The Effects of Noise on Oxidative and Antioxidative Balance in Human Erythrocytes

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ABSTRACT

Reactive oxygen and nitrogen species have been implicated in the pathogenesis of noise-induced hearing loss. In this case-control study, we investigated the oxidative and antioxidative status of erythrocytes from workers in noisy workplace. Blood samples of 127 workers in noisy workplace (WNW) and 117 workers in non-noisy workplace (WNNW) from the same company were taken into tubes with potassium EDTA as anticoagulant in order to obtain hemolysate. Total superoxide dismutase (SOD) and catalase (CAT) activities as the enzymes of antioxidative defense mechanism in the erythrocytes together with malondialdehyde (MDA) as the lipid peroxidation index and total nitric oxide (NO) as an index for nitrogen species analyses were performed by spectrophotometric methods.

SOD activity was found to be 450.0±106.4 U/g Hb in WNW and 443.1±83.1 U/g Hb in WNNW. The difference between two groups were not statistically significant (p= 0.582). CAT activity was found to be 426.0±98.0 k/g Hb in WNW and 432.6±109.0 k/g Hb in WNNW showing statistically insignificant difference (p= 0.621). MDA levels in erythrocytes from WNW was significantly higher than WNNW (39.28±10.22 nmol/g Hb and 32.51±10.73 nmol/g Hb, respectively and p= 0.0001). On the other hand, NO levels were found to be significantly reduced in WNW (0.275±0.187 μmol/g Hb) compared to WNNW (0.382±0.284 μmol/g Hb) (p= 0.001). When we analyzed the hematological parameters, all the cell counts increased in WNW except monocytes and platelets compared to WNNW (p= 0.0001). Related to this changes, hemoglobin, MCHC, and hematocrit also increased in WNW (p= 0.0001).

The oxidative stress, which is possibly propagated by the physical environment, seems to have an important pathophysiological role in hearing loss and lipid peroxidative cellular changes in all of the workers who work in noisy occupations.

Key words: Noise, Worker, Oxidative stress, Superoxide dismutase, Catalase, Malondialdehyde, Nitric oxide

ÖZET

Gürültünün İnsan Eritrositlerindeki Oksidan ve Antioksidan Dengeye Etkileri

Reaktif oksijen ve nitrojen türleri, yüksek ses kaynaklı duyma kayıplarında önemli bir patolojik faktör olarak kabul görmektedir. Bu vaka kontrollü çalışmamızda yüksek sesli ortamlarda çalışan işçilerin eritrositlerinde oksidan ve antioksidan dengeyi araştırdık. Yüksek sesli ortamda çalışan (YSOÇ) 127 işçi ile normal ortamda çalışan (NOÇ) 117 işçinin kanları hemolizat elde etmek üzere içinde antikoagulan olarak potasyum EDTA bulunan tüplere alındı.

Bu hemolizatta antioksidan sistemin eritrosittteki önemli üyelerinden süperoksit dismutaz (SOD) ve katalaz (CAT) enzim aktiviteleri ile lipid peroksidasyon son ürünü malondialdehit (MDA) ve nitrojen bileşiklerinin bir indeksi olan nitrik oksit (NO) spektrofotometrik metodla çalışıldı.

YSOÇ grubunda SOD aktivitesi 450.0±106.4 U/g Hb bulunurken NOÇ grubunda bu miktar 443.1±83.1 U/g Hb olarak bulundu. Bu iki grup arasındaki ortalama farkı istatistiksel olarak anlamsızdı (p= 0.582). YSOÇ grubunda CAT aktivitesi 426.0±98.0 k/g Hb ve NOÇ grubunda 432.6±109.0 k/g Hb olarak bulundu, bu ortalamalar arasındaki fark da istatistiksel olarak anlamsızdı (p= 0.621). YSOÇ grubunda eritrosit MDA düzeyi NOÇ grubundan anlamlı bir şekilde daha yüksek bulundu (sırasıyla 39.28±10.22 nmol/g Hb ve 32.51±10.73 nmol/g Hb, p= 0.0001). Diğer taraftan NO düzeyleri YSOÇ grubunda (0.275±0.187 µmol/g Hb) NOÇ grubuna (0.382±0.284 µmol/g Hb) göre anlamlı bir şekilde düşüktü (p= 0.001). Çalışılan hematolojik parametrelere bakıldığında, YSOÇ grubunda, NOÇ grubuna göre monositler ve trombositler hariç bütün hücre grup sayılarında artış vardı (p= 0.0001). Bu değişikliklere paralel olarak, hemoglobin, MCHC ve hemotokrit düzeyleri de YSOÇ grubunda anlamlı bir şekilde artmıştı (p= 0.0001).

Fiziksel çevrenin etkisi ile artmış olduğu düşünülen oksidatif stresin, gürültülü ortamda çalışan işçilerin duyma kaybından ve hücrelerdeki lipid peroksidasyon kaynaklı değişimlerden sorumlu olabileceği değerlendirildi.

Anahtar Kelimeler: Gürültü, İşçi, Oksidatif stres, Süperoksit dismutaz, Katalaz, Malondialdehit, Nitrik oksit

INTRODUCTION

Noise has been known to be one of the most common reasons for hearing loss. The scientific authorities are debating on how it is classified and what its border are in recent years. Loud noise at work can damage the hearing of workers either temporarily or permanently. This is usually gradual because of prolonged exposure to it and after that period, hearing could be permanently damaged.[1] Therefore, the workers should understand what they need to do under loud noise and how they can protect themselves from noise. Hearing loss is not the only problem with the workers in heavy noisy environment; they may develop tinnitus leading to disturbed sleep as well. It may manifest as increased physiologic stress response, adverse social results, and expensive economic effects.[2] Thus, the researchers are supposed to reveal not only the hearing effects of heavy noise but also the damaging effects on whole body organism even in cellular level to aware them about the harmful effects of noise.

The mechanisms that lead to hearing loss and other harmful effects of noise in terms of physiological, biochemical and genetic aspects have not been fully understood and they are under investigation. The researches demonstrated that the underlying factor for noise-dependent damages is not so simple, but there are multiple factors including oxidative stress, vascular changes, mechanical trauma, and the several others.[3] One of the most popular subjects in noise-related harmful effects that needs to be clarified is the oxidative stress and antioxidant protective enzymes.[4] Recently, there is a trend in literature exploring the role of oxidative stress and genetic aspects of oxidative stress in noise induced hearing loss.[5,6] Reactive oxygen species (ROS) including the members superoxide ($O_2 \cdot^-$), hydroxyl radical ($\cdot OH$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and nitric oxide ($NO \cdot$) can cause cellular injury or subcellular injury when they are generated in huge amount, or the enzymatic antioxidant defense systems are damaged irreversibly. Additionally, as the non enzymatic antioxidant defense system is damaged, the same results can be seen in terms of the harmful effects of ROS. Malondialdehyde (MDA), or as a more general term, thiobarbituric acid reactant substances (TBARS) are produced during the attack of the ROS to the cellular and subcellular membrane lipoproteins and polyunsaturated fatty acids (PUFAs).[7] The measurement of those end products may give us an estimation on how deep the damage is. Superoxide dismutase (SOD) is an enzyme that catalyzes the conversion reaction of superoxide radical to a lesser harmful molecule hydrogen peroxide and molecular oxygen. Glutathione peroxidase (GSH-Px) and catalase (CAT), which are the subsequent antioxidant enzymes in the cellular pathway, catalyses the reaction decomposing of hydrogen peroxide to water. The measurement of these two (SOD and CAT) or three (SOD, CAT and GSH-Px) enzyme series in erythrocytes may help us to understand the antioxidant status in the workers, who work in the noisy environment.

To the best of our knowledge, there has been no detailed study in the literature on antioxidative enzymes and lipid peroxidation parameters as well as NO in the erythrocytes of noise-exposed people. Therefore, we evaluated the erythrocyte antioxidant enzyme activities in workers, who work in noisy environment with different severity, lipid peroxidation and NO end-products in erythrocytes. Additionally, we performed correlation analyses to reveal any possible relationship between these two opposite biochemical pathways in the cellular environment. We might reach some important clues on noise-induced harmful effect in cochlea by using other cellular representative cells which is easy to get and minimally invasive like taking a blood sample.

MATERIALS AND METHODS

Subjects: This study was performed according to the guidelines in the Declaration of Helsinki and approved by the Ethical Committee of Kirikkale University. Informed consent was obtained from all study participants. The study was conducted at Kirikkale University Faculty of Medicine, Department of Public Health, Kirikkale; Hacettepe University Faculty of Medicine, Departments of Biochemistry, Physiology, and; ENT-Audiology and Speech Pathology Laboratory, Ankara; and TCDD Behicbey Health Center, Ankara in 2008. We recruited male volunteers, who had been employed for several years in the unit of equipment repair and maintenance of a railroad company in Ankara. The measured noise exposure was 85-110.6 dBC (Impulse) for the time study conducted. All the workers, who worked in the same company, were invited to participate in this study. They were globally divided into two groups: The case group of this study was the workers (n= 127), who works in noisy workplace (WNW). The control group (n= 117) was the workers, who work in non-noisy workplace in the same company (WNNW). Subjects, who decided not to continue the study, were excluded from the study. Those, who have head injury, otological disease, and other diseases that could affect hearing, treatment with ototoxic drugs, and a family history of congenital deafness were excluded from the study.

Study Design, Blood Sampling and Hemolysate Preparation: The assessments, which were performed during one regular day of work, included structured interviews, the physical examinations by physicians including otoscopic examination. Audiometric and acoustic analysis (Larson Davis 824 Sound Level Meter) was performed by an odiologist. Noise levels in the working environment were measured in decibels (dBA and dBC). Blood from forearm vein was collected into 5 ml Vacutainer tubes containing potassium EDTA. Hematological parameters were tested by routine laboratory technique using an auto analyzer (ERMA PCE-210N, Japan). The blood samples were centrifuged at 1000 x g for 10 min at 4^0C to remove plasma. The buffy coat on the erythrocyte sediment was separated carefully after the plasma was removed. The erythrocyte sediment was washed three times with 10-fold isotonic NaCl solution to remove plasma. After each procedure, erythrocyte-saline mixture was centrifuged at 1000 x g for 10 min at 4^0C. Aliquots of the samples were transferred into polyethylene tubes to be used in the assay of free radical scavenging enzymes and MDA levels. Erythrocyte sediment samples were stored at -80^0C until analysis. After they were thawed, erythrocyte sediments were treated with 4-fold ice-cold deionized water to obtain hemolysate.

The Chemicals, Enzymes, and Instruments Used in the Analyses: Xanthine oxidase, xanthine, nitroblue tetrazolium (NBT), naphthlethylenediamine, sulphanilamide, thiobarbituric acid, 1,1,3,3 tetramethoxy propane were purchased from Sigma Chemical Co (St Louis, MO, USA) and $CuCl_2$, bovine serum albumin, H_2O_2, EDTA, Na_2CO_3, $(NH_4)_2SO_4$, chloroform, ethanol, NaCl, KH_2PO_4, Na_2HPO_4 and H_2O_2 from Merck (Germany). Cd granules were purchased from Fluka, Germany. Shimadzu UV 1601 (Australia) and Shimadzu UV-1800 (Kyoto, Japan) was used to measure SOD, CAT, MDA, and NO analyses. Hematological parameters like Hb, RBC, leukocytes; MHC, MCHC, etc were measured by using ERMA PCE-210N, Japan.

Hemoglobin Assay in Hemolysate: An aqueous solution containing 1 g sodium bicarbonate, 0.05 g potassium cyanide, and 0.2 g potassium ferricyanide per liter was used to lyses red cells and convert hemoglobin to cyanmethemoglobin. 10 μL of he-

molysate within 2.5 mL drabkin solution was incubated 10 minutes in room temperature and read against blank in 540 nm. Results were expressed as g/dL.

SOD activity measurement: Total (Cu-Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method defined by Sun et al.[8] and a slightly modified method by Durak et al.[9] The principle of the method is based on the inhibition of NBT reduction by the xanthine-xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the lyzate after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of the hemolysate and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. SOD activity was also expressed as Units per gram hemoglobin.

CAT activity measurement: CAT (EC 1.11.1.6) activity was determined by the method of Aebi.[10] The principle of the assay is based on the determination of the rate constant k (dimension: s-1) of the hydrogen peroxide decomposition. By measuring the absorbance changes per minute, the rate constant of the enzyme was determined. Activities were expressed as k per gram hemoglobin.

MDA level measurement: The MDA level was determined by reaction with thiobarbituric acid (TBA) at 90-100^{0}C.[11] MDA or MDA-like substances and TBA react to produce a pink pigment with absorption maximum at 532 nm. The sample was mixed with 2 volumes of cold 10% (w/v) trichloroacetic acid to precipitate protein. The precipitate was pelleted by centrifugation and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA at 90^{0}C for 15 min. After cooling, the absorbance was read at 532 nm. The results were expressed as nmol per gram hemoglobin in erythrocyte based on a graph prepared with 1,1,3,3-tetramethoxypropane standards.

NO level measurement: The estimation of total erythrocyte NO using the Griess reaction (nitrate plus nitrite) is much inferior to the direct measurement of NO using a porphyrinic microelectrode impaled in individual cells, but direct measurement of NO in biological samples is very difficult.[12] Therefore, tissue nitrite (NO_2^-) and nitrate (NO_3^-) were estimated as an index of NO production based on the Griess reaction[13], in which a chromophore with a strong absorbance at 545 nm is formed by reaction of nitrite with a mixture of naphthlethylenediamine and sulphanilamide. Tissue samples were deproteinized with Somogy's reagent and total nitrite (nitrite+nitrate) was measured by spectrophotometer at 545 nm after reduction of nitrate to nitrite with copporized cadmium granules. The assay was calibrated with standard solutions (10^{-8} - 10^{-3} mol/L) of sodium nitrite. The equation obtained from the standards was used to calculate the unknown sample concentrations. Results were expressed as μmol per gram hemoglobin.

Statistical Analyses: Data were analyzed by using SPSS for Windows computing program (SPSS for Windows Version 15.0, Chicago, IL, USA). Nonparametric statistical methods were used to analyze all the data. Mann-Whitney U tests were used for pair-wise comparisons. Bivariate comparisons were examined using Pearson rank correlation coefficients (r) and values were corrected for ties. Two-tailed significance values were used. A p value less than 0.05 was accepted as significant.

RESULTS

The characteristics of the workers included in this study were summarized in Table 1. The results of the variables studied were summarized within Tables 2-5. SOD activity was found to be 450.0±106.4 U/g Hb in WNW and 443.1±83.1 U/g Hb in WNNW (Table 2). The difference between the groups were not statistically significant (p= 0.578). CAT activity was found to be 426.0±98.0 k/g Hb in WNW and 432.6±109.0 k/g Hb in WNNW showing statistically insignificant difference (p= 0.621) (Table 2). MDA levels in erythrocytes from WNW was significantly higher, when compared to WNNW (39.28±10.22 nmol/g Hb and 32.51±10.73 nmol/g Hb, respectively and p= 0.0001) (Table 2). NO levels also significantly reduced in WNW (0.275±0.187 μmol/g Hb) compared to WNNW (0.382±0.284 μmol/g Hb) (p= 0.001) (Table 2). When the workers, who work at noisy workplaces, were divided into two groups as the ones who use headset (n= 46) and the ones, who do not use headset (n= 77), we could not notice significant difference between the parameters (Table 3). When we divided the groups into two subgroups according to

Table 1. The characteristics of the workers who included in this study.

	WNW	WNNW
n	127	117
Age (mean SD) (years)	45.0±5.2	45.3±6.4
Sex	All male	All male
Smoking (-/+)	32/95	36/81
Alcohol intake (-/+)	100/27	82/35
Working longevity (years)	15.5±9.4	17.6±9.0
Headset usage (-/+)	77/47	–
Acute or chronic illnesses (-/+)	85/39	58/59

WNW: workers in noisy workplace, WNNW: workers in non-noisy workplace
There is no differences between ages of the groups (p>0.05).

their working periods as 1-18 years period and 19-40 years period for both group subjects (Table 4), there was no difference between the variables within the groups (within WNW and WNNW). However, as we obviously expected, there were significant differences in MDA levels between the same subgroups, i.e., 1-18 years of WNW group and 1-18 years of WNNW group subjects (p= 0.007), it is valid for the other age group, 19-40 years (p= 0.0001). In the case of NO, there was a significant difference between subgroup 1-18 years of WNW and subgroup 1-18 years of WNNW (p= 0.002), but there was no significant difference between subgroups 19-40 years of WNW and WNNW (p= 0.079). In correlation analyses, there was a positive correlation between SOD and CAT activity in WNW group (r= 0.227, p= 0.001), positive correlation between SOD activity and MDA level in WNW group (r= 0.328, p= 0.001), positive correlation between CAT activity and MDA levels in both WNW (r= 0.437, p= 0.001) and WNNW (r= 0.534, p= 0.001) groups, positive correlation between NO and MDA

Table 2. Superoxide dismutase (SOD) and catalase (CAT) activities as well as malondialdehyde (MDA) and nitric oxide (NO) levels in erythrocytes from workers who are working at noisy (case) and non noisy (control) workplaces.

	SOD (U/g Hb)	CAT (k/g Hb)	MDA (nmol/g Hb)	NO (µmol/g Hb)
Case (n= 127)	450.0±106.4	426.0±98.0	39.28±10.22	0.275±0.187
Control (n= 117)	443.1±83.1	432.6±109.0	32.51±10.73	0.382±0.284
P values	0.583	0.621	0.0001	0.001

Table 3. Superoxide dismutase (SOD) and catalase (CAT) activities as well as malondialdehyde (MDA) and nitric oxide (NO) levels in erythrocytes from workers who are working at noisy workplaces with headset (Group 2) or not (Group 1).

	SOD (U/g Hb)	CAT (k/g Hb)	MDA (nmol/g Hb)	NO (µmol/g Hb)
Group 1 (n= 77)	455.7±105.1	419.3±104.8	38.13±10.53	0.293±0.198
Group 2 (n= 46)	441.1±110.2	439.5±83.2	41.46±9.50	0.252±0.168
p values	0.471	0.266	0.089	0.249

Table 4. Superoxide dismutase (SOD) and catalase (CAT) activities as well as malondialdehyde (MDA) and nitric oxide (NO) levels in erythrocytes from workers who are working at noisy workplaces (a and b) and non noisy workplaces (c and d) according to their working years.

	SOD (U/g Hb)	CAT (k/g Hb)	MDA (nmol/g Hb)	NO (µmol/g Hb)
a) 1-18 years (n=63)	457.3±94.4	432.6±84.1	38.54±9.21	0.250±0.170
b) 19-40 years (n=62)	442.3±118.2	419.3±110.6	40.04±11.18	0.301±0.200
c) 1-18 years (n=50)	436.6±83.2	449.1±103.5	33.28±10.53	0.390±0.303
d) 19-40 years (n=65)	447.9±83.4	419.9±112.2	31.91±10.93	0.376±0.271
p values				
a-b	0.441	0.453	0.424	0.122
c-d	0.481	0.155	0.521	0.804
a-c	0.235	0.349	0.007	0.002
b-d	0.763	0.978	0.0001	0.079

levels in WNW group (r= 0.188, p= 0.04). As we looked at the hematological parameters (Table 5), all cell numbers were increased in WNW group except monocytes and platelet compared to WNNW group. White blood cells, lymphocytes, and granulocytes were slightly higher in WNW group. Related to this increase, hemoglobin, MCHC, and hematocrit were also found to be increased in WNW group (p= 0.0001).

DISCUSSION

In this study, we demonstrated the oxidant/antioxidant imbalance in workers, who work in noisy environment. We choose erythrocytes to measure ROS-related enzymes and to estimate what is going on in cellular level for both acoustic cells and the other cells in the body that were possibly affected from the heavy noisy environment. Here, erythrocytes are representative cells of the whole body. They are easy to obtain and processed for further analyses. The elevation of all cell series except platelets in the blood of WNW is pretty interesting and we could not explain the changes of cell numbers with our contemporary information on noise-induced variations in living organisms.

ROS have been implicated in hearing disorders in recent years. It may be suggested that noisy condi-tions may induce noise trauma in both ears and the other parts of the body at cellular level by triggering the formation of ROS to a level that varies with the intensity of exposure. Both superoxide anion and hydroxyl radical have been known to increase in the cochlea following sound exposure.[14,15] Labbe at all recently found the emergence of lipid peroxidation products (8-isoprostanes) in the guinea pig cochlea in a time-dependent and transient manner in response to noise exposure.[16] They also noticed that compared with the sham operated controls, hydropic cochleae showed strong immunostaining for both oxidative stress markers in spiral ganglion cells, in the blood vessels and fibrocytes of the lateral wall, as well as in supporting cells of the organ of Corti. The present study may elicit some important clues about the harmful effects of heavy noise on human erythrocytes besides the cochlea. The main point here is that the noise affects the whole body, not only the cochlea. The magnetic resonance in that kind of environment may even break down the erythrocyte membranes of the workers leading membrane abnormalities. Elevated MDA levels in hemolysate may exactly show this breakdown of membranes. What the mechanism here is the oxidation of double bounds of fatty acids, abundantly found in the membrane structure, by ROS produced by the magnetic field in the environment

Table 5. Hematological parameters from the subjects according to their workplaces as noisy workplace (A) and non noisy workplace (B)

	Groups	n	Mean±Std. Dev.	p values
WBC (x10³/µL)	A	121	7.10±1.71	0.0001
	B	117	6.25±1.96	
LYM (x10³/µL)	A	121	2.55±0.70	0.0001
	B	117	2.25±0.59	
MO (x10³/µL)	A	121	0.45±0.13	0.258
	B	117	0.47±0.17	
GRA (x10³/µL)	A	121	4.09±1.21	0.002
	B	117	3.52±1.56	
LYM (%)	A	121	36.27±6.87	0.514
	B	117	36.89±7.69	
MID (%)	A	121	6.44±1.72	0.0001
	B	117	7.62±2.14	
GRA (%)	A	121	57.28±6.65	0.053
	B	117	55.50±7.48	
RBC (x10⁶)	A	121	5.39±0.55	0.0001
	B	117	5.09±0.37	
Hgb (g/dL)	A	121	15.34±1.45	0.0001
	B	117	14.35±1.28	
HCT (%)	A	121	44.76±4.06	0.0001
	B	117	42.67±3.57	
MCV (fL)	A	121	83.35±4.98	0.811
	B	117	83.54±7.12	
MCH (pg)	A	121	28.49±1.83	0.326
	B	117	28.24±2.02	
MCHC (%)	A	121	34.17±0.96	0.0001
	B	117	33.46±0.74	
RDW (%)	A	121	16.00±0.73	0.008
	B	117	16.29±0.92	
PLT (x10³/µL)	A	121	203.4±44.6	0.023
	B	117	217.7±52.1	

that workers live in daytime. A reduced level of phospholipids and PUFAs in erythrocyte membranes from WNW by the effect of heavy noise is not good for erythrocyte viability. Abnormalities in membrane fatty acids may lead to destruction of erythrocytes wholly. But the abnormalities in fatty acid metabolism are not specific to noise-induced conditions in our study and might also be connected other factors and conditions including, alcohol intake, age, smoking etc. Increased MDA levels in erythrocyte from our study group are consistent with the previous results.[17,18] Membrane PUFAs are more susceptible to peroxidation than the other lipids such as cholesterol and saturated fatty acids. For this aspect, excessive noise-induced ROS generation is very important particularly for the cell membrane. After peroxidation process, PUFAs and phospholipids in erythrocyte membrane are destroyed and reduced in amount, consequently, essential fatty acid depletion in the membrane might result in cellular destruction and impaired antioxidant defense system.

NO is accepted as oxygen radical but it has some other functions like regulation of vasodilatation. It is produced by various cell types by an enzymatic system called NO synthases (NOS) present in a variety of cell types including erythrocytes.[19] Even if the erythrocytes do not have nucleus, the existing NOS in these cells can produce NO from arginine. Some researchers have been alleged NO levels to be increased in the inner ear leading to nitroactive stress and cell destruction upon noise-induced

stress. To cope with this situation, they have tried to diminish NO levels by using vitamin C as an antioxidant, and they found that when the maximum ascorbic acid dose was substituted, NO production was significantly reduced in the lateral wall after noise exposure.[20] In comparison with this study, we did not find the similar result in our experiment, i.e., NO level in erythrocytes of WNW was pretty lower than that of control group. NO has been rather suggested to be an antioxidant by some scientists. Therefore, it might be diminished in response to the elevation of ROS because of the consumption. In other words, ROS generation by electromagnetic stimulation of heavy noise led to decrease in NO and increase in MDA production which clearly shows the harmful effect of heavy noise. As a matter of fact, extracellular ATP-induced NO production in inner hair cells, outer hair cells, and spiral ganglion neurons affect the ATP-induced Ca^{+2} response via the NO-cGMP-PKG pathway in those cells by a feedback mechanism. A cross talk between NO and ATP is suggested in the auditory signal transduction.[21] Our study showed that the noise-induced NO decrease may therefore affect hearing negatively. We need to prove this finding by checking NOS expression in these subjects in the future planning studies. We have no idea about the controversial findings on NO levels when we compare the other studies like Chen et al.[22], which they found increased NO levels after 40 hours noise exposure in guinea pigs. The longevity of the exposure may be the main factor on the level changes of NO or the other factors we do not know yet.

SOD and CAT are important complementary enzymes to cope with superoxide radicals and hydrogen peroxide in terms of antioxidant defense system. These enzymes have been studied by several researchers to see the changes after noise-induced hearing loss. In an experimental study,[23] Samson et al measured SOD and CAT activities in cochlea from C57BL/6 mice 1-21 days after noise exposure and found increase in SOD activity without a concomitant increase in CAT activity. Antioxidant enzyme polymorphisms were studied in noise-induced hearing loss to see whether susceptibility to noise-induced hearing loss is associated with antioxidant enzymes. Male workers from an aircraft factory were enrolled a study in which SOD polymorphism was studied.[24] Even if there was some obstacles li-

ke small sample size and the difficulty in matching cases to controls, the data suggest Mn-SOD (SOD2) polymorphism could predispose to noise-induced hearing loss. The other study was conducted by Chang et al. in which the distribution of Mn-SOD genetic polymorphisms IVS3-23T/G on noise susceptibility in Asians were investigated.[25] Within the 200 factory workers, individuals with T/G genotype were significantly more vulnerable to noise than the individuals with T/T genotype (wild type). The genetic variants of glutathione S transferases, one of the other antioxidant enzymes, were studied in temporary threshold shift rather than permanent threshold shift in a population of occupational noise-exposed workers to see whether these genotypes are associated with the higher susceptibility to noise-induced temporary threshold shift.[26] They found that subjects harboring all three genotypes that investigated had higher susceptibility for developing noise-induced hearing loss. The protective effect of SOD was also investigated in impulse noise-exposed guinea pigs and it was found that acoustic stress induced ROS formation and SOD exerted a protective effect on cochlea when compared to the animals those without pharmacological protection.[27] As seen in above-mentioned biochemical and genetic studies, it is obvious that antioxidant enzymes are related to noise-induced hearing loss. Our results on antioxidant enzymes did not confirm these studies despite erythrocyte oxidative stress, as represented by MDA, was higher in WNW. The other antioxidant enzymes like GST and GSH-Px should be investigated to give the answer for the question about total enzymatic antioxidant system changes in WNW.

As a conclusion, oxidative stress seems to be a pathophysiological factor in noise-induced hearing loss in WNW. The investigation by adding some antioxidants to the diet together with the standard physical protection for the workers may be a good idea to see the alternative ways for the prevention of cellular structures of the body. Further genetic and biochemical studies in which all the single variables of oxidative stress and antioxidant system will be investigated in such workers are needed to clarify the exact mechanism of noise-induced hearing loss.

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