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The role of lead and cadmium elements, thyroid hormone levels, and GJB2 gene polymorphism on hearing loss in Iraqi society

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Abstract:

Deafness and hearing loss, affecting around 5% of the global population, are major sensory disorders with various etiologies, including environmental, physiological, and genetic factors. This study focused on 50 individuals with hearing loss (32 men, 18 women, ages 9-50) and 25 age-matched controls without hearing loss. The research aimed to investigate the correlation between thyroid hormone levels, heavy metal (lead and cadmium) concentrations, and GJB2 gene polymorphisms in relation to hearing impairment. Samples were collected from November 14 to December 20, 2023, and analyzed for thyroid hormones (T3, T4, TSH), lead, cadmium levels, and genetic polymorphisms.

Results indicated a significant increase ($p \leq 0.05$) in lead (0.52±0.157 µg/ml) and cadmium (0.14±0.03 µg/ml) levels in patients compared to controls (lead: 0.087±0.072 µg/ml; cadmium: 0.007±0.01 µg/ml). Additionally, a significant decrease ($p \leq 0.05$) in TSH levels was observed in patients (0.06 ± 0.03 ng/ml) versus controls (0.67 ± 0.39 ng/ml), while T3 and T4 levels showed no significant differences. Genetic analysis revealed polymorphisms in the GJB2 gene among patients with hearing loss.

The findings suggest that heavy metals, particularly lead and cadmium, are critical environmental risk factors for hearing loss. Additionally, disruptions in thyroid hormone levels, particularly TSH, may contribute to the condition. The GJB2 gene polymorphism is also identified as a potential genetic factor linked to hearing impairment.

key words: *Hearing loss , Thyroid hormones, Heavy metals, GJB2 gene, Polymorphisms*

Introduction:

Hearing loss (HL) is a significant global health issue, affecting approximately 430 million individuals worldwide, including 35 million children[1] . HL can range from mild to severe, with varying degrees of residual hearing. Those with mild to severe HL are often referred to as hard of hearing , while those with little or no hearing ability are classified as deaf [2, 3] . The etiology of hearing loss is multifaceted, encompassing congenital factors, environmental exposures, and genetic predispositions [4] .

One critical factor in hearing loss is exposure to heavy metals such as lead and cadmium[5] . These environmental contaminants have been shown to impair auditory function in both humans and laboratory animals , Chronic exposure to these metals can elevate hearing thresholds, delay auditory nerve conduction, and reduce cochlear blood flow, leading to sensorineural damage [6] . For instance, studies have demonstrated that lead exposure can result in the degeneration of inner ear receptor cells [7] , while cadmium exposure causes the death and rearrangement of these cells [8]. The auditory system's development, particularly during childhood, is vulnerable to these toxic effects, which can disrupt normal speech and language development .

Thyroid hormones also play a crucial role in the development and maintenance of the auditory system , Hypothyroidism during critical developmental periods can lead to conductive hearing loss, while hyperthyroidism has been linked to hearing impairment[9]. Thyroid hormones are essential for the proper formation and function of the central and peripheral auditory pathways. Deficiencies or imbalances in these hormones can result in hearing loss, affecting the cochlea and auditory nerve, as well as central auditory processing centers [10] .

Genetics is another major contributor to hearing loss, with over 100 genes identified as associated with hereditary hearing impairment, The GJB2 gene, which encodes the connexin 26 (CX26) protein, is one of the most significant genetic factors in non-syndromic hearing loss (NSHL)[11]. Mutations in this gene can alter the structure and function of the CX26 protein, disrupting cellular communication within the auditory system and leading to hearing loss[12]. The GJB2 gene, located on chromosome 13, is responsible for encoding proteins that are crucial for the growth and development of auditory structures[13]. Mutations or deletions within this gene can lead to malfunctions in the auditory system, resulting in hearing impairment.

Given the high prevalence of hearing loss in the Iraqi population, this study aimed to investigate the relationship between thyroid hormone levels, heavy metal exposure (specifically lead and cadmium), and GJB2 gene polymorphisms in individuals with hearing loss. By exploring these factors, the study sought to identify potential environmental and genetic contributors to hearing impairment, providing insights that could inform prevention and treatment strategies for this widespread sensory disorder. Understanding the interplay between these elements could lead to improved diagnosis and management of hearing loss, ultimately enhancing the quality of life for affected individuals.

Methodology:

Study Population and Sample Collection

The study was conducted between November 14, 2023, and December 20, 2023, at the Al-Amal Institute for the Care of the Deaf and Dumb in Anbar Governorate. Participants were divided into two groups: the disease group, comprising 50 individuals with hearing loss or deafness, aged between 50 and 99 years, and the control group, consisting of 25 age-matched volunteers without hearing loss. Detailed information regarding the nature of hearing loss, whether hereditary or due to other factors, was collected for each participant.

Blood Sample Collection and Preservation

Venous blood samples (5 mL) were drawn from each participant. Of this, 3 mL was transferred to Gel tubes, centrifuged at 3000 rpm for 10 minutes to separate serum, which was then divided into three micro-centrifuge tubes. These serum samples were stored at -20°C until serological analysis. The remaining 2 mL of blood was collected in EDTA tubes containing an anticoagulant and stored at -20°C for subsequent DNA extraction.

Heavy Metal Analysis in Blood Serum

Serum samples were prepared for heavy metal analysis using the wet digestion method (Kjeldahl method). Specifically, blood serum was placed in 250 mL digestion tubes and mixed with 10 mL of concentrated sulfuric acid (95%) and 3 mL of concentrated perchloric acid (70%). The mixture was shaken and allowed to stand at room temperature for 30 minutes before being heated at 320°C for one hour to complete digestion, resulting in a clear solution. The digested samples were then cooled, diluted to 35 mL, and transferred to screw tubes for analysis. The concentrations of lead (Pb) and cadmium (Cd) in the samples were measured using an atomic absorption spectrophotometer.

Thyroid Hormone Assessment

Thyroid hormone levels (T3, T4, TSH) were assessed using the DRG test kit (EIA-4569), a product of Germany. Hormone concentrations were determined according to the manufacturer's instructions, utilizing the DAW 50 Elisa Reader test method for precise measurement.Genetic Tests.

DNA Extraction

DNA was extracted from whole blood samples collected in EDTA anticoagulant tubes, following the protocol approved by the Korean company "Geneaid." This method ensured the preservation of DNA integrity for subsequent analyses.

Polymerase Chain Reaction (PCR)

Designing Specific Primers:

After extracting DNA with a concentration of 176 and a purity of 1.74, specific primers were designed to detect polymorphisms in the GJB2 gene. The primer sequences used were AAGTCTCCCTGTTCTGTCC (forward) and GGAAATGCTAGCGACTGAGCC (reverse), with a target molecular weight of 865 base pairs. The primers were designed using the NCBI database and synthesized by the Korean company BIONEER. The Single-round PCR technique was employed to confirm the presence of GJB2 gene polymorphisms.

PCR Components:

DNA Template

Specific Primers (Forward and Reverse)

Pre-mix solution

Double-distilled, deionized water (DDW)

PCR Reaction Procedure:

The PCR reaction was prepared by first allowing all components, including the working materials and DNA samples, to equilibrate to room temperature for 10 minutes. For each sample, a reaction tube was assembled with the following components:

5 µl of Pre-mix solution

2 µl of the specific primer mixture (1 µl forward, 1 µl reverse)

16 µl of distilled water (DDW)

2 µl of extracted DNA template

The total reaction volume per tube was 25 µl. The thermal cycler was then programmed according to specific temperature and cycle conditions, detailed in the following protocol table (not provided in this summary), to amplify the GJB2 gene regions of interest.

Table (1): The program used in the polymerase chain reaction

NO	Phase	Tm (C)	Time	No. of cycle
1	itial Denaturation	94	5:00	1 Cycle
2	Iain Denaturation	94	1:00	30 Cycles
3	Annealing	59*	0:30	
4	Extension	72	0:30	
5	Final Extension	72	5:00	1 Cycle
6	Cooling	10	5:00	-

After that, the results of the PCR reaction were known by transferring the samples on an agarose gel by adding 7-5 µl of the reaction product, taking into account the placement of the 100 bp ladder indicator, then confirming the success of the process by detecting the transfer using the UV documentation system.

Statistical analysis

Using the Statistical Package for Social Science (SPSS) and Graph Pad Prism V7 statistical programs, the data were statistically analyzed in accordance with the Complete Randomized Design (CRD) model. The T-test was used to compare the arithmetic means of the coefficients at a significance level ($P \leq 0.05$). The program, the Fisher test, and the Hardy-Weinberg equilibrium were also used to assess the polymorphism rates of the GJB2 gene.

Results:

The current study's findings demonstrated that deaf individuals had significantly higher levels of lead and cadmium than did healthy individuals. Additionally, compared to healthy individuals, the study demonstrated a significant decrease in TSH hormone levels in those with hearing loss.

Table (2) Mean and standard deviation of hormonal tests and heavy metal tests in blood serum .

Parameter	**Groups**		***p-Value***
	Hearing loss (No. 50) Mean (± S.D)	**Control (No. 25) Mean (± S.D)**	

Cd	0.14 (±0.03) µg/ml	0.007 (±0.01) µg/ml	0.0054*
Pb	0.52 (± 0.157) µg/ml	0.087 (± 0.072) µg/ml	0.0023*
T3	1.23 (± 0.23)ng/ml	1.28 (± 0.36) ng/ml	0.525 Ns
T4	3.81 (± 1.12) ng/ml	3.32 (± 1.46) ng/ml	0.633 Ns
TSH	0.06 (± 0.03) ng/ml	0.67 (± 0.39) ng/ml	0.047*

NS=Non-significant, * significant at *p value* ≤ 0.05

Discussion:

The results of the current study, Table (2), showed a significant increase ($p \leq 0.05$) in the level of cadmium in the blood of individuals suffering from hearing loss, as its concentration reached 0.14 µg/ml (±0.03) compared to its concentration in the blood serum of individuals in the control group 0.007 (±0.01) µg/ml. The results of the current study are consistent with the results of the study by Wang et al. (2020), which showed that high levels of cadmium in the blood increase the risk of hearing loss in Chinese adults[5] .

One possible risk factor for hearing loss is cadmium exposure, which is mostly absorbed through food, medicine, drinking water, smoking, and certain industries. In the general American population, Choi et al. (2012) similarly discovered a direct correlation between blood cadmium levels and hearing loss, with the likelihood of hearing loss rising steadily as cadmium concentration increased[14]. A study by Yang et al. (2024) showed that high blood cadmium concentrations are associated with hearing loss, which is attributed to the toxic effects of cadmium on the ear[15].

It also leads to decreased blood flow and lipid peroxidation in the cochlear tissues, which leads to a defect or latency in the conduction of the auditory nerve and finally leads to hearing loss. The results of the current study, Table (4), also showed a significant increase ($p \leq 0.05$) in the level of lead in the blood of individuals suffering from hearing loss, as its concentration reached 0.52 (± 0.157) µg/ml compared to its concentration in the blood of individuals in the control group 0.087 (± 0.072) µg/ml.

The current study's findings are in agreement with a study by Yin et al. (2021), which shown a substantial correlation between high blood lead levels and a higher risk of hearing loss[16]. Divalent lead (Pb^{2+}) exposure has also been linked to ototoxicity, which involves physiological changes in the cochlea and hearing loss, according to previous research conducted on humans and experimental animals[17].

The TSH levels in individuals with hearing loss were found to be significantly lower ($p \leq 0.05$) at ng/ml (±0.03) 0.06 in the current study as compared to 0.67 (±0.39) ng/ml in the control group.

Our findings align with the research conducted by Zheng et al. (2021), which found that individuals with moderate to severe hearing loss had lower TSH levels, an independent predictor of the development of hearing loss[18]. While the results of the current study, which indicate an association between TSH levels and hearing loss, conflict with the study by Chen et al. (2023), which indicated through its results that no independent causal relationship was reached between TSH levels and the risk of hearing loss[19].

Hyperthyroidism may be the reason for low TSH since it raises the rate of cellular metabolism and, consequently, the quantity of free radicals, which results in oxidative stress and lipid peroxidation. In addition, hyperthyroidism increases the generation of reactive oxygen species (ROS) and alters the body's antioxidant mechanisms. These effects exacerbate tissue damage brought on by hyperthyroidism and result in the necrosis and death of endothelial cells in blood arteries that supply the inner ear.

Even the formation of microclots in the inner ear, these changes caused by hyperthyroidism are important mechanisms for deafness caused by hyperthyroidism[20, 21]. The results of the current study did not show any significant differences in the level of T3 and T4 hormones between the two groups.

The results of the current study, Table (3), show the occurrence of polymorphism of the GJB2 gene, as the study showed the occurrence of polymorphism in 38 (76%) individuals with hearing loss and 2 (8%) individuals from the control sample.

Table (3) Frequency of the allelic pattern of the GJB2 gene in the study and control sample individuals.

	Patients No. 50(%)		Control No. 25(%)		P-value	Chi-Square (χ^2)
	p (+)	q (-)	p (+)	q (-)		
Primer 1	**12**(24%)	**38**(76%)	**23**(92%)	**2**(8%)	0.003	3.09 **
Allele Freq.	0.37	0.63	0.72	0.28		

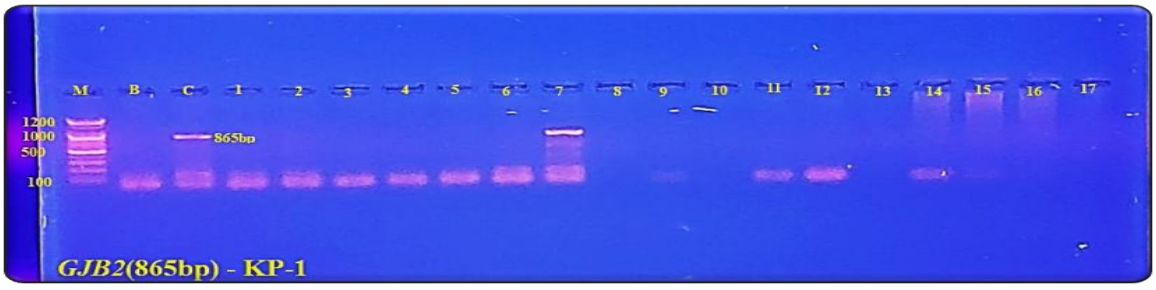

Figure (1) Electrophoresis results of the primer designed for GJB2 gene on 1.5% agarose gel for some study samples at a concentration of 176 70 vol. /cm for 1:15 hour. (Patient 1_17 , C: control).

The GJB2 gene produces the protein connexin 26 (cx26), a member of the gap junction-specific connexin family of proteins. Connexin proteins assemble in the plasma membrane to create hexagonal semi-channels known as connexons, According to Harris and Bevans (2001), these channels are connected to the channels of nearby cells to create functional gap junctions that permit the transfer of ions, molecules, and metabolites with a molecular weight of 1200 Daltons between cells[22].

The GJB2 gene is present in different types of cells and tissues, including cochlear cells, where it has been suggested that gap junctions formed by the CX26 protein play a major role in regulating potassium homeostasis and cochlear inputs in the inner ear (K+) during normal hearing function. Thus, GJB2 gene mutations are responsible for most cases of deafness. Gap junctions in the epidermis also play a role in the growth and differentiation of keratinocytes in a coordinated manner, which explains the skin abnormalities associated with hearing loss in people with GJB2 mutations[23-25]

83 mutations in the GJB2 gene were found in a previous study; these were separated into 36 non-truncating mutations (substitution mutations) and 47 truncating mutations (deletions, insertions, and duplications that result in frame shifts). According to research, people with truncating mutations in the GJB2 gene experience hearing loss that is more severe than people without such mutations [26].

According to recent research, the most common connexin 26 mutation is delG 35 (a truncation mutation) which is a frameshift mutation that involves the deletion of a guanine base at position 35, causing the sequence to shift, thus resulting in a TGA, a premature stop codon and as a result, the coding sequence is terminated resulting in a shorter Cx26 protein[12]. Another important mutation is the non-truncating mutation Cys169Tyr, which is a point mutation that results in a single nucleotide substitution at the coding site, resulting in a codon that codes for a different amino acid. The mutation involves the replacement of the guanine nitrogen base in the codon TGC, which codes for the amino acid cysteine, at position 167, in the 226-amino acid protein sequence, to adenine, which then results in the translation of tyrosine instead of cysteine.

Alterations in the structure of the connexin protein impact intercellular contacts by decreasing the probability of low-molecular-weight substance exchanges between cells and controlling potassium ion stability. According to Zonta, Girotto et al. (2015) and Tlili, Al Mutery et al. (2017), this also interferes with other inner ear functions and eventually results in hearing loss[25, 27].

Conclusions:

The present investigation led us to the conclusion that elevated levels of lead and cadmium are positively correlated with hearing loss in those who have it. Individuals with hearing loss also showed a substantial decrease in TSH hormone levels. Further research is necessary to better understand the pathogenic mechanism underlying hearing loss, as the causal relationship between thyroid hormone levels and hearing loss remains unclear. One of the genetic reasons of hearing

loss is the phenomena of polymorphism of the GJB2 gene, which has also been seen in individuals with hearing loss.

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