Research Report

The correlation between plasma reactive oxygen species and hearing threshold levels in presbycusis patients

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ABSTRACT

Background: Presbycusis is a hearing loss due to the degeneration process in elderly people of 65 years old and beyond, characterized by a decrease in hearing sensitivity in both ears. Reactive oxygen species (ROS) are assumed to have an important role in the pathogenesis of presbycusis. **Purpose:** To find out the correlation between ROS levels in plasma and hearing threshold levels in presbycusis patients. **Method:** The design was cross sectional and conducted at the Outpatient Clinic of Neurotology Division, Geriatric Clinic, Clinical Pathology Installation, and Central Installation of Biomaterials Network Bank of Dr. Soetomo Hospital. Samples were taken by consecutive sampling for audiometric examination and measurement of ROS levels in plasma. **Results:** Fifty samples were collected**,** ROS levels in plasma were found with mean (SD) of 2.46 ng/ml (0.84). The mean hearing threshold level was 47.70 dB (12.42). The Pearson statistical test revealed a significant correlation between ROS levels in plasma and hearing threshold levels, with p=0.003 (p<0.05), and the correlation coefficient (r) 0.41. **Conclusion:** There was a correlation between ROS in plasma and hearing threshold levels in presbycusis patients, with a significant moderate-positive correlation pattern. It indicated that the higher the ROS levels in plasma, the higher the hearing threshold levels in presbycusis patients.

Keywords: presbycusis, reactive oxygen species, hearing threshold, geriatric

ABSTRAK

Latar belakang: *Presbikusis adalah gangguan pendengaran akibat proses degenerasi yang dijumpai pada usia 65 tahun atau lebih, ditandai oleh penurunan kepekaan pendengaran pada kedua telinga.* Reactive oxygen species *(ROS) diduga mempunyai peran penting pada patogenesis presbikusis.* ***Tujuan:*** *Untuk mengetahui hubungan antara kadar ROS dalam plasma dengan nilai ambang dengar pada penderita presbikusis.* ***Metode:*** *Penelitian ini adalah* cross sectional*, dilakukan di Unit Rawat Jalan (URJ) THT-KL Divisi Neurotologi, URJ Geriatri, Instalasi Patologi Klinik, dan Instalasi Pusat Biomaterial Bank Jaringan RSUD Dr. Soetomo. Sampel diambil secara* consecutive sampling*. Diperoleh 50 sampel untuk dilakukan pemeriksaan audiometri dan pengukuran kadar ROS dalam plasma.* ***Hasil:*** *Kadar ROS dalam plasma didapatkan hasil rerata (SD) 2,46 ng/ml (0,84). Nilai ambang dengar didapatkan hasil rerata (SD) 47,70 dB (12,42). Hasil uji statistik dengan korelasi Pearson terhadap kadar ROS dalam plasma dan nilai ambang dengar didapatkan hasil koefisien korelasi (r) sebesar 0,41 dan p=0,003 (p<0,05).* ***Kesimpulan:*** *Terdapat hubungan antara kadar ROS dalam plasma dengan nilai ambang dengar pada penderita presbikusis dengan pola hubungan bersifat positif-sedang yang signifikan, yang berarti semakin tinggi kadar ROS dalam plasma, semakin tinggi nilai ambang dengar.*

Kata kunci: *presbikusis,* reactive oxygen species*, nilai ambang dengar, geriatri*

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INTRODUCTION

Presbycusis is a hearing loss due to degeneration process, found in elderly people of 65 years old or more, characterized by decrease of hearing sensitivity in both ears. The results of the audiogram of presbycusis showed an increase in hearing threshold level with a symmetrical type of bilateral sensorineural hearing loss.[1] The prevalence of presbycusis increases with age. Prevention efforts are expected to reduce the prevalence of presbycusis. Some of the preventions are carried out by limiting calorie intake, providing magnesium nutrition, and antioxidants. Restrictions on calorie intake can reduce oxidative stress, reduce metabolic rates and reduce dietary fat. Magnesium is a vasodilator that can increase blood flow into the cochlea. Some substances which are contained in plants act as anti-aging factor. The prevention efforts mentioned above, could reach maximum results. Giving antioxidants is one of the prevention efforts of presbycusis which shows significant results in some studies.[2,3]

The pathogenesis of presbycusis is not clearly known. Reactive oxygen species (ROS) are assumed to have an important role in the pathogenesis of presbycusis. The knowledge of the role of ROS in the pathogenesis of presbycusis is still very limited.[3] An evidence that oral ACE Mg preserves hearing in an animal model of accelerated age releated hearing loss (ARHL), was shown by improved thresholds and amplitudes in ABR recordings. The oral combination of antioxidant vitamins (A, C, and E) could reduce oxidative stress by scavenging free radicals plus the vasodilator Mg++, to increase strial blood flow, and to produce a clear protective effect of auditory function in an animal model of ARHL. Other study in human also support this evidence, plasma ROS levels were associated with severity of age-related hearing impairment in humans. [4,5]

Risk factors of presbycusis include genetics, aging process, degenerative diseases, and environment. Inherited genetic factors in hearing loss are 35-55%. The gene known to be associated with oxidative stress due to ROS and atherosclerosis is GSTT1 and GSTM1, which are genes that play a role in the formation of glutathione-related antioxidant enzymes, i.e. glutathione S-transferase. Degenerative diseases that increase the risk of presbycusis are cardiovascular diseases (hypertension, atherosclerosis, dyslipidemia), diabetes mellitus and chronic kidney disease. Environmental factors that increase the risk of presbycusis are noise exposure and ototoxic ingredients and drugs. These factors can increase ROS production which causes oxidative stress in the cochlea.[3,4]

Oxidative stress causes mitochondrial DNA mutations, damaging the polyunsaturated fatty acids in cell membranes and damaging nucleic acids. Damage to these cell components causes cell apoptosis. Apoptosis of vascular stria cells, outer hair cells and neurons of spiral ganglia can cause cochlear dysfunction. Cochlear dysfunction causes hearing loss marked by an increase in hearing threshold level.[3] Knowledge of the role of ROS in the pathogenesis of presbycusis is needed as the basic for prevention efforts. Presbycusis prevention is executed by avoiding factors that increase ROS production, and by administrating antioxidant.[6]

Concerning with the above issue, it is necessary to conduct a study to find out the

correlation between ROS levels in plasma and hearing threshold levels in presbycusis patients who came to the Outpatient Clinic of Neurotology Division of Dr. Soetomo, Surabaya. The purpose of this study is to find out the correlation between ROS levels in plasma and hearing threshold levels in presbycusis patients.

METHOD

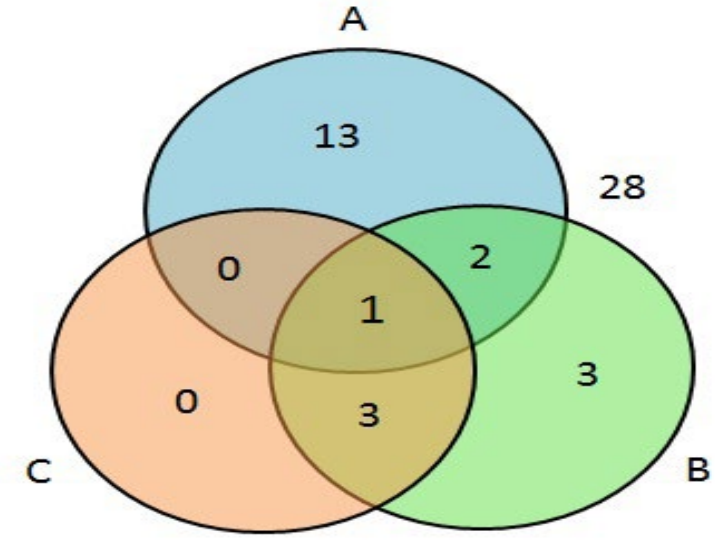
This was an analytic observational study with cross sectional approach. The research was carried out at Outpatient Clinics of Neurotology Division, Geriatric Clinic, Clinical Pathology Installation, and Installation of the Biomaterials Network Bank of Dr. Soetomo Hospital, Surabaya. Research samples were presbycusis patients who came to Outpatient Clinic of Neurotology Division and Geriatric Clinic of Dr. Soetomo Hospital, Surabaya, and meet the research criteria. The acceptance criteria (inclusion) of this study were patients aged 65 years or older with a decrease in auditory sensitivity in both ears which was indicated by the audiogram results showing symmetrical bilateral sensorineural hearing loss, willingness to be examined and signed *informed consent*. The criteria for rejection (exclusion) included external and middle ear diseases, cognitive impairment (difficulty in communicating) and neurological or psychiatric disorders. The sample size were 50 presbycusis patients. The research samples were taken by consecutive sampling. The stages of this study began with the diagnosis of presbycusis patients established by doctors who served in the Outpatient Clinic of Neurotology Division and Geriatric Clinic, Dr. Soetomo Hospital, Surabaya. The prerequisites were based on history, physical examination and pure tone audiometry, and the results were then recorded and reported to the researchers. Patients who were willing to join the study were asked to sign informed consent. The researcher calculated the hearing threshold level from the audiogram. The chosen hearing threshold level was taken from the worse ear. Blood sample was taken from the cubiti median vein blood vessel as much as 2 ml, by a medical analyst officer. The blood sample was centrifuged at 1000 rpm for 15 minutes. The supernatant was sent to the Biomaterials Network Installation Center, Dr. Soetomo Hospital, Surabaya, to be stored in a refrigerator at -80°C, until all 50 samples were collected. Measurement and reading of ROS levels in plasma were carried out by a senior Clinical Pathology Specialist (consultant) at the Clinical Pathology Installation of Dr. Soetomo Hospital Surabaya. The measurement results of ROS levels in plasma were written in ng/ml units.

The obtained data were then statistically analyzed. The correlation between ROS levels in plasma and hearing threshold levels in presbycusis patients was analyzed using Pearson statistical tests. ROS levels in plasma and hearing threshold levels were assessed as ratio-scale variables with significant level (α)=0.05.

RESULTS

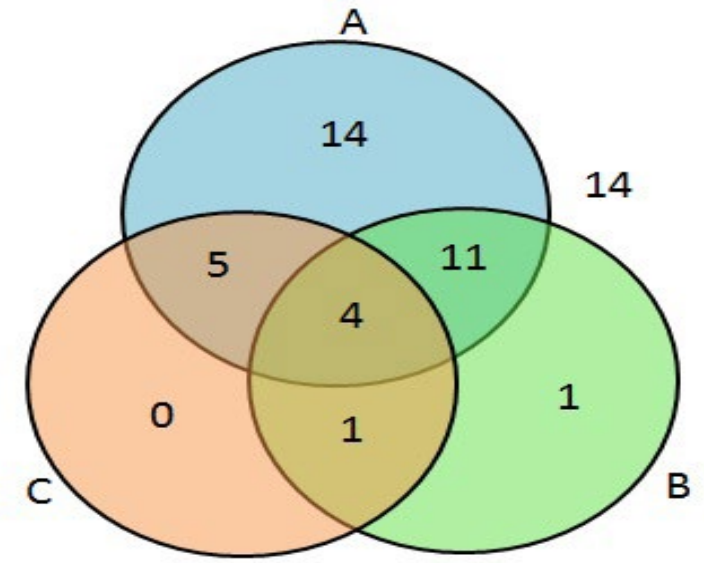
The descriptive data in this study included age, gender, education level, type of work, complaints, family history of hearing loss, environmental factors and degenerative diseases. The youngest was 65 years old and the oldest was 83 years with a mean (SD) 71.78 (4.76). The majority of patients were in the age range of 70-74 years old as many as 22 patients (44%). The majority of patients were 30 females (60%), male were 20 patients (40%). The education level of majority of the patients was high school as many as 26 patients (52%). The highest number of occupations were civil servant retirees as many as 31 patients (62%), and the least number was 1 retired Army and 1 retired Police officers (2 %).

Patient's complaint distribution was shown on Figure 1.



Keterangan
A: hearing loss
B: slow to understand
C: ringing ear

Figure 1 . Venn Diagram of patient's complaints
A: hearing loss, B: slow to understand, C: ringing ear



Keterangan
A: Noise
B: Smoking
C: Alcohol

Figure 2 . Venn Diagram of environmental risk factors
A: Noise, B: Smoking, C: Alcohol

The majority of patients, 36 subjects (72%) came with hearing complaints. The highest complaint was hearing loss in 34 patients (68%), slow to understand conversation in 17 patients (34%), and ringing in the ears as many as 10 patients (20%).

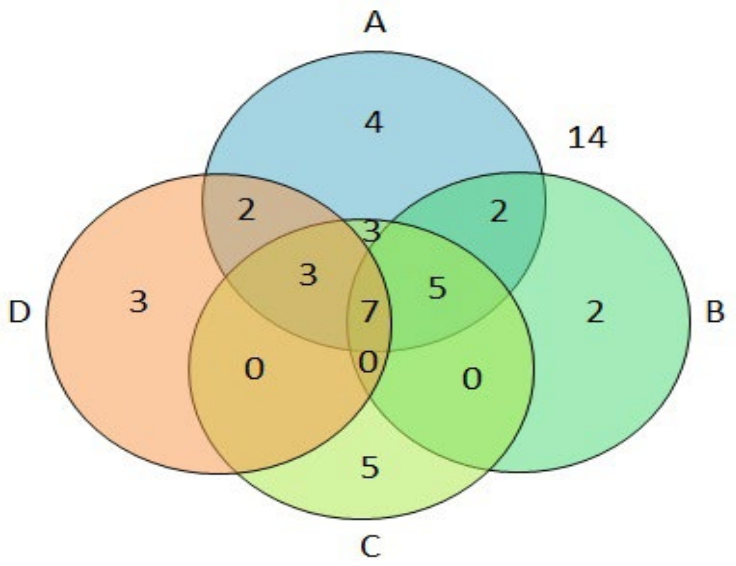
Most of the patients had no history of family hearing loss i.e. 37 patients (74%), while patients with a family history of hearing loss were 13 (26%).

Distribution of patient's environmental risk factors was shown on Figure 2.

Most of the patients in our study had no environmental risk factor i.e. 28 patients (56 %). The highest number of environmental risk factor was noise exposure in 16 patients (32 %), followed by smoking in 9 patients (18%), and alcohol drinking in 4 patients (8%).

Distribution of risk factors for degenerative diseases was shown in Figure 3.

From all patients in our study, there were 36 (72%) who had degenerative diseases risk factors. The highest risk factor



Keterangan
A: Hypertension
B: Dyslipidemia
C: Diabetes Mellitus
D: Coronary Heart Disease

Figure 3 . Venn diagram of risk factors for degenerative diseases
A: Hypertension, B: Dyslipidemia, C: Diabetes Mellitus, D: Coronary Heart Disease

Table 1. Results of measurement of ROS levels in plasma and calculation of hearing threshold level

	ROS (ng/ml)	NAD (dB)	p
N	50	50	0.003*
Mean	2.46	47.70	
Median	2.61	45.50	
SD	0.84	12.42	
Nilai Minimum	0.10	28.00	
Nilai Maksimum	4.29	80.00	

* Significant value ($p < 0.05$)

was hypertension in 26 patients (52%). Diabetes mellitus was in 23 patients (46%), dyslipidaemia in 16 patients (32%), and coronary heart disease in 15 patients (30%).

Data from measurement of ROS levels in plasma and hearing threshold level was shown in table 1.

Measurement level of ROS in plasma showed a minimum value of 0.10 ng/ml and a maximum value of 4.29 ng/ml with a mean (SD) of 2.46 ng/ml (0.84). The calculation of the hearing threshold value showed a minimum value of 28.00 dB and a maximum value of 80.00 dB with average (SD) 47.70 dB (12.42).

Due to the normal distribution data obtained, Pearson correlation test was used to analyse the correlation between ROS levels in plasma and hearing threshold levels. Pearson statistical test revealed a significant correlation between plasma levels of ROS and hearing threshold level (p=0.003) with a moderate positive correlation coefficient (r=0.41). This meant that the higher plasma ROS levels, the higher the hearing threshold level in presbycusis patients.

DISCUSSION

Our study found subjects with the youngest age was 65 years and the oldest was 83 years old with a mean of (SD) 71.78 (4.76). The highest number of patients were in the range of 70-74 years old in 22 subjects (44%). Other study in Taiwan reported that the age range of subjects was 40-77 years with a mean (SD) of 55.4 years (7.8).[5] The

mean age of subjects of our study was higher than the other study because there was a difference in the age limit of subjects on the inclusion criteria of 65 years while in the previous study was 40 years old. Our researchers set the age limit of 65 years old because it fitted the age limit of the presbycusis definition.

The gender distribution found subjects of 30 women (60%), and 20 men (40%). Men have a higher risk of experiencing presbycusis than women. It was assumed that there was a correlation between estrogen hormone receptors and hearing function. Estrogen receptor expression is detected in Reissner membranes, deep hair cells, Hensen and Deiters buffer cells and spiral ganglion neurons. Estrogen receptors function in the regulation of potassium ion homeostasis needed to maintain the function of the cochlea as the auditory organ.[7] Other studies in Taiwan reported that in 302 presbycusis patients, most of the samples were male (60.9%) and female (39.1%).[5] The number of female patients in our study was more than male, which was different from the previous studies, namely more male than female.

Other study in Taiwan reported that in 302 presbycusis patients, most of the samples were male (60.9%), while female were 39.1%.[5] The number of female subjects in our study were higher than male.

The distribution of education levels in our study found that the education level of the majority of subjects was senior high school as many as 26 (52%).

Most of the patients who came to Geriatric Clinic were retired civil servants as many as 31 (62%), with the highest level of education were high school.

The type of work in this study that had a risk factor of hearing loss was one retired Army and one retired Police officers, because there were a history of noise exposure to shotguns (4%).

The distribution of patient's complaints in figure 1 showed that most of the subjects in this study came with hearing complaints as many as 36 (72%). The highest number of complaints were hearing loss in 34 subjects (68%). The most common complaint felt by presbycusis patients was that the hearing sensitivity decrease, slowly progressing in both ears. There was a difficulty in comprehending conversations especially when quickly spoken, and/ or in a noisy environment (*cocktail party deafness*). Pain occurred in the ears when the sound intensity upraised due to an excessive increase of hearing sensitivity above the threshold of hearing *(recruitment)*. Symptoms that often accompanied presbycusis were tinnitus, difficulty in communicating, difficulty of localizing the direction of the sound source, decreased in music appreciation, decreased participation in social activities and depression.[6,8] The most notable complaints of subjects in our study were decreased hearing, this was in accordance with the literature that the complaints most often felt by patients with presbycusis were slowly progressing decrease of hearing sensitivity in both ears.[9]

This study found that in the distribution of family history of hearing loss, 37 subjects (74%) had no family history, while 13 subjects (26%) had history of hearing loss. Inherited genetic predisposing factors of hearing loss were 35-55%. Genetic factors had an important role especially in strial type presbycusis which were characterized by an increase in low frequency hearing thresholds.[1,3] The number of patients with a family history of hearing loss in our study was 26%, which were lower than the genetic factor in the literature (35-55%).

The distribution of environmental risk factor shown in figure 2 found that the majority of subjects in our study had no environmental risk factors i.e. 28

(56 %). The highest risk environmental factor was noise exposure, found in 16 subjects (32 %). Noise exposure included the sound of motorized vehicles, factory engine uproar, blaring music sound, aircraft engine noise, and shotgun explosion. Noise exposure is a major environmental factor that caused hearing loss due to mechanical and metabolic effects. The mechanical effect of noise exposure are in the form of stereocilia end-damage, filamentous protein-damage, actin stereocilia-damage, and reduced stiffness of outer hair cells. The metabolic effects of noise exposure are changes in calcium homeostasis. Continuous noise exposure causes prolonged stereocilia deflection and accumulation of calcium in mitochondria. Calcium plays a role in the stimulation of the respiration chain and activation of the enzyme production of *nitric oxide* (NO) and ROS.[6] Our study found risk factor of noise exposure was higher than smoking and alcohol consumption. This was consistent with the literature that noise exposure is a major environmental factor causing hearing loss.

Distribution of risk factors for degenerative diseases in figure 3 showed that the majority of subjects which had risk factors for degenerative diseases was as many as 36 (72%). The highest risk factor for degenerative disease in our study was hypertension as many as 26 subjects (52%). Some diseases that were risk factors for presbycusis were cardiovascular disorders (hypertension, atherosclerosis and dyslipidemia), diabetes mellitus and chronic kidney disease. Degenerative disorders caused disruption of blood flow to the cochlea resulting in hypoxic conditions, which caused an increase in ROS production and resulting in oxidative stress.[4] A research in Taiwan stated that the highest risk factor for degenerative diseases in presbycusis patients was hypertension as many as 15.9%.[5] In our study the highest risk factor of degenerative diseases for most subjects was hypertension, which was in accordance with the previous study.

Table 1 showed the measurement results of ROS levels in patients' plasma had a minimum value of 0.10 ng/ml and a maximum value of 4.29 ng/ml, with a mean (SD) of 2.46 ng/ml (0.84). Other study in Taiwan measured ROS levels in plasma of presbycusis patients with two luminometry techniques, i.e. *luminol-dependent chemiluminescence* (LmCL) and *lucigenin-dependent chemi-luminescence* (LcCL). With LmCL the measurement results produced a mean (SD) of 101.8 (165.0) counts per ten second, and with LcCL the results showed a mean (SD) of 1088.7 (789.4) counts per ten seconds.[5] In our study, the measurement result of ROS levels in plasma were different from the previous study due to dissimilar techniques.

The calculation results of patients' hearing threshold shown in table 1, exhibited a minimum value of 28.00 dB and a maximum value of 80.00 dB, with an average (SD) of 47.70 dB (12.42). Other study in Taiwan reported a mean (SD) of low frequency hearing threshold levels (250 Hz, 500Hz, and 1 kHz) of 17.2 dB (8.6), and of high frequency (2 kHz, 4 kHz, and 8 kHz) of 27, 4 dB (16.9).[5]

The results of the hearing threshold level in our study were higher than the previous study because there were differences in sample inclusion criteria which included subjects with normal audiogram results in Taiwan's study samples.

The hearing threshold level in our study was calculated from the mean of the hearing threshold in frequency of 500 Hz, 1 kHz, 2 kHz, 4kHz, and 8 kHz because in presbycusis the hearing threshold increased more often in high frequencies.

To analyse the correlation between ROS levels in plasma and hearing threshold levels, our study employed Pearson correlation test

because the data were ratio-scale. The data normality test showed a normal distribution. The statistical test results revealed a significant correlation between levels of ROS in plasma and hearing threshold levels (p=0.003), with a coefficient moderate positive (r=0.41). This meant that the higher plasma ROS levels, the higher the hearing threshold levels in presbycusis patients. Positive correlation meant that the correlation between two variables was unidirectional. The magnitude of the correlation coefficient (r) showed the strength of the correlation between variables.[10]

The research in Taiwan aimed to analyze the correlation between ROS levels in plasma and hearing threshold levels in presbycusis patients. The analysis method was multivariate linear regression. The variables consisted of ROS levels measured by LmCL and LcCL and hearing threshold levels that were calculated by differentiating low and high frequencies. The results showed that there was a significant positive correlation between ROS levels in plasma and hearing threshold level at low frequencies and high frequencies.[5]

The results of our study reinforced the presumption that there were a correlation between ROS levels in plasma and hearing threshold levels in presbycusis patients.

Reactive oxygen species (ROS) are compounds that have unpaired electrons, which are chemically reactive and toxic to cells and intracellular structures. ROS types include superoxide anions (O_2-), hydroxyl radicals (OH^-), hydrogen peroxide (H_2O_2) and hypochlorite acid (HOCl/ OCl). Superoxide anions (O_2-) are very unstable. Hydroxyl radical (OH^-) is very unstable and it can damage all types of human macromolecules, while hydrogen peroxide (H_2O_2) is relatively stable, relatively live longer, and can diffuse freely through cell membranes.[3] In our study the measurement of ROS was total, without differentiating the types, adjusting with the available tools and reagents in Clinical Pathology Installation, Dr. Soetomo Hospital.

Reactive oxygen species are the result of various metabolic pathways through the partial reduction of oxygen molecules. Mitochondria are the main source of ROS production from respiratory metabolism during ATP formation. High ROS levels in the cochlea cause a condition of oxidative stress which is a major role in the pathogenesis of presbycusis.[3,11] Risk factors for presbycusis include genetic, aging, degenerative and environmental diseases. These factors can increase ROS production which causes oxidative stress in the cochlea. Oxidative stress is a stressful condition in cells due to an imbalance between the increasing amount of ROS production and the decreasing antioxidant system.[12]

Oxidative stress in the cochlea causes macromolecular damage that can ruin the main cell components i.e. core *deoxyribonucleic acid* (DNA), mtDNA, membranes and proteins. Oxidative stress in mitochondria causes mitochondrial dysfunction, which in turn causes a decrease in ATP synthesis and increase ROS production, which results in cells apoptosis.[13] Oxidative stress on cell membranes causes peroxidative reactions of proteins and lipids, which produce polyunsaturated fatty acids, which also result in cell apoptosis. Oxidative stress in the nucleus causes mutations in the core of DNA, which will activate p53 factors, which result in cell apoptosis. Cell apoptosis causes cochlear dysfunction. Cochlear dysfunction causes hearing loss. The result of audiometric examination showed an increase in hearing threshold levels, with a symmetric type of bilateral sensorineural hearing loss.[14]

Our research had several limitations. ROS levels in plasma could be affected by other unknown conditions such an inflammatory process, history of consuming antioxidants, and controlled comorbid diseases (hypertension, diabetes mellitus,

dyslipidemia). In our study, the ROS levels measured were plasma levels. Measurement of ROS levels in the cochlea were not possible due to ethical constraints. The disadvantage of the spectrophotometric technique for measuring ROS levels was that it had lower sensitivity and specificity than luminometry.

The results of our study will further reinforce the role of ROS in the pathogenesis of hearing loss due to cochlear dysfunction in presbycusis patients. The outcome of this research could be used to support the prevention of presbycusis by avoiding risk factors which can increase ROS production, and by advocating antioxidant intake, which were relevant with the previous study.[15]

Our study found that there was a correlation between ROS levels in plasma and hearing threshold levels in presbycusis patients with a significant moderate-positive to positive correlation pattern (r=0.41), which meant that the higher the ROS level in plasma, the higher the hearing threshold level. Further research should be carried out to compare ROS levels in plasma and hearing threshold levels of subjects given antioxidants compared to controls. Also, another study is suggested to compare ROS levels in plasma and in the cochlear tissue of experimental animals.

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