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Original Article

Prestin, otolin-1 Regulation, and Human 8-oxoG DNA Glycosylase 1 Gene Polymorphisms in Noise-induced Hearing Loss

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Abstract

Background: Noise induces free radicals release and can damage the cochlear epithelium. The outer hair cell motor protein prestin is necessary for sharp frequency tuning and cochlear function. Otolin-1 is a glycoprotein; its mRNA expression is restricted to the inner ear. Genes involved in repairing the oxidative damage as human 8-oxoG DNA glycosylase 1 (*hOGG1*) can affect the cochlea susceptibility to noise. Prestin upregulation may represent a response to compensate for noise-induced hearing loss (NIHL). **Objectives:** We investigated the association between exposure to noise, the blood perstin and otolin-1level, *hOGG1* polymorphisms, and oxidative DNA damage as indicated by serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) concentrations. **Materials and Methods:** In 300 patients with NIHL and 200 controls with normal hearing, blood prestin, otolin-1, and 8-OHdG levels were studied by ELISA; the *hOGG1* polymorphism was genotyped by polymerase chain reaction amplification followed by digestion with restriction endonucleases. **Results:** The prestin, otolin-1, and 8-OHdG levels were significantly elevated in patients compared to controls ($P < 0.05$). Regression analysis showed that *hOGG1* Cys/Cys genotype showed a significantly increased risk of hearing loss compared with the other genotypes exposed to the same environmental factors (95% confidence interval = 1.1–2.3, adjusted odds ratio = 1.5). This was associated with increased prestin, otolin-1, and 8-OHdG levels and the duration of noise exposure in months. **Conclusion:** These findings are consistent with the notion that prestin increases in an attempt to compensate for missing outer hair cells. Otolin-1 could be a circulatory biomarker for otoconia damage caused by noise, and the *hOGG1* Cys/Cys genotype could be a susceptibility marker for NIHL. The hOGG1 Cys/Cys gene variant was more frequent in patients compared to controls exposed to the same environmental factors and more frequent in severe cases confirmed by elevated prestin, otolin-1, and 8-OHdG l levels.

Keywords: Glycosylase 1 gene polymorphisms, noise-induced hearing loss, otolin-1, prestin

Introduction

Noise-induced hearing loss (NIHL) is the most common form of sensorineural hearing damage caused by exposure to noise. Stimulation with sound of high intensity alters outer hair cell plasma membrane fluidity. Prestin, the motor protein in the outer hair cells, generates electromotility and the absence of prestin results in the lack of electromotility and hearing loss.[1-3] Otolin-1 is a secreted glycoprotein; its mRNA expression is restricted to the vestibular maculae, semicircular canal cristae, organ of Corti, and marginal cells of the stria vascularis.[4] Its functions include interaction with other inner ear proteins such as prestin to maintain inner ear function,[5] so they can serve as circulatory biomarkers for NIHL.

Overstimulation of tissues by noise causes excess production of reactive oxygen species (ROS), which oxidize cellular targets such as proteins and DNA. The 8-hydroxy-2'-deoxyguanosine (8-OHdG) DNA damage caused by ROS – that leads to transversion mutation – is the most common form of oxidative damage to DNA. DNA damage to cochlear hair cells is essential for the development of NIHL. The activity of ROS is antagonized by protective enzymes as human 8-OHdG DNA glycosylase 1 (hOGG1) – an important enzyme in the base excision repair pathway that eliminates 8-oxoG. Previous studies have suggested that the Ser326Cys polymorphism in exon 7 of hOGG1 gene may affect the enzyme activity so it may be NIHL susceptibility genes.[6]

Therefore, we have undertaken this study to investigate the role of prestin and otolin-1 in cochlear dysfunction in NIHL, test the

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association of *hOGG1* polymorphisms with NIHL in patients compared with normal hearing individuals, and to evaluate 8-OHdG as a sensitive biomarker for oxidative DNA damage.

Materials and Methods

This study included 300 NIHL patients referred to Manfalout hospital from Mankabad Industry City between January 2000 and January 2016 and 200 normal hearing volunteer workers (both were matched by age, sex, and occupational exposures). All patients had their hearing tested at presentation (postexposure tinnitus and hearing loss were the main presenting symptoms) and 1 month after treatment. They had not been exposed to physical or chemical factors causing hearing loss (e.g., organic solvents, heat, and drugs or diseases that affect hearing). They did not use hearing protection. This study protocol was approved by Assiut University Ethical Committee, and a written informed consent was obtained from all participants.

History taking included demographic characteristics, the exposure level (mean 87.1 ± 7.7 dB measured using noise level meter [Simpson 890]), and the duration of noise exposure in months (mean 18.6 ± 7.6, Table 1), smoking habits of each patient (most of them were mild smokers as smoking is not allowed in their work, the average number was 3 cigarettes per day), hearing protection use, history of ototoxic drug use, physical and chemical factor exposure, family history of deafness, work history, and disease history. Five hundred, 1000, 2000, 3000, 4000, and 6000 pure-tone air hearing threshold tests (Amplaid audiometer) were carried out in a sound-attenuating chamber with a background noise level of <25 dB (a noisy environment was avoided 48 h before the test). For each test frequency, the initial presentation level was 30 dB HL after which the intensity was decreased in 5 dB steps until the participant failed to respond. Ascending runs using 5 dB increase were repeated three times, and the threshold was detected as the lowest level at which responses were obtained on 2 out of 3 ascending runs.[7] Hearing loss was classified as mild (average threshold 25–40 dB), moderate (41–60 dB), severe (61–80 dB), and profound (>80 dB, usually resulting from a known cause other than noise and removed from the noisy environment and the study as they were lost in the follow-up).[7]

Table 1: The values (mean ± standard variation) of the clinical characteristics and main outcome measures in patients and controls

Variables	Patients (*n*=300)	Controls (*n*=200)	*P*
Age (years)	40.5±5.2	40.3±3.9	0.9
Male/female (%)	93.2	92.3	0.6
Smoker/nonsmoker (%)	77.3	68.3	0.5
Exposure time (months)	18.6±7.6	18.2±7.4	0.4
Exposure level (dB[A])	87.1±7.7	87.0±7.6	0.8
Prestin level (pg/mL)	169.0±88.4	100.9±16.7	0.04*
Prestin level (pg/mL) after 1 month	114.0±99.2	-	0.04*
Otolin-1 level (pg/mL)	200.8±93.5	70.9±15.1	0.02*
Otolin-1 level (pg/mL) after month	199.8±94.2	-	0.36
8-OHdG (pg/mL)	160.8±16.7	52.3±10.1	0.00**

8-OHdG: 8-hydroxy-2'-deoxyguanosine

Ten milliliter venous blood was taken into tubes containing ethylenediaminetetraacetic acid, centrifuged for 10 min at 3000 rpm and plasma was stored at − 80°C until assay. The level of prestin was determined by ELISA (MyBioSource, California, San Diego, USA, Cat No: MBS282125). This assay employs a two-site sandwich ELISA. A specific antibody has been precoated onto a microplate. Samples and standards are pipetted into the wells. After removing any unbound substances; a biotin-conjugated antibody is added. After washing, streptavidin-conjugated horseradish peroxidase is added. Following a wash to remove the unbound avidin-enzyme reagent, a substrate solution is added and color develops. The intensity of the color was measured at 450 nm (by Beckman Coulter DU 7400 Spectrophotometer). Otolin-1 was measured using ELISA kit (MyBiosource.com, San Diego, USA, Cat No: MBS9312379) similar to the previous technique, and the optical density in the wells of the ELISA microplate was measured at 450 nm.

The level of 8-OhdG was determined by enzyme-linked immunosorbent technique (Abcam, Kendall Square, Cambridge, USA, Cat No: ab. 201734). Briefly, the monoclonal antibody for 8-OHdG was precoated onto microplates. Standards and samples were added to the wells, after washing with phosphate-buffered saline; biotinylated anti-8-OHdG antibodies were added. After washing the excess antibodies away, horseradish peroxidase-conjugated streptavidin was added; final washing with PBS, 3,3',5,5'-tetramethylbenzidine substrate solution was pipetted into the wells and the color intensity was measured at a wavelength of 450 nm.

For DNA extraction, DNA extraction kit (Qiagen, Cat No. ID: 69504) was used. Polymerase chain reaction (PCR)-restriction fragment length polymorphism was used to detect the *hOGG1* gene polymorphisms. The PCR primers were 5'-GGAAGGTGCTTGGGGAAT-3' f and 5'-ACTGTCACTAGTCTCACCAG-3' r, B-actin: 5'-ATCATGTTTGAGACCTTCAACA-3'f, 5'-CATCTCTTGCTCGAAGTCCA-3'r. The initial denaturation was at 95°C for 3 min., followed by 35 cycles at 94°C for 30 seconds then annealing was at 52°C for 30 s. The final extension was at 72°C for 5 min. The products were digested by the *Fnu*4HI restriction enzyme (Waltham, MA, USA) for 12 h separated on 2% agarose gel containing ethidium bromide. The three possible genotypes were 200 bp for Ser/Ser genotype, both 100 bp and 200 bp fragments for Ser/Cys genotype, while only 100 bp fragments for Cys/Cys genotype [Figure 1].

Statistical analysis

The statistical analysis was performed using the statistical package for the Social Sciences, Version 13 (SPSS, Inc., Chicago, IL, USA). The mean and standard deviation were

Figure 1: Analysis of human 8-oxoG DNA glycosylase 1 Ser326Cys gene polymorphism detected by polymerase chain reaction-restriction fragment length polymorphism

calculated. Statistical significance was compared using ANOVA. Prestin, otolin-1, and 8-OHdG concentrations of control- and noise-exposed patients were compared with a *t*-test. Pearson correlation coefficient was determined to assess the correlation between various variables. Continuous data were analyzed by independent-sample two-sided *t*-tests. Categorical data were computed by two-sided Chi-square tests. Multivariate logistic regressions were used to compute odds ratios (ORs), 95% confidence intervals (95% CIs), and to test the associations of various genotypes with noise damage. Adjusted ORs and 95% CIs were computed by multivariate logistic regression adjusted for age, sex, and smoking status. The sensitivity and the specificity of the studied parameters are shown in Table 2. $P < 0.05$ was considered statistically significant.

Results

The demographic characteristics

The demographic characteristics are shown in Table 1. There were no significant differences in the distribution of age, sex, smoking, time of exposure, and level between the patients and the controls as the majority of the smokers were light smokers (the average was three cigarettes per day as it was not allowed during work). Prestin, otolin-1, and 8-OHdG levels were significantly increased in patients compared with controls following noise exposure [$P < 0.05$, Table 1]. After 1 month, the mean prestin concentrations were 55% lower in the patients than that was measured right after the noise exposure. A one-tailed *t*-test showed this difference to be significant ($t = 4.3$, $P = 0.02$). The sensitivity and specificity of the different studied parameters are shown in Table 2; prestin showed the highest levels compared with the other parameters.

Comparison of the three human 8-oxoG DNA glycosylase 1 genotypes

No significant differences were detected among the three genotypes regarding age ($P = 0.3$), sex ($P = 0.09$), body mass index ($P = 0.1$), regarding noise exposure duration in months ($P = 0.04$), family history ($P = 0.2$), and smoking (0.0585 as smoking was not allowed during work and most of the patients were mild smokers [<5 cig/day]). There was a statistically

Table 2: The sensitivity and the specificity of the main outcome measures

Parameter	Sensitivity (%)	Specificity (%)	Area under the curve
Prestin level (pg/mL)	94.3	88.5	0.9
Otolin-1 level (pg/mL)	79.2	72.4	0.8
8-OHdG (pg/mL)	86.1	86.7	0.8

8-OHdG: 8-hydroxy-2'-deoxyguanosine

Table 3: The outcome measures of the patients and control in different human 8-oxoG DNA glycosylase1 genotypes

Index (mean±SD)	Ser/Ser (*n*=121)	Ser/Cys (*n*=117)	Cys/Cys (*n*=62)	*P*
Age (years)	61.7±11.2	60.3±9.3	60.7±9.8	0.31
Male/female ratio	86/35	85/32	50/12	0.09
BMI (kg/m^2)	21.4±8.1	22.3±7.1	23.3±7.1	0.13
Noise exposure duration (months)	67.7±13.9	73.1±14.3	70.2±13.1	0.04
Positive family history (%)	25 (21)	21 (18)	15 (18)	0.2
Smoking, *n* (%)	33 (25)	27 (23)	17 (21)	0.059
Genotype (*hOGG1*/*β*-actin)	45.7±18.9	44.7±17.4	35.5±13.9	0.01
Prestin level (pg/mL) at diagnosis	150.0±76.3	152.6±68.3	199.4±40.3	0.03
Prestin level (pg/mL) 1 month later	144.0±66.2	112.2±78.1	179.2±42.13	0.02
Otolin-1 level (pg/mL)	190.7±43.2	193.7±53.4	223.7±43.5	0.01
Otolin-1 level (pg/mL) 1 month later	191.8±41.1	192.4±33.8	221.5±33.3	0.01
8-OHdG (pg/mL)	152.6±14.3	153.8±11.7	162.4±13.2	0.04

8-OHdG: 8-hydroxy-2'-deoxyguanosine, SD: Standard deviation, BMI: Body mass index, *hOGG1*: Human 8-oxoG DNA glycosylase 1

significant difference in the level of Prestin (both at diagnosis and after one month), Otolin-1 and 8-OHdG among the three genotypes. Cys/Cys genotype showed significantly higher levels than the others genotypes (q = 2.3, q = 3.1, $P < 0.05$), while no significant difference was observed between Ser/Cys genotype and Ser/Ser genotype (q = 1.3, $P > 0.05$, Table 3). Significant positive correlations were detected between prestin level and the severity of NIHL ($r = 0.971$**), otolin-1 level ($r = 0.776$**), 8–OhdG ($r = 0.556$**), and Cys/Cys genotype ($r = 0.828$**). Significant positive correlations were detected between otolin-1 level and the severity of NIHL ($r = 0.776$**), 8–OhdG ($r = 0.866$**), and Cys/Cys genotype ($r = 0.778$**). Significant positive correlations were detected between 8-OhdG level and the severity of NIHL ($r = 0.627$**), and Cys/Cys genotype ($r = 0.968$**), $P < 0.01$ for all.

Gene polymorphism and noise-induced hearing loss severity

The percentage of severe cases in Cys/Cys genotype was 74.1% and was 61.1% and 54.9% in Ser/Ser genotype and

Ser/Cys genotypes, respectively. Mild cases constituted 25.8% of Cys/Cys genotype whereas they were 47.0% and 38.8% in Ser/Ser and Ser/Cys genotypes, respectively [Figure 2].

Discussion

The present study demonstrated that after exposure to high levels of noise, prestin and otolin-1 levels were significantly higher than that of control levels. It is a proof of the concept for a blood biomarker specific for inner ear noise-induced injury in human for the first time. The current diagnostic tools remain limited in early detection of hearing loss.

The development of a serological marker will help treat the condition, before the hearing is permanently affected by noise. Normal appearance of cochlea does not necessarily mean intact function.[8] An outer hair cell biomarker as prestin and otolin-1, which can be measured in the blood, could serve as effective markers assessing the efficacy of new therapy designed to protect the cochlea against NIHL and gaining insights into inner ear function.

In this study, prestin concentrations in normal individuals were detected because of the normal turnover in the outer hair cells membrane.[3] Here, the circulating prestin concentration in controls was used as an estimate of baseline levels. The NIHL is associated with increased prestin level in the remaining outer hair cells as detected in the current study and prestin in residual cells decreases 1–4 weeks later. Hence, prestin level shows changes after NIHL[9-14] about time and so similar changes in circulating prestin level may be detected. Shortly after damage, the outer hair cells undergo apoptosis, and prestin concentration is expected to rise, but as the apoptotic mechanisms stop, prestin concentrations drop reflecting a decrease in the number of surviving outer hair cells as the current study revealed so it can be used as a marker of acute and chronic hair cell damage that may affect the management. The presence of otolin-1 in the blood of controls is in agreement with the notion that there is turnover in otoconia and so otolin-1 level could be the product of increased turnover with degeneration in NIHL in patients as detected in the current study.[15-17]

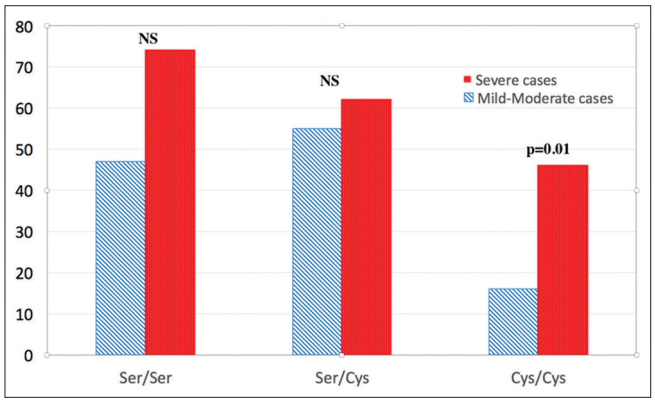

Figure 2: Association between human 8-oxoG DNA glycosylase 1 gene polymorphism and the severity of noise-induced hearing loss

A major contributing mechanism of NIHL is through oxidative stress metabolic damage. The hair cells respond to noise by generating ROS that can overcome the cellular antioxidant mechanism causing DNA oxidative damage and consequently hair cell death and hearing damage.[18] Oxygen free radicals attack the guanine of DNA and lead to the production of an oxidative adduct: 8-OhdG leading to DNA mutation.[19] *hOGG1* is a DNA repair enzyme; it can specifically remove 8-OHdG and repair the damage. Previous studies have confirmed that the activity of *hOGG1* is affected by the *hOGG1* gene polymorphisms.[20,21] In the present study, the percentage of severe cases in Cys/Cys genotype was higher and that of mild cases was lower associated with an increase in the serological indices compared with the other genotypes. Many researchers reported that *hOGG1* Cys326 is a weaker polymorphism as compared to *hOGG1* Ser326 in the ability to repair DNA mutation.[22] The overall differences observed among the three genotypes may be due to the fact that *hOGG1* is a specific 8-OHdG excision repair enzyme, and that mutation polymorphisms lead to the loss of the cells ability to repair 8-OhdG[23,24] as a correlation between the *hOGG1* Ser326Cys polymorphism and the amount of 8-OHdG was observed in the patients. These findings agree with the lower levels of the serological indices, especially 8-OhdG found in the Cys/Cys genotype than in the Ser/Ser genotype and Ser/Cys genotypeIt is proved that the *hOGG1* gene polymorphisms affect the *hOGG1* promoting the initiation and progression of NIHL.[25]

Conclusion

Based on these results, serum prestin and otolin-1can be used in identifying individuals at high occupational risk, especially of the Cys/Cys genotype, monitoring ototoxic agents such as cancer chemotherapy and the possibility of the return of function in response to therapy after a sudden hearing loss. In chronic cases as presbycusis, they may correlate with the degree of dysfunction and response to treatment. The combined use of serological and functional indices could increase the ability to diagnose and manage hearing loss.

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Authors' contribution

Both authors contributed to the conception, planning, conduct of the study, drafting and revising of the manuscript, and approval of its last version.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Compliance with ethical principles

Ethical approval was granted by the Research Committee, University of Assiut. Participants provided a written informed consent.

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