

RESEARCH ARTICLE

Evaluating plasma oxidative stress markers in prelingual profound sensorineural hearing loss

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Abstract

Background and Aim: The etiopathogenesis of sensorineural hearing loss (SNHL) is an essential contributing factor to its morbidity, which cannot be explained entirely so far. The current study aimed to determine the oxidative stress (OS) status by comparing the total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) values in patients with pediatric prelingual profound SNHL. We also evaluated the correlation between OS parameters and audiological test results.

Methods: The study included 25 participants (9 females; age range: 6–34 months) diagnosed with pediatric prelingual profound SNHL and 25 healthy subjects (10 females; age range: 9–28 months). Their TAS, TOS, and OSI levels were measured in the plasma of both groups. We evaluated the correlation between OS parameters and audiological test results in the patient group.

Results: We found significantly higher serum TOS levels and OSI values in the patient group (mean \pm SD of TOS: $16.08 \pm 1.88 \mu\text{mol H}_2\text{O}_2 \text{ eq/L}$, $p < 0.001$; mean \pm SD of OSI: 1.71 ± 0.48 arbitrary units, $p < 0.001$), compared to the

controls. Moreover, we found lower serum TAS levels in the patient group (mean \pm SD of TAS: $0.99 \pm 0.20 \text{ mmol Trolox eq/L}$), compared to the controls. There was a strong correlation between OS parameters and audiological test results of the patient group.

Conclusion: We detected significantly higher TOS, OSI, and lower TAS levels in pediatric patients with SNHL, compared to the healthy subjects. The obtained data indicated that pediatric SNHL is under OS influence.

Keywords: Sensorineural; hearing loss; oxidative stress; total oxidant status; total antioxidant status

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Introduction

Pediatric sensorineural hearing loss (SNHL) is the most frequently reported neurosensory health problem in newborns. The prevalence of bilateral SNHL in newborns is approximately 0.1% to 0.2%, and this ratio may increase to 1% in infants at risk [1-3]. Hearing loss in infants may cause delayed speech and language development and disrupted psychosocial maturation and social cohesion, leading to

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several individual and social problems [3,4]. SNHL is a significant cause of morbidity; its adverse effects can be prevented by early diagnosis and appropriate treatment methods [3]. Genetic mutations, environmental factors (noise exposure), ototoxicity (tobacco smoke exposure, heavy metal toxicity, cisplatin, and gentamycin), infectious factors (cytomegalovirus, meningitis, mumps, and measles), prematurity and asphyxia account for this condition. However, the etiopathogenesis of childhood prelingual profound SNHL has not been fully elucidated. There are issues with treatment methods. A better understanding of the underlying pathophysiology and molecular mechanisms of pediatric prelingual profound SNHL may contribute to the development of new treatment modalities. Adverse oxidative reactions may occur following an increase in reactive oxygen species (ROS), such as hydroxyl radical, superoxide radical, and hydrogen peroxide. These agents are secreted in metabolic and physiological processes in the organism or due to inadequate enzymatic and non-enzymatic antioxidant mechanisms that detoxify the toxic effects of ROS [5-7]. Oxidative stress (OS) can trigger cell death by damaging deoxyribonucleic acid (DNA), proteins, and lipids [5-7]. Oxidative stress markers, such as total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) are markers to recognize the oxidant/antioxidant balance in individuals [7]. Oxidative stress is characterized by increased ROS, which results in injury to intracellular biochemical metabolism. It is a significant determinant in the pathophysiology of various kinds of SNHLs, including Meniere's disease, tinnitus, age-related hearing loss, noise-induced hearing loss, and drug-induced hearing loss [8-14]. By different mechanisms, ROS-induced oxidative damage may disturb the inner ear cells such as hair cells and auditory nerve cells [8,15]. Experimental studies suggest that superoxide anion radicals may damage the inner ear structures of experimental animals [11,14]. Ciorba et al. revealed the presence of ROS in the perilymph fluid of the inner ear during cochlear implantation [16]. Another study investigated TAS,

TOS, and OSI in patients with idiopathic sudden sensorineural hearing loss (ISSNHL) [17]. However, these studies are conducted on adults or have experimental designs. The studies mentioned above provide evidence to the possible role of OS in the etiology of pediatric prelingual SNHL. OS, as in many different types of SNHL, may be a common underlying factor in the etiopathogenesis of pediatric prelingual SNHL. The available data on the relationship between pediatric prelingual SNHL and OS are scarce. We hypothesized that TAS, TOS, and OSI could be used in evaluating oxidative damage in pediatric prelingual SNHL patients.

The current study aimed to compare the TAS, TOS, and OSI levels in pediatric subjects with bilateral prelingual profound SNHL, with a healthy control group. According to the literature, no studies have investigated OS concerning TAS, TOS, and OSI values in pediatric subjects diagnosed with bilateral prelingual profound SNHL. In addition, we evaluated the correlation between the OS parameters and hearing loss. Our study is of importance, as it is the first original investigation in this area.

Methods

Participants

This research was a prospective, controlled clinical study in subjects with pediatric bilateral prelingual profound SNHL. The Ethics Committee of Harran University Medical Faculty approved the study protocol (Code: 02.08.2018; 08/02). Signed written informed consents were obtained from the parents of all study participants. The study procedure was designed in accordance with the Helsinki declaration.

The patient group were 25 pediatric cases with bilateral severe and profound prelingual SNHL (mean \pm SD age: 15.32 \pm 4.35 mos), diagnosed and followed up from August 2018 to November 2018 in the Harran University Medical Faculty, Department of Otolaryngology. The control group comprised 25 healthy children (mean \pm SD age: 12.35 \pm 3.82 mos) who underwent hearing screening and standard audiological tests.

Detailed clinical history was obtained from the

parents of the patients and healthy subjects. The otoscopic examinations were performed on all study participants. All of the study participants underwent tympanometry (tympanometry: Interacoustics AT 235h; e3 Diagnostics represents, Interacoustics, Denmark), acoustic reflex measurements, otoacoustic emission (OAE: Screening program= EZ- Screen 2. TEOAE = ILOv6. Otodynamics, The United Kingdom), and auditory brainstem response (ABR: Interacoustics EP25; e3 Diagnostics represents, Interacoustics, Denmark) tests. A specialized audiologist evaluated all audiological data of the subjects. All patients underwent thin-slice axial and coronal computed tomography (CT) scan and temporal magnetic resonance imaging (MRI) examinations. Tympanometry measurements were approved as normal, with a static compliance value of 0.39–1.30 mL and a peak pressure value of -100 to +50 daPa. All study subjects were infants with prelingual severe and profound SNHL. The patient and control group subjects were matched in terms of age. All subjects were subjected to tympanometry, acoustic reflex measurements, otoacoustic emission (OAE), auditory brainstem response (ABR), and free field audiometry (FFA) tests. The control group was referred to our center for a hearing screening. Their hearing was normal; they obtained ≤ 15 dB values in the ABR test. Those who failed to pass the OAE test and their ABR test was ≥ 91 dB were diagnosed as profound SNHL. FFA was performed on the cases diagnosed with severe and profound SNHL.

The exclusion criteria were acute/chronic suppurative otitis media or otitis media with acute/chronic effusion in otoscopic examination, unilateral SNHL, postlingual SNHL, congenital inner ear anomalies, syndromic subjects, untreated infectious conditions, histories of neuro-otologic surgery, as well as liver, renal, hematological, cardiovascular, metabolic, and neurological diseases.

Biochemical analysis

The blood samples were obtained from the peripheral veins after 6–8 hours during the operational preparation fasting period. The samples

were maintained at -80 °C after being centrifuged at 3000 g for 10 minutes. The stored samples were evaluated for oxidative status by TOS, TAS, and OSI. TAS values determined by using Rel Diagnostics Assay kit (Rel Assay Diagnostics kit, Mega Tip, Gaziantep, Turkey) were specified as $\mu\text{mol Trolox eq/L}$ [5-7]. The free radical reactions induced by the Fenton reaction were followed by the absorbance of dianisidyl radicals. Free dianisidyl radicals were applied in relative amounts for measuring the antioxidative effect. TOS values detected using Rel Assay Diagnostics kit (Rel Assay Diagnostics kit, Mega Tip, Gaziantep, Turkey) by Erel method were specified as $\mu\text{mol H}_2\text{O}_2 \text{ eq/L}$. In this method, the iron ion-o-dianisidine complex is oxidized to ferric ions by oxidation reactions that are increased by glycerol molecules. The color intensity of the iron ions resulting from the complex formed by the xylenol orange indicates the total amount of oxidants. Hydrogen peroxide was used for calibrating the test. To determine the OSI value, OSI (arbitrary unit) = TOS (mmol $\text{H}_2\text{O}_2 \text{ eq/L}$) / TAS (mmol Trolox eq/L) formula was used.

Statistical analysis

In our study, evaluations of all normally distributed data and its statistical analyzes were performed with SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Mann-Whitney U test was used to compare continuous variables, which were recorded and presented as mean (SD) between the groups. The Spearman correlation analysis was used to analyze the correlations between the parametric variables (oxidative stress values and audiological tests). $P < 0.05$ was considered as statistically significant.

Results

The patient group consisted of 25 patients (16 [64%] male, 9 [36%] female), aged 6–34 months (mean \pm SD age: 15.32 ± 4.35 mos). The control group included 25 individuals (15 [60%] male, 10 [40%] female) aged 9–28 months (mean \pm SD age: 12.35 ± 3.82 mos). There was no significant age- and gender-wise differences between the two groups (Table 1).

Table 1. The demographic data of patients with prelingual profound sensorineural hearing loss and the control group

Variable	Patient group (n = 25)	Control group (n = 25)
Age (months)		
Mean ± SD	15.32 ± 4.35	12.35 ± 3.82
Gender (male/female)	16/9	15/10

The otoscopic exam of all study participants was normal. The tympanometry of all study participants was type A. The ipsilateral and contralateral acoustic reflexes of the patient group could not be obtained. The mean ± SD ABR measurement values of the patient group were as follows: right: 96.4 ± 11.4 dB nHL and left: 95.6 ± 9.7 dB nHL. The OAE and ABR test results of the control group were normal. Complete blood counts, biochemical tests, bleeding time, clotting time, PT, aPTT, temporal CT, ear, and brain MRI were normal in all study participants. Table 2 presents the audiological test results of the patient and control groups. Table 3 presents all the obtained OS marker values. We found significantly higher serum TOS levels and OSI values in the patient group (mean ± SD TOS: 16.08 ± 1.88 μmol H₂O₂ eq/L, p < 0.001; mean ± SD OSI: 1.71 ± 0.48

arbitrary units, p < 0.001), compared to the controls. However, we found lower serum TAS levels in the patient group (mean ± SD TAS: 0.99 ± 0.20 mmol Trolox eq/L, p > 0.05), compared to the controls. As per Table 4, there was a positive correlation between OS values and audiological test results, including the mean scores of right and left ear measurements. There was a strong correlation between the ABR test results, and TOS and OSI values in the patient group (Right ear: ABR-TOS (p = 0.001) and ABR-OSI (p = 0.001); Left ear: ABR-TOS (p = 0.016) and ABR-OSI (p = 0.003)). There was a significant correlation between FFA results, and TOS (p = 0.002), and OSI (p = 0.001) values in the patient group.

Table 2. The audiological test results of patient group and control group

Hearing tests		Patient group	Control group
Tympanometry	Bilateral	Type A	Type A
TEOAE (dB)	Bilateral	Refer	Pass
Acoustic reflex	Bilateral	NA	X present
ABR (dB nHL)	Right	96.4 ± 11.4	14.6 ± 2.8
	Left	95.6 ± 9.7	13.4 ± 3.1
FFA (dB)	Binaural	98 ± 10.3	13.1 ± 2.8

TEOAE; Transient evoked otoacoustic emissions, NA; No Answer ABR; auditory brainstem response, dB; decibel, dBnHL; decibel normalized hearing level, FFA; free field audiometry

Discussion

According to the literature, this was the first original study to investigate OS in TAS, TOS, and OSI markers in pediatric patients with SNHL. Thus, our research is of importance. We found that the TOS and OSI levels were significantly higher in the pediatric bilateral prelingual severe and profound SNHL group, compared to the controls. A study on ISSNHL in adults achieved similar TOS and OSI levels to ours [17]. In our study, TAS levels were not statistically significant. However, in another study conducted on patients with ISSNHL, TAS levels were not significantly higher in the patient group, compared to the controls [17]. The etiopathogenesis of SNHL is not precisely recognized; it has a multifactorial origin. The etiopathogenesis of SNHL is quite complicated. The disturbance of the medial cortex to the inner ear is due to free radical-induced oxidative

Table 3. Results of oxidative stress markers in the patient and control groups

Markers	Patient group (mean ± SD)	Control group (mean ± SD)	Mann-Whitney U	Eta-square (η^2)
TAS	0.99 ± 0.20	1.11 ± 0.23	238.0	0.042
TOS	16.08 ± 1.88*	11.98 ± 1.50	29.0	0.605
OSI	1.71 ± 0.48*	1.12 ± 0.25	65.0	0.461

* It is statistically different from control group. $P < 01$, NOTE; Mann Whitney U test, which is one of the non-parametric tests, was used to compare the differences between the two groups. TAS; total antioxidant status (mmol Trolox eq/L), TOS; total oxidant status ($\mu\text{mol H}_2\text{O}_2$ eq/L), OSI; oxidative stress index (arbitrary units).

injury, biochemical metabolic disorders, and chronic inflammatory processes [8-14]. It is well known that ROS is produced in hair cells in many inner ear pathologies; where stress formation in the cochlea and sensorineural hearing loss is observed, including cisplatin, aminoglycosides, or noise. Under normal conditions, there are defensive mechanisms of antioxidant vitamins and enzymes in the cochlea [7,18]. In some inner ear diseases, antioxidant defense systems inadequately function against the toxic effects of ROS; thus, OS develops [7,18,19]. ROS mediated oxidative stress triggers both

necrosis and apoptosis in auditory hair cells of the acoustic system and labyrinth [7,18-22]. This triggering mechanism initiates and maintains the activation of a series of metabolic pathways responsible for lipid, protein, and DNA damages in the inner ear cells [19-22]. The sensorineural epithelium of the cochlea is more susceptible to free radical damage (due to different reasons), compared to other body systems. Antioxidants reduce the level of reactive oxygen types and reactive nitrogen types produced by OS in response to acoustic trauma, aminoglycoside, and platinum-based drugs [19]. ROS, affecting the etiopathogenesis of many inner ear diseases with SNHL may also impact the etiopathogenesis of profound prelingual SNHL.

Studies suggest that oxidative stress affects the development of endolymphatic hydrops. In animal studies that endolymphatic hydrops are created experimentally, ROS-mediated-cellular damage, and apoptotic cell death may contribute to the development of SNHL in Meniere's disease [9,23].

Another study was conducted with the idea that when the excitotoxic process is interrupted at any stage, the hearing loss associated with endolymphatic hydrops may be prevented. They observed that ROS-mediated-oxidative damage, a part of the excitotoxic pathway, would contribute to the development of hearing loss-associated endolymphatic hydrops. The same study also suggested that the anti-inflammatory and antioxidant intervention would protect hearing ability [24].

The exposure to noise increases ROS levels in

Table 4. The correlation between the oxidative stress levels of patient group and the audiological test results of patient group

	TAS	TOS	OSI
ABR-R			
SC	.395	.623**	.745**
p	.050	.001	.000
ABR-L			
SC	.300	.478*	.573**
p	.145	.016	.003
FFA			
SC	.275	.582**	.628**
p	.184	.002	.001
N	25	25	25

* Correlation is significant at the 0.05 level (2-tailed).
 ** Correlation is significant at the 0.01 level (2-tailed).
 TAS; Total antioxidant status (mmol Trolox eq/L), TOS; total oxidant status ($\mu\text{mol H}_2\text{O}_2$ eq/L), OSI; oxidative stress index (arbitrary units), ABR; auditory brainstem response, R; right, L; Left, SC; Spearman correlation

the cochlea. They also suggested that increased ROS would critically affect hair cells deaths in the inner ear with necrotic and as well as apoptotic mechanisms [13].

In an experimental study, Ewert et al. [25] analyzed the therapeutic effects of antioxidants in the rat model. They induced hearing loss by damaging internal ear through exposing rats to excessive pressure as a result of the explosion. They concluded that antioxidant treatment, shortly after the explosion, reduced the hearing damage and significantly improved hearing loss.

The superoxide anion radicals are essential in gentamicin ototoxicity in experimental animals. These data support that various ototoxic agents may lead to the initiation and enhancement of ROS production in cochlear tissues [14]. The presence of ROS increased in perilymphatic fluid collected during surgery from 98 patients who underwent cochlear implantation due to profound SNHL, as well as in the specimens collected from 7 patients who underwent stapedotomy surgery due to otosclerosis diagnosis with a spontaneous perilymphatic leak. The obtained data revealed the presence of ROS in the inner ear perilymph of profound SNHL patients. They suggested that ROS-mediated-oxidative damage leads to pathological processes in the inner ear hair cells [16].

A study investigating the effects of ROS production on cochlear functions suggests that ROS production in perilymphatic space can severely disrupt the formation and transmission of afferent cochlear signals [26].

Therefore, the prolonged duration of ROS-mediated oxidative destruction leads to the activation of apoptotic metabolic pathways and the onset and progression of necrosis-induced cellular damage. This process damages the sensory components or neuronal components of the inner ear. Essential factors impact the pathophysiology of all types of SNHL, including presbycusis, noise trauma, and drug ototoxicity. TOS, OSI, and TAS are reliable parameters, indicating the clinical reflections of the molecular-level OS.

A limitation of our study was the small gender-wise sample size. In addition, the age difference between the two groups was considerable. Eventually, it was difficult to perform direct examination and tissue study due to the deep localization of the cochlea in the temporal bone.

Conclusion

In patients with pediatric prelingual profound SNHL, the TOS and OSI levels increase, and the TAS levels decrease, compared to the controls. This data suggests that patients with pediatric prelingual profound SNHL are exposed to OS. Therefore, the identification of ROS sources in the cochlea and the elucidation of the mechanisms of ROS-induced cochlear injury may contribute to design novel techniques for the inner ear diseases. The obtained results should be examined by future studies with larger sample sizes.

Conflicts of interests

The authors have no conflicts of interests to be declared.

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