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Analysis of Serum Inflammatory Markers in Infants Under 6 Months of Age with Non-Syndromic Moderate and Severe Hearing Loss Associated with *GJB2* Gene Mutations

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Background: The *GJB2* gene is reported to be the main hereditary factor responsible for non-syndromic hearing impairment in infants. Several kinds of hearing loss have been linked to elevated inflammatory markers. This study aimed to evaluate serum levels of IL-2, IL-4, IL-6, IL-10, IL-17, α-TNF, and γ-IFN and the severity of hearing loss.

Material/Methods: Ninety newborns were divided into 3 groups: severe hearing impairment (31 infants), moderate hearing impairment (30 infants), and normal hearing (29 infants). Hearing screening was performed using otoacoustic emissions test. Mutations of the *GJB2* gene were detected with Sanger sequencing. The patients had DNFB1 mutation. Seven blood inflammatory markers were tested using Cytometric Bead Array. We performed the *t* test to examine differences in expression of 7 inflammatory markers between sexes in the groups. The correlation between indicators within groups was studied using the Pearson correlation test. Correlation of different indicators among groups was studied using the Spearman correlation test.

Results: When compared among the 3 groups (severe, moderate hearing impairment, and normal hearing group), we found that IL-10 had a positive correlation with the severity of *GJB2*-associated hearing loss, which was statistically significant ($P<0.05$).

Conclusions: This research aimed to assess the relationship of 7 serum inflammatory markers with *GJB2*-associated hearing loss in infants. Inflammatory marker IL-10 had a positive correlation with the severity of *GJB2*-associated infant hearing loss, and it might have the potential to become a future therapeutic target.

Keywords: GJB2 Protein, Human • Hearing Loss • Infant • IL6 Protein, Human • IL10 Protein, Human

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Background

One in 500 infants is affected by hearing impairment, which makes it the most prevalent inherited sensory disorder [1,2]. The severity of loss of hearing ranges from mild to profound at birth and highly depends on the genotype [3]. There are various causes, more than half of which are genetic, and some are non-genetic [4]. Hearing loss due to genetic mutations can either be syndromic or non-syndromic [5]. Most non-syndromic hearing loss is progressive and genetically diverse [6]. Non-syndromic hearing impairment is not accompanied by other signs or symptoms [7] The *GJB2* gene (Gap Junction Protein Beta 2), present on chromosome 13q12, has been reported to have a direct impact on non-syndromic hearing loss in infants and adults [8,9]. The pattern of inheritance for DFNB1 is autosomal recessive, which accounts for nearly for half of all autosomal recessive non-syndromic hearing loss cases, resulting ultimately in mild to profound hearing loss that is present prelingually, and all frequencies are affected. This hearing impairment does not become more severe over time [10]. DFNA3 has an autosomal dominant inheritance pattern. It is associated with prelingual hearing loss or hearing loss that begins as a child starts to speak. This type of hearing loss can be mild to profound, becoming more severe as time goes by and has been reported to affect the ability to hear high-frequency sounds [11].

Connexins play a vital role in the intracellular communication of cells [12]. They consist of a variety of proteins that form functional channels at the non-junctional sites of the cell membrane and serve as a communication link between the cell cytoplasm and the space surrounding the internal environment of the cell [13]. Connexin 26 is encoded by *GJB2*, which has an important impact on inner ear development by facilitating ionic and metabolic homeostasis [14]. Most of the mutations observed in Connexin 26 correlated with non-syndromic deafness are either nonsense or deletions leading to early truncation of proteins and frameshifts, respectively. Therefore, most of the mutations occurring in *GJB2* in homozygous patients result in gene knockout and complete destruction of Connexin 26 functioning, which ultimately results in hearing impairment. The genetic ablation of Connexin 26 gap junctions has a particular impact in the inner ear. This effect could be the modification of potassium ion transport in the cochlea, which ultimately results in the breakdown of auditory processes. The accumulation of overabundant potassium around hair cells may lead to cellular degeneration and cytotoxicity [15].

It was reported that hearing loss can cause inflammation and physiological defects in various parts of the brain and inner ear. Various non-sensory cells and hair cells of the cochlea could be impaired by the acoustic injury which would result in an inflammatory response with the production of pro-inflammatory cytokines [16]. Animal studies have proved the damaging effects on hearing loss due to inflammation in the cochlea, leading to permanent cell injuries [17]. The cochlea's lateral wall contains macrophages derived from bone marrow. The macrophages become active when exposed to noise, surgical stress, and ischemia [18]. Generation of pro-inflammatory cytokines is crucial in the process of cochlear repair [19]. In vivo studies discovered the production of IL-6 (Interleukin 6) and α-TNF (tumor necrosis factor alpha) after the ear was infected with a certain antigen, leading to an enhanced immune response in the cochlea. α-TNF, being associated with cochlear microcirculation, has been observed to be activated in certain hearing loss cases through its impact on arterial vasoconstriction [20].

High plasma levels of IL-2 (Interleukin 2), IL-4 (Interleukin 4), γ-IFN (Interferon gamma), α-TNF, IL-6, IL-17A (Interleukin 17A), and IL-10 (Interleukin 10), among other inflammatory markers, are indicators of aging and related chronic diseases and physiological disorders [21]. They have been observed to be the significant contributors to inflammation and are now prospective therapeutic targets in certain circumstances [22,23]. Some studies found increased production of pro-inflammatory cytokines due to noise-overstimulated cochlea [24]. IL-6 was reported to be present in stria vascularis, spiral ganglion neurons, and ligaments. Limiting signals from IL-6 can suppress the cochlear inflammatory response [25]. The expression can result in activation of the NF-κB signal pathway, since there is a possible positive feedback loop involving NF-κB and IL-6. This leads to production of certain pro-inflammatory factors that can play a significant role in cochlear injuries associated with NF-κB [26]. Elevated expression of IL-6 can result in abnormal expression of NF-κB in the lateral walls of the cochlea through a positive feedback loop of NF-κB stress response system, as it is both an activation factor of NF-κB and a transcriptional target [27].

Elevated inflammatory markers have been observed and reported in various types of hearing impairment [28]. The extent in adults is strongly associated with the markers of inflammatory status [25]. Hence, we hypothesized that blood inflammatory biomarkers also can affect hearing loss in infants. Our study aimed to investigate the possible correlation between the degree of *GJB2*-associated hearing loss and blood inflammatory markers in infants.

Material and Methods

Ethics

Ethics approval (No. 2020KY882) was acquired from the Ethics Committee of Ningbo Women's and Children's Hospital. We obtained written informed consent from guardians of all participants. The principles of the Helsinki Declaration were followed in the execution of this investigation.

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Subjects

A total of 90 infants under the age of 6 months were enrolled for hearing loss evaluation in Ningbo Women's and Children's Hospital from December 2020 to January 2022. All of them were from the Ningbo region of China and were born in our hospital. Genetic screening and otoacoustic emission testing (OAE) were performed. The infants' parents provided complete medical history by completing a questionnaire and gave written consent before the study started. Doctors and genetic advisors were responsible for explaining the screening outcomes. We collected information on gender, birth date, family medical history, and other related medical conditions.

Hearing Screening

The OAE Screening of the infants was done within 2 to 3 days after birth by using Madsen AccuScreen. A sound is played after placing a probe in the patient's ear canal and the sound that comes back is measured by the device. The results are shown on the display of the OAE hearing screener. Cochlear function is measured (severe >56 dB HL, moderate 16 to 55 dB HL). The infants showing abnormal OAE results were tested again at an interval of 42 days. The hearing loss in these infants was verified by brain auditory-evoked potential (BAEP) followed by audiological diagnostic examinations in case the infant failed to pass through the second screening [29].

Genetic Screening of GJB2 Gene

The coding region of *GJB2* was assessed via Sanger sequencing technique using a DNA sequencer (Applied Biosystems, USA). NCBI's application was utilized to compare reference genome with the obtained sequence of *GJB2*. WES data were used for the control group. Exome capture was then carried out. The TruSeq® DNA Sample Prep Kit v2-Set A (Illumina) was used to prepare a genomic library. Q30 standard was utilized to filter the reads. Burrows-Wheeler aligner (BWA) software was utilized for aligning. Polymerase chain reaction (PCR) was performed for amplifying the amplicons of the *GJB2* gene. The obtained hybridized mixture having specific tag probes was then affixed on to the microarray chip. The chip signals were then analyzed by scanning and imaging using the LuxScan Microarray Scanner and detection system. *GJB2*-Exon2- F: 5'-TTGGTGTTTGCTCAGGAAGA-3' *GJB2*-Exon2-R: 5'-GGCCTACAGGGGTTTCAAAT-3'. Procedures used were: 1) UNG treatment at 50°C for 2 min, 2) Pre-denaturation at 95°C for 10 min, and 3) Drop cycle program 95°C, 15 s; 65°C, 15 s (1°C drop per cycle); 76°C, 20 s (10 cycles). The melting curve analysis program was 95°C for 1 min, 35°C for 3 min, and 40°C to 80°C, set to collect channel fluorescence signal at this stage.

Sample Collections

A genomic DNA extraction system kit (Chengdu Biotech, China) was used to extract DNA from whole blood of each subject by following the manufacturer's instructions. Within 1 week after birth, blood spots with a diameter of at least 8 mm were collected from venous blood drawn from the infants' heel. These blood spots were naturally dried followed by stocking in preparation.

Cytometric Bead Array

BD Cytometric Bead Array (CBA BD Biosciences, CA, USA) is a flow cytometry application which allows users to quantify multiple proteins. IL-2, IL-4, IL-6, IL-10, IL-17, α-TNF, and γ-IFN were measured using the Cytokine Combination Assay Kit (Xiamen Zhishan Biotechnology Co, Ltd) according to the directions in the instruction manual. Then, samples were acquired on a dual-laser flow cytometer. The kit is based on immunofluorescence technology. The microspheres in the kit contained 7 types of microspheres with different fluorescence intensities, and the microspheres were coated with antibodies specific for IL 2, IL 4, IL 6, IL 10, IL 17, interferon γ, and tumor necrosis factor α. We took the microsphere mixture, centrifuged it at 200 g for 5 min, carefully aspirated the supernatant with a pipette and discarded it, then resuspended the microspheres with an equal amount of microsphere buffer. Next, it was placed in a vortex shaker for 3 to 5 s and incubated for 15 to 30 min without light. We removed the calibrator tube from the kit and added 2 ml of sample dilution. Nine test tubes labelled 1: 2, 1: 4, 1: 8, 1: 16, 1: 32, 1: 64, 1: 128, 1: 256, and 1: 512 were taken and 300 uL of sample diluent was added to each tube. Then, we drew 300 uL of the sample from the highest concentration calibration tube into the 1: 2 tube and gently mixed it, then drew 300 uL of sample from the 1: 2 tube into the 1: 4 tube and mixed it, then up to the 1: 512 tube. We added 25 uL to all calibration tubes and sample tubes and added 25 uL of fluorescent detection reagent to all tubes. After incubation for 3 h, 1 mL of PBS solution was added to each tube, centrifuged at 200 g for 5 min, then the supernatant was discarded. We added 100 uL of PBS solution to each tube, then they were prepared for the machine. Data were analyzed using FCAP Array multiplex analysis software.

Statistical Analysis

The data were analyzed using IBM SPSS 25, Microsoft Excel 2021, and R programming software. Mean and standard deviation values of continuous variables were calculated. The *t* test was performed to examine differences in the expression of different inflammatory markers between genders in severe, moderate, and no hearing loss groups. The correlation between indicators within groups was assessed using the Pearson's

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Table 1. Demographic data of the infants.

Cohort	Percentage share	
	Male	Female
Cohort 1	42%	58%
Cohort 2	41%	59%
Cohort 3	57%	43%

correlation test. The correlation of different indicators among groups was assessed via Spearman's correlation test. We considered $P<0.05$ as statistically significant.

Results

Infants with hearing loss had the DFNB1 mutation. In the severe hearing loss group, 42% were male and 58% were female. In the moderate hearing loss group, 41% were male and 59% were female. In the normal hearing control group, 57% were male and 43% were female (**Table 1**). **Table 2A-2C and Figures 1-6** show the mean and standard deviation values of 7 blood inflammatory markers in the 3 groups.

The relationships of the 7 blood indicators between different genders within severe, moderate, and no hearing loss groups were studied. **Table 3** shows that in the group with severe hearing loss, the difference of the expression of IL-2, and IL-4 was statistically significant between females and males ($P<0.05$). In the group with moderate hearing loss, the difference of the expression of IL-10 was statistically significant between females and males.

The correlation between indicators was studied using the Pearson's correlation test. Results of the correlation of blood indicators within the specific groups are shown in **Table 4A-4C**. Data revealed that blood indicators were no significantly associated. In the severe hearing loss group, none of the blood indicators were found to be correlated significantly except for indicator

Table 2A. Values (mean, standard deviation) of 7 blood inflammatory markers in the severe hearing impairment group.

Gender	Statistic	A1	B1	C1	D1	E1	F1	G1
Male	Mean	0.55	0.48	80.55	52.61	3.37	5.25	3.81
	Standard deviation	0.47	0.56	138.64	133.08	5.39	17.39	5.56
Female	Mean	1.84	2.74	46.39	106.46	4.00	2.65	5.72
	Standard deviation	1.86	2.73	66.00	170.79	5.66	2.18	10.47

A – IL-2; B – IL-4; C – IL-6; D – IL-10; E – IL-17A; F – α-TNF; G – γ-IFN.

Table 2B. Values (mean, standard deviation) of 7 blood inflammatory markers in the group of moderate hearing impairment.

Gender	Statistic	A1	B1	C1	D1	E1	F1	G1
Male	Mean	0.84	0.72	3.10	3.37	5.63	1.25	1.21
	Standard deviation	0.76	0.81	2.19	2.63	6.98	0.95	0.96
Female	Mean	0.42	0.94	2.72	1.29	3.93	0.97	0.82
	Standard deviation	0.34	1.12	2.37	0.96	4.04	0.80	0.67

A – IL-2; B – IL-4; C – IL-6; D – IL-10; E – IL-17A; F – α-TNF; G – γ-IFN.

Table 2C. Values (mean, standard deviation) of 7 blood inflammatory markers in the group of infants with normal hearing.

Gender	Statistic	A1	B1	C1	D1	E1	F1	G1
Male	Mean	1.31	1.18	3.04	3.94	1.23	1.43	1.76
	Standard deviation	1.19	1.03	1.69	2.70	1.52	1.59	1.57
Female	Mean	1.68	1.09	4.82	3.25	1.38	2.20	8.15
	Standard deviation	1.28	0.61	3.71	1.70	1.93	1.63	17.58

A – IL-2; B – IL-4; C – IL-6; D – IL-10; E – IL-17A; F – α-TNF; G – γ-IFN.

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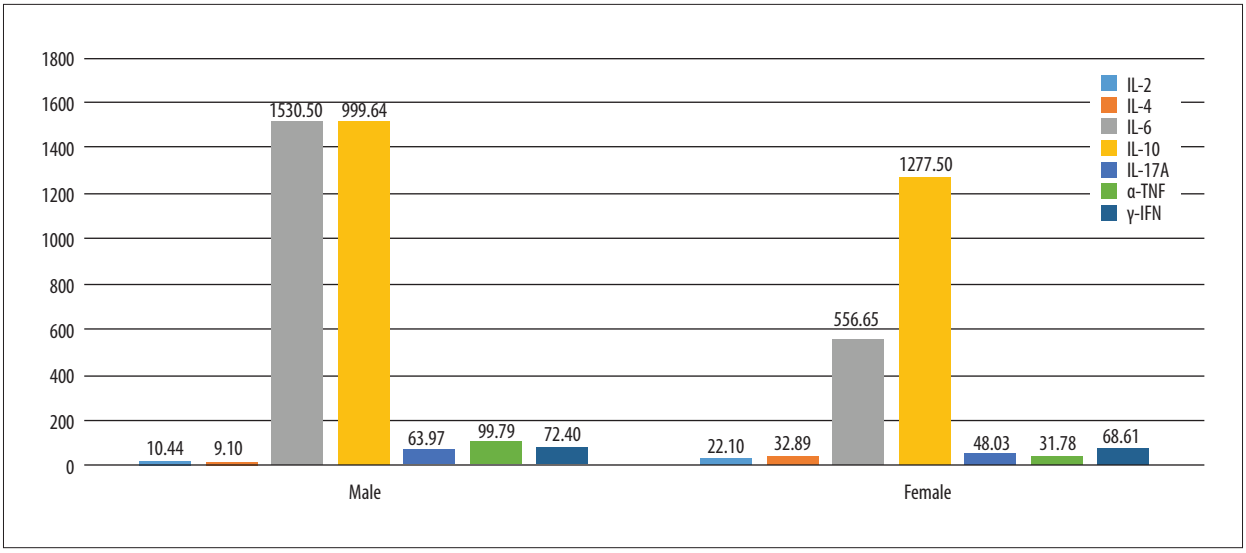

Figure 1. Different serum inflammatory markers distribution in the severe hearing loss group between males and females (Excel 2021, Microsoft).

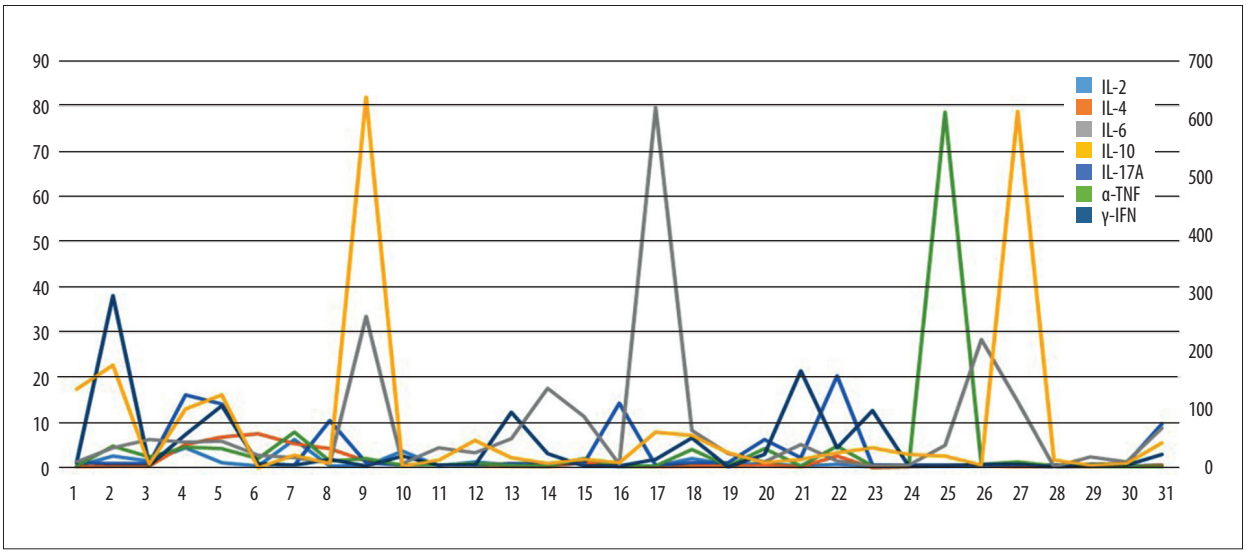

Figure 2. Value of 7 serum inflammatory markers in each patient of the severe hearing loss group.

IL-4 and IL-17A, having a maximum correlation coefficient at 0.44, followed by IL-6 and IL-10 at 0.28. In the moderate hearing loss group, IL-2 and γ-IFN had a coefficient of 0.77. IL-2 and α-TNF had a coefficient of 0.63. IL-6 and γ-IFN had a coefficient of 0.57. In the normal hearing group, IL-6 and γ-IFN had a maximum correlation coefficient of 0.77. IL-4 and α-TNF had a coefficient of 0.63.

The correlations between indicators among different groups were studied using Spearman's correlation test. Spearman correlation coefficient was calculated. Serum inflammatory markers used for this study were the same as above. Results are shown in **Table 5A and 5B**. Blood inflammatory markers IL-6 (0.574) and IL-10 (0.533) had statistically significant relationships with the severity of hearing loss. However, IL-6 failed to exhibit a statistically significant difference between the moderate and no hearing loss groups ($P>0.05$), while IL-10 showed a statistically significant difference among severe, moderate, and no hearing loss groups ($P<0.05$).

Discussion

In this research, we studied the correlation of 7 serum inflammatory markers with the severity of *GJB2* gene-associated infant hearing loss using PCR and cytometric bead array experiments. We found that IL-6 and IL-10 were significantly associated with the degree of hearing impairment. Nevertheless, the difference in the expression of IL-6 between normal hearing and

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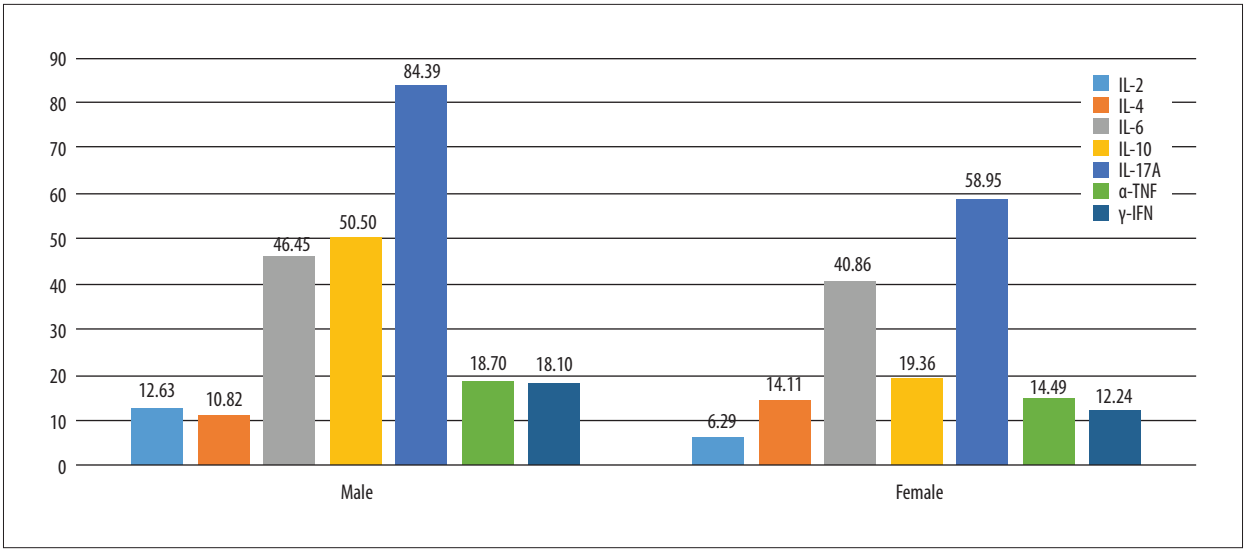

Figure 3. Distribution of serum inflammatory markers in the moderate hearing loss group between males and females.

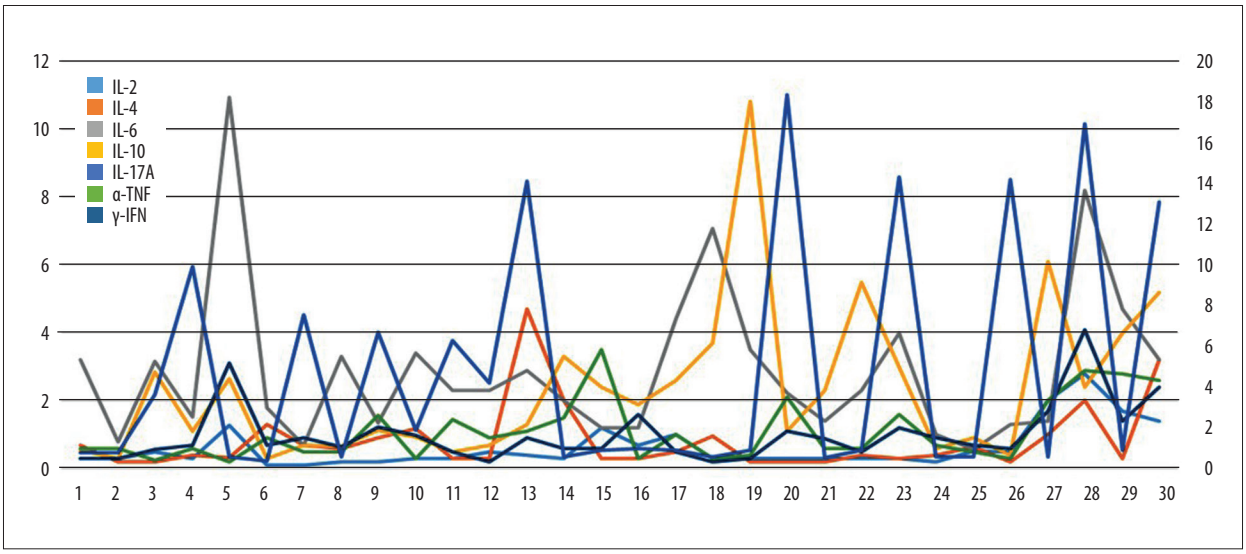

Figure 4. Values of 7 serum inflammatory markers in each patient of the moderate hearing loss group.

moderate hearing loss groups was not statistically significant, while IL-10 showed a statistically significant difference among severe, moderate, and no hearing loss groups.

IL-6 has been observed to be involved in various immune responses. It induces acute phase reactive protein production, activates killer T cells, differentiates macrophages, and plays a crucial part in the process of antibody synthesis [30]. The complexes formed by IL-6 and its receptors bind to gp130 proteins, undergo dimerization, and lead to the onset of intracellular signals via Janus kinase transducer and result in activation of the transcription process [31].

IL-10 is substantially generated from the immune cells. Expression of IL-10 receptors is mainly found in the lateral cochlear wall. Deficiency of IL-10 led to several types of hearing loss in experiments [32]. IL-10 signaling is essential to protect the cochlea from tissue damage caused by inflammation. The molecular pathway for IL-10-induced cochlear inflammation inhibition is still unclear [33].

Lassle et al found that severity of hearing impairment in elderly subjects was closely correlated with white blood cells [21]. Yoon et al found a strong association between TNF-α and sudden sensorineural hearing loss [32]. Diao et al found a correlation between platelet/lymphocyte ratio and hearing loss [34]. Chien et al found that IL-1 was closely correlated with sudden sensory-neural hearing impairment [35].

Despite remarkable genetic heterogeneity, *GJB2* gene mutations are present in up to 50% of individuals with autosomal recessive

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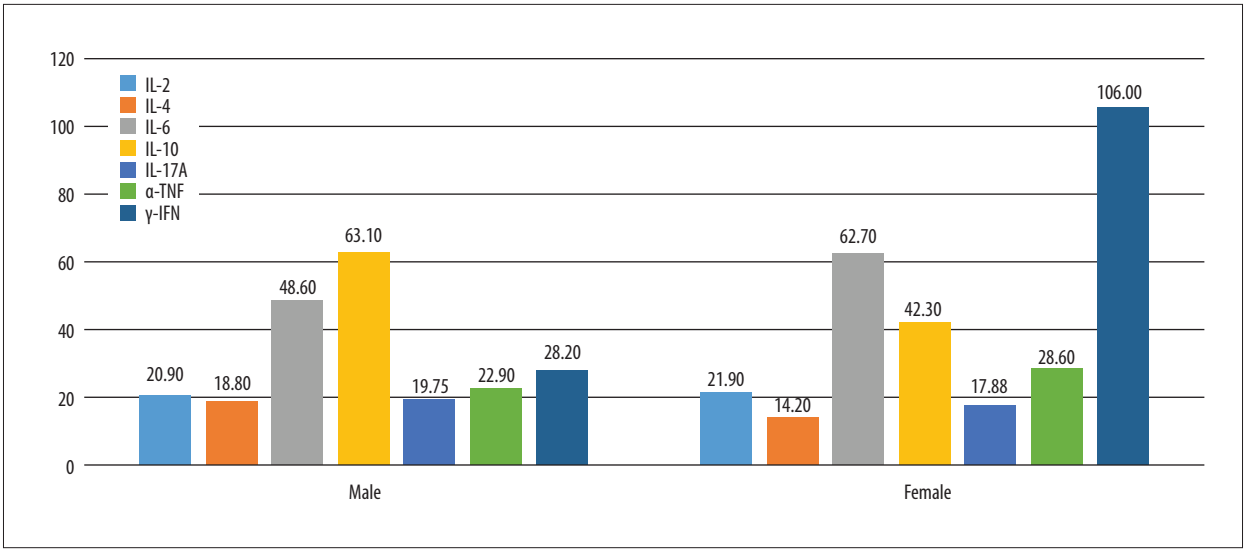

Figure 5. Differences between males and females in distribution of serum inflammatory markers in the normal hearing group.

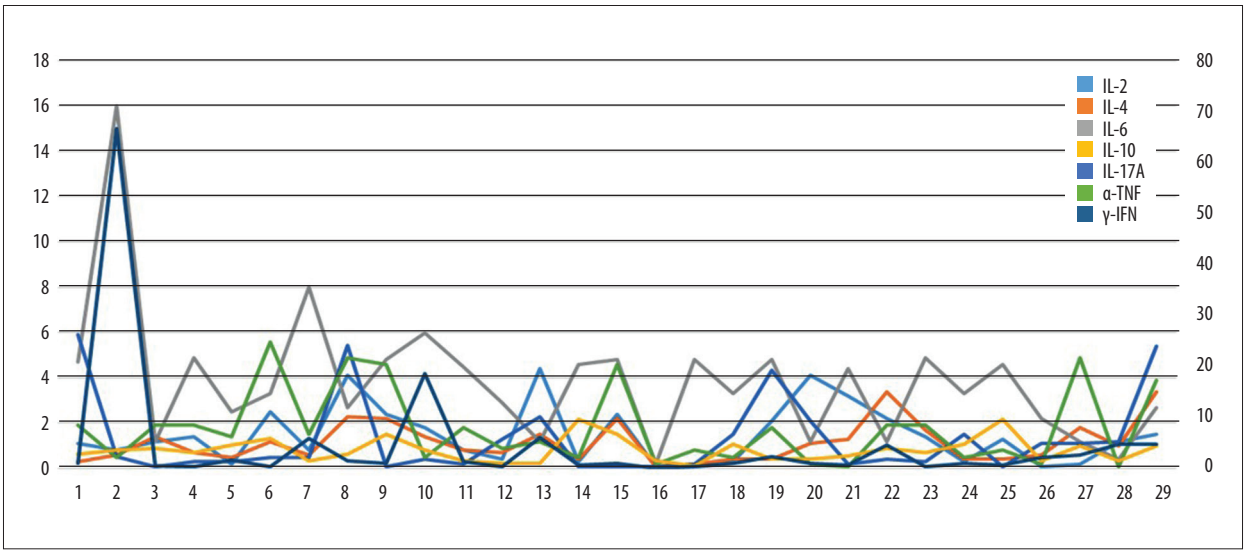

Figure 6. Value of 7 serum inflammatory markers in each patient of the normal hearing group.

non-syndromic hearing impairment. *GJB2* codes for the Connexin 26 protein, which is important in inner ear homeostasis [36]. Given the high incidence of *GJB2* mutations, there are numerous diagnostic tests that can be used to identify this gene [37].

The *GJB2* gene has been reported to be the main hereditary factor accounting for infants' non-syndromic hearing loss [38]. *GJB2 is* expressed regularly for signal transmission between inner ear sensory cells and the cells that surround it, thus benefiting the development of hearing ability [39]. Four *GJB2* mutations – p.W44C, p.R75Q, p.C202F, and p.R75W – leading to the autosomal dominant form of hearing loss have been found [40]. Around 80% of inherited hearing impairment is autosomal recessive, 12% to 15% is dominant, and 1% to 2% is X-linked inherited or mitochondrial [41].

Mutations in the *GJB2* gene are classified as truncating and non-truncating [42]. Truncating mutations, also known as inactivating mutations or nonsense mutations, inhibit protein synthesis and lead to severe hearing loss. They form a premature stop codon which interferes with the protein expression and stops their formation [43]. Non-truncating mutations, also known as missense mutations, alter the amino acid sequence of proteins, resulting in mild or moderate hearing impairment [44]. Previous research reported that c.35delG accounts for about 60% of the mutated alleles and is linked with various hearing loss phenotypes [45]. It is distinguished by a substantial loss in high-frequency hearing, which gradually develops into full-frequency hearing loss before middle age [46]. Mutations in *GJB2* are also linked to some hearing impairment phenotypes, including ichthyosis syndrome, Vohwinkel syndrome, and hyperkeratosis Palmaris [47].

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Table 3. Results of the *t* test performed to examine differences in expression of inflammatory markers between genders and the severity of hearing loss.

Sample	Parameter	T	dof	Alternative	p-val	CI 95%	Cohen-d	BF10	Power	Significant
Cohort 1	A1	2.26	11.88	Two-sided	0.04	[0.04, 2.54]	1.03	2.18	0.77	Yes
	B1	2.71	11.57	Two-sided	0.02	[0.44, 4.09]	1.25	4.70	0.90	Yes
	C1	-0.89	27.61	Two-sided	0.38	[-112.59, 44.26]	0.28	0.468	0.12	No
	D1	0.89	19.07	Two-sided	0.38	[-72.32, 180.02]	0.35	0.468	0.15	No
	E1	0.30	22.36	Two-sided	0.77	[-3.77, 5.04]	0.11	0.359	0.06	No
	F1	-0.63	18.92	Two-sided	0.54	[-11.3, 6.09]	0.18	0.403	0.08	No
	G1	0.56	14.84	Two-sided	0.59	[-5.39, 9.2]	0.24	0.39	0.09	No
Cohort 2	A2	-1.74	17.51	Two-sided	0.10	[-0.91, 0.09]	0.66	1.061	0.41	No
	B2	0.54	25.84	Two-sided	0.59	[-0.57, 0.98]	0.20	0.389	0.08	No
	C2	-0.32	26.99	Two-sided	0.75	[-2.1, 1.54]	0.12	0.36	0.06	No
	D2	-2.68	16.02	Two-sided	0.02	[-3.82, -0.45]	1.02	4.34	0.76	Yes
	E2	-0.91	20.27	Two-sided	0.38	[-6.72, 2.65]	0.34	0.48	0.14	No
	F2	-0.86	25.18	Two-sided	0.40	[-1.01, 0.42]	0.32	0.461	0.13	No
	G2	-1.36	22.77	Two-sided	0.19	[-1.11, 0.23]	0.51	0.693	0.26	No
Cohort 3	A3	0.79	24.79	Two-sided	0.44	[-0.61, 1.37]	0.30	0.442	0.12	No
	B3	-0.26	25.06	Two-sided	0.80	[-0.74, 0.58]	0.09	0.36	0.06	No
	C3	1.55	15.96	Two-sided	0.14	[-0.66, 4.24]	0.62	0.84	0.36	No
	D3	-0.81	25.65	Two-sided	0.43	[-2.44, 1.06]	0.29	0.448	0.12	No
	E3	0.21	22.45	Two-sided	0.84	[-1.27, 1.55]	0.08	0.356	0.05	No
	F3	1.23	25.38	Two-sided	0.23	[-0.52, 2.05]	0.46	0.616	0.22	No
	G3	1.26	12.15	Two-sided	0.23	[-4.68, 17.47]	0.52	0.629	0.27	No

A – IL-2; B – IL-4; C – IL-6; D – IL-10; E – IL-17A; F – α-TNF; G – γ-IFN.

In European countries, many people are affected; they possess a pathogenic variation in *GJB2* gene coding region with several deletions. Deletion can either be in a homozygous state or heterozygous and impairs transcriptional level gene expression by excluding regulatory factors preceding the *GJB2* gene [48,49].

Various studies have reported vulnerability of the cochlea to systemic inflammation [50]. It has also been observed that cochlear microcirculation is regulated by tight junctions which link vascular endothelial cells, resulting in formation of the blood-labyrinth barrier [51,52], which performs an important function by acting as a barrier to pathogen infiltration, transporting certain essential nutrients to the cochlea, and regulating ion homeostasis [53]. Pericytes and melanocytes resembling macrophages residing in the perivascular region are the alternative fence of the blood-labyrinth barrier [18]. Permeability of the barrier increases due to local inflammation in the cochlea as it activates the perivascular region. Pericytes may also result in an increased expression of pro-inflammatory factors via the tight junction barrier [34].

The role of various cytokines and chemokine signals in damaged cochlea leading to hearing impairment is enhanced by inflammatory factors [21]. Various studies have been conducted to investigate cochlear inflammation, showing that there is a potential role of IL-6 and α-TNF regarding monocyte infiltration in cochlear inflammation [20].

Specific or non-specific immune responses and synthesis of certain inflammatory markers are primary responses following inner-ear inflammatory injury [35].

Activated T cells and adhesion molecules can access the inner ear, causing a number of immunological effects, via capillary walls in the brain [54]. Increased T lymphocyte infiltration from the inner ear's lymphatic sac occurs when the immune system reacts, and these T cells and the cytokines they release contribute to the mechanism of inner ear damage [55].

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Table 4A. Correlation of indicators within the specific groups (severe hearing loss group).

Indicator	A1	B1	C1	D1	E1	F1	G1
A1		0.38	-0.16	-0.06	0.09	0.00	0.16
B1			-0.13	-0.02	0.44	0.02	0.00
C1				0.28	-0.18	-0.06	-0.1
D1					-0.07	-0.05	0.04
E1						-0.05	0.06
F1							-0.06

A – IL-2; B – IL-4; C – IL-6; D – IL-10; E – IL-17A; F – α-TNF; G – γ-IFN.

Table 4B. Correlation of indicators within the specific groups (moderate hearing loss group).

Indicator	A2	B2	C2	D2	E2	F2	G2
A2		0.2	0.47	0.3	0.16	0.63	0.77
B2			0.11	0.04	0.37	0.26	0.29
C2				0.23	0.07	0.05	0.57
D2					-0.15	0.16	0.11
E2						0.35	0.37
F2							0.41

A – IL-2; B – IL-4; C – IL-6; D – IL-10; E – IL-17A; F – α-TNF; G – γ-IFN.

Table 4C. Correlation of indicators within the specific groups (normal hearing group).

Indicator	A3	B3	C3	D3	E3	F3	G3
A3		0.49	-0.13	-0.08	0.3	0.29	-0.06
B3			-0.21	0.14	0.19	0.59	-0.04
C3				0.11	-0.12	-0.08	0.79
D3					-0.22	0.29	-0.04
E3						0.24	-0.07
F3							-0.17

A – IL-2; B – IL-4; C – IL-6; D – IL-10; E – IL-17A; F – α-TNF; G – γ-IFN.

Chronic inflammation has been found to pose a significant threat of ischemia by causing athero-genesis and various microvascular injuries [28]. The cochlear blood supply depends mainly on the labyrinthine artery alone [24]. Cochlear cells require more oxygenation because they are quite vulnerable to hypoxia. Therefore, alterations in the microcirculation could have an important effect on the sac cells [56]. In this way, factors such as chronic inflammation that could lead to damage to the microcirculation in the inner ear are profoundly correlated with the pathology of hearing impairment [27].

A limitation of this research is that the sample size was relatively small since hearing loss is relatively rare compared to other more common diseases, which could limit generalization of our results. Further studies with larger sample sizes are needed. In addition, the PCR methods we used have limitations. The kit we used uses the fluorescent PCR melting curve method, which can detect sequence variations that occur within the coverage area of the probe, but not variations outside the range of the probe. Since this test only screens nucleic acid sequences and not amino acid sequences, due to

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Table 5A. Spearman correlation among hearing loss severity and blood markers.

ln_a	-0.159
ln_b	-0.24
ln_c	0.574
ln_d	0.533
ln_e	0.273
ln_f	-0.04
ln_g	-0.031

Table 5B. Linear model results on the relationship among hear loss severity and blood markers.

Severity	Marker	Estimate	Std. error	t value	Pr(>|t|)	Signif.	R2
Normal	ln_a	-0.089	0.182	-0.488	0.627	ns	0.088
Moderate	ln_a	-0.739	0.256	-2.892	0.005	**	0.088
Severe	ln_a	-0.414	0.254	-1.633	0.106	ns	0.088
Normal	ln_b	-0.182	0.187	-0.971	0.334	ns	0.038
Moderate	ln_b	-0.441	0.262	-1.683	0.096	ns	0.038
Severe	ln_b	-0.391	0.26	-1.504	0.136	ns	0.038
Normal	ln_c	1.048	0.189	5.551	0.000	****	0.569
Moderate	ln_c	-0.232	0.265	-0.875	0.384	ns	0.569
Severe	ln_c	2.29	0.263	8.714	0.000	****	0.569
Normal	ln_d	1.075	0.206	5.207	0.000	****	0.528
Moderate	ln_d	-0.645	0.289	-2.23	0.028	*	0.528
Severe	ln_d	2.043	0.287	7.114	0.000	****	0.528
Normal	ln_e	-0.568	0.241	-2.355	0.021	*	0.152
Moderate	ln_e	1.288	0.338	3.806	0.000	****	0.152
Severe	ln_e	0.962	0.336	2.866	0.005	**	0.152
Normal	ln_f	0.04	0.215	0.188	0.851	ns	0.012
Moderate	ln_f	-0.252	0.301	-0.837	0.405	ns	0.012
Severe	ln_f	0.025	0.299	0.084	0.933	ns	0.012
Normal	ln_g	0.372	0.227	1.639	0.105	ns	0.062
Moderate	ln_g	-0.629	0.318	-1.978	0.051	ns	0.062
Severe	ln_g	0.044	0.315	0.14	0.889	ns	0.062

the complexity of human genes, polymorphisms or synonymous mutations may still be classified as mutants.

Conclusions

We analyzed the relationship between the degree of *GJB2*-associated deafness and 7 inflammatory markers in infants. The results showed a statistically significant correlation between expression of IL-10 and severity of infant hearing loss, indicating that it might be a potential therapeutic target in the future. Our study has shed new light and provides important information for further research into this topic.

Declaration of Figures' Authenticity

All figures are original with no duplication, and have not been published previously in whole or in part.

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