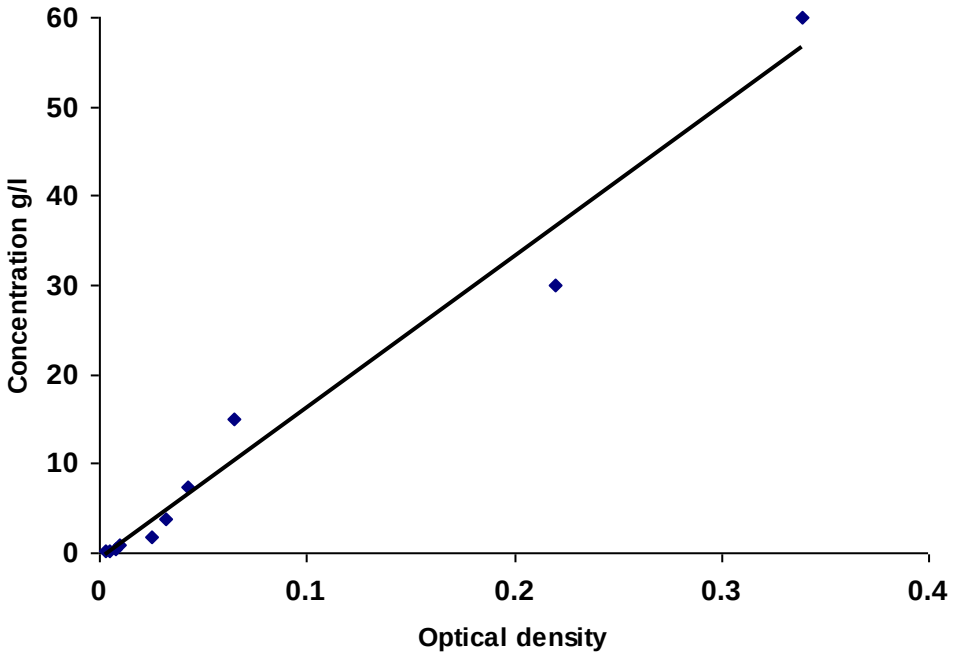
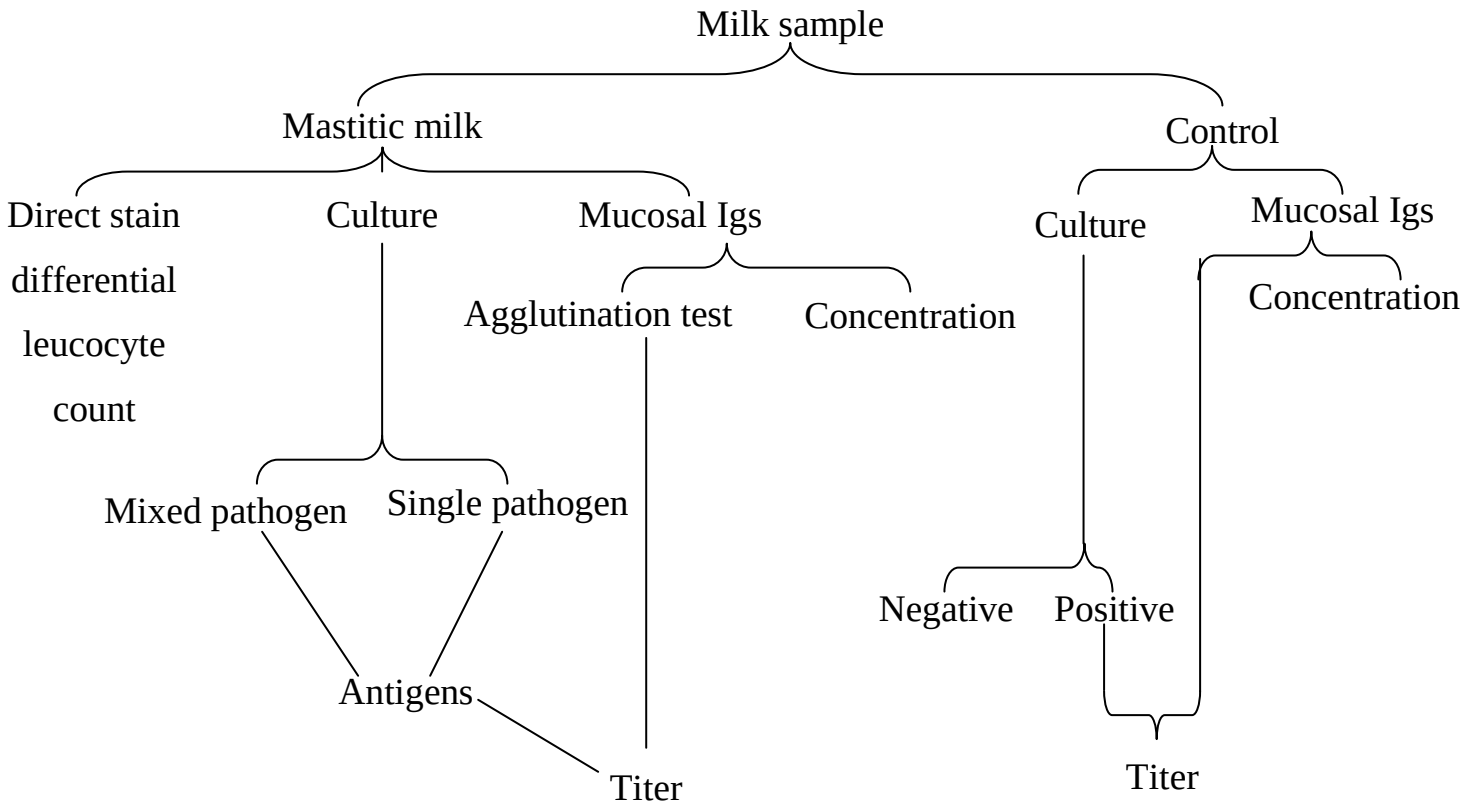


Fig ١. Standard curve for the Albumine solution and optical density

۳-۱۵ Study Menu



CHPTER FOUR

Results

٤-١ The Nature of Mammary Infection

٤-١-١ Age Distribution

The age range of the Lactating women that are suffering mastitis, in this study, was from ١٥-٤٥. It was divided into three age groups. High occurrence was noticed in the age group of the ٢٥-٣٤ years which constitutes of ٦٦.٧٪ of the patients, whearse the low occurrence was noticed in those of ٣٥-٤٥ years which constitutes ١٥٪ of the patient (Table ٢).

Table ٢: The age group distribution of mastitis among lactating women

Age groups	*Occurrence*	
١٥-٢٤	١١:٦٠	١٨.٣٪
٢٥-٣٤	٤٠:٦٠	٦٦.٧٧٪
٣٥-٤٥	٩:٦٠	١٥٪

٤-١-٢ Infected Breast

The affected breast was either right or left, of the Lactating women patients. It has been found that high occurrence was ٥٣٪ of the cases involve right breast, while the low occurrence was ٤٧٪ of the left breast. (Table ٣).

Table ٣: Occurrence of the effected breast in lactating women

Affected breast	*Occurrence*	
Right	٣٢:٦٠	٥٣%
Left	٢٨:٦٠	٤٧%
Right and Left	٠:٦٠	٠

٤-١-٣ Leucocyte Response

Table ٤ shows inflammatory cell in mastitic milk for ten cases. In which differential count was performed. The percentage of Nutrophiles, Lymphocytes, Monocytes Eosinophiles, and Basophiles in the milk smear were; ٤٤.٢٪, ٣١٪, ٢٣.١٪, ١.٣٪, and ٠.٤٪ respectively.

Table ٤: Infiltrated inflammatory cell of the mastitic milk.

Inflammatory	*Case number*										*Mean Percentage %*
	١	٢	٣	٤	٥	٦	٧	٨	٩	١٠	
Nutrophiles	٣٥	٣٠	٥١	٤٠	٥٠	٥٢	٥٧	٤٥	٤٠	٤٥	٤٤.٢
Lymphocytes	٣٧	٤٥	٣٠	٤٥	١٢	٣١	٣٠	١٨	٢٣	٣٧	٣١
Monocytes	٢٣	٢٢	١٧	١٥	٣٧	١٥	١٣	٣٦	٣٥	١٦	٢٣.١
Eosinophiles	٤	٢	٢		١	١		١		١	١.٣
Basophiles	١	١				١				١	٠.٤

٤-١-٤ Microbial Profile

Table ٥ explains the associated pathogens of mammary infection in the lactating women. Microbial pathogen was as follows: The first group is for single infecting microbes. It includes *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Kelebseilla pneumoniae*, *Staphylococcus epidermidis*, *Escherichia coli*, *Sterptococcus viridans* and *Prototheca* spp. High occurrence was ٢٦.٦٪ of *Staphylococcus aureus*, while the low occurrence was ١.٦٪ of *Sterptococcus viridans* and *Prototheca* spp. The second group is mixed infecting bacteria which includes *Staphylococcus aureus* and *K. pheumoniae* for one mastitis case, *Staphylococcus epidermdis* and *Pseudomonas aeruginosa* for two other mastitis cases, as well as *Staphylococcus aureus* and Actinomyces for one mastitis case. In third group, however the associated pathogens couldn't be recovered. This group constitutes of ٦.٦٪ of the patients.

Table ٥: Mastitis associated pathogens

I- Single Infecting Microbes.

Infecting microbes	***Occurrence***	
Staphylococcus aureus	١٦:٦٠	٢٧%
Pseudomonas aeruginosa	١٣:٦٠	٢١.٨%
Kelebseilla pneumoniae	١٠:٦٠	١٦٪
Staphylococcus epidermidis	٩:٦٠	١٥٪
Escherichia coli	٢:٦٠	٣.٣٪
Sterptococcus viridans	١:٦٠	١.٦٪
Prototheca spp	١:٦٠	١.٦٪
		٨٦.٩%

II- Mixed infecting bacteria

Infecting microbes	*Occurrence*	
Staphylococcus aureus and *Kelebseilla pneumoniae*	١:٦٠	١.٦٪
Staphylococcus epidermidis and *Pseudomonas aeruginosa*	٢:٦٠	٣.٣%
Staphylococcus aureus and *Actinomyces*	١:٦٠	١.٦٪
		٦.٥٪

III- No growth ٤:٦٠ ٦.٦٪

٤-٢ Mucosal Immunology of Lactational Mastitis

٤-٢-١ Monomcrobic Gram Positive Infection

٤- ٢- ١- ١ ***Staphylococcus aureus Mastitis***

As in (Table ٦) there were Sixteen cases of *Staphylococcus aureus* mastitis. Only one case didn't possess mammary immunoglobulin. The MMIg concentration was measured in (g\l) and considered as Xi value. On other hand, MMIg titers were considered as Yi values. Statistical features of *Staphylococcus aureus* mastitis were calculated by Linear regression analysis in which b value was ٧٢.٥٥, a value was -١.٠٤, from these constant values of a and b. Straight Line equation could be obtained. The correlation factor value was ٠.٧٦ that was debicated the linear relationship between concentration and titer of MMIg (Figure ٢). The correlation between concentration and titer was significant up to ٧٦٪ as r indicated.

Table ٦: Staphylococcus aureus mastitis and the associated mucosal immune response

I. Observation

Case number	*Age*	*Affected breast*	*MMIg*		
			Concentration (g\l)	*Titer without ٢-ME*	*Titer with ٢-ME*
١	٣٨	L	٠.٣	١٦	٨
٢	٤١	R	٠.٥	٣٢	٣٢
٣	٢٧	R	٠.٤	١٦	١٦
٤	٢١	L	١	٦٤	٦٤
٥	٢٨	L	٠.٤	٣٢	٣٢
٦	٢٨	R	٠.٧	٣٢	٣٢
٧	١٨	L	١.٤	١٢٨	١٢٨
٨	٣٠	R	٠.٢	٨	٨
٩	٢٦	L	٠.٦٧	١٦	١٦
١٠	٣٤	R	١.٧	٦٤	٦٤
١١	٣٠	L	١.٦	١٢٨	١٢٨
١٢	٢٨	R	١.٣	٣٢	٣٢
١٣	٣١	R	٠.٨٤	١٢٨	١٢٨
١٤	٢٨	R	٠.٨	٣٢	٣٢
١٥	٣٣	L	٠.٣	٣٢	٣٢
١٦	٣١	R	-	-	-

II. Statistical Features

$$b = \frac{\sum xy - \left(\sum x \frac{\sum y}{n} \right)}{\sum x^2 - \left[\frac{\left(\sum x \right)^2}{n} \right]} = \frac{246.53}{3.3977} = 72.55$$

$$\bar{a} = \bar{y} - b\bar{x} = -1.04$$

$$\hat{y} = -1.04 + 72.55xi$$

$$r = \frac{\sum xy - \left(\sum x \frac{\sum y}{n} \right)}{\sqrt{\sum x^2 - \left[\frac{\left(\sum x \right)^2}{n} \right] \sum y^2 - \left[\frac{\left(\sum y \right)^2}{n} \right]}} = \frac{246.53}{323.696} = 0.76$$

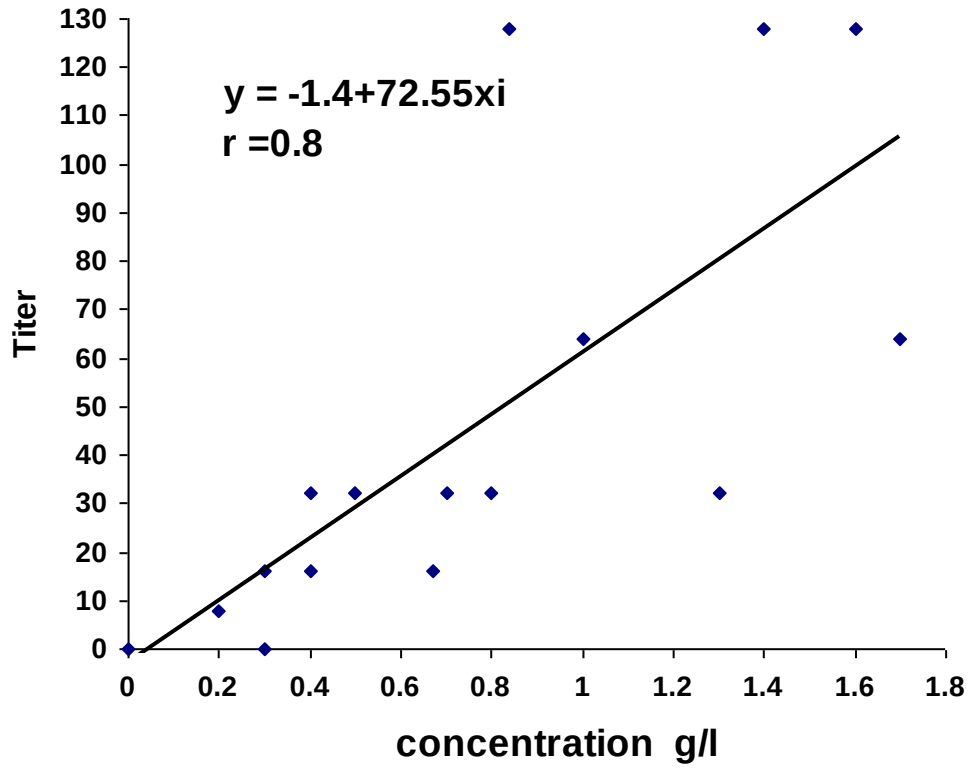


Fig. ٢ The Relationship between Titers and Concentration of Separated Mucosal Immunoglobulin associated with Staphylococcus aureus mastitis

٤- ٢- ١- ٢ ***Staphylococcus epidermidis Mastitis***

There were nine cases of *Staphylococcus epidermidis* mastitis. (Table ٧). MMIg concentrations were measured in (g\l) and considered as Xi value. Whearse the (MMIg) titers were considered as yi values. Statistical features were calculated by liner regression analysis, where the b value was (٩٢.٦), a value was (-٤.٨), the straight line equation could be obtained by the constant value of a and b. The correlation factor value was (٠.٧٧٨), which debicated the linear relationship between concentration and titer of MMIg (Figure ٣). The correlation between concentration and titer was significant up to ٧٧.٨٪ as r indicated.

Table ٧: Staphylococcus epidermidis mastitis and the associated mucosal immune response

I. Observation

Case number	***Age***	***Affected breast***	***MMIg***		
			Concentration (g\l)	***Titer without ٢-ME***	***Titer with ٢-ME***
١	٣٧	L	١	١٢٨	١٢٨
٢	٢٨	R	١.٧	١٢٨	١٢٨
٣	٣٣	R	٠.٣٦	٣٢	٣٢
٤	٢٨	L	٠.٩	٦٤	٦٤
٥	١٨	R	٠.٧	٦٤	٦٤
٦	٢٣	L	٠.٦	٣٢	٣٢
٧	٢٠	L	٠.٩	١٢٨	١٢٨
٨	٢٥	R	٠.٧	٣٢	٣٢
٩	٣٠	R	٠.٦	٣٢	٣٢

II. Statistical Features

$$b = \frac{\sum xy - \left(\sum x \frac{\sum y}{n} \right)}{\sum x^2 - \left[\frac{\left(\sum x \right)^2}{n} \right]} = \frac{105.48}{1.1465} = 92.6$$

$$\bar{a} = \bar{y} - b\bar{x} = -4.8$$

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

$$\hat{y} = -4.8 + 92.6xi$$

$$r = \frac{\sum xy - \left(\sum x \frac{\sum y}{n} \right)}{\sqrt{\sum x^2 - \left[\frac{\left(\sum x \right)^2}{n} \right] \sum y^2 - \left[\frac{\left(\sum y \right)^2}{n} \right]}} = \frac{105.48}{135.49} = 0.778$$

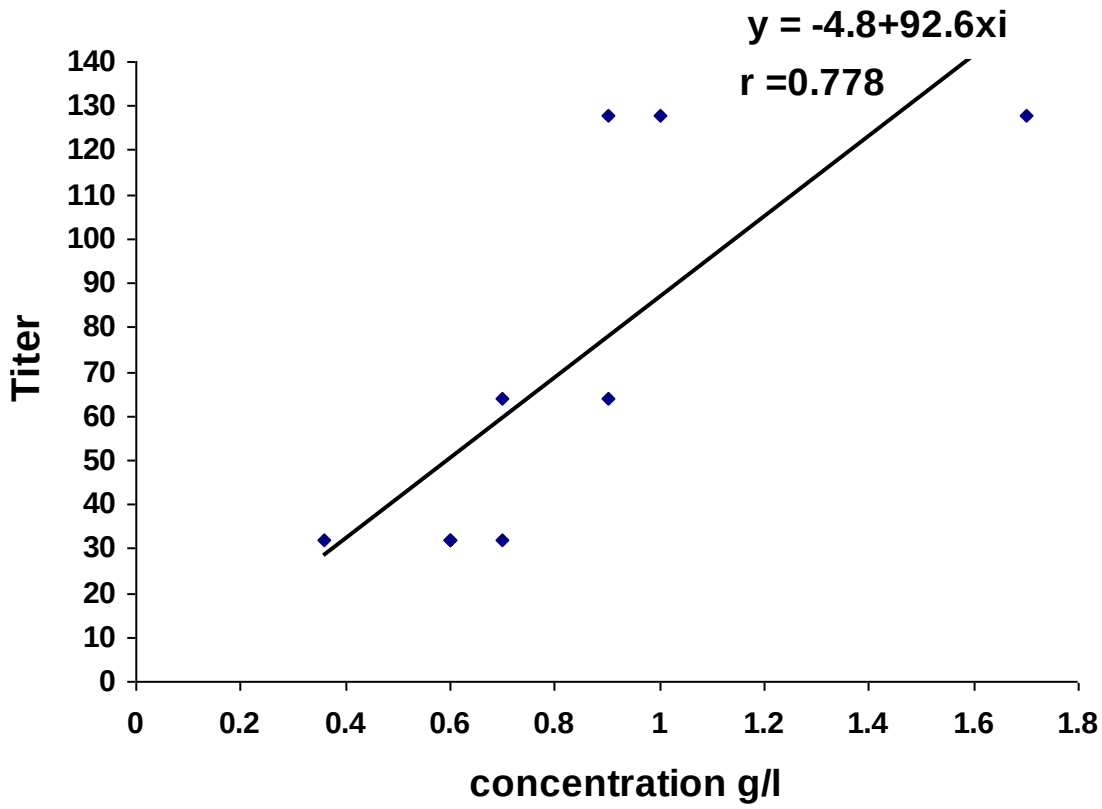


Fig. ٣ The Relationship between Titers and Concentration of Mucosal Immunoglobulin associated with Staphylococcus epidermidis mastitis

٤-٢-٢ Monomicrobic Gram-negative Infection

٤- ٢- ٢- ١ *Pseudomonas aeruginosa Mastitis*

Thirteen cases of *Pseudomonas aeruginosa* mastitis had been found (Table ٨). Only one case didn't MMIg, but had positive culture. The MMIg concentrations were measured in (g\l) and considered as xi value, while MMIg titers were considered as Yi value. (Table ٨).

Statistical features of *Pseudomonas aeruginosa* mastitis were calculated by Linear regression analysis, where the b value was (٥٦.٦١٧) and a value was (-٦.٤٦٢). Straight line equation was also obtained by using of the constant values of a and b. Correlation factor value was ٠.٧١ which was debicated the linear relationship between concentration and titer of MMIg (Figure ٤). The correlation between the concentration and titer was significant up to ٧٠.١٪ as r indicated.

Table ٨: Pseudomonas aeruginosa mastitis and the associated mucosal immune response

I. Observation

Case number	*Age*	*Affected breast*	*MMIg*		
			Concentration (g\l)	*Titer without ٢-ME*	*Titer with ٢-ME*
١	٤٠	R	٠.٧	٣٢	٣٢
٢	٣٤	L	١.٣٥	٦٤	٣٢
٣	٢٥	L	٠.٧٦	١٦	١٦
٤	٢٣	R	٠.٤	١٦	١٦
٥	٢٧	L	١.٤	١٢٨	١٢٨
٦	٢٦	R	٠.٧	١٦	١٦
٧	٢٤	R	٠.١٧	١٦	١٦
٨	٣٢	R	٠.٥	١٦	١٦
٩	٣٥	L	٠.٥	٣٢	٣٢
١٠	٢٣	L	٠.٥	٣٢	٣٢
١١	٢٧	L	٠.٧	٣٢	٣٢
١٢	٣٨	R	-	-	-
١٣	٣٨	R	٠.٣٤	٣٢	٣٢

II. Statistical Features

$$b = \frac{\sum xy - \left(\sum x \frac{\sum y}{n} \right)}{\sum x^2 - \left[\frac{\left(\sum x \right)^2}{n} \right]} = \frac{101.04}{1.7846} = 56.617$$

$$\bar{a} = \bar{y} - b\bar{x} = -6.462$$

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

$$\hat{y} = -6.462 + 56.617xi$$

$$r = \frac{\sum xy - \left(\sum x \frac{\sum y}{n} \right)}{\sqrt{\sum x^2 - \left[\frac{\left(\sum x \right)^2}{n} \right] \sum y^2 - \left[\frac{\left(\sum y \right)^2}{n} \right]}} = \frac{101.04}{142.182} = 0.71$$

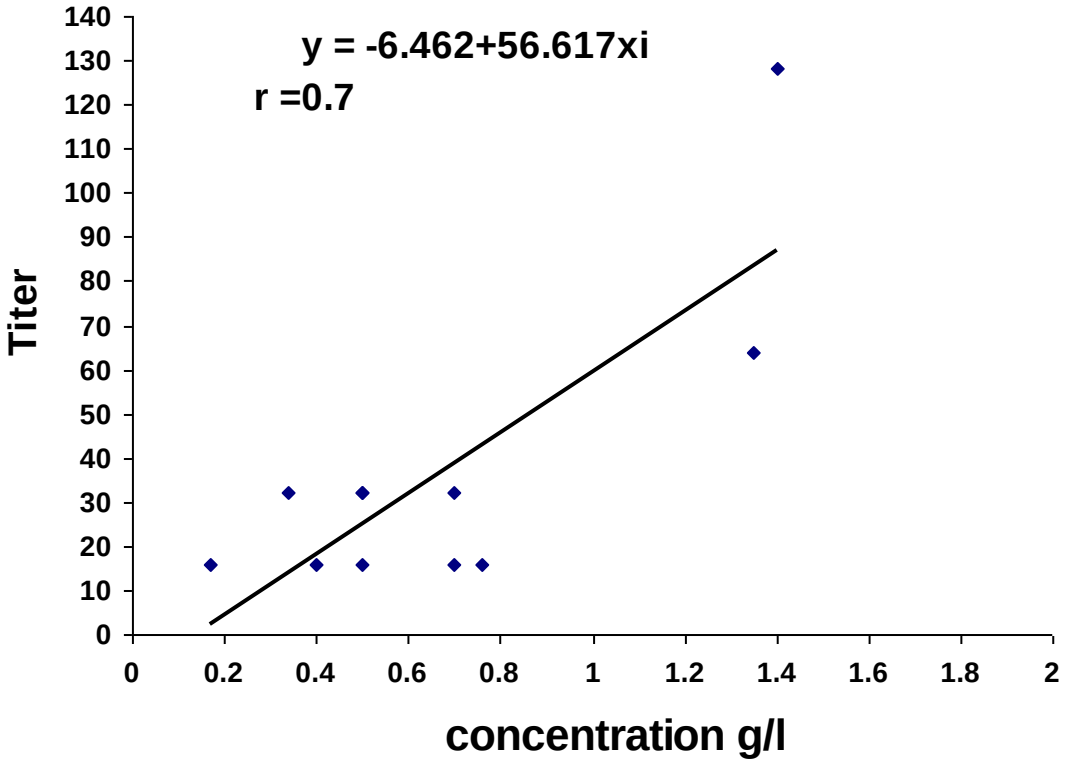


Fig. ٤ The Relationship between Titers and Concentration of Separated Mucosal Immunoglobulin associated with Pseudomonas aernginosa Mastitis

٤- ٢- ٢ ***Klebasiella pneumoniae* Mastitis**

Ten cases of *Kelebseilla pneumoniae* mastitis had been found (Table ٩). MMIg concentrations were measured in (g\l) and considered as xi values, while the MMIg titers were considered as yi values. Their statistical features were calculated by linear regression analysis, where the b value was (٩٤.١) and a value was (-٢٢.١). Straight line equation could be obtained by the b and a constant values. In addition the correlation factor value was ٠.٧٣ that was debicated the MMIg, (Figure ٥). The correlation between concentration and titer was significant up to ٧٣٪ as r indicated.

Table ٩: Kelebseilla pneumoniae mastitis and the associated mucosal immune response

I. Observation

Case number	*Age*	*Affected breast*	*MMIg*		
			Concentration (g\l)	*Titer without ٢-ME*	*Titer with ٢-ME*
١	٢٧	R	٠.٥	٨	٨
٢	٣٥	R	١.٣٥	١٢٨	١٢٨
٣	٢٤	L	٠.٦٧	٨	٨
٤	٢٧	R	٠.٥	٦٤	٦٤
٥	٣٤	L	١.٤٣	١٢٨	١٢٨
٦	٢٥	R	١.٤٣	٦٤	٦٤
٧	٣١	L	٠.٣	٨	٨
٨	٢٥	L	٠.٧	٣٢	٣٢
٩	٣١	R	٠.٧	٨	٨
١٠	٢٥	R	٠.٩	١٢٨	١٢٨

II. Statistical Features

$$b = \frac{\sum xy - \left(\sum x \frac{\sum y}{n} \right)}{\sum x^2 - \left[\frac{\left(\sum x \right)^2}{n} \right]} = 94.1$$

$$\bar{a} = \bar{y} - b\bar{x} = -22.1$$

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

$$\hat{y} = -22.1 + 94.1xi$$

$$r = \frac{\sum xy - \left(\sum x \frac{\sum y}{n} \right)}{\sqrt{\sum x^2 - \left[\frac{\left(\sum x \right)^2}{n} \right] \sum y^2 - \left[\frac{\left(\sum y \right)^2}{n} \right]}} = \frac{145.872}{198.6} = 0.73$$

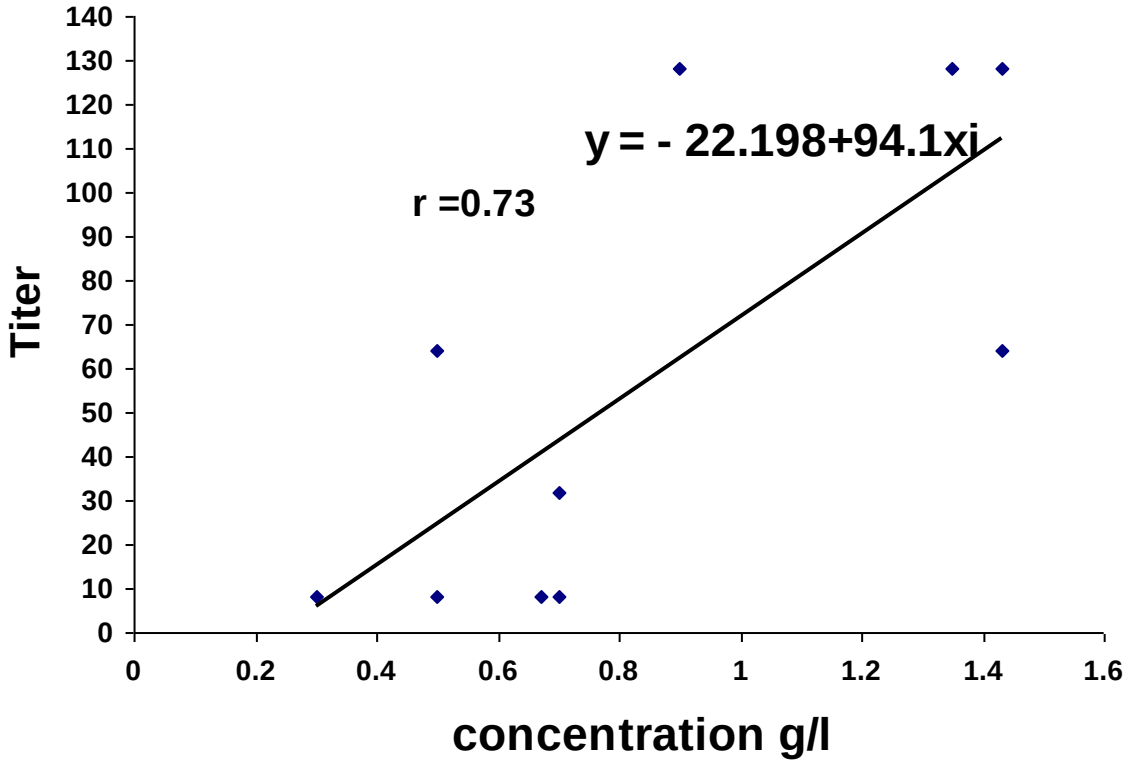


Fig. ٥ The Relationship between Titers and Concentration of Mucosal Immunoglobulin associated with Kelebseilla pneumoniae Mastitis

٤- ٢- ٣ ***Escherichia coli* Mastitis**

Two *Escherichia coli* mastitis cases were demonstrated. It was not given statistical features.

Table ١٠: Escherichia coli mastitis and the associated mucosal immune responses

Case number	***Age***	***Affected breast***	***MMIg***		
			Concentration (g\l)	***Titer without ٢-ME***	***Titer with ٢-ME***
١	٣٠	L	٠.٧	٣٢	٣٢
٢	٢٧	R	١.٤٣	١٢٨	١٢٨

٤-٢-٣ Dimicrobic Infection

There were four cases of mixed infection mastitis. One case was *Staphylococcus aureus* and *Kelebseilla pneumoniae*, two infection cases were *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, and one infection cased by *Staphylococcus aureus* and *actinomyces* as shown in (Table ١١).

Table ١١: Dimicrobic mastitis and the associated mucosal immune response

Case number	***Age***	***Affected breast***	***Associated pathogens***	***MMIg***		
				Concentration (g\l)	***Titer without ٢-ME***	***Titer with ٢-ME***
١	٣٣	R	*Staphylococcus aureus* and *Kelebseilla pneumoniae*	٠.٥	٣٢ and ١٦	٣٢ and ١٦
٢	٣٠	R	*Staphylococcus epidermidis* and *Pseudomonas aeruginosa*	٠.٣	١٢٨ and ١٦	١٢٨ and ١٦
٣	٤٥	L	*Staphylococcus epidermidis* and *Pseudomonas aeruginosa*	٠.٣٤	٤ and ٦٤	٤ and ٦٤
٤	٣٤	L	*Actinomyces* and *Staphylococcus aureus*	١.٤	٦٤ and ٣٢	٦٤ and ٣٢

٤-٢-٤ Miscellaneous Infection

Table ١٢ shows two types of microbes, which were associated with mammary infection , in Lactating women. One of it was *Streptococcus viridans* and the other was *Prototheca* spp.

Table ١٢: Miscellaneous Pathogens and the associated mucosal immune response

Case number	*Age*	*Affected breast*	*Pathogen*	*MMIg*		
				Concentration (g\l)	*Titer without ٢-ME*	*Titer with ٢-ME*
١	٢٧	L	*St. viridans*	١.٣٥	٦٤	٦٤
٢	٢١	R	*Prototheca* spp	٠.٥	٦٤	٦٤

٤-٢-٥ Culture Negative Mastitis

Four cases of the mastitis were the associated pathogens couldn't be identification, (Table ١٣).

Table ١٣: Mastitis of undetermined cause and the associated immune response

Case number	*Age*	*Affected breast*	*MMIg Concentration (g\l)*
١	٢٩	R	٠.٣
٢	٢٨	L	٠.٤
٣	٣٢	L	٠.٩٥
٤	٢٠	L	٠.٦٢

٤-٢-٦ Mastitic Ig as Diagnostic Probe

Table ١٤ explains the means, median, and range for each of the mastitic MMIg which was stimulated by the infecting bacteria like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Kelebseilla pneumoniae*, *Staphylococcus epidermidis*, *Escherichia coli* respectively, and of the MMIg normal milk (control subject).

Table ١٤: Mean, mediun, range of concentrations and titers of MMIg for each of mastitic milk Ig and of the normal milk Ig.

Associated pathogen	***Mean***		***Mediun***		***Range***	
	C	***T***	***C***	***T***	***C***	***T***
Staphylococcus aureus	٠.٨	٥٧	٠.٩	٦٨	٠.٢-١.٧	٨-١٢٨
Pseudomonas aeruginosa	٠.٧٥	٣٦	٠.٧٨	٧٢	٠.١٧-١.٤	١٦-١٢٨
K. pneuminiae	٠.٨٤	٥٧.٦	٠.٨٦٥	٦٨	٠.٣-١.٤٣	٨-١٢٨
Staphylococcus epidermidis	٠.٨٢	٧١	١.٠٣	٨٠	٠.٣٦-١.٧	٣٢-١٢٨
Escherichia coli	١.٠٦٥	٨٠	١.٠٦٥	٨٠	٠.٧-١.٤٣	٣٢-١٢٨
Normal milk	٠.٤٠٦	٣	٠.٥٧٥	٣	٠.٠٥-١.١	٢-٤

C: concentration, T: titer

٤-٣ Control Subject

The control subject (normal milk) was studied in comparison with mastitic milk. There were twenty sample of normal milk. Two milk samples were positive culture and titers were in respective ٢ and ٤. Low value of MMIg concentration was ٠.٠٥ (g\l), whears the high value of MMIg concentration was ١.١(g\l) as shown in (Table ١٥)

Table ١٥: Normal subject, bacteriologic immunologic investigation

Case number	***Culture***	***MMIg***		
		Concentration (g\l)	***Titer without ٢-ME***	***Titer with ٢-ME***
١	No growth	٠.٣	-	-
٢	-	١.١	-	-
٣	-	٠.٦	-	-
٤	-	٠.٠٥	-	-
٥	-	٠.٣	-	-
٦	-	١.١	-	-
٧	-	٠.٠٨	-	-
٨	-	٠.٢	-	-
٩	*Staphylococcus epidermidis*	٠.٥	٢	٢
١٠	-	٠.٤	-	-
١١	-	٠.٢	-	-
١٢	-	٠.٧	-	-
١٣	-	٠.٣	-	-
١٤	-	٠.١	-	-
١٥	-	٠.٥	-	-
١٦	*Staphylococcus epidermidis*	٠.٧	٤	٤
١٧	-	٠.٤	-	-
١٨	-	٠.٠٩	-	-
١٩	-	٠.٣	-	-
٢٠	-	٠.٢	-	-

CHAPTER FIVE

Discussion

٥-١ Natural of Mammary Infection

٥-١-١ Age Distribution

High infection rate was noted among the ٢٥-٣٤ year age group, which constituted ٦٦.٧٪, whearse the infection rate decrease when the age become down to ٢٥ year and ages above ٣٤ years. This result was agreement with that of other workers as Vogel *et al.* (١٩٩٩) and Al-Gebori (٢٠٠٣) which were constituted ٦٨.٧٪, ٦٥.٧٪ respectively. In addition, such finding was confirmed by Jonsson and pulkinen (١٩٩٤) who found reduces infection rate in the ages under ٢١ years and above ٣٤ years. This result did not match with Kaufmann and Foxman (١٩٩١) result, who found higher infection rate in the older women ages.

٥-١-٢ Affected Breast

It has been shown that the incidence of affected right breast is higher (٥٣٪) than that of the left breast (٤٧٪). This result was disagreed with that of WHO (٢٠٠٠) where ٣٧-٥٢٪ of cases involve the right breast and ٣٨-٥٧٪ of cases involve the left breast while the bilateral mastitis in ٣-١٢٪. However which of the each side is affected have been conducted no significant differences has been observed. Breast has been affected as a result of milk stasis. It has been observed that many mothers find it easier to attach their infant to the breast on one side than on the other and it has been found suggested that poor attachment leading to milk stasis and mastitis occur on the side that was more difficult (WHO, ٢٠٠٠).

٥-١-٣ Leucocyte Response

Differential leucocyte count was done and the result has been observed. It was ٤١.١٪ for neutrophils, ٣١.١٪ for lymphocytes, ٢٣.١٪ for monocytes, ١.٣٪ for eosinophiles and ٠.٤٪ for basophiles. The neutrophiles infilteration is pathognomic acute of infection, while the monocyte with neutrophiles infilteration reflectes for subacute infection. A predominantly monocyte cellular response indicate chronic infection, whearse the lonely appearance of lymphocyte in cell infilteration has been observed in resident infection (Walter *et al.*, ١٩٩٦; Rook *et al.*, ١٩٩٨). The Leucocytes in milk consist of Lymphocytes, neutrophiles, polymorphonuclear Leucocyte (PMN), and macrophages. Lymphocytes together with antigen-presenting cells function in the generation of an effective immune response. In the normal mammary gland, macrophages are the predominant cells which act as sentinels to invading mastitis causing pathogens. Macrophages release chemical messenger called chemoattractant that cause migration of PMN to influmation site (Paap *et al.*, ٢٠٠٠). Local plasma cells also increase in number late in pregnancy and during lactation (Roux *et al.*, ١٩٧٧; Brandtzaeg., ٢٠٠٢). Another subsets of Lymphocyte is T-cell. It migrates to the mammary gland to increase ٧-١٠ fold with onest of lactation and remained high during the first ٢ weeks post partum (Manning and Parmely, ١٩٨٠). Eosinophils showed increase in one mastitis case that may related with regulation on activation normal T-cell expressed and Secreted (RANTES). This have been found in relatively high concentration in human milk. RANTES a chemokine involved in the chemotaxis of monocytes, eosinophiles, and memory CD_4 has been detected in human milk (Michie *et al.*, ١٩٩٨).

٥-١-٤ Microbial Profile

The monomicrobic infection was predominant (Table-٥) which constitutes ٨٥.٣٪, whearse the dimicrobic infection was less common. It constitutes ٦.٥٪. This result agreed with that of Thomsen (١٩٨٢) who suggests that mammary gland infection was tool place commenly by single infecting microbe. This result was disagreed with that of Niebyl *et al*. (١٩٧٨) who was found that multimicrobic growth in mammary infection and constituted ٤٢٪. There was third status that was Steril culture. The causes of the culture negative mastitis were:

١. Infection is nonbacterial. It was termed nonbacterial infection, and caused by Cryptococcus or viruses (WHO, ٢٠٠٠).

٢. Mastitis may be caused by *Chlamydia, Mycoplasma* and Mycobacterium that are not grown in the culture conditions, used in this study (Thomsen *et al.*, ١٩٨٣).

٣. Non infectious mastitis can be caused by trauma or blocked duct which are regarded as a predisposing factors for mastitis (Marchant, ٢٠٠٢).

٥-١-٥ Mastitis Associated Pathogens

١. ***Staphylococcus aurueus*** was most frequent isolate of the mastitis in Lactating women. This result is consistent with Bulmstein *et al.* (٢٠٠٣), who suggested that *S. aurueus* is most common cause and typically originating from the nursing child. Moreover this result was supported by Semba and Neville (١٩٩٩) who referred that *S. aurueus* is most common pathogen in the mammary infection, where it constitutes ٥٠٪. It is also associated with breast abscess infection. Non lactating breast abscess is uncommon. In the pureperium, breast abscess tend to occure in the setting of epidemic of *Staphylococcus*

infections involving the newborn infant and nursery. Pathogenic *Staphylococci* initially colonize the nasopharynx of the newborn leading to a high rate of the colonization of the milk and ducts of the nursing mothors. Maternal infection may develop from ١٠-١٤days postpartum. *S. aurueus* is affending pathogen in the vast majority of cases which can be confirmed on culture and gram stain of the breast milk (Wyne and Cohen, ٢٠٠٠) Blumstein *et al.* (٢٠٠٣) who have been shown that *S. aureus* and *Streptococcus* spp are the most common organism isolated in puerperal breast abscess. While the nonpuerperal abscess typically contain mixed infectious (*S. aureus, Streptococcus* spp) and anaerobes.

٢. *P. aeruginosa*

Thirteen cases of *P. aeruginosa* mastitis (٢١٪) were detected (Table ٧). In the previous study of Al-Gebori (٢٠٠٣), four cases of the *P. aeruginosa* mastitis were diagnosed (٤.٠٤٪). It important cause of mammary infection. Pathogenicity of this bacteria may increase in the presence of bacteria in the damaged tissue as a result of the opportunistic adherence (Thomsen *et al.*, ١٩٨٤). As well as attributed of pathogenicity that includes a polysaccharide slime layer, which increases adherence to tissue making them less susceptible to phagocytosis (Johnson *et al.*, ١٩٩٦).

٣. *Klebsiella pneumoniae*

Ten isolate (١٦٪) were obtained. This result is consistent with that of Al-Gebori (٢٠٠٣), who diagnosed thirteen cases of *K. pneumoniae* mastitis (١٣٪). It is also associated with the hospital-acquired mammary infection (Fetherston, ٢٠٠١).

٤. *Staphylococcus epidermidis*

It is presents as, normal flora, but also becomes apportunistic pathogen which causes mastitis when mammary gland immunity becomes reduced (Thomsen, ١٩٨٢). Infection rate of this bacteria was (١٥٪) This result was agreed with that of Al-Gebori (٢٠٠٣) which was ١٥.١٥٪. In experimental study, *S. epidermidis* which was isolated from mastitis milk of the Lactating women, was inoculated in the mammary gland of the lactating mouse. As a result ٧٨-٩٣٪ of the mastitis was observed (Thomsen *et al.*, ١٩٨٥).

٥. *Escherichia coli*

Mammary infection with *E. coli* is less common (Blumstein *et al.*, ٢٠٠٣), where two isolate were diagnosed. This result was agreed with that of the previous study of each of Thomsen *et al.* (١٩٨٣) and Al-Gebori (٢٠٠٣), where the infection rate of *E. coli* mastitis were ٢٪ and ٥.٠٥٪ respectively. Low Incidence of this bacteria may return to the activity of S-IgA against *E. coli* that is found in human milk (May, ١٩٩٤).

٦. *Streptococcus viridans*

There bacteria are normal flora. They are found on the skin and in the nazopharynx. In addition, they are opportunistic pathogen caused in mammary infection when the individual immunity reduced. *S. viridans* has been linked in few cases to neonatal streptococcal infection (WHO, ٢٠٠٠). Infection rate of the *S. viridans* mastitis was ١.٦٪, they was also isolated by other workers like Al-Gebori (٢٠٠٣) who reported of ٦.٠٦٪.

٧. *Actinomyces* spp

There is one case of the mixed infection with *Actinomyces* spp and *S. aureus*. The genus *Actinomyces* comprises several species of

facultatively anaerobic, gram positive, branching rod that are numerically significant autochthonous bacteria in the oral cavity of the human and other animals. Several species of *Actinomyces* are opportunistic species, exogenous and endogenous pathogen. Mammary infection with *Actinomyces* spp is rare in human. *Actinomyces* increase numerically in oral cavity of the infant after teeth eruption (Cole *et al.*, ١٩٩٨). Tilson (١٩٩٣). Who found an increase in incidence of mastitis after eruption teeth of the infant. So the possible infection rout may be exogenous by the nipple fissure as may be Lymphatogenous whe it is not associated with teeth eruption. In an experimental model *A. bovis* antigens stimulate both humoral and cellular immune responses in rabbit (Shnawa and Al-Shakery, ١٩٩١).

٥-٢ Humoral Mucosal Immunology of Lactational Mastitis:

٥-٢-١ Monomicrobic Gram-positive Infection

٥-٢-١-١ *Staphylococcus aureus Mastitis*

The source of mammary infection may exogenous or endogenous (Haematogenous or Lymphatogenous) (WHO, ٢٠٠٠). *S. aureus* on gaining port of entry to mammary gland; logged their in, multiplied, produce quorum sensing signal (William, ٢٠٠٠). By producing quorum sensing signal (QSS), *S. aureus* avoid mammary local defense mechanism like innate soluble factors as: Lactoferrin, Lysozyme (May., ١٩٩٤) and complement (Bjorksten, ١٩٧٩; WHO, ٢٠٠٠). The net result of struggle between virulence factors of *S. aureus* as, coagulase, hacmolysin, Leucocidin, protein A, enterotoxin, and exofoliatius toxin (Brook *et al.*, ١٩٩٨; Ljungh, ١٩٩٨; Tudar, ٢٠٠٢) and mucosal immune defense mechanism. The immunodominant epitopes of *S. aureus* may

succeed in either direct B-cell activation or indirect activation Th_2 then activates B-cell through cytokines action to produce specific Abs (Zubler, ١٩٩٨).

Such specific Abs may be immunoprotective or immunodiagnostic (Musher and Mckenzie, ١٩٧٧). ٢-ME treatment reduced MMIg of one tube. This may be an indication for transudated components (Sheldrake *et al.*, ١٩٨٤, Brandtzaeg and Farstad, ١٩٩٩).

In this study, it has been observed that one case of *S. aureus* mastitis was not possessed MMIg that may be associated with impair or deficiency IL-٦ which is important in mucosal immune response regulation where it causes reduction in IgA-producing B-cell (Ada and Ramsay, ١٩٩٧). There are also other causes that lead to the deficiency in mucosal humoral immune response, as riboflavin deficiency which impair the ability to generate Abs (Giraldo, ٢٠٠٣). Melnikova *et al.* (١٩٨٦) rendered its cause to a relative deficiency in the cellular and humoral of immunity in patient with sever course of suppurative lactational mastitis.

٥-٢-١-٢ ***Staphylococcus epidermidis* Mastitis**

S. epidermidis is skin commensal bacteria. However, it represents the most common isolate foreign body infections and it is a weak inducer of inflammation compared to *S. aureus*, therefore it is responsible for the indolent and chronic course of *S. epidermidis* biomaterial infections. *S. epidermidis* strains isolated either from granulation tissue covering infected hip prosthesis or from normal skin flora are interacting with neutrophiles (Augustinsson *et al.*, ٢٠٠٤). *S. epidermidis* induct innate immune response of the mucosal immune system because it is induced different levels of human B-defensin٢ (HBD-٢). The human B-defensins are small, cationic antimicrobial epitopes that are made by epithelial cell

and play a role in mucosal and skin defenses. Human B-defesin١ (HBD-١) is expressed constituvely epithelial tissue, where as hBD-٢ and hBD-٣ are expressed in response to bacterial stimuli or inflammation (Chung *et al.*, ٢٠٠٤). *S. epidermidis* also stimulates humoral immunity as determined by an-enzyme-linked immunosorbent assay, was evaluated in experimental, *S. epidermidis* infections in rabbit. Antigens of clinical *S. epidermidis* strain detected significant Ab production in rabbit that immunized with different strain of *S. epidermidis* (Espersen *et al.*, ١٩٨٦). This study has been shown that high titers of mammary immunoglobulins against surface antigens of *S. epidermidis* and without reduction in the activity of mammary immunoglobulins that were treated with ٢-ME. This indicated local mucosal synthesis of MMIgs.

٥-٢-٢ Monomicrobic Gram Negative Infection:

٥- ٢- ٢- ١ ***Pseudomonas aeruginosa Mastitis***

P. aeruginosa possesses many virulence factors that are responsible for the pathogenic effect as pilli, mucopolysa-ccharide of alginate, exotoxinA and extracellular enzymes like elastase, protease and haemolysins (Baron *et al.*, ١٩٩٤). Source of infection with *P. aeruginosa* is exogenous when nipple fissure or damaged and in skin breaking (Thomsen *et al.*, ١٩٨٤). So it gets port of entery by Lymphatogenou rout (WHO, ٢٠٠٠). *P. aeruginosa* stimulate humoral and cellular immune response. Humoral immunity was elicited against bacteria and enterotoxin (William, ٢٠٠٠), while the cellular immune response was elicited against exotoxin A. This toxin has a direct cytotoxic effect on the macrophage and other cells in the immune response (Holt and Misfeldt, ١٩٨٤). It is also involved in cell damage such as mammary cell and the cell target by Psendomonas exotoxin (PE) appear to be participate in the antigen presentation and response (Duagherty *et al.*, ٢٠٠٠). MMIgs that

were treated with ٢-ME are undergoing reduction to a rate of one tube. That may return to slective. Transported or transudated of serum derived IgA in mammary secretions (Sheldrake *et al.*, ١٩٨٤; Brandtzage and Farstad, ١٩٩٩). It was noted (Table ٨) that one case of *P. aeruginosa* mastitis didn't possess MMIgs. This could be a case of deficiency in the hummoral immunity in the patient with server course of suppurative Lactational mastitis (Melnikova *et al.*, ١٩٨٦).

٥- ٢- ٢- ٢ ***Klebseilla. Pneumoniae Mastitis***

K. pneumoniae have virulence factors that are responsible for the harmful effect of the infection like: pilli, capsule, Lps, sidrophore and haemolysin (Fader and Davis, ١٩٨٢; Mizuta *et al.*, ١٩٨٣; Podschun *et al.*, ٢٠٠٠). These virulence factors enable bacteria to avoid local mechanism. Immunodominant epitopes of *K. pneumoniae* induce local immunity by either direct B-cell activation or T-cell activation that stimulate B-cell to produce specific Abs (Strans, ١٩٩٨).

K. pneumoniae infection stimulates macrophage inflammatory protein-١ production (MIP-١α) which is a chemokine produced by a variety of host cells as epithelial cell and macrophage. It mediates humoral and cellar mucosal immunity. It is involved in the production of Ag-specific mucosal secretory IgA (Lillard *et al.*, ٢٠٠٣). In this study, it has been conducted to Chek titers of the MMIgs to surface *K. pneumoniae* antigens were estimated. Treatment with ٢-ME didn't reduce the agglutination activity of the specific MMIgs. This indication local mucosal synthesis of MMIgs.

٥- ٢- ٢- ٣ ***Escherichia coli Mastitis***

E. coli possess some virulence factors as; pilli, haemolysin, collicin, K and O antigens, endotoxin, and cytotoxic necrotizing factor

(CNF). (Svanborg-Eden and Svennerholm, ١٩٧٨; Deodhare, ١٩٩٤; Andreu *et al.*, ١٩٩٧) which are responsible for the pathogenic effect. During infection, *E. coli* have overcome innate soluble immune factors like complement (Reiter *et al.*, ١٩٧٨), Lysozyme and Lactoferrin (May. *et al.*, ١٩٩٤). In vitro it has been found that Lysozyme in combination with complement and S-IgA, exhibited bacteriocidal activity to *E. coli* (Gordon, ١٩٧٩). In another report, it has been shown that Lysozyme is effective against *E. coli* in concern with IgA (Losnedahl *et al.*, ١٩٩٦). Experimental intramammary infection with *E. coli* induce innate immune response as increase levels of IL-١ beta, IFN-gamma, IL-١٢, $ScD_{١٤}$, LBPand high levels of $C_{٥}a$ and anti inflammatory cytokin IL-١٠. As well as increases in IL-٨ and TNF-α (Douglas *et al.*, ٢٠٠٤). *E. coli* infection induces the local synthesis of Abs to O and K antigens (Smith and Baijser, ١٩٧٦) and antipilus antibody (Smith *et al.*, ١٩٨١). Hanson *et al.* (١٩٧٧) coduct that S-IgA against $K_{١}$ antigens of *E. coli* is usually found in human milk. The MMIgs are *E. coli* specific and percent in a high titer. ٢-ME treatment doesn't reduce agglutinant activity of MMIg. Indicating it's mucosal origin.

٥-٢-٣ Dimicrobic Infection

On dimicrobic infection, induces mucosal Abs rise to clinical limit for both potential pathogen or for one (Table ١١). One associated pathogen can induce mucosal antibodies to high agglutination titer and other associated pathogen give low agglutination titer. Immunocompetent cell in the inflammation site exposed to two type of bacterial antigens, one type of bacterial antigen stimulate humoral immune response greater than that of other bacteria. Bacteria that induce high immune response may possess immunodominant epitope, so it gives high agglutinin titer. However, other pathogen, have second role in infection. This phenomena

has a possible explanation. It is "antigenic competition" (Tuassig, ١٩٧٥; Borphans *et al.*, ١٩٩٩). Two associated pathogen, that have immunodominant epitope induce humoral immune response, so two associated pathogens elicit high agglutination titer. Borphans *et al.* (١٩٩٩) have shown competition between T-cell, that were competiting for antigens (Ags), they are also competiting for antigenic site on the APC, where T-cell can bind to their specific Ags without being disturbed by surrounding T-cell. T-cell competition occur when the bacterial antigenic epitopes that are found on the different immunogenic molecule (Intermolecular competition). T-cell competition occurs by either sequential competition, in which the immune response was induced by the first given antigen and not by second antigen and by T-cell competition on antigen that are appeared on the macrophage surface space.

This type of competition, when antigens given as mixture. It depending on the relative amount of competiting antigens. The dominant antigen of this competition is the antigen that has succeeded for in stimulation most T and B-cell co-operation (Taussing, ١٩٧٥). Many characters of antigens as flexibility of conformation and ٣D conformation are very important for interaction between Abs and Ags. The researches can demonstrate this fact by doing "Competition" experiment. If one raises antibodies against hen egg white Lysozyme (HEL) loop, then a certain amount of the antibody will precipitate a certain fraction of the HEL. If experimenter added something else to the mixture that the Ab can bind to instead of the Ag, he will reduce the amount of Ag that gets precipitated. The experimenters added a fixed amount of antibodies and a fixed amount of radioactive HEL loop epitop to a test tube. Keeping the amount of Ab and radioactive Ag constant, they added increasing amount

of other stuff. Ab can also bind with nonradioactive HEL (Stuff). If the experimenter instead the amino acid in this epitope and adapted whatever shape they wanted. This molecule don't inhibit the binding of Ab to HEL. Thus they concluded that Ab don't recognize the amino acid sequence aloe, but rather the sequence held in particular three-dimensional structure (Reiness, ٢٠٠٤).

٥-٢-٤ Miscellaneous Infection

٥- ٢- ٤- ١ ***Streptococcus viridans Mastitis***

Clinically important *S. viridans* specific mucosal antibody titer was noted (Table-١٢). *S. viridans* involvement in mammary infection lactating women has been documented (Thomsen *et al.*, ١٩٨٣; Al-Gebori, ٢٠٠٣). Meanwhile, *S. viridans* specific salivary mucosal Abs were induced infant (Cole *et al.*, ١٩٩٨) since mammary gland as slivary gland is considered as one of the compartment of mucosal immune system (Brardtzage *et al.*, ١٩٩٨).

٥- ٢- ٤- ٢ ***Prototheca*** **spp Mastitis**

Prototheca spp was recovered from mastitic milk of lactating women together with its own specific Ab (Table ١٢). Prototheca specific serum Abs has been noted in mastitis cow (Kwon-Chung and Bennett, ١٩٩٢). So far, no mentioned their presence in the milk of mastitic women.

٥-٢-٥ Culture Negative Mastitis

Some of the cases of women lactational mastitis, pathogens not recover by the media used in this study these may be due to (antibody coated bacteria, cryptic infection, tuber bacilli, (TB), Virus and fungus). In these cases. It has been found that MMIg concentrations were low, medium, high, (Table ١٣) the last two cases may have rising titer of

antibacterial specific mucosal Abs, but they specificity couldn't be identified due to the lack of the associated bacterial antigen. Or they were cases of viral mastitis causing rising of MMIg but they couldn't be identified by the allowed conditions. They have found that colostral IgA antibody activity may be enhanced by the locally available viral antigens and further proliferation of previosely homed antigen reactive IgA cell in the mammary gland (Dhar and Orga, ١٩٨٥).

٥-٢-٦ Mastitic Ig as Diagnostic Probe

In normal subject, the base line titers of the specific mucosal Abs to mammary potential pathogens were ranging from ٢-٤. In mild or early infection, however, mammary specific Igs titers were ranging between ٤-٨. Acute to sever mammary infection specific MMIgs titers were ranging between ٨-١٢٨, while the chronic infection also rise up to ٨-١٢٨.

Entity	Titer	Number of fold
١. Normal subject	٢-٤	-
٢. Mild to early infection	٤-٨	٢
٣. Acute or sever infection	٨-١٢٨	٣-٥
٤. Chronic infection	٨-١٢٨	٣-٥

Thus MMIgs could be used as diagnostic probe for mammary infection (Shnawa and Mahdi, ٢٠٠٤).

٥-٣ Control Subject

S. epidermidis is normal flora that are colonized in human breast. It was isolated from normal milk that were taken from health women. In the

previous studies Marchall (١٩٧٥) and Al-Gebori (٢٠٠٣) could isolated *S. epidermidis* and Diphtheriod spp from normal milk. Concentrations of immunoglobulin of the normal milk are lower than that of mastitic milk MMIg concentrations obtained by this study were consistent with those of where the IgA concentration was ٠.٢ g/L. Healthy women possess different levels of MMIgs. This may be related with age factor of lactating women (Groer, ٢٠٠٤) and nutritional factor (Filtean *et al.*, ١٩٩٩). MMIgs of normal milk present in low titers because the *S. epidermidis* didn't induce mammary gland immunity. The data of Gleeson *et al.* (١٩٨٧) Showed only low level of S-IgA antibodies reacted with this commensal enteric bacterium were detected during the first ٤ years of life despite the colonization of large intestines of neonate by *E. coli*.

CHAPTER FIVE

Discussion

٥-١ Natural of Mammary Infection

٥-١-١ Age Distribution

High infection rate was noted among the ٢٥-٣٤ year age group, which constituted ٦٦.٧٪, whearse the infection rate decrease when the age become down to ٢٥ year and ages above ٣٤ years. This result was agreement with that of other workers as Vogel *et al.* (١٩٩٩) and Al-Gebori (٢٠٠٣) which were constituted ٦٨.٧٪, ٦٥.٧٪ respectively. In addition, such finding was confirmed by Jonsson and pulkinen (١٩٩٤) who found reduces infection rate in the ages under ٢١ years and above ٣٤ years. This result did not match with Kaufmann and Foxman (١٩٩١) result, who found higher infection rate in the older women ages.

٥-١-٢ Affected Breast

It has been shown that the incidence of affected right breast is higher (٥٣٪) than that of the left breast (٤٧٪). This result was disagreed with that of WHO (٢٠٠٠) where ٣٧-٥٢٪ of cases involve the right breast and ٣٨-٥٧٪ of cases involve the left breast while the bilateral mastitis in ٣-١٢٪. However which of the each side is affected have been conducted no significant differences has been observed. Breast has been affected as a result of milk stasis. It has been observed that many mothers find it easier to attach their infant to the breast on one side than on the other and it has been found suggested that poor attachment leading to milk stasis and mastitis occur on the side that was more difficult (WHO, ٢٠٠٠).

٥-١-٣ Leucocyte Response

Differential leucocyte count was done and the result has been observed. It was ٤١.١٪ for neutrophils, ٣١.١٪ for lymphocytes, ٢٣.١٪ for monocytes, ١.٣٪ for eosinophiles and ٠.٤٪ for basophiles. The neutrophiles infilteration is pathognomic acute of infection, while the monocyte with neutrophiles infilteration reflectes for subacute infection. A predominantly monocyte cellular response indicate chronic infection, whearse the lonely appearance of lymphocyte in cell infilteration has been observed in resident infection (Walter *et al.*, ١٩٩٦; Rook *et al.*, ١٩٩٨). The Leucocytes in milk consist of Lymphocytes, neutrophiles, polymorphonuclear Leucocyte (PMN), and macrophages. Lymphocytes together with antigen-presenting cells function in the generation of an effective immune response. In the normal mammary gland, macrophages are the predominant cells which act as sentinels to invading mastitis causing pathogens. Macrophages release chemical messenger called chemoattractant that cause migration of PMN to influmation site (Paap *et al.*, ٢٠٠٠). Local plasma cells also increase in number late in pregnancy and during lactation (Roux *et al.*, ١٩٧٧; Brandtzaeg., ٢٠٠٢). Another subsets of Lymphocyte is T-cell. It migrates to the mammary gland to increase ٧-١٠ fold with onest of lactation and remained high during the first ٢ weeks post partum (Manning and Parmely, ١٩٨٠). Eosinophils showed increase in one mastitis case that may related with regulation on activation normal T-cell expressed and Secreted (RANTES). This have been found in relatively high concentration in human milk. RANTES a chemokine involved in the chemotaxis of monocytes, eosinophiles, and memory CD_4 has been detected in human milk (Michie *et al.*, ١٩٩٨).

٥-١-٤ Microbial Profile

The monomicrobic infection was predominant (Table-٥) which constitutes ٨٥.٣٪, whearse the dimicrobic infection was less common. It constitutes ٦.٥٪. This result agreed with that of Thomsen (١٩٨٢) who suggests that mammary gland infection was tool place commenly by single infecting microbe. This result was disagreed with that of Niebyl *et al*. (١٩٧٨) who was found that multimicrobic growth in mammary infection and constituted ٤٢٪. There was third status that was Steril culture. The causes of the culture negative mastitis were:

١. Infection is nonbacterial. It was termed nonbacterial infection, and caused by Cryptococcus or viruses (WHO, ٢٠٠٠).

٢. Mastitis may be caused by *Chlamydia, Mycoplasma* and Mycobacterium that are not grown in the culture conditions, used in this study (Thomsen *et al.*, ١٩٨٣).

٣. Non infectious mastitis can be caused by trauma or blocked duct which are regarded as a predisposing factors for mastitis (Marchant, ٢٠٠٢).

٥-١-٥ Mastitis Associated Pathogens

١. ***Staphylococcus aurueus*** was most frequent isolate of the mastitis in Lactating women. This result is consistent with Bulmstein *et al.* (٢٠٠٣), who suggested that *S. aurueus* is most common cause and typically originating from the nursing child. Moreover this result was supported by Semba and Neville (١٩٩٩) who referred that *S. aurueus* is most common pathogen in the mammary infection, where it constitutes ٥٠٪. It is also associated with breast abscess infection. Non lactating breast abscess is uncommon. In the pureperium, breast abscess tend to occure in the setting of epidemic of *Staphylococcus*

infections involving the newborn infant and nursery. Pathogenic *Staphylococci* initially colonize the nasopharynx of the newborn leading to a high rate of the colonization of the milk and ducts of the nursing mothors. Maternal infection may develop from ١٠-١٤days postpartum. *S. aurueus* is affending pathogen in the vast majority of cases which can be confirmed on culture and gram stain of the breast milk (Wyne and Cohen, ٢٠٠٠) Blumstein *et al.* (٢٠٠٣) who have been shown that *S. aureus* and *Streptococcus* spp are the most common organism isolated in puerperal breast abscess. While the nonpuerperal abscess typically contain mixed infectious (*S. aureus, Streptococcus* spp) and anaerobes.

٢. *P. aeruginosa*

Thirteen cases of *P. aeruginosa* mastitis (٢١٪) were detected (Table ٧). In the previous study of Al-Gebori (٢٠٠٣), four cases of the *P. aeruginosa* mastitis were diagnosed (٤.٠٤٪). It important cause of mammary infection. Pathogenicity of this bacteria may increase in the presence of bacteria in the damaged tissue as a result of the opportunistic adherence (Thomsen *et al.*, ١٩٨٤). As well as attributed of pathogenicity that includes a polysaccharide slime layer, which increases adherence to tissue making them less susceptible to phagocytosis (Johnson *et al.*, ١٩٩٦).

٣. *Klebsiella pneumoniae*

Ten isolate (١٦٪) were obtained. This result is consistent with that of Al-Gebori (٢٠٠٣), who diagnosed thirteen cases of *K. pneumoniae* mastitis (١٣٪). It is also associated with the hospital-acquired mammary infection (Fetherston, ٢٠٠١).

٤. *Staphylococcus epidermidis*

It is presents as, normal flora, but also becomes apportunistic pathogen which causes mastitis when mammary gland immunity becomes reduced (Thomsen, ١٩٨٢). Infection rate of this bacteria was (١٥٪) This result was agreed with that of Al-Gebori (٢٠٠٣) which was ١٥.١٥٪. In experimental study, *S. epidermidis* which was isolated from mastitis milk of the Lactating women, was inoculated in the mammary gland of the lactating mouse. As a result ٧٨-٩٣٪ of the mastitis was observed (Thomsen *et al.*, ١٩٨٥).

٥. *Escherichia coli*

Mammary infection with *E. coli* is less common (Blumstein *et al.*, ٢٠٠٣), where two isolate were diagnosed. This result was agreed with that of the previous study of each of Thomsen *et al.* (١٩٨٣) and Al-Gebori (٢٠٠٣), where the infection rate of *E. coli* mastitis were ٢٪ and ٥.٠٥٪ respectively. Low Incidence of this bacteria may return to the activity of S-IgA against *E. coli* that is found in human milk (May, ١٩٩٤).

٦. *Streptococcus viridans*

There bacteria are normal flora. They are found on the skin and in the nazopharynx. In addition, they are opportunistic pathogen caused in mammary infection when the individual immunity reduced. *S. viridans* has been linked in few cases to neonatal streptococcal infection (WHO, ٢٠٠٠). Infection rate of the *S. viridans* mastitis was ١.٦٪, they was also isolated by other workers like Al-Gebori (٢٠٠٣) who reported of ٦.٠٦٪.

٧. *Actinomyces* spp

There is one case of the mixed infection with *Actinomyces* spp and *S. aureus*. The genus *Actinomyces* comprises several species of

facultatively anaerobic, gram positive, branching rod that are numerically significant autochthonous bacteria in the oral cavity of the human and other animals. Several species of *Actinomyces* are opportunistic species, exogenous and endogenous pathogen. Mammary infection with *Actinomyces* spp is rare in human. *Actinomyces* increase numerically in oral cavity of the infant after teeth eruption (Cole *et al.*, ١٩٩٨). Tilson (١٩٩٣). Who found an increase in incidence of mastitis after eruption teeth of the infant. So the possible infection rout may be exogenous by the nipple fissure as may be Lymphatogenous whe it is not associated with teeth eruption. In an experimental model *A. bovis* antigens stimulate both humoral and cellular immune responses in rabbit (Shnawa and Al-Shakery, ١٩٩١).

٥-٢ Humoral Mucosal Immunology of Lactational Mastitis:

٥-٢-١ Monomicrobic Gram-positive Infection

٥-٢-١-١ *Staphylococcus aureus Mastitis*

The source of mammary infection may exogenous or endogenous (Haematogenous or Lymphatogenous) (WHO, ٢٠٠٠). *S. aureus* on gaining port of entry to mammary gland; logged their in, multiplied, produce quorum sensing signal (William, ٢٠٠٠). By producing quorum sensing signal (QSS), *S. aureus* avoid mammary local defense mechanism like innate soluble factors as: Lactoferrin, Lysozyme (May., ١٩٩٤) and complement (Bjorksten, ١٩٧٩; WHO, ٢٠٠٠). The net result of struggle between virulence factors of *S. aureus* as, coagulase, hacmolysin, Leucocidin, protein A, enterotoxin, and exofoliatius toxin (Brook *et al.*, ١٩٩٨; Ljungh, ١٩٩٨; Tudar, ٢٠٠٢) and mucosal immune defense mechanism. The immunodominant epitopes of *S. aureus* may

succeed in either direct B-cell activation or indirect activation Th_2 then activates B-cell through cytokines action to produce specific Abs (Zubler, ١٩٩٨).

Such specific Abs may be immunoprotective or immunodiagnostic (Musher and Mckenzie, ١٩٧٧). ٢-ME treatment reduced MMIg of one tube. This may be an indication for transudated components (Sheldrake *et al.*, ١٩٨٤, Brandtzaeg and Farstad, ١٩٩٩).

In this study, it has been observed that one case of *S. aureus* mastitis was not possessed MMIg that may be associated with impair or deficiency IL-٦ which is important in mucosal immune response regulation where it causes reduction in IgA-producing B-cell (Ada and Ramsay, ١٩٩٧). There are also other causes that lead to the deficiency in mucosal humoral immune response, as riboflavin deficiency which impair the ability to generate Abs (Giraldo, ٢٠٠٣). Melnikova *et al.* (١٩٨٦) rendered its cause to a relative deficiency in the cellular and humoral of immunity in patient with sever course of suppurative lactational mastitis.

٥-٢-١-٢ ***Staphylococcus epidermidis* Mastitis**

S. epidermidis is skin commensal bacteria. However, it represents the most common isolate foreign body infections and it is a weak inducer of inflammation compared to *S. aureus*, therefore it is responsible for the indolent and chronic course of *S. epidermidis* biomaterial infections. *S. epidermidis* strains isolated either from granulation tissue covering infected hip prosthesis or from normal skin flora are interacting with neutrophiles (Augustinsson *et al.*, ٢٠٠٤). *S. epidermidis* induct innate immune response of the mucosal immune system because it is induced different levels of human B-defensin٢ (HBD-٢). The human B-defensins are small, cationic antimicrobial epitopes that are made by epithelial cell

and play a role in mucosal and skin defenses. Human B-defesin١ (HBD-١) is expressed constituvely epithelial tissue, where as hBD-٢ and hBD-٣ are expressed in response to bacterial stimuli or inflammation (Chung *et al.*, ٢٠٠٤). *S. epidermidis* also stimulates humoral immunity as determined by an-enzyme-linked immunosorbent assay, was evaluated in experimental, *S. epidermidis* infections in rabbit. Antigens of clinical *S. epidermidis* strain detected significant Ab production in rabbit that immunized with different strain of *S. epidermidis* (Espersen *et al.*, ١٩٨٦). This study has been shown that high titers of mammary immunoglobulins against surface antigens of *S. epidermidis* and without reduction in the activity of mammary immunoglobulins that were treated with ٢-ME. This indicated local mucosal synthesis of MMIgs.

٥-٢-٢ Monomicrobic Gram Negative Infection:

٥- ٢- ٢- ١ ***Pseudomonas aeruginosa Mastitis***

P. aeruginosa possesses many virulence factors that are responsible for the pathogenic effect as pilli, mucopolysa-ccharide of alginate, exotoxinA and extracellular enzymes like elastase, protease and haemolysins (Baron *et al.*, ١٩٩٤). Source of infection with *P. aeruginosa* is exogenous when nipple fissure or damaged and in skin breaking (Thomsen *et al.*, ١٩٨٤). So it gets port of entery by Lymphatogenou rout (WHO, ٢٠٠٠). *P. aeruginosa* stimulate humoral and cellular immune response. Humoral immunity was elicited against bacteria and enterotoxin (William, ٢٠٠٠), while the cellular immune response was elicited against exotoxin A. This toxin has a direct cytotoxic effect on the macrophage and other cells in the immune response (Holt and Misfeldt, ١٩٨٤). It is also involved in cell damage such as mammary cell and the cell target by Psendomonas exotoxin (PE) appear to be participate in the antigen presentation and response (Duagherty *et al.*, ٢٠٠٠). MMIgs that

were treated with ٢-ME are undergoing reduction to a rate of one tube. That may return to slective. Transported or transudated of serum derived IgA in mammary secretions (Sheldrake *et al.*, ١٩٨٤; Brandtzage and Farstad, ١٩٩٩). It was noted (Table ٨) that one case of *P. aeruginosa* mastitis didn't possess MMIgs. This could be a case of deficiency in the hummoral immunity in the patient with server course of suppurative Lactational mastitis (Melnikova *et al.*, ١٩٨٦).

٥- ٢- ٢- ٢ ***Klebseilla. Pneumoniae Mastitis***

K. pneumoniae have virulence factors that are responsible for the harmful effect of the infection like: pilli, capsule, Lps, sidrophore and haemolysin (Fader and Davis, ١٩٨٢; Mizuta *et al.*, ١٩٨٣; Podschun *et al.*, ٢٠٠٠). These virulence factors enable bacteria to avoid local mechanism. Immunodominant epitopes of *K. pneumoniae* induce local immunity by either direct B-cell activation or T-cell activation that stimulate B-cell to produce specific Abs (Strans, ١٩٩٨).

K. pneumoniae infection stimulates macrophage inflammatory protein-١ production (MIP-١α) which is a chemokine produced by a variety of host cells as epithelial cell and macrophage. It mediates humoral and cellar mucosal immunity. It is involved in the production of Ag-specific mucosal secretory IgA (Lillard *et al.*, ٢٠٠٣). In this study, it has been conducted to Chek titers of the MMIgs to surface *K. pneumoniae* antigens were estimated. Treatment with ٢-ME didn't reduce the agglutination activity of the specific MMIgs. This indication local mucosal synthesis of MMIgs.

٥- ٢- ٢- ٣ ***Escherichia coli Mastitis***

E. coli possess some virulence factors as; pilli, haemolysin, collicin, K and O antigens, endotoxin, and cytotoxic necrotizing factor

(CNF). (Svanborg-Eden and Svennerholm, ١٩٧٨; Deodhare, ١٩٩٤; Andreu *et al.*, ١٩٩٧) which are responsible for the pathogenic effect. During infection, *E. coli* have overcome innate soluble immune factors like complement (Reiter *et al.*, ١٩٧٨), Lysozyme and Lactoferrin (May. *et al.*, ١٩٩٤). In vitro it has been found that Lysozyme in combination with complement and S-IgA, exhibited bacteriocidal activity to *E. coli* (Gordon, ١٩٧٩). In another report, it has been shown that Lysozyme is effective against *E. coli* in concern with IgA (Losnedahl *et al.*, ١٩٩٦). Experimental intramammary infection with *E. coli* induce innate immune response as increase levels of IL-١ beta, IFN-gamma, IL-١٢, $ScD_{١٤}$, LBPand high levels of $C_{٥}a$ and anti inflammatory cytokin IL-١٠. As well as increases in IL-٨ and TNF-α (Douglas *et al.*, ٢٠٠٤). *E. coli* infection induces the local synthesis of Abs to O and K antigens (Smith and Baijser, ١٩٧٦) and antipilus antibody (Smith *et al.*, ١٩٨١). Hanson *et al.* (١٩٧٧) coduct that S-IgA against $K_{١}$ antigens of *E. coli* is usually found in human milk. The MMIgs are *E. coli* specific and percent in a high titer. ٢-ME treatment doesn't reduce agglutinant activity of MMIg. Indicating it's mucosal origin.

٥-٢-٣ Dimicrobic Infection

On dimicrobic infection, induces mucosal Abs rise to clinical limit for both potential pathogen or for one (Table ١١). One associated pathogen can induce mucosal antibodies to high agglutination titer and other associated pathogen give low agglutination titer. Immunocompetent cell in the inflammation site exposed to two type of bacterial antigens, one type of bacterial antigen stimulate humoral immune response greater than that of other bacteria. Bacteria that induce high immune response may possess immunodominant epitope, so it gives high agglutinin titer. However, other pathogen, have second role in infection. This phenomena

has a possible explanation. It is "antigenic competition" (Tuassig, ١٩٧٥; Borphans *et al.*, ١٩٩٩). Two associated pathogen, that have immunodominant epitope induce humoral immune response, so two associated pathogens elicit high agglutination titer. Borphans *et al.* (١٩٩٩) have shown competition between T-cell, that were competiting for antigens (Ags), they are also competiting for antigenic site on the APC, where T-cell can bind to their specific Ags without being disturbed by surrounding T-cell. T-cell competition occur when the bacterial antigenic epitopes that are found on the different immunogenic molecule (Intermolecular competition). T-cell competition occurs by either sequential competition, in which the immune response was induced by the first given antigen and not by second antigen and by T-cell competition on antigen that are appeared on the macrophage surface space.

This type of competition, when antigens given as mixture. It depending on the relative amount of competiting antigens. The dominant antigen of this competition is the antigen that has succeeded for in stimulation most T and B-cell co-operation (Taussing, ١٩٧٥). Many characters of antigens as flexibility of conformation and ٣D conformation are very important for interaction between Abs and Ags. The researches can demonstrate this fact by doing "Competition" experiment. If one raises antibodies against hen egg white Lysozyme (HEL) loop, then a certain amount of the antibody will precipitate a certain fraction of the HEL. If experimenter added something else to the mixture that the Ab can bind to instead of the Ag, he will reduce the amount of Ag that gets precipitated. The experimenters added a fixed amount of antibodies and a fixed amount of radioactive HEL loop epitop to a test tube. Keeping the amount of Ab and radioactive Ag constant, they added increasing amount

of other stuff. Ab can also bind with nonradioactive HEL (Stuff). If the experimenter instead the amino acid in this epitope and adapted whatever shape they wanted. This molecule don't inhibit the binding of Ab to HEL. Thus they concluded that Ab don't recognize the amino acid sequence aloe, but rather the sequence held in particular three-dimensional structure (Reiness, ٢٠٠٤).

٥-٢-٤ Miscellaneous Infection

٥- ٢- ٤- ١ ***Streptococcus viridans Mastitis***

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antibacterial specific mucosal Abs, but they specificity couldn't be identified due to the lack of the associated bacterial antigen. Or they were cases of viral mastitis causing rising of MMIg but they couldn't be identified by the allowed conditions. They have found that colostral IgA antibody activity may be enhanced by the locally available viral antigens and further proliferation of previosely homed antigen reactive IgA cell in the mammary gland (Dhar and Orga, ١٩٨٥).

٥-٢-٦ Mastitic Ig as Diagnostic Probe

In normal subject, the base line titers of the specific mucosal Abs to mammary potential pathogens were ranging from ٢-٤. In mild or early infection, however, mammary specific Igs titers were ranging between ٤-٨. Acute to sever mammary infection specific MMIgs titers were ranging between ٨-١٢٨, while the chronic infection also rise up to ٨-١٢٨.

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٤. Chronic infection	٨-١٢٨	٣-٥

Thus MMIgs could be used as diagnostic probe for mammary infection (Shnawa and Mahdi, ٢٠٠٤).

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previous studies Marchall (١٩٧٥) and Al-Gebori (٢٠٠٣) could isolated *S. epidermidis* and Diphtheriod spp from normal milk. Concentrations of immunoglobulin of the normal milk are lower than that of mastitic milk MMIg concentrations obtained by this study were consistent with those of where the IgA concentration was ٠.٢ g/L. Healthy women possess different levels of MMIgs. This may be related with age factor of lactating women (Groer, ٢٠٠٤) and nutritional factor (Filtean *et al.*, ١٩٩٩). MMIgs of normal milk present in low titers because the *S. epidermidis* didn't induce mammary gland immunity. The data of Gleeson *et al.* (١٩٨٧) Showed only low level of S-IgA antibodies reacted with this commensal enteric bacterium were detected during the first ٤ years of life despite the colonization of large intestines of neonate by *E. coli*.

Dedication

To:

My respectable supervisor,

Professor, Ibrahim M.S. Shnawa

My family, who gave me patience and help

Acknowledgement

I am greatly indebted to my supervisor professor Dr. Ibrahim Shnawa, for his continuous help and encouragement.

I would like to thank the dean of the Collage of Science. Grateful thanks are afforded to the staff of unit of the early diagnosis of breast disease Hilla Teaching Hospital; especially Dr. Walaa Noori, Dr. Niemat Abid Al-Kadhim and Miss Rajaa Ali.

Finally I am so grateful to my parents, who support me and to my brother and sisters for their encouragement.

List of Contents

List of Tables

List of Figures

List of Abbreviations

Designation	Meaning
APC	Antigen Presenting Cell
CD	Clasters of designation or claster of differentiation
CNF	Cytotoxic necrotizing factor.
EGF	Epithelial growth factor
GALT	Gastro intestinal-associated lymphreticular tissue
GI tract	Gastrointestinal tract
G-CSF	Gramulocyte-colony stimulating factor
HBD-١, ٢ and ٣	Human beta defensin-١, ٢ and ٣
HEL	Hen egg white Lysozyme
HEV	High endothelial venules
ICAM-١ and ٢	Intercellular adhesion molecule-١ and ٢
IEL	Interaepithelial Lymphocyte
IFN-γ	Interferon-γ
IGFs	Insulin growth factors
IL	Interleukin
LBP	Lipopoly saccharid binding proteins

LFA-١	**Lymphocyte functional antigen-١**

Designation	Meaning
LPS	**Lipopoly saccharide**
MAdCAM-١	**Mucosal Vaslular addrissins cellular adhesion molecule-١**
MALT	**Mucosa associated-lymphoreticular tissue**
M-cell	**Membranous cell**
MG	**Mammary gland**
MIP-١	**Macrophage inflammatory protein-١ alpha**
MMIg	**Mammary mucosal immunoglobulin**
MN	**Mesenteric Lymph node.**
NALT	**Nasopharyngeal-associated Lymphoreticular tissue.**
PE	***Pseudomonas* exotoin**
P-IgA	**Polymeric immunoglobulin A**
P-IgR	**Polymeric immunoglobulin receptor**
PMN	**Polymorphoneuclear leucocyte**
PNAd	**Peripheral lymph node addrisins**
RANTES	**Regulated on activation normal T-cell expressed and secreted**
S-IgA	**Secretory immunoglobulin A**

Designation	Meaning
S-IgM	Secretory immunoglobulin M
SLPI	Secretory leucocyte protease inhibitor
TCR	T-cell receptor
TGf-β	Transforming growth factor-β
Th	T-helper Lymphocyte
٢-ME	٢-Mercaptoethanal
٣D	Three dimensional

References

- Ada, G. and Ramsy, A. (١٩٩٧). Vaccines, Vaccination and the Immune Response. PP: ٧٦-٨٥. Black Well Science
- Al-Amedi, B.H. (٢٠٠٣). Comparative study of mucosal and systemic immune responses for the persistant pyuria patients. Ph. D. Thesis. Babylon university. Babylon.
- Al-Gebori, A.F. (٢٠٠٣). A study on the Associated Bacterial causal of Human mastitis. Thesis. Babylon University. Babylon.
- Aloisi, R.M. (١٩٧٩). Principles of Immunodiagnosis. ٥th ed. PP: ٩٣-١٠٧. Mosby company. London.
- Andreu, A.; Stapleton, E.; Fennell C.; Lockman, H.; Xercavins, M.; Fernandez, f. and Stam, w. (١٩٩٧). Urovirulance determinants in *Escherichia coli* Strains causing prostitis. J. infect. Dis. ١٧٦: ٤٦٤-٤٦٩.
- Bankers, D.D. (١٩٨٠). Modren Practice in Immunization. ٣th. ed, PP١٨٩-١٩١. Popular prakashan private Ltd. Bomby.
- Baron, E.J.; Peterson, L.R. and Fingold, S.M. (١٩٩٤). Bailey and Scotts Medical Microbiology. ٩th ed. The C.V. Mosby company, USA.
- Bjorksten, B.; Gothefors, L. and Sfden vall, R. (١٩٧٩). The Effect of Human Colostrum on Neutrophil Function. Pediat. ٣(٦): ٧٣٧-٧١٧.
- Blumstein, H., Rontal, A. K.; Brown, D. F. M.; Talavera, F.; Sheridan, B. J.; Halmaka, J. and Alder, J. (٢٠٠٣). Breast Abscess and Masses. e. Medicine World Medical library. An Internet Lecture, Annotated with Eligible References. Department of Emergency Medicine.

- Borphans, J. A. M.; Taams, L.; Wauben, M. H. M. and De Boer, R. J. (١٩٩٩). Competition for Antigenic Sites during T cell Proliferation: A mathematical interpretation of *in vitro* data. Proc. Natl. Acad. Sci. ٩٦: ١٠٧٨٩-١٠٧٨٧.
- Brandtzaeg, P. (١٩٩٥). Molecular and Cellular aspect of the Secretory Immunoglobulin System APMIS, ١٠٣:١-١٩.
- Brandtzaeg, P. (٢٠٠٢). Current Understanding of Gastrointestinal Immunoregulation and its Relation to Food Allergy. Annal. Acad. Sci. ٩٦٤: ١٣-٤٥.
- Brandtzaeg, P. and Farstad, N. (١٩٩٩). The Human Mucosal B cell System. Mucosal Immunology. Academic press.
- Brandtzaeg, P.; Farstad, I. N.; Johanson, F. E; Marton, H. c.; Norderhang I. N. and Yamanaka, J. (١٩٩٩). The B cell System of Human Mucosa and Exocrine. Immunol. Rev., ١٧١: ٤٥-٨٧.
- Brandtzaeg., P.; Farstad, N.; Haraldsen, G. and Jahnsen, F. L. (١٩٩٨). Cellular and Molecular Mechanisms for Induction of Mucosal Immunity. In Modulation of The Immune Response to Vaccine Antigens. Brown, F and Hauheim, L. R. (eds.)٢. Dev. Biol. Stand. Basel, Krager. ٩٢: ٩٣-١٠٨.
- Brook, G. F.; Butel, J. S. and Morse, A.S. (١٩٩٨). In: Jawetz, Melin, K, and Adelbergs Medical Microbiology. ٢th ed. Appleton and lung, California.
- Butcher, E. C. and Piker, L. J. (١٩٩٦). Lymphocyte Homing and Homeostasis. Sci. ٢٧٢: ٦٠-٦٦.
- Chung, W.O. and Dale, B. A. (٢٠٠٤). Innate Immune Response of Oral and Foreskin Keratinocytes: Utilization of Different

Signaling Pathways by Various Bacterial Species. Infect. Immunol. ٧٢(١): ٣٥٢-٣٥٨.

- Cole, M.F.; Bryan, S.; Evan, M.K.,pearce, C. L.; Sheridan, M.J.; Sura, P.A.; Weintzen, R. and Borden, G.H.W. (١٩٩٨). Humoral Immunity to Commensal Oral Bacteria in Human Infants: Salivary Antibodies Reactive with *Actinomyces naeslundii* Genospecies ١ and ٢ during Colonization infect. Immunol. ٦٦(٩): ٤٢٨٣-٤٢٨٩.
- Connor, A. E. (١٩٧٩). Elevated Levels of Sodium and Chloride in Milk from Mastitis Breast. Pediatr. ٦٣: ٩١٠-٩١١.
- Coppa, G.V.; Gabrielli, O.; Giorgi, P.; Catassi, C.; Montanari, M.P.; Varaldo, P. E. and Nicholas, B.L. (١٩٩٠). Preliminary Study of Breast Feeding and Bacterial Adhesion to Uroepithelial Cells. Lancet. ٣٣٥: ٥٦٩-٥٧١.
- Daugherty, A. L.; Mckee, M. L.; Fitz Gerald, D. J. and Mrsuny. R. J. (٢٠٠٠). Epithelial Application of *Pseudomonas aeruginosa*, Exotoxin A Results in a Selective Target in The Liver, Spleen, and Lymphnode. Journal of Controlled Release. ٦٦(١): ٢٩٧-٣٠٢.
- Dawed, K.M. and Al-Yas, Z.A. (١٩٩٠). Statistical Methods in Agricultural Researches, PP: ٥٤; Al-Mousel University, Iraq.
- De Araujo, A.N. and Giugliano, L.G. (٢٠٠١). Lactoferrin and Free Secretory Component of Human Milk Inhibite The Adhesion of Enteropathogenic *Escherichia coli* to Hela Cells. BMC. Microbiology. ١: ٢٥.
- Deodhare, S. G. (١٩٩٤). Y. M. Bhends General Pathology PP ٣٥٩-٣٥٥. Popular Praksham Private. Ltd. Bomby.

- Dhar, R. and Orga, P. L. (١٩٨٥). Local Immune Response. British Medical Bulletin. ٤١(١): ٢٨-٣٣.
- Douglas, B.; Max, P.; Jar-Wei, L.; Xin, Z.; Jayne, H. and Pascal, R. (٢٠٠٤). *Echerichia coli* and *Staphylococcus aureus* Elicit Differential Innate Immune Response Following Intramammary Infection. Clinc. Diag. Lab. Immunol. ١٩٥(٢): ٢١١-٢١٣. (Abstract).
- Editorial. (١٩٧٦). Puerperal Mastitis. BR. Med. J.; ١(٦٠١٥): ٢٢٠-٢٢١.
- Editorial. (١٩٨١). Microbial Adhesion, Colonization and Virulance. Lancet; ii: ٥٠٨-١٠.
- Espersen, F.; Wheat, L,J.; Bemis, A. T. and White, A. (١٩٨٦). Enzyme-Linked Immunosorbent Assay for Detection of *Staphylococcus epidermidis* Antibody in Experimental S. Epidermidis Endocarditis. J. Clin. Microbiol. ٢٣(٢): ٣٣٩-٣٤٢.
- European Agency for The Evaluation of Medicinal Products. (١٩٩٨). Hum, Med. Eval. Unite. CPMP/BWP/٤٧٧/٩٧: ١-١٤.
- Fader, R.C. and Davis, C.P. (١٩٨٢). *Kelebsiella pneumoniae* Induced Experimental Pyelitis: The Effect of Piliation on Infectivity. J. Urol. ١٢٨: ١٩٧-٢٠١.
- Farrell, I. D. (١٩٩٦). Brucella, In Collee, J. G.; Fraser, A. G. Marmion, B. P. and Simmons, A(eds.) Mac Kie and McCar They Practical Medical Microbiology. ١٤th ed. PP: ٤٧٧. Churchill Livingston. Newyork.
- Fetherston, C. (٢٠٠١). Mastitis in Lactating Women: Physiology or Pathology? Breast Feed. Rev. ٩(١): ٥-١٢.
- Filteau, S. M.; Rice, A. L.; Ball, J. J.; Chakraborty, J.; Stoltzfus, R; De Francisco, A-and Willumsen, J. J. (١٩٩٩). Breast Milk Immune Factors in Bangladeshi Women Supplemented

Postpartum with Retinal or β Carotene. Am. J. Clin. Nutr. ٦٩: ٩٥٣-٩٥٨.

- Francis, P.G. (١٩٨٥). Bovin Mastitis: Examination of Milk in Control Schemes. In: Collins, C. H. and Grange, J. M. Isolation and Identification of Microorganisms of Medical and Veterinary Importance. Academic press, London, ٣٤٥-٣٤٧.
- Giraldo, R. (٢٠٠٣) Nutritional Therapy for the Treatment and Prevantion of AIDS. Scientific Bases. An Internet Lecture An Notated with Eligible References. SADC., ١-٣٢.
- Glesson, M., Cripps, A. W.; Clancy, R. L.; Wlodarczyk, J. H.; Dobson, A. J. and Hensley, J. H. (١٩٨٧). The Development of IgA-Specific Antibodies to *Escherichia* Coli O Antigen in Ohlidren. Scand. J. Immunol. ٢٦: ٦٣٩-٦٤٣.
- Golding, J.; Emmett, P. M. and Rogers, I. S. (١٩٩٧). Does Breast Feeding Protect Against Non-gastric Infection? Early Hum. Dev. ٤٩(Supp L): S١٠٥-١٢٠.
- Goldman, A. S. (١٩٩٣). The Immune System of Human Milk: Anti Mmicrobial, AntIinflammatory and Immunomodulatory Properties. J. Pediat. Infect. Dis. ١٢: ٦٦٤-٦٧١.
- Gordon, L. I.; Douglas, S. D.; Kay, N. E.; Osmaue, Y.; Jacob, H. S. and Osserman, E. F. (١٩٧٩). Modulation of Nneutrophil Function by Lysozyme-Potential Negative Feedback of Inflammation. J. Clinc. Inverst. ٦٤: ٢٢٦.
- Gould, D. and Brooker, C. (٢٠٠٠). Applied Microbiology for Nurses. PP: ٣٠-٥٩. Macmilianpress. Ltd.
- Groer, M.; Davis, M. and Steele, K. (٢٠٠٤). Association between Human Milk S-IgA and Maternal Immune, Infections,

Endocrine, and Strees Variable. Journal of Human Lactation. ٢٠(٢): ١٥٣-١٥٨. (Abstract).

- Guyer, R. L.; Koshland, M. E. and Knopf, P. M. (١٩٧٦). Immunoglobulin Binding by Mous Intestinal Epithelial Cell Receptors. J. Immunol. ١١٧: ٥٨٧.
- Hanson, L. A.; Ahlstedt, S.; Fasth, A.; Jodal, U.; Kaijser, B.; Larrsson, P.; Lindberg, U.; Olling, S.; Sohl-Akerlund, A. and Svanborg-Eden, C. (١٩٧٧). Antigens *Escherichia coli*, Human Immune Response, and The Pathogenesis of Urinary Tract Infections. J. Infect. Dis. ١٣٦ (Supplemented): ١٤٤-١٤٩.
- Holmgren, J.; Hanson, L. A.; Calsson, B.; Lindblad, B. S. and Rahimtoola, J. (١٩٧٦). Neutralizing Antibodies Against *Escherichia coli* and *Vibrio Cholerae* Enterotoxin in Human Milk from A Developing Country. Scand. J. Immunol. ٥: ٨٦٧.
- Holt, P.S. and Misfeldt. (١٩٨٥). Alteration of Murine Immune Response by *Psendomonas aeruginosa* Exotixin A. Infect. Immun. ٤٥(١)٢٢٧-٢٣٣.
- Johnson, A. G.; Ziegler.; R. J.; Lukasewycz, O. A. and Hawley, L. B. (١٩٩٦). Microbiology and Immunology. Board Review Series. PP: ٨٤-٨٦. Lippincot Williams and Wilkins.
- Johnston, A. and Thorp, R. (١٩٨٢). Immunochemistry In Practice. Pp: ٦٤- ٦٧. Black Well Scientific Publication. Oxford.
- Jonssan, S. and Pulkinen, M. O. (١٩٩٤). Mastitis today: Incidence Prevention and Treatment. Ann. Chir, Gynecol. ٨٣: ٨٤-٨٧.

- Kato, N. (١٩٨٣). Virulance for Mice of Klebsiella Strain Belonging to The OI Group; Relationship to Their Capsular (K). Types. Infect. Immunol. ٤٠(١): ٥٦-٦١.
- KauFmann, R. and Foxman, B. (١٩٩١). Mastitis among Lactating Women: Occurrence and Risk Factors Soc. Sc. Med., ٣٣(٦): ٧٠١-٧٠٥.
- Kaufmann, S. H. E. and Kabelitz, D. (١٩٩٨). Introduction: The Immune Response to Infections agents. In: Methods In Microbiology, Volume ٢٥. PP: ١-١٧. Acad. Press. Ltd.
- Kwon-Chung, K. J. and Bennet, J.E.(١٩٩٢). Medical My Cology. PP: ٧٨٥-٤٩٤. Philadelphia. London.
- Levin, B. R. and Antia, R. (٢٠٠١). Why we Don't Get Sick: The Within-Host Population Dynamic of Bacterial Infection. In: Ecology and Evolution of Infection. Sci. ٢٢٢: ١١١٢-١١١٥. USA.
- Lie Lu, L. and Walker, W. A. (٢٠٠١). Pathogenic and Physiologic Interactions of Bacteria with Gastrointestinal Epithelium. Am. J. Clinc. Nutr. ٧٣(Suppl): ١١٢٤S-٣٠S. USA.
- Lillard, J. W.; Singh, S.; Taub, D. D. and Mc Ghee, J.R. (٢٠٠٣). Mip-١α and Mip-١β Differentially Mediate Mucosal and Systemic Adaptive Immunity. Blood, ١٠١ (٣) : ٨٠٧-٨١٤.
- Ljungh, A (١٩٩٨). Staphylococcus Infection and Immunity. In: Encyclopedia of Immunology. Vol ٤., ٢nd ed. P-J. Delves and I.M. Rottin. (eds.). P: ٢٢٠٨-٢٢١١. Academic. Press, London.
- Lydyard, P. and Grossi, C. (١٩٩٨). The Lymphoid System. In. Roitt, I., Brostoff, J. and Male, D.(eds.). Immunology. ٥th ed. PP ٣١-٣٨. Mosby. International. Ltd. London.

- Mac Sween, R.N. and Whaley, K. (١٩٩٢). Muir's Text-book of Pathology. ٣th ed. PP: ١٣٤-١٩٤. ARNOLD. Oxford, London.
- Macfaddin, J. E. (٢٠٠٠). Individual Biochemical Tests. In: Biochemical Test for Indentification of Medical Bacteria. ٣th ed. Macfaddin, J. E. (ed.) P:٢٧-٤٣٩. Lippincott Williams and Wilkins co., Blatimore. USA.
- Mahdi, M. S. (٢٠٠٠). Role of Urinary Secretory Immunolobulin in Pyuria Patient. M. Sc. Thesis. Babylon University. Babylon.
- Manning, L. S. and Parmely, M. J. (١٩٨٠). Cellular Determinants of Mammary Cell-mediated Immunity in The Rat. I. The Migration of Radioisotopically Labeled T-Lymphocytes. J. Immunol. ١٢٥ Issue ٦: ٢٥٠٨-٢٥١٤.
- Marchant, D-J. (٢٠٠٢). Inflammation of The Breast. Obstet. Gynecol. Clinic. Nor. Am. ٢٩(١): ٨٩-١٠٢.
- Marshall, B. R.; Hepper, J. K. and Zirbel, C. C. (١٩٧٥). Sporadic Puerperal mastitis: An infection that need not interrupt Lactation. J. Am. Med. Asso. ٢٣٣(١٣): ١٣٧٧-١٣٧٩.
- May, J. T. (١٩٨٨). Microbial Contamination and Antimicrobial Properties of Human Milk. Microbiology Sxi. ٥: ٤٢-٤٦.
- May, J. T. (١٩٩٤). Antimicrobial Factprs and Microbial Contaminants in Human Milk. J. Pediatr. Child Health. ٣٠: ٤٧٠-٤٧٥.
- Mc Coy, K. and Kennedy, E. R. (١٩٦٠). Autogenous Vaccine Therapy in Staphylococci Infection. J. A. M. A.; ١٧٤(١): ١١٧-١٢٠.

- Mc Ghee, J.R. and Kiyono, H. (١٩٩٩). The Mucosal Immune System. In : Paul, W.E (ed.). Fundame-ntal Immunology. ٤th ed: PP ٩٠٩-٩٤٠. Lippin Cott-Raven Publisher-Philadelphia.
- Mel nikova, V. N.; Spesivtsev. I.U.A.; Andreev, A.V.; Volkova, S.D. and Simrnova, A. I. (١٩٨٦). Immunologic Status and Leucocyte Function of Patients with Suppurative Lactational Mastitis. Vestn. Khir. Im II. Grek. ١٣٧(١٢): ٤٧-٥٠.
- Michie, C. A.; Tanstscher, E.; Schall, T. and Rot, A. (١٩٩٨). Physiological Secretion of Chemokines in Human Breast Milk. Eur. Cytokin. Nelw. ٩: ١٢٣-١٢٩.
- Mizuta, K.; Ohta, M.; Meri, M.; Hasegawa, T.; Nakashima, I. and Kato, N. (١٩٨٣. virulence for Mice of *Klebsiella strains* Belonging to the O'I group; Relationship to their capsular (K) types. Infec. Imm. ٤٠(١): ٥٦-٦١.
- Musher, D. M. and Mckenzie, S. O. (١٩٧٧). Infection due to *Staphylococcus aureus*. Medicine. ٥٦(٣): ٣٨٣-٤٠٩.
- Neville, M. C. Allen, J. C.; Archer, P. C.; Casey, C. E.; Seacat, J.; Keller, R. P.; Lutes, V.; Rasbach, J. and Neifert, M. (١٩٩١). Studies in Human Lactation Milk Volume and Mutrient Composition During Weaning and Lactogenesis. Am. J. Clinc. Nutr. ٥٤: ٨١-٩٢.
- Niebyl, J. R.; Spence, M. R. and Parmley, T. H. (١٩٧٨). Sporadic (nonepidemic) Puerperl Mastitis J. Rep. Med. ٢٠(٢): ٩٧-١٠٠.
- Nildotted- Augstinsson, A.; Wilsson, A.; Larsson, J.; Stendahl, O and Lundqvist-Gustafsson, H. (٢٠٠٤). *Staphylococcus aureus*, but not *Staphylococcus epidermidis*, Modulate the Oxidative Response and Induces a Poptosis in Human

Neutrophiles. Apims. ١١٢(٢): ١٠٩-١١٨(١٠). Black Well Publishing (Abstract).

- Noguera-Obenza, M.; Ochoa, T. J. Gomes, H. f. Guerrero, M. L.; Horrera-Insua, I.; Morrow, A. L.; Ruiz-Palacios, G.; Pickering, L. K.; Guzman, C. A. and Clleary, T. G. (٢٠٠٣). Human Milk Secretory Antibodies Against Attaching and Effacing *Escherichia coli* Antigens. Emerg. Infect. Dis. ٩(٥): ٢-٤٤١.
- Paap, M. J.; Shafer-Weaver, K.; Capuca, A. V.; Van. Oostveldt, K. and Burvenich, C. (٢٠٠٠). Immune Surveillance of Mammary Tissue by Phagocytic Cells. Adv. Exp. Med. Biol. ٤٨٠: ٢٥٩-٧٧٠.
- Playfiar, J. H. L. and Chain, B. M. (٢٠٠١). Immunology At Glance. ٧th ed. Black Well Science.
- Podschun, R.; Fischer, A. and Ulluman, U. (٢٠٠٠). Expression of Putative Virulance Factors by Clinical Isolated of *Klebsiella Planticala*. J. Med. Microbiol. ٤٩: ١١٥-١١٩.
- Reiness, G. (٢٠٠٤). Antigens. An overview. An Internet Lecture, an Notated with Eligible References. Department of Microbiology and Immunology. UK.
- Rieter, B. (١٩٧٨). Review of Nonspecific Antimicrobial Factors in Colostrum. Ann. Rech. Vet. ٩: ٩-٢٠٥.
- Roitt, I. And Rabson, A. (٢٠٠٠). Really Essential Medical Immunology. PP: ٣-٦٤. Black Well Science.
- Roitt, I.; Brostoff, J. and Male, D. (٢٠٠١). Immunology. ٦th ed. Mosby. W.B. Saunders. pp: ٣٧- ٤٤.
- Rook, G.; and Balk will, F. (١٩٩٨). Cell-Mediated Immunity Reactions. In: Roitt, I.; Brostoff, T. and Male, D. (eds).

Immunology. ٥th ed. PP ١٢١-١٣٥. Mosby International. Ltd. London.

- Ross, D. L. (١٩٨٥). Proteins. In: Bishop, M. C.; Dben-Vo, L. J.; Fddy, E. P. and Thirty Three Contributors (eds). Clinical Chemistry Principle, Procedures and Correlation. PP: ١٨٠-١٨١. The Murray Printing Company. Philadelphia.
- Rossen, R.D.; Butter, W.T. and Woldman, R.H. *et al.* (١٩٧٠). The Proteibs in NASA-٢ Secretions. II. A Longitudinal Study of IgA and Neutralizing Antibody Levels in Nasal Washings from Men with Infleunzal Virus. J.A.M., ٢١١: ١١٥٧-١١٦١.
- Roulin, A. and Heep, P. (١٩٩٩). The Immunological Function of Allosuckling. Ecology Letters. ٢: ٣١٩-٣٢٤. Black Well Science. Ltd.
- Roux, M. E; Mc Williams, M.; Philips-Quagliata, J. M.; Weisz-Carrington, P. and Lamm, M. E. (١٩٧٧). Origin of IgA-Secreting Plasma Cell in The Mammary gland. J. Exp. Med. ١٤٦: ١٣١١-١٣٢٢.
- Sait, L.; Galic, M.; Strugnell, R. A. and Hanssen, P. H. (٢٠٠٣). Secretory Antibodies do not Effect The Composition of The Bacterial Microbiota in The Terminal Ileum of ١٠-Week-Old Mice. AEM. ٦٩(٤): ٢١٠٠-٢١٠٩.
- Semba, R. D. and Neville, M. C. (١٩٩٩). Breast Feeding, Mastitis, and HIV Transmission: Nutritional Implication. Nutri. Rev. ٥٧(٥): ١٤٦-١٥٣.
- Semba, R. D. Kamwende , N.; Taha, T. E, Hoover, D.; Lan, Y.; Eisinger, W.; Mitmavalye, L; Brood Head, R.; Mortti, P.; Vander Hoeven, L. and Chiphangwi, I. D. (١٩٩٩). Mastitis

and Immunological Factor in Mreast Milk of Lactating Women in Malawi. Clin. Diag. Lab. Immunol. ٦(٥): ٦٧١-٦٧٤.

- Sheldrake, R. F.; Husband, A. J.; Watson, D. L. and Cripps, A. W (١٩٨٤). Selective Transport of Serumderived IgA into Mucosal Secretions. J. Immunol. ١٣٩ (issue ١): ٣٦٣-٣٦٨.
- Shnawa, I. M.S. and Mahdi, M.S. (٢٠٠٤). Mucosal Agglutinating Antibodies to The Antigens of Bacteria Associated with Urinary Tract Infection in Human Adult. ٩: ٧٧٣-٧٨١. Babylon. University, Iraq.
- Shnawa, I.M.S. and. Al-Shakery, M.A.N. (١٩٩١). Lapin Humoral and Cellural Immune Responses to *Actinomyces bovis* Antigens. J.Vet. Sci.
- Smith, J.w. and Kaijser, B. (١٩٧٦). The Local Immune Response to Escherichi coli O and K Antigen in Experimental Pyelonephritis. J. clinc. Invest. ٥٨ (٢): ٢٧٦-٢٨١.
- Smith, J.W.; Wagner, S. and Swensan, R.M. (١٩٨١). Local Immune Response to *Escherichia coli* Pili in Experimental Pyelonephritis. Infect. Immune. ٣١ (١): ١٧-٢٠.
- Smits, E.; Burvnich, C.; Guidry, A.J. and Roets, E. (١٩٩٨). In Vitro Expression of Adhesion Receptors and Diapedesis by Polymorphonuclear Neutrophils During Experimentally Induced *Streptococcus uberis* Mastitis. Infect. Immune. ٦٦٨: ٢٥٢٢-٢٥٣٤.
- Sordillo, L. M.; Shafer-Weaver, K. and De Rosa, D. (١٩٩٧). Immunobiology of The Mammary Gland. J. Dairy. Sci. ٨٠: ١٨٥١-١٨٦٥.

- Strans, D. C. (١٩٩٨). Klebsiella Tnfection and Immunity. In: Encyclopedia of Immunology. Vol ٣. ٢nd ed. P. J. Delves and I. M, Roitt (eds.). P: ١٥٢٢-١٥٢٣.
- Strober, W. and James, S. P. (١٩٩٤). The mucosal immune system. In: Basic and Clinical immunology. ٣rd ed. D. P. Stitis.; A. I. Terr. And T. G. Parslow. (eds.). P: ٥٤١-٥١٥. Prentice-Hall. International, Inc. London.
- Svanborg-Eden, G.; Kalhary, R.; Marlid, S.; Prince, S.J. and Mestecky, J. (١٩٨٥). Urinary Immunoglobulin in Healthy Individual and Children with Acute Pyplonephritis. Scand. J. Immunol. ٢١: ٧٩٠- ٧٩٧.
- Taussing, M. J. (١٩٧٥). Antigenic Competition. In: Hobart. M. J. and Mc Cannetl, I. (eds.). The Immune System. A Course on The Molecular and Cellular Basis of Immunity. PP: ١٦٥-١٧٨. Black Well Scientific Publications. Oxford.
- Thomsen, A. C. (١٩٨٢). Infections Mastitis Occurrence of Antibody-Coated Bacteria in Milk. Am. J. obstet. Gynecol. ١٤٤(٣): ٣٥٠-٣٥١.
- Thomsen, A. C.; Espersen, T. and Maigaard, S. (١٩٨٤). Course and Treatment of Milk Stasis, Non Infections Inflammation of The Breast, and Infections Mastitis in Nursing Women. Am.J. Obstet. Gynecol. ١٤٩ (٥): ٤٢٩-٤٩٥.
- Thomsen, A.C.; Hansen, K.B. and Moller, B.R. (١٩٨٣). Leucocyte Count and Microbiological Cultivation in The Diagnosis of Puerperal Mastitis. Am.J. obstet. Gynecol. ١٤٦(٨): ٣٩٨-٩٤١.
- Thomsen, A.C.; Magensen, S.C. and love-Jepsen, F. (١٩٨٥). Experimental Mastitis in Mice Induced by Coagulase Negative Staphylococci Isolated from Cases of Mastitis in

Nursing Women. Acta. Obstet. Gynecol. Scand.٦٤ (٢): ١٦٣-٦.

- Tilson, B.(١٩٩٣). Mastitis-Plugged Duct and Breast Infections. J. Leaven. ٢٩ (٢): ١٩-٢١.
- Tlaskalova-Hogenova, it.; Tuckova, L.; Londinova, Zadnikova, R.; Stepankova, R.; Cukrowska, B.; Funda, D., P.; Striz, I.; Kozakova, H.; Trebichvasky, I.; Sokol, D.; Rehakova, Z.; Sinkora, J.; Fundova, P.; Horakova, D.; Jelinkova, L. and Scanchez, D. (٢٠٠٢). Mucosal Immunity: Its role in Defense and Allergy. Allergy and Immunology. ١٢٨(٢): ٧٧-٨٩.
- Tomasi, T. B. (١٩٧٦). The Immune System of Secretion. Prentic-Hall. Inc. Engle Wood. Clifts, New Jersey.
- Tudar, K. (٢٠٠٠). Mechanisms of Bacterial Pathogenicity: Bacterial Defense Against The Host Immune Responses. Bacteriology ٣٣٠ UW Madison. ١:٥.
- Van Cott, J.L.; Yamamoto, S. and Mc Ghee, J.R. (٢٠٠٠). Mucosal Immunity. In: M.W. Cunningham; R.S. Fujinami. (eds). Effect of Microbes on The Immune System. Lippincott Williams and Wilkins. Philadelphia. PP, ٢٣٣-٢٤٩.
- Vercellotti, G.M.s Mccarthy, J.B.; Lindholm, P., Peterson, P.K.; Jacob, H.S. and Furcht, L. (١٩٨٥). Extracellular Matrix Proteins (Fibronectin, Lamnin, and type IVCollagen). Bind and Aggregate Bacteria. Am. J. Pathol. ١٢٠: ١٣-٢١.
- Virella, C. (١٩٩٧). Microbiology and Infections Diseases. ٣rd ed. Williams and Wilkins Awaverly Company.
- Vogel, A.; Hutchison, L. and Edward, A. (١٩٩٩). Mastitis in the first year postpartum. Birth. ٢٦(٤): ٢١٨-٢٢٥.

- Wagner, C.L.; Forsythe, D.W. and Pittard, W.B. (١٩٩٥). Variation in the Biochemical forms of transforming growth factor-alpha present in human milk and secreted by human milk microphages. Boil. Neonate. ٦٨(٥): ٣٥٢-٣٣.
- Walter, J. B.; Talbot, I. C.; Gardner, H.; Halloran, P. F.; Zuckerman, M.; Bird, A. G. and Fobes, A. (١٩٩٦). Walter and Israel General Pathology. ٧th ed. Churchill Livingstone, New York.
- Wayne, R. Cohen, M. D. (٢٠٠٠). Cherry and Merkatzs Complications of Pregnancy. PP: ٧٥٥-٧٥٦. Lippincott Williams and Wilkins. Philadeliphia.
- Weir , D.M(١٩٩٢). Immunology . ٦th .ed ,pp ٨٦ –١٣٦. Longman Group UK Limited . London.
- WHO ,(٢٠٠٠). Mastitis : Causes and Mangement. WHO /FCH/ ٠٠.١٣ . Geneva.
- Williams , P; Comara, M. Hardman, A.; Swift, S.; Milton , D.; Hope , V.J.; Winzar, K.; Middleton, B.; Pritchard , D.I. and Bycroft , B.W.(٢٠٠٠). Quorum Seasing and The Population Dependent Control of Virulance. Phi. Trans, R.soc. Land B. ٣٥٥:٦٦٧-٦٨٠.
- Wold, A.F.; Mestecky, J. and Tomana, M. *el al.*(١٩٩٠) Secretary Immunoglobulin A Sarries Oligosaccharide Receptors for Escherichia Coli Type ١ Fimbrial Lectin . Infect. Immune. ٥٨ : ٣٠٧٣.
- Wold, A.E. and Adlerberth, I. (٢٠٠٠). Breastfeeding and The Intestinal Micro Flora of The Infact-Implications for Protection Against Infection Diseases. Adv. Exp. Med. Biol. ٤٧٨: ٧٧-٩٣.

- Zubler, R.H. (١٩٩٨). Antigens, T dependent and independent. In: Delves, R.T. and Roitt, I. (eds.) Encyclopedia of Immunology. Vol I. Academic Press, London. PP: ٢١٤-٢١٨.