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Evaluation Of Galectin-3 , IL-6 And Cadmium In Adult Patients With Chronic Otitis Media In Hilla – Iraq

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Abstract: Otitis media is a first intermediary of health care visits across the world. The aims of the present study were to evaluate Galectin-3 , IL-6 and cadmium (Cd) levels in patients with COM. The study population consisted of 100 participants: 50 healthy controls (Group 1) and 50 patients with COM (Group 2). Galectin-3 and IL‑6 were determined using the ELISA, Cd measurement was performed in a graphite furnace atomic absorption spectrophotometer. Galectin-3 and IL‑6 had the highest value in patients with COM (Group 2) compared to the healthy control (Group 1) at (29 ± 9.5 ng/ml vs. 8.6 ± 2.3 ng/ml) and (69.5 ± 22 pg/ml vs. 11.2 ± 2.4 pg/ml), Cd levels were significantl y higher in group 2 than in group 1 at (1.9 ± 0.85 ng/ml vs. 0.5 ± 0.4 ng/ml). Our results could propose that Galectin-3, IL-6 and Cd excess could play a crucial and additive role in the etiopathogenesis of COM. However, further investigations with larger numbers of patients are warranted to determine the exact role of these marker.

Keywords: otitis media , Galectin-3 , interleukins, Cadmium .

1. INTRODUCTION

Chronic otitis media (COM) is a chronic inflammatory disease of the middle ear mucosal lining and airy spaces of the temporal bone characterized by persistent perforation of the tympanic membrane, conductive-type hearing loss, and recurrent ear discharge lasting more than 3 months. The disease is considered to be a significant health problem due to its long duration, irreversible sequelae, and serious complications [(1, 2)]. Although the etiology of COM is still unknown, certain reasons have been suggested, such as infection (viral and bacterial), genetics, congenital abnormalities, anatomic factors such as eustachian tube dysfunction, immature or impaired immune status, and environmental variables.such as smoking and pollution of the air [(3, 4)]. Also, patients with COM, otitis media with effusion, and tympanosclerosis have been demonstrated to have oxidative stress-related middle ear damage [(5, 6)]. Neutrophils and bacteria are the generators of free oxygen radicals (FORs) in the pathophysiology of OM [(7, 8)]. Damage to the mucosa caused by oxidative stress may slow recovery and lead to middle ear effusion and recurring or chronic infection [7].

Galectins are a group of lectins that bind to -galactosides and have one evolutionary conserved carbohydrate recognition domain (CRD) [(9)]. There are currently 15 galectins

identified in mammals, which are classified into three types based on domain organization: I prototype galectins with one CRD; ii) tandemrepeat galectins with two CRDs; and iii) chimeratype galectins with a single CRD connected to a long, flexible Nterminal domain [9,10] It's worth noting that Galectin3 is the only chimeric galectin. Human galectin 3 is a 35-kDa protein encoded by LGALS3, a single gene on chromosome 14 that codes for it [10]. All types of immune cells (macrophages, monocytes, dendritic cells, eosinophils, mast cells, natural killer cells, and activated T and B cells) as well as epithelial cells, endothelial cells, and sensory neurons express galectin-3 [9,11]. Galectin3 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 bodily fluids [9]. Galectin3's diverse functions are influenced by its numerous sites.Galectin3 interacts with several survival-associated proteins in the cytoplasm, including Bcl2 and activated guanosine5'triphosphate (GTP)bound KRas, which are both critical for cell survival. Galectin3 increases premRNA splicing and regulates gene transcription in the nucleus, whereas galectin3 modifies cell-cell interactions in the extracellular matrix, especially between epithelial cells and the extracellular matrix. As a result, it plays a role in cell differentiation, inflammation, fibrogenesis, and host defense [9,12]. As a result, galectin3 plays a critical role in a variety of biological processes, including cell proliferation, apoptosis, and premRNA splicing, differentiation, transformation, angiogenesis, inflammation, fibrosis, and host defense [13].

Interleukin-6 (IL-6) is a pleiotropic pro-inflammatory cytokine generated by mesangial cells, adipocytes, keratinocytes, fibroblasts, endothelial cells, smooth muscle cells, T and B cells, monocytes, dendritic cells (DCs), macrophages, and osteoblasts. B-cells, osteoclasts, regulatory T cells (T-Regs), hematopoietic stem cells, and keratinocytes are among the cells that are affected (14,15). IL-6 acts upon binding to IL-6 receptor (IL-6R) and glycoprotein 130 (gp130) [16]. IL-6 stimulates the development of certain cellular and humoral immune responses, including end-stage B cell differentiation, immunoglobulin production, and T cell activation, in addition to acute phase reactions [18]. 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[17].

Synthesis of IL-6 , Nuclear factor-kappa B (NF-B) stimulates mRNA transcription of IL-6 and other pro-inflammatory cytokines, such as 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 [19].

Cadmium (Cd) is a hazardous heavy metal that is one of the most common sources of contamination in the environment due to industrial and agricultural processes. High levels of Cd are ingested by the air (smoking, ambient air), food (shellfish, offal, vegetables), and water [20]. Because there is limited excretion in the organism due to the lack of an effective Cd elimination pathway, it has a lengthy biological half-life and is hazardous to multiple organs [21]. Recent studies have found a link between OM and ambient air pollution or tobacco exposure in the environment that is characterized by high amounts of hazardous heavy metals as Cd [22,23]. Lee et al. [24] discovered a link between blood Cd levels and the prevalence of COM in Korean adults in a recent investigation. Cd is thought to impact the beginning and course of illnesses through systemic and organ-specific inflammation, as well as oxidative stress [25]. Cd-induced ototoxicity has been demonstrated in vitro and in vivo experimental models via reactive oxygen species (ROS) production and apoptosis [26]. Cd exposure has recently been linked to a decrease in cell viability, as well as apoptosis and

necrosis in middle ear cells [27]). According to earlier investigations in various tissues [28,29], the middle ear damage produced by Cd could be related to oxidative stress and inflammation of middle ear cells.
The aim of this study was to examine Galectin-3 , IL-6 and Cadmium levels in patients with COM, taking into account that Cd via oxidative, inflammatory, and immune mechanisms could have an ototoxic effect.

2. MATERIALS AND METHODS

Study Design
50 patients with COM and 50 healthy control subjects who were similar to the study group in terms of age and body mass index (BMI) were included in the study. This study was conducted in College of Medicine, University of Babylon, Department of of Biochemistry. Research ethics approval was obtained from the Ethics Committee of University of Babylon before the initiation of the study.
Study Population
The study was carried out at the department of biochemistry / college of medicine / Babylon University and ENT department in Imam Sadiq Hospital The study included (100) subjects that were involved divided into two groups: Group 1: 50 healthy (29 male and 21 female) with age range (15-65) years. Group 2: 50 chronic otitis media patient (29 male and 21female) with age range (15-65) years. The 50 patients attended with symptoms of COM longer than 3 months and diagnosed with COM after a detailed otorhinolaryngologic examination was included in the study. The control group consisted of 50 subjects who were admitted to the otorhinolaryngology clinic with no symptoms related to ear and proven to be normal by otoscopic examination.
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 3000 rpm 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.
Galectin-3 and IL-6 measurement
The ELISA technique used specific antibodies and antigens and different dilutions. Gal-3 was determined using the Human (GAL-3) ELISA Test Kit produced by MyBioSource (Cat.No: MBS722196), IL‑6 was determined using the Human IL‑6 ELISA Test Kit produced by MyBioSource (Cat.No: MBS021993)
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.

Statistical analysis:
Data analysis by used SPSS version 20. results that presented as mean±standard division) (M±SD) were performed by t-test (P<0.05 was considered as significant).

3. RESULTS

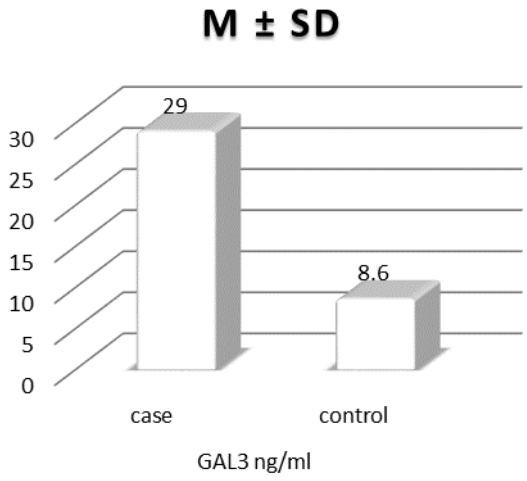
The clinical characteristics and the laboratory results of the groups are given in **Table 1**.

Table 1 The clinical/demographical characteristics and laboratory results of groups

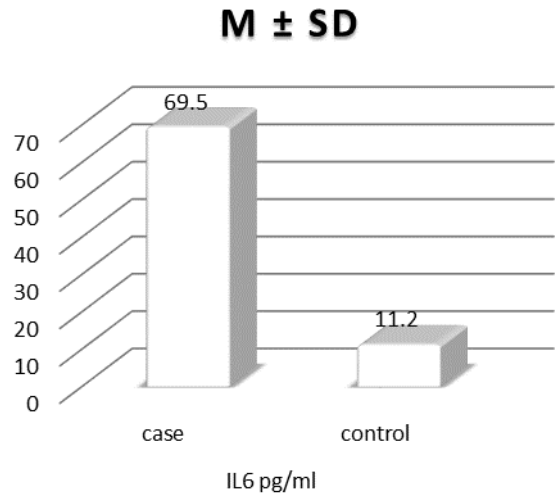
	Group	N	Mean ± SD	P-value
Age	*Group2* *Group1*	50 50	39 ± 11 32 ± 7	> **0.05**
GAL3 ng/ml	*Group2* *Group1*	50 50	29 ± 9.5 8.6 ± 2.3	< **0.001**
IL6 pg/ml	*Group2* *Group1*	50 50	69.5 ± 22 11.2 ± 2.4	< **0.001**
Cd ng/mL	*Group2* *Group1*	50 50	1.9 ± 0.85 0.5 ± 0.4	< **0.001**

All parameters are given mean ± standard deviation values in **Table 1** n subject number, p values statistically evaluated as p > 0.05 not significant, p < 0.05 significant

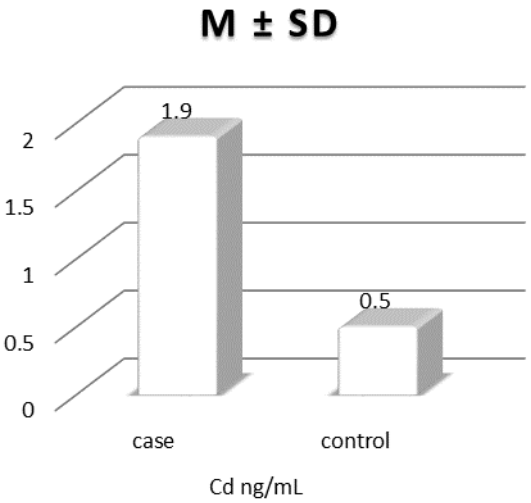
Demographic feature (age) in both groups were similar (p > 0.05). there was in serum levels significant (P<0.001) increase in GAL3, IL6 and Cd levels of Group2 (69.5 ± 22 , 29 ± 9.5 , 1.9 ± 0.85) compared with Group1 (11.2 ± 2.4 , 8.6 ± 2.3 , 0.5 ± 0.4). (Table 1; Fig.1).



(A)



(B)



(C)

Fig.1 The Gal-3 **(A)** ,IL-6 **(B)** and Cd **(C)** levels of the groups. Values are expressed in means + standard deviation. Serum GAL3, IL6 and Cd levels were significantly different between the groups ($p < 0.05$).

During our study, we found that there is significant positive correlation between GAL3 and IL6($r=0.656$, $p=0.001$)(Fig. 2A), significant positive correlation between Cd and GAL3 ($r= 0.293$, $p=0.039$)(Fig. 2B), Cd and IL6($r=0.378$, $P=0.007$)(Fig. 2C).

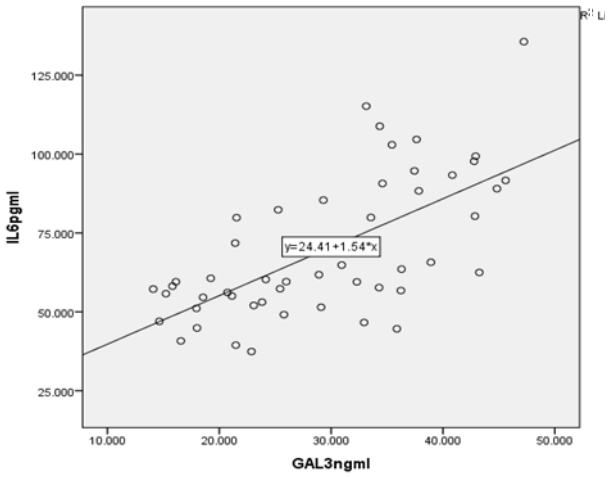


Fig. 2A correlation between GAL3 and IL6

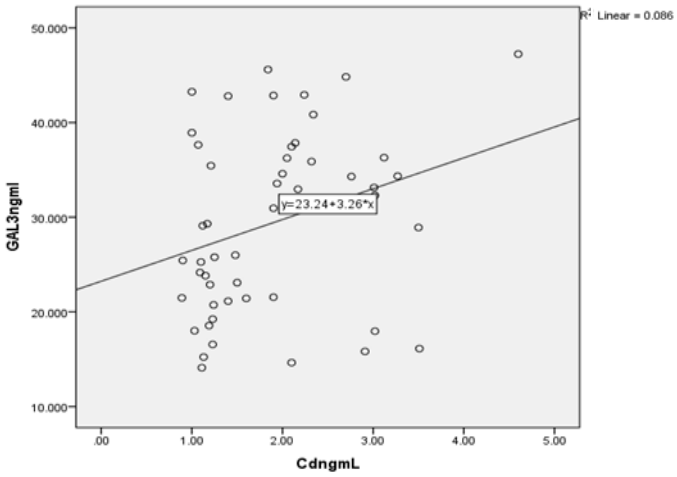


Fig. 2B. correlation between Cd and GAL3

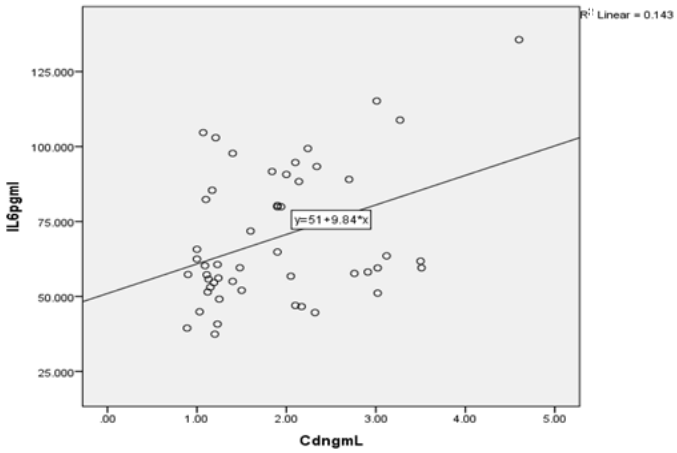


Fig. 2C. correlation between Cd and IL-6

Fig.2(A)-The significant positive correlation between GAL3and IL6, **(B)**- The significant positive correlation between Cd and GAL3, **(C)**-The significant positive correlation between Cd and IL-6, Values are expressed in means + standard deviation. Significantly different between the groups ($p < 0.05$).

4. DISCUSSION

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 When compared to healthy people, epithelial Gal-3 was higher in chronic disease[30].Gal-3 (either membrane associated or free) is involved in a wide range of processes, including pathogen immunity and acute and chronic inflammation[31].

The findings of this research indicated that the level of IL-6 in patients higher than controls, P-value (< 0.001) 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[32]. 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[33].The findings were comparable to those of Nofal and Kwatly [34], who found a greater blood level of IL6 in patients with AOM and CSOM when compared to a healthy control group[35]. 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 [36].

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) whose founds that cadmium exposure in the environment is associated with an elevated risk of COM[4]. Cd2+ decreases cell viability and promotes apoptosis in 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 [37]. Mucin secretion and inflammatory cytokines(including IL-6) play essential roles in the development of OM[38]. Middle ear inflammation and the pathophysiology of COM have been linked to oxidative stress and the production of excessive free radicals[39].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[40].Another role of Cd is that it affects the function of the Eustachian tube, which is the most important factor in the pathogenesis of middle ear illness in all age groups and causes COM. The nose, palate, nasopharynx, middle ear, and mastoid air cells are all connected via the Eustachian tube, which is a system of contiguous organs. When the Eustachian tube is damaged, nasopharyngeal discharge can occur, and allowing viruses and germs from the nasopharynx to enter into the middle ear[39].High levels of Cd activate the NF-kB protein, which promotes inflammation and compromises immunological function[34]. 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[41]. higher Cd levels identified in our study could contribute to the production of COM, contribute to disease creation processes that are similar, such as increased oxidative stress and reduced immunological responses[39].

During our study, we found that there is significant positive correlation between (GAL3 and IL6) ,and significant positive correlation between Cd (GAL3 and IL6), 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[42].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)[43].Galectin-3 interacts selectively through its CRD with activated K-Ras (K-Ras-GTP)[44]. 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) [45]. MEK/ERK signaling pathways will stimulate S1P-promoted IL-6 production[46].

In rats, 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[47]. 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 livers of the cadmium exposed rats. These results suggested that galectin-3 secretion in cadmium exposure was more likely related with tissue inflammation levels[48]. 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[49].IKK and the conventional NF-B pathway are activated by ROS[50].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[55]).NF-B interacted with the galectin-3 promoter to boost galectin-3 expression [49].

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[51].In bronchial epithelial and renal glomerulus endothelial cells, cadmium increases IL-6 is expression and secretion[51].Cadmium-induced IL-6 is mediated in the peripheral system by the ERK1/2 and NF-B pathways [53].IL-6 is produced by a variety of cell types in response to several signal transduction pathways activated by distinct stimuli [54]. Mitogen-activated protein kinases (MAPKs) are a type of mitogen-activated protein kinase (MAPK)[55].The production of reactive oxygen species(ROS) by Cd promotes MAPK signaling in many cell[56].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[57]. The activation of MAPKs by Cd-induced ROS enhances IL-6 release[58]. By decreasing MAPK phosphatase activity, ROS such as H2O2 upregulate the activity of the JNK pathway, activating MAPKs such as p38[59].ROS trigger signal transduction pathways such as NF-kB, causing proinflammatory mediators to be produced[60].

In conclusion, high serum levels of GAL-3 and Il‑6 were recorded in all otitis media groups compared to the healthy group, increased Cd levels in sera from patients with COM. Gal-3 induced IL-6 production and secretion, Cadmium-induced IL-6 is mediated in the peripheral system as well as an increase in galectin-3 levels. However, further investigations with larger numbersof patients are warranted to determine the exact role of Cd in the etiopathogenesis of COM.

Ethical Clearance:
From research ethic committee in Department of Biochemistry, College of Medicine, University of Babylon.

Source Of Funding:
Self.

Conflict Of Interest:
None

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