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Research Article

Comparative study of oxidative stress in individuals with and without age related hearing loss

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ABSTRACT

Background: Age related hearing loss is an universal feature of mammalian ageing and refers to sensorineural hearing impairment in elderly individuals. It was reported that increased generation of free radicals during cellular metabolism plays a major role in age related disorders. Therefore, the occurrence of age related hearing loss depends mainly on antioxidant status of the body. Hence, this study was undertaken to investigate the status of Glutathione and Superoxide dismutase in age related hearing loss.
Methods: This study was conducted after the approval from institutional ethical committee. The study group included 25 patients diagnosed for age related hearing loss between the age group of 55-80 years of either sex and 25 healthy age and sex matched individuals as controls. Estimation of Glutathione was done by DTNB method and Superoxide Dismutase using standard procedures. The data was analysed using One Way ANOVA. P value less than 0.05 will be considered the level of significance.
Results: The results showed that, the Glutathione level in Hearing Loss patients was increased significantly (p=0.0001) as compared to normal controls. The SOD Activity has declined significantly (p=0.001) in Hearing Loss patients as compared to normal controls.
Conclusion: We found an association between the level of Glutathione and Super Oxide Dismutase in age related hearing loss. Thus the serum Glutathione and Super Oxide Dismutase level can be used as a biomarker for the assessment of age related hearing loss.

Keywords: Presbycusis, Glutathione, Super Oxide Dismutase, DTNB method

INTRODUCTION

Age related hearing loss known as Presbycusis is a universal feature of mammalian ageing and refers to sensorineural hearing impairment in elderly individuals. Among community-dwelling elderly people, the prevalence of hearing loss is ≈24% in those aged 65–74 years and ≈40% among those who are 75 years of age. The Molecular mechanism underlying Age related hearing loss is about 24%. Along with heart problems, hypertension and arthritis the hearing impairment is one of the four leading chronic health conditions experienced by the elderly people.[1,2]

It is characterised by an age dependent decline of auditory function associated with loss of sensory hair cells, spiral ganglion neurons and stria vascularis cells in the cochlea of inner ear.[3] It is also associated with difficulty in speech discrimination and central auditory processing of information.

Hearing loss occurs when the tiny hairs inside the ear are damaged or die. The hair cells do not re-grow, so most hearing loss is permanent. Stria vascularis is the highly vascular tissue in the lateral wall of the cochlea. The spiral or cochlear ganglion is the group of nerve cells that serve the sense of hearing by sending a representation of

sound from the cochlea to the brain. Degeneration of cochlear sensory neurons is an important cause of hearing loss, but the mechanisms that maintain the survival of adult cochlear sensory neurons are not clearly defined.

There is strong evidence that increased generation of free radicals during cellular metabolism plays a major role in age related disorders.[4] A free radical is a molecule or molecular fragment that contains one or more unpaired electrons in its outer orbital. Important characteristics of free radicals are Extreme reactivity, Short life span, Generation of new free radicals and Damage to various tissues.

Oxidative stress has also been implicated in Presbycusis by reports of increased susceptibility of superoxide dismutase. Oxidative stress represents an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA . The oxidative stress theory of ageing offers the best mechanistic elucidation of the ageing phenomenon and other age related diseases. The occurrence of age related hearing loss depends mainly on antioxidant status of the body. Other possible causes are dismutation of superoxide, catalase etc. which truly participate in controlling the ill effects caused by reactive oxygen species.[5-8]

Glutathione (GSH) is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced (active) forms. It is a tripeptide with an unusual peptide linkage between the amine group of cysteine, which is attached by normal peptide linkage to a glycine) and the carboxyl group of the glutamate side-chain. It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides.[9] Superoxide dismutase is one of the main reactive oxygen species in the cell. Superoxide dismutase's (SOD) are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. Thus, they are an important antioxidant defence in nearly all cells exposed to oxygen.[10]

But as per the literature, till now data were not well documented on the effect of Glutathione, and superoxide dismutase in age related hearing loss. Hence, this study was undertaken to investigate the status of these antioxidants in age related hearing loss.

METHODS

This study was conducted after the approval from institutional ethical committee. The study group included 25 patients reporting to the Speech and Hearing department diagnosed for age related hearing loss between the age group of 55-80 years of either sex and 25 healthy age and sex matched individuals as controls. An informed consent was taken duly signed by each participant. Patients who were diagnosed for other systemic or metabolic disorders and those who are not willing to participate in the study were excluded.

Under aseptic precautions blood samples approximately 5ml was collected without anticoagulant in appropriate sterile vials by venous arm puncture. The serum was separated by centrifugation at 1500 rpm for 15 minutes and stored at 4^0C.

Estimation of Glutathione was done by DTNB method.[11] 500 µL of whole blood anticoagulated with fluoride is centrifuged and 100 µL of the erythrocytes are taken and diluted to 1 mL with distilled water. (Dilution 1:10). The diluted samples are treated with 1.5 mL of precipitating solution and kept for 10 minutes for the precipitation to complete. The solutions are then filtered through a whatmann No.1 filter paper. 500 µL of the filtrate is taken and to this 2 mL of phosphate solution and 250 µL of DTNB solution is added. Simultaneously a blank is maintained containing 200 µL of distilled water, 300 µL of precipitating solution, 2 mL of phosphate solution and 250 µL of DTNB. The intensity of the yellow colour formed is spectrophotometrically read immediately (within ten minutes) at 412 nm against the blank. The optical densities obtained are plotted against the standard graph. The concentration of glutathione is calculated graphically and multiplied with the respective dilution factors and the total glutathione in the sample is expressed as µg/mL.

Estimation of Superoxide Dismutase:[12] 500 µL of drawn heparinised blood is centrifuged at1800rpm for ten minutes and the upper plasma layer is separated out, and about 500µL of normal saline is added to the erythrocyte layer, mixed well and centrifuged, again discarding the upper layer and adding fresh normal saline to the erythrocytes, this step is repeated two more times in order to wash the erythrocytes. Finally 200µLof the washed erythrocytes is taken and lysed with 600 µL of cold distilled water to lyse the erythrocytes and release the SOD enzyme contained. The thus prepared 1:4 diluted erythrocytes can be stored at 0-4^o C until analysed. The test was performed according to the manufacturer's guidelines.

Statistical Analysis

The data was analysed using One Way ANOVA for statistical significance using Graph Pad Prism software. P value less than 0.05 will be considered the level of significance.

RESULTS

In the present study, 25 patients reporting to the Speech and Hearing department who are diagnosed for age

related hearing loss between the age group of 55-80 years of either sex 25 healthy age and sex matched individuals used. The results showed that, the Glutathione level in Hearing Loss patients was increased significantly (p=0.0001) as compared to normal controls (Table 1, Figure 1). The results of SOD Activity has shown that, it was declined significantly (p=0.001) in Hearing Loss patients as compared to normal controls (Table 1, Figure 2).

Table 1: The level of glutathione and SOD activity in normal individuals and hearing loss patients. Values are expressed as mean ± standard deviation. N=25 in each group.

Parameters	Groups		
	Normal	Hearing Loss	P Value
Glutathione (µg/mL)	248.955±67.311	342.568±88.051	0.0001
SOD Activity (U/g Hb)	48.321±10.395	44.627±10.978	0.001

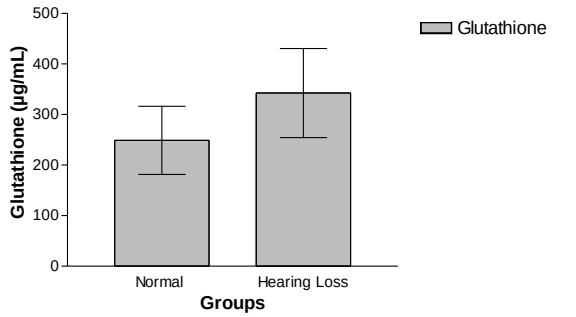

Figure 1: The glutathione level in normal individuals and hearing loss patients. N=25 in each group. The glutathione level was increased significantly (p=0.0001) in hearing loss patients as compared to normal controls.

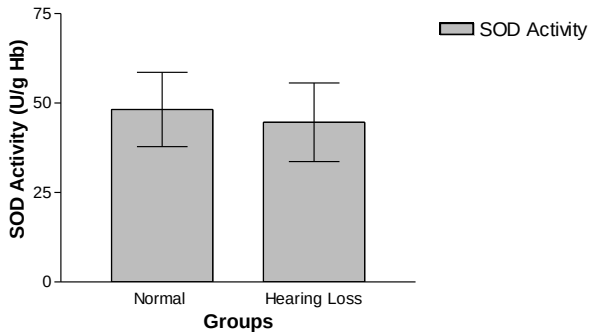

Figure 2: The SOD activity in normal individuals and hearing loss patients. n=25 in each group. The SOD activity was declined significantly (p=0.001) in hearing loss patients as compared to normal controls.

DISCUSSION

Aging is associated with a wide array of physiological, biochemical, and molecular changes including progressive DNA damage, deterioration of mitochondrial function, and reduction of cellular water concentrations, ionic changes, vascular insufficiency, and decreased elasticity of cellular membranes.

Age-related hearing loss, known as *presbyacusis*, is characterized by the progressive deterioration of auditory sensitivity associated with aging and is the most common cause of adult auditory deficiency in the United States. Experimental evidence suggests that mitochondrial dysfunction associated with reactive oxygen species (ROS) plays a central role in the aging process of cochlear cells.[13]

This was evidenced in the present study, in which we observed a significant increase in the level of Glutathione and a slight but significant decline in the SOD activity. A very high increase in the level of one antioxidant might have suppressed the activity of another antioxidant, SOD in the present study. But, there was an overall increase in the antioxidant status. The increase in the antioxidant status recorded in the present study clearly indicates the increased oxidative stress.

It is clear that in the aging cochlea there is a significant reduction of blood supply and the ongoing need for energy generation through oxidative phosphorylation.[14] Thus, these two processes, as well as others, allow for the generation of ROS within the cochlea.

Among the various mechanisms that are postulated to result in age-related hearing loss in the current literature, the one that is arguably the most intriguing, is the membrane hypothesis of aging (MHA), also known as the mitochondrial clock theory of aging, which states that with aging, hypo-perfusion of the cochlear tissue occurs, leading to ischemia and the formation of ROS. These species are highly toxic substances that adversely affect the auditory neuro-epithelia. More specifically, these ROS damage mitochondrial DNA (mtDNA), resulting in both the production of specific mtDNA deletions, as well as reducing the mitochondrial membrane potential.[15] However, in this study we tried to exclude the confounding variables and results obtained can be taken as the standard normal in our environment.

CONCLUSION

The significant increase in the level of Glutathione and significant decline in the SOD activity observed in the present study illustrates the increased oxidative stress. A very high increase in the level of one antioxidant (Glutathione) might have suppressed the activity of another antioxidant (SOD). Since there is an association between the level of Glutathione and Super Oxide Dismutase in age related hearing loss, these parameters

can be used as a biomarker for the assessment of age related hearing loss. With a thorough investigation on the level of antioxidant enzymes, it is possible to monitor from such complications of age related hearing loss.

Conflict of interest: None declared
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Ethical approval: The study was approved by the Institutional Ethics Committee

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