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Analysis of NFKB1 and NFKB2 gene expression in the blood of patients with sudden sensorineural hearing loss

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ABSTRACT

Objectives: Sudden Sensorineural Hearing Loss (SSNHL) is an increasingly common health problem today. Although the direct mortality rate of this disorder is relatively low, its impact on quality of life is enormous; this is why accurate identification of pathogenesis and influencing factors in the disease process can play an essential role in preventing and treating the disease. Acute inflammation, which leads to chronic inflammation due to aberrant expression of inflammation-mediating genes, may play a significant role in the pathogenesis of the disease. The essential Nuclear factor kappa B (NF-kB) pathway genes, *NFKB1* and *NFKB2*, serve as prothrombotic agents when expressed abnormally, compromising the cochlea by disrupting the endolymphatic potential and causing SSNHL.

Methods: This study investigates the expression levels of *NFKB1* and *NFKB2* in peripheral blood (PB) through a quantitative polymerase chain reaction in 50 Iranian patients with SSNHL, and 50 healthy volunteers were of the same age and sex as controls.

Results: As a result, *NFKB2* expression levels in patients were higher than in controls, regardless of sex or age (posterior beta = 0.619, adjusted P-value = 0.016), and *NFKB1* expression levels did not show significant differences between patients and controls. The expression levels of *NFKB1* and *NFKB2* had significantly strong positive correlations in both SSNHL patients and healthy individuals (r = 0.620, P = 0.001 and r = 0.657, P 0.001, respectively), suggesting the presence of an interconnected network.

Conclusion: NFKB2 has been identified as a significant inflammatory factor in the pathophysiology of SSNHL disease. Inflammation can play an essential role in developing SSNHL, and our findings could be used as a guide for future research.

1. Introduction

Sudden sensorineural hearing loss (SSNHL) is a complication defined by at least 30 dB of sensorineural hearing loss at three consecutive frequencies in fewer than three days. It is rare, affecting 5 to 20 people in every 100,000 [1], with about 66,000 new cases per year in the United States [2]. In Japan [3] and Germany [4], there are 60.9 and 160 new cases, respectively, of SSNHL per 100,000 individuals per year. The prevalence of SSNHL prevalence is highest in patients between the ages of 25 and 60, reaching its peak in individuals between the ages of 46 and 49. SSNHL in children is uncommon, and the reason is unknown [5,6]. SSNHL patients were reported to be 6.6% under the age of 18, 3.5%

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under the age of 14, and just 1.2% under the age of nine [6]. In up to 90% of cases, SSNHL is idiopathic, and 95% of SSNHL patients present unilaterally [1]. In contrast, bilateral SSNHL was rare and accounted for about 5% of cases [7]. The pathogenesis of SSNHL is not fully understood. Vascular occlusion, autoimmune diseases, inner ear pathology, viruses, and central nervous system abnormalities have the potential to cause SSNHL [8]. Hearing loss can be caused by damage to hair cells or other cochlear structures in the inner ear. The inner ear is the target of many of these disease-causing conditions, and is irreversible. Additional damage can be prevented if diagnosed and treated promptly [9]. In general, vascular disorders with a notable effect on inner ear circulation can be considered the final common route for sudden idiopathic hearing loss [10]. The internal auditory artery is a minimally protected functional terminal artery responsible for supplying blood to the inner cochlea and labyrinth. On the other hand, high oxygen consumption in cochlear hair cells reduces the threshold of hypoxia tolerance. This condition makes the inner ear very susceptible to changes in blood circulation and oxygen supply pathways and increases the chance of this area being vulnerable to ischemia [11]. Thus, the pathophysiology of SSNHL may depend more on vascular and inflammatory changes in the inner ear, many of which may be due to genetic factors.

Inflammation can be part of the body's biological response to harmful stimuli such as pathogens, damaged cells, or various factors, a response that consists of immune cells, molecular mediators, and blood vessels [12]. The function of inflammation begins with eliminating the underlying cause of cell damage and clearing necrotic and damaged cell debris, followed by a reduction in the inflammatory process and tissue repair [13]. Nuclear factor kappa B (NF-KB) is a pleiotropic transcription factor (TF) that controls inflammation, as well as innate and adaptive immune responses [14]. This transcription factor family consists of five members: *RELA* (p65), *RELB*, *REL* (c-Rel), *NF-KB1* (p105), and *NF-KB2* (p100) [15]. It is worth noting that p105 and p100 are pro-forms that are proteolytically converted to p50 and p52. The N-terminal Rel homology domain (RHD) of all members of the NF-KB family interacts with DNA sites and facilitates homo-and heterodimerization. The combination p65–p50 is the most prevalent form of the NF-KB family heterodimer, and it remains inactive in the cytoplasm by binding to IkB family proteins, which are NF-KB inhibitors [16].

Inflammation generally activates platelets, leukocytes, and endothelial cells, and the interaction of these cells may lead to endothelial damage and endothelial cell dysfunction (ECD), disrupting the physiological anticoagulant process and normal endothelial vessels [17]. This process is regulated at the molecular level through various pathways, the most important of which is regulating the expression of inflammatory genes. The malformation created in the genes of this pathway can turn primary inflammation into chronic and permanent inflammation [18]. Several chronic inflammatory diseases closely related to the NF-KB pathway can be listed, including rheumatoid arthritis, atherosclerosis, chronic obstructive pulmonary disease, asthma, and multiple sclerosis [19]. *NFKB1*, as a member of this pathway, is the most frequently expressed transcription factor in macrophages (16), which are critical initiators of chronic inflammatory illness. It also plays a vital role in determining macrophage polarization and innate immunological memory responses. Furthermore, *NFKB2* can aggravate inflammation by complementing latent *RELA* dimers to the canonical NF-KB module [20]. As a pathogenic effect of inflammation, chronic inflammation can be linked to endothelial dysfunction by acting as a prothrombotic, resulting in decreased blood flow due to the thickening of the vascular wall, even at the surface of the inner ear [21]. When the thickened endothelium is unable to function due to chronic inflammation, blood flow to the inner ear decreases, leading to a loss of endolymphatic potential, which ultimately reduces the strength of the cochlea, which can lead to deafness. Blood inflammation molecules have also been found to harm cochlear vascular tissue, both next to the modular spiral artery and between the capillaries that comprise the stria, located outside the cochlea [22].

In this study, we examine the two influential factors of *NFKB1* and *NFKB2* on the inflammation response that has the ability to change the pathophysiological characteristics of the increase in the incidence of SSNHL in a case-control study.

2. Materials and methods

2.1. Patient recruitment

Fifty patients with SSNHL were diagnosed between June 2019 and March 2020 by well-trained medics specializing in ear, nose, and throat at Tabriz University of Medical Sciences/Imam Reza hospital. Patients set out the following diagnostic criteria: >30 dB sensorineural hearing loss on a pure tone audiogram at more than three consecutive frequencies, onset within three days and no involvement of cranial nerves other than the eighth cranial nerve. Fifty healthy Iranian volunteers who underwent physical examinations and showed no clinical signs of SSNHL or other disorders were used as controls. SSHNL patients in the acute stage of hearing loss had blood samples taken. The study's purpose was explained to all patients and healthy volunteers, and each participant gave informed consent. This study was approved by the Research Ethics Committee (REC) of Tabriz University of Medical Sciences (TUOMS) (64866).

2.2. Collection and preparation of samples

Five ml of PB were collected from all SSNHL patients and healthy subjects. Blood was drawn in ethylenediaminetetraacetic acid (EDTA) tubes to prevent clotting. Phosphate-buffered saline (PBS) was used to wash the EDTA tubes after centrifugation. Using ACK lysis buffer, the erythrocytes were removed twice (Gibco, Grand Island, NY, USA).

2.3. RNA isolation and quantitative real-time PCR

Total cellular RNA was extracted from whole blood cells using TRIzol (RiboEx, GeneAll Biotechnology Co. Ltd, Korea). Following a NanoDrop determination (Thermo Scientific, Wilmington, DE) determination of RNA concentration and quality, RNA was incubated with DNase to remove genomic DNA contamination. The cDNA was synthesized using the cDNA Synthesis Kit (Geneall, HyperScript™ First Strand Synthesis Kit) according to the manufacturer's instructions. For further research, the obtained cDNA was stored at −20 °C. Designing specific primers for *NFKB1*, *NFKB2*, and ubiquitin C (*UBC*) was carried out by applying Primer3 (version 2.6.0) and Oligo7 (version 7.60) software. The primers used for *NFKB1*, *NFKB2*, and *UBC* as the housekeeping gene are shown in Table 1. A quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed using QIAGEN Rotor-Gene Q (Corbett Rotor-Gene 6000) and the RealQ Plus2x Master Mix Green without ROX™ (Ampliqon, Odense, Denmark). The average expression of *UBC* was used to normalize the expression levels of each gene.

2.4. Statistical analysis for qPCR

The R v.4 software packages brms, stan, pROC, and GGally were used to analyze the data. The Bayesian regression model was used to evaluate the relative expressions of *NFKB1* and *NFKB2* in SSNHL patients and healthy controls. The effects of age and gender have been adjusted. Adjusted P-values of less than 0.05 were considered significant. The expression of the genes mentioned above was also investigated across age groups and between males and females. The Spearman correlation coefficients were used in the study to assess the strength and direction of the monotonic association between two variables. A receiver operating characteristic (ROC) curve analysis was used to estimate the diagnostic power of the genes.

Table 1
List of primers used in this study.

Gene name	Gene reference ID	Primer sequences (5′-3′)	Product size	Tm
NFKB1	NM_001382627.1 NM_001382625.1 NM_001382628.1 NM_001382626.1 NM_001382626.2 NM_001319226.2 NM_001165412.2 NM_003998.4 XM_024454069.1 XM_024454068.1	Forward primer: TGATTTACTAGCACAAGGAGACAT; Reverse primer: TTGCTTCGGTGTAGCCCATT	254 nt	60
NFKB2	NM_001261403.3 NM_002502.6 NM_001322934.2 NM_001322935.1 NM_001077494.3 NM_001288724.1	Forward primer: CGAGAACGGAGACACACCAC; Reverse primer: CTGAGTCTCCATGCCGATCC	233 nt	61
UBC	NM_021009.7	Forward primer: CAGCCGGGATTTGGGTCG; Reverse prime: CACGAAGATCTGCATTGTCAAGT	72 nt	60

3. Results

3.1. General demographic data

We examined 50 patients (male/female: 25/25) with an age range of 5–14 years and (mean ± standard deviation (SD)) of 9.36 ± 2.49 and 50 healthy controls (male/female: 26/24) with an age range of 4–14 years and (mean ± SD) of 9.16 ± 2.54 who were all Iranian.

3.2. qPCR data analysis

Fig. 1 shows the relative expression levels of *NFKB1* and *NFKB2* in SSNHL patients and controls. *NFKB2* expression levels were higher in patients than controls, regardless of participants' gender and age (posterior beta = 0.619, adjusted *P*-value = 0.016). Considering the gender of study participants, the analysis showed that *NFKB2* expression levels were significantly higher in female patients than in healthy female controls (posterior beta = 0.894, adjusted *P*-value = 0.042) and in male patients than in healthy male controls (posterior beta = 0.513, adjusted *P*-value = 0.048). The *NFKB1* expression levels demonstrated no significant differences in PB samples among SSNHL patients and healthy controls (adjusted *P*-value = 0.223). Tables 2 and 3 provide detailed information on the relative expression of *NFKB1* and *NFKB2*, respectively.

3.3. Correlation analysis

NFKB1 and *NFKB2* expression were correlated with both cases and controls. The expressed levels of *NFKB1* and *NFKB2* were correlated significantly both among SSNHL patients and among healthy controls ($r = 0.620$, $P < 0.001$ and $r = 0.657$, $P < 0.001$, respectively). The expressed levels of *NFKB1* and *NFKB2* were correlated significantly considering sexes both among males and females ($r = 0.547$, $P < 0.001$ and $r = 0.576$, $P < 0.001$, respectively) (Fig. 2).

3.4. ROC curve analysis

We assessed the diagnostic power of *NFKB1* and *NFKB2* to distinguish SSNHL patients from healthy controls at different threshold settings. We classified them as candidates for biomarkers according to the decreasing area under the receiver operating characteristic (ROC) curve (AUC) and graphed the results. *NFKB1* transcript level presented a diagnostic power of 0.555 (95% confidence interval, 0.45–0.65), Youden index J = 0.14 (95% confidence interval, 0.08–0.2), and optimal

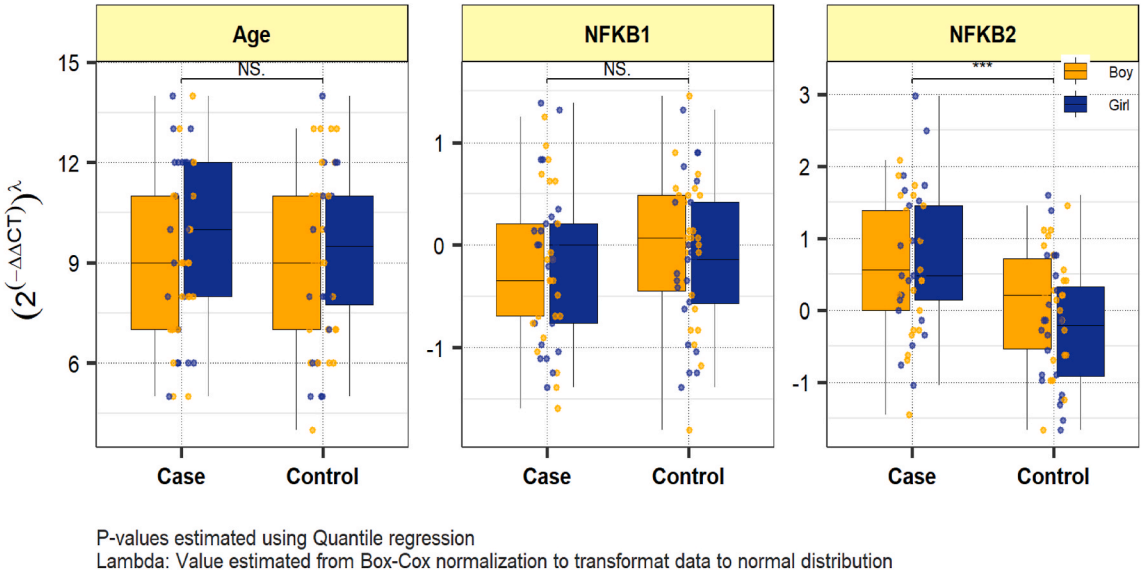

Fig. 1. This figure depicts the expression of *NFKB1* and *NFKB2* in blood samples from patients and controls. Gray dots represent the values. The interquartile range and the average of the expression levels are displayed.

Table 2
The association between SSNHL and *NFKB1* relative gene expression: The results of Bayesian quantile regression model.

	NFKB1	Posterior Beta of $(2^{(-ddct)})^{\lambda}$	SE	Adjusted P-Value[a]	95% Crl for Beta
Total	Group, Case vs. control	−0.160	0.15	0.223	[-0.46, 0.164]
	Sex, Female vs. Male	0.059	0.15	0.259	[-0.26, 0.35]
	Age (years)	−0.027	0.03	0.578	[-0.09, 0.035]
	Group[a] Sex	−0.482	0.28	0.08	[-1.04, 0.09]
Male	Case vs. control	−0.359	0.2	0.378	[-0.75, 0.06]
	Age	−0.001	0.05	0.435	[-0.1, 0.09]
Female	Case vs. control	0.1	0.22	0.422	[-0.35, 0.52]
	Age	−0.052	0.05	0.999	[-0.15, 0.04]

50 patients (male/female: 25/25) with age (mean ± SD) of 9.36 ± 2.49.
50 healthy controls (male/female: 26/24) with age (mean ± SD) of 9.16 ± 2.54.
[a] Estimated from frequentist methods; CrI: Credible interval, λ: Power transformation value estimated from Box-cox or Yeo-Johnson methods.

Table 3
The association between SSNHL and *NFKB2* relative gene expression: The results of Bayesian quantile regression model.

	NFKB2	Posterior Beta of $(2^{(-ddct)})^{\lambda}$	SE	Adjusted P-Value[a]	95% Crl for Beta
Total	Group, Case vs. control	0.619	0.18	0.016	[0.258, 0.98]
	Sex, Female vs. Male	−0.265	0.18	0.280	[-0.62, 0.108]
	Age (years)	0.012	0.04	0.510	[-0.07, 0.088]
	Group[a] Sex	−0.403	0.39	0.849	[-1.19, 0.36]
Male	Case vs. control	0.513	0.25	0.048	[0.02, 0.97]
	Age	0.001	0.06	0.573	[-0.11, 0.11]
Female	Case vs. control	0.894	0.29	0.042	[0.3, 1.45]
	Age	0.006	0.05	0.999	[-0.1, 0.11]

50 patients (male/female: 25/25) with age (mean ± SD) of 9.36 ± 2.49.
50 healthy controls (male/female: 26/24) with age (mean ± SD) of 9.16 ± 2.54.
[a] Estimated from frequentist methods; CrI: Credible interval, λ: Power transformation value estimated from Box-cox or Yeo-Johnson methods.

cut-off: <-3.4 (95% confidence interval, <-4.7, <-2.1). *NFKB2* transcript level presented a diagnostic power of 0.703 (95% confidence interval, 0.603–0.79), Youden index J = 0.32 (95% confidence interval, 0.18–0.44), and optimal cut-off: <-4.1 (95% confidence interval, <-5, <-2.2) (Fig. 3), based on the area under the ROC curves.

4. Discussion

SSNHL's pathophysiology has several possible origins, including vascular problems, viral infections, ruptured labyrinthine membrane endolymphatic hydrops, and inner ear immunity-mediated disorders [23]. The common denominator of many pathogens is permanent hearing loss caused by the destruction of hair cells or other cochlear structures. Prompt diagnosis and treatment can help to avoid further harm [24]. However, the idiopathic sudden sensorineural hearing loss (ISSNHL) mechanism is unclear. Remarkably, vascular malfunction has lately been hypothesized as the last common mechanism for sudden idiopathic hearing loss in terms of inner ear-specific blood circulation [25]. The internal auditory artery is a functioning terminal artery with limited protection that delivers blood to the inner cochlea and the labyrinth. The blood and oxygen delivery channels make this area more vulnerable to ischemia [11]. Thus, the pathophysiology of ISSNHL may depend more on vascular and inflammatory abnormalities in the inner ear, many of which may be related to hereditary factors.

Inflammation is regulated at the molecular level by multiple pathways, the most significant of which is regulating the expression of inflammatory genes. The abnormality caused by the genes of this pathway can transform acute inflammation into chronic and persistent inflammation [18]. Chronic inflammation can act as a prothrombotic (a factor that causes thrombosis) and is associated with endothelial dysfunction that reduces blood flow due to the thickening of the vascular wall, even at the level of the inner ear [21]. The endothelium of the inner ear arteries thickens due to the pathophysiology of inflammation-causing thrombosis. Inflammation generally activates platelets, leukocytes, and endothelial cells, and the interaction of these cells may lead to endothelial damage and endothelial cell (ECD) dysfunction, leading to the loss of physiological anticoagulant properties and normal endothelial vessels. Chronic inflammation can significantly cause endothelial damage with thrombosis [17]. When the thickened endothelium cannot function due to chronic inflammation, blood flow to the inner ear decreases, leading to a loss of endolymphatic potential, which ultimately reduces the strength of the cochlea, which can lead to deafness [22]. Furthermore, it has been demonstrated that circulating inflammatory mediators have a deleterious effect on cochlear vascular tissue close to the modular spiral artery and distally between the capillaries of the striae (the outer wall of the cochlea).

Cochlear hair cells can also lead to apoptosis by other factors. The first Gasdermin E (*GSDME*) mutation was discovered in a Dutch family in 1998 as the etiology of a particular kind of autosomal dominant, progressive, non-syndromic, sensorineural hearing loss [26]. At least nine *GSDME* mutations related to hearing loss have been identified, six of which are found in the introns surrounding exon eight and three of which are found inside the exon [27–30]. Even though various *GSDME* mutations vary at the genomic DNA level, they all result in exon eight skipping during splicing, contributing to a frameshift and premature termination of the protein [31]. The truncated protein has an inherent necrotic function due to releasing the inhibitory C-terminal domain and subsequent activation of the necrotic N-terminal due to the deletion of exon eight during *GSDME* mRNA transcription [26,28,32,33]. Because *GSDME* has an N-terminal domain that induces apoptosis, it can cause hearing loss by increasing the death of cochlear hair cells [31], which are critical to hearing and are especially susceptible to the negative consequences of mitochondrial damage [34]. A study found that mutant *GSDME* dramatically enhanced ROS production and elevated multiple cytochrome *c* oxidase (COX) genes in yeast and 293T cells, consistent with its apoptosis-relevant activity [32]. Additional evidence showed that the mutant *GSDME* interacted with mitochondria or the endoplasmic reticulum, which was linked to apoptosis [33]. Interestingly, a cancer-related study confirmed that *GSDME* is regulated by one of the NF-KB (AMPK/SIRT1/NF-KB) inflammatory pathways affected by metformin, leading the cell to progress in apoptosis progress [35].

NF-KB activation includes two key signaling pathways, canonical and non-canonical (or alternative), which are critical for regulating immunological and inflammatory responses despite their variations in signaling mechanisms [36,37]. The canonical NF-KB pathway is activated by stimuli such as T- and B-cell receptors [38], in addition to numerous cytokine and pattern recognition receptor ligands, including the TNF receptor (TNFR) superfamily. The fundamental mechanism for conventional NF-KB activation is the inducible degradation of *IkBα*, initiated by the site-specific phosphorylation of *IkBα* by a multi-subunit IkB kinase (*IKK*) complex [39,40]. Each subunit of IKK (*IKKα* and *IKKβ*) is responsible for a different part of the catalytic process. The regulatory subunit is known as the essential modulator NF-KB (NEMO) [41]. Cytokines, growth factors, mitogens, microbes, and stress agents are

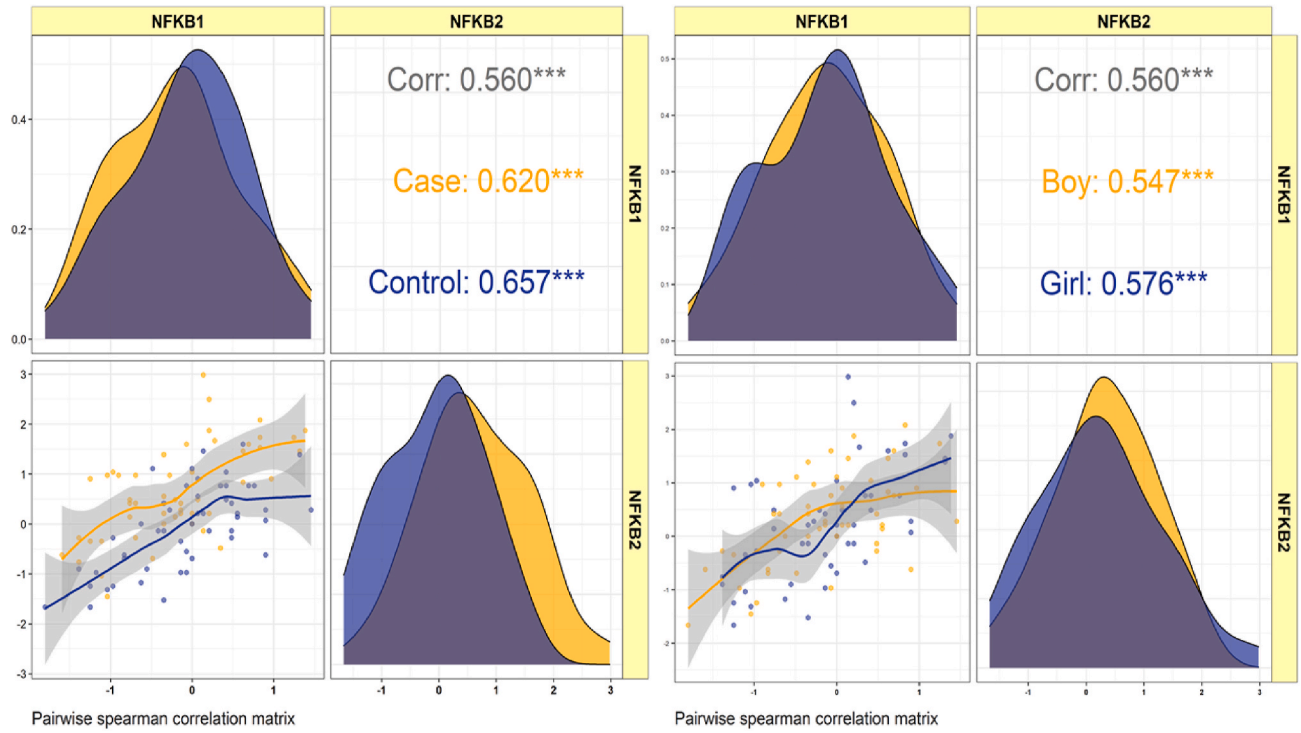

Fig. 2. Correlations between the expression levels of the *NFKB1* and *NFKB2* genes in cases and controls, as well as boys and girls. The variable distribution is depicted on the diagonal. Bivariate scatter plots with a fitted line are shown in the lower portion of the diagonal. Correlation coefficients and P-values are shown in the upper part of the diagonal. The correlation coefficients plus the significance level as stars are shown. *, **, and *** is significant correlation at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

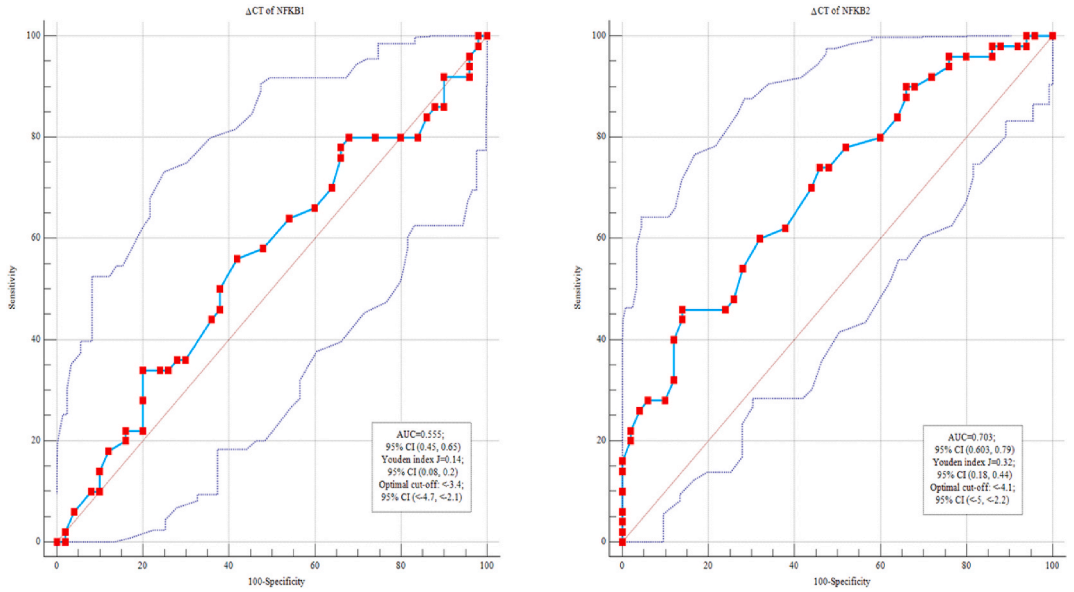

Fig. 3. ROC curve analysis. *NFKB1* and *NFKB2* transcript levels displayed diagnostic power of 0.555 and 0.703, respectively.

among the stimuli that can activate IKK [42]. When IKK is activated, it phosphorylates *IkBα* at two N-terminal serines, triggering ubiquitin-dependent *IkBα* breakdown in the proteasome, resulting in fast and transitory nuclear translocation of canonical NF-KB members, primarily the p50/RelA and p50/c-Rel dimers [43,44].

The non-canonical NF-KB pathway reacts to a subset of TNFR superfamily ligands such as LTR, BAFFR, CD40, and RANK instead of responding to all stimuli, and this is different from the canonical NF-KB pathway [45]. Furthermore, non-canonical NF-KB activation depends on processing NF-KB2 precursor protein, p100, rather than IB degradation [46]. An NF-KB-inducing kinase (NIK) is an essential signaling protein in this pathway, activating and functionally cooperating with *IKKα* to cause p100 phosphorylation, leading to p100 ubiquitination and processing [47]. p100 is processed by degrading its IkB-like C terminal, which produces mature *NFKB2* p52 and nuclear translocation of the non-canonical NF-KB complex p52/RelB [36,46]. The canonical NF-KB route is involved in nearly all aspects of immune responses. However, the non-canonical NF-KB pathway appears to have developed as an

additional signaling axis following the canonical NF-KB pathway to regulate certain activities of the adaptive immune system [45]. Regulation of inflammatory reactions is a well-known function of NF-KB. NF-KB regulates the activation, differentiation, and effector function of inflammatory T cells and regulates the expression of several proinflammatory genes in innate immune cells [48]. Recent research shows that NF-KB is also involved in regulating inflammasome activity [49]. Not unexpectedly, chronic inflammatory disorders are characterized by dysregulated NF-KB activation. As a result, a better knowledge of the mechanism behind NF-KB activation and proinflammatory action is critical for developing therapeutic methods for inflammatory disorders or diseases such as SSNHL, in which inflammation can play a role in their pathogenesis.

In this study, we investigated the expression of *NFKB1* and *NFKB2* in SSNHL patients in a case-control study with a sample size of 50 SSNHL patients and 50 healthy controls. *NFKB2* expression levels in patients were higher than in controls, regardless of gender or age (posterior beta = 0.619, adjusted P-value = 0.016). When the gender of study participants was considered, the analysis revealed that *NFKB2* expression levels were significantly higher in both female (posterior beta = 0.894, adjusted P-value = 0.042) and male (posterior beta = 0.513, adjusted P-value = 0.048) subjects. There were no significant differences in *NFKB1* expression levels in PB samples between SSNHL patients and healthy controls (adjusted P-value = 0.223). In both cases and control groups, the expression of *NFKB1* and *NFKB2* was correlated. There was a significant correlation between the expressed levels of the *NFKB1* and *NFKB2* genes in SSNHL patients and healthy controls ($r = 0.620$, $P < 0.001$ and $r = 0.65$, $P < 0.001$, respectively). The expressed levels of *NFKB1* and *NFKB2* were significantly correlated between the sexes in both males and females ($r = 0.547$, $P < 0.001$ and $r = 0.576$, $P < 0.001$, respectively). According to the findings, one of the likely explanations is related to the overexpression of the *NFKB2* gene as one of the primary mediators of inflammation in SSNHL patients, which causes acute inflammation to become chronic inflammation and, as a prothrombotic factor, promotes vascular wall thickening. It decreases the flow of the inner ear, which affects the strength of the cochlea by changing the endolymphatic potential, finally resulting in SSNHL. In particular, the impact of hyperbaric oxygen treatment (HBOT) on inflammation and as a treatment for SSNHL has been investigated. HBOT reduces hearing loss in the SSNHL by reducing inflammation generated by the inflammatory response induced by the NF-KB and TLR4 pathways [50].

The direct effect of *NFKB2* on other components of the NF-KB signaling pathway has also been confirmed in other studies. According to a mechanistic analysis, Tonic non-canonical *NFKB2* signaling replenished latent RelA: p50 and RelA: p52 dimers by causing simultaneous processing of p105 and p100. During the onset of experimental colitis, *NFKB2*-dependent regulation of latent RelA dimers induced a hyperactive canonical response in epithelial cells, exacerbating intestinal inflammation. Even the non-canonical control of the *NFKB2* pathway of latent *NFKB2* dimers presents a therapeutic target in RelA-based inflammatory diseases [20]. On the other hand, in a study the specific profiles of cells and immune factors of acute SSNHL patients were mentioned. In patients with acute SSNHL, the percentage of proinflammatory CD40, TNF-α, cyclooxygenase-2, or CD38-positive T or B lymphocytes was significantly increased [51]. *NFKB2* could play a key role in regulating the proliferation and population of B cells, although the immune profile of the study participants was not studied in this study. The non-canonical NF-KB pathway activator, the B-cell activating factor (*BAFF*), delivers essential survival signals during B cell maturation and leads to B cell proliferation. B cell maturation requires the NFKB family member RelB ex vivo, whereas cRel is essential for proliferation. When *BAFF* uses *NFKB2* p100 at low levels in developing B cells, it forms the RelB: p52 dimer, but when synthesized at high levels, it forms multimeric IκBsome complexes that *BAFF* neutralizes to increase cRel activity and B cell proliferation [52].

One research indicated that the *NFKB* gene variations *rs4648011* and *rs3774937* have a long-term effect on the progression of hearing loss [53]. Another study utilizing an NF-KB reporter mouse indicated that NF-KB exhibited a protective function in connective tissue cells within the spiral ligament. Thus, type II fibrocytes are activated in the spiral ligament in response to systemic inflammatory stress, and immune-mediated SSNHL in humans may be partly caused by type II fibrocyte sensitivity [54]. Because type II fibrocytes play a vital role in K+ ion absorption from perilymph, an aberrant function of these cells would probably have a considerable influence on hearing thresholds. Furthermore, the fact that steroids are potent NF-KB pathway inhibitors may explain the observed response to systemic steroids in individuals with SSNHL [53]. It is vital to highlight a significant correlation between the expression levels of the *NFKB1* and *NFKB2* genes in patients and healthy controls. *NFKB1* and *NFKB2* were also correlated in both sexes of patients. Although *NFKB1* expression in patients was not statistically significant compared to healthy controls, *NFKB1* with a positive correlation can affect *NFKB2* expression.

Our study design has some limitations. It would have been preferable to investigate the *NFKB1* gene variations *rs3774937* and *rs4648011*. On the other hand, the primary and practical variations of the *NFKB2* gene were studied in patients and controls from a more comprehensive statistical sample. It could also be used to monitor blood flow to the inner ear in patients and record any changes in blood supply for future research. It was also not possible to assess the neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio of SSNHL patients compared to controls. However, it is strongly recommended that this rate be considered in future studies of genes and inflammatory factors.

5. Conclusion

This study evaluated the expression of inflammatory factors *NFKB1* and *NFKB2* in SSNHL patients. *NFKB2* can be identified as one of the significant inflammatory factors in the pathogenesis of SSNHL disease. Inflammatory factors play an essential role in the pathophysiology of this disease, and our findings might serve as a suitable starting point for further research.

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