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Evaluation of dynamic thiol/disulphide homeostasis and ischemia-modified albumin levels in sudden sensorineural hearing loss

Ani sensörinöral işitme kaybında dinamik tiyol/disülfid homeostazisi ve iskemi modifiye albümin düzeylerinin değerlendirilmesi

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ABSTRACT

Objectives: This study aimed to investigate the role of ischemia-modified albumin and thiol/disulfide hemostasis in idiopathic sudden sensorineural hearing loss (ISSNHL).

Patients and Methods: This prospective study was conducted with volunteers diagnosed with ISSNHL and healthy individuals between November 2018 and June 2019. The study group included 46 patients (27 males, 19 females; mean age: 42.4±12.3 years; range, 18 to 69 years), and the control group included 41 healthy individuals (25 males, 16 females; mean age: 37.8±11.7 years; range, 18 to 65 years). Serum thiol, disulfide, and ischemia-modified albumin were calculated in ISSNHL patients and control groups. The relationship between prognostic factors of disease and blood values was investigated.

Results: The native thiol/total thiol ratio was significantly higher in the control group. Disulfide levels, disulfide/native thiol ratio, and disulfide/total thiol ratio were significantly higher in the patient group.

Conclusion: Oxidative stress that is not balanced by an adequate antioxidant defense may play an important role in the etiopathogenesis of ISSNHL.

Keywords: Audiometry, ischemia-modified albumin, oxidative stress, sudden sensorineural hearing loss, thiol/disulfide homeostasis.

ÖZ

Amaç: Bu çalışma, iskemi modifiye albümin ve tiyol/disülfid hemostazının idiyopatik ani sensörinöral işitme kaybı (İASNİK)'daki rolünü araştırmayı amaçladı.

Hastalar ve Yöntemler: Bu prospektif çalışma, Kasım 2018-Haziran 2019 tarihleri arasında İASNİK tanısı konan gönüllüler ve sağlıklı bireyler ile yürütüldü. Çalışma grubu 46 hastadan (27 erkek, 19 kadın; ort. yaş: 42.4±12.3 yıl; dağılım, 18-69 yıl), kontrol grubu ise 41 sağlıklı bireyden (25 erkek, 16 kadın; ort. yaş: 37.8±11.7 yıl; dağılım, 18-65 yıl) oluştu. İdiyopatik ani sensörinöral işitme kaybı hastaları ve kontrol gruplarında serum tiyol, disülfid ve iskemi modifiye albumin hesaplandı. Hastalığın prognostik faktörleri ile kan değerleri arasındaki ilişki araştırıldı.

Bulgular: Native tiyol/toplam tiyol oranı kontrol grubunda anlamlı olarak daha yüksekti. Disülfid düzeyleri, disülfid/native tiyol oranı ve disülfid/toplam tiyol oranı hasta grubunda anlamlı olarak yüksekti.

Sonuç: Yeterli antioksidan savunma ile dengelenmeyen oksidatif stres İASNİK etyopatogenezinde önemli rol oynayabilir.

Anahtar sözcükler: Odyometri, iskemi modifiye albümin, oksidatif stres, ani sensörinöral işitme kaybı, tiyol/disülfit homeostazı.

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Idiopathic sudden sensorineural hearing loss (ISSNHL) is defined as sensorineural hearing loss that develops in three days or less at three consecutive frequencies in pure tone audiometry.[1] Fifteen thousand new cases are reported each year in the world, and approximately 4,000 new ISSNHL cases are defined each year in the USA.[1,2] Its etiopathogenesis is uncertain, many cases are idiopathic, and a specific cause can be revealed in only 10% of cases. Viral infections, circulatory disorders, labyrinth membrane rupture, autoimmune reactions, metabolic events, and toxic causes are the leading etiological causes.[3-5]

Oxidative stress, defined as the excess of proxidant species that are not sufficiently balanced by endogenous and exogenous antioxidant defense systems, is a serious risk for microvascular damage. Oxidative stress associated with cellular damage, and apoptotic cell death can contribute to sensorineural hearing loss.[6] Thiols can oxidize with oxidants and form disulfide bonds. Dynamic thiol disulfide hemostasis has a critical importance in antioxidant protection, detoxification, and apoptosis. Thus, the determination of dynamic thiol disulfide homeostasis can provide valuable information about various normal or abnormal biochemical processes.[7]

Ischemia-modified albumin (IMA) is an oxidatively modified form of albumin, and blood levels rise due to oxidative stress developing after acute ischemia. Ischemia-modified albumin has been studied in many different diseases and was considered a sensitive marker for the diagnosis of oxidative stress.[8]

In this study, we aimed to demonstrate the role of IMA and thiol/disulfide hemostasis in ISSNHL, considering the prognostic factors and hearing levels of ISSNHL patients.

PATIENTS AND METHODS

This prospective study included volunteers diagnosed with ISSNHL in our clinic and healthy individuals over the age of 18 who agreed to participate in the study between November 2018 and June 2019. The study was conducted at the University of Health Sciences, Konya Health Application and Research Center. The study group included 46 patients (27 males, 19 females; mean age: 42.4±12.3; range, 18 to 69 years), and the control group included 41 healthy individuals (25 males, 16 females; mean age: 37.8±11.7; range, 18 to 65 years). Patients who had one or more of the following criterias; history of autological surgery and ototoxic drug use, acoustic trauma and barotrauma, genetic sensorineural hearing loss, evidence of retrocochlear disease in magnetic resonance imaging, malignancy and autoimmune disease, smokers, those with upper respiratory tract infection in the last four weeks, fluctuant hearing loss, those with any systemic additional disease (e.g., diabetes mellitus, kidney failure, cardiovascular disease, and chronic obstructive pulmonary disease).

The same treatment protocol was applied to all patients participating in this study. This protocol included intravenous 1 mg/kg methylprednisolone (Precort-Liyo 40 mg; Kocak Farma, Istanbul, Türkiye) with decreased doses within two to three weeks and hyperbaric oxygen therapy. Hyperbaric oxygen treatment was performed at 2 ATA (atmosphere absolute) for 1 h, for a total of 20 sessions without weekends.

Audiological evaluation

Pure tone audiometry test was performed to all patients both before and after treatment (AC33 Clinical Audiometer; Interacoustic Company, Middelfart, Denmark) one to three months after diagnosis on average. Hearing thresholds at frequencies of 250, 500, 1000, 2000, 4000, 6000 Hz were detected in pure-tone (PTA) audiometry. Pure-tone average was calculated by calculating the average of the hearing thresholds at 500, 1000, 2000, 4000 Hz. Hearing impairment was classified according to international standards set by the World Health Organization (26-40 dB, mild; 41-60 dB, moderate; 61-80 dB, severe; >81 dB, profound hearing loss).[9] Degree of recovery was classified into four categories: complete recovery (hearing is at the same level with unaffected ear or less than 30 dB), good recovery (hearing return within 15 dB of unaffected ear or PTA improved more than 30 dB but not within normal hearing limits), fair recovery (hearing level increased more than 10 dB but did not return to 15 dB from unaffected ear), poor recovery (hearing level worsened, did not change, or improved less than 10 dB).[10]

Evaluation of blood parameters

Fasting blood samples were obtained from volunteers to plain tubes. Sera were separated after centrifugation at 1600 g for 10 min and stored at -80 C° until the analyzing time.

Measurement of thiol-disulphides homeostasis parameters

Thiol/disulfide homeostasis tests were measured by automatic spectrophotometric method defined by Erel and Neselioğlu.[7] Disulfide bonds were reduced with sodium borohydride, and then free thiol groups were formed. Unused reductant sodium borohydride was finished and extracted to prevent DTNB [5,5'-dithiobis-(2-nitrobenzoic) acid] reduction. Afterward, natural

thiol groups were assigned. The natural thiol content is subtracted from the total thiol content, and half of the difference obtained gives the amount of disulfide bond. Disulphide/total thiol percent ratios, disulfide/native thiol percent ratios, and native thiol/total thiol percent ratios were calculated after calculating natural and total thiols and disulfide amounts.

Albumin cobalt binding test

Ischemia-modified albumin was measured by albumin cobalt binding test. Ischemia-modified albumin, a marker of ischemia, provides its ability to bind elements such as cobalt and copper in the N-terminal of albumin. Ischemia-modified albumin test was performed with blood prepared from tubes separated from serum. Specimens were frozen at -80°C. Frozen samples were gently vortexed after thawing. In the albumin cobalt binding test, 95 µL of a patient sample and 5 µL of cobalt chloride [Co(II)] are incubated for 5 min. During incubation, the Co(II) binds to the N-terminus of unaltered albumin in the sample; albumin, for which the N-terminus is altered as a result of ischaemic processes, binds to the Co(II) to a far lesser extent. After incubation, 25 µL of dithiothreitol is added to the mixture. Dithiothreitol forms a coloured complex with Co(II) that is not bound at the N-terminus of albumin, and this complex is measured spectrophotometrically at 480 nm.

Statistical analysis

All analyses were done with the IBM SPSS version 25.0 software (IBM Corp., Armonk, NY, USA). Variables were evaluated after normality and homogeneity of variance preconditions were checked (Shapiro Wilk and Levene test). Comparisons of two groups was made with Student's t test, and the Mann Whitney-U test was used when the prerequisites were not met. Comparison of three or more groups was made with one-way analysis of variance and the Tukey honestly significant difference test. The relationship between two variables was evaluated by the Pearson correlation coefficient. If it did not satisfy the parametric test prerequisites, it was evaluated with the Spearman correlation coefficient. Categorical data were analyzed by Fisher exact test and the chi-square test. In cases where the expected frequencies were less than 20%, evaluations were made with the Monte Carlo simulation method to include these frequencies in the analysis. A *p*-value of <0.05 was considered statistically significant.

RESULTS

There was no significant difference between the two groups in terms of age and sex (p>0.05). The PTA of the study group was 61.3 dB, while the PTA of the control group was 12 dB at the time of admission. In the study group, nine patients had vertigo, and 33 patients had tinnitus (Table 1).

When the blood parameters were evaluated, there was no significant difference between the two groups in terms of native thiol, total thiol, and IMA levels; however, the control group had a significantly higher native thiol/total thiol ratio. There was a significant difference between the two groups in terms of disulfide

Table 1
Distribution of blood values and disease parameters according to the groups

	Control group (n=41)			Patient group (n=46)			
Parameters	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (year)			37.8±11.7			42.4±12.3	0.080
Sex							0.829
Male	25	61.0		27	58.7		
Female	16	39.0		19	41.3		
Native thiol (µmol/L)			359.4±59.1			352.5±63.8	0.600
Total thiol (µmol/L)			409.7±71.3			410.7±72.8	0.950
Disulfide (µmol/L)			25.1±8.2			29.1±8.0	0.030
Disulfide/native thiol (%)			6.9±1.7			8.3±2.1	0.001
Disulfide/total thiol (%)			6.1±1.3			7.1±1.5	0.001
Native thiol/total thiol (%)			87.9±2.7			85.9±3	0.001
IMA (ABSU)			0.8±0.1			0.8±0.1	0.740
PTA (dB)			12±5.4			61.4±24.5	0.001

SD: Standard deviation; IMA: Ischemia-modified albumin; ABSU: Absorbance units; PTA: Pure tone average.

Table 2
Distribution of blood values according to disease and audiogram parameters

Parameters	Native thiol (μmol/L) Mean±SD	Total thiol (μmol/L) Mean±SD	Disulfide (μmol/L) Mean±SD	Disulfide/ native thiol (%) Mean±SD	Disulfide/ total thiol (%) Mean±SD	Native thiol/ total thiol (%) Mean±SD	IMA (ABSU) Mean±SD
Hearing loss							
Mild (n=8)	371.8±77.8	428.1±93.5	28.2±9.0	7.4±1.4	6.5±1.1	87.1±2.1	0.8±0.1
Moderate (n=18)	355.6±52.0	415.8±56.1	30.1±8.1	8.6±2.4	7.3±1.8	85.5±3.6	0.7±0.1
Severe (n=11)	349.0±73.6	405.9±86.2	28.5±8.0	8.1±1.6	7.0±1.2	86.1±2.4	0.7±0.1
Profound (n=9)	333.3±65.0	390.7±73.3	28.7±8.0	8.7±2.2	7.3±1.6	85.3±3.3	0.8±0.1
P value	0.67	0.75	0.92	0.55	0.59	0.59	0.49
Beginning of HL							
≤10 days (n=40)	346.7±65.7	403.6±75.0	28.4±8.2	8.2±2.1	7.0±1.6	86.0±3.1	0.8±0.1
>10 days (n=6)	390.6±30.1	457.9±28.4	33.6±4.3	8.7±1.6	7.4±1.1	85.3±2.2	0.7±0.1
P value	0.120	0.090	0.140	0.630	0.600	0.600	0.610
Audiometry type							
Descending (n=9)	327.2±81.4	384.4±94.1	28.6±8.2	8.8±1.8	7.4±1.3	85.2±2.6	0.8±0.1
Ascending (n=14)	367.2±61.1	423.1±69.7	28.0±8.3	7.6±2.1	6.6±1.6	86.9±3.2	0.7±0.1
Flat (n=19)	358.7±38.3	420.8±40.2	31.1±7.0	8.8±2.2	7.4±1.6	85.2±3.2	0.8±0.1
P value	**0.03**	**0.02**	0.49	0.27	0.27	0.27	0.37
Recovery							
Complete (n=21)	360.8±62.1	418.1±70.8	28.7±7.8	8.0±2.0	6.8±1.5	86.3±3.0	0.7±0.1
Good (n=13)	363.5±61.6	421.0±71.5	28.8±8.0	7.9±1.8	6.8±1.4	86.4±2.7	0.8±0.1
Fair (n=6)	331.0±65.9	389.0±75.0	29.0±7.3	8.9±1.9	7.5±1.4	85.0±2.7	0.8±0.1
Unchanged (n=6)	321.1±73.3	383.8±88.6	31.4±10.7	9.7±2.6	8.0±1.9	84.0±3.8	0.9±0.1
P value	0.42	0.62	0.91	0.26	0.29	0.29	0.2

IMA: Ischemia-modified albumin; ABSU: Absorbance units; SD: Standard deviation; HL: Hearing loss.

levels and disulfide/native thiol and disulfide/total thiol ratios, and all of these markers were significantly higher in the study group ($p<0.05$, Table 1).

Table 2 shows the distribution of blood parameters of the patient group according to the severity of hearing loss, duration of hearing loss, audiogram type, and degree of recovery. There was no significant relationship between the examined blood values and the parameters of the disease and audiometry except for audiogram type ($p>0.05$, Table 2). Native thiol and total thiol levels of patients with descending type audiogram were lower than those with flat and ascending type audiogram, and this difference was statistically significant [$p<0.05$; the bowl-type audiograms was not taken into consideration due to its very low number (n=4)]. Furthermore, although not statistically significant, native thiol and total thiol levels gradually decreased as the severity of hearing loss increased. In addition, as the recovery rate increased, the level of native thiol tended to increase, but this rate was not statistically significant ($p>0.05$).

In the correlation analysis, there was no relationship between age, pre- and post-treatment PTA, hearing gain, and blood parameters, while there was a negative relationship between hearing gain and IMA levels ($r=-0.324$, $p=0.028$). Additionally, there was a negative relationship between serum IMA levels and native thiol ($r=-0.677$, $p<0.01$), total thiol ($r=-0.659$, $p<0.01$), and disulfide ($r=-0.303$, $p<0.01$) levels.

DISCUSSION

Although the mechanism underlying ISSNHL is not fully understood, it is known that oxidative stress has a critical role in the etiopathogenesis of this disease. Oxidative stress is described as a disequilibrium between reactive oxygen species (ROS) and the intracellular antioxidant system. Destruction of inner ear hairy cells may also occur due to ROS-mediated damage. The presence of ROS was identified in the inner ear perilymph of human subjects treated with cochlear implantation due to profound hearing loss.[11]

Thiols are the main part of total body antioxidants and have a critical role in the defense mechanism against ROS. In high oxidative stress situations, thiol levels decrease to neutralize ROS and sulfhydryl groups of thiols play a critical role. The natural thiol content is subtracted from the total thiol content, and half of the difference obtained gives the amount of disulfide bond. In other words, when thiol molecules are oxygenated, disulfide bonds are produced in a recyclable way. In this way, dynamic thiol/disulfide hemostasis is preserved.[12-14]

Although there are many reports investigating the role of oxidative stress in ISSNHL etiology, the number of studies investigating thiol/disulfide hemostasis in this disease is limited. Dinc et al.,[15] in a study with the limited number of patients, found that native and total thiol levels were significantly lower in ISSNHL patients compared to the control group. The average disulphide levels were lower, whereas disulphide/native thiol and disulphide/total thiol ratios were higher in the study group, but these differences were not significant. Gul et al.[16] stated that the total oxidant status and oxidative index were significantly higher in ISSNHL patients and the disulfide levels and TOS were associated with ISSNHL. However, in this study, it was revealed that there was no difference in terms of total antioxidant capacity, paraoxanase, native thiol, and total thiol levels. In a study examining autosomal recessive non-syndromic hearing loss and thiol-disulfide balance, it was reported that thiol levels increased and disulfide levels decreased in these patients. The authors claimed that there may be an inverse relationship between autosomal recessive non-syndromic hearing loss and oxidative stress.[17] In our study, although there was no significant difference between the two groups in terms of native thiol and total thiol values, the native thiol/total thiol ratio was significantly higher in the control group. In addition, disulfide levels, disulfide/native thiol and disulfide/total thiol ratios were significantly higher in the patient group. This result indicates that the imbalance between ROS and antioxidants plays an important role in the etiopathogenesis of ISSNHL. As known, there are some publications reporting that ISSNHL patients with ascending type audiograms have a better prognosis than the descending type.[18] In this study, we also obtained some results supporting this information, patients with descending type audiograms had lower serum native and total thiol levels compared to patients with flat and ascending type audiograms. Native and total thiol levels of patients with prognostically poor audiogram configuration were also low compared to other types.

To the best of our knowledge, there is only one report that investigates the relationship between ISSNHL and IMA. Cırık et al.,[19] in their study on 17 patients, reported that the serum IMA value in ISSNHL patients did not differ from the control group. In our study, there was no difference between the patient group and the control group in terms of IMA values. In addition, we did not find a relationship between various diseases and audiogram parameters and IMA. However, there was a negative correlation between hearing gain calculated at the end of treatment and IMA levels. In addition, we revealed that there is

a negative relationship between serum IMA levels and native thiol, total thiol, and disulfite. The blood parameters in this study were evaluated after a period. Therefore, it should be kept in mind that IMA is sensitive to storage at low temperatures and its levels may increase after freezing.[20]

The main limitation of this study is the low number of patients, and when we consider the factors related to the disease and patient, the number of patients in the subgroups is insufficient. In addition, if the blood values of patients after the treatment were evaluated, both changes in blood values and a comparison of these changes with changes in hearing levels could be made. Finally, since blood taken from patients was not evaluated immediately, blood values may have been affected.

In conclusion, this study is the first study examining the relationship between both thiol/disulfide hemostasis and IMA levels in ISSNHL. In this study, disulfide levels and disulfide/native thiol and disulfide/total thiol ratios were significantly higher, and native thiol/total thiol ratios were significantly lower in the patient group. Studies with a larger number of patients are needed to make more accurate comments in terms of patient and disease factors.

Ethics Committee Approval: The study protocol was approved by the Necmettin Erbakan University Ethics Committee (date: 2018, no: 1547). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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