Downregulation of GJB2 and SLC26A4 genes induced by noise exposure is associated with cochlear damage

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Abstract

Background Noise-induced hearing loss (NIHL) is one of the major causes of acquired hearing loss in developed countries. Noise can change the pattern of gene expression, inducing sensorineural hearing impairment. There is no investigation on the effects of noise frequency on the expression of GJB2 and SLC26A4 genes involved in congenital hearing impairment in cochlear tissue. Here we investigated the impacts of white and purple noise on gene expression and pathologic changes of cochlear tissue.

Methods In this study, 32 adult male Westar rats were randomly divided into experimental groups: WN, animals exposed to white noise with a frequency range of 100-20000 Hz; PN, animals exposed to purple noise with a frequency range of 4–20 kHz, and control group, without noise. The experimental groups were exposed to a 118–120 dB sound pressure level for 8 h per 3 days and 6 days. 1 h and 1 week after termination of noise exposure, cochlear tissue was prepared for pathology and gene expression analysis.

Results Both white and purple noises caused permanent damage to the cortical, estrosilica systems of hair cells and ganglion of the hearing nerve. GJB2 and SLC26A4 were downregulated in both groups exposed with white and purple noise by increasing the time of noise exposure. However, differences are notably more significant in purple noise, which is more intensified. Also, 1 week post noise exposure, the downregulation is remarkably higher than 1 h.

Conclusions Our findings suggest that downregulation of GJB2 and SLC26A4 genes are associated with pathological injury in response to noise exposure in cochlear tissue. It would be suggested the demand for assessment of RNA and protein expression of genes involved in noise-induced hearing loss and subsequently the practice of hearing protection programs.

Keywords GJB2 & SLC26A4 genes · Real-time RT-PCR · Hearing impairment · Cochlear tissue · White & Purple noise
Introduction

Noise-induced hearing loss (NIHL) is the most common form of acquired hearing impairment in developed countries, and therefore, it is considered a public health issue [1]. A wide range of workers is exposed to noise pollution in the workplace, which has been shown to have detrimental effects, such as hearing impairment [2]. Limited studies have been conducted on the impact of high-frequency noises on the expression of genes involved in hearing impairment. One type of noise with a high-frequency range is purple noise (PN), and the other, which has a range of audible frequencies, is white noise (WN). The frequency range of white noise (100-20000 Hz) and purple (4000-20,000 Hz) are both in the range of audible, but depending on the application, white or octave band noises centered on low frequency and purple noise centered on high frequency [3]. In sound engineering, it has been shown that the color of noise is related to the sound intensity level (SIL). Indeed, in different colors of noise such as purple, pink, red, and blue, the numerical values of sound intensity level (audio intensity) at each frequency are different, and the energy isn’t equally distributed across them. For example, the purple-noise audio signal in the frequency range between 6000 and 7000 Hz has a different sound intensity level (SIL) value compared to the frequency range between 8000 and 9000 Hz. Purple noise’s power density increases 6.02 dB per octave with increasing frequency (density proportional to f^2) over a finite frequency range, so purple noise generates very high energies at higher frequencies. On the other hand, white noise is the only color noise in which the sound intensity level (SIL) spectrum is the same and equal at different frequencies, giving it a constant power spectral density. According to the International Organization of Employers, the physical characteristic of purple sound is very similar to industrial sounds and are considered as one of the essential physical harmful factors in the workplace. Also, it probably has more destructive effects than white noise because of the higher energy and sound intensity. On the other hand, white noise is a mixture of all audible frequencies people expose in daily lives and work environment. However, according to different studies, this noise can cause irreversible damage to the human’s hearing system if it is exposed to a high sound pressure level (SPL). Accordingly, it is important to investigate the harmful effects of these sounds, which could be helpful in prevention in the community who are exposed to [1, 4, 5].

It is well documented that hearing impairment could be induced by these noises or after exposure to high-intensity noises [6]. Studies were demonstrated that exposure to sound pressure level (SPL) of 90–120 dB and a frequency range of 1–7 kHz could induce upregulation of heat shock protein genes [6], Protein 45β (GADD45β), CDK-interacting protein 1 (p21^Cip1) [7], CIITA, MHCII, [8] and Connexin 26 (Cx26) [9] in the atrium of inner ear suggesting them as biomarkers of audible frequencies noise response. Moreover, exposure to a sound pressure level of 120 dB and frequency range of 7 kHz resulted in the upregulation of C1s and Cfi proteins of the Corti system [10]. Stimulation with ultrasonic noises (16 kHz) and sound pressure level (SPL) = 130 dB significantly increased the expression of CRH (Corticotropin-Releasing Hormone) genes and its CRH-R1 receptor in rats; also, the physical stress caused by these noises had a severe effect on microglial cells [11]. Moreover, acute exposure to high-frequency noise (10–20 kHz) and SPL = 110–120 dB resulted in upregulation of different cochlear tissue proteins such as β-ACTIN, PRESTIN, and β-SPECTRIN [12]. Indeed, abnormal noise at different frequencies and times exposure can induce mutation and changes in the gene expression pattern in ganglion and cochlear tissue. In this manner, noise can cause morphological and physiological changes leading to hair-cell death and disorders in different sections of the human auditory system [7, 13, 14]. Inner and outer hair cells (IHC and OHC, respectively), especially those located in the basal turn of the cochlea in the
auditory system, are susceptible to noise damage. Thus, the disruption of stereocilia leads to a severe alteration in HC structural integrity that has been correlated to permanent threshold shifts (PTS) in many species [15–20].

It was documented that several genes are involved in the physiological response of the cochlea to noise-induced damage. These genes are associated with various biological processes such as oxidative stress and transcriptional signaling [7, 21]. The gap junction beta-2 (GJB2) encoding for gap junction protein connexin-26 (Cx26) is one of the most important proteins involved in the regulation of potassium ions in the cochlea, which is located within the spiral cells of the limbus and the spiral ligament fibroblasts [22, 23]. The solute carrier family-26 member-4 (SLC26A4) gene encodes an anion transporter, pendrin, essential for normal inner ear function. It consists of the DFNB4 locus, a recessive chromosomal locus that causes sensorineural hearing impairment in both non-syndromic and syndromic forms (pendred syndrome, PDS) [24]. Pendred syndrome is most often diagnosed with nerve deafness associated with goiter. Sensorineural hearing impairment due to pendred syndrome is progressive in some cases and causes varying degrees of deafness [24]. Since no research has been performed directly on the effects of white and purple noises exposure on the expression of SLC26A4 and GJB2 genes involved in congenital hearing impairment, in the present study we investigate their expression pattern after exposure with white and purple noise at a different time in the rat. Given that cochlear tissue is the most important sensorineural receptor of sound when exposed to noises, the pathologic changes in this tissue were also investigated.

**Fig. 1** Schematic representation of the experimental design and schedule of the protocols. Total population involving 32 male rats, including 8 control, 12 rats exposed with white noise (WN) and 12 exposed with purple noise (PN). Animal divided into five main groups: (1) control group (n = 8), without noise exposure; (2) WN-3D (n = 6), animals exposed to white noise for 3 days with a frequency range of 100-20000 Hz; (3) WN-6D (n = 6) animals exposed to white noise for 6 days with the same frequency; (4) PN-3D (n = 6), animals exposed to purple noise for 3 days with a frequency range of 4–20 kHz; (5) PN-6D (n = 6), animals exposed to purple noise for 6 days with the same frequency range. All experimental groups were exposed to a sound pressure level (SPL) of 118–120 dB for 8 h per day. At two time points, 1 h and 1 week after termination of noise exposure, 3 rats of each experimental group and 4 rats of the control group were anesthetized, and the samples were collected for pathologic and gene expression analysis.
Materials and methods

Study group selection

In this experimental study, total population involving 32 male rats, including 8 control rats, 12 rats exposed with white noise (WN) and 12 exposed with purple noise (PN) for 3 (3D) and 6 days (6D). In detail, 32 adult male Westar rats were randomly divided into five main groups: (1) control group (n = 8), animals not exposed to any noise; (2) WN-3D (n = 6), animals exposed to white noise for 3 days with a frequency range of 100-20000 Hz; (3) WN-6D (n = 6) animals exposed to white noise for 6 days with the same frequency; (4) PN-3D (n = 6), animals exposed to purple noise for 3 days with a frequency range of 4–20 kHz; (5) PN-6D (n = 6), animals exposed to purple noise for 6 days with the same frequency range. The experimental groups were exposed to a sound pressure level of 118–120 dB for 8 h per day (Fig. 1).

To determine the effect of the long time-point after termination of noise exposure on gene expression and pathologic changes, rats were analyzed at a two-time point. For this purpose, rats in each experimental group were divided into two equal subgroups (n = 3 each); one subgroup was anesthetized at 1 h (1 h) and the other at 1 week (1w) after cessation of noise exposure. In the control group (n = 8), 4 rats were analyzed at 1 h and 4 at 1-week post noise exposure (Fig. 1). Rats were sacrificed, and after preparing the biopsy from the cochlea, the samples were subjected to pathologic and gene expression analysis.

In this study, the sample size, time, and type of exposures in the experimental groups were obtained based on our preliminary research and similar studies in this field [6, 9, 25]. The study was done under the principles of the Ethics Research Committee of the School of Public Health, Tehran University of Medical Sciences (Code: IR.TUMS.SPH.REC.1398.220).

Laboratory conditions

Rats used in this study with a specific weight and age (7–8 weeks, 190-200 g) were placed in a high-performance four-cell audio chamber. One week before the start of exposure, they adapted to the conditions of the experimental environment (12 h of darkness and 12 h of light, temperature of 25 ± 2 °C and humidity of 45–52%) and then were exposed to noises at a specific time from 10 am to 6 pm [6, 9] and during the period the necessary tests, water, and food were sufficiently provided to the rats. Exposure to white and purple noise took place inside a four-cell high-performance audio chamber measuring 60 × 52 × 40 cm³. The sound transmission loss from inside the chamber to the outside was 50 decibels. The walls of this chamber are composed of layers of iron sheet, cellulose, air layer, cellulose, iron sheet, and cellulose, respectively. Two holes on the chamber walls were used for ventilation of the chamber; and to supply enough oxygen for animal samples inside this chamber, a peripheral blowing pump with a flow of 70 L per minute was used.

Noise exposure

The noise pollution was generated using computer software (ADOBE AUDITION with version 13.0.7) and distributed inside the chamber by an amplifier (Crown D75A, Elkhart IN) and 2 speakers (RadioShack Corp, Ft Worth TX). Frequency and sound pressure level monitoring were used with SPECTRUM ANALYZER software (version 5.0.2.). Also, to check the uniformity of the sound pressure level in different parts of the chamber, the sound pressure level in different parts was measured by an analytical sound meter (CELL491 catalog model).

Histological assessment

First, the rats were anesthetized by exposure to carbon dioxide in a cubic chamber, then the skull bone was dissected, and the brain of the specimens was removed. The cochlear tissue, which includes the inner ear and is located inside the pterygium, was removed from the tympanic and temporal sections of the auditory system by surgical instruments. The cochlear tissue was fixed with 4% paraformaldehyde (PFA) for 24 h at 4°C and dehydrated in ascending ethylic alcohols (30–100%), cleared with 100% xylene, and embedded in 100% paraffin. Afterward, the samples were cut with a microtome in thickness of 5 to 10 μm and stained with hematoxylin-eosin (H&E) and mounted on a glass slide for microscopic observation. In the end, morphological and pathologic changes of cochlear tissue were checked using light microscope images at a 200-micron scale [13, 26].

RNA extraction

Total RNA was extracted from cochlear tissue using Tripure reagent (Roche, Mannheim, Germany) according to the manufacturer’s instructions. Briefly, 50 mg of tissue was homogenized and lysed using Tripure reagent. The lysate was mixed with chloroform and vortexed vigorously and then centrifuged at 12,000 g for 15 min at 4°C. Afterward, the colorless upper phase was precipitated by adding
isopropanol and washed with 75% ethanol. Finally, RNA was dried at room temperature and dissolved in RNase-free water. The quantity and quality of RNA were evaluated by nanodrop (ND-1000, Thermo Scientific Fisher, Waltham, Massachusetts, USA) and gel electrophoresis, respectively. RNA was treated with DNase to avoid genomic contamination (Qiagen, Hilden, Germany) according to the manufacturer’s instructions.

cDNA synthesis

Complementary DNA (cDNA) was synthesized using 1 µg RNA, 2.5 µmol/µl oligo-dT (Roche, Mannheim, Germany) and 60 µmol/µl random hexamers (Roche, Mannheim, Germany) incubated 10 min at 65 °C followed by addition of 10 mM of dNTP mix (Deoxy Nucleoside Triphosphate mix) (Roche, Mannheim, Germany) 40U/µl RNase inhibitor (Roche, Mannheim, Germany), 4uL of 5X reverse transcriptase (RT) buffer (Roche, Mannheim, Germany) and 20 U/µL reverse transcriptase enzyme (Roche, Mannheim, Germany) and then incubated at 25 °C for 10 min followed by incubation at 50 °C for 60 min. The integrity of cDNA was checked with GAPDH (Glyceraldehyde 3-Phosphate Dehydrogenase) primers (Table 1) as a housekeeping gene. The PCR condition is as follows: an initial denaturing step of 94 °C for 5 min and 35 repetitions of denaturation at 94 °C for 30 s, annealing at 49 °C for 30 s, and extension at 72 °C for 45 s with a final extension of 72 °C for 5 min.

Real-time RT-PCR analysis

To investigate the differences in the RNA expression level of target genes among exposure and control groups, real-time reverse transcriptase-PCR (RT-PCR) was used. Specific primers were designed by Primer 3 software version 0.4.0 (https://www.ncbi.nlm.nih.gov/tools/primer-blast) (Table 1). Two independent RNA preparations were used for each real-time RT PCR experiment. The experiments were performed in 20 µl reactions containing 1 µl cDNA target, 100 nM forward and reverse primers, and 1x SYBR® Premix Ex Taq™ II (Takara, Tokyo, Japan). RT-PCR was performed in duplicate for target genes and GAPDH as a housekeeping gene using a Real-Time System (Applied Biosystems [ABI], Foster City, CA, USA). The PCR condition was as follows: activation at 95 °C for 3 min, amplification at 95 °C for 10 s, 60 °C for 32 s for 40 cycles followed by a melting curve, temperature increments of 0.2 °C every 30 s to determine amplification of the expected product [27].

Table 1 Primer sequences of target genes used in real-time RT-PCR

| Oligo Name | seq 5 – 3 | TM |
|------------|-----------|----|
| GJB2 Forward | 5´-TCACT-GTCTCTTCTCATCTTCCG-3´ | 59.51 |
| GJB2 Reverse | 5´-CTTCCGGTCTTTCTCTGT-3´ | 57.88 |
| SLC26A4 Forward | 5´-CATCATGCCTGCTGGTTCT-3´ | 60.40 |
| SLC26A4 Reverse | 5´-TGGACACACATTCCGTCA-3´ | 59.53 |
| GAPDH Forward | 5´-AAGTTCACGCAGCAGTCAAGG-3´ | 61.58 |
| GAPDH Reverse | 5´-CATACTCAGCACCAGCATCACC-3´ | 61.32 |

Analysis of gene expression

The expression level of target genes is reported as the gene fold in the noise-exposure samples normalized to the internal control gene (GAPDH) and relative to the control group using the $2^{-\Delta\Delta CT}$ method as previously described [28].

Statistical method

Statistical calculations were performed by prism Graph pad software with version 9.0.0.121. Relative gene expression value data are mean ± standard deviation and were normalized to the internal control. The normal distribution hypothesis for quantitative variables was evaluated by the Shapiro-Wilk test and P-value < 0.05 was considered statistically significant. Differences among the exposure & control groups were assessed by Tukey’s Multiple Comparison Test, followed by post hoc pairwise comparisons to determine which differences account for the result of the more inclusive test.

Results

Chamber sound pressure level measurement reports

The measurement results showed that the arrangement of sound systems in the chamber was such that the sound pressure level was constant in all parts of the chamber, and all samples were in contact with white and purple noise and with increasing distance from the floor surface of the chamber, the sound pressure level has also increased. Also, the SPECTRUM ANALYZER software results showed that the frequency range and produced sound pressure level in the chamber were quite like the frequency range and target sound pressure level for the present study.
Results of pathological studies

Microscopic examination of tissues and their imaging showed that 1 week after cessation of noise exposure, neurons in the samples of groups exposed to white noise (WN-3D and WN-6D) were damaged more seriously than 1 h after exposure termination (Fig. 1). Also, the nerve cells of the samples exposed to purple noise (PN-3D and PN-6D)

![Microscopic images](image)

**Fig. 2** Hematoxylin and Eosin staining of cochlea tissue of different exposure group samples, 1 h and 1 week after cessation of noise exposure. WN-3D, group were exposed to white noise for 3 days; WN-6D, exposed to white noise for 6 days; PN-3D, group exposed to purple noise for 3 days; PN-6D; exposed to purple noise for 6 days. Abnormal morphology of auditory nerve ganglion and Reisner membrane was observed following noise exposure.

**Fig. 3** Expression analysis of GJB2 gene using real-time RT-PCR after white & purple noise exposure in cochlea tissue of rat. The expression level of target genes is reported as the gene fold in the noise-exposure samples normalized to the internal control gene (GAPDH) and relative to the control group using the \(2^{-\Delta\Delta CT}\) method. In all experiment group the expression of GJB2 is significantly downregulated at 1 week post noise exposure compared to 1 h. Data are expressed as mean ± SD of three independent experiments. * p < 0.05; ** p < 0.01, *** p < 0.001. Two-way ANOVA followed by the Tukey multiple-comparison test
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Expression pattern of SLC26A4 gene

Gene expression analysis showed that 1 h after cessation of noise exposure, SLC26A4 gene was significantly \( (p\text{-value}<0.05, \text{Tukey’s Multiple Comparison Test}) \) downregulated in exposure groups WN-3D, WN-6D, and PN-6D by 0.42-, 0.09- and 0.28-fold, while it was slightly upregulated in PN-3D \((1.21\text{-fold}, p\text{-value}>0.05)\) as compared to control group. On the other hand, 1 week after termination of noise exposure, the RNA level of SLC26A4 was significantly \( (p\text{-value}<0.05, \text{Tukey’s Multiple Comparison Test}) \) decreased in exposure groups WN-3D, WN-6D, PN-3D, and PN-6D by 0.31-, 0.019-, 0.43- and 0.04-fold, respectively. Additionally, 1 week after cessation of noise exposure, the average expressions of SLC26A4 exhibited more downregulation than 1 h after cessation of noise exposure in all the exposure groups (Fig. 4).

Expression pattern of GJB2 gene

The results showed that 1 h after cessation of noise exposure, the transcript level of GJB2 was significantly \( (p\text{-value}<0.001, \text{Tukey’s Multiple Comparison Test}) \) decreased in exposure groups WN-3D and WN-6D by 0.51- and 0.12-fold, while it was increased in PN-3D \((1.26\text{-fold}, p\text{-value}>0.05)\) as compared to control. 1 h after cessation of noise exposure, the PN-6D exposure group was slightly downregulated which was not significantly \((0.72\text{-fold}, p\text{-value}>0.05)\). Also, 1 week after cessation of noise exposure, the transcript level of GJB2 was significantly \( (p\text{-value}<0.001, \text{Tukey’s Multiple Comparison Test}) \) decreased in exposure groups WN-3D, WN-6D, PN-3D, and PN-6D by 0.34-, 0.02-, 0.51- and 0.12-fold, respectively. Overall, 1 week after cessation of noise exposure, the average mRNA levels of GJB2 were significantly lower when compared to 1 h after cessation of noise exposure in all the exposure groups (Fig. 3).

Discussion

The cochlear tissue and hair cells of the Corti system are the most important sensorineural receptors for sound in the human auditory system. Noise exposure could trigger point mutation and alteration in gene expression, resulting in many sensorineural hearing defects [14]. Therefore, it is hypothesized that the cochlear tissue sample can be used as an ideal tissue sample to study the gene expression and understand the mechanisms that induced syndromic/non-syndromic sensorineural hearing impairment. Accordingly, in this study, the effects purple and white noise, which had different frequency ranges, were investigated on the RNA
expression of GJB2 and SLC26A4 genes in rat’s cochlear tissue. Purple noise has a high-frequency range, and white noise has a range of audible frequencies. Most studies in the field of gene expression and its relationship with noise have examined only the effects of noises with a certain frequency on the expression of genes [10, 11, 25, 29], while in this study, sound with a different frequency range in the human hearing range (20-20000 Hz) were analyzed.

To determine the gene fold, GAPDH (Glyceraldehyde 3-Phosphate Dehydrogenase) as a housekeeping gene was selected to normalize the data. The expression of GAPDH is consistent and stable in different tissue, so it has been used as an internal control for relative gene expression analysis in cochlear tissue [30, 31]. In a study by Melgar-Rojas et al., it was demonstrated that in Wistar Rat in response to NIHL, the expression of housekeeping genes such as GAPDH in cochlea tissue during noise exposure, 1 day and 10 days after noise-exposure was constant and stable relative to control animals. However, in permeant damage of cochleae, 30 days after noise exposure, the expression of some housekeeping was changed [32]. In our study, given that the noise exposure is short (3–6 days), and the RNA extraction was 1 h and 1 week after termination of noise-exposure it seems that the GAPDH is a stable and proper choice for normalization of data.

The results showed that the RNA expression level of GJB2 and SLC26A4 genes were decreased in both groups exposed to white and purple noise. Also, these genes exhibited remarkable downregulation after 6 days exposure to white and purple noises compared to 3 days exposure, suggesting that increases in the duration of time exposure could induce more alteration in gene expression. Additionally, when comparing two time-points of RNA extraction after the termination of noise exposure, it was revealed that the average expression of GJB2 and SLC26A4 genes displayed more notable downregulation 1 week after the termination of noise exposure as compared to 1 h in all the experiment groups. In harmony with gene expression results, in pathologic examination of cochlear tissues, it was demonstrated that neurons were damaged more seriously 1 week after cessation of white and purple noise exposure compared to 1 h after experiments. These results propose that more stable differences in gene expression and more pathologic damage could be seen at a longer time-point after the termination of noise exposure.

There is a lack of enough knowledge regarding the expression of GJB2 and SLC26A4 genes after white and purple noise exposure. However, recently several studies have evaluated the expression of some cochlear tissue and hair cells proteins, such as β-ACTIN, PRESTIN, and β-SPECTRIN after contact with sound pressure level between 110 and 120 dB, and the results demonstrated that gene expression was significantly increased in exposure groups [6, 10, 12, 25]. Furthermore, it was documented that after exposure of rats with white noise (4 kHz) for 2 days, the expression of connexin 26 (CX26) protein in the dorsal wall of the cochlear tissue increased significantly, and the amount of protein in ligament fibrocytes was increased [29].

In this context, a similar study showed that the expression of heat shock proteins (HSP 27, 70) increased after 1 h of noise exposure (100 dB), and the level of the hearing threshold was raised following 4 h of exposure with the noises, suggesting that changes in HSP gene expression could lead to rapid physiological changes at the level of the auditory threshold [33]. Consistent with the former studies, Gratton et al. [7] stated that a sound pressure level of 105 dB (for 1 h per day) induces overexpression of HSP70, HSP40, growth arrest and DNA damage-inducible protein 45β (GADD45β), and CDK-interacting protein 1 (p21cip1) genes in cochlear tissue of mouse. However, the discrepancy between the results of these studies and the present study may be due to the difference in a time exposure, type of noise, and false-positive and negative results of the real-time reverse transcriptase-PCR technique. Also, 1 h after cessation of exposure, the expression of both GJB2 and SLC26A4 genes in one group exposed to purple noise (PN-3D) upregulated and the other groups exposed to white and purple noise down-regulated significantly. These results are in harmony with a study by Alagramam et al., [2] in which two groups of mice were exposed to noise with sound pressure levels of 110 and 116 dB for 1 h per day. The results showed that exposure to 110 dB reduced the expression of 221 genes and increased the expression of 55 genes in the cochlea tissue, while after exposure to 116 dB, the expression of 61 Genes was decreased and 243 genes enhanced. Additionally, the expression of Fos Proto-Oncogene in the cochlea increased significantly after exposure to 116 dB, while the expression of calcium-related genes decreased significantly after exposure to 110 dB suggesting mitogen-activated protein kinase (MAPK) signaling was the major pathway in both levels of noise exposure.

It was demonstrated that acute and chronic noise exposure could change gene expression differently. In agreement with this notion, a study by Eraslan et al. [34] revealed that after acute noise exposure, the expression of both CRH-R1 and CRH-R2 (corticotropin-releasing hormone receptor) genes were downregulated, while after chronic noise-exposure the expression of CRH-R1 and CRH-R2 genes increased and decreased, respectively. These results are consistent with the results of the effects of purple noise on the expression of GJB2 and SLC26A4 genes in the present study, in which the gene expression is lower after 6-day exposure compared to 3-day exposure. The results of this study are also in harmony with the findings of a similar study [8] that
have shown a significant relationship between changes in gene expression pattern and the incidence of sensorineural hearing impairment. Furthermore, it was documented that GJB2, SOD2, and CAT genes may be involved interactively or independently in the development of NIHL [35]. Moreover, exposure of mice to 110 dB for 8 h a day for 5 consecutive days decreased the expression of connexin 26 (CX26) in cochlear tissue and led to impairment of hair cells in the cortical system and triggered the risk of NIHL [9].

Another important result in this study is that exposure to both white and purple noises has adverse effects such as axonal changes of nerve neurons and the destruction of cochlear tissue and hair cells on the samples of the auditory system. These injuries have intensified with the increasing of time-exposure; the cochlea tissue was also damaged more significantly 1 week after cessation of noise exposure compared to 1 h after challenge. In agreement with our finding, it was reported that the cochlea damage increased with longer time points after the noise exposure [32].

Also, our result revealed that purple noise due to the higher energy and frequency has more destructive effects than white noise. Likewise, it was reported that exposure to loud noises can lead to increased production of nitric acid in the spiral ganglion neuron of pregnant women leading to alteration in the gene-expression pattern of ganglion cell and thereby cell death, and development of congenital hearing impairment [36, 37]. It is well documented that after damage and destruction of the auditory system, restoration and reconstruction of nerve cells such as hair cells in mammals is not possible and subsequently can cause cell death in the Corti system, the development of sensorineural hearing impairment, and permanent hearing threshold (PTS) changes [14]. In terms of pathology, cochlear injury induced by noise exposure is a multifactorial degradation process that occurs due to inflammation and direct mechanical responses contributing to apoptosis and necrosis [21]. In this line, changes in the neuron cell body’s golico kanghogahse after noise exposure have been reported in ear tissue and other tissues such as the heart [38]. Evidence has also shown that changes in the expression level of proteins related to the auditory system noises cause permanent damage to the auditory nerve ganglion and cochlear tissue neuron tissue to lead to non-syndromic hearing loss or sensorineural syndrome [9, 14, 26]. Given that both white and purple noises have a significant effect on the expression of GJB2 and SLC26A4 genes involved in sensorineural hearing impairment, research on this issue should be concerned. The discovery of genes that are affected by noises and cause sensorineural hearing impairment can be useful in pathophysiological studies and the development of an appropriate solution for prevention [39].

Conclusions

Our findings revealed that exposure to both white and purple noises induces structural and cellular damage in cochlear tissue. Moreover, the RNA expression level of GJB2 and SLC26A4 genes were decreased in both groups exposed to white and purple noise. However, the differences in gene expression and pathologic damages are notably more significant in purple noise, which has a higher frequency. Also, the injuries and downregulation have intensified with increasing time exposure and longer time-point after the noise exposure. Further study on cochlea tissue through transcriptome and proteomics analysis along with pathophysiological studies could be helpful to find potential genes that are linked to noise-induced hearing loss. Appropriate training and advice should be suggested at the community level to prevent the effect of noise on genetic changes in the auditory system; also, using engineering or therapeutic control methods could decrease the risk of injury to the hearing system.

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Authors’ contributions AAG, MRME and EK collected and analyzed data. AAG and EK wrote the manuscript. MK, MMH and SM reviewed and revised the manuscript. EK and MRME supervised the entire processes. All authors read and approved the final manuscript.

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Availability of data and materials Data will be available on special request to corresponding author.

Declarations

Conflict of interest/Competing interests The authors declare that they have no competing of interest.

Ethical approval and consent to participate The study was approved by the Ethics Research Committee of the School of Public Health, Tehran University of Medical Sciences (Code: IR.TUMS.SPH.REC.1398.220).

Consent for publication All authors read and approved the final manuscript.

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