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Original Article

Thiol–Disulfide Homeostasis in Noise–Induced Hearing Loss in Rats

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BACKGROUND: This study was designed to assess if thiol-disulfide homeostasis could be used as diagnostic biomarker in noise-induced hearing loss (NIHL) in a laboratory animal model.

METHODS: The study was carried out with a total of 28 female albino rats in 4 groups: group 1 (control group) included rats that were not exposed to noise or any study treatment; in group 2, following noise exposure, rats received 2 mg of dexamethasone per kilogram of body weight via the intramuscular route for 5 days; in Group 3, rats were exposed to noise and received a saline solution for 5 days, in a volume (0.15 cc) matched to that of dexamethasone administered in group 2; and in group 4, rats were exposed to noise, and blood samples were collected during the early phase to assess thiol-disulfide homeostasis without administering any treatment. Rats in groups 2, 3, and 4 were exposed to 120 dB noise in the 4 kHz octave band for 4 hours. The auditory brainstem response (ABR) test was performed in all groups on day 1 after noise exposure and was repeated in groups 1, 2, and 3 on day 5. Auditory brainstem response thresholds were recorded at 8, 12, 16, 20, and 32 kHz frequencies. Groups 1, 2, and 3 rats were sacrificed on day 5, and group 4 rats were sacrificed by exsanguination on day 1 after noise exposure. Venous blood samples collected from the caudal vena cava were centrifuged and sent to the corresponding laboratory for thiol-disulfide homeostasis studies. After sacrificing the rats, the right and left temporal bones of each rat were removed and stained with hematoxylin eosin for histological studies to explore any pyknotic changes in spiral ganglion cells.

RESULTS: Intergroup comparisons by frequency on day 5 of noise exposure showed statistically significantly lower responses in ABR measurements at 8 kHz, 12 kHz, and 16 kHz in group 2 compared to group 3 ($P = .003$, $P = .006$, and $P = .002$). Improvements were observed with dexamethasone administered for therapeutic purposes, particularly if the hearing loss was induced by low-frequency noise. In the assessment of the parameters of thiol-disulfide homeostasis, disulfide/native thiol and disulfide/total thiol ratios and ischemia-modified albumin (IMA) levels were higher in group 4 than in other groups, although only the differences between group 1 and group 4 reached statistical significance.

CONCLUSION: According to this study, thiol-disulfide homeostasis and IMA can be shown as diagnostic biomarkers in NIHL, especially in the early period. The results from our study suggest that these markers may be used as adjunctive diagnostic tools in NIHL, in addition to audiological studies. However, this issue can be clarified with further clinical studies.

KEYWORDS: Noise-induced hearing loss, rats, thiol-disulfide homeostasis

INTRODUCTION

Noise is characterized by unwanted and disturbing sound elevations that may be harmful to health.[1,2] Noise-induced hearing loss (NIHL) occurs as a result of exposure to noise of varying duration and intensity. Reports from industrialized countries indicate an increasing incidence of NIHL.[3] Occupational NIHL is a common cause of hearing loss today.[4,5]

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The inner ear is directly affected by mechanical damage and histological changes associated with noise exposure occur in the inner ear.[6-8] The outer and inner hair cell degeneration (gaps, disorganization, and disruptions) and cell loss may occur in the organ of Corti due to mechanical damage.[9] Another mechanism that may lead to NIHL is the apoptosis and necrosis induced by oxidative stress that results from increased free radicals. This mechanism leads to the dysfunction of the outer hair cells, which are sensitive to high-frequency sounds, and increased hearing thresholds make it difficult to hear, especially in noisy environments.[10,11] In living organisms, noise-induced oxidative damage is known to trigger antioxidant defense mechanisms.[12-14] Thiol is an organosulfur compound and plays a role in stabilizing oxidative stress. Thiol groups provide this compensation by reacting with reactive oxygen species (ROS) that occur after oxidative stress. Disulfide bonds are converted to thiol groups over time, and this reaction continues in both directions, ensuring thiol-disulfide homeostasis. Thiol-disulfide homeostasis can be demonstrated with high precision in the laboratory by measuring thiol and disulfide levels in serum.[15]

Vascular changes in the inner ear may occur due to noise. As a result of these vascular changes in the inner ear, cochlear ischemia may occur.[16] Ischemia-modified albumin (IMA) levels may be expected to increase in response to oxidative stress and acidosis resulting from this ischemia in the inner ear. Ischemia-modified albumin is caused by changes in the molecular structure of albumin as a result of ischemia.[17]

In the medical literature, thiol-disulfide homeostasis has been suggested as a biomarker in sudden hearing loss.[18] A study evaluating IMA in hearing loss has not yet been conducted. Considering these reports, thiol-disulfide homeostasis and IMA may also be used as biomarkers in ototoxic or noise-induced cochlear damage associated with oxidative stress.

This study aimed to determine if thiol-disulfide homeostasis and IMA could be used as diagnostic biomarkers in an experimental animal model of NIHL.

METHODS

The study was initiated with the approval of the Dokuz Eylul University Animal Experiments Local Ethics Committee (Protocol No. 49/2021). The study was conducted with 28 female outbred albino Wistar rats (young adults) weighing approximately 300 g.

All rats were examined otoscopically, earwax was removed, and the study was continued after the eardrums were judged to be normal. Four groups of 7 rats each were randomized.

MAIN POINTS

- Thiol-disulfide homeostasis is a marker of oxidative stress.
- Noise causes hearing loss by creating oxidative stress in the inner ear.
- Thiol disulfide-homeostasis can be used as a biomarker in noise-induced hearing loss.

Group 1 (control group): Rats not exposed to noise or any study treatment were included.

Group 2: Rats received 2 mg of dexamethasone per kilogram of body weight via the intramuscular route for 5 days after noise exposure.

Group 3: Rats were exposed to noise and received a saline solution in a volume (0.15 cc) matched to that of dexamethasone administered to group 2 for 5 days.

Group 4: Rats were exposed to noise, and blood samples were collected to assess thiol-disulfide homeostasis without administering any treatment.

Audiological Examinations

Following intraperitoneal general anesthesia, an auditory brainstem response (ABR) test was performed in all groups to determine hearing thresholds. Rats in the experimental groups were kept in a cage with 4 kHz octave band noise at 120 dB sound pressure level for 4 hours. They were given free access to food and water in this cage. The ABR test was repeated 1 day after the noise exposure in all study groups and 5 days after the noise exposure in groups 1, 2, and 3. During the ABR test, hearing thresholds were analyzed at frequencies of 8, 12, 16, 20, and 32 kHz.

Biochemical Tests

Blood samples were collected from the caudal caval vein on day 5 after noise exposure in groups 1, 2, and 3, and in day 1 after noise exposure in group 4. Blood samples were centrifuged, and collected sera were sent to the corresponding laboratory for thiol-disulfide homeostasis studies in Eppendorf tubes in −20°C cold chain. Sera were analyzed for biochemical parameters in the corresponding laboratory.

Histological Exams

Groups 1, 2, and 3 rats were sacrificed on day 5, and group 4 rats were sacrificed by exsanguination on day 1 after noise exposure. Then, the right and left temporal bones of each rat were dissected and removed and stored in a 10% formalin solution with a volume 10 times higher than the temporal bone. The bones were fixed using a solution called formalin at ambient temperature for 48 hours. Tissue samples were decalcified in a 5% glacial acetic acid solution for 5 days. Following decalcification, cross-sections were obtained, checked, and washed under tap water for 1 hour. The samples were then placed in cassettes, and paraffin blocks were formed. Sections taken from paraffin blocks were stained with hematoxylin-eosin stain for histopathological evaluations. Any pyknotic changes in spiral ganglion cells were evaluated in each group.

Statistical Analysis

All ABR data were summarized descriptively using means ± SD. Measurable data were compared using Kruskal–Wallis analysis of variance. The determination of the group causing the difference was made by Mann–Whitney U-test with Bonferroni correction. Within-group comparisons were made by Wilcoxon signed-rank test with Bonferroni correction. The normality of the distribution of quantitative data for thiol-disulfide homeostasis, IMA, and histologic studies was checked by the Shapiro-Wilk test. Variables were compared with 1-way ANOVA test (Bonferroni test for pairwise comparisons) if they

fit the normal distribution, and with Kruskal–Wallis test (Dunn test for pairwise comparisons) if they do not fit the normal distribution.

RESULTS

Auditory Brainstem Response Findings

Hearing thresholds at baseline hearing evaluations were within normal limits in all rats ($P > .05$). Auditory brainstem response measurements made on the day after the noise exposure showed that the noise caused severe hearing loss ($P = .0000$). Auditory brainstem response measurements made on the day following the noise did not show any difference in the noise groups ($P > .05$). Day 5 measurements were performed in all groups except group 4, in a total of 21 rats. On day 5 ABR measurements, hearing thresholds were statistically significantly higher in the noise-treated groups compared to the non-noise-treated group ($P = .0000$). When the comparison was made according to frequencies, it was observed that the threshold values at frequencies of 8 kHz, 12 kHz, and 16 kHz in group 2 were statistically significantly lower than the threshold values in group 3 ($P = .003$, $P = .006$, and $P = .002$). However, there was no significant difference between groups 2 and 3 in threshold values at 20 kHz and 32 kHz ($P = .164$ and $P = .150$) (Table 1). Improvements were observed with dexamethasone administered for therapeutic purposes, particularly in low-frequency hearing.

Intragroup comparisons revealed that no significant differences were found among baseline, day 1 and day 5 measurements of group 1, regardless of the frequency of the sound ($P > .05$). In group 2, hearing thresholds were significantly higher on day 1 and day 5 after noise exposure compared to baseline measurements at all frequencies; however, day 5 measurements were significantly lower and improvements were observed compared to day 1 measurements after noise exposure.

(Table 2). In group 3, hearing thresholds were significantly higher on days 1 and 5 after noise exposure compared to baseline measurements at all frequencies. Measurements at 8 kHz and 12 kHz did not differ significantly between day 1 and day 5 ($P = .317$, $P = .075$, respectively). Day 5 measurements are statistically significantly lower than day 1 measurements at 16 kHz, 20 kHz, and 32 kHz ($P = .013$, $P = .003$, $P = .005$) (Table 3). Group 4 had significantly higher hearing thresholds at all frequencies compared to baseline measurements made the day after noise exposure.

Table 1. Day 5 Auditory Brainstem Response Measurements (ms) ± SD and *P*-Values for Group 2 and Group 3 after Noise Exposure

Frequency (kHz)	Group 2	Group 3	*P*P<.05
8	69.64 ± 12.92 (40-85)	82.86 ± 8.01 (70-95)	.003
12	61.07 ± 13.75 (20-75)	75.00 ± 10.56 (60-95)	.006
16	56.43 ± 12.92 (20-70)	70.71 ± 10.35 (55-95)	.002
20	58.57 ± 10.08 (30-75)	65.00 ± 14.67 (40-85)	.164
32	50.36 ± 12.92 (30-70)	62.50 ± 24.07 (25-95)	.150

Biochemical Findings

As a result of intergroup comparisons of disulfide, total thiol, and native thiol levels, it was observed that native thiol levels were lower in group 2 (162.17 ± 11.14), group 3 (179.39 ± 13.20), and group 4 (162.87 ± 42.82) compared to group 1 (195.73 ± 27.49) without statistical significance ($P > .05$). In parallel with native thiol levels, total thiol levels were lower in group 2 (195.79 ± 13.06), group 3 (216.56 ± 15.61), and group 4 (199.87 ± 48.61) compared to group 1 (231.81 ± 31.34) without statistical significance ($P >. 05$). When the measured disulfide values were analyzed, it was found that the noise did not cause a significant change in the disulfide levels ($P> .05$). When the disulfide/native thiol ratio was analyzed, it showed an increase in group 4, i.e., on day 1 after the noise, but this increase only reached significance against group 1 ($P > .05$) (Figure 1). When the disulfide/total thiol ratio was analyzed, a statistically significant increase was found in group 4 as a result of the decrease in total thiol compared to group 1 (Figure 2). Native thiol/total thiol ratio was found to be low in group 4 and this low ratio was statistically significant only when compared with group 1.

When IMA levels were analyzed, they were found to be higher in Group 4, but this increase was significant only when compared with Group 1 ($P = .006$) (Figure 3).

Histological Findings

Pyknotic changes in spiral ganglion cells were more common in group 3 compared to other groups. While there were significantly more pyknotic changes in group 3 compared to group 1 and group 4 ($P = .000$, $P = .001$), no difference was found between group 3 and group 2 ($P = .129$).

DISCUSSION

Reactive oxygen species are endogenous substances released as a result of various biochemical reactions in oxygen metabolism in the

Table 2. Changes in Day 1 and Day 5 Hearing Thresholds After Noise Exposure Compared to the Baseline by Statistical Significance in Group 2 (*$P < .05$)

	8 kHz (*P*)P<.05	12 kHz (*P*)P<.05	16 kHz (*P*)P<.05	20 kHz (*P*)P<.05	32 kHz (*P*)P<.05
Baseline vs. day 1	.001	.001	.001	.001	.001
Baseline vs. day 5	.001	.001	.001	.001	.001
Day 1 vs. day 5	.001	.001	.001	.001	.001

Table 3. Changes in Day 1 and Day 5 Hearing Thresholds After Noise Exposure Compared to the Baseline by Statistical Significance in Group 3 (*$P < .05$)

	8 kHz (*P*)P<.05	12 kHz (*P*)P<.05	16 kHz (*P*)P<.05	20 kHz (*P*)P<.05	32 kHz (*P*)P<.05
Baseline vs. day 1	.001	.001	.001	.001	.001
Baseline vs. day 5	.001	.001	.001	.001	.001
Day 1 vs. day 5	.317	.075	.013	.003	.005

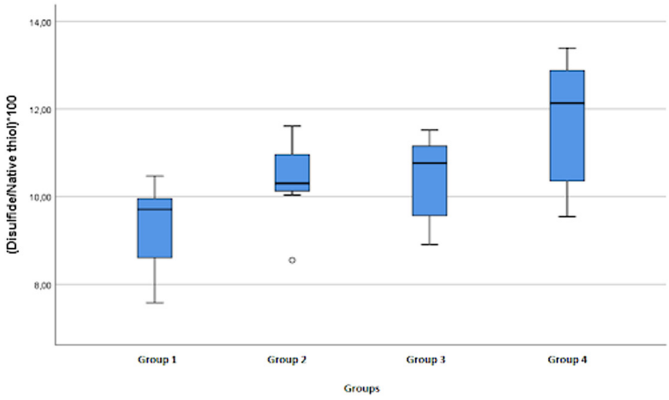

Figure 1. Disulfide/native thiol ratios: Candlestick chart representation of disulfide/native thiol ratios according to groups. The disulfide/native thiol ratio was the highest in group 4, but only the difference between group 4 and group 1 reached statistical significance ($P > .05$).

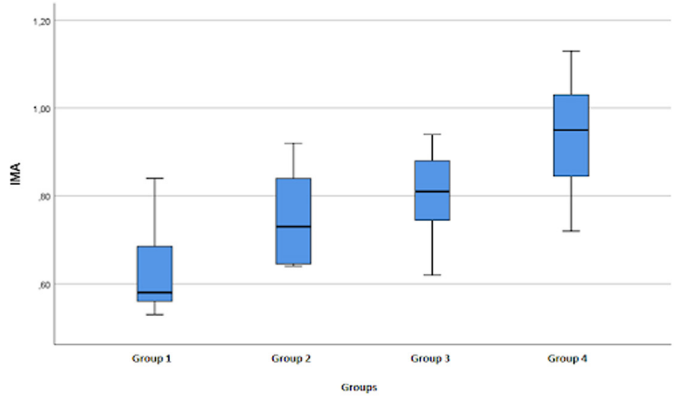

Figure 3. IMA values: Candlestick chart representation of IMA values according to groups. Ischemia-modified albumin (IMA) levels were significantly higher in group 4 compared to group 1 ($P = .006$) while no significant differences were observed between group 4 and group 2 or group 3 ($P > .05$).

body. ROS may be produced and released into the medium by key cells involved in host defense (monocytes and macrophages) that are attracted to sites of inflammation or ischemia for purposes of damage repair. However, excessive ROS release may lead to further tissue damage. Based on studies investigating ROS in hearing loss, ROS are thought to result from apoptosis and necrosis caused by damage to hearing cells. Increased potent oxidants have been reported to cause endothelial damage in the inner ear.[19]

The presence of ROS induces antioxidant molecules, and increased antioxidant molecules play a role in cell renewal and apoptosis and regulate cellular enzymatic activity. Thiol-based compounds are important representatives of antioxidants and show activity in oxidative stress situations. Increased free oxygen radicals lead to the oxidation of thiol-based compounds and the formation of disulfide bonds. This process is reversible, and thiol compounds form again from disulfide bonds. This process is known as thiol-disulfide homeostasis. Thiol-disulfide imbalance has been reported in acute and chronic conditions. It is known that thiol levels may decrease and indirectly disulfide levels may increase in various systemic diseases and malignancies.[20]

In a study, it was found that thiol levels decreased in response to increased oxidative stress in sudden hearing loss.[21] Temiz et al[22] evaluated thiol-disulfide homeostasis in chronic otitis media and found that the balance changed in favor of disulfide in chronic otitis media.

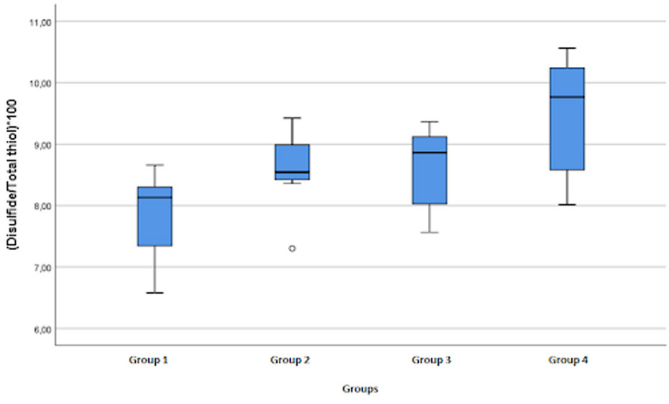

Figure 2. Disulfide/total thiol ratios: Candlestick chart representation of disulfide/total thiol ratios according to groups. The disulfide/native thiol ratio was higher in group 4 compared to other groups, but only the difference between group 4 and group 1 was statistically significant.

In this study, the ratios of disulfide values to total thiol and native thiol values were found to be statistically significantly higher in group 4, where measurements were made on day 1 after noise exposure, compared to group 1, where no study procedure was performed. These findings suggest that antioxidant mechanisms are activated during the early phase after oxidative stress. Statistically non-significant results in group 2 or group 3 vs. the control group may be attributed to the length of the time interval after noise exposure. In a study that assessed thiol-disulfide homeostasis parameters after cardiopulmonary bypass surgery, changes in thiol-disulfide homeostasis parameters were found to occur 6 hours after surgery and continue up to 24 hours after the surgery.[23] These findings were in parallel with our study data and provided further support to our assumption on the effect of the length of the time interval after noise exposure in group 2 and group 3. Despite years of investigation, the etiology underlying NIHL remains unclear. Any disorder of cochlear blood flow may explain temporary or permanent threshold shifts induced by noise. Several studies have demonstrated that a decreased cochlear blood flow (vasoconstriction and cochlear hypoxia), leukocytic infiltration, and increases in adhesion molecules after noise exposure might cause oxidative stress leading to albumin modifications.[17,24,25] This modified albumin form has been referred to as IMA.[26]

There are studies showing that IMA can be used as a biomarker, especially in diseases such as myocardial infarction due to ischemia, cerebrovascular infarction, and metabolic diseases such as diabetes, renal failure, hypothyroidism, and hyperthyroidism. Studies have observed significant changes in IMA values in the early period.[27-30] In a study, IMA was used as a biomarker in acute ischemic stroke. In acute ischemic stroke, IMA has been shown to increase in the early stage of stroke and decrease within a week.[31] Only in group 4, IMA levels were higher than the control group in our study. The detection of significantly higher IMA levels exclusively during the early phase in group 4 suggests that IMA may be an early and reversible biomarker. In the literature, there are no studies investigating IMA levels following hearing loss, and this makes our study the first in this area of investigation.

To date, the therapeutic efficacy of various pharmacological agents in NIHL has been investigated in animal models and clinical trials. These therapeutic options were selected to target various phases of the formation mechanism of NIHL, with the most used being steroids.[32] Dexamethasone and methylprednisolone have been the most used steroids mainly to act by suppressing inflammatory responses. Wang et al[33] showed the therapeutic efficacy of intraperitoneal dexamethasone in an animal model of NIHL, while Takemura et al[34] showed the therapeutic efficacy of intratympanic dexamethasone injections also in an animal model of NIHL. However, in another NIHL model generated by exposing study rats to noise at 120 dB Sound Pressure Level (SPL), investigators failed to demonstrate any otoprotective effect of dexamethasone.[35] Another clinical study demonstrated the otoprotective effects of intratympanically administered dexamethasone in addition to oral steroids in patients presenting with NIHL.[36] In our study, group 2 rats that received dexamethasone after noise exposure showed improvements at low frequencies compared to group 3 rats that received a saline solution. In our study, serum was used to evaluate thiol-disulfide homeostasis and IMA in NIHL.

Both thiol-disulfide homeostasis and IMA are biomarkers affected by various systemic changes that are not specific to the inner ear.[20,27-30] By designing our study as an animal experiment, we aimed to minimize the problems arising from this situation. There are animal experiments in the literature using cochlea tissue to reveal inner ear damage in NIHL.[37] Within the current possibilities, thiol-disulfide homeostasis and IMA can only be analyzed with serum. To correlate changes in biomarkers with damage to the inner ear, samples taken directly from the inner ear or from a fluid in contact with the inner ear may provide more reliable results. Although sampling from inner ear fluids provides more reliable results, it is not an easy method to apply clinically.[38,39] Collection of serum samples is a common, non-invasive diagnostic tool that is easy to implement in clinical practice. There are studies showing that inner ear-specific biomarkers such as otolin-1, prestin, and matrilin-1 can be detected in serum.[40,41] Considering that the biomarkers we studied are not specific to the inner ear, this can be considered a shortcoming of our study. Thiol-disulfide homeostasis and IMA are directly related to oxidative stress and ischemia in the body and may be important biomarkers in elucidating the pathophysiology of NIHL and providing treatment options.

CONCLUSION

Based on the results of our study, thiol-disulfide homeostasis and IMA represent early biochemical markers of NIHL. We believe that these markers, in addition to audiological studies, may aid in the diagnosis of NIHL and shed light on the pathogenesis of NIHL. More extensive clinical studies are needed to clarify this issue.

Ethics Committee Approval: This study was approved by the by the Dokuz Eylül University Animal Experiments Local Ethics Committee University (Approval No: 49/2021, Date: 19/01/2021).

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Author Contributions: Concept – E.A., A.D.; Design – E.A., Ö.E.; Supervision – O.Y., A.D.; Resources – E.A., S.N.; Materials – S.D., G.K.; Data Collection and/or Processing – E.A., S.A.; Analysis and/or Interpretation – S.D., A.D.; Literature Search – E.A., Y.O.; Writing – E.A; Critical Review – Ö.E., A.D.

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REFERENCES

1. Basner M, Babisch W, Davis A, et al. Auditory and non-auditory effects of noise on health. *Lancet*. 2014;383(9925):1325-1332. [CrossRef]
2. Stansfeld S, Haines M, Brown B. Noise and health in the urban environment. *Rev Environ Health*. 2000;15(1-2):43-82. [CrossRef]
3. Ahn JH, Kang HH, Kim YJ, Chung JW. Anti-apoptotic role of retinoic acid in the inner ear of noise-exposed mice. *Biochem Biophys Res Commun*. 2005;335(2):485-490. [CrossRef]
4. Chen GD. Effect of hypoxia on noise-induced auditory impairment. *Hear Res*. 2002;172(1-2):186-195. [CrossRef]
5. Nelson DI, Nelson RY, Concha-Barrientos M, Fingerhut M. The global burden of occupational noise-induced hearing loss. *Am J Ind Med*. 2005;48(6):446-458. [CrossRef]
6. Cascella V, Giordano P, Hatzopoulos S, et al. A new oral otoprotective agent. Part 1: electrophysiology data from protection against noise-induced hearing loss. *Med Sci Monit*. 2012;18(1):BR1-BR8. [CrossRef]
7. Le Prell CG, Yamashita D, Minami SB, Yamasoba T, Miller JM. Mechanisms of noise-induced hearing loss indicate multiple methods of prevention. *Hear Res*. 2007;226(1-2):22-43. [CrossRef]
8. Lu J, Li W, Du X, et al. Antioxidants reduce cellular and functional changes induced by intense noise in the inner ear and cochlear nucleus [published correction appears in J Assoc Res Otolaryngol. 2014 June;15(3):373-4]. *J Assoc Res Otolaryngol*. 2014;15(3):353-372. [CrossRef]
9. Henderson D, Bielefeld EC, Harris KC, Hu BH. The role of oxidative stress in noise-induced hearing loss. *Ear Hear*. 2006;27(1):1-19. [CrossRef]
10. Puel JL, Ruel J, Gervais d'Aldin C, Pujol R. Excitotoxicity and repair of cochlear synapses after noise-trauma induced hearing loss. *NeuroReport*. 1998;9(9):2109-2114. [CrossRef]
11. Park JS, Jou I, Park SM. Attenuation of noise-induced hearing loss using methylene blue. *Cell Death Dis*. 2014;5(4):e1200. [CrossRef]
12. Ates I, Ozkayar N, Topcuoglu C, Dede F. Relationship between oxidative stress parameters and asymptomatic organ damage in hypertensive patients without diabetes mellitus. *Scand Cardiovasc J*. 2015;49(5):249-256. [CrossRef]
13. Dean RT, Fu S, Stocker R, Davies MJ. Biochemistry and pathology of radical-mediated protein oxidation. *Biochem J*. 1997;324(1):1-18. [CrossRef]
14. Villanueva I, Alva-Sánchez C, Pacheco-Rosado J. The role of thyroid hormones as inductors of oxidative stress and neurodegeneration. *Oxid Med Cell Longev*. 2013;2013:218145. [CrossRef]
15. Erel O, Neselioglu S. A novel and automated assay for thiol/disulfide homeostasis. *Clin Biochem*. 2014;47(18):326-332. [CrossRef]
16. Quirk WS, Seidman MD. Cochlear vascular changes in response to loud noise. *Am J Otol*. 1995;16(3):322-325.
17. Levine RL. Ischemia: from acidosis to oxidation. *FASEB J*. 1993;7(13):1242-1246. [CrossRef]
18. Dinc ME, Ulusoy S, Is A, et al. Thiol/disulfide homeostasis as a novel indicator of oxidative stress in sudden sensorineural hearing loss. *J Laryngol Otol*. 2016;130(5):447-452. [CrossRef]
19. Quaranta N, De Ceglie V, D'Elia A. Endothelial dysfunction in idiopathic sudden sensorineural hearing loss: a review. *Audiol Res*. 2016;6(1):151. [CrossRef]
20. Kundi H, Ates I, Kiziltunc E, et al. A novel oxidative stress marker in acute myocardial infarction; thiol/disulfide homeostasis. *Am J Emerg Med*. 2015;33(11):1567-1571. [CrossRef]
21. Çetin YS, Bozan N, Avcı K, Aslan M, Erel Ö. The relationship between thiol-disulfide balance and idiopathic sudden sensorineural hearing loss. *Braz J Otorhinolaryngol*. 2022;88(6):948-953. [CrossRef]

22. Temiz H. Kronik Otitis Media'da Tiyol-Disülfid Homeostazının değişikliği. *Dicle Tıp Dergisi*. 2021;48(1):197-204. [CrossRef]
23. Ozgunay SE, Ozsin KK, Ustundag Y, et al. The effect of continuous ventilation on thiol-disulfide homeostasis and albumin-adjusted ischemia-modified albumin during cardiopulmonary bypass. *Braz J Cardiovasc Surg*. 2019;34(4):436-443. [CrossRef]
24. Lee P, Wu X. Review: modifications of human serum albumin and their binding effect. *Curr Pharm Des*. 2015;21(14):1862-1865. [CrossRef]
25. Roy D, Quiles J, Gaze DC, Collinson P, Kaski JC, Baxter GF. Role of reactive oxygen species on the formation of the novel diagnostic marker ischaemia modified albumin. *Heart*. 2006;92(1):113-114. [CrossRef]
26. Bal W, Sokołowska M, Kurowska E, Faller P. Binding of transition metal ions to albumin: sites, affinities and rates. *Biochim Biophys Acta*. 2013;1830(12):5444-5455. [CrossRef]
27. Bhagavan NV, Lai EM, Rios PA, et al. Evaluation of human serum albumin cobalt binding assay for the assessment of myocardial ischemia and myocardial infarction. *Clin Chem*. 2003;49(4):581-585. [CrossRef]
28. Bilgi M, Keser A, Katlandur H, et al. Evaluation of the relationship between microalbuminuria and urine ischemia-modified albumin levels in patients with diabetic nephropathy. *J Clin Lab Anal*. 2017;31(3):e22058. [CrossRef]
29. Collinson PO, Gaze DC. Ischaemia-modified albumin: clinical utility and pitfalls in measurement. *J Clin Pathol*. 2008;61(9):1025-1028. [CrossRef]
30. Reddy VS, Bukke S, Mahato K, et al. A meta-analysis of the association of serum ischaemia-modified albumin levels with human hypothyroidism and hyperthyroidism. *Biosci Rep*. 2017;37(1):BSR20160268. [CrossRef]
31. Menon B, Ramalingam K, Krishna V. Study of ischemia modified albumin as a biomarker in acute ischaemic stroke. *Ann Neurosci*. 2018;25(4):187-190. [CrossRef]
32. Sakat MS, Kilic K, Bercin S. Pharmacological agents used for treatment and prevention in noise-induced hearing loss. *Eur Arch Otorhinolaryngol*. 2016;273(12):4089-4101. [CrossRef]
33. Wang B, Liu Y, Chi F, Zhang Y, Yang M, Zhu X. Dexamethasone suppresses cochlear Hes1 expression after noise exposure. *Acta Otolaryngol*. 2013;133(3):233-238. [CrossRef]
34. Takemura K, Komeda M, Yagi M, et al. Direct inner ear infusion of dexamethasone attenuates noise-induced trauma in guinea pig. *Hear Res*. 2004;196(1-2):58-68. [CrossRef]
35. Bas E, Martinez-Soriano F, Láinez JM, Marco J. An experimental comparative study of dexamethasone, melatonin and tacrolimus in noise-induced hearing loss. *Acta Otolaryngol*. 2009;129(4):385-389. [CrossRef]
36. Zhou Y, Zheng G, Zheng H, Zhou R, Zhu X, Zhang Q. Primary observation of early transtympanic steroid injection in patients with delayed treatment of noise-induced hearing loss. *Audiol Neurootol*. 2013;18(2):89-94. [CrossRef]
37. Miao L, Zhang J, Yin L, Pu Y. Metabolomics analysis reveals alterations in cochlear metabolic profiling in mice with noise-induced hearing loss. *BioMed Res Int*. 2022;2022:9548316. [CrossRef]
38. Alawieh A, Mondello S, Kobeissy F, Shibbani K, Bassim M. Proteomics studies in inner ear disorders: pathophysiology and biomarkers. *Expert Rev Proteomics*. 2015;12(2):185-196. [CrossRef]
39. Peter MS, Warnecke A, Staecker H. A window of opportunity: perilymph sampling from the round window membrane can advance inner ear diagnostics and therapeutics. *J Clin Med*. 2022;11(2):316. [CrossRef]
40. Gomaa NA, Jimoh Z, Campbell S, Zenke JK, Szczepek AJ. Biomarkers for inner ear disorders: scoping review on the role of biomarkers in hearing and balance disorders. *Diagnostics (Basel)*. 2020;11(1):42. [CrossRef]
41. Mulry E, Parham K. Inner ear proteins as potential biomarkers. *Otol Neurotol*. 2020;41(2):145-152. [CrossRef]