THE STUDY OF IMMUNE RESPONSES IN PATIENTS WITH BACTERIAL CHRONIC OTITIS MEDIA

A Thesis

Submitted to the Council of the College of
Science, Babylon University,
in Partial Fulfillment of the Requirements for the Degree of
Doctorate of Philosophy in Biology/Microbiology

By

Salman Aziz Adoos M.Sc. College of
Science University of Baghdad ١٩٨٧

May ٢٠٠٥

بِسم الله الرحمن الرحيم

((قُلْ لَوْ كَانَ البَحر مِداْداً لِكَلِماْتِ
رَبيْ لَـَفِذَ البَحرُ قَبلَ أنْ تَنْفِذَ كَلِماتُ
رَ ْيّ وَ لَو جِئنْا بَمَثلِه مَدَدَا))

صَدَق الله أَلْعَلِي العَ ظْيم

((الكهف ١٠٩))

CERTIFICATION

I certify that this thesis was prepared under my supervision at the Department of Biology, College of Science, Babylon University, in partial requirements for the Degree of Doctorate of Philosophy of Science in Microbiology and this work has never been published anywhere.

Signature:
Name:Dr. Ibrahim MS. Shnawa
Title: Professor
Address:
Department of Biology College of Science Babylon University

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

Signature:
Name: Dr.Ali S. M. AIaraji

Title: Professor

Address: Head of Department of Biology College of Science **Babylon University**

Date:

Examination Committee

We, the examination committee, after reading this thesis and examining the student Salman Aziz Adoos, in its content, have found that it meets the standard and requirements as a thesis in fulfillment for the Degree of Ph.D.in Biology/Microbiology.

Signature :
Name: Dr. Ibrahim M.S.Shnawa
(Supervisor)
Title :Professor
Address: Department of Biology College of Science University Of Babylon

Signature :
Name:Dr. Jaafar K.Naama
(Member)
Title :Assistant Professor
Address: Head of Medical Microbiology
College of Medicin ,
Univ.Of Kufa

Signature:
Dr. Hadi R. Al-Masoody
(Member)
Assistant Professor
Department of Biology
College of Education
University of Kerballa

Signature
Name: Qasim, N.
(Member)
Title :Assistant Professor
Address: Dept. of Biology
College of Science,
University Of Babylon

Signature :
Wesam, A. Al-Taher
(Member)
Assistant Professor
Babylon Technical Institute

Signature
Name: Dr.Hussain S. Al-Janabi
C H A I R M A N
Title : Professor
Address: College of Medicine(Dean)
University of Babylon

Approved for the College Committee of Graduate Studies

Signature

Dr. Oda M.Y. Al- Zamely

Title: Assistant Professor

Adress:Dean of College of Science, University of Babylon.

DEDICATION

Dedicated to

My Wife……..

Sons……

And Daughter

Salman…….٢٠٠٥

ACKNOWLEDGMENT

I would like to express my deepest gratitude to my supervisor, Professor Dr. IBRAHIM MOHAMAD SAEED SHNAWA, for introducing me to the field of immunology research, and for his great support and expert guidance at various stages of my study. I greatly admire his creative scientific attitude which helped me throughout this study.

I would like to express my gratitude to university of kufa for offering me the study leave.

My deepest thank to Babylon University and College of Science for their scientific support , education and guidance.

I am especially grateful to Dr. Jaafar, K. Naama, Head of the department of Microbiology, College of Medicine. University of Kufa, for his unlimited support throughout the course of my study.

My sincere gratitude is addressed to all the physicians in E.N.T. Unit in Teaching Hospital in Najaf, specially, Dr.Yasser Hassoon; Dr.Saad Mahboba; and Dr. Kahtan AI-Zeber for the positive atmosphere that has been created by them throughout of my research.

I would like to express my thanks to all lab. Staff of teaching hospital in Najaf for their kind help in specimens collection and diagnosis of samples in their Laboratories.

I am greatly indebted to Dr. Abdul Karim Abdullah, Head of the department of Community Medicine, College of Medicine. University of Kufa, for his statistical expert guidance.

Finally I express my gratitude to staff of department of Microbiology, College of Medicine. University of Kufa, especially, Miss Ekhtiar Thabit for her skillful assistance in the collection of references and printing of some data for my study.

SUMMARY

At present time, bacterial infection predisposing to chronic ear discharge has been thoroughly investigated in this area. Work on immunology of ear in cases of chronic ear discharge; has been scarce or nill. The aim of the present work was to investigate mucosal as well as systemic immune responses of chronic ear discharge patients.

٢٤٥ patients with chronic ear discharge who attended to otorhinolaryngology unit at teaching hospital in Al- Najaf were subjected to a retrospective study for determination of the bacterial profile of chronic ear discharge. The most widely associated bacteria, in chronic otitis media were *Pseudomonas aeruginosa* ٣٦٪ , *Proteus mirabilis* ٢٠٪ **,** *Staphylococcus aureus* ١٩٪, *E. coli*, ١٠%, B- hemolytic *Streptococci* were ٥٪ and *Klebsiella* species were ٣٪.

New ١٠٠ patients were enrolled in a prospective study just to reaffirm the bacterial profile and the study indicate the most common three bacterial associated with chronic ear discharge were *Ps. aeruginosa* ٤٦٪; *P. mirabilis* ١٩٪, *S. aureus* ١٧٪.

Surface antigens(whole cell antigens) were prepared from these species by heat killing of Gram negative bacterial isolates of *Ps. aeruginosa* and *P. mirabilis* and by benzalchonium chloride for Gram positive *S. aureus***.** Immunological studies were accomplished on top

three bacterial isolates *Ps. aeruginosa*, *P. mirabilis* and *S.aureus*.

Humoral and cellular immune responses of CED patients to these most common bacterial isolates at both systemic and mucosal compartment. Patients with other species were not included.

Natural humoral immunity of these patients was assayed by measuring total serum protein, serum Ig and secretory Ig (mucosal Ig). Cases of CED patients caused by the three bacterial *Ps. aeruginosa, P. mirabilis* and *S. aureus* were as follows:

In *Ps. aeruginosa* patients the total serum protein increased with mean value of ٦٥.٦g/L. Serum with Ig mean value was increased to ٣٥.٨٦g/L. Serum albumin slightly decreased to ٣٢.٣٥g/L. Mucosal Ig increased with mean value ٠.٣٧g/L.

In *P. mirabilis* patients, the total serum protein raised up with mean value ٦٤.٤g/L; serum Ig increased to ٣٦.٦١/L,.serum albumin decreased to ٣٠.٢g/L, mucosal Ig increased to ٠.٤١g/L.

Total patients serum protein in cases of *S. aureus* increased with mean value ٦٦.g/L, serum Ig raised up to ٣٥.٥, serum albumin came down to ٢٧. ٨٤ g/L. the mucosal Ig was ٠.٤٨ g/L.

Specific humoral immunity was determined in those patients affected by bacteria mentioned above at the systemic and mucosal system.

In ١٠ patients with CED that were caused by *Ps.aeruginosa*, the titers of specific antibodies was measured. Mean serum value was ٢٤٠ when whole cell antigen were used as counter antigen, and the mucosal mean titer was ١٩.٢ using whole cell antigen (by standard

tube agglutination method).

In ١٠ patients with *P. mirabilis* chronic ear discharge the mean systemic titer was ٢٢٤ and mucosal titer mean value was ٢٤ when somatic antigens were used in standard tube agglutination method. These values were characterized by increased titers mean value in mucosal and peripheral blood.

In case of *S. aureus* ten patients with CED were studied for serum and mucosal specific immunoglobulin. The serum mean value was ٣٣٦ and mucosal Ig titer was ٢٤. The titer at systemic and mucosal raised up more than normal value.

Phagocytic activity of polymorph nuclear cells was determined by nitroblue tetrazolium reduction test (NBT) at the peripheral blood and local system.

In *Ps. aeruginosa*, the number of PMNs as measured by NBT test at the peripheral blood raised up with mean value ٣٠. ١٪ and mucosal mean value ٣٧٪.

P. mirabilis patients NBT systemic value was ٢٧. ٤٪ with no significant increase observed. At the mucosal system the mean value for NBT increased to ٣٧.٤٪.

In patients with *S. aureus* the systemic NBT increased to ٣٥.١٪ and mucosal ٤٠.٨٪ compared with normal subject.

For the determination of cellular immune responses, two parameters were performed, Leukocyte inhihibitory factor and E-rosette formation .Ten patients were taken for each parameter in each case, as fallows:

In *Ps. aerugiosa* the LIF percentage raised up with systemic LIF of ٦٣٪ and at the mucosal it was ٤٩٪ when CFCF were used as a sensitizer.

E-rosette formation was increased at local and peripheral blood. It was ٣٢.٢ and ٤٦.١% at the systemic and mucosal respectively.

P. mirabilis did not induce systemic and mucosal cellular immune response when LIF was used as a parameter. It was ٨٧٪ at the systemic as ٨٢٪ at the mucosal one when cell free culture filtrate was used as a sensitizer. The systemic E-rosette formation also did not increase, it was ٢٦.٦٪, and it was raised up at the mucosal system and reached up to ٣١.٧.

S. aureus ear infection was characterized by a significant increase of LIF and E-rosette formation as the systemic and mucosal system, the LIF was ٥١٪ and ٤١٪ at systemic and mucosal respectively. E-rosette formation was ٣٨.٥٪ at the systemic and ٤١٪ at the mucosa! system.

Lipopolysaccharide was extracted and purified from Gram negative bacteria in CED patients and the study was done by using LPS as a sensitizer in LIF.

In ١٠ patients with Ps. aeruginosa CED, LIF was measured by using LPS as a sensitizer. The LIF significantly increased at both systemic and mucosal system. It was ٥٢٪ and ٤١٪ at peripheral and local system respectively.

In ١٠ patients with CED by P. mirabilis the LIF was measured at

peripheral blood and cells from ear discharge by using LPS as sensitizer. The LIF significantly increased. It was ٥١٪ as the peripheral blood and ٤٣٪ at the mucosal one as compared with normal subject.

LIST OF CONTENTS

List of Tables

LIST OF FIGURES

LIST OF ABBREVIATIONS

Ags	Antigens
APC	Antigen Presenting Cell.
AS	Alsever Solution
BCED	Bacterial Chronic Ear Discharge
BEM	Basal Eagle Medium
CED	Chronic Ear Discharge.
CEDP	Chronic Ear Discharge Patient
CFCF	Cell Free Culture Filterate.
CFTR	Cystic Fibrosis Trans Membrane.
CMI	Cell Mediated Immunity.
CTLs	Cytotoxic T-Lymphocyte.
D.W	Distilled water
ET	Eustachian Tube.
FS	Formal Saline
HEV	High Endothelial Veinules.
IG	Immunoglobulin.
IL	Interleukin.
INF-a	Interferon Alpha.

LBP	LipoPolySaccharide binding Protein
LFA-٣	Lymphocyte function associated antigen ٣
LIF	Leucocytes Inhibitory Factor
LOS	LipoOligoSaccharide
LP	Lamina Properia.
LPS	LipoPolySaccharide
MALT	Mucosa Associated Lymphoid Tissue.
ME	Middle Ear.
MEC	Middle Ear Cavity.
MHC	Major histocompatibility complex
NALT	Nasal-Associated Lymphoid Tissue
NBT	Nitro-Blue Tetrazolium Stain
NO	Nitric Oxide.
NS	Normal Saline
OM	Outer Membrane.
OMP	Outer Membrane Protein.
PIgR	Polymeric Immunoglobulin Receptor.
PMN	Polymorphnuclear cell
PP	Pyery's Patch
RA	Rheumatoid Arthritis

Sc	Secretory Component.
SIgA	Secretory Immunoglobulin A.
SIgM	Secretory Immunoglobulin M.
SIRS	Systemic Inflammatory Response Syndrome
TLR	Toll-Like Receptor
TNF	Tumor Necrotic Factor.

INTRODUCTION

١-١.OVERVIEW

Injury, infection, degenerative disease and cancer are the four main causes of human death at the age before childbearing (Playfair and Chain, ٢٠٠١). Infection from the immunology point of view can be vaccine preventable and nonvaccine preventable (Bluestone, ١٩٩٨). Among the nonvaccine preventable are gut, genitourinary, integument as well as respiratory infections. A rather common complaine an ear discharge can be seen mostly in childhood in our community the mid-Euphrates as well (AI-Mola *et al,* ١٩٩٨).

Otitis media, however, are medically classified as upper respiratory tract affections. Several studies have been conducted on microbial profile of Otitis media at mid-Euphrates (Aubaidy, ١٩٩٨; and AI-Tallah, ٢٠٠٠).

١-٢ Reasoning:

The mucosal versus systemic immune state of chronic ear discharge patients were not tackled by Iraqi researchers so far our knowledge had been indicated. A mucosal immunological studies of

Otitis media have been tackled on by other workers (Daly, *et al*, ١٩٩٩; and Straetemans, *et al*, ٢٠٠١). Thus the present work has been planned

١-٣. AIM OF THE STUDY

The main aim of the present work has been the investigation of humoral and cellular immune response on patients with chronic ear discharge both at mucosal and systemic levels. To verify this aim several steps have been followed, as shown below:

١-Performing a retrospective bacterial screen on chronic ear discharge.

٢-Doing prospective study on ١٠٠ cases of chronic ear discharge (CED) patients to fix up the bacterial profile.

٣-Selection of the three commonly associated bacterial causals and making immunologic assay using whole cell antigen and cell free culture filtrates.

٤-Purification and assay of Lipopolysaccharides of the gram negative bacteria , then using this pure LPS for assaying humoral and cellular immune responses.

٥-Comparing results of the immune function test for step ٣ and ٤ to draw correlation and conclusions.

LITERATURES REVIEW

٢-١: Ear: a structure function relationship:

The ear has three major parts: the outer ear, middle ear with Eustachian tube ET and an inner ear. Both the ET and middle ear ME are important in relation to the accumulation of fluid behind the tympanic membrane. ME is an air-filled chamber which contains the auditory ossicles. The ET connects the ME with the nasopharynx. The nasopharyngeal two thirds of the tube are cartilaginous, while the tympanic one third is bony and blends into the ME Cavity (Proctor, ١٩٦٧; Holborow, ١٩٧٠; and Prades, *et al.* ١٩٩٨).

The mucosal lining of the ET consists of epithelium and sub epithelial connective tissue, which contain numerous blood capillaries and nerve fibers (lim, ١٩٧٤). The nasopharyngeal end of the ET has a squamous epithelial lining. This lining is mucociliary and continuous with two strand of ciliated cells on the floor of the ME cavity. The mucocilliary epithelium is covered with mucus blanket this provides the basis for tubotympanic mucociliary transport which clears the ME cavity (Sade, ١٩٦٧; lim, ١٩٧٤). ET has three important functions with respect to the ME: ventilation, protection and clearance. ME is protected by the functional anatomy of ET and by immunological and mucocilliary defense of the mucosal lining. Clearance of ME is achieved by

mucociliary transport in the ET. Ciliary movement is directed towards the nasopharynx and transport ME secretion via mucus blanket on top of the cilia (Sade, ١٩٦٧).

The mucocilliary system is the primary defense of the tubotympanum to keep middle ear sterile. The dysfunction of ET is considered to be an etiological factor in the development of otitis media (Takahashi, *et al.* ١٩٨٩). Studies on different population of children indicate that ET function is impaired in children with otitis media with effusion or otitis-prone children compared to healthy children (Stenstrom, *et al.* ١٩٩١; Bylander and stenstrom, ١٩٩٨)

Impairment of clearance function can result from epithelial damage by viral or bacterial infection originating in nasopharynx e. g. non typable *Haemophilus influeuzae* and influenza A virus (Wilson, *et al.* ١٩٨٥; and Ohashi, *et al.* ١٩٩١).

٢-٢-A: Ear affections an overview

The source of ear affection could be exogenous, endogenous of oral, lymphatic or haematogenic origins (Hellstrom, *et al.* ١٩٩٧). Ear affection can be classified on basis of the temporal sequence of the disease process (not severity). The terms acute, subacute and chronic are recommended. Acute otitis media (AOM) is an inflammation of middle ear that presents with a rapid onset of signs and symptoms, such as pain, fever, irritability, anorexia, or vomiting (Bluestone and Klein, ١٩٨٣).

Chronic disease implies middle ear fluid that has been present for three months or longer and has had many synonyms, including serous OM, secretory OM, and ((glue ear)). The sub acute is the time in between (Kenna, ١٩٩٨).

٢-٢-B: Ear potential pathogens.

The profile of potential ear pathogens covers virus, bacteria and/or fungi (EI-Gamal, *et al.* ١٩٩٣; Heikkinin, *et al.* ١٩٩٩). Studies abroad showed the following profile: the bacteria are most frequently cultured from effusions during ongoing acute otitis media and/or secretory otitis media are *Streptococcus pneumoniae, Hemophilus influenzae , Moraxella catarrhalis* that gain access through ET from nasopharynx. In chronic OM, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are frequent pathogen (Stenfors, ١٩٩٩), While Iraqi and vicinity studies have the following profile: *Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli, Klebsiella spp., and Ps. aeruginosa* (Al-Toraihi, ٢٠٠٠; Al-Snafi, *et al*. ١٩٩٩).

٢- ٣: Ear potential bacterial pathogens.

It was focused on the top of three ear potential bacterial pathogens as in the following:

٢-٣- ١: Pseudomonas aeruginosa.

Ps. aeruginosa typically causes an array of diseases only in individual with impaired host immune defenses and it is referred to as opportunistic pathogen and the host as immune

compromised individual which include patients undergoing immunosuppressive therapies (e.g. cancer treatment) or those receiving treatment for traumatic skin damage (burn wound) and those with human immunodeficiency virus infection and those with cystic fibrosis (Kielhofner, *et al.* ١٩٩٢; Govan and Deretic, ١٩٩٦). Recent clinical data indicated that *Ps. aeruginosa* was the fourth leading cause of nosocomial infection (Jarvis and Marion, ١٩٩٢). *Ps aeruginosa* may produce three colony types: rough, smooth and mucoid colonies, the later is frequently obtained from respiratory and urinary tract secretions which is attributed to production of alginate slime. The smooth and mucoid colonies are presumed to play a role in colonization and virulence (Kenneth, ٢٠٠٢).

Most Pseudomonas infections are both invasive and toxinogenic .The ultimate infection may be seen as composed of three distinct stages: (١) bacterial attachment and colonization (٢) local invasion (٣) disseminated systemic disease (Kenneth, ٢٠٠٢). Ps. *aeruginosa* has a series of virulence associated antigens such as fimbrae, slime layer proteases, cytotoxins (Todar, ٢٠٠٢). These antigen and their specific host responses will be mentioned with ear mucosal response to infections

٢-٣-٢: *Proteus spp* as pathogen.

The genus *Proteus* comprises three medically important species *Proteus mirabilis**,** P. vulgaris and P. penneri**,*** they cause mainly wound and urinary tract infections.

P. mirabilis is the most common pathogen which may be explained by its high carriage rate (٢٥٪) in human intestine (Chow, *et al.* ١٩٧٩). This part of the body is the major reservoir of these bacteria in human and this can result in auto infections or transmission of bacteria from patient to patient in hospital (Warren, et al. ١٩٨٢).

P. mirabilis has been described as opportunistic etiological agents in many infections including respiratory tract, ear, eyes, nose and throat (Cooper, *et al.* ١٩٧١; Pennar, ١٩٩٢).

P. *mirabilis* has several virulence associated antigens such as fimbrae, outer membrane proteins, lipopolysaccharide (LPS), capsular antigen, IgA protease and other proteinases (Rozalski, *et al.* ١٩٩٧).

This will be thoroughly discussed in ear mucosal immune response to specific affections.

٢-٣-٣; *Staphylococcus aureus*.

S. aureus is a pyogenic gram positive human pathogen. It was involved in several infections including that of respiratory tract. This is with an emphasis on otitis media and chronic ear discharge (Stenfors, ١٩٩٩). As a pathogen, it gains assess ear either as a result of exogenous or endogenous routes. *S.aureus* has several virulence associated antigens such as surface proteins, surface factors protein A, couagulase, membrane damaging toxins and exotoxins (Todar,٢٠٠٢). These will be thoroughly discussed in ear mucosal immune responses to

infection as well as systemic response.

٢- ٤: Ear versus systemic affections.

The infections of nose, tonsils, teeth, gum periodontal area as well as the infection of buccal cavity may be transmitted to the ear through Eustachian canal (Kenna, ١٩٩٨). Blood and /or lymph circulations may be a possible vehicle for potential mucous invading pathogens which may find their way to Eustachian canal then to the middle ear causing disease. Common cold and influenza may have such complications (Tarlow, ١٩٩٨).

Reversal of ear affection may be possible from middle ear cavity through mucous transported from middle ear cavity to nasopharynex (Block, ١٩٩٧).

٢- ٥: Mucosal, humoral and cellular immune system.

Mucosa -associated lymphoid tissues (MALT) aggregation of noncapsulated lymphoid tissue is found especially in lamina properia (LP) and submucosal areas of gastrointestinal, respiratory and genitourinary tract. The lymphoid cells are present as either solitary or aggregated nodules containing germinal center (secondary follicles). In humans, the tonsils contain considerable amount of lymphoid tissue often with large secondary follicles and intervening T-cell zones with high endothelial venules. Similar accumulations of lymphoid tissue are seen lining the bronchi and along the genitourinary tract, the digestive, respiratory and genitourinary mucosa contain

dendtritic cells for uptake processing and transport of antigen to the draining lymph nodes(Kuby, ١٩٩٧).

The mucosa is bombarded immediately after birth by a large variety of microorganisms as well as by protein antigens from the environment. Mucosal immune system has generated two arms of adaptive defense to handle these challenges: (i) antigen exclusion performed by secretory IgA (SIgA) and secretory IgM (SIgM) antibodies to modulate or inhibited colonization of microorganisms and dampen penetration of potentially dangerous soluble luminal agents. (ii) suppressive mechanisms to avoid local and peripheral over reaction (hypersensitivity) against innocuous substances bombarding the mucosal surface. The latter arm is referred to as "oral tolerance" when induced via the gut against dietary antigens (Brandtzaeg, ١٩٩٦). Secretory immunity is preferentially stimulated by pathogens and other particulate antigen taken up through thin M cells located in the dome epithelium covering inductive mucosa associated lymphoid tissue. M cells contain deep invagination of the basolateral plasma membrane which forms pockets containing B and T lymphocytes, dentritic cell and macrophages (Hathaway and Kraehenbuhl, ٢٠٠٠). Many enteropathogenic infectious bacteria and viral agents use the M cell as portal of entry and reach professional antigen presenting cells (APC) including B-cells and follicular dentritic cell .In addition, mucosal dentritic cell may capture antigen and migrate via draining lymph

to regional lymph node where they become active APCs which stimulate T-cell for positive or negative (down regulatory) immune response (Brandtzaeg , ٢٠٠٢).

Mucosal inductive sites in human, such as peyers patches in the intestinal tract and the nasal associated lymphoreticular tissue in the oropharyngeal cavity, stand as sentinels to the intestinal and respiratory tracts and represent the major site where mucosal immune response are initiated. Common features of these inductive sites are microfold or M-cell. Although the precise function of M-cell has not yet been established, recent studies have indicated that they are involved in uptake, transport, processing and possibly presentation of microbial antigens (Allan, *et al.* ١٩٩٣; and Neutra, *et al.* ١٩٩٦,a, b). The interaction of epithelial cell to differentiate in to M-cell in vitro (Kernels, *et al.* ١٩٩٨), indicating the importance of lymphocyte-epithelial cell interactions for maintaining M-cells in follicle-associated epithelium of the peyers patches. These lymphocyte M-cell interaction which can occur in the pocket of the M-cells are mediated through thin cellular extensions, indicating that cell-ceil interactions are intricate part of M-cell function and that they may facilitate transfer of luminal antigen, virus, bacteria and other protein component (Neutra, *etal.* ١٩٩٦).

M-cells are well suited for efficient endocytosis and transcytosis, they lack the rigid brush border cytoskeleton of the enterocyte of their neighbors, and their apical surfaces have

broad membrane microdomains from which endocytosis occur. The M-cell basolateral membrane is directly invaginated to form large intraepithelial "pocket" containing T- Lymphocytes (including CD٤+ cells and CD٤٥Ro+ memory cells) B-Lymphocytes and Macrophages (Neutra, *et al.* ١٩٩٦). This structural specialization brings the basolateral cell surface to within a few microns of the apical and greatly shortens the distance that transcytotic vesicles must travel to cross the epithelial barrier. Endocytic or phagocytic uptake of foreign antigens or particles is followed by rapid transcytosis to the intraepithelial pockets, with little or no retention in M-cell lysosomes. After M-cell transport, antigens are processed and presented by macrophages, dentritic cells, and B-cell within and between epithelium, resulting in generation of IgA-committed, antigen specific B-lymphoblast that proliferate locally in the germinal center of organized MALT and migrate via blood stream to distant mucosal area and glandular tissues where they differentiate into plasma cells. The dimeric or polymeric IgA antibody thus produced are selectively bound by epithelial polymeric immunoglobulin receptors. Transcytosis across-epithelial cells and released into glandular and mucosal secretions (Apodaca, *et al.* ١٩٩١). M cell transcytosis is an important first step in initiation of a secretory immune response.

The importance of mucosal immune system was quickly realized after the discovery that SIgA is the most abundant

isotype of antibody in secretions and the elucidation of its unique structural properties. SIgA is the major isotype in human digestion tract and milk, as evidenced by both Ig levels and the presence of Ig-producing cells (Brandtzaeg and Farstad, ١٩٩٩). SIgA structure affords the molecule resistance to most proteases and increases its functional affinity for corresponding antigens. Unlike serum, IgA which is highly sensitive to protease, the Fc region of S-lgA is wrapped within a secretory component (Sc) molecule, which renders associated chains protease resistant (Mestecky and Russell, ١٩٨٦). In addition, the hing region of IgA is either absent in the lgA٢ or replaced by a pseudohing structure with low flexibility, which is also protected from many enzymes by the presence of special carbohydrate chains (Mattu, *et al*. ١٩٩٨). The functional capacity of the S-lgA molecule is increased by its dimeric and even tetrameric status, as demonstrated for other polymeric immunoglobulin (Hornick and Karush, ١٩٧٢). It has been clearly established that the secretory immune system is compartmentalized and independent from the systemic immune system (Lue, *et al.* ١٩٩٤). When antigen penetrates the epithelial layer through M-cell in mucosa-associated lymphoid tissue, it triggers the immune response (Neutra, *et al.* ١٩٩٦).The activated cells undergo a circulating cycle via blood and lymph where they mature and reach the "high" endothelial veinules (HEV), and then disperse to areas of the subepithelial stroma.

It has been found that cells of the secretory system often, but not always, tend to migrate toward their tissue of origin (Brandtzaeg &Farstad ١٩٩٩). In the stroma, these Ig-producing cells synthesize polymeric IgA through epithelium covalently linked with adjoining (J) chain required for Ig binding to the transmembrane precursor of Sc (Brandtzeag and prydz, ١٩٨٤), called the polymeric Ig receptor (PIgR) (Mostov, *et al.* ١٩٨٠).

The PIgR is initially localized to the basolateral surface of epithelial cells and allows active transcytosis of IgA through the epithelium and its release into the lumen as SIgA after cleavage of the ectoplasmic domain of the PIgR. Covalent disulfide bridges between IgA and the PIgR appeared during transport. This well designed system ultimately leads to "immune exclusion" on the mucosal surface (Brandtzaege, ١٩٩٦) i.e. preventing the entry of new pathogen through the mucosal barrier .In addition, transcytosis results in immune elimination (Brandtzaeg, ١٩٩٥) which consists of the active transport of IgA-bound pathogens from either the stroma (Kactzel, *et al.* ١٩٩١) or the epithelium where it is ultimately released (Mazanec, *et al.* ١٩٩٢). The sequences of events of mucosal immune response are:

Mucosal immune response

Inductive phase

-Priming of lymphocyte =antigen presentation

-Migration of lymphocyte from inductive site in mucosa (e.g.peyers patches).

↓

Regional lymph nodes

↓

Blood circulation

↓

Mucosal effectors sites (lamina properia)

↓

Effectors phase

Production of antibody predominately of SIgA

All isotype expressed in lamina properia

Beside the humoral immune mechanisms, mucosal immune system contains also cellular immune mechanisms such as $CD^{٨+}$ cell mediated immunity(CMI) and $CD^{٤+}$ cytokine production, and forms early line of defense. Beside supporting humoral immunity, $CD^{٤+}$T- helper cells function in CMI as producer of cytokines ,which mediate delayed type

hypersensitivity and support cytoxic T- lymphocyte (CTLs) which as such are critical components of the CMI responses to intracellular pathogens. Both antigen specific and non specific (Natural-killer cells, antibody-dependent cytotoxicity) cytotoxic cell types can control growth of intracellular pathogens by two distinct mechanisms. First they can respond to the infection by secreting a number of cytokines such as IFN-gð and TNF-að (Chiasari, ١٩٩٧) or chemokines such as Rantes, macrophage inflammatory protein-lao (Yang, *et al.* ١٩٩٧; Fehniger, *et al.* ١٩٩٨). These soluble factors inhibit growth of intracellular pathogens such **as** virus without destroying the host cell. Second, cytotoxic cells can effectively and efficiently recognize and lyse infected cells and prevent multiplication of virus.

Cytotoxic T-lymphocyte plays an important role in elimination of cells infected with various intracellular pathogens by recognizing specific antigen\MHC complexes. Antigen - specific CTLs inhibit further spread of pathogens and help to terminate infections. Compartmentalization of pathogen specific CTL responses have been reported and located at the site of initial infection, for example, CTLs preferentially compartmentalize in mucosa- associated lymph reticular tissues after pulmonary and intestinal infection (Frederik, *et al.* ٢٠٠٠).

٢-٦: Ear as a compartment of mucosal immune system.

In any circumstances where the ear becomes in a pathophysiological state, an impaired mucociliary clearance

which results from epithelial damage by viral or bacterial infection originating in nasopharynx can contribute to the accumulation of secretions in the middle ear (Karja, *et al.* ١٩٨٣).

As a consequence of ET dysfunction, pathogens can enter the normally sterile ME cavity, and the presence of bacteria and virus in the ME initiate an inflammatory response which subsequently may change the gas composition and also tissue characteristic.

What is typical of the non-specific protective modalities of middle ear cavity (MEC) is that they are present at birth, react promptly against a broad spectrum of microorganisms, have no memory system for their action and are present for life; the mucociliary clearance system is unceasingly transporting mucus containing trapped foreign material from the MEC to the nasopharynx. The viscous properties of the mucus film definitely play an important role in entrapping bacteria which have advanced in to MEC. In addition, the mucous layer contains several substances having antibacterial effect like lactoferin lysozyme, fibronectin, lactoperoxidase (Stenfors, ١٩٩٩).

Although the role of complement is not clear in MEC, the final phase of activation of complement cascade is the formation of membrane attack complex (Walpart, ١٩٩٣). The best known opsonins are the complement cleavage product C٣b, C-reactive protein and, of course, antibodies (lgG١, lgG٣, IgM) (Prellner, *et al.* ١٩٨٩). Leukocytes are very few in healthy MEC, and these

cells are attracted to mucous membrane whenever an inflammatory reaction is induced. Ultimately many pathogenic bacteria invading the MEC are killed by phagocyte. The rapid release from macrophage of cytokines such as TNF and IL-١ leads to systemic activation of phagocytic cells and their adhesion to endothelium facilitating their passage to inflamed tissue.

The upper respiratory tract is the site of initial exposure to ingested and inhaled antigens. The nasopharynx is part of the upper respiratory tract and is closely related to the ME and ET. Walleye's ring (predominantly represented by the adenoid) which belongs to the mucosa- associated lymphoid tissue (MALT), which forms, the primary defense against pathogens at the port of entry of upper respiratory tract. The lymphoid cells of the ring are able to sample, recognize and destroy any inhaled pathogens. In addition, effectors and memory lymphocytes are produced here which may migrate to a neighboring mucosal site (i.e. the middle ear) to reinforce the local immune system (Van Kempen, *et al.* ٢٠٠٠)

Another mechanism against infection in the upper respiratory tract is the production of SIgA antibody by mucosal lining and lymphoid ring e.g. the adenoid. SIgA in nasopharyngeal secretions inhibits bacterial adherence and reduced nasopharyngeal bacterial colonization, both important factors in the protection against otitis media (Lim and

Mogi,١٩٩٤),

٢-٦-١: *Pseudomonas aeruginosa* immunogens versus ear mucosal and systemic immune responses during infections as in the following:

٢-٦-١-١.Fimbrae.

The fimbrae of *Pseudomonas* will adhere to the epithelial cells of the upper respiratory tract and by interence to other epithelial cells as well .These adhesins appear to bind to specific galactose or mannose or sialic acid receptors on epithelial cells. Colonization of the respiratory tract by *Pseudomonas* requires fimbrial adherence and may be aided by production of a protease enzyme that degrades fibronectin in order to expose the underlying fimbrial receptors on epithelial cell surface. Tissue injury may also play a role in colonization of respiratory tract (Fick, ١٩٨٩). Mucoid strains, which produce an exopolysaccharide (alginate) have an additional or alternative adhesion which attaches tracheobronchial mucin (N-acetyl glucosamine) .Beside pili and mucoid polysaccharide, there are possibly two other cell surface adhesins utilized by *Pseudomonas* to colonize respiratory epithelium or mucin in addition, it is likely that surface bound exoenzyme S can serve as an adhesin for glycolipid on respiratory cells

٢-٦-١-٢: Bacterial slime layer

The ability of *P. aeruginosa* to invade tissues depends upon it resistance to phagocytosis and to the host immune

defense, and the extracellular enzymes and toxin that break down physical barriers and contribute to bacterial invasion. Bacterial capsule or slime layer effectively protect cells from opsonization by antibodies; complement deposition, and phagocyte engulfment.

٢-٦-١-٣: Proteases

Two extra cellular proteases have been associated with virulence that exerts their activities at the invasive stage: elastase and alkaline protease .Elastase has several activities that relate to virulence. The enzyme cleaves collagen, IgG, IgA and complement. Alkaline protease interferes with fibrin formation and will lyse fibrin. Elastase & alkaline protease together are also reported to cause the inactivation of ga'ma interferon (IFN) and tumor necrosis factor (TNF) (Liu, ١٩٦٦ ; Liu, ١٩٧٩).

٢-٦-١-٤: Cytotoxin:

Ps. aeruginose produce three other soluble proteins involved in invasion: cytotoxin and two hemolysin (phospholipase and lecithinase). Cytotoxin (pore-forming protein originally named Leukocidin) have effect on neutrophiles but also appeare to be cytotoxic for most eukaryotic cell. Ps euroginosa produce two extracellular protein toxins, Exoenzyme S and exotoxin A.

the first exototoxin impairs the function of phagocytic cell in blood stream and internal organs to prepare for invasion by *Ps. aeruginosa.* Exotoxin A has exactly the same mechanism of action as diphtheria toxin, it causes the ADP ribosylation of eukaryotic elongation factor ٢ (Stanley, *et al.* ١٩٨٣). Also Weber, *and* Osborn (١٩٨٢) found that toxin -A and elastase inhibit protein synthesis in macrophage of mice thus indirectly affect phagocytosis.

Immunological response to *Ps. aeruginosa* antigen are various cell associated and secreted antigens of *Ps. aeruginosa* have been studied, among pseudomonas antigen, the mucoid substance, which is an extracellular slime consisting predominantly of alginate, was found to be heterogeneous in terms of size and immunogenicity. It was found that crude slime extracted from *Ps. aeruginosa* has activity against phagocytosis in animal but their effect on phagocytosis in human is not well known (Laharague, *et al.* ١٩٨٤). In addition, Garzelli, *et al.* (١٩٧٩) found that bacteria killed by heat inhibit humoral immune response in mice by impairing production of antibody and, that heat-killed bacterial cell inhibit B-lymphocyte response. Rubin, *et al.* (١٩٨٣) found that *Ps. aeruginosa* inhibit proliferation of lymphocyte and DNA synthesis in this cell.

Effect of secreted substances by *Ps. aeruginosa* are well studied by different scientist .It was found that the effect of these substances depend on their type, their molecular weight and biological activities. Nonoyama, *et al.* (١٩٧٩) found that substances with high molecular weight inhibited phagocytosis and killed polymorphnuclear cell. The scientist purified these substances and described them as protein in nature. Sorensen, *et al.* (١٩٨٣) found that substances with low molecular weight like pyocyanin inhibit lymphocyte proliferation. Kharazmi, *et al.* (١٩٨٤) found that the effect of alkaline protease and elastase stopped chemotaxis in agarose.

٢-٦-١-٥: Lipopolysaccharide

LPS molecules are located in the cell wall and thus play an important structural role which mediates interaction with neighboring environment. The tripartite nature of LPS divides the molecule into hydrophic lipid-A region, central core oligosaccharide region and repeating polysaccharide referred to as ٠-antigen or ٠-polysaccharide. The terms "smooth" and "rough" are often used to describe the LPS phenotype. Attachment of the ٠-antigen to core-lipid -A results in smooth LPS phenotype, while core-lipid A lacking ٠-antigen is referred to as rough LPS. The contribution of smooth LPS to virulence has been demonstrated repeatedly by using various animal model systems (Rocchelta, *et at.* ١٩٩٩).

Cryz, *et al.* (١٩٨٤) showed that a wild-type strain of *Ps.*

aeruginosa with smooth LPS was more virulent than were isogenic mutants. In a burned -mouse infection model, the mutant, which has rough LPS, has ٥٠٪ lethal dose more than ١,٠٠٠-fold higher than that of the wild type strain .Recent studies by Preston, *et al.* (١٩٩٥) with mouse cornea infection model and with neonatal -mouse challenge model confirm that intact smooth LPS is required for *Ps. aeruginosa* virulence . In vitro experiments have shown that rough mutants of *Ps. aeruginosa* deficient in ٠- antigen synthesis are sensitive to the killing effects of human serum while wild- type strain with smooth LPS are serum resistant (Hancock and Speert , ١٩٨٣ ; Dasgupta, *et al.* ١٩٩٤).

When LPS is shed by bacteria into host tissues, it is usually bound by LPS binding protein (Lamping, *et al.* ١٩٩٦), which is transferred to the CD١٤ receptor on macrophage, thereby inducing secretion of cytokines including tumor necrosis factor alpha,interleukin-١ (١L-١),١L-٦,١L-٨,and ١L-١٠(Lynn and Golenbock,١٩٩٢). These cytokines are known markers of inflammatory responses. The lipid A region of LPS composed of a phosphorylated diglucosamine moiety substituted with fatty acid, is thought to be responsible for most of the biological activities of LPS through the induction of these immunomodulating molecules. Release of this inflammatory mediator enhances host defense against bacterial infection. Excessive LPS stimulation of the immune system can occur,

whereby elevated levels of activated and recruited immune cells results in septic shock and even death (Lynn and Golenbock, ١٩٩٢). *Ps. aeruginosa* is one of the top three pathogens responsible for sepsis due to gram-negative bacteria, and LPS from this organism is capable of overstimulating the immune system (Dulley, ١٩٩٠). Interestingly, *Ps. aeruginosa* lipid A is less toxic than that of entric organisms, probably due to the presence of mostly pentaacyl rather than hexoacyl chain (Kulshin, et al. ١٩٩١). Attached to the lipid A is the core oligosaccharide region of LPS, which can be subdivided into inner and outer core. The outer core region of *Ps. aeruginosa* LPS has recently been proposed to function as bacterial legand for association and entry of the organism into corneal cells during the infection stage of keratitis (Zaidi, et al. ١٩٩٦). Recently Pier, *et al.* (١٩٩٦a), and Pier, et al. (١٩٩٦b) assessed the role of cystic fibrosis transmembrane regulator protein (CFTR) as a receptor for the binding of *Ps. aeruginosa* to host epithelial surface. In these studies CFTR - minus epithelial cell lines showed poor ingestion and uptake of *Ps. aeruginosa* compared to cell lines transfected with CFTR. These authors have proposed that the presence of CFTR in normal host could be a defense mechanism whereby efficient epithelial-cell ingestion of *Ps. aeruginosa* followed by cellular desquamation and swallowing, could facilitate bacterial killing by the digestive system of the host.

The O-antigenic region is highly immunogenic and elicits

a strong antibody response from the host. Resistance to serum is conferred by the presence of O-antigen on the cell surface, and the extent of this serum resistance is influenced by O-antigen structure, chain length, and the amount of O-antigen substituted on core lipid A (Joiner, ١٩٨٥). *Ps .aeruginosa* produces two forms of O-antigen known as A band (homopolymer), and B band (heteropolymer). The A band O-polymersaccharide region is composed of D-rhamnose (D-Rha) residues (approximately ٧٠ D-Rha residues) (Yokota, *et al.* ١٩٨٧). This is shorter than B-band O-antigen which is thought to mask under lying A-band O-polysaccharide, since A^+B^+ strains are not agglutinable with A-band specific monoclonal antibodies (Lam, *et at.* ١٩٨٩). Several studies have demonstrated that *Ps. aeruginosa* LPS confers serum resistance and elicits protective immune response (Hancock and Speert, ١٩٨٣; Cryz, *et al.* ١٩٨٤; Engels, *et al.* ١٩٩٤). Recently, a panel of *Ps. aeruginosa* LPS-deficient mutants was used to determine that B- band LPS confers serum resistance to the organism while A-band LPS is not protective against serum- mediated lysis (Dasgupta, *et al.* ١٩٩٤). Studies have also indicated that while antibodies directed toward B-band LPS are highly protective of neutropenic mice upon challenge with smooth LPS strains, antibodies against A band LPS are not protective (Hatano, *et al.* ١٩٩٥].

٢-٦-٢: *Proteus mirabilis* immunogens versus ear mucosal

and systemic responses during infection as in the following:

٢-٦-٢-١: Fimbriae and adherence ability.

Bacterial adhesions to epithelial surface is thought to be one of the most virulent factors (Reid and Sobel, ١٩٨٧). The data obtained by several authors suggest that adhesion of bacteria to epithelial cells is very important in the process of infection (Savoia, *et al.* ١٩٨٣ ; Cellini, *et al.* ١٩٨٧). Fimbrated *Proteus* are easily phagocytosed while partially or unfimbriated, they are resistant to phagocytosis (Silverblate and Ofek, ١٩٧٨).

Ultrastructural studies of *Proteus* strain have shown two types of fimbriae thick and thin. The first type which is also known as type IV fimbriae, was found mannose resistant and named *Proteus* -like fimbriae (MR\P). The second type III fimbriae, which is mannose resistant *Klebsiella* -like fimbriae (MR\K). These types of fimbriae are associated with their ability to hemagglutinate untreated (MR\P) or tanic acid treated (MR\K) erythrocyte from several animal species (Old and Adegbola, ١٩٨٥; Clegg and Geriach, ١٩٨٧; and Gabidullin and Ishkidin, ١٩٨٩).

Hornick, *et al.* (١٩٩١) showed that these types of fimbrae are absent in *P. mirabills* isolates. Silverblatt and Ofek (١٩٧٨) demonstrated that MR\P fimbriae are more important for the adherence of bacteria to epithelial cell than MR\K hemagglutinin. Strains possessing MR\K hemagglutinines adhere no better to these cells than do depiliated bacteria. It was

found that rat infected intraurethrally with strains of *P. mirabilis* expressing either MR\P or MR\K hemagglutinins, a higher frequency of cortical abscess was observed within one week with strains having MR\P fimbriae than in those infected MR\K strain (Silverblatt and Ofek, ١٩٧٨). MR\K hemagglutinin is completely different from the MR\P fimbriae in the tissue binding pattern.

٢-٦-٢-٢: Outer membrane protein (OMP).

OMP possesses immunogenic properties and mitorgenic activity for B-cells (Metchers, *et al.* ١٩٧٥; Chen, *et al.* ١٩٨٠). Furthermore outer membrane lipoproteins and their synthetic analogs function as adjuvant and can also activate macrophage to produce a tumor necrosis factor (TNF) (Hoffman and Bessler, ١٩٨٨). The outer membrane of *P. mirabilis* contains three major proteins. OMPA which is effective as immunomodulator of immune response to LPS, and greatly enhances the level ٠-specific IgG (Karch, *et al.* ١٩٨٣).

This protein is also a mitogen for murine B-cells *in vitro*. The other part of protein (sqkDa protein) inhibite oxygen radicals, as well as (١L-١) production, and enhance TNF secretion by LPS-stimulated macrophage (Weber, *et al.* ١٩٨٣).

٢-٦-٢-٣: Lipopolysaccharide (endotoxin).

Proteus is an antigenically heterogeneous genus, principally because of structural differences of its ٠-specific polysaccharide chain of LPS (٠-antigen) as well as its H-antigen. The virulent factors of these bacteria are the LPS (endotoxin)

which function as surface antigen and exert a number of pathophysiological activities (Rozalski, *et al.* ١٩٩٧). Carbohydrate part of the LPS, the ٠-specific polysaccharide (٠-antigen) and the core oligosaccharide are the targets of specific antibodies induced by LPS in the host immune system, whereas lipid-A is responsible for the endotoxic activities (Mamat, *et al.* ١٩٩٩).

B*ased on the ٠-antigens two species, P.mirabilis and P. vulgaris* were classified in to ٦٠-٠-serogroups (Penner and Hennessy, ١٩٨٠; Larsson, ١٩٨٤).

A peculiar feature of *Proteus* ٠-specific polysaccharides is their acidic nature due to the presence of hexuronic acids, their amides with amino acids, phosphate groups and other acidic non sugar components, which often serve as epitopes recognized by specific antibodies (Knirel, *et al.* ١٩٩٣; Knirel, *et al.* ١٩٩٩). Most *Proteus* O-antigens have a unique structure, but some of them possess marked structural similarities to another, which accounts for the serologica! cross - reactivity of strains. LPs from smooth and rough form proteus strains induce production of nitric oxide (NO) and TNF by macrophage isolated from C٣H\HeN but not C٣\HeJ mice. Lipid-A from proteus strains also induced NO and TNF production, although lipid-A was less potent than LPS. The effects of LPs were mainly dependent on CD١٤ (Swierzko, *et al.* ٢٠٠٠).

٢-٦-٢-٤: Capsular antigen:

For along time, it was believed that proteus strains did not

produce the typical capsule antigens characteristic of some gram negative bacteria .The capsule structure also termed as slime material or glycocalyx, was demonstrated to be a potential pathogenic factor of proteus strains because of its positive effect on struvit crystal growth and stone formation (Beynon, *et al.* ١٩٩٢). The acidic character of proteus capsule due to the presence of uronic acid, pyruvic acid or phosphate groups enable them to bind metal cataion (e.g. Mg+٢) via electrostatic interactions.

٢-٦-٢-٥: IgA١ protease:

Human immunoglobulin A (IgA) which occurs in two isotypic forms, lgA١, and lgA٢, is unlike other immunoglobulin in that it exists in a variety of molecular forms each with a characteristic distribution in various body fluids (Kerr, ١٩٩٠, Mestecky, *et al.* ١٩٩٩). Serum IgA, is predominantly a monomer of lgA١ (١٦٠ KDa). This IgA is composed of two α١ chains, each of ٦٠KDa and contains one variable domain, a hinge region, and three constant domains (CH١, CH٢ and CH٣). The α١ chains are linked by disulfide bonds to each other and to two light chains (λ or K chains) that are identical to those found in other immunoglobulin. In normal human serum, ١٠٪ of the IgA comprises dimeric and higher polymeric forms .The proportion of IgA in these forms increases in a number of disease states (Roberts and Shepherd, ١٩٩٠).Dimeric and polymeric forms of IgA contain an additional protein known as J chain, which links

the IgA monomers via the tail piece -an ١٨ amino acid extension of the α-chain (Mestecky, *et al.* ١٩٩٩). Secretory IgA(S-lgA) is the form of IgA synthesized at mucosal surfaces to protect them from microbial attach. S-lgA is dimeric or polymeric IgA complexed with heavily glycosylated protein called secretary component (Sc). Sc is part of a cell surface receptor that mediates the transport of polymeric IgA across the epithelial barrier (Mostov, ١٩٩٤). Sc is thought to provide stability to the structure of SIgA to increase its resistance to proteolytic degradation (Kerr, ١٩٩٠). Colostral S-lgA is composed of approximately equal proportions of S-lgA١ and S-lgA٢ although the ratio differs in other secretion. The protinase that are produced are highly specific and cleave, almost exclusively, IgA١ from human and from higher apes at the hinge (Qiu, *et al.* ١٩٩٦). Human lgA١, in its secretory form is also cleaved by them, suggesting that the hing is not protected by Sc. These lgA١, proteinases are unable to cleave lgA٢ because it lacks the sequence of the lgA١ hing. Although these lgA١ proteinases are extremely specific, recent studies have identified other nonimmunoglobulin substrates susceptible to cleavage by them (Hauck and Meyer, ١٩٩٧; lin, *et al.* ١٩٩٧; Senior, *et al.* ٢٠٠١). The lgA١ proteinases are believed to be virulent factors because they are produced *in vitro* (Insel, *et al* .١٩٨٢; Kilian and Russell, ١٩٩٩). Most strains of *Proteus mirabilis* of diverse type produce an E D T A -sensitive metallo-proteinase, which, unlike the lgA١

proteinase previously described is able to cleave not only lgA١ but also lgA٢, IgG and other immunoglobulin substrates (Senior, *et al.* ١٩٨٧; loomes, *et al.* ١٩٩٠).

٢-٦-٣: *S. aureus* immunogens versus ear mucosal and systemic immune responses during infections as in the followings:

S.aureus expresses many potential virulence associated antigens such as:

Surface protein that promote colonization of host tissues.

Invasin that promote bacterial spread in tissues include leukocidin, kinases, and hyaluronidase (Sheagren, ١٩٨٤).

Surface factors that inhibit phagocytic engulfment like capsule and protein-A (SPA) that contain five homologus extracellular Ig-binding domains in tandem, designated E, D, A, B, and C which exists in both secreted and membrane associated forms possesses two distinct Ig-binding activities each domain can bind Fc (the constant region of IgG involved in effectors functions) and Fab (the Ig fragment responsible for antigen recognition (Boyle, ١٩٩٠).

Biochemical properties that enhance their survival in phagocytes are Carotinoids, and catalase production (Brooks, *et al.* ١٩٩٨).

Immunological disguises include protein-A, and coagulase.

Membrane damaging toxins that lyse eukaryotic cell membrane are hemolysin, leukotoxin, and leukocidin (Sheagren,

١٩٨٤).

Exotoxins that damage host tissues or otherwise provoke symptoms (SEA-G, TSST, and ET).

Virulent forms of *Staphylococci* of which *S. aureus* is perhaps the most common, resist phagocytosis. This may be partly due to capsule formation *in vivo* and partly to the elaboration of factors such as coagulase enzyme which could protect the bacterium by a barrier of fibrin. It has been suggested that the ability of cell wall component, protein -A, to combine with the FC portion of IgG (other than subclass IgG_3) is responsible for inhibition of phagocytosis by virulent strains, but IgG-proteinA complex fix complement and one study reports that protein -A actually increases complement mediated phagocytosis (Roitt, ١٩٩٧). It seems to be accepted that *S. aureus* is readily phogocytosed in the presence of adequate amounts of antibody but a small proportion of the ingested bacteria survive and they are difficult to eliminate completely. Where the infection is inadequately controlled, sever lesions may occur in the immunized host as a consequence of type IV delayed hypersensitivity reactions. Thus, *staphylococci* were found to be virulent when injected into mice passively immunized with antibody, but caused extensive tissue damage in animal previously given sensitized T- cells (Bloom and Zinkernagel, ١٩٩٦, Blackwell, ١٩٩٦).

٢-٦-٤ Pathogenesis and ear mucosal responses to

endotoxins:

Endotoxins are high molecular weight complexes of lipopolysaccharide (LPS), that constitutes the major cell wall component in all gram-negative bacterial families (Mecuskey, *et al.* ١٩٩٦). These molecules have been intensively investigated because of increasing appreciation of their potentially pathogenic role in wide variety of human disease states (Rietschel, *et al.* ١٩٩٦).

Purified endotoxin is a complex glycolipid composed of a biologically active lipid (lipid A) linked to a polysaccharide region (Glauser, et al. ١٩٩١, Reitschel, *et al.* ١٩٩٦). In addition to LPS, other endotoxic cell membrane molecules have been identified, including lipooligosaccharide (LOS, as short chain endotoxin) and certain lipoproteins.

The basic endotoxin molecular structure consists of two distinct regions; hydrophilic carbohydrate (polysaccharide) portion which include an O-specific side chain and an inner and outer core region, and hydrophobic toxic lipid-A component, (Glauser, *et al.* ١٩٩١). Although the general structure is highly conserved among gram- negative bacteria, there is considerable variability at the ٠-specific chain between bacterial species.

Since the ٠- specific chain is enzymatically constructed by the sequential addition of oligosaccharides, the endotoxin of a given bacterium at a given point in time is a heterogeneous mixture of molecules with short, inter mediate and long ٠-specific

chains thus, there is no precise or standard way to measure molecular composition or molecular weight of endotoxin. Evolutionary pressure exerted by phagocytic cell and macrophages on Gram-negative bacteria may account for some of this heterogeneity (Nikaido, ١٩٧٠).

The structural variation in the ٠-specific side chains produces two distinct morphological types of Gram-negative bacterial growth in culture ,the "rough" (or short ٠- specific chain-containing LPS) and the "smooth" (long chain containing LPS). Variants in laboratory and in animal experiment, the smooth phenotype emerges as an important virulence factor, conferring resistance to complement mediated serum killing of bacteria (McCallum, *et al.* ١٩٨٩). Smooth strains of salmonella demonstrate accelerated rates of proliferation and mortality in mouse model infection (Lyman, *et al.* ١٩٧٦) .The lipid A component of endotoxin, is highly conserved from one Gram-negative bacterial family to another and give the endotoxin molecules its toxicity (Rietschel, et al. ١٩٩٦), whether as a component of a viable microorganism or when shed from the cell wall. The most powerful evidence implicating lipid A as biologically-active portion of endotoxin involves upper respiratory tract. The lymphoid cells of the ring are able to sample, recognize and destroy an inhaled pathogen. In addition effectors and memory lymphocyte are produced here which may migrate to neighboring mucosal sites i.e. the ME to reinforce the local

immune capacity (Van Kempen, *et al.* ٢٠٠٠). Another defense mechanism against infection in the upper respiratory tract is the production of secretory IgA (SIgA). Antibody by mucosal lining and lymphoid ring e.g. the adenoid-SlgA in nasopharyngeal secretions inhibit adherence and reduces nasopharyngeal bacterial colonization, both important factors in the protection against otitis media (Lim and Mogi ١٩٩٤).

Studies using synthetic molecules, showed that lipid A independent from all carbohydrate constituents, is as toxic as its naturally occurring endotoxin counterpart (Ulevitch and Tobias, ١٩٩٥). The actual endotoxic activity of LPS is believed to be dependent up on specific conformation of lipid A portion of the molecule. At high concentrations this conformation appears to be three- dimensional non- lamellar structure (Shenep, *et al.* ١٩٨٨). It is believed that this conformation enables endotoxin to maximally interact with humoral and cellular host factors, triggering the inflammatory cascade.

٢-٦-٤-١ Routs of exposure to endotoxin:

Human may be exposed to endotoxin via two routs: the first and most widely appreciated is systemic or localized Gram-negative infection of exogenous source as a consequences of infection by specific pathogen. Bacteria and bacterial cell wall fragments can cause local or systemic inflammatory response. However even when the bacteria are successfully killed, their residual endotoxin can continue to fuel an inflammatory

response. It was found that antibiotic treatment highly increase endotoxin load and exacerbate the inflammatory response.

The second, less well-recognized rout of endotoxin exposure is bacterial translocation from the gut (Lemair, *et al.* ١٩٩٧; Van leeuwen, *et al.* ١٩٩٤).The gastrointestinal tract normally contains a population of non pathogenic bacterial flora, primarily gram negative facultative anaerobes. Outer membrane fragment of Gram negative bacteria including endotoxin, are continuously produced within the normal gut, without apparent harm to the host (Van leeuwen, *et al.* ١٩٩٤). In fact, there is a considerable evidence showing that minute amount of endotoxin are constantly being shed in to the portal circulation by the healthy gut and cleared by cells in the liver (Mathison and Ulevitch, ١٩٧٩ ; Nolan, ١٩٨١). During this process, LPS is normally taken up by endothelial and kupffer cell, and possibly hepatocyte, via pinocytosis or receptor-dependent mechanism (Van leeuwen, *et al.* ١٩٩٤) and is thereby rendered harmless to the host before reaching the systemic circulation. Pathologic translocation may lead to endotoxin-related illness when one or more of the natural host controls of this process fail, allowing excessive quantities of bacteria or endotoxin to exit the gut via lymphatic or vascular channels (Dietch, ١٩٩٠, Van Deventer, *et al.* ١٩٩٨).

Endotoxin exerts its highly complex array of pathophysiologic effects by interacting in the host with naturally-

occurring cellular and humoral element (Bone, *et al.* ١٩٩٢; Bone, *et al.* ١٩٩٥; Rietschel, *et al.* ١٩٩٦). These elements routinely mediate the normal host response against infectious agent. In systemic inflammatory response syndrome (SIRS) or sepsis, the host normal homeostatic mechanisms break down and the inflammatory response manifests as fever, vascular leakage, and shock (Suffredeni, *et al.* ١٩٨٩).

٢-٦-٤-٢ Activation of cellular mediator.

Endotoxin interacts with virtually all components of the cellular immune system. It is taken by neutrophil leading to cell activation and the subsequent enhancement of the phagocytic ability of these cells. Further, it may activate neutrophils to express cell adhesion molecules which mediate neutrophil -to-neutrophil, neutrophil-to-vascularendothelial cell and neuterophil -to-tissue binding, causing local inflammation and vascular leakage (Glauser, *et al.* ١٩٩١). LPS also appear to affect various populations of lymphocyte, stimulating B-cell proliferation and antibody production, activating T-cells to secrete cytokines and down regulating T-suppressor cells (Rietchel, *et al.* ١٩٩٦).

The most widely studied and probably the most significant cellular effects of endotoxin involve the interaction with cells of monocyte /macrophage lineage which express a membrane receptor known as CD١٤ (Remick, ١٩٩٥). Circulating LPS is bound by glycoprotein serum factor, lipopolysacchride binding protein (LBP), which a facilitate to its principal cellular

receptor, the CD١٤ molecule (Ulevitch & Topias, ١٩٩٥). The importance of this interaction is demonstrated by experiments in which preventing LPS-LBP, binding to monocyte blocks the activity of endotoxin (Remich, ١٩٩٥). Binding of LPS-LBP to CD١٤ induces monocytes to produce and secrete apro-and anti-inflammatory cytokines, including interleukins (١L-١, ١L-٦, ١L-٨, ١L-١٠), macrophage migration inhibitory factors and TNF (Remick, ١٩٩٥).

Recognition of bacterial endotoxin (LPS) elicit multiple host responses, including activation of cells of innate immune system .LPS exposure occur repeatidly during septicemia, making strict regulation of gene expression necessary. Such regulation might prevent, for example the continuous production of pro-inflammatory cytokines such as tumor necroting factor (TNF), which could lead to vascular collapse. Tolerance to LPS is characterized by a diminished production of TNF during prolonged exposure to LPS, and is therefore likely to represent an essential control mechanism during sepsis (Bohuslav, *et al.* ١٩٩٨).

LPS preparations are known to contain substances which activate cells through Toll-like receptor ٢(TLR-٢).and it is suspected that bacterial lipoproteins responsible for this activation (Muroi, *et al.* ٢٠٠٣). TLR٢ has recently been associated with cellular responses to numerous microbial products, including LPS and bacterial lipoprotein. However

many preparations of LPS contain low concentrations of highly bioactive contaminant described previously as "endotoxin protein" suggesting that these contaminants could be responsible for the TLR٢ -mediating signaling observed upon LPS stimulation (Hirschfeld, *et al.* ٢٠٠٠). Although CD١٤ and TLR٤ have been identified as the key molecule involved in LPS inducing signal transduction in innate immune system ,accumulating evidence indicates that multiple receptor are also involved. Recently has been identified a cluster of receptor involving heat-shock proteins ٧٠ and ٩٠, chemokine receptor ٤ as well as growth differentiation factor ٥ that are formed after following LPS stimulation (Triantafillow and Triantafillow ٢٠٠٣).

٢-٦-٤-٣ Stimulation of other humoral mediator.

Gram-negative bacteria activate the complement system through two separate pathways; bacteria and bacterial cell wall components complexed with antibodies activate the classical (anti-body dependent). Complement pathway intended to kill the bacteria, while the bacteria and endotoxin directly activate the alternative (non-antibody) pathway (Glauser, *et a*/. ١٩٩١). The resulting complement cascade induced by LPS produces, among other mediators, anaphylotoxins C٣a and C٥a, which contribute to vasodilatation, increased vascular permeability, and circulatory collapse. In addition complement components induce

adhesion and activation of platelet aggregation, release of lysosomal enzymes and arachidonic acid metabolites and microvascular injury. The damage from inappropriate (pathologic) complement consumption is interlinked with over production of cytokines .

٢-٧. Mucosal versus systemic immune Responses during ear affections:

In systemic immune response much research into immunological defense against MC infection has been focused on *Streptococcus pneumonia* one of the main pathogens for otitis media. Effective defense by means of phagocytosis against infection with pneumococci depend on opsonization by specific antibodies and complement (Prelliner, *et at.* ١٩٨٩). Specific antibodies directed towards the polysaccharide capsule of this bacteria protect children from upper respiratory tract infections including otitis media. The antipneumococcal activity predominantly belongs to the lgG١, and lgG٢ subclasses. Although the colonization of pathogen stimulate the production of mucosal as well as serum antibodies, IgA mucosal antibody limit the duration and frequency of colonization. Serum IgG anti body protect children against the development of otitis media but does not affect colonization. Antibody detected in the middle ear often

reflects passive transfer from serum rather than local production (Faden, ٢٠٠١). Although secretory IgA is critical for protection of the nasopharynx, its function in the middle ear has still not been resolved. The evidence strongly suggests that lgG١ and lgG٣ sub classes are responsible for eradication of middle ear pathogens (Bernstein, ٢٠٠١).

The nasopharyngeal tonsil, or adenoid, is a major inductive site for the synthesis of J-chain-positive B-cells that may migrate to other areas of the upper respiratory tract, such as the nasal mucosa, middle ear and parotid gland during inflammation. The production of SIgA by both nasopharyngeal tonsil and nasal mucosa play a major role in local immune protection against bacteria and viruses. The release of cytokines from TH١ and TH٢ lymphocyte must be appropriate for B cells to produce IgA. The factors or mechanism responsible for this are not, at present, known, but it appear that there is a difference in the profiles of cytokine secretion by Th١ and Th٢ lymphocytes in adenoid in both otitis-prone, as well as non otitis-prone children. There are other modalities of therapy to protect the nasopharynx from colonization with pathogenic bacteria or viruses which include the production of specific antibodies against bacterial surface proteins that have been identified as mucin-binding

protein (Bernstein, *et al.* ١٩٩٧).

The nasal mucosa is the first site of contact with inhaled antigens. The nature of local immune responses and role of nasal -associated lymphoid tissue (NALT) in those responses have been rarely studied .To characterize the cell involved in mucosal derived immune responses, NALT and peyer's patch (PP) from normal mice, and mice immunized intragastrically or intranasaly with cholera toxin were isolated and analyzed (Philippa, *et al.* ١٩٩٧). Compared with PP cells, unstimulated NALT cells contained a higher proportion of T-cells. The CD٤:CD٨ ratio in NALT cell preparations was less than that observed in PP and more closely resembled that seen in spleen. (Zuercher, *et al.* ٢٠٠٢). Additionally the total B-cell frequency in NALT cell isolates ٢٠٪ lower than that observed in PP cell preparations. Although NALT and PP cell isolates contained both mature B- cells and cells undergoing activation to express surface IgA, unlike PP, NALT showed no significant frequency of IgA switched cells. (Kodama, *et al.* ٢٠٠٠).

Materials and Methods

٣-١: Solutions

٣-١-١: Normal Saline (NS):

This solution was prepared at concentration ٠.٨٥٪ by dissolving ٠.٨٥g of sodium chloride (NaCl), (BDH Company,) in small amount of distilled water (D.W.), then complete to final volume of ١٠٠ml with distilled water and autoclaved for ١٥min at ١٢١c. Normal saline was used in titration of antibodies in systemic and mucosal with causative agent antigens of otitis media and preparation of antigen of Gram positive (McCoy and Kennedy, ١٩٦٠, Garvey, et al. ١٩٧٧).

٣-١-٢: Formal saline (FS):

The solution was prepared by adding ٠.٥ml of formaldehyde (H-CHO), (BDH Company), to ٩٩.٥ml sterile normal saline, the final concentration of formalin in this solution is ٠.٥٪. The solution used as a solvent for immunoglobulin from otitis media and serum immunoglobulin (Pears, ١٩٨٥) and in preparation of Gram negative bacterial antigens (Lehman, et al. ١٩٦٨).

٣-١-٣: Basal Eagle Medium (BEM):

Prepared following Sigma - company instruction as: one gram of Eagle medium was dissolved in sterile distilled water (١٠٠ml). This solution was membrane filtered through usually ٠.٤٥ biurat followed by ٠.٢٢µm sterile Millipore filter by syringe filter device. The solution preserved at sterile plastic universal AFMA-Dispo in refrigerator ٤C°. This BEM was used

as cell nutritive solution helpful for measuring migration inhibitory factors of leucocytes in blood and otitis media exudates

٣-١-٤: Alsever Solution (AS)

The solution was prepared by dissolving:

٢٠.٠٥ g Dextrose .

٠٨.٠٠ g trisodium citrate (dehydrated)

٠٤.٢٠ g NaCl.

Dissolved in ١٠٠٠ mL D.W., PH was adjusted to ٦.١ by ١٥٪ of citric acid. The solution was membrane filtered through ٠.٢٢µm Millipore filter in syringe type device, and used as leucocytes preservative (Garvey, et al. ١٩٧٧).

٣-١-٥: Ammonium sulfate:

This solution was prepared at concentration of (٤٠٪) by dissolving ٤٠g of Ammonium Sulfate $(NH_4)_2SO_4$, (BDH company), molecular weight ٣٢.١٣ in small amount of distilled water. Then the volume completed to ١٠٠ml in volumetric flask. This solution was used to separate immunoglobulin (Keay, et al. ١٩٩٧).

٣-١-٦: Biuret solution:

Prepared by dissolving ٣g of Copper Sulfate $CuS_{٠٤.٥}H_{٢٠}$ (M.W= ٢٤٩.٥) in ٥٠٠ml of distilled water then ٩g of Sodium Potassium tartarate and then ٥g of Potassium Iodide and after complete dissolving ١٠٠ml of Sodium Hydroxide ٠.٦M was added and the volume completed to final volume ١L by adding distilled water. This solution was used for measuring the total protein and serum and secretory

immunoglobulin (Bishop, et al. ١٩٨٥).

٣-١-٧: Standard albumin solution:

The solution prepared by dissolving ٦٠g of bovine albumin in small amount of Sodium Hydroxide ٠.٦ N and then the volume was completed to I liter by using the same solution to final concentration of albumin ٦٠g/L. A serial double dilutions was prepared as ١:١, ١: ٢, ١: ٤, ١: ٨, ١:١٦ ,١: ٣٢ ,١: ٦٤ ,١:١٢٨ g\L respectively. The dilution made by ٠.٦N Sodium Hydroxide for preparation of standard curve for determination of total protein and immunoglobulin concentrations (Bishops, et al. ١٩٨٥).

٣-١-٨: Benzalconium chloride (١:١٠٠٠);

This solution was prepared in concentration (١/١٠٠٠) by adding ٠.٢ml of Alkyl Benzyl Dimethyl Ammonium Chloride C٢٢H٤oCIN (the concentration inside the bottle ٥٠٪) to small amount of sterile distilled water with agitation for homogenization then the volume was completed to ١٠٠ml. This solution was used in preparation of antigen of Gram positive bacteria (Banker, ١٩٨٠).

٣-٢: Culture Media

٣-٢-١: Mannitol salt agar.

This medium used for diagnosis of Staphylococcus aureus and prepared using instruction of company.

٣-٢-٢: MacConKey agar.

Used for Isolation of Gram negative bacteria

٣-٢-٣: Pepton water:

Used for preparation of cell free culture filtrates for

causative organism in otitis media.

٣-٢-٤: Brain-heart infusion broth:

Prepared following the instructions of company and used in cultivation of Gram negative bacteria for the preparation of dry weight and isolation of endotoxins of this microorganisms.

٣-٢-٥: (Master) Agar- A:

Prepared by dissolving ٢g in ١٠٠ml of distilled water sterilized by autoclave. Used for study of Leucocyte Inhibitory Factor (LIF) in blood and exudates of otitis media.

٣-٢-٦: Nitrobluetetrazolium:

Prepared by dissolving (٠.١) g of stain powder in ٢ml methanol alcohol, then add ٥٠ml of phosphate buffer saline. Kept at ٤C° away from light and used in NBT test (Metcalf, et al. ١٩٨٦).

٣-٣: Ear discharge samples;

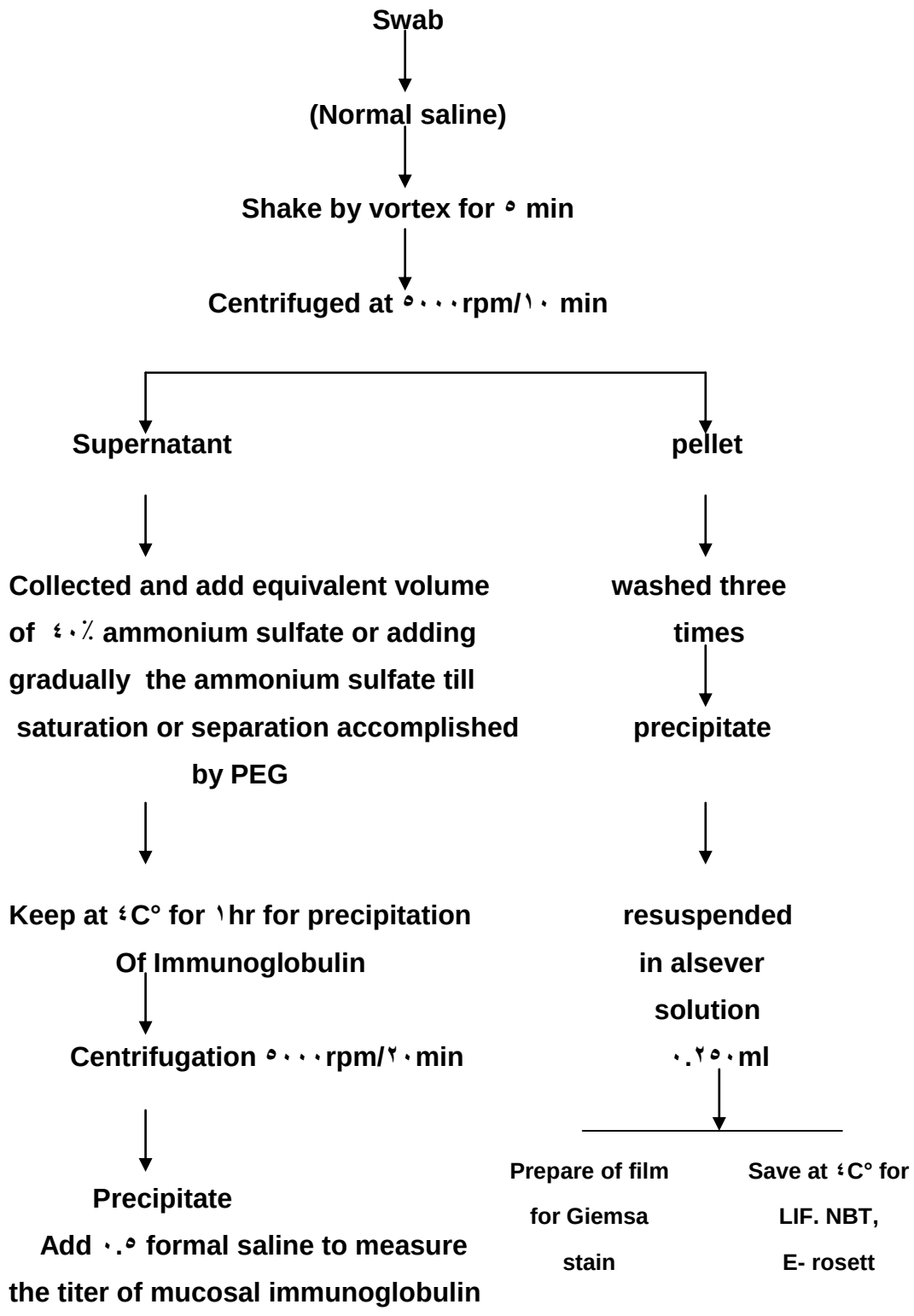
٣-٣-١: Bacteriological evaluation of chronic ear discharge patients:

From ١٠٠ patients of chronic otitis media samples were taken using sterile disposable swabs immediately cultured on blood agar, MacConKey agar and Mannitol salt agar for isolation and biochemical identifications of causative bacteria and determination the top three dominant organism in case of chronic otitis media (Crucshank, et al. ١٩٧٥; and MacFaddin , ٢٠٠٠).

٣-٣-٢ Immunological aspects of patients with chronic otitis media:

Inflammatory cell preparation:

From the ١٠٠ ear swabs, after culturing they were taken and transferred to a universal sampling tube containing ٢-٥ml sterile normal saline, shaked well by vortex and centrifuged at ٥٠٠٠rpm for ١٠min. The precipitate was washed three times with normal saline and preserved at ratio of ١:١ in, alsever solution, for studying LIF as showed in figure (١).



Figure(١):Flow chart for the investigation of chronic ear discharge patient swab sample (Shnawa and THwaini, ٢٠٠٠).

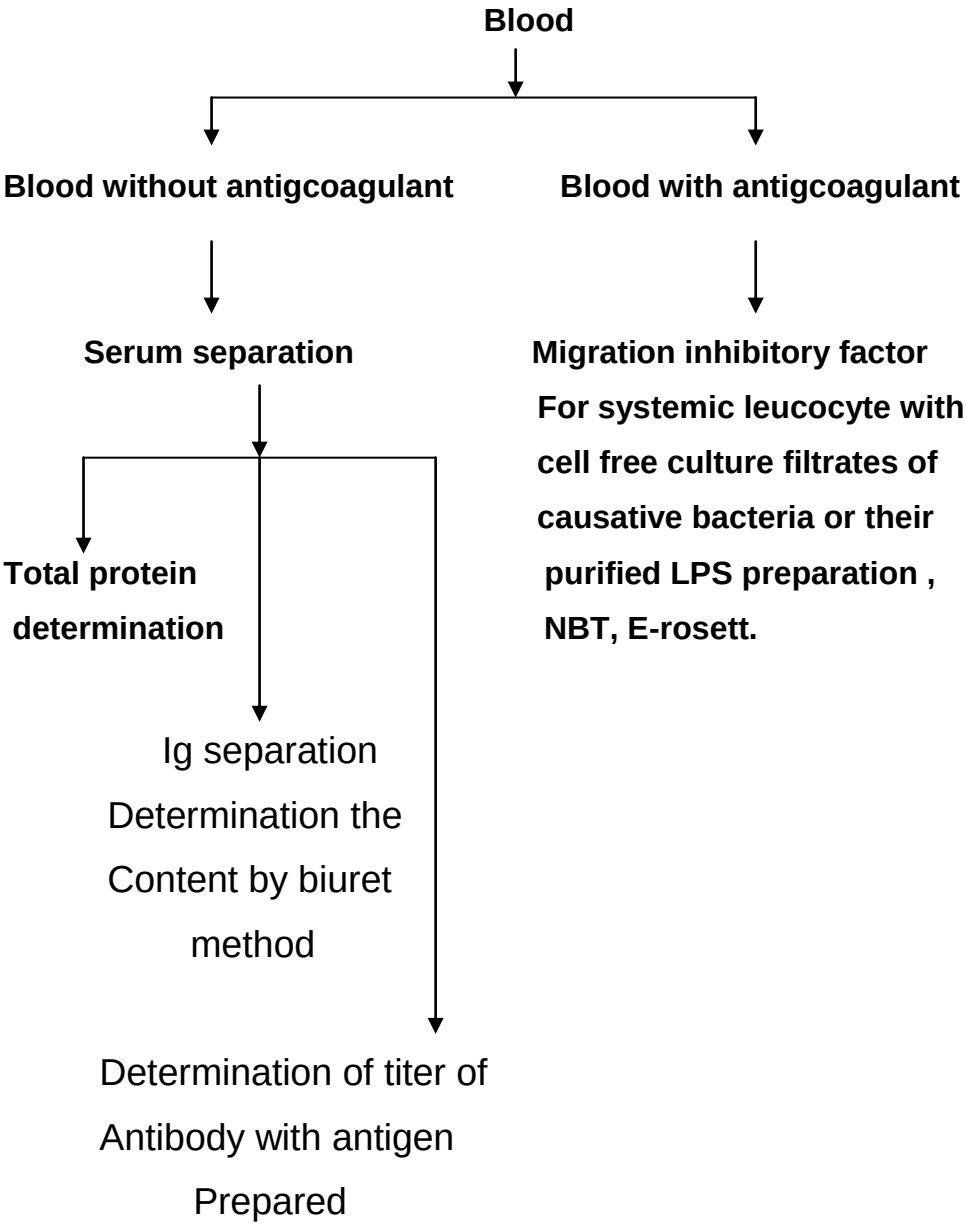
٣-٤, Blood Samplings

٣-٤-١: Blood without anticoagulant:

Half of the blood samples were collected from healthy and patients from veins by disposable syringes in ٥ml volume without anticoagulant. The blood left at room temperature for ١٠ min and then centrifuged for ٥ min at ٥٠٠٠ rpm. The serum was collected by Pasteur pipette and decomplementized and preserved at freeze in tube at -١٨C° until used.

٣-٤-٢ Blood with anticoagulant:

The second half of blood sample that was colleted in previous section (٢-٤-١) was tubed in AFMA-Dispo-EDTA tube that contains EDTA as anti coagulant. The sample was mostly processed for LIF (Soberg, ١٩٦٩). At critical rare occasion it was preserved in refrigerator for overnight then processed for measuring systemic leucocytes migration inhibitory factor as showed in figure(٢),



Figure(٢):Flow chart for the investigation of chronic ear discharge patient blood sample.

٣-٥: Separation of immunoglobulin:

٣-٥-١: Separation of serum immunoglobulin:

١- To a ٥ml of serum, equal volume of ٤٠٪ Ammonium Sulfate was added. The mixture left in refrigerator for ٣٠min at ٤c.

٢-Centrifuged at ٥٠٠٠rpm for ٢٠min

٣-supernate was discarded and the precipitate was taken.

٤-٥ml of formal saline & ٥ml of Ammonium Sulfate was added mixed well and left at room temperature for ١٠-١٥min.

٥-Centrifuged at ٣٥٠٠rpm for ٢٠min.

٦-Discard the supernatant and the tube overlapped on filter Paper to dry the precipitate

٧-Disolve the pellet at ٠.٢٥ml formal slain .

٣-٥-٢: Separation of secretory immunoglobulin of middle ear

Heavy swab from cases of otitis media was taken and immediately emerged in universal bottle containing ٢-٥ml of sterile normal saline shaked well by vortex ,the swab was removed and the solution was centrifuged for ٢٠min at ٥٠٠٠rpm, the supernatant was taken and equal volume of ٤٠٪ of Ammonium Sulfate was added or gradually adding ammonium sulfate powder until saturation. The mixture was kept at refrigerator ٤C° for ١hr for precipitation of immunoglobulin (Shnawa and Thwaini, ٢٠٠٢), and resuspended in formal saline.

٣-٦: Measurement of protein concentration:

٣-٦-١: Measurement of total protein in serum:

Biuret method is used in measurement of total protein in serum according to Bishops, et al. (١٩٨٥) as follows:

١- To ٥ml of Biureat solution in a test tube ٠.١ml of serum was added and regarded as test sample.

٢- ٠.١ml of distilled water was added to ٥ml of Biureat solution in another test tube (control)

٣- The test tubes in (١ and ٢) were mixed by vortex and leaved for ٣٠min at room temperature, in dark

٤- The optical density was measured at a wave length ٥٤٠nm by using spectrophotometer (Spectronic ٢٠).

٥- The equation of standard curve was applied when optical density, a, b is constant; y is the concentration of protein in sample. The standard curve was prepared by using standard dilution of bovine albumin.

$$\check{y} = -٤.٠٩٧ + ١٤٧.٢٦٦ \times ٠.١٢٩$$

٣-٦-٢: Measurement of serum immunoglobulin;

The separated immunoglobulin at (٢-٥-١) measured by (Bishop, et al. ١٩٨٥) method.

٣-٦-٣: Measurement of secretory immunoglobulin in otitis media:

Biuret method was used in measuring the concentration of secretory immunoglobulin with some modification since the concentration of immunoglobulin was low.

١-To ٥ml of Biuret solution ٠.٢ml of immunoglobulin solution was added this represent the immunoglobulin to

be measured

٢- ٠.٢ml of distilled water added to ٥ml of Biuret in a test tube used as control.

٣- The two test tube were mixed by vortex and leaved for ٣٠ min at room temperature.

٤- The optical density was measured for the test and control using the wave length ٥٤٠nm.

The concentration of immunoglobulin was measured using the equation of standard curve.

٣-٦-٤: Measurement of albumin in blood sample:

Add about ٣ml ether to the rest of serum -sulfate-sulphite mixture, stopper and shake ٤٠ times, twice each second for ٢٠ second using a movement of the arm of about ٤٠cm. It is important not to shake more vigorously other wise the albumin may be denatured. The time of shaking should be between ١٥-٢٥ S-centrifuges for ٥min that is just long enough for a firm globulin layer to form. Cap the tube during the centrifuging. The air space in the tubes was less than ٣ml. If it is larger some of the albumin may be denatured and low results be obtained. After centrifuging, tilt the tube and insert a pipette in to clear solution below the globulin layer. Care was taken not to disturb the precipitate. Asparate ٢ml and add to ٥ml of biuret reagent in a test tube (Wooten and freeman,١٩٨٢).

٣-٧: Preparation of surface antigens for sharing bacteria in otitis media:

٣-٧-١: Preparation of surface antigens of Gram positive bacteria:

The procedure of McCoy and Kennedy, ١٩٦٠, was depended with some modification:

١- A pure ٢٤hr culture of causative Gram positive bacteria was prepared on blood agar medium.

٢- ٦ml of sterile normal saline was added on the surface of the plate and growth was harvested.

٣- The harvested suspension mixed well by vortex for ٣min.

٤- ٥ml of this suspension was centrifuged at ٤٠٠٠rpm for ٥min.

٥- The pellet was resuspended by ٥ml of normal saline and mixed well by vortex, centrifuged at ٣٥٠٠rpm for ٥min.

٦- The supernatant was discarded and the pallet resuspended in ٥ml of benzalconium chloride (١:١٠٠٠) and mixed well by vortex.

٧-This suspension was taken and tubed in a tube of opacimeter W.O.H. International References and the volume ١ ml was completed by adding another benzalconium chloride until the opacity of the suspension become equal to the opacity of the standard tube. The concentration of tested suspension was ١٠ IU.

٨- By vortex the suspension mixed well and incubated at ٣٧C for ٣٠min.

٩-Sterility test was done for antigenic suspension by

culturing on nutrient solid medium .

٣-٧-٢: Heat killed whole cell bacterial antigen:

The surface antigen of causative Gram negative bacteria was prepared as mentioned by Smith, ١٩٧٥ and Svanborg-Eden, et al. ١٩٨٥, with some modifications:

١-A ٢٤hr pure cells of causative Gram negative on solid MacConekey medium

٢-Formal saline (٦ml) was added on surface of solid media, and the growth was harvested by loop.

٣-The washed cell was mixed by vortex for ٣min.

٤-٥ml of this suspension centrifuged ٤٠٠٠rpm for ٥min.

٥-The sediment washed by ٥ml of formal saline and centrifuged after mixing by vortex at ٣٥٠٠rpm for ٥min.

٦-The supernatant was discarded and the sediment was resuspended in ٥ml of formal saline and mixed by vortex.

٧-١ ml of the suspension (from the step ٦) was taken and transferred to tube of (opacimeter) and completed by adding formal saline until the opacity of the suspension in the tube was equal to opacity of the standard tube , now the final concentration for the suspension ١٠ I.U.

٨-The suspension mixed well by vortex for ٣min.

٩-The suspension was heat treated in water bath at ٦٠C° for ١.٥hr.

١٠-Sterility test was made for antigenic suspension on solid nutrient medium. Growth of bacteria indicate contamination and bacterial growth should be absence to exclude contamination and proves stirity.

٣-٨: Test for mucosal and systemic immunity:

٣-٨-١: Test for mucosal and systemic humoral immunity:

٣-٨-١-١: Standard tube agglutination method (Garvey et al,١٩٧٧):

٣-٨-١-١ A: Mucosal:

Immunoglobulin solution was serially double diluted in which ٠.٢٥ml of the ear mucosal immunoglobuline was made in a set of agglutination tubes containing ٠.٢٥ formal saline to obtain the dilutions ١:٢, ١:٤, ١:٨, ١:١٦, ١:٣٢ and so on then ٠.٢٥ml of the antigenic suspension was added to each tube .

٣-٨-١-١ B: Systemic:

The serum immunoglobulin was diluted in serial decimal-double dilution manner, in which ٠.١ml serum was used to obtained the dilutions ١:١٠, ١:٢٠, ١:٤٠, ١:٨٠ , ١:١٦٠, ١:٣٢٠, ١:٦٤٠ and so on, then to each tube of serum dilution in the set, ٠.٥ml of the antigenic solution was added.

The reaction mixtures in section ٢-٨-١-١ were shaken gently and incubated at ٣٧C° for ٢٤hr then results were scored as the reciprocal of the highest dilution that gives clear positive results (Titer).

٣-٨-٢: Test for mucosal and systemic cellular immunity:

٣-٨-٢-١: Preparation of the leucocytes sensitizers:

The preparation of cell free culture filterate by cultivation of causative bacteria for otitis media on broth for ٢٤ hr and the liquid media was centrifuged at ٥٠٠٠ rpm for ١٥ min this culture supernatant was membrane filterated through Millipore filter in syringe device, the resulted filtrate was collected and

distributed in sterile bottle and preserved in refrigerator until use as sensitizer (Shnawa and Thwaini, ٢٠٠٢).

٣-٨-٢-٢: Leucocytes inhibitory factor (LIF):

a- Mucosal LIF:

Measurement of LIF in otitis media discharge system (Mucosal) was done for measurement the factor that inhibit the migration of leucocyte in case of otitis media that caused by 'bacteria following Soberg, (١٩٦٩) method as follow:

١-Agar-A medium was prepared in sterile plastic plate (٢٪ agar), and two wells were done with ٢ cm in diameter on plate of agar.

٢-From cells of exudates of otitis media in step (٢-٣-٢) that preserved in alsever solution, capillary tube filled and put in the well after centrifugation by Haematocrit for ١٠min

٣-In each well ٠.١ml of Eagle Basal medium was added and one of the well used as control.

٤-Addition of ٠.١ml of cell free culture filterate prepared in step (٢-٨-١) for one well and the well leaved as control

٥- The plate incubated at ٣٧C in jar in a humid environment for ٢٤hr, and then inhibition of migration was measured.

٦- Measuring the factor of inhibition of Macrophage by oculometor.

b- Peripheral blood LIF:

Measurement of migration inhibitory factor in systemic blood:

١-Preparation of Agar -A medium in sterile plastic plates and ٢ wells were made as in (٢-٨-١)

٢- Capillary tube containing systemic blood from patients with otitis media was put in each well after centrifugation by haematocrite for ١٠min (Leucocyte and buffy coat).

٣-Eagle basal medium (٠.١) ml was put in each well , one of the well was control.

٤- ٠.١ml of antigen (cell free culture fillerate) of causative bacteria was added in one well.

٥- Incubation at ٣٧C° for ٢٤hr in humid environment.

٦- Measurement of LIF by oculometer. Same steps were used for control and normal saline was added instead of culture filtrate as mentioned by Soberg, (١٩٦٩). LIF was measured as follow:

$$\text{Factor of inhibition} = \frac{\text{Diameter circle of migration of cell with antigen}}{\text{Diameter cicle of migration without antigen}} \times 100$$

٣-٨-٣: The E- rosette test for T cell Enumeration:

The procedure applied as following Burrell method (١٩٧٩) with some modifications:

١- ٣ ml of blood was drown from patients and transferred to EDTA tube.

٢- Carefully layered ٢ ml of this blood on to the surface of tube containing gradient medium (lymphopreb).

٣- Centrifuge at ٤٠٠g for ٣٠ min at R°.

٤- The leukocytes will appear as a fluffy white coating at the plasma-medium interface while the erythrocyte will be on the bottom of the tube. Carefully remove as much of the upper plasma layer possible and save.

٥- Remove the white cells with capillary pipettes taking as little of the lympho-prep from the lower layer as possible.

٦- Centrifuge at ٤٠٠g for ٥ min and discard the supernatant.

٧- Wash three times in buffered saline at ٤٠٠g for ٦ min each. Resuspend of saline to which ٠.٠٥ ml autologous plasma has been added.

٨- To a tube containing ٠.١ ml of plasma-lymohocyte mixture, add ٠.١ ml of sheep RBCs (١٠٪suspension).

٩- Mix gently, incubate at ٣٧C for ١٥ min, and spin immediately at ٢٠٠g for ١٥ min.

١٠- Incubate for ١hr at ٤C with the supernatant still on the pellet.

١١- Very gentle resuspen the cell in supernatant by gentle tilting the tube back and forth ٢-٣ times.

١٢- Make smear on the slide from this mixture, leave to dry.

١٣- Fix by methylalcohol for ١٠ min.

١٤- Stain the smear with Giemsa stain for ١٥ min.

١٥-Wash with tap water and leave to dry.

١٦- Examine under light microscope with oil emersion lens.

٣-٨-٤: Nitroblue tetrazolium reduction test (NBT test):

Blood was obtained from patient with otitis media and healthy individuals. ١ml of blood was drawn into a plastic syringe, transferred to EDTA containing tube, mixed thoroughly by gentle shaking. ٠.١ml of this blood was transferred into a clean, siliconesed tube and mixed with equal amounts of NBT solution the tube was incubated at ٣٧C° for ٣٠min with humidity source at the end of this period

the blood/NBT mixture was again mixed and blood film was made. The films were dried in air and stained with lishman stain. The slide examined under the microscope with oil immersion lens and ١٠٠ neutrophiles were counted. Only those neutrophiles with a large black deposit were classified as NBT positive and the percentage of these cells was recorded (Park, et a/. ١٩٦٨),

٣-٩: Endotoxin preparation:

٣-٩-١: Preparation of bacterial dry weight:

Brain-heart infusion broth was inoculated by *Proteus mirabilis* or *Pseudomonas aeruginosa* that were isolated from cases of otitis media. And by using shaking incubators for over night to obtain growth at the log phase; the culture was cooled centrifuged at ٥٠٠٠ rpm for ٢٠ min The precipitate which represents the cells of bacteria was dried at incubators for ٤٨ hrs at ٤٥C°. The dried cells were mixed with ethanol alcohol, mixed well and centrifuged at ٥٠٠٠ rpm for ٢٠ min the precipitate was mixed well with acetone and centrifuged again; the precipitates was washed twice with ether and the precipitate was dried in incubator or oven at ٤٥C° for ٤٨ hrs and this represent the dry weight of bacterial growth.

٣-٩-٢: Lipopolysaccharide preparation:

The method, of LPS preparation devised by Westphals, et al. (١٩٥٢) was followed. Bacteria (١٠ g of dry weight) are suspended in ١٦٠ ml of distilled water and mixed with ٢٦٥ ml of ٧٥٪ phenol(prepared by adding ٦٥ ml of water to ٢٠٠g of liquid phenol) the mixture left at ٣٥C° for ٣٠ min with

occasional shaking then centrifuged at ٣٠٠٠ to ٤٠٠٠ rpm. The upper aqueous phase should be collected. The remaining material was washed with water, centrifuged and the upper layer combined with first supernatant and this fluid was dialyzed for ٢ to ٣ days against tap water and ١ day against distilled water, and then concentrated to a ٤٠-٥٠ml volume. LPS is precipitated from dialyzed with six volumes of acetone mixed with a small volume of saturated alcoholic solution of sodium acetate. The sediment was collected by centrifugation with alcohol and acetone and dried in vacum.

٣-٩-٣: Purification of lipopolysaccharide:

A sephadex G٥٠ was used for purification of LPS extracted from Gram negative bacteria (*Ps. aeruginosa*, and *P. mirabilis*). The dimensions of the column were selected according to the following equation (scopes, ١٩٨٢):

$$\text{Column diameter} = \sqrt[3]{\frac{\text{mg of LPS}}{10}}$$

١٠٠ mg of LPS was dissolved in ١٠ ml of normal saline according to this equation the diameter of column was ١,٨ cm

Column length= ٣٠ X Column diameter

=٣٠X ١.٨= ٥٤ cm

٣-١٠. Studying of standard curve for protein concentration.

Standard Albumin was used at a concentration ٦٠g/L and a series of dilution was made and the optical density was estimated by Biuret method:

x	Y	X٢	Y٢	XY
٠.٣٨٣	٦٠.٠٠	٠.١٤٦٦	٣٦٠٠	٢٢.٩٨
٠.٢٨٣	٣٠.٠٠	٠.٠٨٠٠	٩٠٠	٨.٤٩
٠.١٧٠	١٥.٠٠	٠.٠٢٨٩	٢٢٥	٠٢.٥٥
٠.٠٧٠	٧.٥٠٠	٠.٠٠٤٩	٥٦.٢٥	٠.٢٥٢
٠.٠٥٠	٣.٧٥٠	٠.٠٠٢٥	١٤.٠٦	٠.١٨٧٥
٠.٠٣٤	١.٨٨٧٠	٠.٠٠١١٥٦	٣.٥	٠.٠٦٣٥٨
٠.٠٢٩	٠.٩٣٧	٠.٠٠٠٨٤١	٠.٨٧	٠.٠٢٧١٧٣
٠.١٤	٠.٤٦٨	٠.٠٠٠١٩٩٦	٠.٢٢	٠.٠٠٦٥٥٢
Sum ١.٠٣٣	١١٩.٥٢٥	٠.٢٦٥٠٩	٤٧٩٩.٩	٣٤.٨٢٩٨٠٥
Everg:٠.١٢٩	١٤.٩ |			

.

Statistical features for standard curve equation for albumine concentration and their dilutions

$$Ssx=\Sigma x^{2} - \frac{(\Sigma x)^{2}}{N} = ٠.٢٦٥٠٩ - \frac{(١.٠٣٣)^{2}}{٨} = ٠.١٣٧١$$

$$Ssy=\Sigma y^{2} - \frac{(\Sigma y)^{2}}{N} = ٤٧٩٩.٩ - \frac{(١١٩.٥٢٥)}{٨} = ٣٠١٤.١$$

$$SSxy = sxy - \frac{(\Sigma x)(\Sigma y)}{N} = ٣٤.٨٢٩٨ - \frac{(١.٠٣٣)(١١٩.٥٢٥)}{٨} = ١٩.٣٩٥$$

$$b = \frac{Ssxy}{Ssx} = \frac{١٩.٣٩٥}{٠.١٣١٧} = ١٤٧.٢٦٦$$

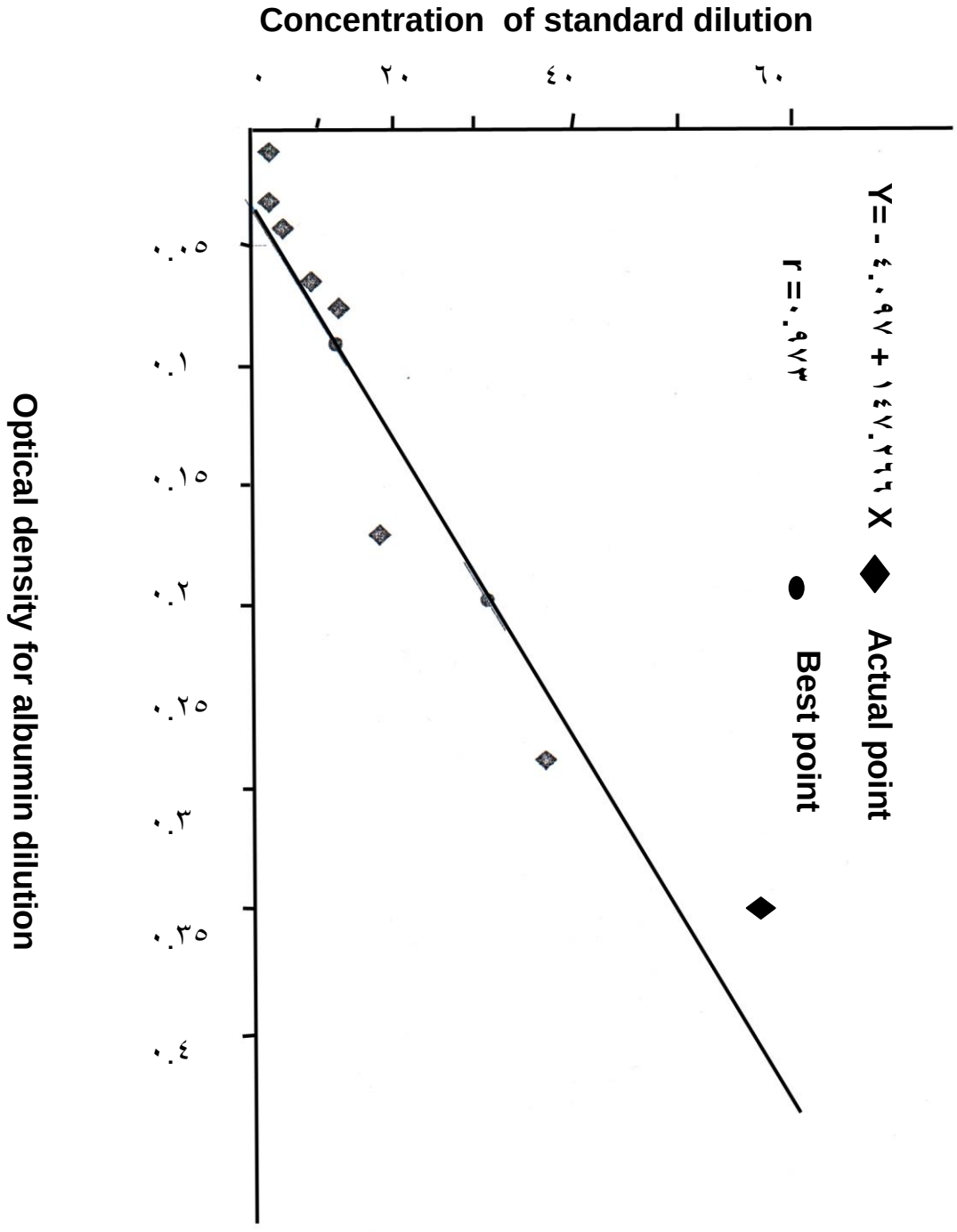
$$a = \hat{Y} - b\,x = ١٤.٩ - (١٤٧.٢٦٦)\,(٠.١٢٩) = -٤.٠٩٧$$

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

$$\hat{Y} = -٤.٠٩٧ = ١٤٧.٢٦٦ x ٠.١٢٩$$

$$= -٤.٠٩٧ + ١٨.٩٩٧٣١٤$$

$$r = \frac{SSxy}{\sqrt{(SSx)(SSy)}} = \frac{١٩.٣٩٥}{\sqrt{(٠.١٣١٧)(٣٠١٤.١)}} = ٠.٩٧٣$$



Protein determination standard curve . The X axis represent albumin concentration with Y axis state the optical density. Best fit points from regression analysis were affixed.

٣-١١: Chronic Ear Discharge Patients (CEDP):

The study patients were the attendance of otolaryngology consultation units at Najaf (ENT). Three series of patients were investigated. Retrospective conservations for ٢٤٥ patients constitute the series number one. Series number two; however, ١٠٠ patients were for prospective study. The third series consisted ٥٠ of CEDP due to the top three bacterial pathogens.

٣-١٢: Study Plane:

The plane includes the following sections:

Retrospective study.

Prospective study.

Immune function parameters

Immunologic investigation using whole cell antigens and cell free culture filtrate.

Immunologic studies using purified LPS preparations.

Comparative immunology of BCED.

٣-١٣: Study Battery:

For humoral serum antibody agglutination assay was used. Nitrobluetetrazolium reduction test (NBT) was performed for assessing phagocytosis. E-rosette was done to detect T cell and leukocytes inhibitory factor and delayed type hypersensitivity was attempted.

٣-١٤: Scores:

Normal, value for immune function parameters, Abnormal and subnormal values were noted among BCED patients

٣-١٥: Text presentation:

Statistical trends were followed for presenting value as absolute observation. Mean, median and ranges values were emphasized in testing values. Comparative views to the obtained facts were followed.

٤-٥. The immune status of patients with chronic otitis media caused by *Pseudomonas aeruginosa*:

When purified lipopolysaccharide (LPS) was used as antigen, the extracted LPS demonstrated a high humoral immune response at the systemic and local immunity.

Non specific immunity was also investigated and the data are listed in the (table ٤-١١). The total serum protein concentration slightly increased with control value ٦٣.٩ g/L. The immunoglobulin slightly increased and ranged between ٢٩.٨٨-٤٠.٠١ g/L compared with normal value ٣١.١ g/L. In specific humoral immune response the titer of antibody increased at the systemic immunity and ranged between ١٦٠- ٦٤٠, with mean and median value ٣٨٤, ٣٢٠ respectively. At the mucosal level the titer ranged between ٣٢-١٢٨ with mean and median values ٦٠.٨, ٦٤ respectively (using standard tube agglutination method).

LPS was able to detect nonspecific cellular immune response when phagocytic activity was measured by NBT test That increased at the peripheral blood ٢٦-٣٩% and in local (mucosal) system was ٢٨-٤٣%.

In specific cellular immune response the systemic LIF ranged between ٤١-٦٣٪ with inhibition of migration ranged ٣٧-٥٩٪. At the

mucosal immune response the LIF ranged between ٣٤-٥٢٪ compared with normal value ٩٣٪.

E-rosette formation also increased at systemic and mucosal immunity as compared with normal value at the healthy patients. It ranged between ٢٨-٤٠% at systemic and ٤٢-٥٠% at the mucosal response. The normal value was ٢٦%.

Table ٤-١١: Pseudomonas aeruginosa CBED immune status using LPS as antigen and sensitizer.

Sequences	Humoral							Cellular					
	Systemic					Mucosal		Systemic			Mucosal		
	Titer	TPC g/L	Ig g/L	Albumin g/L	Ig / Al	Titer	Ig g/L	LIF%	NBT%	E-rosette %	LIF%	NBT%	E-rosette %
١	١٦٠	٥٨.٩	٢٩.٨٨	٢٦.٨٢	١.١١	٣٢	٠.٢٧	٤١	٢٦	٢٨	٣٤	٢٨	٤٢
٢	١٦٠	٦٠.٨٨	٣٣.٣٠	٢٧.٨٠	١.٢١	٣٢	٠.٢٩	٤٢	٢٦	٣٠	٣٧	٢٨	٤٤
٣	٣٢٠	٦٢.٩٠	٣٣.٨١	٢٩.٨٨	١.١٣	٣٢	٠.٣٢	٥٠	٢٨	٣٠	٤٠	٢٩	٤٤
٤	٣٢٠	٦٣.١٦	٣٣.٩٠	٣٤.٢٤	٠.٩٩	٦٤	٠.٣٣	٥١	٢٨	٣٠	٤٠	٣٠	٤٦
٥	٣٢٠	٦٥.٨٠	٣٤.١٧	٣٦.٦٠	٠.٩٣	٦٤	٠.٣٣	٥٢	٢٩	٣١	٤١	٣٦	٤٦
٦	٣٢٠	٦٦.٦٠	٣٦.٧١	٣٦.٩٠	٠.٩٩	٦٤	٠.٣٨	٥٢	٢٩	٣٢	٤١	٤٠	٤٦
٧	٣٢٠	٦٨.٢٠	٣٧.٨٩	٣٨.٩٠	٠.٩٧	٦٤	٠.٤٠	٥٤	٣٠	٣٣	٤٣	٤٢	٤٧
٨	٦٤٠	٦٩.٦٠	٣٨.٨٣	٣٩.١٦	٠.٩٩	٦٤	٠.٤١	٥٧	٣١	٣٣	٤٤	٤٢	٤٨
٩	٦٤٠	٦٩.٩٠	٣٩.٢١	٤٠.١٢	٠.٩٧	٦٤	٠.٤٨	٥٨	٣٥	٣٥	٤٧	٤٣	٤٨
١٠	٦٤٠	٧٠.١٠	٤٠.٠١	٤٠.٩٠	٠.٩٧	١٢٨	٠.٥٢	٦٣	٣٩	٤٠	٥٢	٤٣	٥٠
Mean	٣٨٤	٦٥.٦٠	٣٥.٨٦	٣٢.٣٥	١.٠٣	٦٠.٨	٠.٣٧	٥٢	٣٠.١	٣٢.٢	٤١	٣٧	٤١.١
Medium	٣٢٠	٦٦.٦٠	٣٦.٧١	٣٦.٩٠	٠.٩٩	٦٤	٠.٣٨	٥٢	٢٩	٣٢	٤١	٤٠	٤٦
Range	١٦٠-٦٤٠	٥٨.٩-٧٠.١	٢٩.٨٨-٤٠.٩٠	٢٧.٨٢-٤٠.٩٠	٠.٩٣-١.٢١	٣٢-١٢٨	٠.٢٧-٠.٥٢	٤١-٦٣	٢٦-٣٩	٢٨-٤٠	٣٤-٥٢	٢٨-٤٣	٤٢-٥٠

٤-٦.The specific and nonspecific immunity to *Proteus mirabilis* CBED enlisted in Table (٤-١٢).

The nonspecific humoral immunity was characterized by slight increase in total serum protein ٥٧.٢١- ٧٠.٠١g/L in comparison with normal value ٦٣.٩g/L. The immunoglobulin concentration also slightly increased in systemic (peripheral blood) and mucosal (local) system with mean value ٣٦.٦١ and ٠.٤١g/L respectively. The systematic mean value was ٣٨.٨g/L whereas the mucosal value was ٠.٣٩g/L.

At the specific humoral immunity level, it was demonstrated that systemic and mucosal immune response represented in increase of titer of specific immunoglobulin ١٦٠-٦٤٠ in systemic immune response and ٣٢-٦٤ in mucosal when pure LPS were used as antigen. The mean value for titers at the systemic ٣٨٤ and ٤١.٦ at the mucosal system of middle ear. The median titer was ٣٢٠ and ٣٢ in systemic and mucosal respectively.

At the cellular level, purified LPS extracted from *P.mirabilis* induce cellular immunity at both systemic and mucosal compartments. The tests NBT, LIF and E-rosette formation were used as parameters for cellular nonspecific and specific immune response.

Nonspecific cellular immune response, using NBT test for measuring phagocyteic activity, increased in comparison with control ٢٢%. The systemic NBT test ranged ٢٣-٣٢% and mucosal

NBT ranged ٢٨-٤٥%.

Specific cellular immune response was measured by LIF. It gave the following results: the systemic LIF was ٤٣-٦٠٪ and ٣٧-٥٢٪ at the mucosal level. The latter showed more inhibition of migration of leucocytes than the first one in comparison with normal value ٩٣٪.

In E-rosette formation by T-lymphocyte with sheep RBCs, the data showed increase in E-rosette formation value that ranged in systemic ٢٢-٣٣% and ٢٦-٣٧% in mucosal when compared with control ٢٦%.

Table ٤-١٢: Proteus mirabilis CBED immune status using LPS as antigen and sensitizer**.**

Sequences	Humoral							Cellular					
	Systemic					Mucosal		Systemic			Mucosal		
	Titer	TPC g/L	Ig g/L	Albumin g/L	Ig / Al	Titer	Ig g/L	LIF%	NBT%	E-rosette %	LIF%	NBT%	E-rosette %
١	١٦٠	٥٧.٢١	٢٩.١٩	٢٧.٨٧	١.٠٤	٣٢	٠.٢٨	٤٣	٢٣	٢٢	٣٧	٢٨	٢٦
٢	١٦٠	٥٩.٩٢	٣٠.٨٩	٢٧.٩٠	١.١١	٣٢	٠.٣٠	٤٥	٢٤	٢٣	٣٩	٣٠	٢٩
٣	٣٢٠	٦٠.٨٨	٣٢.٨٢	٢٨.٨٠	١.١٣	٣٢	٠.٣٧	٤٧	٢٥	٢٤	٣٩	٣٣	٢٩
٤	٣٢٠	٦٢.٨٢	٣٣.١١	٢٩.٨٢	١.١١	٣٢	٠.٣٨	٥٠	٢٦	٢٥	٤٠	٣٦	٣٠
٥	٣٢٠	٦٣.٣٣	٣٦.٢٢	٢٩.٩٣	١.٢١	٣٢	٠.٣٨	٥٠	٢٦	٢٦	٤١	٣٦	٣٠
٦	٣٢٠	٦٥.١١	٣٨.٨٠	٣٠.١١	١.٢٨	٣٢	٠.٣٩	٥٢	٢٨	٢٦	٤٣	٣٨	٣٢
٧	٣٢٠	٦٦.٦٠	٣٩.٧٠	٣٠.٣٥	١.٣٠	٣٢	٠.٤٢	٥٥	٢٩	٢٨	٤٥	٤٠	٣٣
٨	٦٤٠	٦٨.٩٨	٤٠.١٨	٣٢.٢٢	١.٢٢	٦٤	٠.٤٤	٥٧	٣٠	٢٩	٤٧	٤٤	٣٥
٩	٦٤٠	٦٩.٢٢	٤١. ١٨	٣٢.٨٠	١.٢٥	٦٤	٠.٥٥	٥٩	٣١	٣٠	٥١	٤٤	٣٦
١٠	٦٤٠	٧٠.٠١	٤٤.٠١	٣٣.١٦	١.٣٢	٦٤	٠.٥٩	٦٠	٣٢	٣٣	٥٢	٤٥	٣٧
Mean	٣٨٤	٦٤.٤٠	٣٦.٦١	٣٠.٢٩	١.١٩	٤١.٦	٠.٤١	٥١	٢٧.٤	٢٦.٦	٤٣	٣٧.٤	٣١.٧
Medium	٣٢٠	٦٥.١١	٣٨.٨٠	٣٠.١١	١.٢٨	٣٢	٠.٣٩	٥٢	٢٨	٢٦	٤٣	٣٨	٣٢
Range	١٦٠-٦٤٠	٥٧.٢١-٧٠.٠١	٢٩.١٩-٤٤.٠١	٢٧.٨٧-٣٣.١٦	١.٠٤-١.٣٢	٣٢-٦٤	٠.٣٠-٠.٥٩	٤٣-٦٠	٢٣-٣٢	٢٢-٣٣	٣٧-٥٢	٢٨-٤٥	٢٦-٣٧

RESULTS

٤-١: Retrospectives investigation:

It was evident that *Ps. aeruginosa* was the major gram negative ear associated pathogens (٣٦٪). This was followed by *Proteus spp* (٢٠٪). Meanwhile S. *aureus* was the major gram positive (١٩٪) ear etiogen (Table ٤-٢).

٤-٢: Prospective investigation:

The bacteriologic investigation of ١٠٠ BCED patients showed that *Ps. aeruginosa* was (٤٦٪), *P. mirabilis* (١٩٪) and *S. aureus* (١٧٪). These were the top three major ear associated bacterial pathogens (Table, ٤-٣). So these were elected for the detailed immunologic studies.

Table ٤-١: Normal. Subnormal and abnormal values for chronic ear discharge patients.

Parameter Name	Normal Value	Subnormal Value	Abnormal Value
NBT %	٢٤.٢٥ ± ١.١١	٢٣	٤٦
E-rosette %	٢٦.٥٠ ± ٠.٢٩	٢٢	٥٠
LIF %	٩٣ ± ٠.٦٥	٣٤	٩٥
Total Serum Protein(g/L)	٦٣.٩	٥٧.٢١	٧٢.٠٣
Serum Ig (g/L)	٣١.١	٢٩.١٩	٤٤.٠١
Serum albumin (g/L)	٣٢.٢	٢٢.٩٥	٤٠.٩

Table ٤-٢: Retrospective view in chronic ear discharge

Etiologic agent in CED	Occurrence%	NO. of cases
Pseudomonas aeruginosa	٣٦	٩٠
Proteus spp	٢٠	٥٠
Staph. aureus	١٩	٤٨
E. coli	١٠	٢٥
ß-hemolytic streptococci	٥	١٢
Streptococcus pneumoniae	٥	١٢
Klebsiella spp	٣	٨
Total		٢٤٥

Table ٤-٣: Prospective investigation in chronic ear discharge.

Bacterial agent	Occurrence %	NO. of Cases
Ps. aeruginosa	٤٦	٤٦
P. mirabilis	١٩	١٩
S. aureus	١٧	١٧
ß-hemolytic streptococci	٦	٦
Klebsiella spp.	٧	٧
S. pneumonae	٣	٣
α-hemolytic streptococci	٢	٢
Total		١٠٠

٤-٣: Immunology:

٤-٣-١: Bacterial chronic ear discharge serology:

The study of serum specific agglutinins showed titer ranges between ١٦٠-٦٤٠ for *S.aureus,* ١٦٠-٣٢٠ for *Ps. aeruginosa* and ١٦٠-٣٢٠ for *P. mirabilis,* while ١٦-٣٢ were for each of the *Ps. aeruginosa, P. mirabilis* and S. *aureus* specific mucosal antibodies. One to two fold rises in titers when LPS was used in the assay for Ps. *aeruginosa* and P. *mirabilis* using standard tube agglutination method. The specific antibody titer means are presented in Table٤.٤.

٤-٣-٢: NBT in chronic bacterial ear discharge

Nitroblue tetrazolium reduction test assessment showed high mucosal phagocytic activity in all etiological agents tested. From the data presented, NBT mean value was ٤٠.٨% in S. *aureus, Ps. aeruginosa* ٣٧% and ٣٧.٤% *in P. mirabilis.* Systemic increase in NBT activity was also noted slightly more than normal value, it was ٣٠.١% in *Ps.aeruginosa,* ٢٧.٤% in P. *mirabilis* and ٣٥.١% in S. *aureus* (Table, ٤-٥).

Table ٤-٤: Humoral systemic and mucosal bacterial specific antibody titer mean in CED patients, using standard tube agglutination method

Bacterial agent	Systemic titer mean		Mucosal titer mean	
	Whole cell antigen	LPS	Whole cell antigen	LPS
Ps. *aeruginosa*	٢٤٠	٣٨٤	١٩.٢	٦٠.٨
P. mirabilis	٢٢٤	٣٨٤	٢٤	٤١.٦
S. aureus	٣٣٦	-	٢٤	-

Table ٤-٥: Nitroblue tetrazolium reduction test activity in bacterial chronic ear discharge patients.

Bacterial agent	Systemic NBT Mean %	Mucosal NBT Mean%
Ps. *aeruginosa*	٣٠.١	٣٧
P. mirabilis	٢٧.٤	٣٧.٤
S. aureus	٣٥.١	٤٠.٨

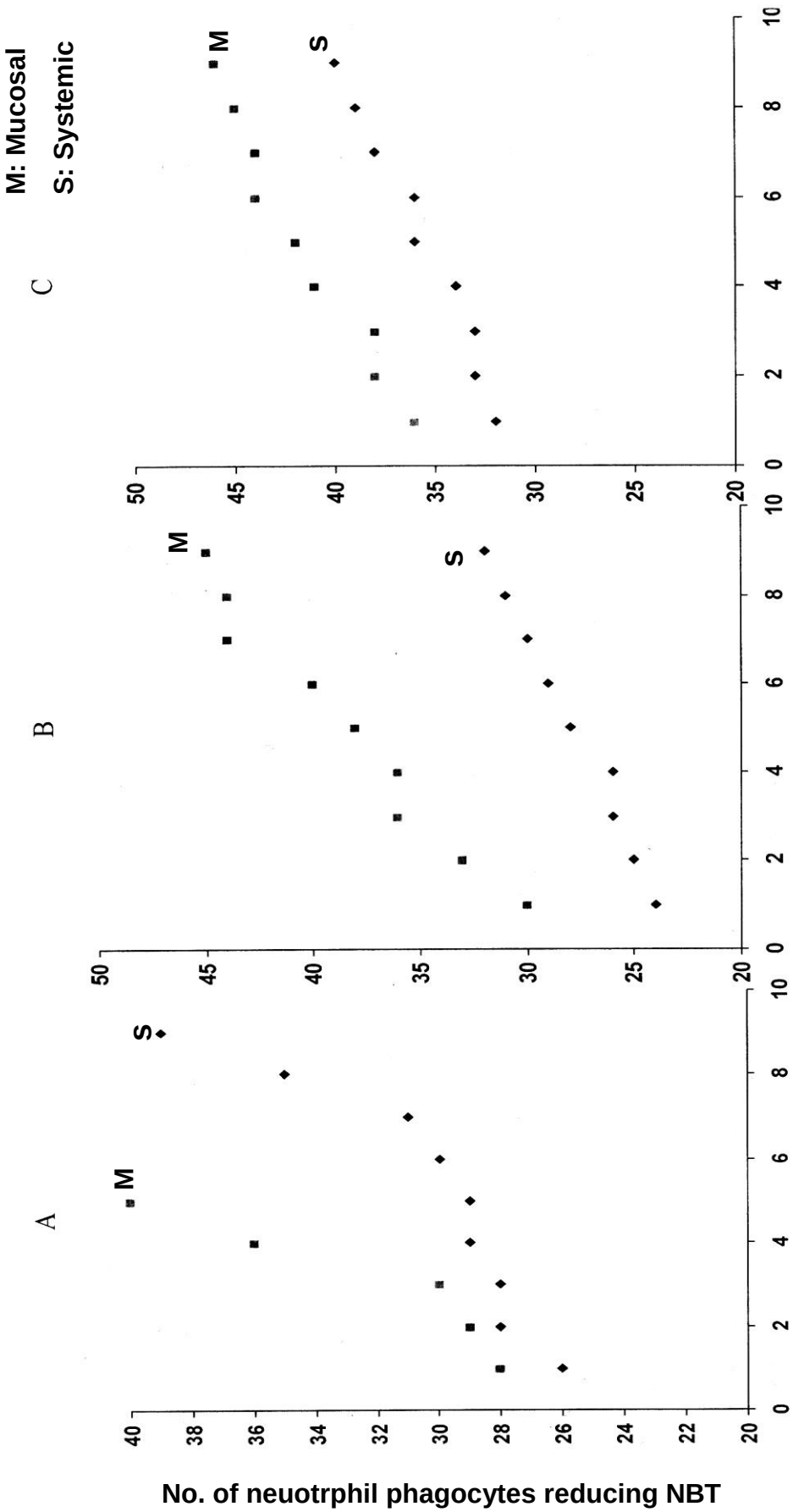


Fig.(3): NBT Test for the top three casuals of CED patients
A- *Ps. auerogenosa* B- *P. mirabilis* C- *S. aureus*

٤-٣-٣: Leucocytes inhibition test (LIF):

The mucosal LIF assessments showed highly significant LIF indices than in peripheral blood LIF indices. Systemic LIF was ٦٣٪ for Ps. *aeruginosa* using CFCF as a sensitizer and ٥٢٪ when using LPS. The mucosal LIF was ٤٩٪and ٤١٪ when CFCF and LPS were used as a sensitizer respectively. For *P. mirabilis* CFCF sensitizer gave non significant LIF value. Systemic and mucosal were ٨٣-٩٥٪ and ٧٢-٩٠٪ respectively. On using LPS as a sensitizer ٤٣-٦٠ and ٣٧-٥٢٪ were noted for systemic and mucosal accordingly. In S. *aureus* CED, LIF values ranged from ٤٦-٥٩٪ and from ٣٦-٤١٪ for systemic and mucosal LIF respectively. LIF% mean is presented in Table (٤-٦).

Table ٤-٦: LIF% mean in patient with bacterial chronic ear discharge

Bacterial agent	LIF% mean			
	Systemic		mucosal	
	CFCF	LPS	CFCF	LPS
Ps.aeruginosa	٦٣	٥٢	٤٩	٤١
P. mirabilis	٨٧	٥١	٨٢	٤٣
S. aureus	٥١	-	٤١	

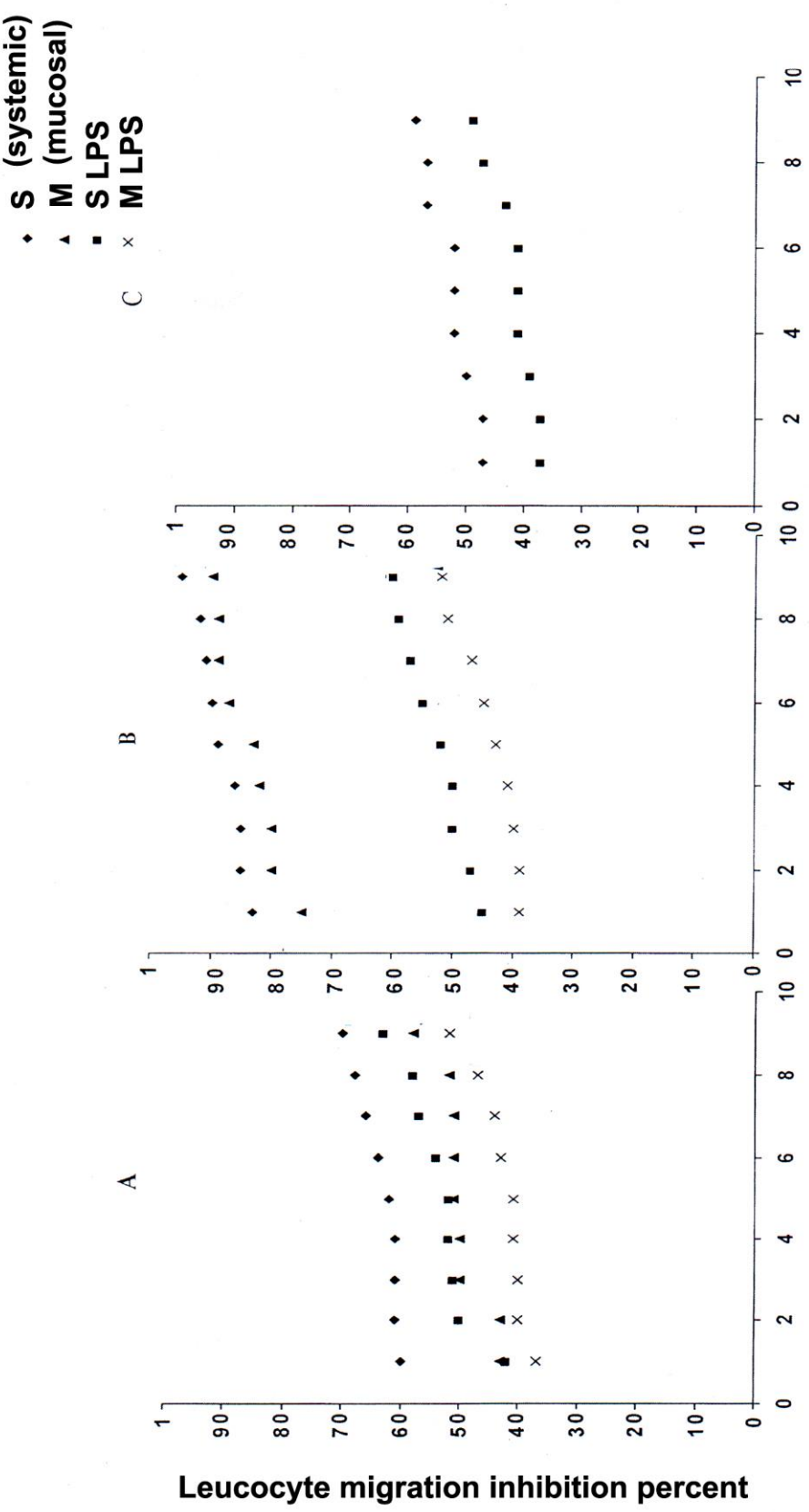


Figure (٤): Leucocyte inhibition factor test for the major three causals of CEDP

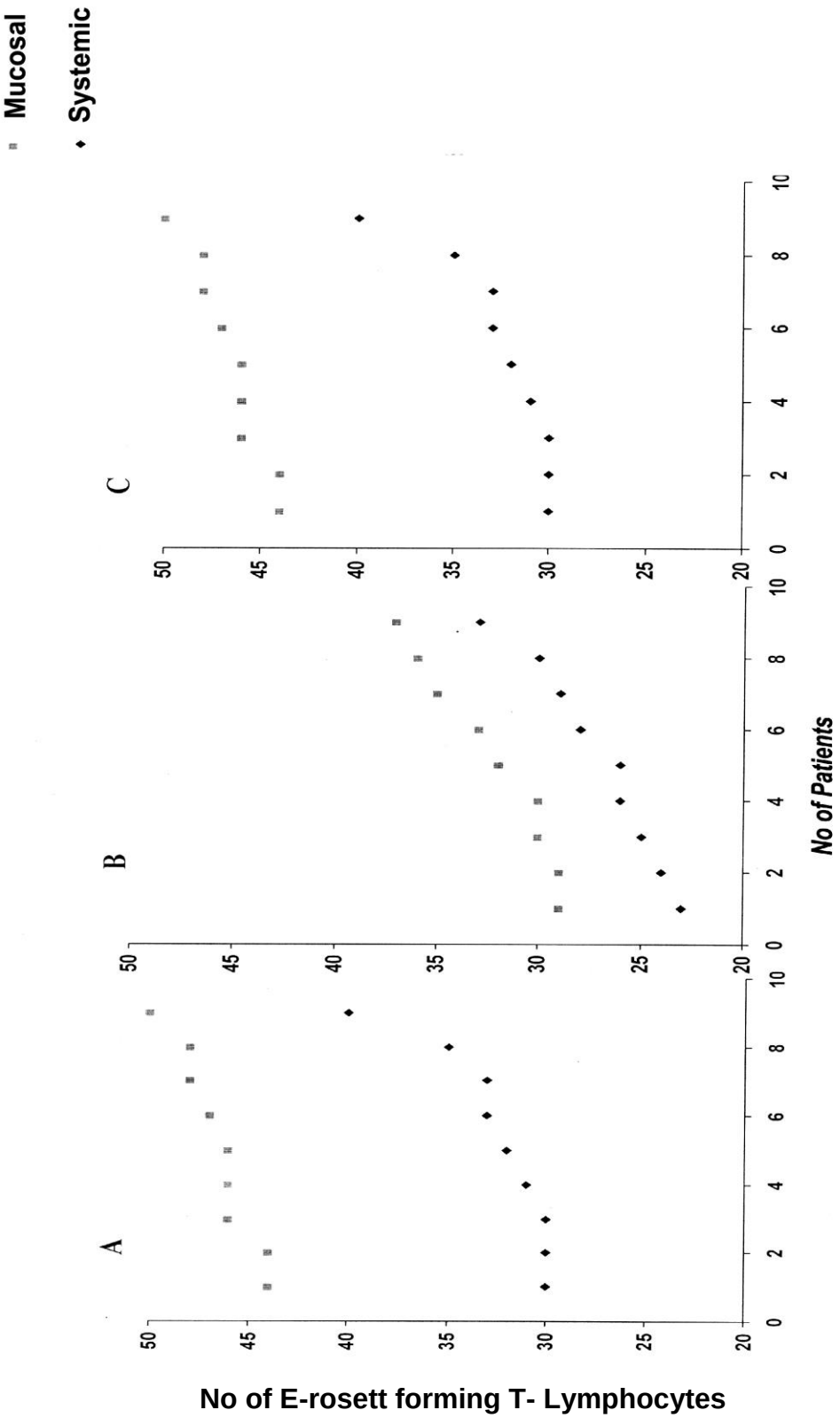
A. *Ps. aeruoginosa* . B.*P.mirabilis*, C. *S. aureus*

٤-٣-٤ E-rosette test:

The mucosal E-rosette was ٤٦.١% which was higher than that of systemic E-rosette, ٣٢.٢% for *Ps. aeruginosa* while the mucosal E-rosette was ٣١.٧% and ٢٦.٦% for systemic E-rosette in *P. mirabilis.* In case of *S. aureus,* it was ٤١% for mucosal and ٣٨.٥% for systemic E-rosette. The E-rosette mean values of these patients group are enlisted in Table ٤-٧.

Table ٤-٧: E-rosette Mean % in patients with chronic ear discharge.

Bacterial agent	E-rosette Mean %	
	Systemic	Mucosal
Ps. *aeruginosa*	٣٢.٢	٤٦.١
P. mirabilis	٢٦.٦	٣١.٧
S. aureus	٣٨.٥	٤١



Figure(٥). E-rosett test for the major three causals of CED patients

A. *Ps. aeruoainosa* . B.*P.mirabilis*. C. *S. aureus*

٤-٤: Immunology of specific groups in chronic ear discharge patients.

٤-٤-١ *Staphylococcus aureus* immunological status:

The assay antigens were whole cell antigen (WCA) and cell free culture filtrate (CFCF). The immunology of *S. aureus* CED, both of humoral and cellular immune parameters was investigated (Table ٤-٨.).

The non specific humoral response was studied and the mean value of total protein in the serum ranged between ٥٩.٧-٧٢.٠٣g/L. The normal value was ٦٣.٩. The mean and median value were ٦٥.٧, ٦٦.١ respectively.

The specific humoral immune response was assessed by the determination of antibody titers in serum and it ranged between ١٦٠-٦٤٠, the highest liter value was ٦٤٠.

The local, however, humoral immune parameters were assessed by determination of mucosal antibody titers. The titer ranged between ١٦-٣٢; the highest titer was ٣٢ whereas the lowest one was ١٦ (table ٤-٨).

At the cellular level different parameters were taken including phagocytic activity (NBT), LIF and E rosette formation by T-lymphocyte.

Non specific cellular immune response was studied represented by NBT test and gave the following values: At the systemic immune system it ranged between ٣٠-٤٠٪ with mean and median value ٣٥.١٪ and ٣٦ % respectively. The mucosal value ranged between ٣٤-٤٦٪ the median value was ٤٢٪ and mean value was ٤٠.٨٪.

Specific cellular immune response was studied and the LIF at both of the systemic and mucosal ranged between ٤٦-٥٩٪ at the systemic and ٣٦-٤٩٪ at the mucosal system. The mean and median values were ٥١% and ٥٢٪ respectively at the mucosal system the mean and medina value were ٤١٪ for each one.

E-rosette formation by T- lymphocyte was also investigated as specific cellular immune response and it ranged at systemic between ٣٠-٤٦%and mucosal as ٣٢-٤٨٪ the median and mean value was ٤٣٪ and ٤١٪ respectively. At the systemic, the mean and median values were ٣٨.٥ and ٣٨٪.

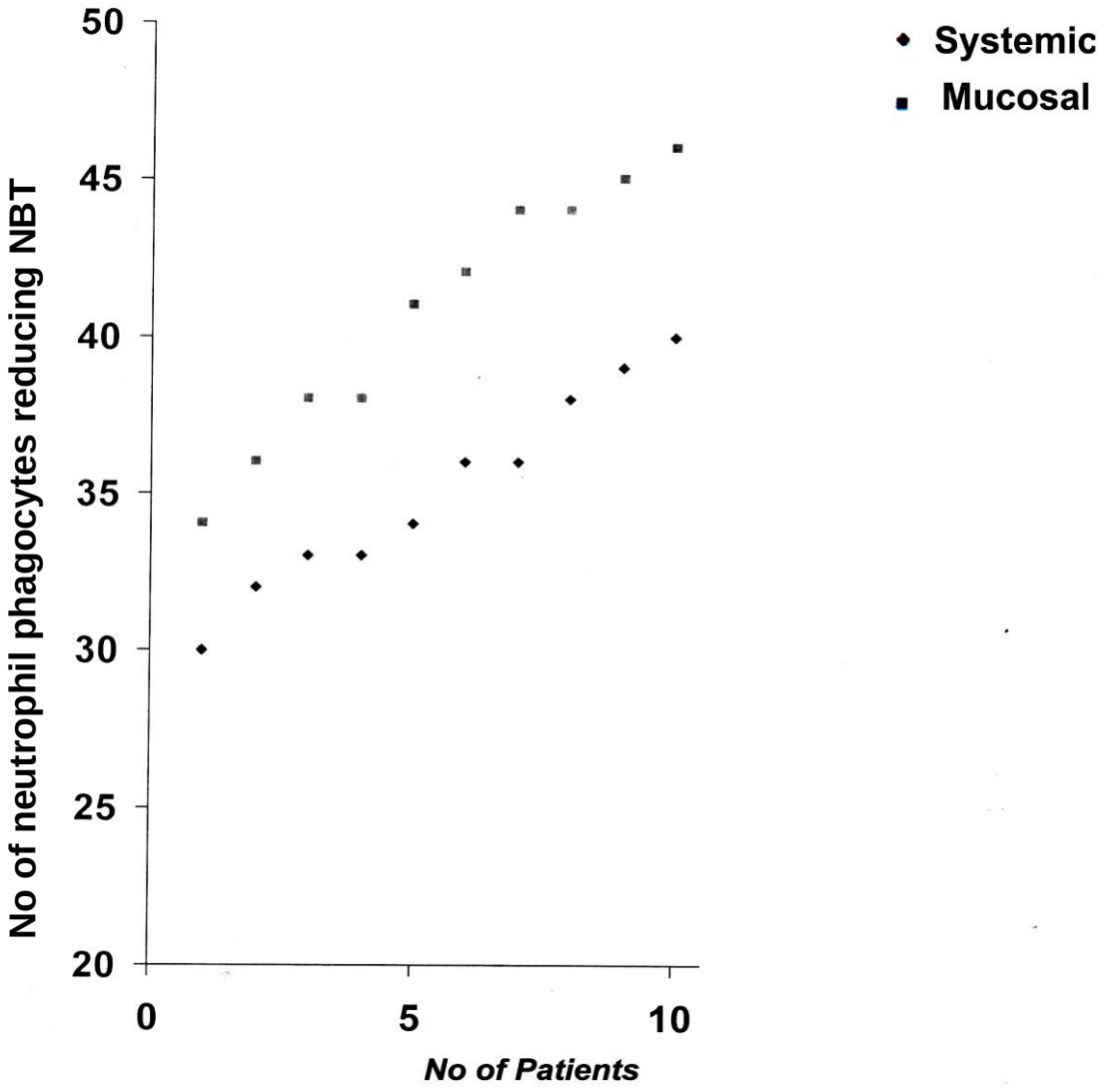


Fig. (6): The Nitroblue Tetrazolim reduction Test (NBT) for *S. aureus* CED patients

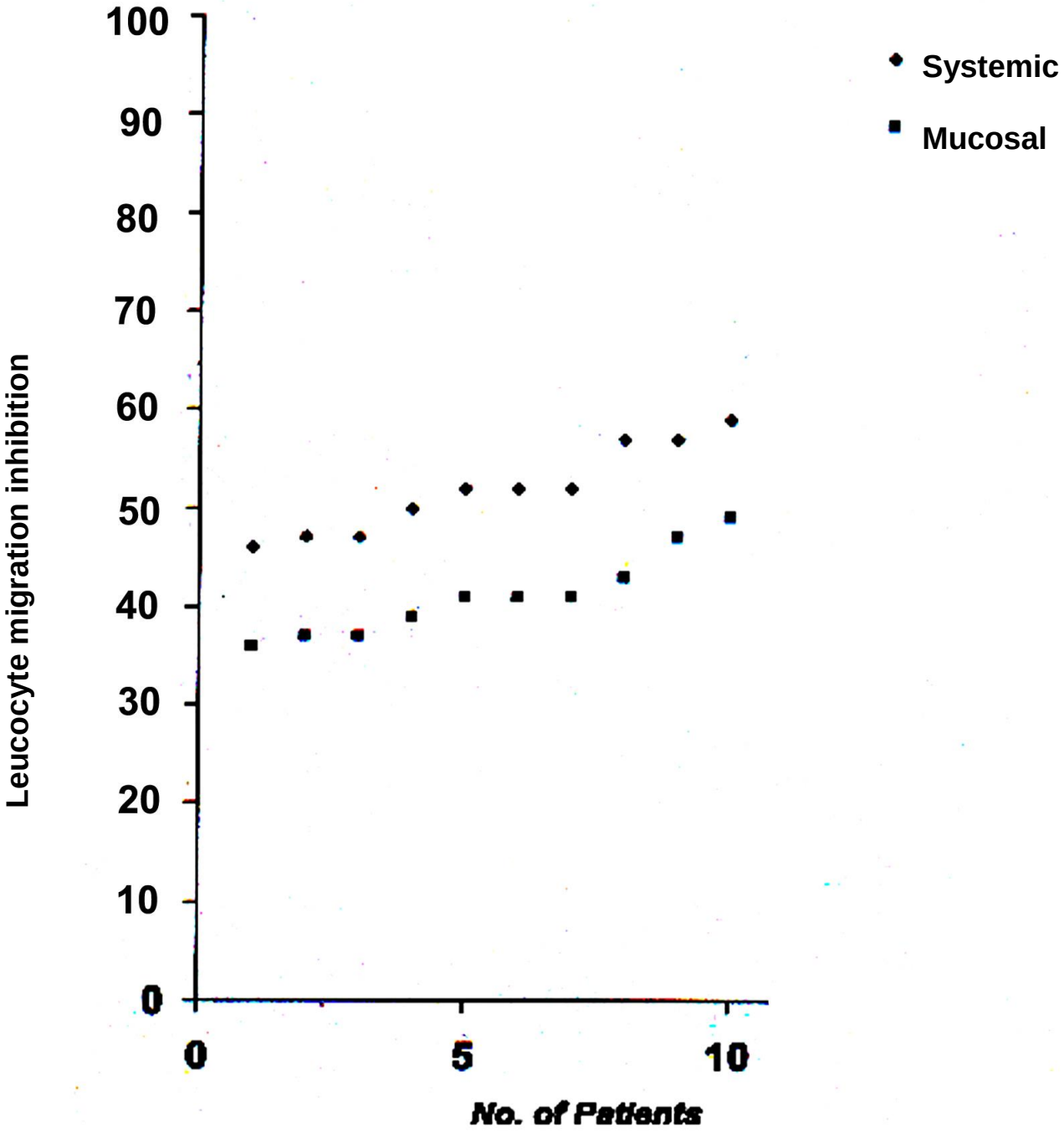


Figure (٧). The leucocyte migration inhibition test for *S.aureus* CED patients

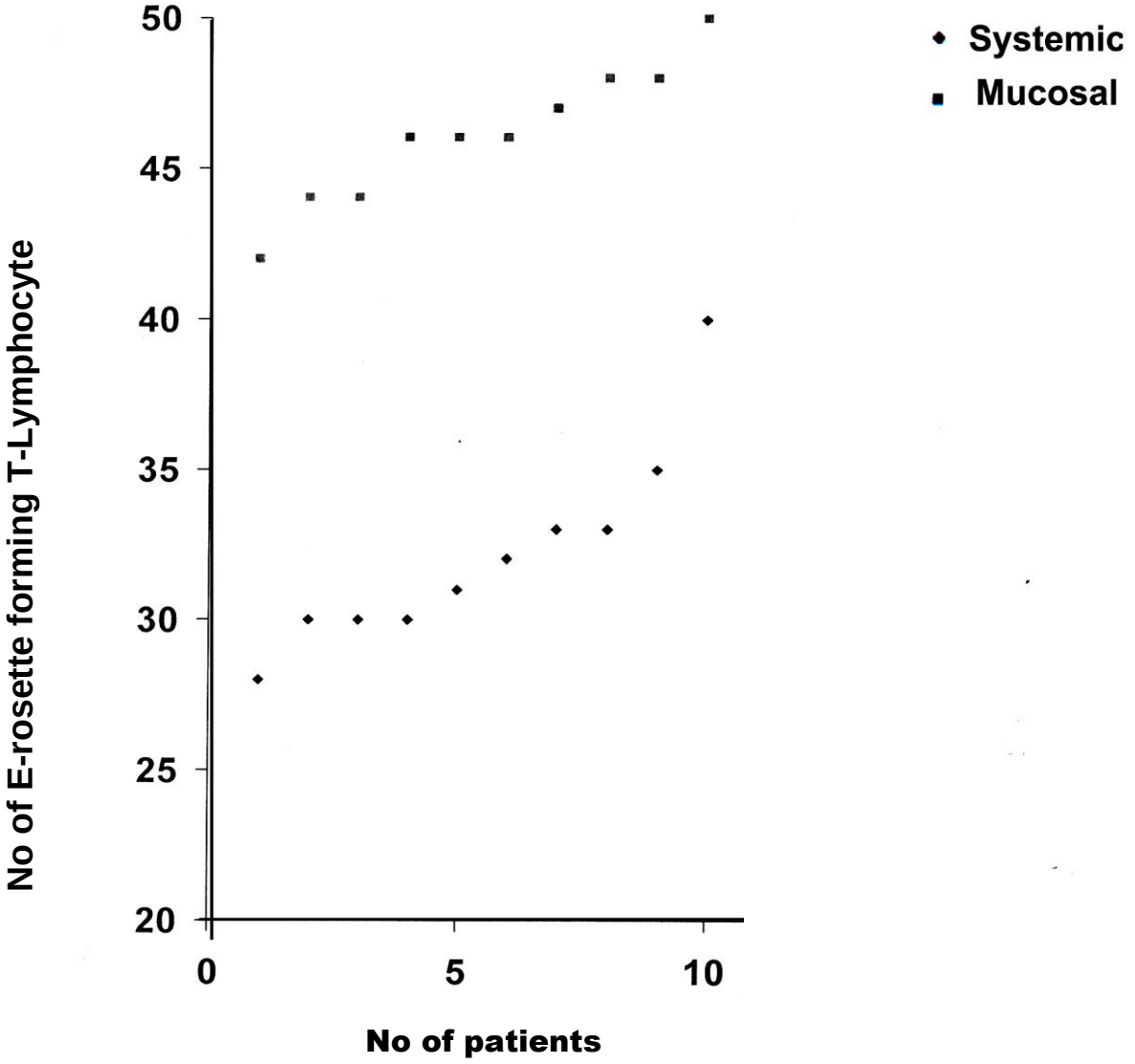


Fig. (8): E rosett test for *S. aureus* CED patients

Table (٤-٨): Immune status of patients with chronic otitis media caused by *S. aureus*

Sequences	Humoral							Cellular						
	Systemic					Mucosal		Systemic				Mucosal		
	Titer	Conc. Total Protein g/L	Con. Immun Globuli g/L	Albumin	Al/gl	Titer	Con. Immun-Globuli g/L	Sequences	LIF%	NBT%	E-rosette%	LIF%	NBT%	E-rosette%
١	١٦٠	٥٩.٧	٣٠.٠٤	٢٢.٩٥	٠.٧٦	١٦	٠.٣١	١	٤٦	٣٠	٣٠	٣٦	٣٤	٣٢
٢	١٦٠	٦٢.٨	٣٠.١٦	٢٥.٢٢	٠.٨٣	١٦	٠.٣٣	٢	٤٧	٣٢	٣٣	٣٧	٣٦	٣٤
٣	١٦٠	٦٣.٢	٣٢.١٢	٢٥.٨٢	٠.٨٠	١٦	٠.٣٧	٣	٤٧	٣٣	٣٦	٣٧	٣٨	٣٤
٤	٣٢٠	٦٤.١	٣٤.٧٢	٢٦.٦٢	٠.٧٦	١٦	٠.٤٢	٤	٥٠	٣٣	٣٧	٣٩	٣٨	٣٩
٥	٣٢٠	٦٥.٦	٣٦.١٧	٢٧.٣٠	٠.٧٥	١٦	٠.٤٤	٥	٥٢	٣٤	٣٨	٤١	٤١	٤٠
٦	٣٢٠	٦٦.١	٣٦.٥٥	٢٧.٨٠	٠.٧٦	٣٢	٠.٤٨	٦	٥٢	٣٦	٣٨	٤١	٤٢	٤٣
٧	٣٢٠	٦٨.١	٣٧.٢٠	٢٨.٢١	٠.٧٥	٣٢	٠.٥٠	٧	٥٢	٣٦	٤٠	٤١	٤٤	٤٦
٨	٣٢٠	٦٨.٤	٣٨.١١	٣٠.١٨	٠.٧٩	٣٢	٠.٦١	٨	٥٢	٣٨	٤٣	٤٣	٤٤	٤٦
٩	٦٤٠	٧٠.٠١	٣٨.٥٠	٣١.١٠	٠.٨٠	٣٢	٠.٧٣	٩	٥٧	٣٩	٤٤	٤٧	٤٥	٤٨
١٠	٦٤٠	٧٢.٠٣	٤٠.٢٢	٣٣.٢٠	٠.٨٢	٣٢	٠.٧٧	١٠	٥٩	٤٠	٤٦	٤٩	٤٦	٤٨
Mean	٣٣٦	٦٦.٠	٣٥.٥٠	٢٧.٨٤	٠.٧٨	٢٤	٠.٤٩		٥١	٣٥.١	٣٨.٥	٤١	٤٠.٨	٤١
Medium	٣٢٠	٦٦.١	٣٦.٥٥	٢٧.٨٠	٠.٧٦	٣٢	٠.٤٨		٥٢	٣٦	٣٨	٤١	٤٢	٤٣

٤-٤-٢: *Ps. aeruginosa* immunological status.

The humoral and cellular immunity of *Ps. aeruginosa* was investigated and results are listed in table (٤-٩), the data showed inductions of humoral immunity at both of the systemic and mucosal level. The titer at serum ranged between ١٦٠-٣٢٠ and ١٦-٣٢ at the mucosal immunity when whole cell antigen was used.

At the cellular immune function levels *Ps. aeruginosa* induced non-specific cellular immune response.

In NBT test it ranged between ٢٦-٣٩% at the systemic and ٢٨-٤٣% at the mucosal system, the mean and median values were ٣٠.١% ,٢٩% at the systemic and ٣٧%, ٤٠% at the mucosal respectively.

In specific immune response at the cellular level *Ps.aeruginosa* induced cellular immunity when LIF and E-rosette were used as parameters. LIF value ranged between ٥٧ - ٧٠٪ with mean and median values ٦٣% , ٦٢% respectively at the systemic immunity. LIF rang at the mucosal immunity between ٤١-٥٨% with mean and median value ٤٩% , ٥١% respectively.

E- rosette formation also increased more than normal value (٢٦%) and ranged ٢٨-٤٠% at the systemic level and ٤٢-٥٠% at the mucosal system.

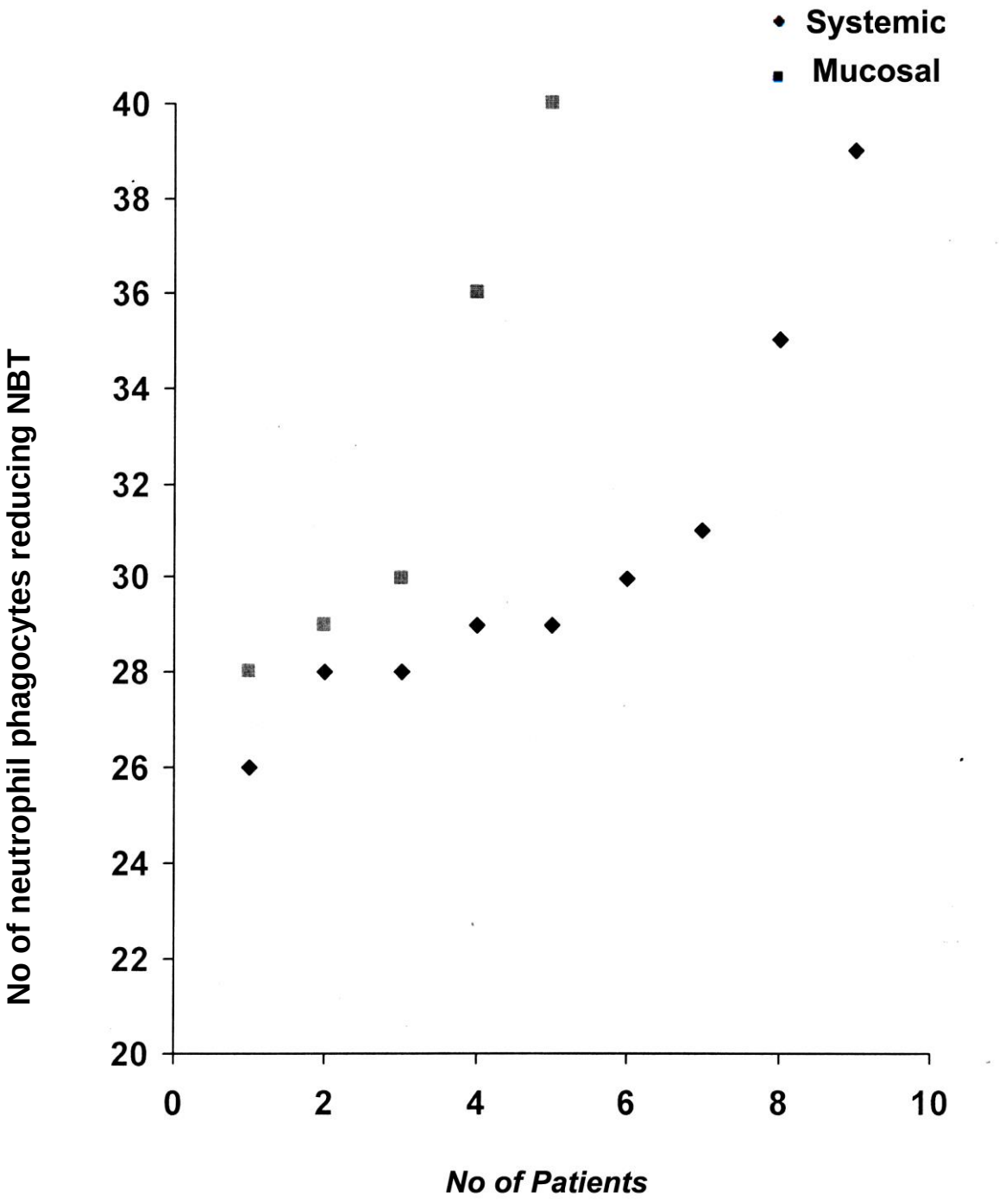


Figure (٩). The nitroblue tetrazolium reduction test (NBT) for *Ps. aeruginosa* CED patients

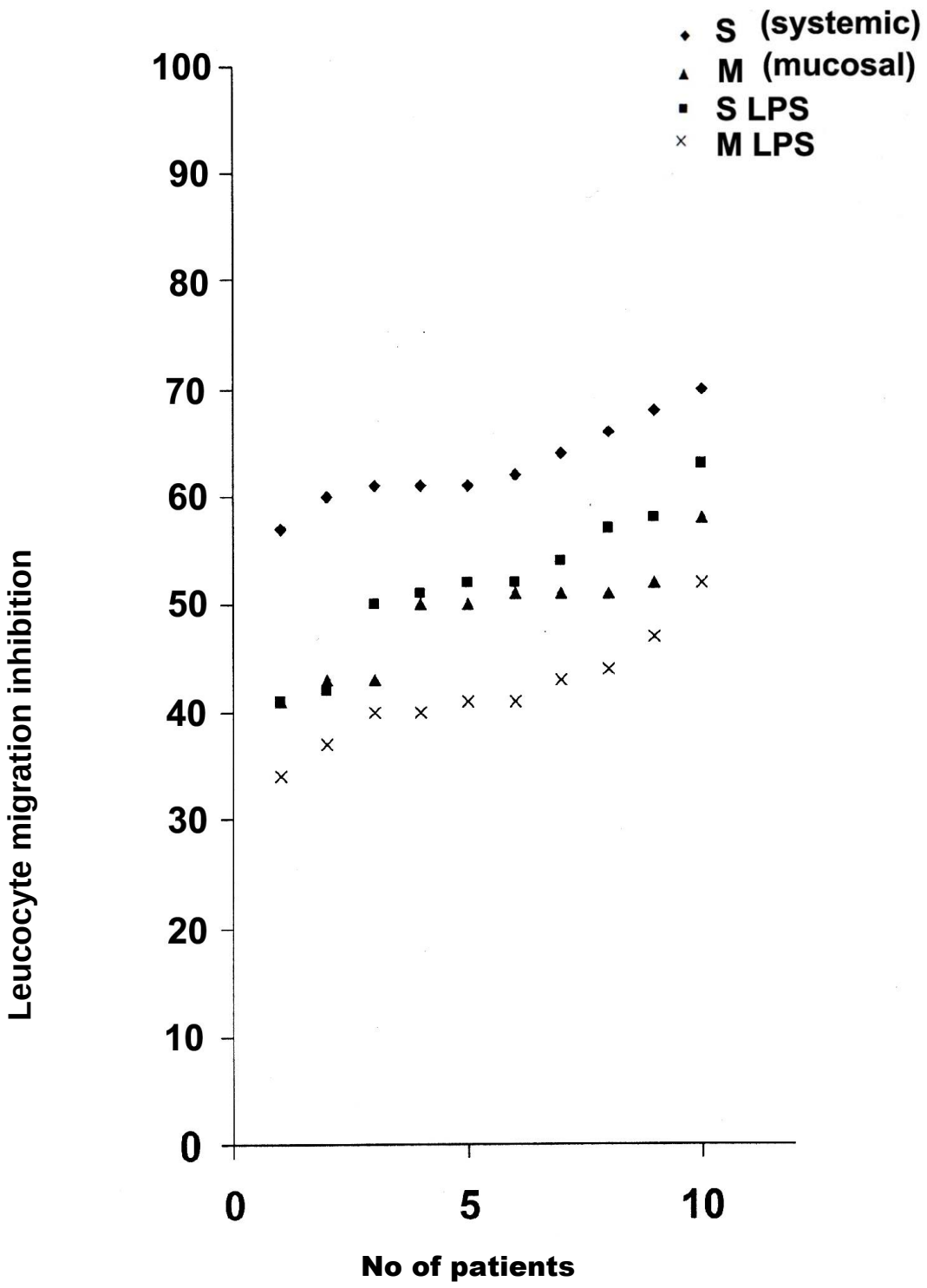

S (systemic)
M (mucosal)
S LPS
M LPS
100
90
80
70
60
50
40
30
20
10
0
0
5
10
Leucocyte migration inhibition
No of patients

Figure (١٠). The leucocyte migration inhibition test for *Ps. aeruginosa* CED patients

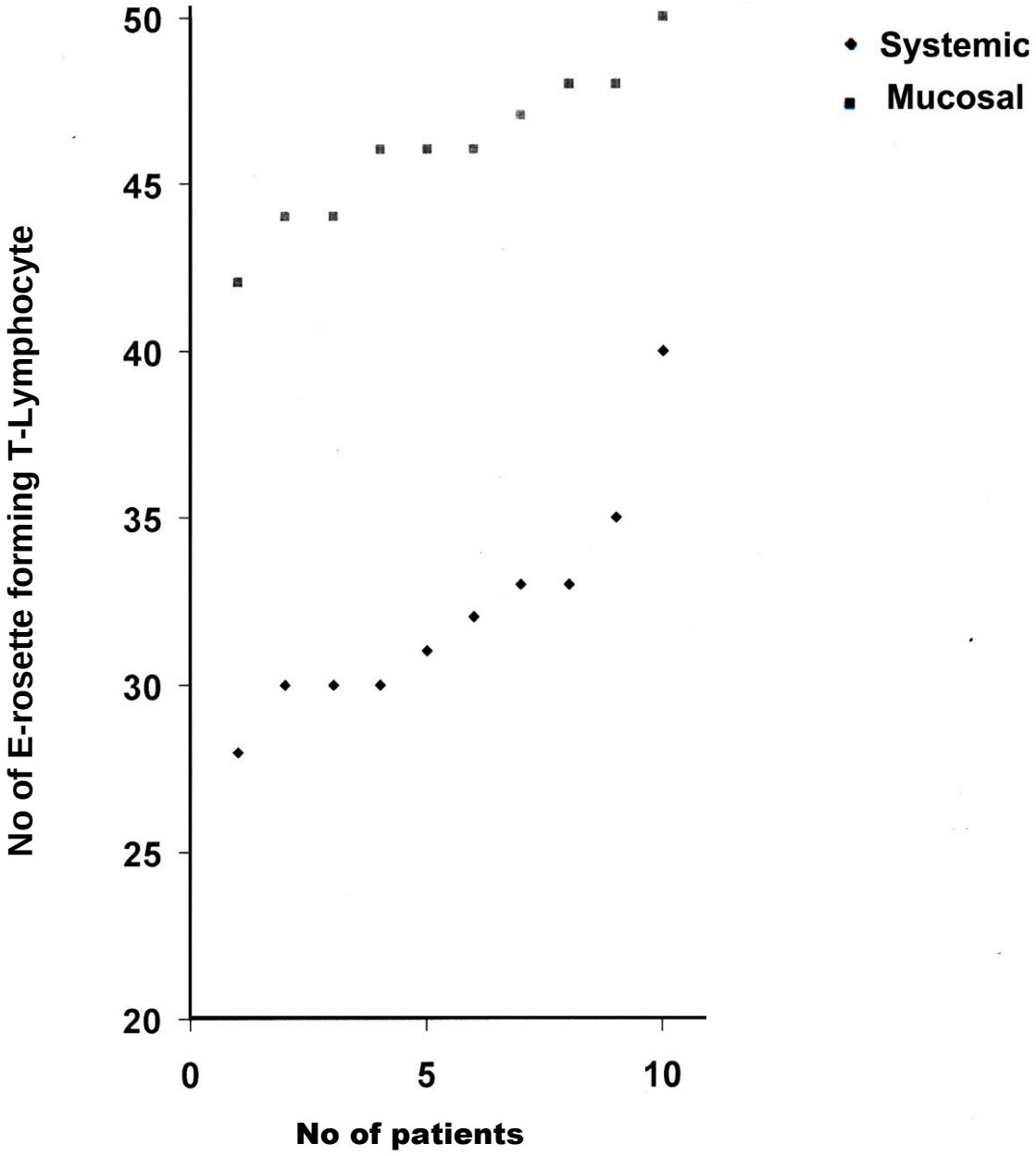


Figure (١١). E-rosette test *for Ps. aeruginosa* CED patients

Table ٤-٩: Immune status of patients with chronic otitis media caused by *Pseudomonas aeruginosa*.

Sequences	Humoral		Cellular					
	Systemi	Mucosal	Systemic			Mucosal		
	Titer/ WCA	Titer/ WCA	LIF %	NBT %	E-rosette	LIF %	NBT %	E-rosette
١	١٦٠	١٦	٥٧	٢٦	٢٨	٤١	٢٨	٤٢
٢	١٦٠	١٦	٦٠	٢٦	٣٠	٤٣	٢٨	٤٤
٣	١٦٠	١٦	٦١	٢٨	٣٠	٤٣	٢٩	٤٤
٤	١٦٠	١٦	٦١	٢٨	٣٠	٥٠	٣٠	٤٦
٥	١٦٠	١٦	٦١	٢٩	٣١	٥٠	٣٦	٤٦
٦	٣٢٠	١٦	٦٢	٢٩	٣٢	٥١	٤٠	٤٦
٧	٣٢٠	١٦	٦٤	٣٠	٣٣	٥١	٤٢	٤٧
٨	٣٢٠	١٦	٦٦	٣١	٣٣	٥١	٤٢	٤٨
٩	٣٢٠	٣٢	٦٨	٣٥	٣٥	٥٢	٤٣	٤٨
١٠	٣٢٠	٣٢	٧٠	٣٩	٤٠	٥٨	٤٣	٥٠
Mean	٢٤٠	١٩.٢	٦٣	٣٠.١	٣٢.٢	٤٩	٣٧	٤٦.١
Median	٣٢٠	١٦	٦٢	٢٩	٣٢	٥١	٤٠	٤٦
Range	١٦٠-٣٢٠	١٦-٣٢	٥٧ -٧٠	٢٦-٣٩	٢٨-٤٠	٤١-٥٨	٤٢-٥٠	٤٢-٥٠

٤-٤-٣: *P. mirabilis* immunological status.

The immune features of *P. mirabilis* in patients with BCED are enlisted in (table ٤-١٠). The humoral and cellular immunity was investigated.

The humoral immunity at the systemic and mucosal showed increase in the titer of specific immunoglobulin which ranged between ١٦٠-٣٢٠ at the systematic and ١٦-٣٢ at mucosal system, with mean and median value ٢٢٤, ١٦٠ respectively at the systemic and ٢٤, ٣٢ at the mucosal when whole cell antigens were used. Thus, *P. mirabilis* induce humoral local and systemic immunity.

In case of non specific cellular immune response NBT test showed slightly increase in phagocytic activity and ranged between ٢٣-٣٢% at the systemic and ٢٨-٤٥% at the mucosal system in comparison with normal value ٢٤%.

P. mirabilis, however, did not induce cellular immunity, when LIF used as parameter, so that LIF ranged ٨٣-٩٥% at the systemic and ٧٢- ٩٠٪ at the mucosal which were near to the normal value ٩٣%.

E-rosette formation ranged between ٢٦- ٣٧% at the mucosal and ٢٢-٣٣% at the systemic immune system which indicate an increase in T-cell rosette formation in mucosal system in comparison with normal value ٢٦%.

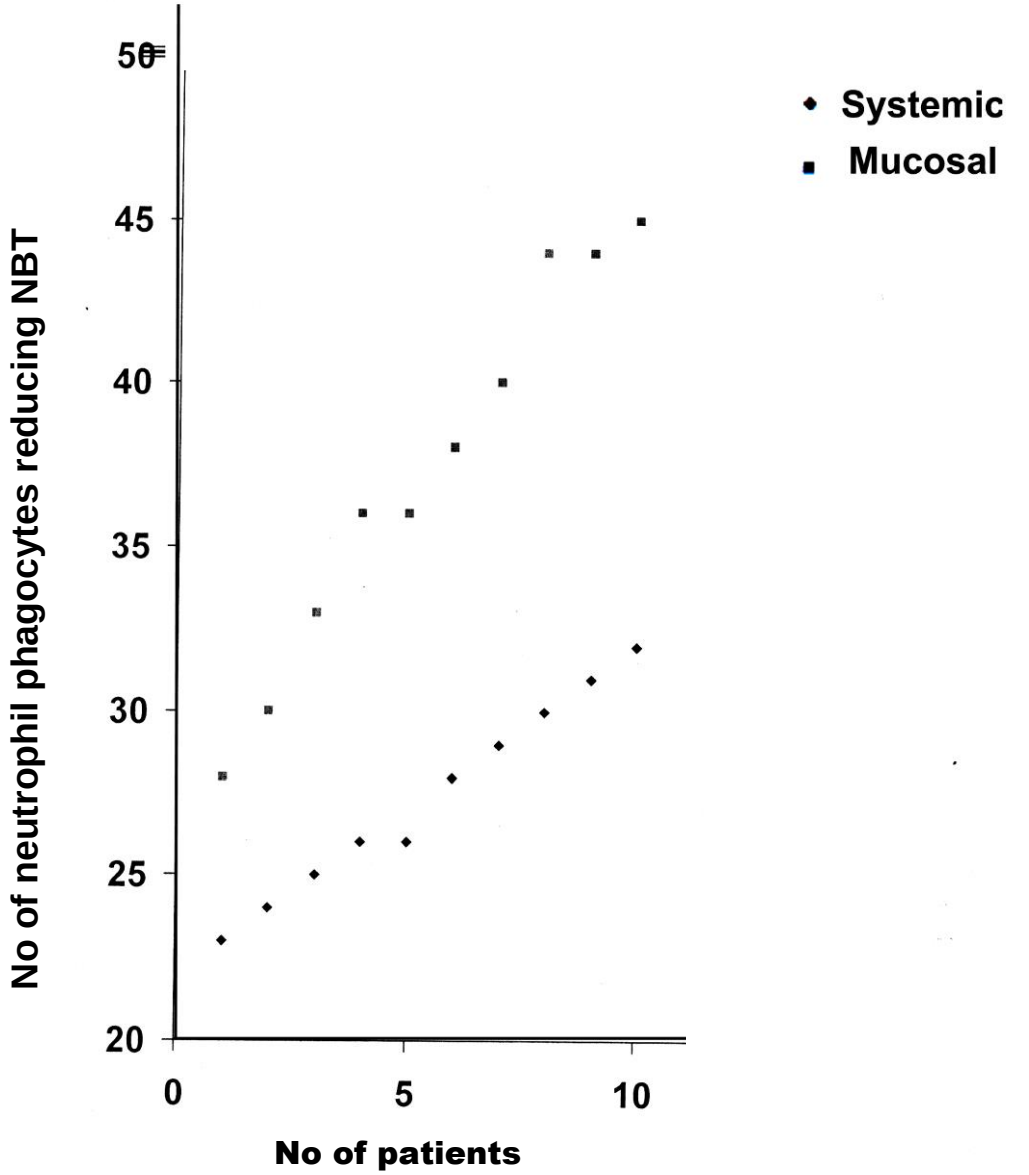


Figure (١٢). The nitroblue tetrazolium reduction test (NBT) for *P. mirabilis* CED patients

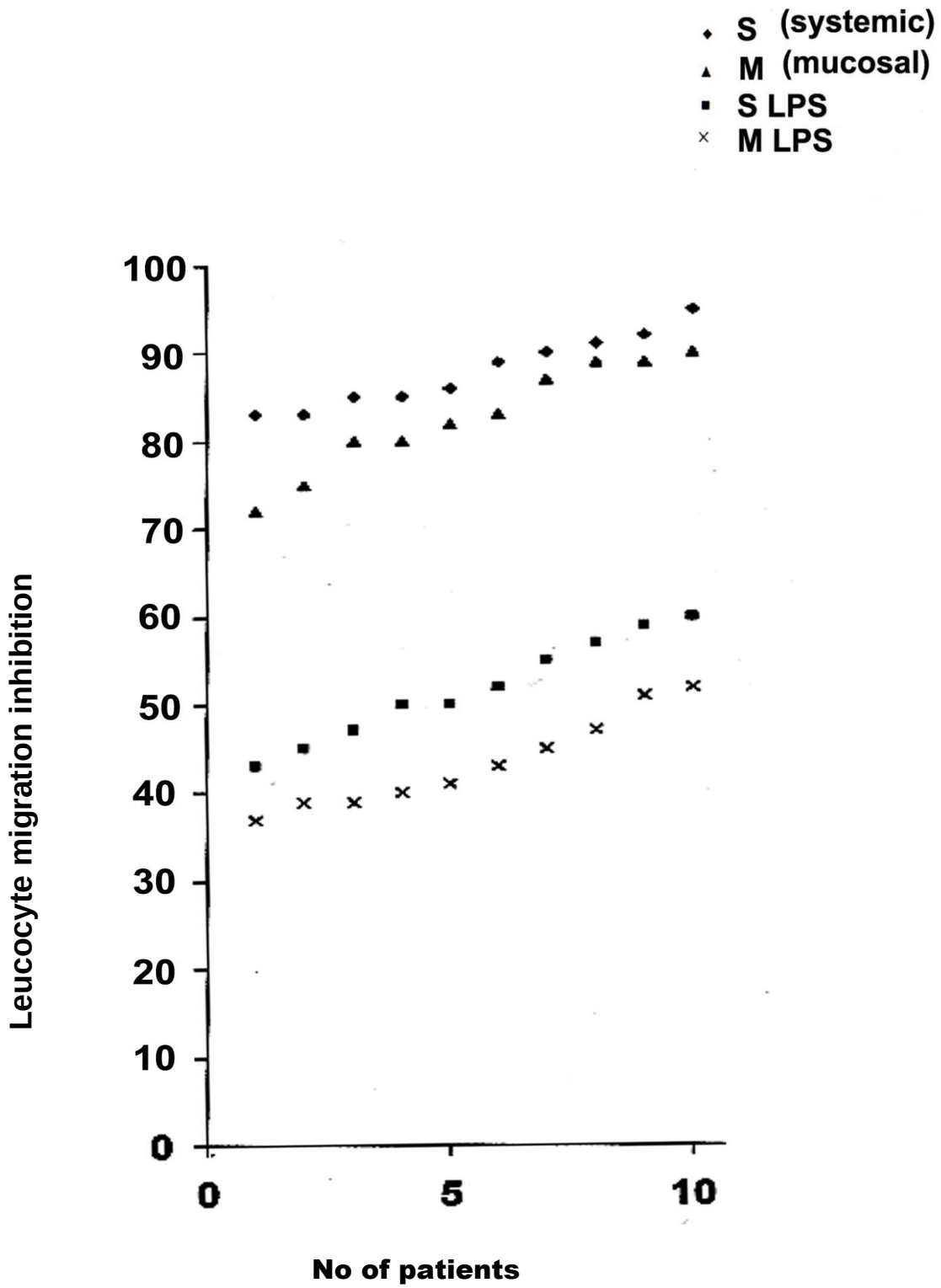


Figure (١٣). The leucocyte migration inhibition test for *P. mirabilis* CED patients

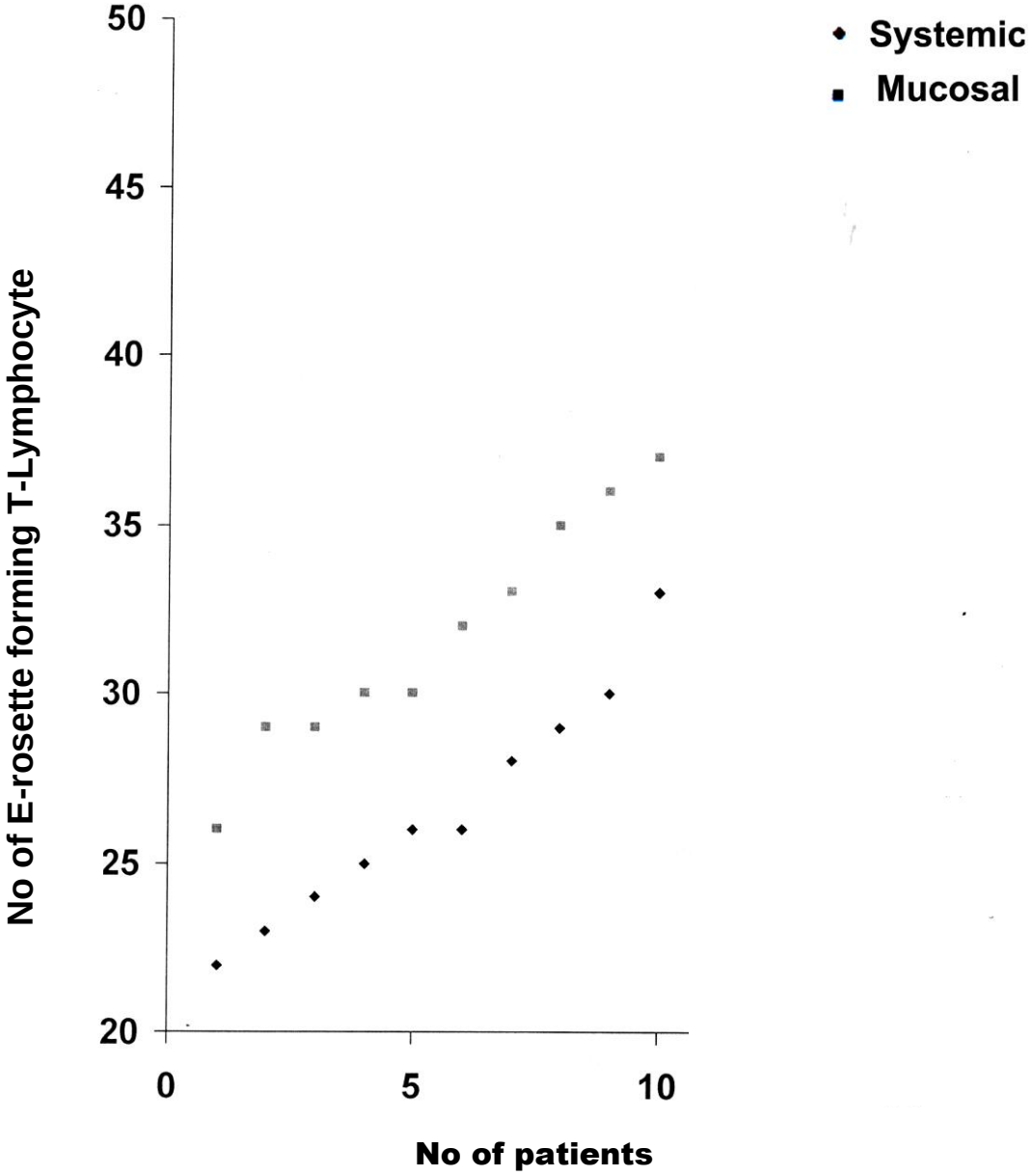


Figure (١٤). E-rosette test for *P.mirabilis* CED patients

Table ٤-١٠: Immune status of patients with chronic otitis media caused by *Proteus mirabilis*.

Sequences	Humoral		Cellular					
	Systemi	Mucosal	Systemic			Mucosal		
	Titer/ WCA	Titer/ WCA	LIF %	NBT %	E-rosette %	LIF %	NBT %	E-rosette %
١	١٦٠	١٦	٨٣	٢٣	٢٢	٧٢	٢٨	٢٦
٢	١٦٠	١٦	٨٣	٢٤	٢٣	٧٩	٣٠	٢٩
٣	١٦٠	١٦	٨٥	٢٥	٢٤	٨٠	٣٣	٢٩
٤	١٦٠	١٦	٨٥	٢٦	٢٥	٨٠	٣٦	٣٠
٥	١٦٠	١٦	٨٦	٢٦	٢٦	٨٢	٣٦	٣٠
٦	١٦٠	٣٢	٨٩	٢٨	٢٦	٨٣	٣٨	٣٢
٧	٣٢٠	٣٢	٩٠	٢٩	٢٨	٨٧	٤٠	٣٣
٨	٣٢٠	٣٢	٩١	٣٠	٢٩	٨٩	٤٤	٣٥
٩	٣٢٠	٣٢	٩٢	٣١	٣٠	٨٩	٤٤	٣٦
١٠	٣٢٠	٣٢	٩٥	٣٢	٣٣	٩٠	٤٥	٣٧
Mean	٢٢٠	٢٤	٨٧	٢٧.٤	٢٦.٦	٨٢	٣٧.	٣١.٧
Median	١٦٠	٣٢	٨٦	٢٨	٢٦	٨٣	٣٨	٣٢
Range	١٦٠- ٣٢٠	١٦-٣٢	٨٣-٩٠	٢٣-٣٢	٢٢-٣٣	٧٠-٩٠	٢٨- ٤٥	٢٦-٣٧

CONCLUSIONS

١- The retro-and prospective studies have shown that the three top bacterial species in chronic ear discharge were Ps. aeruginosa, P. mirabilis and S. aureus.

٢- The three bacterial in CED patients induce systemic humoral immunity characterized by increasing the antibody titers in patients' serum.

٣- Mucosal humoral immune response was also induced in CED patients with increasing antibody titer specific to the three bacterial species.

٤- Cell free culture filterate of *Ps. aeruginosa* and *S. aureus* sensitize cellular immune response in both systems (systemic and mucosal) of CED patients.

٥- Cell free culture filterate did not sensitize cellular immune response at both systems in case of P. mirabilis CED patients.

٦- LPS extracted from Gram negative species have large potency to express humoral and cellular immune response at both systems.

٧- Purified LPS extracted from *P. mirabilis* showed a high significant immune response at both level cellular and humoral immune system in local and peripheral blood as compared with the culture filterates.

٨- All these bacterial species studied induce non-specific humoral immune response at serum represented by increasing total serum proteins, serum Ig and decreased serum albumin.

RECOMMENDATIONS

The study of pro-inflammatory, inflammatory and IL-١٠ cytokines in management of chronic infections like CED and cancer disease.

DISCUSSION

٥-١: The profile of bacteria associated with chronic ear discharge:

Otitis media is an infection or inflammation of middle ear. This inflammation begins when infections that cause sore throats, colds or other respiratory problems spread to the middle ear. These can be viral or bacterial infection. Children are more likely to suffer from otitis than adults. This is because children have more trouble fighting infections as their immune system are still developing. Another reason has to do with the closed Eustachian tube (Gates, ١٩٩٦).

One more factor that makes children more susceptible to otitis media is that the adenoids in children are larger than they are in adults. Adenoids are composed largely of cells (Lymphocytes) that help in fighting infections. Enlarged adenoids can interfere with Eustachian tubes opening.

In addition, adenoid may also become infected, and the infection spread in to the Eustachian tubes. Other factors that contributed to otitis media are cold climate, high altitude and exposure to cigarette smokes.

Bacteria reach the middle ear through the lining or passage way of the Eustachian tubes and can then produce infection, which causes blocking of Eustachian tube, swelling of lining of middle ear and migration of white cells from blood stream to help overcome the infection. In this process the white cells

accumulate often killing bacteria and die themselves, leading to the formation of pus, a thick yellowish white fluid in the middle ear(Berman *et al,* ١٩٩٧).

In middle ear infections, microorganisms ascending from nasopharynx via the Eustachian tube in the middle ear cavity play a central role. In chronic suppurative otitis media with continuously discharging ear, indicating a drum perforation, **S.** *aureus,* coagulase negative *Staphylococci, Ps. aeruginosa* and *Proteus* species are the predominant microorganism (Johnson, *et al.* ١٩٨٦; Karma, *et al* ١٩٧٨).

Following the above information, we dealt with a case that was characterized with chronic ear infection that was associated with bacterial agent only. All the regarded cases were accompanied by ear discharge that was used for culture and isolation of mucosal immunoglobulin.

٥-١-١: Retrospective study:

In retrospective study (Table, ٤-٢) the *Ps. aeruginosa* was the majority dominant microorganism in case of BCED and contribute in ٣٦٪ of incidence, the other two dominant bacteria were Proteus species gains ٢٠٪ of occurrence and the *S.aureus* was equal to ١٩٪.

٥-١-٢: Prospective study:

The present prospective results, showed the same observations that *Ps. aeruginosa* was the dominant bacteria ٤٦٪ followed by *P.mirabilis* ١٩٪ and *S. aureus* ١٧٪. In case of

BCED (table, ٤-٣), similar findings have been documented about the profile of dominant bacteria in chronic discharging ear by other workers (Johnson, et al.; ١٩٨٦, Karma et al. ١٩٧٨).

٥-٢: Immune function parameters of CED.

٥-٢-A: Humoral (mucosal and systemic) immunity:

The mucosal surface is protected specifically by secretory IgA (SIgA) and to lesser extent IgM through external translocation of locally produced dimeric IgA and pentameric IgM. Their active transport is mediated by the epithelial polymeric receptor (pigR) also called transmembrane secretory component. Paracellular passive external transfer of systemic and locally produced antibodies also provide mucosal protection, making the biological importance of secretory immunity difficult to assess (Johansen *et al.* ١٩٩٩;and Roe, *et al.* ١٩٩٩).

Induction of a secretory immune response is often associated with elevation of corresponding serum IgG and IgA (in human mainly monomeric) (Brandtzaege, *et al.* ١٩٩٩). These antibodies can reach external secretion by passive paracellular diffusion and may thus contribute to immune exclusions (Johansen, *et al.* ١٩٩٩).

The enlisted data (table, ٤-٤) indicate increasing of titer of antibodies in systemic and mucosal for each of bacterial agent in the two cases whole cell antigen and for LPS separated from gram negative bacteria. When whole cell antigens were

used for determination of antibody titers indicate in *Ps. aeruginosa* was ٣٢٠ and in *P.mirabilis* was ١٦٠ and for *S.aureus* was ٣٢٠. Using LPS separated and purified, the titer of antibodies also increased in patients with BCED to reach ٣٢٠ in *Ps. aeruginosa* and to ٣٢٠ in *P.mirabilis* titers which were more than the base titers in normal one.

At the mucosal humoral response the titer measured was ١٦ in *Ps*. *aeruginosa* and ٣٢ for each of P. *mirabilis* and of *S. aureus* when whole cell antigen were used. The titers scored high values when LPS used in titration of antibodies present in ear discharge, it was ٦٤ in *Ps. aeruginosa* and ٣٢ in case of *P.mirabilis.*

Although over ٩٠٪ of all human infection begins at mucosal sites the total number of infections occurring in population is relatively low. This is in part the result of secretion of a complex array of local and systematically produced immunoglobulin (Bouvets and Fischett, ١٩٩٩).

The importance of the mucosal immune system was quickly realized after the discovery of that SIgA was the most abundant isotype of antibody in secretions as evidence by both Ig levels and presence of Ig-producing cells (Brandtzaeg and Farsted, ١٩٩٩).

In addition to response to inflammation and transient increase in mucosal permeability serum IgG can also translocate toward the lumen by a physiological mechanism associated

with the normal catabolism of Igs (Persson, *et al.* ١٩٩٥), the possibility of locally synthesized IgG different from its serum counterpart has been suggested by the observed higher specific activity of local IgG during intestinal infection (MacPherson, *et al* ١٩٩٦; Gordby, *et al* ٢٠٠٣).

٥-٢-B: Cell-mediated immunity:

The following parameters were applied:

٥-٢-B-١.NBT: Non-specific cellular immunity by measuring the phagocytic activity through NBT test. Nitroblue tetrazolium is an electron acceptor used to detect indirectly the production of superoxide by stimulated polymorphonuclear cells (PMN) as outlined in the following equation:

$$NBT + O_2 \text{ ———— } Formazan + O_2$$

By this reduction, yellow, soluble NBT is converted to blue-black formazan an insoluble material that precipitates intracellularly. The formazan can be seen microscopically, thereby providing superoxide.

The NBT slide test provides an easy method to screen PMNs for the capacity to undergo oxidative metabolism. PMNs that fail to reduce NBT including those with chronic granulomatous disease (CGD) (no oxidase activity), glucose-٦-phosphate deficiency (NADPH store rapidly depleted) and those PMNs with specific receptor defects for the stimulus

employed (Julia, ***etal.*** ١٩٨٦).

Although the failure of PMNs to reduce NBT clearly implies that there is a defect in super oxide generation, other study has suggested that the ability to reduce NBT does not exclude defective oxidative metabolism (Lew, ***etal.*** ١٩٨١).

Phagocytosis has been recognized as an important mechanism of host antimicrobial defense and serum factors (opsonins) play a cooperative role in promoting phagocytosis of pathogenic bacteria and other organism (Root and Cohen, ١٩٨١).

Inflammatory cells constitute an essential basis for the defense mechanism against microbial infections, of these polymorphnuclear leukocyte and large monocytes/ macrophage (MP) are considered to be nonspecific cells that phagocytose bacteria , whereas lymphocyte (LC) are specific inflammatory cells. In middle ear effusions all these cells have been documented (Bryan and Bryan, ١٩٧٦; and Lim, *et* al. ١٩٧٩). PMN and MP have some intrinsic ability to phagocytose bacteria by enveloping them within the cytoplasm. The phagocytotic process is potentiate, however, if the microorganism are subsequently opsonized, i.e, if the microbes are coated with immunoglobulin, in particular IgG (Rayfield and challacombe, ١٩٨٧).

The opsonization is further enhanced if complement cleavage product C٣b is added to the bacteria-antibody complex (Bernstein, ١٩٧٨).

Stenfors and Raisanen, ١٩٩٢ findings suggest that the mucosal defense system of middle ear in the early course of acute otitis media is highly non-specific. However, the finding is consistent with the circumstance that the middle ear mucosa is poor immunocompetent tissue and devoid of aggregates of immunological tissue, such as adenoid tissue of the nasopharynx, palatine, tonsils or the peyer patches of the gut(Albiin, *et al* ١٩٨٦).

The antimicrobial property of the inflammatory cell was not so much in the form of phagocytosis as by means of other antimicrobial modalities, viz, myeloperoxidase, cathepsin G, lysozyme, lactoferrin, elastases or collagenases possibly secreted from the intracellular granules of the phagocytes (Lehrer and Ganz, ١٩٩٠).

There was significant increase in nitroblue tetrazolium reduction (Table, ٤-٥) by neutrophil in peripheral blood (systemic) and in local (mucosal) system, as compared with control ٢٤.٢٥±١.١١ .

Middle ear effusion obtained from discharging ear, culture-positive for **S. *aureus,*** showed an intense phagocytosis in addition to subsequent immunoglobulin and complement coating of microorganisms (Stenfors and Raisanen, ١٩٩٢). These observations were clearly seen with highly significant increase (P<٠.٠٥) in phagocytic activity in mucosal system of middle ear.

Although *Ps .aeruginosa* could destroy the inflammatory cells completely due to the production of a large number of extracellular products, such as alkaline proteases, elasteases, exotoxin A, which can cleave both IgG and complement and inhibite the function of the cells of the immune system(Kharazmi, ١٩٩١). It shows an increase in phagocytic activity in comparison with control at the systemic and mucosal responses. In all bacteria tested (Table ٤-٥) for phagocytic activity showed a significant increase in this phenomenon at the mucosal more than that at the systemic response and in highly significant increase P<٠.٠٥ compared with control.

*P***.** *mirabilis* induce only phagocytic activity at the mucosal system but not at the peripheral blood. The P value was less than ٠.٠٥ (P<٠.٠٥), but at the systemic or peripheral blood there was no significant increase in the number of leukocyte performing phagocytic activity P>٠.٠٥(P=٠.٠٨٩).

٥-٢-B-٢. E-rosette test

The embryonic stem cells are originated in the embryonic yolk sac. During embryogenesis they are conveyed to embryonic liver then to embryonic bone marrow. Thereby they are developed to myeloid, lymphoid and erythroid series precursors. From lymphoid series precursors pro B and pro T cells are differentiated. Pro T cells migrate to thymus and developed to mature T cells by thymic hormones and thymic

factors via positive and negative selection procedures. T-cells, have several surface markers that display several biological functions among which CD٢ which acts as receptors for heterologus animal erythrocyte such as sheep erythrocyte (Binns, et al. ١٩٨٨; Hammerberg and Shuring, ١٩٨٦; and Roitts, ١٩٩٧). Such phenomenon is important as differentiation criteria for T-cells.

Binding of the cell receptor to the MHC/peptide antigen is not, by itself, sufficient to activate T-cells efficiently. T-cell needs simultaneously to receive signals via other cell surface receptors which bind legands on APC. An example of such co-stimulatory interaction is that between CD٢ on the T-cell and LFA(CD٥٨) on APC. It has important implications for the induction of tolerance, absence of right co-stimulation T-cell unresponsive to future encounters with antigen (Playfair and Chain, ٢٠٠١). Rosette formation were increase in certain disease condition such as Hodgkin disease which increases in spleen and depleted at the peripheral blood (Gupta and Tan, ١٩٨٠). It also increases in case of Rheumatoid arthritis (Utsinger, ١٩٧٥).

In case of BCED it was found that E-rosette formation value increase in peripheral blood and middle ear mucosa in patients with *S. aureus* and *Ps. aeruginosa and P. mirabilis.* The mean value at the mucosal system were ٤١, ٤٦.١, ٣١.٧ respectively (Table,٤-٧), while at the peripheral blood, the two

dominant bacteria *S. aureus and Ps. aeruginosa* showed significant increase which was higher than normal subjects ٢٦. *P. mirabilis* did not increase T-cell rosette formation in peripheral blood compared with control.

٥-٢-B-٣. LIF test.

A cell-mediated response is characterized by a sequence of reactions triggered by T-lymphocytes coming into site where antigen is present. The cell becomes activated by interaction with the antigen through the presence of specific cell receptors (sensitization). Following subsequent contact with antigen (challenge), the cell produce and release a variety of effectors molecules called lymphokines. These are biochemical mediators of a number of widely studied in vitro phenomena , but it is believed that similar activity is responsible for the immune response seen in tissues. About thirteen invitro activities have been identified as lymphokine-mediated, but the best characterized lymphokine is LIF the subject of present assay (Table, ٤-٦). Upon release from lymphocytes, LIF can be identified by this ability to trap macrophages and inhibited their migration. The macrophage is the target cell responding to the product LIF of lymphocyte which is the effecter cell.

*LIF is a secreted protein that activate macrophage, neutrophil and T-cell. These cytokines elicited biological responses by

activating specific cell surface receptors.

*LIF has along association with delayed-type hypersensitivity reactions (Bernhagen, *et al.* ١٩٩٦), while LIF shares many similarities with cytokines; some dissimilarities have also been noted.

*LIF is performed in cells and lacks signal sequences in contrast to most cytokines which are produced in response to stimuli and secreted using the signal sequence pathway, LIF was also different from cytokines in that it catalyzed chemical reaction (Rosengren, *et al.* ١٩٩٦). ,

*LIF was firstly described as a factor produced by T-lymphocyte that was associated with the migration of macrophages during delayed-type hypersensitivity responses (Bloom and Bennett, ١٩٦٦; David, ١٩٦٦). More recent findings have identified the mononuclear phagocyte system as well as anterior pituitary glands as a major source of LIF(Bucala,١٩٩٦).

Bacher, *et al.* (١٩٩٦) found that anti-LIF antibody inhibite proliferation of T-cells, yet LIF does not appear to be a T-cell mitogen. One explanation for these results is that LIF biological activity requires co-factors. Antibodies to LIF may therefore inhibite biological activity but to induce biological activity the cofactor and LIF are required. The suggestion that an enzymatic activity underlies LIF biological activity indicates that the essential cofactor may be a substrate for chemical reaction.

The data presented in Table (٤-٦) indicate that *S. aureus* and *Ps. aeruginosa* induce specific cellular immunity when culture filtrates were used as a sensitizer. The mean values were ٥١ and ٦٣ respectively at the systemic (peripheral blood of patients). The LIF of mucosal system was induced by CFCF of these two organisms The value of inhibition of migration were more than that at the systemic. The mean value at the mucosal were ٤٩ and ٤١% in *S. aureus* and *Ps. aeruginosa* respectively when compared with the normal value (٩٣%). There is a significant increase in inhibition of migration P<٠.٠٥ when compared the mucosal and systemic LIF value with control. But in case of *P. mirabilis* the LIF value indicate that this microorganism did not induce cellular immune response at the peripheral blood and mucosal system when cell free culture filtrate was used as a sensitizer. This indicates that culture filtrate antigens did not induce lymphocyte for production of lymphokines. The mean value were ٨٢ and ٨٧% at the mucosal and systemic P>٠.٠٥.

The mucosal system as in other two previously mentioned organisms, *S. aureus* and *Ps. aeruginosa* induce LIF more than that at the systemic due to sensitization of lymphocyte by culture filtrate. The same results were found by previous study on systemic and mucosal system in case of urinary tract infection by AL-Amide ٢٠٠٣, which found that *proteus* species isolated from urinary tract infection did not induce cellular immune response at the systemic and mucosal response of urinary tract. The present study also showed that

P. mirabilis did not induce local and systemic immune response when culture filtrates were used as a sensitizer. Other studies on proteus species also indicate that cellular immunity was not induced by these organisms antigens (Brooks *et al*,١٩٩٨; Heimer and Mobley, ١٩٩٨).

٥-٣: Immunology of *S. aureus*

There was increase in total serum protein, total serum immunoglobulin, total ear mucosal immunoglobulin normal or reduced serum albumin in staphylococcal chronic ear discharge patients (Table ٤-٨) in comparison with normal subjects. This may be a direct and or an indirect consequence of *S. aureus* ear affection (Wooten ١٩٨٢). The *S. aureus* specific antisomatic antigen agglutinins titers were found to rise during the infection processes both at ear mucosal and systemic antibody responses in comparison with that of normal subjects. This may indicate the presence of *S. aureus* antigenic epitope(s) either of direct B-lymphocytic activation potential or an epitope that activates the helper T-cell (Th٢) that activate B-lymphocyte to proliferate, expand, synthesis of specific *S.aureus* specific antibodies (Zubler ١٩٩٨; Roitt, *et al.* ٢٠٠١). Meanwhile the local mucosal epitope stimulation lead to both mucosal and systemic antibody responses (Johansen, *et al.* ١٩٩٩). The phagocytic activity was measured by NBT both for ear mucosal and peripheral blood phagocytes which were found as ٣٥.١ and ٤٠.٨ for mucosal and systemic respectively in patient in comparison with ٢٤.٢٥ in normal subjects .Such increase was mediated by

S.aureus ear infection, since it has been suggested that phagocytosis of bacteria during otitis media and it is highly species specific mechanism and that both natural and adapted immune mechanisms are taking part in protection against middle ear infections (Stenfors and Raisanin, ١٩٩٢ and Nibbering, *et al* ١٩٩٦).

The cell free culture filtrate of *S. aureus* as a sensitizer induce secretion of leukocyte inhibitory factor LIF from the already sensitized lymphoid cells in peripheral blood and in the inflammatory cells of ear discharge. This LIF cytokines were found as significant inhibition in the leukocyte migration (٥١, ٤١%) in comparison to LIF of normal subjects (٩٣%). It also indicates the involvement of delayed type hypersensitivity reactions (Bernhagen, *et al.* ١٩٩٦).

T-lymphocytes of human have several types of surface markers or recognition molecules (Cuby, ١٩٩٧; and Roitt, ٢٠٠١). Among which, we can identify CD٢ which is responsible for E-rosett formation with xenogenic erythrocytes such as sheep erythrocytes (Binns, *et al.* ١٩٨٨). E-rosett formation increased in both peripheral blood and ear discharge leukocyte and more in patients than in those of normal subjects. Such increase was statistically significant at P<٠.٠٥ level. It was found higher at mucosal than of systemic E-rosett formation. Such findings were supported by the studies of other people working on pleural effusion, meningitis (Playfair and Chain, ٢٠٠١).

٥-٤: Immunology of *Ps. aeruginosa* CED

The Ps. *aeruginosa* ear infection led to chronic ear discharge, and it was associated with high total serum protein, high total serum immunoglobulin, and high ear mucosai immunoglobulin in comparison with normal control subjects (Table ٤-١١). This could be a direct or an indirect squale of the ear infection (Braudae, ١٩٨٢; and AI-Amedi, ٢٠٠٣).

Monoclonal antibodies specific to the virulence associated antigens show that there are ٢٠ LPS serotype, ١٤ pilin serotype and five flagellin servars. On the other hand, the outer membrane proteins Oprf, OprL and OprI and LPS rough core, lipid A and O-antigens have only one or two antigenic variant types (Hancock and Lam, ١٩٩٨).

Outer membrane protein (OMP), peptidoglycan-associated lipoprotein, Braun liprotein, lipopolysacchride core, flagella and the exotoxin A are immunogens of Ps. *aeruginosa* that stimulate strong antibody responses (Hancock and Lam, ١٩٩٨). In this case, ear discharge *Ps. aeruginosa* somatic antigen and LPS separately detects rather high mucosa and serum antibodies and higher in serum in comparison to mucosa. Meantime pure LPS detect higher antibodies than somatic ٠-antigen preparations (Table, ٤-٩) antitoxin to exotoxin A is detectable in some human who were recovered from serious *Ps. aeruginosa* infection (Brooks, *et al* ٢٠٠٤).

T-cell responses constitute an important component of host defense against *Ps. aeruginosa* (Stevenson, *et al.* ١٩٩٦) *Ps. aeruginosa,* during infection may modulate T-cell and macrophage functions. The quorum sensing signal ٣-OXO-

C١٢-HSL potently down regulates IL١٢ production a T helper supportive cytokine and such signal also inhibits lymphocyte proliferation of LPS stimulated macrophage (Telford, *et al.* ١٩٩٨; Williams, *et al.* ٢٠٠٠). Thus *Ps.aeruginosa* antigenic epitopes can be T-cell dependent type (Zubler, ١٩٩٨) with immunomodulating potentials. In accordance with these results, significant LIF and increase of E-rosett forming T-cell percentage were noted in CED patient with *Ps. aeruginosa* This is also in agreement with *Ps.aeruginosa* urinary tract infection (AI-Amedi, ٢٠٠٣).

Therefore, the immunodominant epitope (Zubler, ١٩٩٨) of *Ps.aeruginosa* may be of T dependent type with potential activation for both Th١ and Th٢. Possibly switch on from Th٢ antibacterial to Th١ cellular response can happen (Williams,٢٠٠٠).

٥-٥: Immunology of *P. mirabilis* CED

Motile strain of Proteus contains H antigen in addition to somatic O-antigen. Certain strains share specific polysaccharide with Rickettsial antigens (Weil-Felix test). Proteus specific antibodies develop in systemic infections but it is uncertain whether significant in immunity and infection follow (Brooks, *et al.* ١٩٩٨, Brooks, *et al.* ٢٠٠٤).

Serum from active rheumatoid arthritis (RA) reacts with *P. mirabilis* antigens. *P. mirabilis* secretes an immunoglobulin A degrading protease. *P. mirabilis* haemolysins contains hexameric peptide with immunogenic properties. The role of

such hexameric peptide in stimulation of autoimmune responses in rheumatoid arthritis remains to be explored.

It was found that IM injection of purified outer membrane protein of *P mirabilis* can protect against experimental challenge with the infectious dose to mice.

Vaccination of mice with purified fimbriae omp as well as LPS preparation gives partial immunity following the administration in mice (Heimer and Mobley ١٩٩٨). Thus with the formation of serum antibodies in systemic infection, OMP vaccine protects against experimental infection in mice and the possible role of the haemolysin hexameric peptide in rheumatoid arthritis autoimmune responses give an indication of involvement of humoral immunity in *Proteus* infection (Brooks *et al.* ١٩٩٨, Brooks *et al.* ٢٠٠٤, Heimer and Mobley ١٩٩٨). Mean time *Proteus* possesses an immune escape mechanism through IgA proteas splitting enzymes. No evidence so far is known about the involvement of cell mediated immunity in Proteus infection.

P. mirabilis chronic ear discharge induce rise up of total serum protein, total serum globulin, mucosal globulin, phagocytic activity, non significant LIF with CFCF (table٤-١٠) and significant with LPS pure preparation as well as increase of E-rosette forming T cells at mucosal but not peripheral blood(table ٤-١٢). Generally speaking all these are sequale of *P. mirabilis* infection of middle ear causing chronic ear discharge . In the immunological sense ; however CFCF

contains either T independent antigenic epitope or Th٢ lymphocyte activating epitopes. Pure LPS can contain epitopes that activate both Th٢ and Th١ cells. Th١ activating epitope appeared to be missing from CFCF antigens (AI-Amedi ,٢٠٠٣). Thus LPS exhibit significant LIF results and with CFCF showed non significant LIF result.

Hence*, P. mirabilis* CED provide rather new prospect to the cellular immune reactions in *Proteus* clinical infection, a finding that has never been mentioned by other workers In the immunology of Proteus infections and especially those of Proteus CED patients.

The major immune features of Proteus infection are

١-Induces both mucosal and systemic specific antibodies

٢-increase NBT phagocytic activity at mucosal system

٣-CFCF triggers non significant LIF at the mucosal and systemic (AI-Amedi ,٢٠٠٣)

٤- Pure LPS trigger significant LIF at the mucosal and systemic

٥-Increase T-Erosett formingcells at the mucosal system (William et al. ,٢٠٠٠, Sousa,١٩٨٠)

REFERENCES

- AL-Amedi, B. H. (٢٠٠٣). Comparative study of mucosal and systemic immune response for the pyuria patients. Ph.D. thesis Babylon University, College of Science.
- Albiin, W, Hellstrom, S, Stenford, L.E. (١٩٨٦). The mucosa in rats and humans. Ann. Otol RhinolLaryngol. ٩٥(suppl. ١٢٦)٢-١٥.
- Allan, C. H.; Mendrick, D. L.; Trier, J. S. (١٩٩٣). Rat intestinal M-cells contain acidic endosomal lysosomal compartments and express class II Major Histocompatibility complex determinants. Gastroenterology. ١٠٤: ٦٩٨-٧٠٨.
- Al-Mola, G., Jassim, T.M., Abhami, S.(١٩٩٨).Bacteriological study of otitis media among children in Saddam General Hospital in Tikrit City.The Medical Tikrit University. ٥:٢٢٩-٢٣٤.
- Al- Snafi, A.E., Al-Saadi, A.A., Al-Samarrai, G.A.(١٩٩٩). Bacterial etiology of acute and chronic suppurative otitis media. The medical J. Tikrit University. ٥:٢٢٩-٢٣٤.
- AL-Tallah, M.S. (٢٠٠٠). Microbiology of chronic suppurative otitis media with cholesteatoma. Saudia Medical Journal. ٢١(١٠);٩٢٤-٩٢٧. ٥٩٩ -٦٠٦
- Al-Toraihi, T. S. (٢٠٠١). Bacteriological study of acute otitis media. M.Sc. thesis university of Kufa.
- Apodaca, G., Katz, L.A., and Mostove, K.E.,(١٩٩٤). Receptor-mediated transcytosis of IgA in MDCK cell, via apical

recycling endosomes. J. Cell Biol. ١٢٥, ٦٧.

- Aubaid, Q.N. (١٩٩٨). Aerobic bacteria associated with chromic ear discharge. J. Babylon University. ٣(٣): ٢٨١-٢٨٤
- Bacher, M.C.; Met, Z.; Calandra, T.; Mayer, K. (١٩٩٦). An essential role for MIF is a pituitary derived cytokine that potentates lethal endotoximia Nature ٣٦٥:٧٥٦-٧٥٩.
- Banker, D. (١٩٨٠). Modern practice in immunization programes .Jyepe Brothers India.
- Berman, S.; Byrns, PJ. ; Bony, J.; Smith, PJ. ; Lezotte, D. (١٩٩٧). Otitis Media-related, prescribing patterns, outcomes and expenditures in Pediatric Medicaid. Pediatrics. ٤ Oct. ١٠٠(٤): S٨٥-٥٨٥.
- Bern Hagen, J.; Bacher, M.; Calundra, T. (١٩٩٦). An essential role for macrophage MIF in the tuberculin DTH reaction J. Exp. Med. ١٨٣, ٢٧٧-٢٨٢.
- Bernstein. J.M. ١٩٧٨. Complement activity in ME effusions. Clin EXP. Immunol. ٣٣:٣٤٠-٣٤٦.
- Bernstein, J. M. (٢٠٠١). Immunological aspects of otitis media (Report). Current Allergy and Asthma Reports ٢:٣٠٩-٣١٥.
- Bernstein, J.M.; Reddy, M. S.; Scannapieco, F.A.;Faden, H. S.; and Ballow, M. (١٩٩٧).The microbial ecology of the adenoid: implications for otitis media Annals of the New York academy of science, Vol ٨٠٣, Issue ١ ١٩-٣١ copyright ١٩٩٧ by New York Academy of sciences.
- Beynon, L.M.; Dumanski, D.J.; Mclean, R.J.C.; Maclean, L.L;

and Perry, M.B. (١٩٩٢). Capsule structure of Proteus mirabilis (Atcc ٤٩٥٦٥) J. Bact. ١٧٤: ٢١٧٢-٢١٧٧.

- Binns, R. M.; Pabst, R.; Licence S.T. (١٩٨٨) subpopulation of T- lymphocytes emigrating in venous blood draining pig thymus labeled in vivo with fluorochrome. Immunol. ٦٣(٢): ٢٦١-٢٦٧.
- Bishops, M.C., Deon-Vanlaufer, I.I.; Fodys, E.P.; and thirty three contributors (١٩٨٥) In: Clinical Chemistry Principles, Procedure And Correlation, the Murvay printing company, Philadelphia.
- Blackwell J.M. (١٩٩٦). Structure and function of the natural resistance associated macrophage (Nramp١) a candiddate protein for infectious disease susceptibility . Molecular Medicine Today ٢:٢٠٥.
- Block, S.L. (١٩٩٧). Causative pathogenesis , Antibiotic resistances, and therapeutic consideration in acute otitis media. Pediatr infect.Dis. J. ١٦: ٤٤٩-٥٦.
- Bloom, B.R. and Bennett, B. (١٩٦٦). Mechanism of reaction in vitro associated with DTH, Science ١٥٣, ٨٠-٨٢ [Medline].
- Bloom, B. and Zinkenagel, R. (Eds.) (١٩٩٦). Immunity to infection. Current opinion in Immunology ٨; ٤٦٥.
- Bloom, B.R. and Bennett, B. (١٩٦٦). Science. ١٥٣: ٨٠-٨٢[Medline].
- Blueston, C.D. (١٩٩٨).Epidemiological and pathogenosis of chronic suppurative otitis media : implication for prevention

and treatment . Int. J. Pediatr otorhinolaryngol ٤٢: ٢٠٧-٢٤٧.

• Blueston, C.D. and KIien, J.O. (١٩٨٣). Otitis media witheffusion, Atelectasis, and ET Dysfunction pediatric otolaryngology. Blueston and stool. ٣٥٦-٥١٢.

•Bohuslav, J. ; Vladimir, V. ; Kravchenko ; Jonathan, H. ;Gerondakis, S.; Ulevitch, R. (١٩٩٨). Regulation of an essential innate immune response by the Pso subunit of NFKB. J. Clin. Invest. ١٠٢(٩): ١٦٤٥-١٦٥٢.

•Bone, R.; Balk, R.; Fein, A. Peri, T.; and Schein, R. (١٩٩٢). A second large controlled clinical study of E٥, a monoclonal antibody to endotoxin: result of prospective multi center, randomized, control trial. Crit Care Med. ٢٣(٦):٩٩٤-١٠٠٦[c].

•Bone, R.; Balk, R.; Cerra, F.; Fein, A.; and Sibbald, W. (١٩٩٥). Definition for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Chest ١٩٤٤-٥٦. [b].

• Bouvet, J. P.; and Fischetti, V.A. (١٩٩٩) .Diversity of antibody mediated Immunity at the mucosal barriers. Infect .Immune. June ٢٦٨٧-٢٦٩١ Vol. ٦٧ No. ٦

• Boyle, M. ٠, P. (١٩٩٠) .In bacterial immunoglobuline-binding protein. (Ed.) Boyle, M.P.D (Academic San Diego) Vol. I pp: ١٧-٢٨.

•Brandtzaeg, P. (٢٠٠٢). Current understanding of gastrointestinal immuno-regulation and its relation to food allergy. Annals of the New York Academy of sciences. ٩٦٤: ١٣-٤٥ ٢٠٠٢ New York Academy of science.

- Brandtzaeg, P., (١٩٩٦). History of oral tolerance and mucosal immunity. Ann. N.Y. Acad. Sci ٧٧٨:١-٢٧.
- Brandtzaeg, P. (١٩٩٥). Molecular and cellular aspects of the secretory Immunoglobulin system Acta Pathol. Microbiol. Scand. ١٠٣: ١-١٩.
- Brandtzaeg, P. and Farsted. I. N. (١٩٩٩). The human mucosal B-cell system, P. ٤٣٩ - ٤٦٨. In Ogra, P.L; Mestecky, J.; Lamm, M.E.; Strober, W.; McGhee, J.R.; and Bienenstock, J. (ed)Handbook of Mucosal Immunology. Academic press. Inc. SanDiego, Calif.
- Brandtzaege, P.; Farsted, I.N.; Johansen, F.E.; Morton, H. C,; Norderhang, I.N.; and T. Yamanaka, (١٩٩٩). The B-cell system of human mucosa and exocrine glands. Immunol. Rev.
- Brandtzaeg, P., and Prydz, H. (١٩٨٤). Direct evidence for an integrated function of J-chain and secretory component in epithelial transport of immunoglobulin. Nature, ٣١١:٦١-٧٣.
- Braudae I. I. (١٩٨٢). Microbiology .W.B. Saunders company, London.
- Brooks, G.F; Butel, J.S.; and Morse, S.A. (٢٠٠٤).*Pseudomonas* in Jawetz, Milnike, Adelbergs Medical Microbiology ٢٣rd ed. McGrawtill, London.
- Brooks, G.F.; Janel, S.; Stephen, A.; (١٩٩٨). Staphylococcus in Medical Microbiology ٢١ edition, pp: ١٩٧-٢٠٢.
- Bryan, M. P.; and Bryan, W. T. K. (١٩٧٦). Cytological and

immunological response revealed in ME effusions Ann. OtolRhinol. Larynogol. (Suppl.٢٥) ٨٥: ٢٣٨-٢٤٤.

•Bucala, R.(١٩٩٦). MIF rediscovered:Cytokine, pituitary hormone and glucocorticoid- induced regulator of the immune response. FASEB j. ١٠:١٦٠٧-١٦١٣.

• Burrell, R. (١٩٧٩). Experimental immunology "٥th ed". Burgess publishing company, Minnesota, ٢٤-٢٥.

• Bylander-Groth, A. and Stenstrom, C. (١٩٩٨). Eustachian tube function and otitis media in children. Ear, nose throat J. ٧٧:٧٦٢ -٧٦٤; ٧٦٦,٧٦٨ -٧٦٩.

•Cellini, L.; Piccolomini, R.; Allocati, N.; and Ravagnan, G. (١٩٨٧). Adhesive properties of Proteus genus related to antimicrobial agents resistance. Microbiologica. ١٠: ٢٩١-٢٩٩.

•Chen, Y.H.U.; Haucock, R. E. W.; and Meshill, R. I. (١٩٨٠). Mitogenic effect of purified outer membrane, from Pseudomonas aeruginosa Infect Immune ٢٧:١٧٨-١٨٤.

•Chisari, F. V. (١٩٩٧). Cytotoxic T cells and viral hepatitis. J. Clin. Invest. ٩٩:١٤٧٢-٧.

•Chow, A. W.; Taylor, P.R.; Yoshikawa, T.T.; and Guzc, L.B. (١٩٧٩). A chromosomal out break of infection due to multiply resistant P. mirabilis. role of intestinal colonization as a major reservoir. J. Infect. Dis. ١٣٠: ٦٢١-٦٢٧

•Clegg, S.; and Geriach, G.F. (١٩٨٧). Enterobacterial fimbriae. J. Bacterial. ١٦٩:٩٣٤-٩٣٨.

•Cooper, K, E.; Davis, J.; and Wieseman, J. (١٩٧١). An investigation of an out break of food poisoning associated of to Proteus group. J. Pathol. Bacterial ٥٢:٩١-٩٨.

•Cruckshank, R., Daguid, J.P.; Marmion, B.P. and Swain, R.H.A. (١٩٧٥). Medical microbiology vol. ٢(١٢ th ed) P:٤٥٩. The English language bock society London.

•Cryz, S.J.; Jr, Pit, T.L; .Furer, E. (١٩٨٤). Role of LPS in virulence of Ps. aeruginosa. Infect. Immune. ٤٤: ٥٠٨-٥١٣ .

Cuby, J. (١٩٩٧). Immunology "٣rd ed" cells and organs of Immun system. New York: WH Freeman and Co. ٤٧-٨٣.

•Daly, K.A., Hunter, L-L, Giebink, G.S., (١٩٩٩). Chronic otitis media with effusion. Pediatrics in Review. March ٢٠(٣):٨٥-٩٤.

•Das gupta, T.; de Kievit T.R.; Masoud H.; Altman, E.;and lam. J. S. (١٩٩٤). Characterization of LPS-deficient mutants of Ps, aeruginosa derived from serotypes ٠٣, ٠٥ and ٠٦. Infect Immune. ٦٢:٨٠٩-٨١٧

• David, R. (١٩٦٦). Proc. Natl. Acad. Sci USA ٥٦,٧٢-٧٧.

•Deitch, E. (١٩٩٠). Bacterial translocation of the gut flora. J. Trauma. ٣٠(١٢):S١٨٤-S١٨٩

•Dulley, M. N. (١٩٩٠). Over view of gram -negative sepsis. Am. J. Hosp. Pharm. ٤٧:S٣ -S٦ .

• El-Gamal, S. Rabia, S., Ahmed, R.,(١٩٩٣). Viral and bacterial infection among children with acute otitis media. The new Egyptian J. Med.٨(٥): ١٢٩٣- ١٢٩٥.

•Engel's, W.; Endert, J.; Kemps, M.A.F.; and Van Boven, C.P.A. (١٩٨٥). Role of LPS in opsonization and phagocytosis of Ps. aeruginosa. Infect. Immun. ٤٩: ١٨٢-١٨٩

•Faden, H. (٢٠٠١). The microbiological and immunologic basis for recurrent otitis media In children. Eur .J. Pediatr. ١٦٠(٧): ٤٠٧-١٣.

•Fehniger , T. A. ; Herbein , G. ; Yu , H. ; Para, M. I. ; Bernestein, Z. P. O. Brien, W. A., (١٩٩٨). Natural Killer cells from HIV-١+ patient produce c-c chemokines and inhibit H IV infection. J. Immunol. ١٦١:٦٤٣٣-٨

•Fick, R.B. (١٩٨٩). Pathogenesis of Pseudomonas lung lesion in cystic fibrosis. Chest ٩٦:١٥٦-١٦٤.

•Frederik, W.; Ginkel, Van. ; Huan, H.; Guyen, N.; and Jerry R. McGhee. (٢٠٠٠). Vaccines for mucosal immunity to combat emerging infections disease. J. National center for infections disease. Center for disease control and prevention Vol. ٦. No. ٢.

•Gabidullin, Z.G. ; and Ishkildin, I.B.(١٩٨٩). Morphological properties and adhesiveness of bacteria of the genus bacteria of the genus Proteus. J. Microbiol. Epidemiol Immunol. ٦: ٨٣-٨٦.

• Gardby, E.; Wrammert, J.; Schon, K. (٢٠٠٣). Strong Differential regulation of serum and mucosal IgA responses revealed in CD-٢٨ Deficient mice using cholera toxin adjuvant. Immunol. ١٧٠: ٥٥-٦٣.

•Garrey, J.S., Gemer, N.E. and Sussdrof, D.H.(١٩٧٧). Method in immunology. (٣rd ed.)P ٥١٧-٥٣٤.Addison –Wosley publishing company Inc. Masachusetts.

•Garzelli, C.; Collizi, V.; Campa, M.; Bozzi, L.; and G. falcon (١٩٧٩). Infec. Immune. ٢٤: ٣٢-٣٨

• Gates, GA. (١٩٩٦). Cost-effectiveness in otitis media treatment Otolaryn. Head and Neck Sur. April ١١٤(٤):٥٢٥-٥٣٠.

•Glauser, M., Zanetti, G. and Cohen, J. (١٩٩١).Septic shock pathogenesis. Lancet ٣٣٨:٧٣٢-٧٣٦

• Govan, J.R.W., and Deretic, V. (١٩٩٦). Microbial pathogenesis in cystic fibrosis: mucoid Pseudomonas aemginosa and Burkholdria cepacia. Microbiol. Rev. ٦٠:٥٣٩ -٥٧٤.

• Gupta, S.; and Tan, C.T.C.(١٩٨٠). Subpopulation of human Xiv Abnormality of T-cell locomotion and of distribution of subpopulations of T and B lymphocyte in peripheral blood and spleen from children with untreated Hodgkin's disease. Clin .immunol. Immunopath ١٥,١٣٣.

• Hammerberg; C.; and Schurng G.G.(١٩٨٦). Characterization of monoclonal antibodies directed against swine leucocytes. Vet. Immunol. Immunopathol. ١١(٢): ١٠٧-١٢١.

• Hancock, R.E.W. and Lam, J.S. (١٩٩٨). Ps aeruginosa infection and Immunity. In Delves and Roiit, I. (Eds).Encyclopedia of immunology ٢nd academic press London, vol. ٤; ٢٠٤٠-٢٠٤٥.

•Hancock, R.E.W. (١٩٩٨). Resistance mechanism in Ps.

aeruginosa and other non fermentative gram-negative bacteria. Clin. Infec. Dis.١:S٩٣-S٩٩.

- Hancock, R.E.W.; and Speert, D.P. (١٩٨٣). Ps. aeruginosa isolates from patients with cystic fibrosis: A class of serum - sensitive, non typeable strains deficient in LPS-٠ side chains. Infect. Immun. ٤٢:١٧٠-١٧٧ .
- Hatano, K.; Goldberg, J. B.; and Pier, G.B. (١٩٩٥). Biologic activities of antibodies to the neutral-polysaccharides component of Ps. aeruginosa LPS are blocked by ٠ side chains and mucoid exopolysaccharide (alginate). Infect. Immun ٦٣: ٢١-٢٦.
- Hathaway, L.J.; and Kraehenbuhl, J. P. (٢٠٠٠). The role of M-cell in mucosal immunity. Cell Mol. Life Sci. ٥٧: ٣٢٣-٣٣٢.
- Hauck,C.R. and Meyer, T.F. (١٩٩٧). The lysosomal phagosomal membrane protein h-Lamp-١ is a target of IgA١ protease of N. gonorrhoeae. FEBS Lett. ٤٠٥: ٨٦-٩٠.
- Heikkinen, T., Thint, and Chonmaitree, T. (١٩٩٩). Prevalence of various respiratory viruses in the middle ear during acute otitis media. N. Engl.J. Med. ٣٤٠: ٢٦٠-٢٦٤.
- Heimer, S.P. and Mobley, H.L.T. (١٩٩٨). Proteus infection and immunity in: Encyclopedia of immunology Vol. ٤, (٢nd -Ed.) Delves, P. J. and Roitt, I.M.(eds) P: ٢٠٣٩-٢٠٤٢.
- Hellstrom, S.; Eriksson, P.O.; Yoon, Y.J and Johansson, U. (١٩٩٧). Interaction between the middle ear and the inner ear: bacterial product .Annals of New York Academy of sciences

. Vol ٨٣٠, Issue I: ١١٠-١١٩

• Hirschfeld, M.; Ma, Y.; John, W.; Stefanie, V.; and Janis, W. (٢٠٠٠). Cutting edge: Repurification of lipopolysaccharide Eliminates signaling through both human and murine toll-like receptor٢. J. Immunol. ١٦٥:٦١٨-٦٢٢.

• Hoffman, P.; Bessler, W.G. (١٩٨٨). Tumor cytotoxicity involving TNF induced in bone marrow derived macrophages by tow lipopeptide analogues of bacterial lipoprotein. Immunology. ١٧٨: ٩٤-١٠١.

•Holborow, C. (١٩٧٠). Eustachian tube function. Changes in Anatomy and function with age and the relationship of these changes to aural pathology. Arch, Otolaryngol. ٩٢: ٦٢٤- ٦٢٦.

•Hornick C.L; and Karush, F. (١٩٧٢) Antibody affinity ٣.the role of multivalence. Immunochemistry ٩: ٣٢٥-٣٤٠.

•Insel, R. A.; Alleu, P. Z.; and Berkowitze, ١.٠. (١٩٨٢). Types and frequency of Haemophilus influenzae IgA, proteases. Semin infect, Dis. ٤:٢٢٥-٢٣١.

• Jarvis, W.R.; and Marion, W.J. (١٩٩٢). Predominant pathogens in hospital infection.J. Antimicrob. Chemother٢٩:S١٩-S٢.

•Johansen, F.E.; Pekna, M.; Norderhany, I.N.; Haneberg, B.; Hietala, M.A (١٩٩٩). Absence of epithelial IgA transport with increased mucosal leakiness, in polymeris lgR\SC deficient in mice. J. of Exp . Med.V١٩٠N.٧oct٤

• Johnson, L.; Schwan, A.; Thomander, L; and Fabian, P. (١٩٨٦). Aerobic and anaerobic bacteria in chronic Suppurative otitis

media. Acta Otolaryngol. ١٠٢, ١٤٠-٤١٤.

• Johnston, A. and Thrope (١٩٨٢). Immunochemistry in practice p: ١-٧٢. Blackwell Scientific Publication.

•Joiner, K.A. (١٩٨٥). Studies on the mechanism of bacteria resistance to complement- mediating killing and on the mechanism of action of bactericidal antibody. Curr. Top. Microbiol. Immunol. ١٢١:٩٩-١٣٣.

•Julia, A. M, Gallin, J. I.; Nauseef, W.M. (١٩٨٦), Laboratory Manual of Neutrophil Function. Raven press books ltd. New York.

•Kactzel, C.S.; Robinson, J.K.; Chantal, K.R.; Charuru, J. P.; Vaerman, and Lamm, M.E. (١٩٩١). The polymeric immunoglobulin. Receptor (secretory component) mediates transport of immune complex across epithelial cells a local defense function for IgA. Proc. Nat!. Acad. SCI- USA ٨٨: ٨٧٩٦-٨٨٠٠ .

•Karch, H.; Gmeiner, J.; and Wixdorff, K. (١٩٨٣). Alteration of the immunoglobuline G subclass response in mice to LPS effects of non-bacterial protein and bacterial phospholipids or outer membrane proteins of Proteus mirabilis Infect. Immune ٤٠:١٥٧-١٦٥.

•Karja, J.; Nuutinen, J.; Karjalainen, P. (١٩٨٣). Mucocilliary function in children with secretory otitis media. Acta Otolaryngol. ٩٥: ٥٤٤ -٥٤٦.

•Karma, P.; Jokipii, L; Ojala, K.; and Jokipii, A.M.M. (١٩٧٨).

Bacteriology of the chronically discharging ear. Acta Otolaryngol. ٨٦. ١١٠-١١٤.

• Keay, K.S.; Rezal, C.O.; Trifillis, A.L; Hebel, J.R. , Jacobs, S.C. and Warren, J. W. (١٩٩٧). Urine autantibodies in interstitial cystitis. J. Urol. ١٥٧:١٠٨٣-١٠٨٧.

•Kenneth, T. (٢٠٠٢). Bacteriology, Ps. aeruginosa. p٣٣٠, University of Wisconsin-Madison

• Kerna, M .A. (١٩٩٨). Otitis media with effusion. Head and Neck surgery- otolaryngology ٢nd ed. B.J. Baily. P ١٢٩٧-١٣١٠.

•Kerneis, S.; Bogdauova, A.; Krueheubuhl, J. P.; Pringaut, E . (١٩٩٨). conversion by Peyer's patches lymphocyte of human enterocytes in to M-celi that transport bacteria. Science. ٢٧٧: ٩٤٩-٢٥.

•Kerr, M.A. (١٩٩٠). The structure and function of human IgA. Biochem J. ٢٧: ٢٨٥-٢٩٦.

•Kharazmi, A.G.; Doring, N.; Hgiby and Valerius (١٩٨٤). Interaction of Ps. aeruginosa alkaline protease and elastase with human polymorphnuclear leukocyte in vitro. Infec. Immune. ٤٣: ١٦١-١٦٥

•Kharazmi, A. (١٩٩١). Mechanism involved in the evasion of the host defense by Ps. aeruginosa. Immunol. Lett. ٣٠:٢٠١-٢٠٦.

•Kielhofner, M.; Atmar, R.L; Hamill, R .J.; and Musher, D.M. (١٩٩٢). Ps. aeruginosa infections in patients with human immunodeficiency virus infection. Clin. Infect. Disease ١٤:٤٠٣-٤١١.

•Kilian, M and Russell, M.W. (١٩٩٩). Microbial evasion of IgA functions, p. ٢٤١-٢٥١. In Ogra, P.L, et al. (Ed) mucosal immunology ٢nd ed. Acad. Press Inc. San Diego, Calif.

•Knirel, Y.A.; Kaca, W. A. Rosalski, Z.; Sidorezy, K. (١٩٩٩). Structure of the ٠-antigenic polysaccharides of Proteus bacteria. Pol. J. Chem. ٧٣: ٨٩٥-٩٠٧.

•Knirel, Y.A.; Vinogradov, E. V. ; Shashkov, S. A. ; Sidorezyk, Z. ; Rosalski, A.; Radziesewsk-lebrecht, I. and Kaca, W. (١٩٩٣). Structural study of ٠-specific polysaccharide of Proteus. J. Carbohydrate Chem. ١٢: ٣٧٩-٤١٤.

•Kodama, S.; Suenaga, S.; Hirano, T.; Suzuki, M.; and Mogi, G. (٢٠٠٠). Induction of specific IgA and Th٢ immune response to p٦ outer membrane protein of non-typeable H. influenzae in middle ear mucosa by intranasal immunization. Infect. Immun., April ١. ٦٨(٤): ٢٢٩٤-٢٣٠٠.

• Kuby, J. (١٩٩٧). Immunology ٣rd ed. Cells and Organ of Immun System. New York : WH freeman and Co. ٤٧-٨٣.

•Kulshin, V. A.; Zahringer, U; Lindner, B.; and Rietschel, E.T. (١٩٩١). Structural characterization of the lipid A component of Ps. aeruginosa wild- type and rough mutant LPS. Eur. J. Biochem. ١٩٨:٦٩٧-٧٠٤

•Laharague, P.F.; Corberna, J.X.; Filial, I.G.; Glazes B.J.; Fontaillis A.M.; and Gyrade, E. (١٩٨٤). Infec. Immune ٤٤: ٧٦٠- ٧٦٢

•Lam, M.Y.C.; McGroarty, E.J; Kropinski, A.M.; and Lam, J.S.

(١٩٨٩). Occurrence of common LPS antigen in standard and clinical strains of Ps. aeruginosa. J. Clin. Microbiol. ٢٧:٩٦٢-٩٦٧.

•Lamping, N.; Hoess, A.; Yu, B.; Park, T.C.; Kirschning C.J.; and Schumann R.R. (١٩٩٦). Effect of a site-directed mutagenesis of basic residues [Arg ٩٤,LYS as , LYS ٩٩] of LPS- binding protein on binding and transfer of LPS and subsequent immune cell activation J. Immunol. ١٥٧: ٤٦٥٨-٤٦٥٦.

•Larsson, P. (١٩٨٤). Serology of Proteus mirabilis and Proteus vulgaris Methods, Microbiol. ١٤: ١٨٧-٢١٤.

•Lehman, J.D.; Smith, J.W.; Miller, T.E.; Bornett, J.A-Stanford. J.P. (١٩٦٨). Local immune response in experimental pyelonephritis. J. Clin. Invest. ٤٧:٢٥٤١-٢٢٥٠.

•Lehrer, R. I and Ganz, T. (١٩٩٠). Antimicrobial polypeptides of human neutrophiles. Blood. ٧٦: ٢١٦٩-٢١٨١.

•Lemaire, L; Vanlanschot, J.; Stoutenbeek, C.; Van Deventer, S,; Gouma, D. (١٩٩٧). Bacterial translocation in multiple organ failure. Br. J. Surg. ٨٤:١٣٤٠-٥٠.

.Lew, P.D, SouthWick, F. S.; Stoosel. T.B. (١٩٨١). A variant CGD: deficient oxidative metabolism due to a low affinity NADPH oxidase. N. Engl. J. Med. ٣٠٥: ١٣٢٩-١٣٣٣

•Lim, D.J. (١٩٧٤). Functional morphology of the lining membrane of the middle ear and Eustachian tube an overview. Ann. Otol. Rhinol. laryngol.٨٣:(suppl II) :٥-٢٦

•Lim, D. J. Mogi, G. (١٩٩٤.) Mucosal immunology of middle ear

and Eustachian tube. In Ogra P.L.; Mestecky, J.; Lamen, M. E.; Strober, W.; Bienerstock, J. (eds.) Handbook of mucosal immunology. Santiago. Academic press.

• Lim, D.J., Lewis, D.M.; Proctor, B. (١٩٦٧). Embryology and anatomy of Eustachian tube. Arch. Otolaryngol. ٦٨:٣٠٥-٣١٤

•Lim, D.J., Lewis, D. M.; Schram, J.L.; and Birck, H. G. (١٩٧٩). Otitis media with effusion cytological and microbiological correlates. Arch. Otolaryngol. ١٠٥; ٤٠٤-٤١٢.

•Lin, L.; Ayala, P.; Larson, J.; Mulks, M.; Fuku, M; Carlsson, S.R.; Euns, C.; and So, M.(١٩٩٧). The Neisseria type٢ IgA, protease cleaves LAMPI and promotes survival of bacteria within epithelial cells. Mol. Microbiol. ٢٤: ١٠٨٣-١٠٩٤.

•Liu, P. (١٩٧٩), Toxins of Ps. aeruginosa. ٦٣-٨٨; In Ps. aeruginosa by Doggett

•Liu, P. (١٩٦٦). The role of various fraction of Ps. aeruginosa in it is pathogenesis. J. Infect. Dis ١١٦:٤٨١-٤٨٩.

•Loomes, L.M.; Senior, B-W.; and Kerr, M.A. (١٩٩٠). Aproteolytic enzyme secreted by Proteus mirabilis degrades immunoglobuline of the lgA١, lgA٢, and IgG isotypes. Infect. Immune. ٥٨:١٩٧٩-١٩٨٥.

•Lue, C.A.; Van Den Wall Bake, W.L; Prince, S.J. et al. (١٩٩٤) Intra peritoneal immunization of human subjects with tetanus oxide induces specific antibody- secreting cells in the peritoneal cavity and in the circulation, but fails to elicited secretory IgA response. Clin. Exp. Immunol. ٩٦:٣٥٦-٣٦٣ .

•Lyman, M.; Steward, J.; and Roantree, R. (١٩٧٦). Characterization of the virulence and antigenic structure of Salmonella typhimurium strains with LPS core defect. Infect -Immun .١٣(٦); ١٥٣٩-٤٢.

•Lynn, W.A.; and Golenbock, D.T. (١٩٩٢). LPS antagonists. Immunol. Today ١٣:٢٧١-٢٧٦.

•Macfaddin, J.E.(٢٠٠٠). Individual biochemical test . In Biochemical test for identification of medical Bacteria(٣rd ed) Macfaddin, J.E.(ed) P: ٢٧-٤٣٩ Lioincott Williams and Wilkins Co., Blatimore, U.S.A.

•Macpherzon, A.; Khoo, U.Y.; Forgacs, I.; Pilpott-Houavds, J.; and Bjarnason, I. (١٩٩٦). Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacteria. Gut. ٣٨:٣٦٥-٣٧٥.

• Mamat, U.; Seydel, U.; Grimmecke, D.; Hoist, O.; Rietschel, E.T. (١٩٩٩). LPS, in comprehensive natural products chemistry, Vol. ٣, carbohydrates and their derivatives including Tauuis, cellulose, and , related Lignins (Pinto, B. M. ed) pp. ١٧٩-٢٣٩ Elsevier, Amsterdam ,The Netherlands.

•Mathison, J., Ulevitch, R. (١٩٧٩). The clearance, tissue distribution, and cellular localization of intravenously injected LPS in rabbits. J. Immunol. ١٢٣(٥): ٢١٣٣-٤٣.

•Mattu, T.S, Pleass, R.J. ; Willis, A.C. ; Kiline, M. ;Wormald, M.R. ; Leilouch, A.C.; Rudd, P.M. ;Woof, J.M. ; and Dwek, R.A. (١٩٩٨). The glycosylation and structure of human serum

lgA١, fab and fc regions and the role of N-glycosylation on fc receptor interaction. J. Biol. Chem. ٢٧٣: ٢٢٦٠-٢٢٧٢.

•Mazanec, M. B.; Kaetzel, C.S.; Lamm, M. E.; Fletcher, D.; and Nedrud, J. G. (١٩٩٢). Intracellular neutralization of virus by immunoglobulin A antibodies. Proc. Nat .Acad. Sci. U.S.A. ٨٩: ٦٩٠١-٦٩٠٥.

•McCallum, K.; Schoenhals, G.; Laakso, D.; dark, B.; and Whitfield, C. A. (١٩٨٩). high molecular- weight fraction of smooth LPS in Klebsiella serotype ٠١:K٢ contains a unique ٠-antigen epitope and determines resistance to nonspecific serum killing. Infect. Immune. ٥٧:٣٨١٦-٢٢.

•McCoy, K. L; and Kennedy, E.R. (١٩٦٠). Autogenous Vaccine therapy in staphylococcing infection. J.A.M. ١٧٤(١): ١٧٧-١٢٠.

•Mecuskey, R. , Urbaschek, R. and Urbaschek, B.(١٩٩٦). The microcirculation during endotoxemia . Cardiovas. Res. ٣٢:٧٥٢-٧٦٣.

• Mestecky, J.; Moro, I.; and Underdown, B.J. (١٩٩٩). Mucosal immunoglobulin, P.١٣٣-١٥٢ -In Ogra, P.L. et al. (ed), Mucosal immunity, ٢nd ed .Academic press Inc., SanDiego, Calif.

• Mestecky, J.; and Russell, M.W. (١٩٨٦). IgA Subclass .Monoge Allergy. ١٩:٢٧٧-٣٠١.

•Metcalf, J.; Gallin, J.; Nauseef, W.; and Root, A. (١٩٨٦). Transduction mechanism receptor expression. In Lab manual of Neutrophil function Raven Press New York p: ٧٨-

٧٩.

•Mostov, K.E. (١٩٩٤). Transepithelial transport of immunoglobulines .Annu. Rev. Immunol. ٣٧;.٦٠٣-٦٢٢.

•Mostov, K. E.; Kraehenbuhl, J.P.; and Blobel, G. (١٩٨٠). Receptor-mediated transcellular transport of Ig: synthesis of secretory component as multiple and larger transmembrane forms. Proc. NatI Acad. Sci. USA. ٧٧:٧٢٥٧-٧٢٦١.

•Muroi, M.; Takahiro, ٠.; Satoko, M.; and Ken, T. (٢٠٠٣). LPS-Mimetic activates of toll-like receptor٢ stimulating substance(s) in Enterobacterial LPS preparation. Infect. Immunity. ٧١(٦):٣٢٢١-٣٢٢٦

•Neutra, M.R.; Pringauit, E.; and Kraehenbuhl, J.P. (١٩٩٦). Antigen sampling across epithelial barriers and induction of mucosal immune response. Annu: Rev. Immunol. ١٤: ٢٧٥-٣٠٠ .

•Neutra, M.R.; Frey, A. Kraechenbuhl, J.P. (١٩٩٦). Epithelial M-cells: gate ways for mucosal infection and immunization. Cell. ٨٦: ٣٤٥-٨.

• Nibbering, P.H., Brong-Holub, E.; Bezmer, A.C. (١٩٩٦). Phagocytosis and intracellular killing of serum opsonized S. aureus by mous fibroblast expressing human . FCYR type IIa (CD٣٢) Forontiers in bioscience (١)٢٥-٣٣.

•Nikaido, H., (١٩٧٠). LPS in the taxonomy of enterobacteriaceac. Int J. Syst. Bacteriol. ٢٠(٤):٣٨٣-٤٠٦.

• Nolan, J. (١٩٨١). Endotoxin, reticuloendothelial function and

liver injury. Hepatol. ١(٥):٤٥٨-٦٥.

•Nonoyama, S.; Kojo, H.; Mine, Y.; and Nishida, M. (١٩٧٩). Inhibitory effect of Ps, aeruginosa on phagocytic and killing activities of rabbit PMN leucocytes purification and characterization of an inhibitor of PMN function. Infec. Immune. ٢٤:٣٩٤-٣٩٨

•Ohashi, Y.; Nakai, Y.; Esaki, Y.; Ohno', Y.; Sugiura, Y; Okamoto, H. (١٩٩١). Influenza A virus -induced otitis media and mucociliary dysfunction in guinea pig. Acta Otolaryngol. Suppl. (SuppI ٤٨٦) (١٣٥ -١٤٨).

•Old, D.C.; and Adegbola, R. (١٩٨٥). Antigenic relationship among type-٣ fimbriae of enterobacteriaceae revealed by immunoelectron microscopy. J. Med. Microbiol. ٢٠: ١١٣-١٢١.

•Park, B.H., Fikrig, and Smithwick, E.M.(١٩٦٨). Infection and nitroblue-tetrazolium reduction by neutrophils. Lancet, Sept. ٧, ٥٣٢-٥٣٣.

•Pears, A. (١٩٨٥). Histochemistry (^ed) pp: ٤٥٠ Churchill-Livingston, New York.

•Penner, J.L. (١٩٩٢). The genera Proteus, Providensia, and Morganella . P. ٢٨٤٩-٢٨٥٣ In: Balows, A.; Truper, H.G. . Harder, W and Schleifer, K.H. (Ed), The Prokaryotes, Vol. II. Springer Verlag KG, Berlin, Germany.

•Penner, J.L. (١٩٩١). The genera Proteus, Providencia and Morganella. In: the prokayotes: Handbook on the biology

of bacteria: Ecophysiology, identification, Applications (Balows, A. Truper. H.G., Dworkin, M. Harder, W. and Schleifer, K. H., Eds) pp. ٢٨٤٩- ٢٨٦٢ springer Verlag, Heidelberg, Germany.

•Penner, J. L; Hennessy, C. (١٩٨٠). Separate ٠-grouping schemes for serotyping clinical isolate of Proteus mirabilis and P. vulgaris. J. Clin. Invest. ١٢:٧٧-٨٢.

•Persson, C.G, Audwson, M.; Grieff, L.; Sueusson, C. (١٩٩٥). Airway permeability. Clin. Exp. Allergy, ٢٣:٨٠٧-٨١٤.

•Phillipp, A.L.H.; Brian, J.; Underdown, A.; Larry Arsenault, A.; Denis snider, P.; and Mark, R. (١٩٩٧). Comparison of Murin nasal associated lymphoid tissue and peyer's patches. Am. J. Respir. Crit Care Med. Vol. ١٥٦ No.٤ October, ١٢٥٦-١٢٦٢.

•Pier, G.B.; Grout, M.; Zaidi, T.S.; Olsen, J .C. and Gold berg, J.B. (١٩٩٦).Role of mutant CFTR in hypersusceptibility of cystic fibrosis patient to lung infection science ٢٧١:٦٤-٦٧.

•Pier, G.B.; Grout, M.; Zaidi, T.S.; and Gold berg, J.B. (١٩٩٦). How mutant CFTR may contribute to Ps. aeruginosa infection in cystic fibrosis. Am. J. Respir. Crit. Care Med. ١٥٤: S١٧٥-١٨٢.

•PlayFair, J.H.L and B.M. Chain. ٢٠٠١. Immunology at a Glance. ٧th edition.

•Prades, G.M. ; Dumollard, G.M.; Calloch, F; Merzougui, N; Veyref, C.; Martin, C . (١٩٩٨). Descriptive anatomy of human auditory tube. Surg. Radiol. Anat. ٢٠:٣٣٥ -٣٤٠.

•Prellner, K.; Kalam, ٠.; Harsten, G.; Heldrup, J.; Oxelius, V. (١٩٨٩). A phemococal serum antibody concentration during the first three years of life. A study of otitis -prone and non otitis prone children. Int. J. Pediatr. Otorhinolaryngol. ١٧:٢٦٧ - ٢٧٩.

•Preston, M. J.; Fleiszig, S.M.; Zaidi, T.S.; Goldberge, J. B; and Pier, G.B. (١٩٩٥). Rapid and sensitive method for evaluating P. aeruginosa virulence factors during corneal infection in mice. Infect immune. ٦٣:٣٤٩٧-٣٥٠١.

• Proctor, B. (١٩٦٧) Embryology and anatomy of Eustachian tube. Archotolarngol. ٨٦: ٣٠٥-٣١٤.

•Qiu, J.; Brackee, G.P. and Plaut, A.G. (١٩٩٦). Analysis of the specificity of bacterial immunoglobulin A (IgA) protease by a comparative study of ape serum IgAs as substrates. Infec. Immune. ٦٤:٩٣٣-٩٣٧.

• Rayfield, L.S.; and Chalcocomb, S.J. (١٩٨٧). Basic immunology in ٥th ed Scott-Browns otolaryngology Vol. ١ Basic science (Wright, D.A., Kerr, A.G. eds) Butter-worth's: London P٤٢٦-٤٥١.

•Reid, G., and Sobel, J.D. (١٩٨٧). Bacterial adherence in the pathogenesis of U.T.I. : a review. Rev. Infect. Dis. ٩:٤٧٠-٤٨٧

• Remick, D. (١٩٩٥). Applied molecular biology of Sepsis. J. Crit. Care. ١٠(٤): ١٩٨-٢١٢

•Rietschel, E.; Hoist, D.; Brade, L; Muller-Loennies, S.;and Beckmann, F. (١٩٩٦). Bacterial endotoxin: chemical

constitution biological recognition, host response, and immunological detoxification. Curr. Top. Microbiol. Immunol. ٢١٦:٣٩-٨١

•Roberts-Thomson, P.J.; and Shepherd, K. (١٩٩٠). Molecular size heterogeneity of immunoglobulin in health and disease. Clin. Exp. Immunol. ٧٩:٣٢٨-٣٣٤.

•Rocchetta, H.L; Burrows, L.L. and Lam, J.S. (١٩٩٩). Genetics of ٠-Ag Biosynthesis in Ps. aeruginosa. Microbiol. and Molecular Biology Reviews Vol. ٦٣ No.٣: ٥٢٣-٥٥٣.

•Roe. M.; Inger, N. N.; Brandtzeage, P. and Johansen, F. E. (١٩٩٩). Fine specificity of ligand-Bending Domain I. in PIgR: importance of CDR٢-Containing Region for IgM interaction. J. Immunol . ١٦٢: ٦٠٤٦٦-٦٠٥٢.

•Roitt, I.(١٩٩٧). Roitt's Essential immunology. ٩th ed. Black well Science.

•Roitt, I; Brostoff, J; and Male, D.(٢٠٠١). Immunology. ٦th ed., Mosby, London.

•Root, R. K. and Cohen, M. S. (١٩٨١). The microbicidal mechanisms of human neutrophil and eosinophilis. Rev. Infect. Dis. ٣:٥٦٥-٥٩٨.

•Rosengren, E.; Bucala, R.; Arnan, P. (١٩٩٦). The immunoregulatory macrophages MIF catalyze a tautomerization reaction .Mol .Med. ٢: ١٤٣-١٤٩.

•Rozalsk, A.; Sidorczyk, Z. and Kotellxo, K. (١٩٩٧). Potential virulence factors of Proteus bacilli In: Microbidogy and

Molecular biology Reviews, Mar, P. ٦٥-٨٩ American society for Microbiology.

•Rubin, H.R.; Sorensen, R.M.; and Klinger, J. D. (١٩٨٣). Suppression of in vitro lymphocyte DNA synthesis by Killed Ps. aeruginosa Infect. Immune. ٣٩: ٦٣٠-٦٣٧.

•Sade, J. (١٩٦٧). Ciliary activity and middle ear clearance. Arch. Otolaryngol. ٨٦:١٢٨ - ١٣٥

•Savoia, D.; Martenito, P.; Achino, A.; and Puglies, A. (١٩٨٣). Adhesion of *Proteus* species to various cell types. Eur. J. Clin. Microbiol ٢: ٥٧١-٥٧٦

•Senior, B.W.; Albrechtsen, M. and Kerr, M.A. (١٩٨٧). *Proteus mirabilis* strains of diverse type have IgA protease activity .J. Med. Microbiol. ٢٤:١٧٥-١٨٠.

•Senior, B.W.; Stewart, W.W.; Gallowuy, C.; And Kerr, M.A. (٢٠٠I). Cleavage of the hormone human chorionic gonadotropin by the type I lgA١ protease of *Neisseria gonorrhoeae* and its implications. J. Infect. Dis. ١٨٤:٩٢٢-٩٢٥.

• Sheagren, J.N. (١٩٨٤). *Staplylococeus aureus* the persistant pathogen (two-parts). N. Engl. J. Med. ٣١٠:١٣٦٨, ١٤٣٧.

•Shenep, J.; Barrett, P.; Stidham, G.; and Westenkirchner, D. (١٩٨٨). Serial quantitation of endotoximia and bacterimia during therapy for Gram-negative bacterial sepsis. Infect. Dis ١٥٧(٣):٥٦٥-٨

•Shnawa, I.M.S. and Thwaini, Q.N. (٢٠٠٢). Lapin mucosal humoral versus systemic humoral and cellular immune

response post to intrateticular administration of heat Killed C. feutus J. Baby. Univ. ٧(٣): ٥٣٨-٥٤٣

•Silverblatt, F.J.; and Ofek, I. (١٩٧٨). Effect of pili on susceptibity of Proteus mirabilis to phagocytosic and on adherence to bladder cell, p. ٤٩-٥٩ in Kass, E.H. and Brumfitt, W. Infection of urinary tract, University of Chicago press Chicago III

•Silverblatt, F.J., and Ofek, I. (١٩٧٨). Influence of pili on the virulence of Proteus mirabilis in experimental hematogenous pyelonephritis. J. Infect. Dis ١٣٨: ٦٦٤-٦٦٧

•Smith, J.W. (١٩٧٥). Local immune response in experimental pyelonephritis in the rabbit. Immunol. ٢٩: ١٠٧٧-١٠٨٤

•Soberg, M. (١٩٦٩). In vitro Migration inhibition of peripheral blood leucocytes in delayed type hypersensitivity. Acta Medica. Secand. ١٨٤:١٣-٢٥.

•Sorensen, R.U.; Klinger, J.D.; Cash, H.A.; Chase, P .A; and Dear Dorn, D.G. (١٩٨٣). In vitro inhibition of lymphocyte proliferation by Ps. aeruginosa phenajin pigments. Infect. Immune. ٤١: ٣٢٤ - ٣٣٠.

•Sousa, M., (١٩٨٠). Lymphocyte circulation, experimental and clinical aspects. John Wlley and Sons.

•Stanley, J. C.; Furer, JR. F.; and Germanir, R. (١٩٨٣). Passive protection against Ps. aeruginosa in experimental Leukopenic mouse model. Infect. Dis. ١٢٩:١٠١-١٠٩.

•Stenfors, L.E. and Raisanen, S. (١٩٩٢). Opsonization and phagocytosis of bacteria during various middle ear

infections. Int. J. of pediatric otorhinol. ٢٧: ١٣٧-١٤٥.

•Stenfors, L.E.; and Raisanen, S. (١٩٩١). SIgA and IgG-coated Bactria in chronically discharging ears. J. Laryngol. Otol. Vol. ١٠٥: ٥١٥-٥١٧.

•Stenfors, L.E. (١٩٩٩). Nonspecific and specific immunity to bacterial invasion of the middle ear cavity. Int. J. Pediatr. Otorhinol . ٤٩(suppl. ١) S٢٢٣ -S٢٢٦.

•Steustrom, C. and Bylander -Groth, A. Ingrarsson, L. (١٩٩١). Eustachian tube function and otitis media in otitis pron and healthy children. Inter. J. Pediatr. Otorhinolaryngool. ٢١:١٢٧ - ١٣٨

•Stevenson M.M; Kondratera T.K.; Apt, As.; Tarn, M.F.; and Skarmeme, E. (١٩٩٦). In vitro and in vivo T cell responses in mice during bronchopulminary infection with mucoid *Ps. aeruginosa*. Clin. Exp. Immunol .٩٩: ٩٨-١٠٥.

•Straetemans, M. , VanHeerbeek,N., Tonnaer, E.Ingels, K.J.A.O. and Rijkers, G.T. (٢٠٠١). A comprehensive model for the aetiology of otitis media with effusion . Medical hypotensis .٥٧(٦): ٧٨٤-٧٩١.

•Suffredini, A.; Fromm, R.; Parker, M.; and Parrillo, J. (١٩٨٩). The cardiovascular response of normal humans to the administration of endotoxin. N. Engl-J. Med٣٢١(٥):٢٨٠-٧

•Swierzko, A. S. ; Kirikae, T.; Kirikae, F.; Hirata, M. Ziolkowski, H. and Nakano, M.(٢٠٠٠). Biological activities of LPS of Proteus species and their interactions with polymyxin B and

an ١٨KDa Cationic antimicrobial protein (CAP١٨) derived peptide. J. Med. Microbiol. vol.٩:١٢٧-١٣٨.

•Takahashi, H.; Hayashi, M.; Sato, H.; Honjo, I. (١٩٨٩). Primary deficits in Eustachian tube function in patients with otitis media with effusion. Arch. Otolaryngol. Head. Neck .Surg. ١١٥:٥٨١ -٥٨٤.

•Tarlow, M.(١٩٩٨). Otitis media pathogenesis and medical sequelae. ENT J. Suppl. June Vol. ٧٧ No.٦ ٣-٦.

•Telford G.; Wheeler; D. P.; Willams, R.G.; Tomkins, P.; Appreby, H.; Seweli, G.S.A.B.; Stewart , B.W.;Bycrofy ; and Pritechard, D.I. (١٩٩٨). Ps. aeruginosa quorum sensing signal molecule N-OKO L homoserine lactone has immunomodulatory activity. Int. Immune. ٦٦: ٣٦-٤٢

•Todar, K., (٢٠٠٢). Staphylococcus in Bacteriology. ٣٣٠ Home pages.

•Triantafilou, M.; and Triantafilou, K. (٢٠٠٣). Receptor cluster formation during activation by bacterial product. J. Endotoxin Res. ٩(٥):٣٣١-٥.

•Ulevitch, R.; Tobias, P. (١٩٩٥). Receptor- dependant mechanism of cell stimulation by bacterial endotoxin. Ann. Rev. Immunol. ١٣:٤٣٧-٤٥٧

•Utsinger, P.D. (١٩٧٥). Synovial fluid lymphocytes. in RA. Arthr. Rheum. ١٨, ٥٩٥.

• Van Deventer, S.; Tencate, J.; and Tytgat, G. (١٩٩٨). Intestinal endotoximia- clinical significance. Gastroenterology

٩٤:٨٢٥-٣١.

•Van Kempen, M. J.; Rijkers, G. T.; Vancauwenberge, P.B. (٢٠٠٠). The immune response in adenoid and tonsils. Int. Arch Allergy Immunol. ١٢٢:٨ - ٩.

•Van leeuwen, P.; Boermeester, M.; Houdijk, A.; and Wesdrop, R. (١٩٩٤) Clinical significance of translocation. Gut. S٢٨-S٣٤.

•Wallport, M. (١٩٩٣). Complement. In: 'Roitt, I;Brostoff, J.; Male, D. (Eds) .Immunology ٣rd ed. Mosby, London, pp. ١٢.١٧.

•Warren, J.W.; Tenny, J. H.; Hoopes, J. M.; Juncie, H.J.; and Anthony, W.C. (١٩٨٢). A prospective microbiological study of bacteria in patients with chronic indwelling urethra! catheters J. Infect. Dis. ١٤٦:٧١٩-٧٢٣.

•Weber, G., Link, F. Farber,E. and Muder, P.G.(١٩٨٣). Deferential modulation of the effect of LPS on macrophage by a major outer membrane protein of Proteus mirabilis . J. Immunol. ١٥١: ٤١٥-٤٢٤.

• Weber, K .and Osborn, M. (١٩٨٢). The reliability of molecular weight determination by dodeyl sulfate -polyacrylamide Gel electrophoresis. In physical Biochem.

•Westphals, O. and Jann, K.(١٩٦٥). Bacterial LPS extraction with phenol water and further applications of the procedure, Methods Carbohydr Chem. ٥,٨٣-٩١.

•Wooton, I.D.P. and Freeman, H.(١٩٨٢). In " Microanalysis in Medical Biochemistry" ٦th ed.

• Williams, P. M.; Camara, A.; Hardman, S.; Swift, D.; Milton, N.J.; Hope, K.; Winzer, B.; Middleton,.;Pritchard, D.I; and Bycrofy, B.W. (٢٠٠٠). Quorum sensing the population dependent-control of Virulence. Phil. Tran .R. Soc .Lond .B ٣٥٥: ٦٦٧-٦٨٠.

•Wilson, R.; Robert, D.; Cole, P. (١٩٨٥). Effect of bacterial products on human ciliary's function in vitro. Thorax. ٤٠:١٢٥ - ١٣١.

•Yang, ٠. ٠; Kalams, S. A.; Trocha, A.; Cao, H.; Luster, A. ;Donsol , R. P. (١٩٩٧). Suppression of human immunodeficiency virus type I replication by CD٨'١" cells. Evidence for HLA class ١-restricted triggering of cytolytic and noncytolytic mechanisms. J. Virol. ٧١:٣١٢٠-٨.

•Yokota, S; Kaya, S.; Sawada, S.; Araki, Y.; and Ito, E. (١٩٨٧). Characterization of a polysaccharide component of lipopolysaccharide from P. aeruginosa II D ١٠٠٨[AtCC٢٧٥٨٤] as D. rhamnan. Eur.J. Biochem. ١٦٧:٢٠٣-٢٠٩.

•Zaidi, T- S.; Fleiszig, S.M.J; Preston, M.J.; and Pier, G.B. (١٩٩٦). LPS outer core is alignad for corneal cell binding and ingestion of P. aeruginosa. Investing .Ophthalmol .Visual Sci. ٣٧:٩٧٦-٩٨٦.

•Zubler, R.L.T. (١٩٩٨). Antigens: T dependent and T

independent. In, Delres, R.J. and Roitt, I. (Eds). Encyclopedia of Immunology. Vol. I Academic press, London,٢١٤-٢١٨.

• Zuercher, A.W.; Coffin, S.E; Thurnheer, M.C.; Fundova, P.; and Cebra, J.J. (٢٠٠٢). NALT is a mucosal inductive for virus specific humoral and cellular immune response. J. Immunol. February ١٥,١٦٨(٤), ١٧٩٦-١٨٠٣.

دراسة الاستجابة المناعية في المرضى المصابين بالتهاب الأذن الوسطى البكتيري المُزمن

أطروحة مقدمة إلى مجلس كلية العلوم- جامعة بابل
وهي جزء من متطلبات نيل درجة الدكتوراه- فلسفة
في
علوم الحياة/ الأحياء المجهرية

من قبل
سلمان عزيز عدوس
ماجستير علوم حياة
كلية العلوم- جامعة بغداد
١٩٨٧

١٤٢٥ هـ ٢٠٠٥ م

الخلاصـــــــــــــــــــــــة

تم في هذه الدراسة فحص المرضى الذين يعانون من طرح أذني مزمن. و جرى التحري عن المسببات المكروبية المشاركة مع هذه الحالات ثم أُختير ثلاث من هذه المسببات البكتيرية الأكثر شيوعا بناءا على مسح رجعي لسجل الحالات و مسح واقعي لمدة سنة اجري من قبل الباحث.

خضع المرضى لدراسة مناعية مستفيضة شملت المناعة الخلطية الموضعية والجهازية والمناعة الخلوية الموضعية والجهازية مع محاولة لتاشير العلاقة بينهما اذ كانت الفحوصات الخلطية تتضمن التلازن البكتيري والخلوية شملت معامل تثبيط هجرة الخلايا LIF والتشكيل الزهري للخلايا اللمفية التائية والنشاط البلعمي للبلاعم الكبيرة NBT، كانت المادة المحسسة لتفاعلات المناعة الخلوية راشح المزرعة الحر من الخلايا ومعتمدد السكريد الشحمي.

كان عدد المرضى ٢٤٥ مريضاً في مرحلة الدراسة الرجعية وفي مرحلة تتبع الباحث لصورة المرضى ميدانياً كان عدد المرضى ١٠٠ مرضاً، وفي دراسة مناعة الطرح الاذنى البكتريا كان عددالمرضى ٥٠ مريضاً، فحصيلة عدد المرضى الذين شملتهم هذه الدراسة بلغ ٣٩٥ مريضاً. هؤلاء المرضى مراجعي وحدة الأنف والإذن والحنجرة في المستشفى التعليمي في النجف.

كانت نسبة تواجد الزوائف الزنجارية *Ps.aeruginosa* هي ٣٦% ٤٦% في الدراسة المرجعية والميدانية على التوالي. وكنت سيادة المتقلبات *P.mirabilis* بنسب تواجد ٢٠% و١٩% في الرجعية والميدانية على التوالي. في ما يخص المكورات العنقودية كانت نسب التواجد ١٩% و١٧% في كل من الرجعية والميدانية على التعاقب.

حضرت المستضدات السطحية لكل من الزوائف الزنجارية والمتقلبات بطريقة المعاملة بكلوريد البنزالكونيوم ٠.٠٥ %.

تم قياس المناعة الطبيعة الخلطية للمرضى وشملت قياس بروتين المصل الكلي وأضداد المصل والضد المخاطي للمرضى المصابين بنضح الإذن المزمن.

ففي مرضى الزوائف الزنجارية زاد معدل بروتين المصل الكلي وبلغ ٦٥.٦ غم أما أضداد المصل فقد زادت وبلغت ٣٥.٨ غم/ لتر أما ألبومين المصل فقد انخفض قليلا لغاية ٣٢.٣٥ غم/ لتر. زاد الكلوبيولين المخاطي وبلغ معدل ٠.٣٧ غم / لتر.

أما في مرض المتقلبات فقد ارتفع بروتين المصل الكلي حتى وصل معدل ٦٤.٤ غم/لتر وانخفض في نفس الوقت ألبومين المصل ووصل معدل ٣٦.٦ غم/لتر في حين زاد الكلوبيولين المخاطي إلى ٠.٤١ غم/لتر.

في حالة المرضى بالبكتريا المكورة الذهبية فأن معدل البروتين الكلي للمصل قد زاد الى ٦٦غم/لتر وكذلك كلوبيولين المخاطي الى معدل٣٥.٥ . في حين إنخفض البومين المصل الى ٢٧.٨٤غم/لتر وأزداد الكلوبيولين المخاطي الى معدل ٠.٤٨غم/لتر مقارنة بمعامل السيطرة.

تمت دراسة المناعة النوعية الخليطية للمرضى المصابين بالانواع البكتيرية المذكورة انفا وفي كلا الجهازين المخاطي والجهازي.

ففي عشرة مرضى مصابين بنضح الاذن المزمن المسبب بالزوائف الزنجارية ارتفع عيار الضد المتخصص لهذة البكتريا حتى بلغ في المصل معدل ٢٤٠ عند استخدام الستضدات السطحية مقابل الضد المتخصص اما عيار الضد المخاطي فقد بلغ معدل ١٩.٢.

اخذ عشرة مرضى اخرين مصابين ايضا بطرح الاذن المزمن المتسبب بالمتقلبات فقد بلغ معدل عيار الضد في الدم ٢٢٤ ومعدل عيار الضد المخاطي ٢٤ عند استخدام المستضدات السطحية. تميزت هذه القيم بزيادة معدلات عيار الضد في الجهازين المخاطي والدموي عن المعدل الطبيعي.

في حالة المرضى المصابين بطرح الاذن المزمن المتسبب بالمكورات العنقودية الذهبية فقد اخذ عشرة مرض ايضا للوقوف على معدل الزيادة في عيارات الضد ولكلأ الجهازين حيث تبين إن هناك زيادة في معدلات عيار الضد في مصل الدم حيث بلغ ٣٣٦ وفي الجهاز المخاطي كانت بمعدل ٢٤. تبين هذه الأرقام بان هناك زيادة واضحة في معدلات عيارات الضد وفي كلا الجهازين.

درست المناعة الخلوية غير المتخصصة في المرض المصابين بالانواع البكتيرية الثلاثة المذكورة وقد شملت الدراسة عملية البلعمة التي تم تحديدها من خلال ملاحظة اختزال صبغة النايتروبلوتترازوليوم من قبل الخلايا العدلة وفي الجهازين الدموي والمخاطي للإذن. ارتفعت قابلية اختزال الصبغة في مرضى الزوائف الزنجارية في الدم وبلغت ٣٠.١% وفي الجهاز المخاطي بلغت ٣٧%. في مرضى المكورات العنقودية بلغت نسبة اختزال الصبغة في الدم ٣٥.١ % وفي الجهاز المخاطي الموضعي للإذن ٤٠.٨ % مقارنة بعامل السيطرة.

أما في حالة المتقلبات فقد بلغت النسبة الجهازية لاختزال الصبغة ٢٧.٤ % حيث لم تطرأ زيادة نوعية في نسبة اختزال الصبغة على عكس الجهاز المخاطي للأذن فقد زادت نسبة اختزال الصيغة الى ٣٧.٤ % مقارنة بالسيطرة

لتحديد الاستجابة المناعية الخلوية المتخصصة فقد تم اعتماد معيارين الأول هو عامل تثبيط هجرة الخلايا البلعمية والثاني التشكيل الزهري التائي. كانت النتائج على الشكل التالي بعد اخذ عشرة مرضى لكل حالة من الحالات البكتيرية الثلاثة ولكل معيار من المعياريين المذكورين.

في مرضى الزوائف الزنجارية زادت نسبة تثبيط هجرة الخلايا الى ٦٣% في الدم وفي الجهاز المخاطي للإذن بلغت ٤٩% عند استخدام راشح النمو الخلايا المحفزة.

أما التشكيل الزهري التائي فقد ازداد في كلا الجهازين حتى بلغ في الدم المحيطي معدل ٣٢.٢% وفي الجهاز المخاطي ٤٦.١%.

في بكتريا المكورات العنقودية المصاحبة للطرح الاذني المزمن، زاد معدل تثبيط هجرة الخلايا بعد استخدام راشح الخلايا البكتيرية المحفزة وبلغ في الدم ٥١% وفي الجهاز المخاطي للاذن ٤١%. اما التشكيل الزهري التائي فقد ارتفع و بلغت نسبته ٣٨.٥% في جهاز الدم وفي الجزء المخاطي للاذن بلغ معدل ٤١%.

اظهرت بكتريا المتقلبات عدم القدرة على التثبيط هجرة الخلايا في الجهازين الدموي والمخاطي عند استخدام راشح الخلايا البكتيرية المحفزة. حيث كانت في جهاز الدم ٨٧% وبلغت في الجهاز المخاطي ٨٢%. اما في حالة التشكيل الزهري التائي في الدم لم تظهر اية زيادة عن المعدل الطبيعي، فقد بلغت ٢٦.٦% في حين زادت في الجهاز المخاطي وبلغت ٣١.٧%.

اظهر الذيفان الداخلي المستخلص والمنقى من البكتريا السالبة لصبغة كرام والمعزولة من مرض الطرح الاذني المزمن، قابلية عالية على التثبيط هجرة الخلايا البلعمية عند استخدامه كمحفز فقد بلغت في بكتريا الزوائف الزنجارية ٥٢% في الجهاز الدموي المحيطي و ٤١% في الجهاز المخاطي للأذن.

في عشرة مرضى مصابين في المتقلبات تم تثبيط هجرة الخلايا تثبيطا معنويا وفي كلا الجهازين فقد بلغ في جهاز الدم ٥١% وفي الجهاز المخاطي ٤٣% مقارنة بعامل السيطرة عند استخدام الذيفان الداخلي المحفز.