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Evaluation of Galectin-3 , IL-6 and Some Trace Elements in Adult Patients with Chronic Suppurative Otitis Media in Hilla – Iraq

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By

Ruqaya Ali Haider Abd AL-Ameer

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Supervised by

Assist .Prof.

Dr. Ban Mahmood Shaker Al-Joda

Prof.

Dr. Safaa Sahib Naji

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ ۚ
وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا

صدق الله العظيم
سورة النساء ١١٣

Supervisor Certificates

We certify that this thesis entitled (**Evaluation of Galectin-3 , IL-6 and some trace elements in adult patients with chronic otitis media in Hilla – Iraq**) has been prepared under our supervision at the Department of Biochemistry, College of Medicine, University of Babylon, in partial requirements for the degree of master in Clinical Biochemistry.

Assistant. Professor
Dr. Ban Mahmood shaker Al-Joda
Supervisor

Professor
Dr. Safaa Sahib Naji
Supervisor

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

Professor
Dr. Abdulsamie Hassan Alta'ee
Head of Department of Clinical Biochemistry
Data / /2022
College of Medicine/Babylon University

Dedication

To the person whom God has entrusted with prestige and reverence
To the person who taught me to give without waiting anything in return
To the person that I bear his name with proud ….
(My dear father)

To my wisdom..... And my knowledge
To my literature..... And my dream
To the source of patience, optimism and hope
To everyone in existence after God and His Messenger….
(My dear mother)

To the ideal man who supported me with all his strength.
To those who encouraged me to continue my scientific career
To the man who stayed awake to help me realize my dreams
To the companion of my path ….
(My Dear husband)

To those whom I see optimism in their eyes and happiness in their laughter
To those with whom I spent the most beautiful moments
To those who preferred me over themselves….
(My brothers and sisters)

To my soul, my eyesight, and my heartbeat.
To my delight and smile in life
To my dear daughter….
(Alkawther)

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Summary

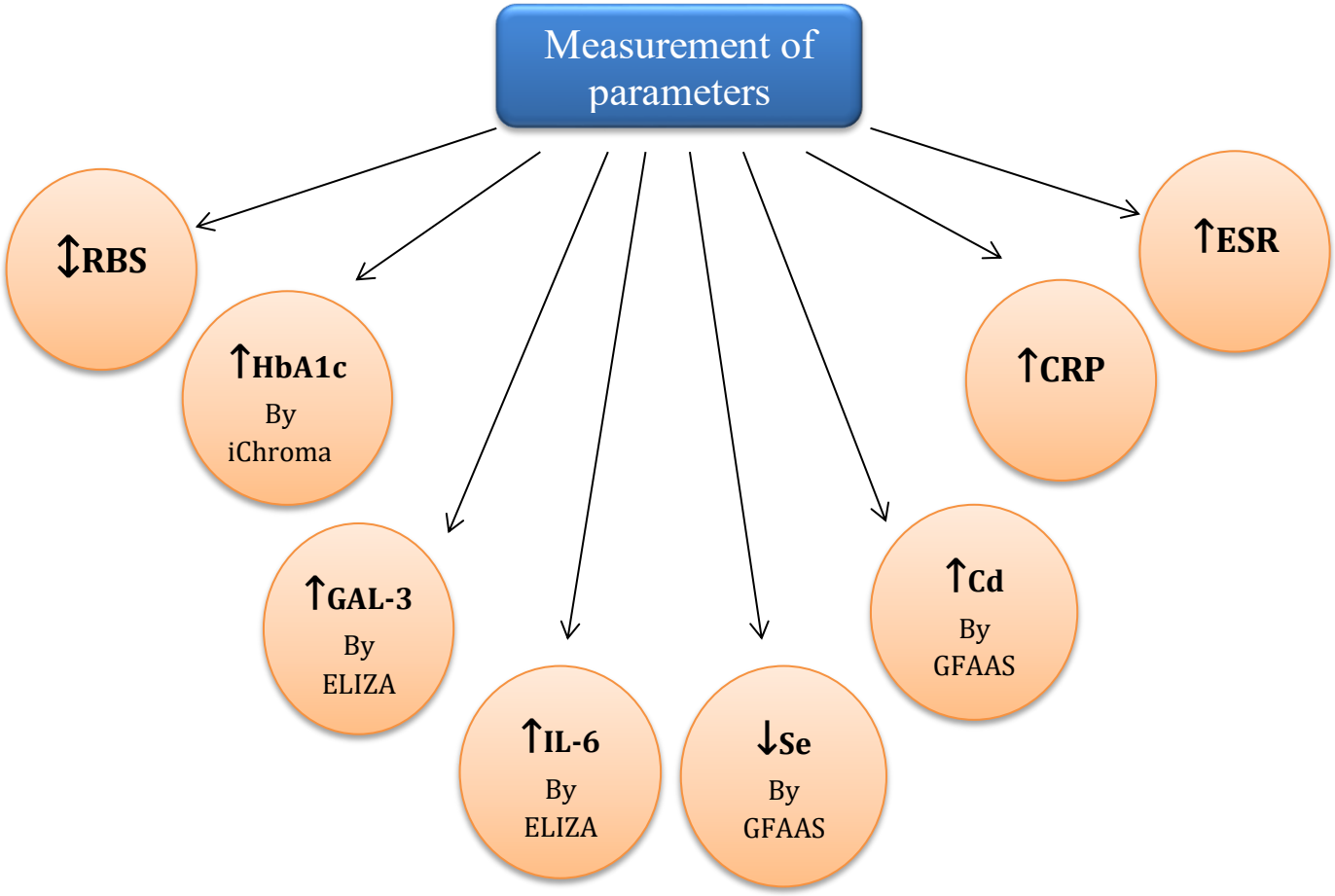
Chronic suppurative otitis media (COM) is a recurrent infection of the middle ear and/or mastoid air cells in the presence of a tympanic membrane perforation. Symptoms commonly associated with chronic ear disease include hearing loss, otorrhea, aural fullness, otalgia, and occasionally true vertigo. The study's objective is to evaluate serum levels of Galectin-3(GAL-3), Interleukin-6(IL-6), Selenium and Cadmium in patients with Chronic otitis media (COM)and healthy controls.

The study involved collecting blood samples from 100 volunteers, 50 healthy subjects (29 men and 21 women) and (29 men and 21 women) suffering from Chronic otitis media. age was (15 –65) years. Patients were subjected to ENT department in Imam Al-Sadiq Hospital.

The levels of GAL-3 & IL-6 was measured by (ELISA) technique whereas the levels of Selenium and Cadmium were measured by (GFAAS) technique, and the levels of other parameters were measured by colorimetric method according to the manufacturer manual.

As the results of the tests that were conducted showed that the levels of GAL-3 ,IL-6 & Cadmium in the people with Chronic otitis media were significantly Higher than healthy people, The levels of Selenium in people with Chronic otitis media were significantly Lower than healthy people, as the value of $P < 0.05$. Also, the relationship between the levels of GAL-3 & IL-6 was studied, where the value of $r = 0.656$, $P<0.001$was found and there was significant positive correlation between them

It can be concluded from the current study that we carried out that the level of occurrence of The GAL-3 ,IL-6 & Cadmium is higher in patients with Chronic otitis media than those healthy control, and the level of Selenium is lower in patients with Chronic otitis media than those healthy control. The presence of a positive relationship between the GAL-3 & IL-6 and between the GAL-3 & Cd, IL-6 & Cd that were performed and inverse relationship between GAL-3,IL-6 &Se, the current study worked on studying the positive relationship and inverse relationship existed between this parameters.



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List of Abbreviation

Abbreviation	Details
ADAM	Adisintegrin And Metalloproteinases
AOM	Acute otitis media
Bcl-2	B-cell lymphoma 2
Cd	Cadmium
CRD	Carbohydrate recognition domain
CRP	C-reactive protein
CSOM	Chronic suppurative otitis media
DAMP	Damage-associated molecular pattern
ECM	Extracellular matrix
ERK	Extracellular signal-regulated protein kinase
ESR	Erythrocyte Sedimentation Rate
Gal-3	Galectin-3
GPx	Glutathione peroxidases
HbA1c	Hemoglobin A1C

HRV	Human rhinovirus
IAV	Influenza A viruses
ICAM-1	Intercellular Adhesion Molecule 1
IKK	IκB kinase
IL-6	Interleukin-6
ILVs	Intraluminal vesicles
IκBs	Inhibitors of NF-κB
JNK	c-Jun N-terminal kinase
KRas	Kirsten rat sarcoma
LGALS3	Gal-3 gene
MAPKs	Mitogen-activated protein kinase
MCP-1	Monocyte Chemoattractant Protein-1
MEE	Middle ear effusion
MVBs	Multivesicular bodies
NF-B	Nuclear factor-kappa B
NK	Natural Killer cells
NSAIDs	Non-steroidal anti-inflammatory medications
OM	Otitis Media
OME	Otitis media with effusion
PAMPs	Pathogen-associated molecular patterns
PRR	Pattern-recognition receptor
ROS	Reactive oxygen species
RSV	Respiratory Syncytial Virus
SAA	Serum amyloid A
Se	Selenium
T3	Triiodothyronine
T4	Thyroxine
TLRs	Toll-like receptors
TNF	Tumor necrosis factor
TrxR	Thioredoxin reductases
URI	Upper respiratory tract infection

Chapter One

Introduction and Literature Review

1.Chronic Otitis Media

1.1.Ear

The ear is divided into three sections: Outer, middle, and inner. The pinna, or outer ear, is made up of ridged cartilage that is covered with skin. The pinna directs sound into the external auditory canal, a narrow tube that leads to the eardrum (tympanic membrane).The eardrum and its small connected bones in the center section of the ear quiver in response to sound, and the vibrations are transmitted to the neighboring cochlea. The cochlea is a spiral-shaped organ in the inner ear that converts sound into nerve impulses that pass to the brain[1]. Inside the ears, the Eustachian tubes connect the middle ear to the back of the nose. They aid in the drainage of fluid, the stabilization of air pressure inside the ear, and the prevention of germs[2].

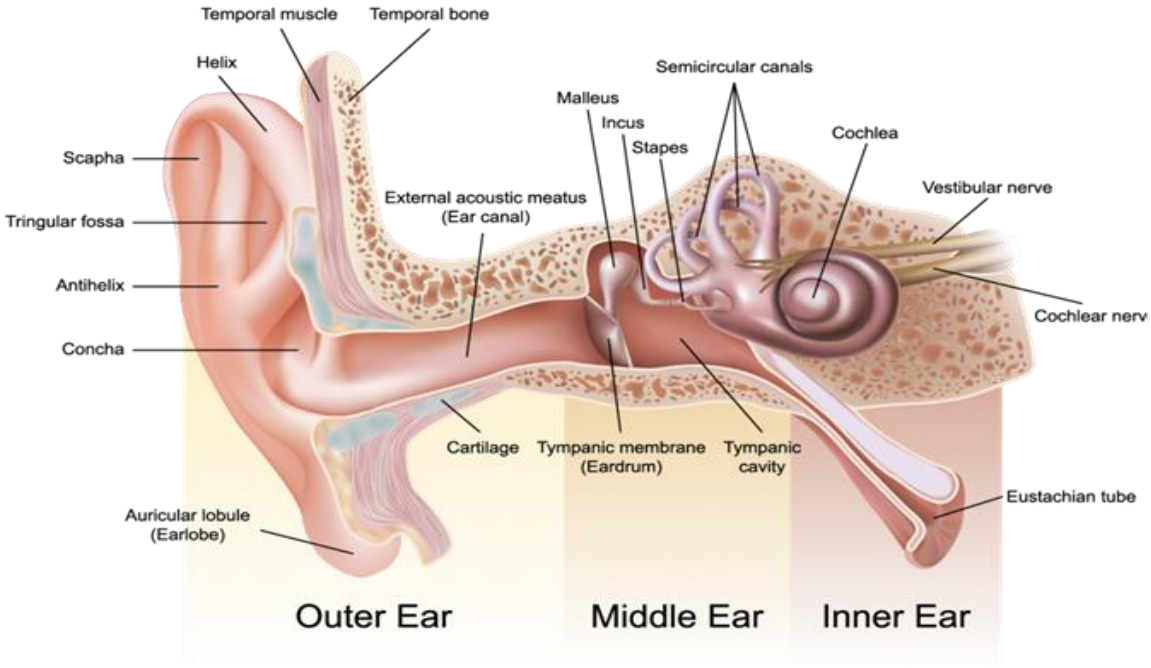


Figure (1-1): Anatomy of the ear[3].

The mucosal membrane that lines the middle ear cavity encompasses all structures, including the ossicles, and is continuous with the mucosal membrane that lines the mastoid antrum, eustachian tube, and nasopharynx[4]. Middle ear epithelial cells, neutrophils, macrophages, fibroblasts, mast cells, and natural killer cells all play a part in protecting the middle ear against pathogens[5].

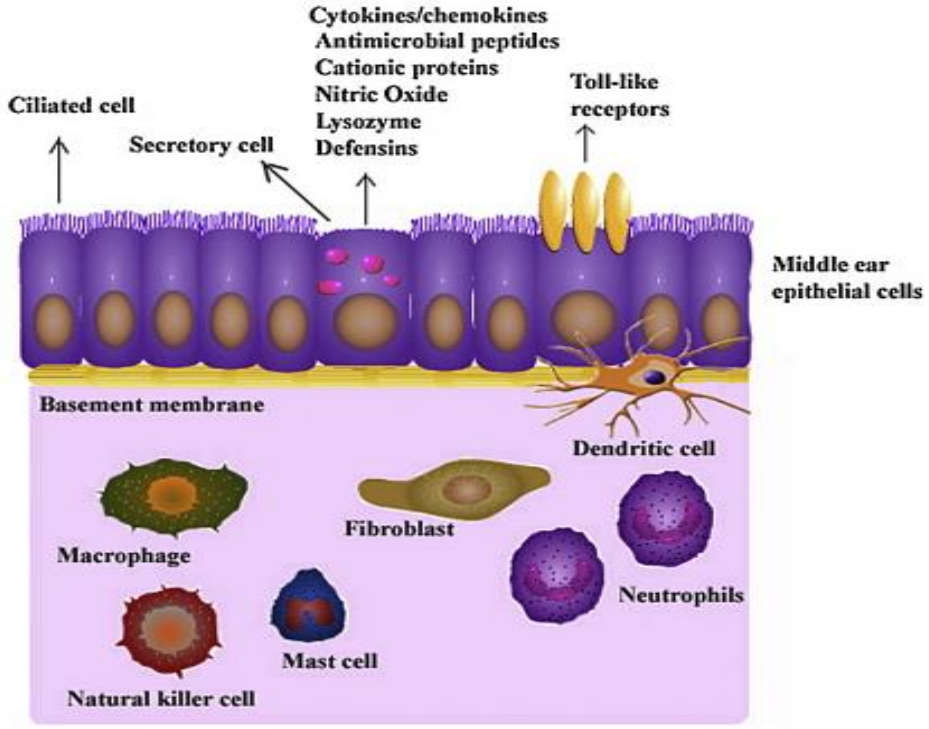


Figure (1-2): The middle ear innate immune cells [5].

The mucociliary apparatus, the ability to trap mucous glycoproteins and surfactants, the ability to secrete innate defense molecules such as defensins, interferons, lactoferrin, and nitric oxide, and antibody production through the adaptive immune response are all present in the Epithelial lining of the middle ear[6].

1.2.Otitis Media

Otitis media is a first intermediary of health care visits across the world, and its complications are enormous causes of preventable hearing loss, predominantly in the developing world.Otitis media is a term used to describe a set of inflammatory and infective disorders affecting the middle ear[7]. Ear inflammatory occure when viruses or bacteria enter the middle ear, which is the space behind the eardrum. The middle ear fills with pus when child gets an ear infection (also known as otitis media). The pus presses against the eardrum, causing severe pain[8]. Acute otitis media (AOM), otitis media with effusion (OME), and chronic suppurative otitis media (CSOM) are all examples of otitis media [9].

1.2.1.Classification of otitis media

1.2.1.1.Acute otitis media (AOM)

Definition, Causes, Pathophysiology, Symptoms, Diagnosis, Treatment.

Acute otitis media is a middle ear infection that is the second most prevalent pediatric diagnosis in the emergency department, after upper respiratory infections. Although acute otitis media can occur at any age, but it is most frequent in children aged 6 to 24 months[10]. AOM characterizes by The presence of fluid in the middle ear (middle ear effusion (MEE)) along with signs and symptoms of an acute infection[11].

Acute otitis media (AOM) is a polymicrobial illness that usually develops as a result of a viral upper respiratory tract infection (URI)[12]. The most common causes of AOM are Streptococcus pneumoniae, non-typable Haemophilus influenzae, and Moraxella catarrhalis[13].

Several respiratory viruses have been extensively studied related to AOM pathogenesis; among the more common and important are Respiratory Syncytial Virus (RSV), Influenza A viruses(IAV), Adenovirus and Human rhinovirus (HRV)[12].

Acute otitis media caused when Eustachian tube becomes bloated or obstructed, fluid is trapped in the middle ear, causing an AOM. It's possible that the confined fluid will become contaminated. The Eustachian tube is shorter and more horizontal in early children than it is in older children and adults. As a result, it's more likely to get contaminated[14]. Causes of blocked Eustachian tubes are sinus infections, allergies, colds, deviated septum in the nose, poor muscle tone around the Eustachian tube, cleft palate, swollen adenoids, damage to the ear, acid reflux, radiation, smoke, the flu and drinking while laying down (in infants)[14,15].

Pathophysiology of Otitis media is an inflammatory condition that affects the mucosa of the nose, nasopharynx, middle ear mucosa, and Eustachian tubes after a viral upper respiratory tract infection. The edema generated by the inflammatory process obstructs the narrowest region of the Eustachian tube, resulting in a decrease in ventilation due to the constrained anatomical space of the middle ear. This causes a chain reaction that results in an increase in negative pressure in the middle ear, increased exudate from the inflamed mucosa, and mucosal secretion accumulation, allowing bacterial and viral species to colonize the middle ear. Viruses can potentially affect the immune system of the host , as well as antibiotic activity[12]. A bulging or erythematous tympanic membrane, as well as purulent middle ear fluid, are clinical signs[16].

The most common symptom of AOM is a middle ear effusion, which is often accompanied by indications of acute sickness such as earache, otorrhea (runny and filled ear), ear tugging, fever, irritability, anorexia, vomiting, or diarrhea[17].

Diagnosis, AOM is distinguished from OME and CSOM On the basis of a complete history and examination findings[7]. Doctor examines ear with an equipment called an otoscope to search for: redness, swelling, blood, pus, air bubbles, fluid in the middle ear and eardrum perforation[14]. The most consistent sign of AOM and the most useful feature for distinguishing AOM from OME is a bulging tympanic membrane, which is associated with a high level of bacterial pathogens in the MEE[9]. Tympanometry, during a tympanometry test, doctor uses a small instrument to measure the air pressure in ear and determine if the eardrum is ruptured[10]. Reflectometry, during a Reflectometry test, doctor makes a sound near his or her ear with a little instrument. By listening to the sound reflected back from the ear, doctor can establish if there is fluid in the ear[14].

Treatment, once an acute otitis media diagnosis has been made, the goal of treatment is to relieve pain while also treating the infectious process with antibiotics. Pain relief can be achieved with non-steroidal anti-inflammatory medications (NSAIDs), such as acetaminophen[18]. Antibiotic concentrations in middle ear fluid were adequate for AOM treatment when an antibiotic-containing (ciprofloxacin) gel was applied to the tympanic membrane[19]. If suppurative AOM is present, oral antibiotics are prescribed to treat the bacterial infection, with high-dose amoxicillin or a second-generation cephalosporin being the first-line medicines[18].

Ceftriaxone for three days, either intravenously or intramuscularly, as an alternative to oral antibiotics in children who are vomiting or in conditions where oral antibiotics cannot be given. Antihistamines and systemic steroids have not been found to provide any substantial benefit[20].

1.2.1.2. Otitis Media with effusion (OME)

Definition, causes, Pathophysiology, symptoms, Diagnosis, Treatment.

Ear fluid, AOM ear infection, serous, secretory, or nonsuppurative otitis media are Synonyms for OME[21]. Affects children between ages 3 to 7 years old[22]. The presence of Middle ear effusion (MEE) behind an intact tympanic membrane characterizes OME; however, unlike AOM, OME is not associated with signs and symptoms of an acute infection[23]. OME can develop as a result of an upper respiratory infection, as a result of impaired eustachian tube function, or as an inflammatory response to AOM[21]. After a viral infection, OME can develop[24]. Many other risk factors that increase the vulnerability to OME include young age, craniofacial defects, allergic rhinitis, passive smoking, daycare attendance, genetic predisposition, and gastric reflux[25]. The adenoids can restrict the Eustachian tube in people with big adenoids, resulting in a poorly ventilated middle ear. Otitis Media with effusion (OME) may occur as a result of this type of obstruction. Because the adenoids are a lymphatic structure, germs may be transmitted into the Eustachian tube, allowing biofilms to develop. This type of bacterial growth can induce inflammation, which can lead to a blockage and fluid build-up in the middle ear[26].

Diagnosis, The majority of OME cases are diagnosed clinically after an otoscopic examination. The physician can use a pneumatic otoscope to check for middle ear effusion and the aspect of the tympanic membrane[27].

Treatment, OME is caused by viral or allergy-related causes, rather than a bacterial infection. As a result, the use of antibiotics is not advised. Furthermore, corticoids used to treat allergies have not been shown to have a substantial impact on OME patient outcomes. Antibiotics and corticoids are therefore not indicated for the treatment of OME. As a first-line treatment for OME patients, watchful waiting for three months is recommended. If OME persists, a specialist referral may be necessary to determine surgical treatment alternatives[28].

1.2.1.3.Chronic suppurative otitis media(CSOM)

Definition, Causes, Pathophysiology, Symptoms, Diagnosis, Treatment.

Chronic suppurative otitis media, also known as chronic otitis media, is a stage of ear disease in which the middle ear is infected on a long-term basis and the tympanic membrane is not intact. This condition is characterized by a long-term inflammation of the middle ear and mastoid cavity[29]. A ruptured tympanic membrane with persistent drainage from the middle ear for more than 2-6 weeks is characterized as chronic suppurative otitis media (CSOM)[30]. In CSOM, the tympanic membrane is punctured. Tubotympanic perforation (in the middle of the tympanic membrane) is commonly regarded as safe, but atticoantral perforation (at the top of the tympanic membrane) is frequently seen as unsafe.The presence of cholesteatoma determines whether it is safe or unsafe[31]. A cholesteatoma in the middle ear will be linked to CSOM[32].

Cholesteatoma, a keratinized mass in the middle ear or mastoid, can arise as a primary lesion or as a result of perforation of the tympanic membrane[33].

Causes of CSOM, AOM can proceed to CSOM, which is defined by a chronic infection and inflammation of the middle ear and mastoid air cells despite antibiotic treatment[34]. The Eustachian tube is significant in this disorder, and it is found to be dysfunctional in 70% of individuals undergoing middle ear surgery. When the Eustachian tube malfunctions, the middle ear pressure equilibration is disrupted, and middle ear aeration is disrupted, resulting in the characteristic symptoms of chronic suppurative otitis media[35]. The most prevalent infections involved in CSOM are Pseudomonas aeruginosa and Staphylococcus aureus[36]. Also, it has been shown that a state of oxidative stress-related middle ear damage is present in patients with COM, otitis media with effusion, and tympanosclerosis[37].

Pathophysiology of CSOM begins with irritation of the middle ear mucosa and subsequent inflammation[30]. Bacterial pathogens infiltrate the mucosa of the middle ear through the external canal in chronic suppurative otitis media. An inflammatory reaction occurs in the middle ear, which is followed by edema and fibrosis, as well as spontaneous tympanic membrane perforation and infection[29,38]. Bacterial biofilms (that is, colonization of bacteria embedded in the extracellular matrix and adherent to a surface), which are known to protect bacteria against antibiotic treatment[39]and the host's immune response, have been demonstrated in the middle ears of patients with CSOM [40]. Inflammation causes mucosal ulcers and epithelial lining disintegration. In the middle ear, granuloma formation can lead to polyps. This process could continue, damaging

nearby structures and resulting in CSOM complications [31]. Oxidative stress-mediated damage of the mucosa may delay recovery and may predispose to persistent middle ear effusion and recurrent or chronic infection[37].

The most common symptom is persistent or recurring ear discharge caused by a tympanic membrane hole or a ventilation tube.CSOM causes conductive hearing loss and may damage the ossicles in the middle ear[41].

Diagnosis of CSOM, When a permanent tympanic perforation is discovered alongside middle ear mucositis with or without continuous otorrhea, CSOM is diagnosed[42]. Chronic suppurative otitis media may be diagnosed with Otoscopy or Otomicroscopy, but may require removal of ear discharge by suctioning for adequate visualization[9].

Treatment of CSOM, In CSOM, various novel adjuvant treatments have been tested aimed at enhancing the repair of tympanic membrane perforation, including biomolecules to stimulate growth of the perforated edges and bioengineered scaffolds [43]. Topical quinolones are the treatment of choice for chronic suppurative otitis media; they are equally or more effective as aminoglycosides and lack the risk of ototoxicity. Quinolones are effective in resolving otorrhoea and eliminating the microorganism [44]. In circumstances where a parental regimen is required, beta-lactam anti-pseudomonal medicines such as ceftazidime are employed. Pseudomonas sp. and Staphylococcus aureus are both susceptible to ticarcillin-clavulanate[29]. Unlike OME and AOM, CSOM is frequently treated surgically, using a variety of methods meant to restore the ear drum and control infection[45].

1.3.Galectin-3

Galectins are soluble small molecules with a preference for β-galactosides. Approximately 15 galectins have been discovered in mammals so far, all of which share a conserved carbohydrate recognition domain (CRD) but differ in their function excellent specificity[46]. Galectins 1, 2, 5, 7, 10, 11, 13, 14, and 15 are prototype galectins with a single CRD; Galectins 4, 6, 8, 9, and 12 are tandem galectins with two distinct but homologous CRDs; and Galectin-3 (Gal-3) is the only member of the chimera-type group, which has a C-terminal CRD and a large N-terminal (NT) protein-binding domain[47]. Galectins are involved in a wide range of biological functions including development, cell polarity, immunology, inflammation, apoptosis, and cancer[48]. Human galectin-3 is a 35-kDa protein encoded by LGALS3, a single gene on chromosome 14 that codes for it. Galectin-3's N-terminal domain is required for multimerization, is vulnerable to proteolysis by matrix metalloproteinases, and may interact with other intracellular proteins[49].

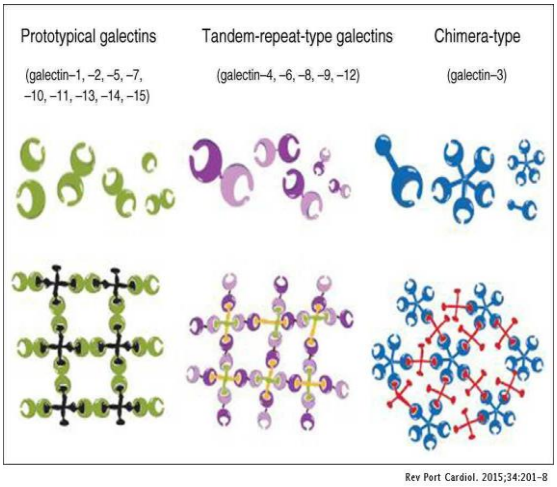


Figure (1-3): Galectin family members[50].

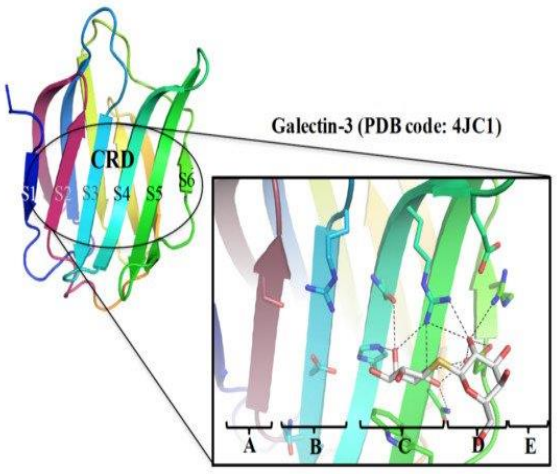


Figure (1-4): Galectin-3 structure[108]

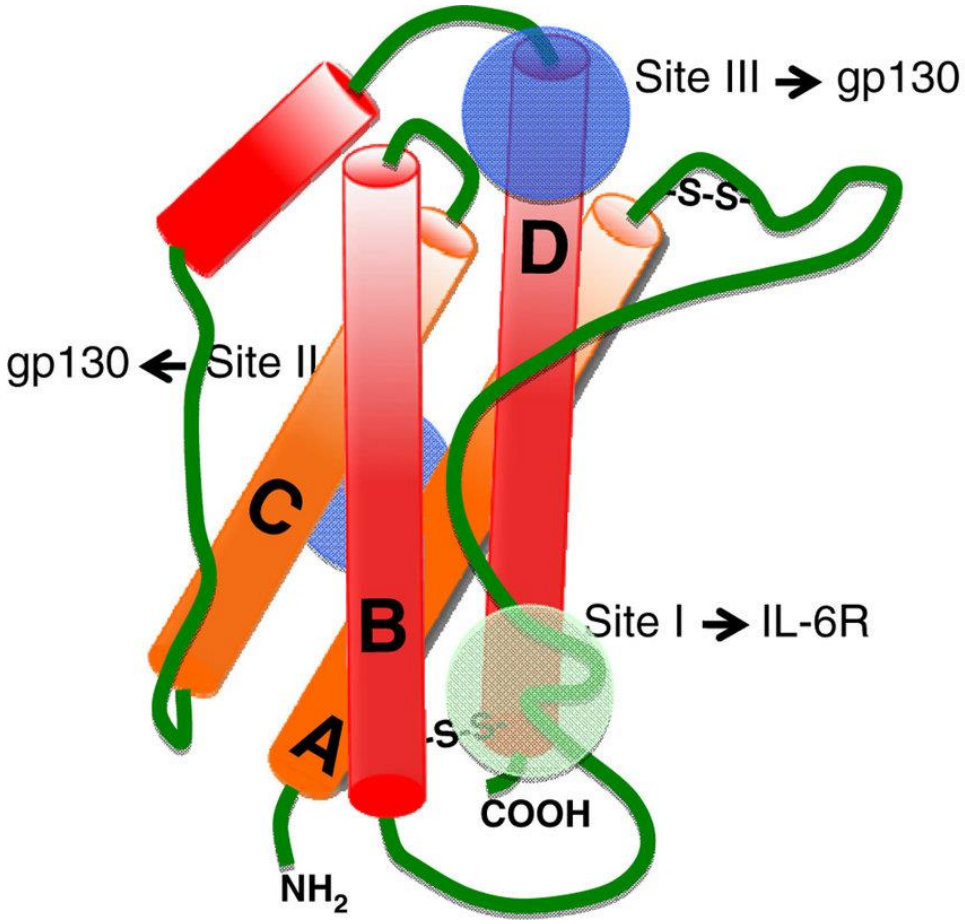
The cytoplasm, nucleus, endosomal compartments, and extracellular space have all been found to contain Galectin-3 (Gal3)[51]. All types of immune cells (macrophages, monocytes, dendritic cells, eosinophils, mast cells, natural killer cells, and activated T and B cells), epithelial cells, endothelial cells, and sensory neurons produce galectin3[52]. Gal-3 is secreted either passively by dying cells or actively by activated cells[53]. Exosomes or microvesicles were found to contain secreted Gal3 depending on the cell type. Exosomes and microvesicles are characterized by their physical and morphological features as well as their origin. Exosome biogenesis begins with the development of intraluminal vesicles (ILVs) in multivesicular bodies, which are special late endosomes (MVBs). ILVs are released into the extracellular area as exosomes when MVBs fuse with the plasma membrane[48]. The Gal-3 gene (LGALS3) in humans is 17 kb long and has 6 exons and 5 introns[47]. Galectin3 expression levels have been found to be elevated and lowered in a variety of diseases, including cardiac, kidney, liver illness, cancer, and infections[54].

Function of Galectin-3 is primarily found in the cytoplasm, however it can also be found in the nucleus. It's also secreted onto the cell surface and into body fluids[55]. In the cytoplasm, Galectin3 interacts with several survival-associated proteins in the cytoplasm, including B-cell lymphoma 2 (Bcl2) and activated guanosine5'triphosphate (GTP)bound KRas, which are both critical for cell survival. In the nucleus, Galectin-3 has the ability to cause pre-mRNA splicing and regulates gene transcription [56]. Extracellular, Because of its propensity to multimerize and bind glycoconjugates on the cell surface or in the ECM, extracellular Gal-3 acts

as an adhesion modulator. Laminin, fibronectin, collagen IV, and elastin are binding partners in the ECM[57]. Extracellular Gal-3 appears to primarily promote apoptosis, but intracellular Gal-3 appears to inhibit cell death[58].

Galectin-3 in immune response; Once in the extracellular medium, Secreted Gal-3 can operate as a pattern-recognition receptor (PRR) and an activator or modulator of innate immunity cells once it reaches the extracellular media, and it is also thought to be a potential damage-associated molecular pattern (DAMP)[53]. Gal-3 effects on immune cell function can be mediated via external Gal-3 binding to cell membrane receptors or intracellular Gal-3 regulating intracellular protein activity[47]. Galectin-3 is a potent pro-inflammatory signal in general. In response to diverse inflammatory stimuli, some cells generate and secrete a considerable amount of galectin-3. Galectin-3, when secreted or externalized, may have an autocrine or paracrine effect on inflammatory cells. It serves as a chemoattractant for monocytes and macrophages, stimulates the adherence of human neutrophils to laminin and endothelial cells[59]. Increased Gal-3 levels enable cytokines like IL-6 to be secreted, which upregulate E-selectin, Intercellular Adhesion Molecule 1(ICAM-1), and integrins on endothelial cells for improved adherence[60]. The anti-apoptotic activity of galectin-3 is apparently dependent on phosphorylation of Ser6. The phosphorylation is also necessary for the export of the protein out of the nucleus when cells are exposed to apoptotic stimuli [50]. Depending on a variety of factors, such as specific inflammatory conditions, the type of targeted cell, or its expression level, galectin-3 can be both a positive and negative regulator of inflammatory response[59].

1.4.Interleukin-6 (IL-6)



Figure(1-5):Schematic representation of the domain structure of human IL-6[62].

Interleukin-6 is a cytokine that has a diverse set of biological functions. It functions as a mediator for immunoglobulin type substitution and acute phase response regulation. It's also a sign of inflammation in the body. IL-6 can also be used as an investigative marker in the case of bacteremia[61]. IL-6 is produced at the site of inflammation and is important in the acute phase response, which is defined by a number of clinical and biological characteristics such as the creation of acute phase proteins[63].

IL-6, which is released quickly and transiently in response to infections and tissue damage, aids in host defense by stimulating acute phase responses, hematopoiesis, and immunological responses[64]. IL-6 elicits not only acute phase reactions but also the development of specific cellular and humoral immune responses, including end-stage B cell differentiation, immunoglobulin secretion and T cell activation. The main switch from acute to chronic inflammation is the recruitment of monocytes to the area of inflammation. IL-6 is important to the transition between acute and chronic inflammation[65]. IL-6 has a detrimental influence on chronic inflammation and autoimmunity, despite the fact that its expression is tightly controlled by transcriptional and posttranscriptional processes[64]. By shifting the type of the leucocyte infiltration (from polymorphonuclear neutrophils to monocyte/macrophages), IL-6, in conjunction with its soluble receptor sIL-6R, regulates the transition from acute to chronic inflammation[63].

The Interleukin 6 receptor (IL-6R) is a transmembrane protein found on hepatocytes and some leukocyte subsets, but soluble isoforms of the IL-6R (sIL-6R) are produced by alternative splicing or limited proteolysis of the ADisintegrin And Metalloproteinases (ADAM) gene family members ADAM10 and ADAM17[66]. IL-6 is produced by the macrophages and monocytes in the initial stage of infectious inflammation immediately after the stimulation of Toll-like receptors (TLRs) with separate Pathogen-associated molecular patterns (PAMPs)[67].

Synthesis of IL-6 , Nuclear factor-kappa B (NF-B) stimulates mRNA transcription of IL-6 and other pro-inflammatory cytokines, such as tumor necrosis factor (TNF) and IL-1, in monocytes and macrophages after

activation of cell surface and intracellular TLRs . TNF or IL-1 signaling also causes IL-6 mRNA transcriptionHepatocytes, neutrophils, monocytes, activated B cells, and CD4 T cells, for example, have high affinity for IL-6 because they express both membrane IL-6R and gp130[68]. Interleukin-6 in acute inflammation, the body's response to infection and injury entails a slew of changes both near and far from the site of inflammation. This physiological state appears at the start of the inflammatory process and lasts for 1-2 days, These events cause an acute-phase systemic reaction, which is characterized by a wide-ranging systemic response [69].

IL-6 travels through the bloodstream to the liver, where it causes a rapid induction of a wide range of acute phase proteins, including C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen, haptoglobin, and 1-antichymotrypsin, IL-6, on the other hand, suppresses fibronectin, albumin, and transferrin synthesis[64]. CRP is utilized to increase bacteria's phagocytic rate, while SAA is used to change the rate of protein gene transcription[70].

Interleukin-6 in chronic inflammation, Chronic inflammation, which involves immunological responses, develops from acute inflammation. Through continual MCP-1 production, angioproliferation, and antiapoptotic actions on T cells, IL-6 plays a negative role in chronic inflammation, favoring mononuclear cell accumulation at the site of injury [71]. This may increase serum levels of IL-6 and provide the basis for the amplification step of chronic inflammatory proliferation[63].

1.5. Selenium

Selenium (Se34 79) is a metalloid that has characteristics similar to sulfur (S).The amount of Se in soil varies depending on the soil type, texture, and organic matter level. and when it rains. The physico-chemical properties of it influence its uptake by plants. soil characteristics (redox status, pH and microbial activity)[72]. Selenium is a mineral that can be found in soil. Selenium can be found in water and certain foods. While only a trace amount is required, selenium is essential for human metabolism[73].

Brazil nuts, seafood, offal, eggs, meat, and grains are all high in selenium[74]. Because the amount of selenium in different crops is strongly reliant on the amount of selenium in the soil, dietary consumption varies greatly between geographical locations[75]. The biological effects of selenium are mediated via selenoproteins. In the human proteome, there are a total of 25 selenoproteins, but not all of them have been functionally characterized[76].

Selenium is incorporated into selenoproteins as selenocysteine, which replaces the sulfur atom of cysteine with a selenium atom. When compared to a thiol group, the functional selenol group has a higher reactivity. Except for selenoprotein P, which has ten selenocysteine residues, all selenoproteins have one selenocysteine residue. This selenoprotein is the most common form of selenium in circulation and storage, and it appears to have a function in antioxidant protection[76]. Several other selenoproteins, such as glutathione peroxidases (GPx) and thioredoxin reductases, have antioxidative characteristics (TrxR). GPx catalyzes the reduction of

hydrogen peroxide(H_2O_2) to water(H_2O) and glutathione disulfide, whereas TrxR catalyzes the reduction of the redox protein thioredoxin as well as other endogenous and foreign chemicals[77].The deiodinases are another type of selenoprotein that regulates thyroid hormone function by catalyzing the conversion of thyroxine (T4) to triiodothyronine (T3, active form)[78]. Selenium is absorbed most effectively in the lower section of the small intestine. This is thought to happen through a variety of membrane transport processes, some of which are similar to those for sulfur[74]. There appears to be no homeostatic regulation of selenium uptake, with absorption ranging from 70 to 90% depending on selenium species. After being absorbed and transported to the liver, dietary selenium (mostly selenomethionine, selenocysteine, selenite, and selenate) is converted to selenoproteins through a series of intermediate stages for which the details remain unknown[77].

Selenomethionine is processed similarly to methionine and can be incorporated into methionine-containing proteins at random[74,75]. Excess selenium must be excreted in order for the body to function properly. Selenium is methylated and eliminated primarily in urine and, to a lesser extent, in breath [particularly at high exposure[74]. diseases are frequently linked to increased oxidative stress. Through its engagement in many antioxidative mechanisms, selenium may be thought to be preventative. For the same reason, it's possible that selenium could protect those who are highly exposed to pro-oxidants like arsenic, cadmium, or mercury[79]. In the immune system, selenium stimulates antibody formation and activity of helper T cells, cytotoxic T cells and Natural Killer (NK) cells[72].

Function,Selenium is a non-essential trace element. It is simply required in little amounts by your body. Selenium aids in the production of antioxidant enzymes, which are particular proteins found in your body[80]. These aid in the prevention of cell damage. According to some study, selenium may help with Certain malignancies can be avoided. Protect the body from the toxicity of heavy metals and other potentially dangerous elements[72]. Selenium is needed for the development of thyroid hormone-metabolizing enzymes, and it is hypothesized that selenium supplementation can help thyrocytes and immune cells work better[81].

1.6.Cadmium

Cadmium is an immunotoxic heavy metal that is utilized as an anticorrosive, stabilizing, or coloring agent in a variety of industries[82]. Cadmium is a widespread toxic environmental metal contaminant and potential toxin, which may adversely affect human health either naturally or because of industrial use. Modes of exposure are either through intake of contaminated food, drinking water, or by inhalation of polluted air or occupational in industries[83]. Tobacco smoke and occasionally occupational exposure also contribute to the body burden of cadmium[84]. Smokers have approximately twice the cadmium body burden than nonsmokers[85]. Regardless of the route of exposure, cadmium is efficiently retained in the organism and remains accumulated throughout life. Cadmium irreversibly accumulates in the human body, particularly in kidneys and liver.

In addition to its cumulative properties, it is also a highly toxic metal that can disrupt a number of biological systems, usually at doses that are much lower than most toxic metals. Cadmium is absorbed mainly through

the respiratory and digestive tracts and under conditions of chronic exposure; it is transported in blood and bounded mainly to metallothionein [86]. Substitutes for zinc and copper in metalloenzymes and has a high affinity for sulfhydryl groups that reducing the effectiveness of metalloenzymes[87]. The reduction of activities of several antioxidant proteins (catalase, glutathione reductase, total glutathione), may cause the accumulation of ROSs in cells[88]. Indirectly, this overproduction of oxidant molecules may be also responsible for the generation of abnormal proteins and lipid peroxidation[89]. A primary mechanism for cadmium toxicity is in its effect on cells which has been ascribed to the oxidative stress[90]. Cadmium induces mitochondrial dysfunction with inhibit respiratory electron transport of complex III[91]. Reduction of ATP; inactivating numerous enzymatic reaction[92]. Other mechanism is the ability of Cd to replace Fe, a redox reactive metal, thereby increasing the availability or free Fe in cells and hence inducing oxidative stress. As a redox-reactive element, Fe in its turn produces highly damaging hydroxyl radicals via the Fenton reaction[93].

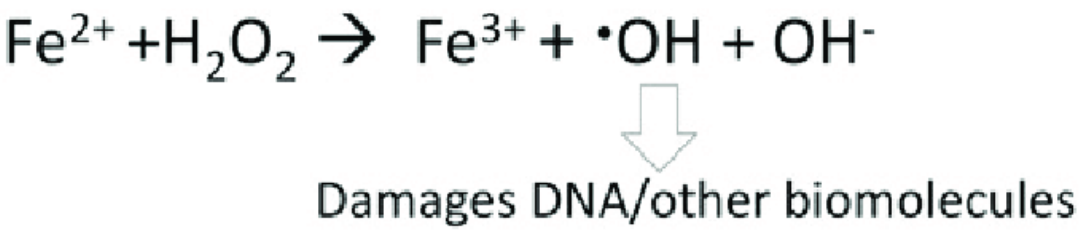


Figure (1-6): Fenton reaction [109].

Cadmium affects cell cycle progression, proliferation, differentiation, DNA replication, and repair as well as apoptotic pathways[94]. In addition to its

role as a generator of ROSs, involved in the occurrence of DNA damage, cadmium may also reduce cellular antioxidants levels[95] . A recent study found that cadmium exposure can reduce cell viability, apoptosis, and necrosis in middle ear cells[96]. According to researchers, an imbalance between oxidative stress and antioxidant enzymes, which could be produced by cadmium exposure, plays a key role in the development of COM[97,98]. As a result, cadmium-induced oxidative stress or cytotoxicity of middle ear cells may increase vulnerability to recurring bacterial infection in the middle ear, leading to COM[84,99].

1.6.1.Interactions Between Selenium And Cadmium

Selenium may protect against the hazardous effects of metals/metalloids like arsenic, cadmium, and mercury, according to certain studies. The toxicity of such elements is mostly due to the formation of reactive oxygen species (ROS)[100], and one of the proposed mechanisms for selenium's beneficial impact is antioxidative protection via selenoproteins like GPx and TrxR. Sequestration into inert conjugates for excretion has also been suggested. Although the development of such complexes may help to reduce metal-induced toxicity, it may also lead to functional selenium insufficiency[101]. Animal research on selenium and cadmium interactions have revealed an antagonistic relationship between the two elements, with selenium enhancing antioxidant defenses and reducing oxidative stress produced by cadmium exposure[102]. Indeed, cadmium has been demonstrated to reducing the antioxidant defense mechanism in various organs, including the brain[103], which could be countered by selenium supplementation[104].

The authors discovered a protective effect of injected selenite in animals exposed to a lethal dosage of cadmium chloride in 1946, indicating that selenium and cadmium have an antagonistic connection. Later, it was revealed that injected selenium might protect rats from developing testicular cancer induced by injected cadmium, a finding that was later verified by others. Nonetheless, other investigations indicated that as selenium levels rose, so did the concentration of cadmium in the testes and blood[105]. Finally, some observational studies have discovered a higher link between cadmium and negative health outcomes in people with the lowest levels of selenium[106].

Others, on the other hand, have found no evidence that selenium protects against cadmium-induced birth defects[107].

1.7.Aim of the study

1. Measuring CRP,ESR,RBS and HbA1C in patients with chronic otitis media and healthy control
2. Measuring serum level of Galectin-3 and IL-6 in patients with chronic otitis media and healthy control
3. Measuring serum level of Selenium and Cadmium in patients with chronic otitis media and healthy control
4. Study the relationship between parameters

Chapter Two

Materials and Methods

2.Materials and Methods

2.1.Materials:

2.1.1. Chemical (kits)

Enzyme-linked immunosorbent assay (ELISA Kits) and Graphite Furnace Atomic Absorption Spectrometry (GFAAS) used in this study.

All kits used in the study are listed in **Table(2-1).**

Table (2-1): List of chemical (Kits) used in the study.

No	The Item	Name of the Company	Manfacuture Country
1	Cadmium	Sigma	Germany
2	C-Reactive Protein	Linear	Spain
3	ESR	Al.malak	China
4	Galectin -3	Mybiosource	USA
5	Glucose	Linear	Spain
6	HbA1c	Ichroma	korea
7	IL-6	Mybiosource	USA
8	Selenium	Sigma	Germany

2.1.2. Instruments and Equipments

The instruments and Equipment's used in this study are listed in the **Table (2-2).**

Table (2-2): The instruments and equipment that used in the study.

NO.	Instruments	Company	Country
1	Atomic Absorption Spectrophotometer(GFAAS)	Shimadzu	**Japan**
2	Centrifuge	kokusan	**Japan**
3	Cotton	Local company	**Iraq**
4	Disposable syringe	Medeco	**Germany**
5	ELISA washer, reader and printer	Human	**Germany**
6	Eppendorf tube (1.5ml)	karlkole	**Germany**
7	Examination Gloves	WRP Asia	**Malaysia**
8	Gel tube	karlkole	**Germany**
9	Incubator	Friocell	**Germany**
10	Multichannel micropipette reservoir	Mybiosource	**USA**
11	Refrigerator	Hitachi	**Japan**
12	Spectrophotometer	Jenway	**Italian**
13	Water Bath	HH-2	**Chain**
14	(10-1000) µL micropipettes with disposable tips.	Mybiosource	**USA**

2.2. Study Group:

The study was carried out at the department of biochemistry / college of medicine/ Babylon University and ENT department in Imam Sadiq Hospital. The data was collected from the study participants directly and filled in prepared questionnaire.The study included 100 Persons that were involved divided into two groups:

1) Group 1: 50 healthy (29 male and 21 female) with age range (15-65) years.
2) Group 2: 50 (100 ear) chronic otitis media patient (29 male and 21 female) with age range (15-65) years.

*Inclusion criteria

1-The criteria of inclusion were patients with chronic otitis media

2-Adult patients with age (15-65)

*exclusion criteria

The criteria of exclusion were patients with

1- obesity

2- rheumatoid arthritis

3- kidney disease

4- other chronic disease

2.3. Methods

2.3.1. Collection of Samples

The study samples were serum.

*Blood collection

Venous blood was collected from each participating subject (control and patient), bloods were taken about 10 mL of the venous blood was obtained by using a 10 mL replaceable syringe. the blood was distributed in a gel tube to separate serum after clotting, venous blood was centrifuged at 1000 xg at the room temperature for a period of time (10 minutes), and then the blood serum was isolated, split into aliquots an eppendorf tube, and put away in a deep freeze at (-20°C) till date of assayed

2.3.2. Biochemical assay:

2.3.2.1. Glucose Measurement

Principle

In the Trinder reaction, the glucose is oxidized to D-gluconate by the glucose oxidase (GOD) with the formation of hydrogen peroxide. In the presence of peroxidase (POD), a mixture of phenol and 4-aminoantipyrine (4-AA) is oxidized by hydrogen peroxide, to form a red quinoneimine dye proportional to the concentration of glucose in the sample.

$$\beta\text{-D-Glucose} + H_2O + O_2 \xrightarrow{GOD} \text{DGluconate} + H_2O_2$$

$$4\text{-AA} + \text{Phenol} \xrightarrow[POD]{H_2O_2} \text{Quinoneimine} + H_2O$$

Reagent Composition

R1	Monoreagent.	Phosphate buffer glucose oxidase peroxidase 4-aminoantipyrine phenol
CAL	Glucose standard.	Glucose 100 mg/dL

Assay Procedure

1.Reagents and samples were brought to room temperature.

2.Pipette into labelled tubes:

Tubes	Blank	Sample	CAL. Standard
R1.Monoreagent Sample CAL.Standard	1.0 mL – –	1.0 mL 10 µL –	1.0 mL – 10 µL

3.Tubes were mixed and let stand 10 minutes at room temperature or 5 minutes at 37ºC.

4. The absorbance (A) of the samples and the standard were read at 500 nm against the reagent blank.

The color is stable for about 2 hours protected from light.

Calculations

$$\frac{A\ Sample}{A\ Standard} \times C\ Standard = mg/dL\ glucose$$

2.3.2.2. HbA1c Measurement

Principle

The test uses a sandwich immunodetection method. The detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip. The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody. Instrument for ichroma™ tests displays the content of glycated hemoglobin in terms of percent of the total hemoglobin in blood.

Assay Procedure

1)100 µL of hemolysis buffer was drawn and transferred it into detection buffer tube.

2) 5 µL of fingertip blood or tube blood was drawn, 5 µL capillary tube was used and put the capillary tube into the detection buffer tube.

3) The lid of the detection buffer tube was closed and mixed the sample thoroughly by shaking it about 15 times.

4) The cartridge half was taken out form i-Chamber slot.

5) 75 µL of the sample mixture was pipetted out and loaded it into a sample well in the test cartridge.

6) The cartridge was inserted into i-Chamber slot.

7) The cartridge was left in i-Chamber for 12 minutes before removing. Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.

8) The sample-loaded cartridge was scanned, it was inserted into the cartridge holder of the instrument for ichroma™ tests.

9) 'Select' button on the instrument for ichroma™ tests was pressed to start the scanning process.

10) Instrument for ichroma™ tests was started scanning the sample-loaded cartridge immediately.

11) The test result was read on the display screen of the instrument for ichroma™ tests.

2.3.2.3. C-reactive protein Measurement

Principle

CRP-Latex test is a rapid slide agglutination procedure based on a modification of the latex fixation method. developed for the direct detection and semi-quantitation of C-reactive protein (CRP) in serum. The assay is performed by testing a suspension of latex particles coated with anti-human CRP antibodies against unknown serum. The presence of a visible agglutination indicates an increase of the CRP level above the upper limit of the reference interval in the samples tested.

Reagent Composition

R	CRP-Latex Reagent. Suspension of polystyrene latex particles coated with specific anti-human C-reactive protein antibodies in a buffered saline solution. Contains 0.95 g/L of sodium azide
CONTROL +	Human serum with a CRP concentration > 15 mg/L. Contains 0.95 g/L of sodium azide.
CONTROL -	Animal serum with a maximum concentration of human CRP of 1 mg/L. Contains 0.95 g/L of sodium azide.

Assay Procedure

1.The test reagents and samples were brought to room temperature.

2.The Reagent vial was mixed gently. Aspirate dropper several times to obtain a thorough mixing.

3. 1 drop (50 µL) of the serum was placed under test into one of the circles on the card. Dispense 1 drop of positive control serum and 1 drop of negative control serum into two additional circles.

4. 1 drop of CRP-Latex Reagent was added to each circle next to the sample to be tested.

5.The contents of each circle were mixed with a disposable stirrer while spreading over the entire area enclosed by the ring. Separate stirrers were used for each mixture.

6.The slide means of a mechanical rotator (100 r.p.m) were Rotate for a period of 2 minutes.

7.Agglutination had been Observed immediately under a suitable light source

Reading

Nonreactive: Smooth suspension with no visible agglutination, as shown by negative control.

Reactive: Any degree of agglutination visible macroscopically

2.3.2.4. Erythrocyte Sedimentation Rate (ESR) Measurement

The ESR system consists of a pipette and a tube. An upper sealing cap Was located on the top of the pipette. The tube with a removable and pierceable rubber stopper was supplied with pre-filled 3.8% sodium citrate. Once blood and citrate were mixed together,the pipette was inserted through the stopper and pushed to the bottom of the tube, the blood automatically was reached the 0 level

2.3.2.5 Galectin-3 measurement

Galectin-3 was detected quantitatively in the human serum by ELISA kit.

Principle:

GAL-3 ELISA kit applies the competitive enzyme immunoassay technique utilizing a monoclonal anti-GAL-3 antibody and an GAL-3-HRP conjugate. The assay sample is incubated with GAL-3-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the GAL-3 concentration since GAL-3 from samples and GAL-3-HRP conjugate compete for the anti-GAL-3 antibody binding site. Since the number of sites is limited, as more sites are occupied by GAL-3 from the sample, fewer sites are left to bind GAL-3-HRP conjugate. A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of standards. The GAL-3 concentration in each sample is interpolated from this standard curve

Reagents

NO.	Materials	Specification
1	Microtiter Plate	96 wells
2	Enzyme Conjugate	6.0 mL
3	Standard A	0 ng/mL
4	Standard B	2.5 ng/mL
5	Standard C	5.0 ng/mL
6	Standard D	10 ng/mL
7	Standard E	25 ng/mL
8	Standard F	50 ng/mL
9	Substrate A	6 mL
10	Substrate B	6 mL
11	Stop Solution	6 mL
12	Wash Solution (100 X)	10 mL

Assay Procedure

1) The desired numbers of coated wells were secured in the holder then added 100 µL of Standards or Samples to the appropriate well in the antibody pre-coated Microtiter Plate.

2) 100 µL of PBS (pH 7.0-7.2) was added in the blank control well.

3) 50 µL of Conjugate was added to each well (NOT blank control well). mixed well. Mixing well in this step is important. the plate was

Covered and incubated for 1 hour at 37°C.

4) Automated Washing: plate was washed FIVE times with diluted wash solution (350-400 µL/well/wash) using an auto washer. After washing, the plate was dried as above.

5) 50 µL Substrate A and 50 µL Substrate B were added to each well including blank control well, subsequently. It was Covered and incubated for 10-15 minutes at 37°C.

6) 50 µL of Stop Solution was added to each well including blank control well. Mixed well.

7) The Optical Density (O.D.) was Determined at 450 nm using a microplate reader immediately.

Calculation

The concentration was evaluated employing a calibration curve which is established from the calibrators supplied with the kit. The relative O.D at (450nm) = the O.D at (450nm) of each standard solution (Y) vs. the respective concentration of the standard solution (x). The Galectin-3 level in samples can be interpolated by the standard curve.

2.3.2.6. interleulin-6 measurement

IL-6 was detected quantitatively in the human serum by ELISA kit.

Principle:

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay to assay the level of human Interleukin 6 in samples. IL-6 is added to monoclonal antibody enzyme well which is pre coated with human IL-6 monoclonal antibody, incubation; then, IL-6 antibodies labeled with biotin, and combined with streptavidin-HRP are added to form immune complex, then incubation is carried out and washing again to remove the uncombined enzyme. The chromogen solution A, B, are added, the color of the liquid changed into the blue, and at the effect of acid (stop solution) the color finally became yellow. The chroma of color and the concentration of human substance IL-6 of sample were positively correlated. The optical density was measured under 450 nm wavelength (within 15 minutes after adding the stop solution).

Reagents

NO.	Materials	Specification
1	Microtiter Plate	96 wells
2	Enzyme Conjugate	6.0 mL
3	Standard A	3.12 pg/ml.
4	Standard B	6.25 pg/ml.
5	Standard C	12.5 pg/ml.
6	Standard D	25 pg/ml.

7	Standard E	50 pg/ml.
8	Standard F	100 pg/ml.
9	Substrate A	6 mL
10	Substrate B	6 mL
11	Stop Solution	6 mL
12	Wash Solution (20×)	25 mL

Assay Procedures

1- The Plate and equipment was checked before experiments and made sure they are no problem. the labels and the color of the covers of the vials/bottles were checked and made sure they are matched and no mistake.

2- The Plate, all reagents and samples were allowed to come to room temperature (18°C-25°C) naturally before starting assay procedures.

3- The Plate was removed from the foil pouch. The Plate is detachable, the unused strips were returned to the foil pouch with the desiccant pack, and reseal for preventing damp.

4- Blank wells; Standard wells and Sample wells were set.

5- Nothing was added to all Blank wells; 50 µl Standard (S1, S2, S3, S4, S5, S6) was added to corresponding Standard wells; 50 µl Sample ws added to every Sample well.

6- 100 µl HRP-Conjugate Reagent was added to every well except blank wells;

7- The Plate was covered with a Closure Plate Membrane and incubated for 60 minutes at 37°C.

8- all wells (including all Blank wells) were washed 4 times.

9- 50 µl Chromogen Solution A was added to every well.

10- 50 µl Chromogen Solution B was added to every well.

11- The Plate was mixed gently and incubated for 15 minutes at 37°C.

12- 50 µl Stop Solution was added to every well.

13- The Optical Density (O.D.) was read at 450 nm using an ELISA reader within 15 minutes after adding Stop Solution (around 5 minutes is usually the best time).

Calculation:

1. As the horizontal value ,The standard concentration was plotted and the corresponding OD value was drawn as the vertical value.

2. The resulting calibration curve was utilized to determine values of the samples. The OD values of the samples were correlated with the corresponding IL-6 antibody by interpolation.

3. The OD value was utilized for each sample to determine the concentration of Il-6 in ng/L from the standard curve.

2.3.2.7. Selenium and Cadmium Measurement

This experiment use Graphite Furnace Atomic Absorption Spectrometry (GFAAS) in which has the higher sensitivity and reach low detection limits (in ppb), Samples were digested by transferring (0.5 mL) of serum into a Pyrex test tube .Then (1mL) nitric acid was added to the serum of these samples which placed on vortex for 10 minutes so the solution was mixed vigorously , samples were heated for 15 minutes and finally diluted to 10 mL with deionized water . After cooling, solution was filtered and then appropriate solution volume of 10 µL was injected into the graphite tube for reading. very small amount of samples (10µL -20µL) is injected in a small graphite or paralytic carbon coated graphite tube, which can then be heated by a wide range of temperature to vaporize and atomize the analyst. The atoms absorb the electromagnetic radiation in the ultraviolet or visible region resulting in transitions of electrons to higher electronic energy levels to the excited state and then back to the ground state by emitting it's specific characteristic light which can be measured to determine the samples concentrations(110,111).

Preparation of Selenium and Cadmium Standard Solutions:

Stock Solution of Se and Cd (1000 µg/ml in 2% HNO3)

Five standard solutions of selenium and cadmium were prepared (20 , 40 ,60 , 80 , 100 ppb) , stock standard solutions of selenium and cadmium were prepared by taking (1 ml) of 1000 µg/mL (Se , Cd) and diluting it to

(100 mL) by deionized water, a 10 µg / mL (ppm) standard was formed ,then 10 ml from this stock (10 ppm) was pipetted into 100 mL of deionized water to form stock of (1 ppm = 1000 ppb) which it used to make up five standard above . All stock standards were prepared by using deionized water as diluents.

Condition of Selenium Determination:

Variable	Ideal condition
Lamp current(mA)	23
Wavelength(nm)	196.0
Slit width(nm)	0.5
Slit height	Normal
Read time(s)	3
Replicates	3

Condition of cadmium Determination:

Variable	Ideal condition
Lamp current(mA)	8
Wavelength(nm)	228.8
Slit width(nm)	0.5
Slit height	Normal
Read time(s)	3
Replicates	3

2.5. Statistical Analysis

This study was a case –control research design. Statistical analysis done by SPSS 22, and Microsoft excel software v. 2016 were used for the data analysis and graphs plotting. No missing values were observed for any variable. Frequency and percentage used for categorical data, mean and SD for continuous data. person correlation show the correlation between continuous data. T-test used for evaluation differences between mean and median of continues variables. P-value less or equal to 0.05 is considered significant.

Chapter Three

Results and Discussion

3.Results &Discussion

3.1.General characteristics of studied groups

A total number of one hundred (100) patients and control ,50 patients with COM (21 females and 29 males) have been investigated in this study and 50 control were (21females and 29 males).The clinical and demographic characteristics of them and of age and sex-matched apparently healthy controls are presented in **Table (3-1)**.

BMI was (18.5-24) for patients and control

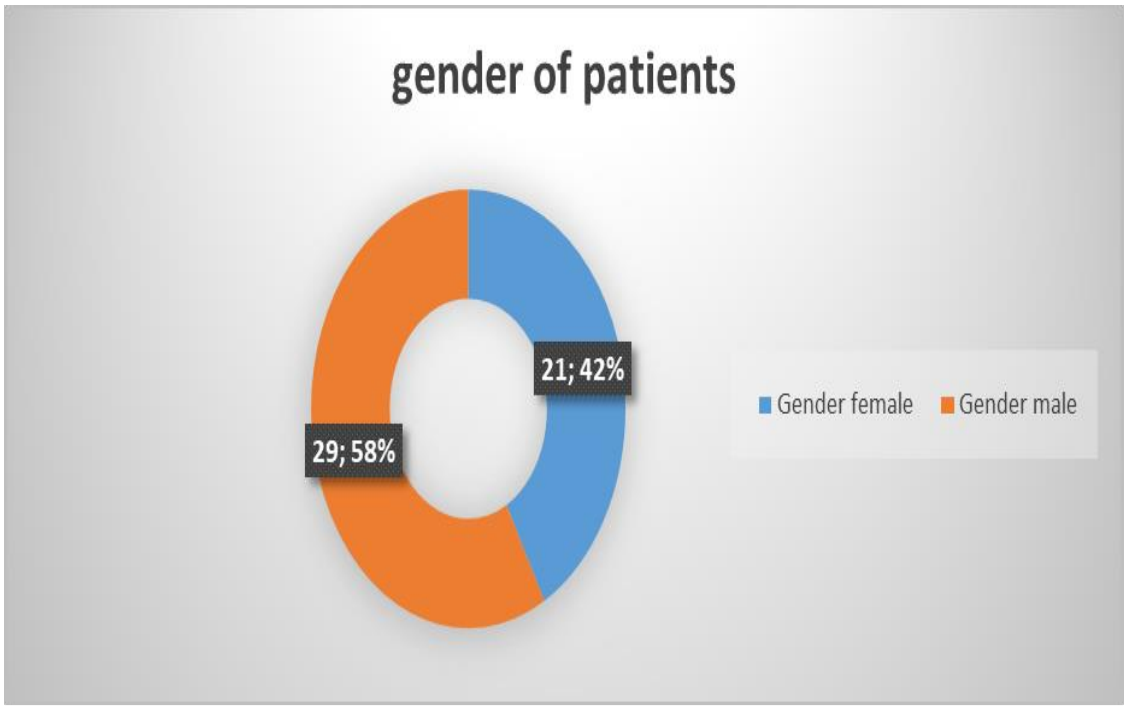
Table (3-1): Clinical and Demographic Characteristics of CSOM Patients and Controls

Characteristic		Patients N=50		Controls N=50		P-Value
		No	%	No	%	
Age (years)	15-65	50		50		0.066
	Mean ±SD	35.9 ± 8.5		33 ± 7.0		
Gender	Female	21	42%	Female	21	
	Male	29	58%	Male	29	
CRP	Negative	38	76%	50	100%	
	Positive	12	24%	0	0	

significant difference ($p \leq 0.05$)

This Table shows the clinical and demographic characteristics of study groups and in which the distribution of patients and control according to gender was 42% as females and 58% as males.The mean age of patients with COM was 35.9 ± 8.5 (15-65) years while was 33.0 ± 7.0 (15-65) years for controls.

There are 24% of patients with CRP positive, while 100% of control with CRP negative.



Fig(3-1):Percentage Distribution of Study Patients According to Gender.

In this study, 50 patients with CSOM , The percentage 42% as females and 58% as males as show in figure (3-1). the male was more than female because of Suggesting that boys may have a slightly higher risk for CSOM [160].

3.2.Descriptive Parameters:

The mean ± SD and the p-value of descriptive parameters in all studied Groups are presented in **Table(3-2)**

There is no significant difference mean of **RBS** according case and control groups, (P> 0.05) but **HbA1c** levels was significantly higher (P<0.01) in patients Compared with control group . **ESR** levels was significantly higher (P<0.01) in patients Compared with control group. **Table (3-2)** showed that there was in serum levels significant (P<0.001) increase in **GAL3**, **IL6** and **Cd** levels of patients compared with control groups and showed that there was a significant decrease (P<0.01) in **Se** level of patients compared with control groups. as shown in the **figure (3-2)**

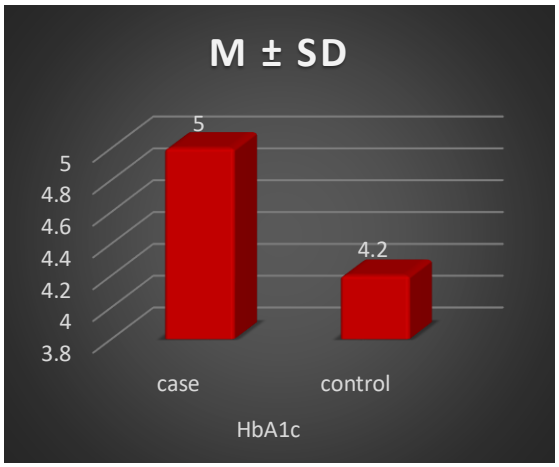
Table(3-2): difference mean all laboratory investigations according case and control groups

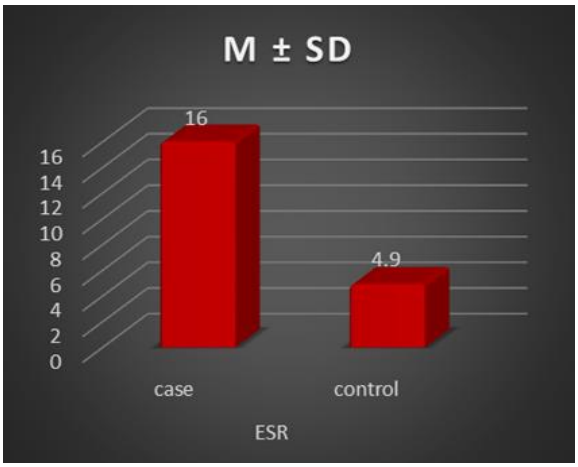
	Group	N	Mean ± SD	P-value
RBS (mg/dl)	***Case***	50	115.4±19.1	$> 0.05^{NS}$
	Control	50	110.3±17.5	
HbA1c	***Case***	50	5 ± 1.4	**< 0.05***
	Control	50	4.2 ± 0.6	
ESR	***Case***	50	16 ± 14	**< 0.01***
	Control	50	4.9 ± 3.2	
GAL3 (ng/ml)	***Case***	50	29 ± 9.5	**< 0.01***
	Control	50	8.6 ± 2.3	
IL6 (pg/ml)	***Case***	50	69.5 ± 22	**< 0.001***
	Control	50	11.2 ± 2.4	

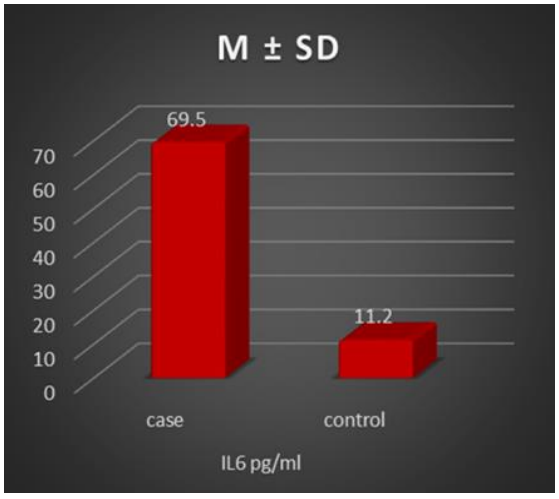
Se (ng/mL)	***Case***	50	28.5 ± 11.9	**< 0.01***
	Control	50	83 ± 15.7	
Cd (ng/mL)	***Case***	50	1.9 ± 0.85	**< 0.01***
	Control	50	0.5 ± 0.4	

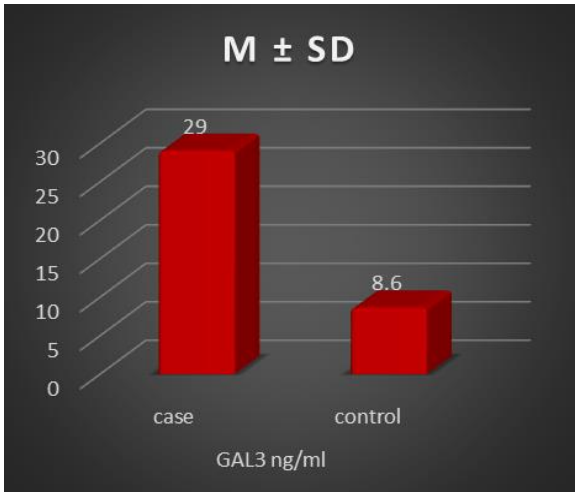
NS non statistical significance (p>0.05)

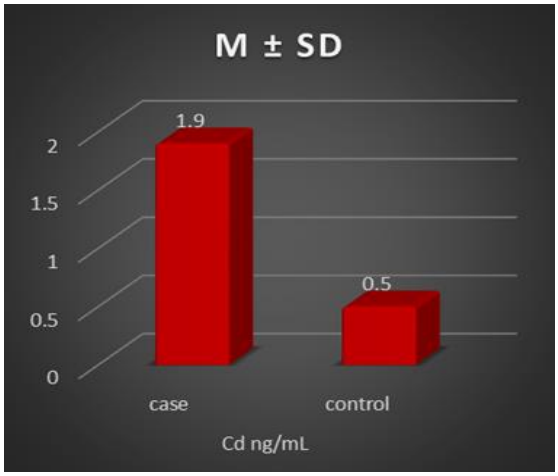
*significant difference (p≤0.05)

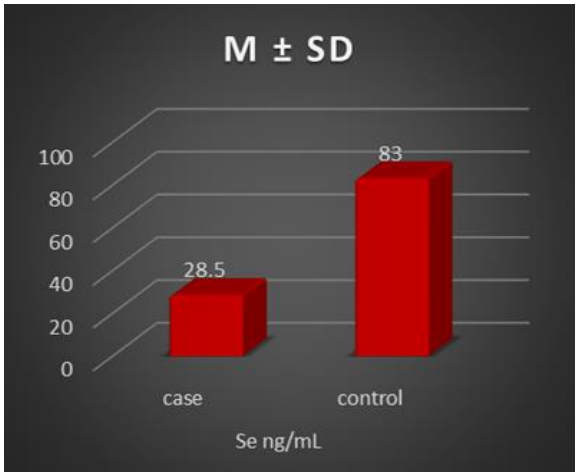












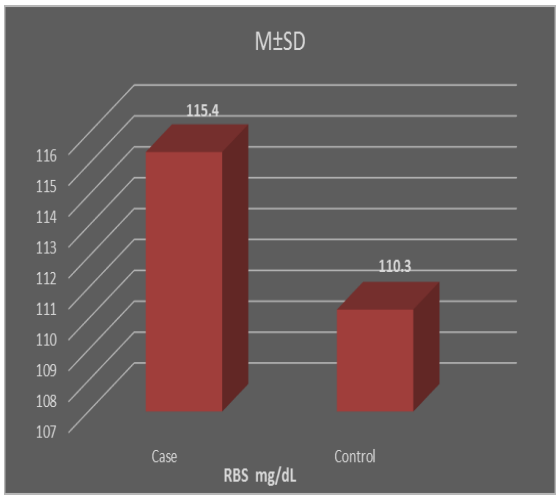


Figure (3-2); difference mean all laboratory investigations according case and control groups

HbA1c test is used to evaluate a person's level of glucose control. The test shows an average of the blood sugar level over the past 90 days and represents a percentage. The test can also be used to diagnose diabetes[157]. People with diabetes are more prone to ear infections over time. This is due to a combination of poor blood flow and high blood sugar, which can lower immune system response and make you more susceptible to infections. Otitis

media is prevalent among people with high blood pressure and diabetes mellitus[158].

ESR and CRP are blood indicators that are used to monitor inflammation. They are higher during acute inflammation as well as chronic inflammation. Although a high ESR/CRP results doesn't tell what's causing the inflammation, They can be a useful tool for monitor inflammation and to assess for improvement in inflammation over time. CRP is quicker- it rises faster and falls more quickly (about 3 days) after inflammation. ESR takes longer to rise and also stays higher for longer after inflammation is resolved [112].Results obtained in this study revealed that ESR/CRP levels in the blood of patients was significantly higher than healthy control groups, This finding is in agreement with that of (ÇeviKer ASA *et al*) demonstrated increase in ESR in patients with CSOM[113].

When comparing the patients group to the control group, GAL-3 levels were found to be significantly higher in the patient group, this study agree with (Pilette, C) .who found that epithelial Gal-3 was higher in chronic disease compared to healthy people[114].Gal-3 (either membrane associated or free) is involved in a wide range of processes, including pathogen immunity and acute and chronic inflammation]115].The current study found that 8 weeks of cadmium exposure increased proinflammatory cytokine production, apoptosis, and galectin-3 levels in the liver of rats[116].

The findings of this research indicated that the level of IL-6 in patients higher than controls, P-value (< 0.01) agreement with (Serban R *et al*), found that IL6 serum levels showed a high mean level in both males and females in patients with COM. It appears to have a important role in otitis

media inflammation. Mucin production in middle ear epithelial cells is regulated by IL6, which is implicated in the etiology of both serous and mucous otitis media[117]. Mucins are responsible for the high viscosity of middle ear effusions, which prevents normal mucociliary clearance and predisposes to chronic otitis media and hearing loss[118].

Endotoxin is a substance found in bacteria's cell walls that causes inflammation and infections in the ear. As well as in response to injury the keratinocyte may produce numerous critical soluble mediators independently from immune cells such as interleukin-6[119].The findings were comparable to those of Nofal, who found a greater blood level of IL6 in patients with AOM and CSOM when compared to a healthy control group[120].T cells, monocytes/macrophages, endothelial cells, fibroblasts, and hepatocytes are among the activated immune cells and stromal cells that produce IL-6,IL-6 has a vital function in the activation of both T and B cells. They are essential cells in the development of many autoimmune disorders and the principal drivers of adaptive immune responses, IL-6 is a crucial mediator of both innate and adaptive immune responses [121].

Interleukin-6 is a proinflammatory cytokine that induce of C-reactive protein (CRP) is a predominantly synthesized by the hepatocytes, under transcriptional control by the cytokine interleukin 6 increased serum levels of IL-6 and CRP in bacterial OM patients with an excellent diagnostic value given by IL-6 concentrations and a good one given by CRP in the differentiation between healthy people and OM patients [159].

The current study found that Se levels in patients were substantially lower than in apparently healthy controls. This research supports by (Arikan TA *et al*) who found that serum Se deficiency could be important and additive in the etiopathogenesis of COM[37]. Se is a trace element with antiproliferative and immune-modulating activities, as well as antioxidant characteristics. This active ingredient is engaged in redox processes, which protect membranes from oxidative damage, and can alter immunological response. This is accomplished by altering cytokine and receptor expression or making immune cells more resistant to oxidative stress[122].Several investigations have found that people with OM had reduced levels of antioxidant enzymes[123].In chronic suppurative OM, (Elemraid et al) found decreased Se levels. As a result, the low level of Se reported in our study could be one of the most important etiopathogenetic agents of COM, increasing oxidative stress and impairing immunological function[124].

Selenoproteins require numerous cofactors for synthesis and depend mostly on Se intake from the diet[125]. Glutathione peroxidase (GPx), a selenoprotein, detoxifies H_2O_2 and lipid peroxidation, removing harmful H_2O_2 that causes ciliary dysfunction and hairy cell death in the inner ear. As a result,H_2O_2 levels and oxidative stress rise in selenium insufficiency[126].

The levels of Cd in patients were found to be considerably higher in this investigation when compared to apparently healthy controls. This research agree with (Lee D-W *et al*) who founds that cadmium exposure in the environment is associated with an elevated risk of COM[84].Cd^{2+} decreases cell viability and promotes apoptosis in Human Middle Ear Epithelial Cells HMEECs. Cd increased the formation of reactive oxygen

species (ROS), as well as inflammation and mucin gene expression. Cadmium has been shown to impair the viability of inner hair cells by increasing oxidative stress. Mucin secretion and inflammatory cytokines(including IL-6) play essential roles in the development of OM[96]. Middle ear inflammation and the pathophysiology of COM have been linked to oxidative stress and the production of excessive free radicals[37].Cadmium, like other air pollutants or smoke, can enter the middle ear space through the Eustachian tube, or it can enter through systemic circulation. Cadmium has been found in ossicles as a result of systemic exposure[84]. Another role of Cd is that it influences the function of the Eustachian tube, the most important factor in the pathogenesis of middle ear disease in all age groups and leads to the development of COM. The Eustachian tube is a system of contiguous organs including the nose, palate, nasopharynx, middle ear, and mastoid air cells. When the Eustachian tube is not intact, reflux of nasopharyngeal secretion can occur, and viruses/bacteria from the nasopharynx can enter into the middle ear[37].

High levels of Cd activate the NF-kB protein, which promotes inflammation and compromises immunological function[128].Similarly to Se deficiency ,high Cd may impair immune processes and increase the risk of infection. By increasing nuclear factor-kB (NF-kB) protein activation, which plays a vital role in inflammatory and immunological responses in COM[129]. both the lower Se levels and the higher Cd levels identified in our study could contribute to the production of COM. Both contribute to disease creation processes that are similar, such as increased oxidative stress and reduced immunological responses[37]. Although the exact mechanism of this effect is unknown, Se may protect tissues against Cd toxicity by an

antioxidative effect that reduces lipid peroxidation, increases antioxidant enzyme activity, and inhibits apoptotic pathways[130]. the development of Se-Cd complexes could be a feasible reason for the reduction in Se [131].as a result of chronic stressor exposure, which may deplete Se and hence the body's ability to neutralize free radicals. Higher ROS and Cd levels may be noticed[37].

3.3.Relationships and correlation coefficients

Table(3-3): correlation between all laboratory investigations with each other

		HbA1c	ESR	IL6 pg/ml	GAL3 ng/ml	Se ng/mL	Cd ng/mL
RBS mg/dl	Pearson Correlation (R)	0.718	0.307	0.206	-0.021	-0.203	-0.136
	Significant	0.021	0.183	0.152	0.888	0.157	0.345
HbA1c	Pearson Correlation (R)		0.413	0.085	-0.182	-0.237	-0.159
	Significant	----	0.291	0.556	0.206	0.098	0.272
ESR	Pearson Correlation (R)			0.245	-0.025	-0.411	-0.207
	Significant	----	----	0.086	0.864	0.528	0.150
IL6 pg/ml	Pearson Correlation (R)				0.656**	-0.364**	0.378**
	Significant	----	----	----	**0.024**	**0.038**	**0.017**
GAL3 ng/ml	Pearson Correlation (R)					-0.389**	0.293*
	Significant	----	----	----	----	**0.015**	**0.039**
Se ng/mL	Pearson Correlation (R)						0.039
	Significant	----	----	----	----	----	0.789
Cd ng/mL	Pearson Correlation (R)						
	Significant	----	----	----	----	----	----

According to **Table(3-3)** There is significant positive correlation between IL6 and GAL3

The MAPK/ERK pathway is involved in Gal-3BP-induced IL-6 production and secretion, which has been demonstrated by various research groups in a variety of cell types. It needs a carbohydrate-mediated contact between Gal-3BP and Gal-3 at the cell surface[138]. The transcriptional increase of IL-6 was caused by a Gal-3BP/Gal-3/Ras/MEK/ERK signaling pathway in bone marrowmesenchymal stem cells (BMMSC)[139].

Galectin-3 interacts selectively through its CRD with activated K-Ras (K-Ras-GTP)[127]. Activated Ras then activates the protein kinase activity of a RAF kinase. The RAF kinase phosphorylates and activates a MAPK/ERK Kinase (MEK1 or MEK2). The MEK phosphorylates and activates a mitogen-activated protein kinase (MAPK) ,MAPKs were originally called "extracellular signal-regulated kinases" (ERKs)[140]. MEK/ERK signaling pathways will stimulate S1P-promoted IL-6 production[141].

During this study found that there is significant positive correlation between Cd (GAL3 and IL6), Also, a clarification of the existence of negative correlation between Se(IL6 and GAL3).

oral Cd treatment causes an increase in TNF- α and IL-6 production in heart tissue, as well as an increase in galectin-3 levels. Cadmium caused cardiomyocytes to apoptose[142].

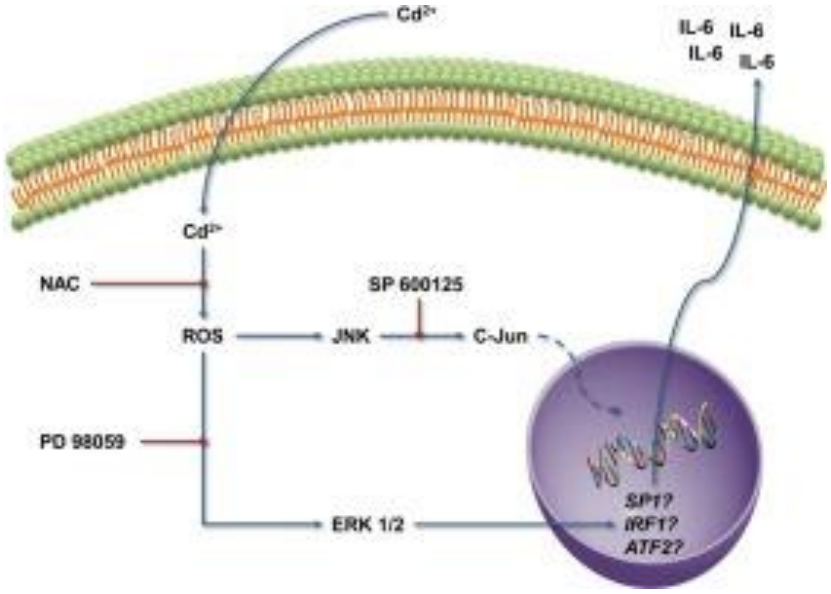
Galectin-3 expression in non -tumor tissues was discovered to be associated with fibrosis and inflammation. The current study showed that galectin-3 production was shown to be increased in liver of the cadmium

exposed rats. These results suggested that galectin-3 secretion in cadmium exposure was more likely related with tissue inflammation levels[116].The expression of galectin-3 is regulated by a number of factors, including nuclear factor KB (NF-kB), inflammatory cytokines, and several intracellular signal pathways[143]. IKK and the conventional NF-B pathway are activated by ROS. The IB kinase (IKK) complex phosphorylates IkB, resulting in IkB breakdown, which activates NF-kB. This frees up NF-kB, allowing it to readily translocate to the nucleus and transactivate target genes(144). NF-B interacted with the galectin-3 promoter to boost galectin-3 expression [143].

Previous research has shown that Cd toxicity, both acute and chronic, results in a systemic inflammatory response and increase in cytokines such TNF-α and IL-6[145]. In bronchial epithelial and renal glomerulus endothelial cells, cadmium increases IL-6 is expression and secretion[146]. Cadmium-induced IL-6 is mediated in the peripheral system by the ERK1/2 and NF-B pathways[147].

IL-6 is produced by a variety of cell types in response to several signal transduction pathways activated by distinct stimuli [148]. Mitogen-activated protein kinases (MAPKs) are a type of mitogen-activated protein kinase (MAPK)[149]. The production of reactive oxygen species(ROS) by Cd promotes MAPK signaling in many cell[150]. The mitogen-activated protein kinase (MAPK) cascades are intracellular signal transduction pathways that respond to a variety of extracellular stimuli and regulate a wide range of cellular processes, including growth, proliferation, differentiation, motility, stress response, survival, and apoptosis [151]. Four distinct MAPK cascades in mammals have been identified and named based on their MAPK

components: ERK1/2, c-Jun N-terminal kinase (JNK), p38, and ERK5 are extracellular signal-regulated kinases [148]. The activation of MAPKs by Cd-induced ROS enhances IL-6 release figure(3-3)[152]. By decreasing MAPK phosphatase activity, ROS such as H_2O_2 upregulate the activity of the JNK pathway, activating MAPKs such as p38[153]. ROS trigger signal transduction pathways such as NF-kB, causing proinflammatory mediators to be produced[154].



Figure(3-3): production of IL-6[161]

Negative correlation between Se ,IL6 and Gal-3, reduced serum selenium concentrations and a negative link between serum Se and interleukin-6 concentrations were found in our investigation[155]. Se deficiency can impair immunological responses and increase the risk of infection By increasing nuclear factor-B (NF-B) protein activation(stimulate IL-6,Gal-3)[156].

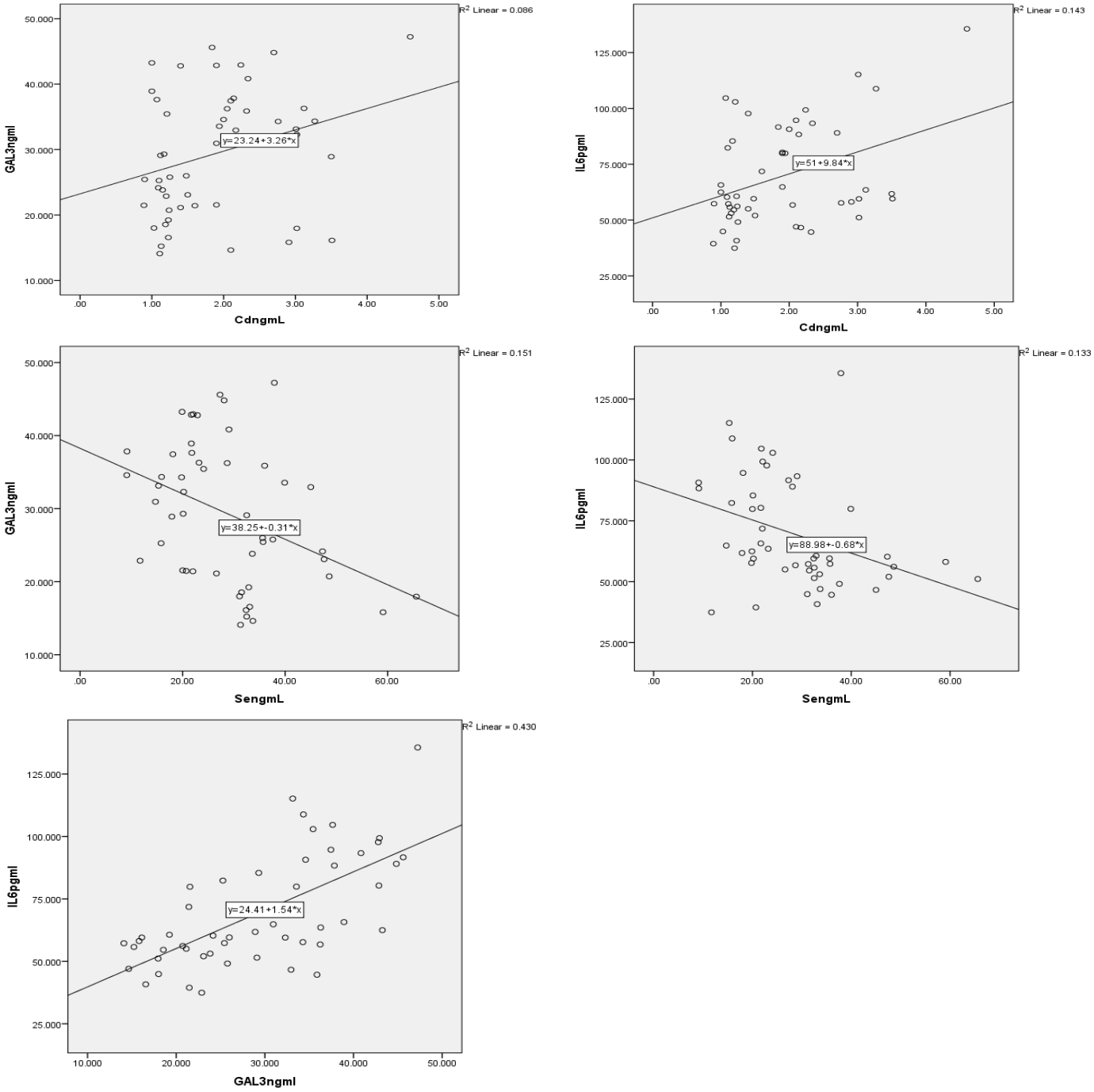


Figure (3-3):correlation between parameters

Conclusions

This study concluded that:

1- Patients with Chronic otitis media had greater levels of

Galectin-3, Interleukin-6 and Cadmium

2- Galectin-3 and Interleukin-6 have a positive relationship.

3- Se deficiency could be important and additive in the etiopathogenesis of COM

4- Cadmium exposure in the environment is associated with an elevated risk of COM

5-ESR elevated in CSOM due to increase in IL-6

6-HbA1C increase in patients with CSOM so People with diabetes are more likely to get otitis media

Recommendations:

1-Preferably taken larger number of subjects. The follow up study give a better result about the variability in the level of GAL-3 and IL-6.

2-Patients with CSOM take Se supplementations

3- Patients should avoid work in place exposure to Cd

4-Some factors like smoking habit contribute to Cadmium accumulation

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APPENDIX A

Questionnaire

Case No. Date:

Hospital name:

Patient name:-

Age: -

Sex:-

Weight: - Kg

Telephone number:-

Other diseases:-

Type of Treatment: -

CRP: -

ESR: -

RBS: -

HBa1c: -

الخلاصة

التهاب الأذن الوسطى المزمن هو عدوى متكررة تصيب الأذن الوسطى و / أو خلايا الهواء الغشاء مع وجود ثقب في الغشاء الطبلي. تشمل الأعراض المرتبطة عادةً بأمراض الأذن المزمنة فقدان السمع ، والإرهاق العظمي ، والامتلاء السمعي ، وآلام الأذن ، والدوار الحقيقي في بعض الأحيان.

الهدف من الدراسة هو تقييم مستويات المصل من الكالكتين٣ والانترلوكين٦ والسيلينيوم والكادميوم في مرضى التهاب الأذن الوسطى المزمن واخرون اصحاء. تضمنت الدراسة جمع عينات دم من 100 متطوع ، 50 شخصًا يتمتعون بصحة جيدة (٢٩رجلاً و ٢١امرأة) و٥٠ شخصًا (٢٩رجلاً و ٢١امرأة) يعانون من التهاب الأذن الوسطى المزمن. كان العمر (١٥-٦٥) سنة. تم ايجاد المرضى في قسم الأنف والأذن والحنجرة في مستشفى الامام الصادق(ع). تم قياس الكالكتين٣ والانترلوكين٦ بتقنية الاليزا بينما تم قياس مستويات السيلينيوم والكادميوم بتقنية الامتصاص الذري (الكرافيت) وتم قياس المعلمات الأخرى بطريقة القياس اللوني وفقًا لدليل الشركة المصنعة. كما أظهرت نتائج الاختبارات التي أجريت أن مستويات الكالكتين٣ والانترلوكين٦ والكادميوم في الأشخاص المصابين بالتهاب الأذن الوسطى المزمن كانت أعلى بشكل ملحوظ من الأشخاص الأصحاء ، كانت مستويات السيلينيوم لدى الأشخاص المصابين بالتهاب الأذن الوسطى أقل بشكل ملحوظ من الاشخاص الاصحاء حيث ان قيمة$p<0.05$

كما تمت دراسة العلاقة بين مستويات الكالكتين٣ والانترلوكين٦ حيث وجدت قيمة$P<0.001$ و $r = 0.656$.ووجدت علاقة ارتباط موجبة بينهما

يمكن الاستنتاج من الدراسة الحالية التي أجريناها أن مستويات الكالكتين٣ والانترلوكين٦ اعلى في المرضى الذين يعانون من التهاب الاذن الوسطى قياسا بالمجموعة المقارنة ومستوى السيلينيوم أقل في المرضى الذين يعانون من التهاب الأذن الوسطى المزمن من تلك المجموعة المقارنة ووجود علاقة إيجابية بين الكالكتين٣ والانترلوكين٦,الكادميوم و الكالكتين٣ والانترلوكين٦.ووجود علاقة عكسية بين السلينيوم الكالكتين٣ والانترلوكين٦, وفي الدراسة الحالية عملنا على دراسة العلاقة الايجابية والعلاقة عكسية بين هذه المعلمات

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل/ كلية الطب

فرع الكيمياء الحياتية السريرية

تقييم الكالكتين٣ ,الانترلوكين٦ وبعض العناصر النزرة لدى الاشخاص البالغين المصابين بالتهاب الاذن الوسطى المزمن في محافظة بابل العراق

رسالة

مقدمة الى مجلس كلية الطب في جامعة بابل

كجزء من متطلبات نيل شهادة الماجستير

في العلوم/ الكيمياء الحياتية السريرية

من قبل

رقية علي حيدر عبد الامير

بكالوريوس تقنيات التحليلات المرضية

٢٠١٦

إشـــراف

ألاستاذ المساعد

د. بان محمود شاكر

٢٠٢٢م

ألاستاذ

د.صفاء صاحب ناجي

١٤٤٣هـ