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**ABSTRACT**

**Background:** Presbycusis is a gradual hearing loss caused by the ageing process. This is a chronic condition that affects the elderly population and sensorineural progressive bilateral symmetry occurs with predominantly high-frequency hearing loss. The ability to discriminate speech decreases; hence, most of the affected patients have conversation problems, especially in noisy environments. This situation is a serious problem among elderly individuals. Social isolation, depression, and paranoia can be related to presbycusis.

**Objective:** The aim of this study was to investigate GPx and the GSH:GSSG ratio as risk factors for presbycusis.

**Methods:** A case-control study was conducted to determine the role of GPx activity with the GSH:GSSG ratio as a presbycusis risk factor in 60 subjects aged 55 to 75 years old during the period of August 2012 - April 2014. All of the subjects passed an ENT examination, pure tone audiometry, and tympanometry. The activity of GPx was measured with the Paglia and Valentine method, and the GSH:GSSG ratio was measured by the calorimetric method.

**Results:** The activity of GPx and the GSH:GSSG ratio were significantly different between the groups (p<0.05), and the odds ratio for high GPx with a low GSH:GSSG ratio was 135 (CI 95%: 5.17–20,028.88).

**Conclusion:** High GPx activity with a low GSH:GSSG ratio is a risk factor for presbycusis.

**Keywords:** Glutathione peroxidase (GPx), glutathione to disulfide glutathione ratio (GSH:GSSG), oxidative stress, presbycusis.

1. **BACKGROUND**

The World Health Organization (WHO) has already made recommendations for developing countries regarding the priority of treating hearing loss, including hearing loss caused by chronic otitis media, noise, ototoxic drugs, congenital conditions, and hearing loss during ageing (presbycusis) (1).

Presbycusis is a gradual hearing loss caused by the ageing process (2). This is a chronic condition that affects the elderly population (1) and sensorineural progressive bilateral symmetry occurs with predominantly high-frequency hearing loss. The ability to discriminate speech decreases; hence, most of the affected patients have conversation problems, especially in noisy environments (1). This situation is a serious problem among elderly individuals. Social isolation, depression, and paranoia can be related to presbycusis (3).

The free radical theory postulated by Harman is the most widely hypothesized theory explaining the ageing process, and this theory is also believed to be the basis of hearing loss during ageing (presbycusis) (4). This theory states that ageing is based on progressive oxidative processes as a consequence of damage by free radicals at the cellular level and an imbalance between free radical production and defence against antioxidants, which would induce damage to the cell structure and integrity. Mitochondria, as a cell’s energy resources, are the primary source of free radicals. The primary target of free radicals is unsaturated fatty acids on cell membranes, which cause fat peroxidation and malondialdehyde formation and could damage the structure and function of the cell nuclei. In addition, these products could induce mitochondrial DNA mutations, and these mutations are related to cellular and tissue dysfunction and death (5). The direct defence mechanism against free radicals is antioxidative defence. This mechanism protects cell membranes from oxidative damage, and the primary target of free radicals is unsaturated fatty acids on cell membranes, which cause fat peroxidation and malondialdehyde formation and could damage the structure and function of the cell nuclei. In addition, these products could induce mitochondrial DNA mutations, and these mutations are related to cellular and tissue dysfunction and death (5). The direct defence mechanism against free radicals eliminates free radicals or converts the aggressive radicals to less aggressive forms (6) which are both accomplished by antioxidants. In the cochlea, free radicals are controlled by superoxide dismutase (SOD), catalase.
Glutathione Peroxidase and Glutathione to Disulfide Glutathione Ratio in Presbycusis: a Case-control Study

2. OBJECTIVE

The aim of this study was to investigate GPx and the GSH:GSSG ratio as risk factors for presbycusis.

3. MATERIAL AND METHODS

A case-control study was conducted from August 2012 to April 2014. The subjects were members of elderly organizations in Bandung city.

The inclusion criteria for case group were subjects aged 55 to 75 years old who had bilateral intact tympanic membranes, type A tympanometry, sensory or metabolic type presbycusis, and were in a good enough condition to perform daily activities, while the exclusion criteria were subjects who had ear abnormalities and disorders, a history of noise exposure, ear surgery, ototoxic drug consumption within the last 6 months, hearing loss since a younger age, head trauma, deafness in their family history, chronic neurological diseases, and diabetes mellitus. The control group were subjects without presbycusis. All subjects had no history of heart diseases, kidney disorders, liver diseases, diabetes mellitus, malignancy, or ear surgery and had not suffered from acute or chronic infection within the last 6 months.

Based on the required sample size formula, 30 subjects for each group were selected by consecutive sampling. The independent variables were erythrocyte glutathione peroxidase (eGPx/cGPx/GPx1) and the GSH:GSSG ratio, with presbycusis as the dependent variable, and the confounding variables were sex, age, nutritional status, blood glucose level, and smoking behaviour.

The hearing threshold was examined according to a standard protocol with a pure tone audiometric examination. The average hearing threshold was calculated at frequencies of 500, 1,000, 2,000, and 4,000 Hz. A hearing loss diagnosis was established when the ear’s hearing threshold was > 30 dB. Blood samples were collected using EDTA anticoagulants. Glutathione peroxidase was examined according to the Paglia and Valentine method (15), while the GSH:GSSG ratio was examined using the Ellman reagent by spectrophotometry at 412 nm (16). GPx activity and the GSH:GSSG ratio between the two groups were compared using the Mann–Whitney test. The extent of the risk was calculated by determining the odds ratio, the significance was determined by the 95% confidence interval and a p value < 0.05. This study was conducted after obtaining approval from the Health
An in Depth Look Into Intracranial Abscesses and Empyemas: a Ten-year Experience in a Single Institute

Research Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran.

2.1. Subject Characteristics
Subjects in the two groups were assessed based on 5 characteristics that could also act as confounding variables (Table 1). According to Table 1, both groups could be considered homogeneous.

3.2. Relationship between eGPx activity and the GSH:GSSG ratio with presbycusis
The cut-off points were obtained through the receiver operating characteristic (ROC) curve. It was > 152.8 mU/mL for eGPx activity, and for the GSH:GSSG ratio, it was ≤ 376.26. The relationship between eGPx activity and the GSH:GSSG ratio with presbycusis was calculated with a chi-squared test (the cut-off point value was used as a boundary value), as shown in Table 2. Table 2 shows that the activity of eGPx was significantly associated with presbycusis (p <0.001), with an odds ratio of 14.0 (95% CI: 2.82–69.56). Additionally, the ratio of GSH:GSSG had a significant relationship with presbycusis (p = 0.003), with an odds ratio of 14.5 (95% CI: 1.72–122.39). To determine the relationship between the activity of eGPx and the GSH:GSSG ratio simultaneously with presbycusis, the calculations shown in Table 3 were performed. Table 3 shows that the combination of high eGPx activity with a low GSH:GSSG ratio has a significant relationship with presbycusis as determined by an OR value of 135 (CI 95%: 5.17–20,028.88), indicating that every individual with high eGPC activity and a low GSH:GSSG ratio was 135 times more likely to have presbycusis than individuals with low eGPx activity and a high GSH:GSSG ratio.

5. DISCUSSION
 Globally, the elderly population has recently increased, so the prevention of ageing-related diseases needs to be promoted by enhancing our understanding of the ageing process (17). Better molecular understanding is fundamental for developing treatments to reduce oxidative stress in the auditory system.

4.1. Subject Characteristics
Sex, age, body mass index (BMI), blood glucose levels, and smoking behaviour did not differ statistically. Based on Table 1, presbycusis affects women more than men, but gender does not statistically affect the incidence of presbycusis (p = 0.488). These studies had the same outcome as studies in America, which revealed that there was no relationship between gender and presbycusis (18), and studies in Norway found that the hearing threshold at a high frequency did not differ by sex (19).

A higher BMI is associated with dyslipidaemia, which will increase the risk of atherosclerosis. In the ear, this condition will cause hypoperfusion, which increases

Table 1 Subject Characteristics Notes * Fisher’s Exact Test ** t-test

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4. RESULTS

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systemic oxidative stress and ultimately reduces hearing ability (20). Health conditions that adversely affect the circulatory system, such as diabetes mellitus and hypercholesterolemia, could affect the cochlea by decreasing nutrient transport due to changes in blood vessels and indirectly decreasing blood flow, resulting in secondary degeneration of the auditory nerve (21).

Smoking increases carboxyhemoglobin in the blood, which decreases oxygen that could be used by the cells. This condition will reduce the energy available to the cochlea and increase the damage to sensory cells. Nicotine also contributes to hearing loss by promoting atherosclerosis of the arteries (22).

4.2. eGPx Activity as a Risk Factor of Presbycusis

Glutathione peroxidase is an antioxidant enzyme that plays a significant role in glutathione metabolism and it is essential for maintaining mitochondrial viability. Mitochondria are abundant in the outer and inner hair cells and the stria vascularis. The outer and inner hair cells in the organ of Corti and cells in the stria vascularis have very high activity; therefore, these cells require much energy to be produced by the mitochondria. High production of free radicals causes oxidative stress. To overcome this condition, many antioxidants are required to reduce or avoid mitochondrial and cell damage.

The ageing process based on membrane hypotheses or the mitochondrial clock theory states that ageing is related to decreased effectiveness of cellular protection and secondary repair mechanism from damage caused by free radicals, which causes lipid peroxidation, polysaccharides depolymerization, nucleic acids damage, and the oxidation of sulfhydryl groups, finally resulting in enzyme inactivation. In mitochondria, the process causes DNA damage, and its accumulation causes oxidative phosphorylation deficiencies and produces bioenergy-deficient cells; therefore, this hypothesis states that cellular membrane structure damage induced by free radicals is the primary mediator of cellular ageing (2, 23, 24). Mitochondria are the producers and targets of reactive oxygen damage. As mitochondria function, free radical production is influenced by their cellular metabolic activity and environmental factors, and free radical formation plays a role in tissue damage during long-term ischaemia, reperfusion, and hypoperfusion phases, similar to what occurs in ageing. It has been proven that during cochlear ageing, there is a decrease in blood flow in the inner ear circulation. This fact supports the free radical theory in ageing as the cause of hearing loss associated with ageing (25). Free radical or reactive oxygen species (ROS) concentrations will increase in almost every essential tissue during ageing, including in the ear and the auditory nervous system, and mitochondrial damage caused by ROS activity increases during ageing. Decreasing mitochondrial function and increasing mitochondrial DNA damage have previously been identified in ageing monkeys; in ageing rat hearts, brains, and livers; and in the muscles of elderly humans (23). A study in Fischer-344 mice and humans observed a relationship between mitochondrial DNA damage and presbycusis (24), and in Fischer-344 mice, presbycusis resulted in high eGPx activity in the cochlear lateral wall and in the organ of Corti (8). A study performed by Coling et al. (8) and Jiang et al. (26) in rats showed that there was an increase in oxidative stress increase during the ageing process that was related to the occurrence of presbycusis. Some studies have shown that presbycusis is related to antioxidants involved in GSH metabolism; therefore, GPx1, as one of the enzymes that plays a role in GSH metabolism, is also related to presbycusis, which is supported by the results of this study where GPx1 activity in presbycusis was found to be significantly higher than in subjects without hearing loss. Literature research has shown obvious differences in antioxidant enzyme activity among healthy individuals, resulting in no standard parameters for clinical use without the "own reference value" of each laboratory. Hubner-Wozniak et al. (27) reported positive correlations between GPx and age. Joziwak and Jasnowska (28) found that GPx was higher in the 65–80 years age group than in the 20–50 years age group. Similar to the theories related to oxidative stress in presbycusis, based on the cut-off point in this study, a significant relationship was found between high GPx1 activity and presbycusis (p <0.001), as shown in Table 2.

4.3. The GSH:GSSG Ratio as a Risk Factor of Presbycusis

Glutathione is a major thiol molecule in mammals that plays a key role in cellular resistance to oxidative and nitrosative damage by eliminating toxic oxidation products and reducing oxidized forms. Measurements of GSH, GSSG, and their relative ratios are indices of oxidation status as biomarkers of oxidative stress status that are sensitive and useful as indicators of the disease risk in humans. Blood and tissue contain concentrations of GSH in millimolar amounts and its oxidized form is in low micromolar concentrations (29) GSH levels decrease with age (30).

The GSH:GSSG ratio shows the large use of NADPH in the GSH and GSSG cycle reactions needed for defence against oxidative stress conditions due to the imbalance between free radical products and cellular defence systems that occur because of high metabolic activity. Thus, this ratio determines the maintenance of several mitochondrial functions, intramitochondrial redox status, calcium homeostasis, and the activity of several mitochondrial enzymes that contain sulfhydryl groups (4, 7). In ageing, a hypoperfusion condition results in high production of free radicals. Cellular glutathione peroxidase, as an intracellular primary antioxidant enzyme, increases its activity under these conditions, and as compensation, there is a decrease in GSH and an increase in GSSG, so the ratio of GSH:GSSG decreases. This was proven in the study by Coling et al. (8), which found that the ratio of GSH:GSSG in the lateral wall of the cochlea of Fisher-344 mice decreased with ageing and was significantly associated with presbycusis events, as well as in studies by Kasapoglu and Ozben (10), which found that ageing in humans correlated with a linear decrease in the ratio of GSH:GSSG. Based on the cut-off point, Table 2 shows that a low ratio of GSH:GSSH was significantly correlated with presbycusis (p = 0.003).

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4.4. Relationship of eGPx Activity and the GSH: GSSG Ratio with Presbycusis

We found that a high eGPx activity accompanied by a low GSH:GSSG ratio will present a 135-fold increased risk of presbycusis (OR = 135; 95% CI: 5.17–20,028.88). The magnitude of the risk from these statistical results reinforces the theory that ageing increases the antioxidant activity of eGPx as compensation for high levels of free radicals. As a result, the GSH concentration decreased followed by an increase in the GSSG concentration, so the ratio of GSH:GSSG decreased. These results indicate that the values of these two parameters in the blood can be considered to reflect their levels in the cochlea, so the serum levels of these two parameters can be used as predictors of presbycusis occurrence.

6. CONCLUSION

High eGPx activity accompanied by a low GSH:GSSG ratio indicates a very strong risk of presbycusis (OR = 135, IK 95%; 5.17–20,028.88). Further research needs to be implemented on gene polymorphisms that play a role in the metabolism of eGPx, GSH and GSSG during ageing. It is necessary to develop presbycusis management and preventative measures, which involves examination of the eGPx and GSH:GSSG ratio and the possibility of giving antioxidants as a therapeutic approach.

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Ethics approval and consent: This study has received ethical approval from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran.

Authors’ contributions: WH performed examination to the subject, analysis, and interpretation of the patient’s data, also was a major contributor in writing the manuscript. All authors had full access to all data in the study ad had final responsibility for the decision on submit for publication.

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