Research

Correlation of malondialdehyde and hearing threshold level at frequency 4000 Hz post gunshot exposure

Nyilo Purnami, Fauzi Helmi, Sri Herawati
Department of Otorhinolaryngology Head and Neck Surgery,
Faculty of Medicine Universitas Airlangga/Dr. Soetomo General Hospital,
Surabaya

ABSTRACT

Background: The incidence of acoustic trauma after gunshot exposure in students of the Sekolah Polisi Negara (SPN) is quite high. Malondialdehyde (MDA) is a compound that can portray oxidative stress due to free radicals. The correlation between the levels of MDA and the hearing threshold frequency post gunshot exposure could be used as the base for administering antioxidants to prevent acoustic trauma. **Objective:** To find out the correlation between the levels of MDA and the hearing threshold frequency post gunshot exposure, in East Java SPN students. **Method:** An observational analytic study with a retrospective cross sectional approach using secondary data of medical records of the East Java SPN students batch 2017/2018. The samples were selected by simple random sampling. **Result:** Out of 50 students, the mean of age was 19.62 years. All samples were male with mean 559.17 and standard deviation (SD) 959.86. The calculation of the 4000 Hz frequency threshold value obtained an average value of 31.52 and SD of 13.4. Hearing loss complaint was found in 1 student (2%). No complaints of tinnitus and vertigo were found. Statistical tests with Pearson correlation between serum MDA levels and 4000 Hz frequency threshold values showed a correlation coefficient (r) of 0.74 and p = 0.00 ($p<0.05$). **Conclusion:** There was a significant correlation between MDA levels and the hearing threshold at 4000 Hz frequency after gunshot exposure in East Java SPN students.

Keywords: acoustic trauma, malondialdehyde, hearing threshold level at frequency 4000 Hz

ABSTRAK

Latar belakang: *Insiden trauma akustik pasca pajanan letusan senjata api pada siswa Sekolah Polisi Negara (SPN) cukup tinggi. Malondialdehid (MDA) merupakan senyawa yang dapat menggambarkan stres oksidatif akibat radikal bebas. Adanya hubungan antara kadar MDA dengan nilai ambang dengar frekuensi 4000 Hertz (Hz) dapat menjadi dasar pemberian antioksidan untuk pencegahan trauma akustik.* ***Tujuan:*** *Membuktikan hubungan antara kadar MDA dengan nilai ambang dengar frekuensi 4000 Hz pasca pajanan letusan senjata api pada siswa SPN Jawa Timur.* ***Metode:*** *Penelitian ini adalah observasional analitik dengan pendekatan retrospektif* cross sectional *menggunakan data sekunder berupa rekam medik siswa SPN Jawa Timur angkatan 2017/2018. Sampel dipilih secara* simple random sampling. ***Hasil:*** *Dari 50 siswa, usia rerata adalah 19,62 tahun. Seluruh sampel penelitian adalah laki-laki, rerata=559,17 dan standar deviasi (SD)=959,86. Penghitungan nilai ambang dengar frekuensi 4000 Hz didapatkan hasil nilai rerata=31,52 dan SD=13,4 Keluhan penurunan pendengaran hanya dijumpai pada 1 siswa (2%). Uji statistik dengan korelasi Pearson antara kadar MDA dalam serum dengan nilai ambang dengar frekuensi 4000 Hz didapatkan hasil koefisien korelasi (r) sebesar 0,74 dan p = 0,00 ($p<0,05$).* ***Kesimpulan:*** *Terdapat hubungan yang signifikan antara kadar MDA dengan nilai ambang dengar frekuensi 4000 Hz pasca pajanan letusan senjata api pada siswa SPN Jawa Timur.*

Kata kunci: *trauma akustik, malondialdehid, nilai ambang dengar frekuensi 4000 Hz*

Correspondence address: Nyilo Purnami. Department of Otorhinolaryngology Head and Neck Surgery, Faculty of Medicine Universitas Airlangga/Dr. Soetomo General Hospital. Jl. Mayjen Prof. Dr. Moestopo No 6-8 Surabaya 60131. Indonesia. E-mail: nyilo@fk.unair.ac.id.

INTRODUCTION

Gunshot exposure could create acoustic trauma to the shooter. The pure tone audiometric of acoustic trauma revealed a sensorineural hearing loss (SNHL) with a dip or increased hearing threshold level at frequency 4000 Hz.[1,2,3] Shooting exercise is a curriculum at Sekolah Polisi Negara (SPN). Acoustic trauma incidence post gunshot exposure at SPN was quite high. A study of 100 East Java SPN students showed an incidence of 15% acoustic trauma.[4] While other study on 100 Bali SPN students showed an incidence of 11% acoustic trauma.[5] SPN had tried to prevent acoustic trauma by using earplugs, although not regularly implemented. Earplugs were also not effective in reducing the exposure intensity, since it could only lessen the intensity exposure by 8 to 25 dB. Thus, other methods were needed to prevent the occurrence of acoustic trauma.[4,6]

Administering antioxidants had become frequently discussed to prevent acoustic trauma.[7] The gunshot exposure could create an oxidative stress in the cochlea as a result of free radicals release.[8] An oxidative stress occurred due to the imbalance between free radicals and antioxidants, where the free radicals were distinctly dominant. An oxidative stress could cause lipid peroxidation process, increased MDA level and hair cells damage which started at a high frequency. The initially most affected frequency was the 4000 Hz.[1] Malondialdehyde (MDA) was a compound that signaling oxidative stress due to free radicals inside cells, hence, MDA could be one of biomarkers of the occurrence of an oxidative stress as a result of free radicals.[9] The correlation between the levels of MDA and the hearing threshold frequency post gunshot exposure, could be used as the base for administering antioxidants to prevent acoustic trauma.

Cochlear damage caused by acoustic energy post gunshot exposure was a mechanical and metabolic process. The gunshot exposure initiated excessive fluid movement inside the cochlea, mechanical process caused rupture of hair cells, basilar membranes and hydrolymphatic membranes. Constant gunshot exposures could cause cochlear damage as a result of metabolic process. It started with prolonged stereociliar deflection, causing calcium accumulation in mitochondria, and cochlear hypoxia. Calcium homeostasis disturbance and cochlear hypoxia, instigated increased free radicals and triggered oxidative stress.[8] Free radicals are particles from atomic cells contain uncoupled electrons, highly reactive and tend to release or bind electrons from surrounding tissues. The most important human body's free radicals in aerobic metabolism are oxygen derivates called Reactive Oxigen Species (ROS).[10,11] ROS could attack polyunsaturated fatty acid (PUFA) causing lipid peroxidation, damaging cell membranes, ending in apoptosis and cochlear hair cells necrosis.[8]

Cochlear damage as a result of voice exposure with high frequency and intensity was centered in 4000 Hz frequency. About 10 mm from foramen ovale, resides the weakest anatomical structure area i.e. 4000 Hz receptors. These 4000 Hz receptors are hair cells with the amplitudo, accepting the biggest energy from noise exposure and it is a Corti locus minors. High intensity noise exposure in high frequency could cause damage to the basal hair cells, while a low frequency could cause damage to apex hair cells.[3] The difference of basal tonotopic and cochlear apex as in the viability condition of outer hair cells (OHC), vascularization, intrinsic vulnerability of basal hair cells towards free radicals had caused the basal receptors more

easily damaged. Antioxidants level in the basal part were lower than in apex, so that the destruction of higher frequency was more often found in basal area.[2,12]

Malondialdehyde (MDA) was a compound that portrayed the activities of free radicals inside cells, the oxidative stress due to free radicals.[9] A study by Singh et al[13] supported that statement by formulating that MDA mediator was an end product of lipid peroxidation, which was used as a biologic biomarker of lipid peroxidation and it could demonstrate the level of oxidative stress.[13] Several recent studies had tried to explain the role of oxidative stress in hearing disturbance due to acoustic trauma, as an effort to find other methods for preventing acoustic trauma. Antioxidants are produced naturally endogenic, to suppress free radicals in a certain condition, for instance in a constant gunshot exposure, where free radicals were still more dominant than antioxidants, thus, arising oxidative stress.[14,15] A research in Turkey in 2008, reported the increased of MDA post gunshot exposure.[1] A study in Bandung in the year 2015 reported MDA increased level and an increase in acoustic trauma incidence in a placebo group compared to a group of orally given peroxidase glutathione antioxidants.[16]

Up to date, the correlation between MDA level and hearing threshold level at frequency 4000 Hz post gunshot exposure in East Java SPN students was still unclear. Therefore, a study to find out the correlation between MDA level and hearing threshold level at frequency 4000 Hz post gunshot exposure in East Java SPN students was necessary to be performed.

METHOD

The design was an observational analytic study with a retrospective cross sectional approach using secondary data of medical records of the East Java SPN students, batch 2017/2018. The samples were selected by simple random sampling. The study was conducted at East Java Sekolah Polisi Negara (SPN) to obtain medical record data of East Java SPN students i.e. anamnesis, physical examination, pure tone audiometry examination, and MDA level measurement. Time of study was from August until November 2018. The included population was East Java SPN students batch 2017/2018. Study sample was all population complying with inclusion and exclusion criteria. The sample was taken by simple random sampling from year 2017-2018 data. The inclusion criteria were personnel with complete medical record such as anamnesis data, physical examination, audiogram, and MDA level measurement. The exclusion criteria were if during the physical examination a perforation of tympanic membrane was found, and the level of MDA measurement could not be assessed. The size of sample was determined by formula Madiyono et al.[17] The researcher had taken 50 samples from the population by simple random sampling from the population adjusting to suitable minimal sample size.

The used instruments to collect data were medical records, and data collection form. The independent variable was MDA level, and the dependent variable was hearing threshold level at frequency 4000 Hz.

The samples were medical record data of East Java SPN students batch 2017/2018 who had underwent 5 months shooting exercise, as many as 200 times shootings consisted of 100 times with long guns (including rifles and shotguns) and 100 times with handguns (including revolvers and pistols). Shooting exercises were carried out in groups, with a very short distance between shooters, whilst one group consisted of 20 students. Ear protector device was not routinely used. Examinations comprised of anamnesis, physical examinations, pure tone audiometry, and measurement of MDA level, were conducted 2 (two) weeks after the last shooting exercise.

The pure tone audiometry was conducted 2 weeks post final gunshot exposure. The result of pure tone audiometry used was the audiometry result of the worse ear.

The examinations were performed in silent room of SPN, where the sound level meter showed a noise pollution inside the silent room at 40 to 45 dB. The audiometry used was a calibrated Interacoustic type AD226. The sound level meter and pure tone audiometry examinations were executed by experts from Dr. Soetomo General Hospital Surabaya.

Blood specimens were taken 2 weeks post final shooting exercise. MDA level was measured at the Pathology Installation of Dr.Soetomo General Hospital Surabaya. The measurement result was read by a senior Pathology Clinic specialist (consultant). The MDA quantity result was in ng/ml.

All data gathered from data collection forms were arranged as a table, and descriptively analyzed. Data processing and analyzing were carried out with computer application. Descriptive data used frequency table covered patients' age, gender, and patients' complaint. The Pearson correlation test was used to find out the correlation between MDA level and hearing threshold level at frequency 4000 Hz. The significant level was identified by (α) equal to 0.05.

RESULT

Patient's characteristic distribution was presented in table 1.

The students' mean age was 19.62 years old, with standard deviation 0.83. The majority students were 20 years old, as many as 21 students (42%), and the minority was 18 years old, as many as 4 students (8%). Most students in this study had no complaints, only one student complained of hearing loss (2%), while the rest 49 students filed no complaints (98%).

Table 1. The East Java SPN students general characteristic distribution

Variable	Number (n = 50)
1. Age	
18 years old	4 (8%)
19 years old	18 (36%)
20 years old	21 (42%)
21 years old	7 (14%)
2. Students' complaint	
Hearing loss	1 (2%)
No complaint	49 (98%)
3. Gender	
Male	50 (100%)

Table 2 showed the result of MDA level measurement and hearing threshold level at frequency 4000 Hz.

Table 2. The East Java SPN students' MDA level and hearing threshold level at frequency 4000 Hz distribution

	MDA ng/ml	NAD freq 4000 Hz dB	
N	50	50	
Mean	959.8	31.5	p=0.00**
SD	559.1	13.4	r=0.74

** *Correlation was significant at the 0.01 level*

The MDA level measurement in this study gave a mean result of 559.17 and standard deviation of 959.86. The hearing threshold level at frequency 4000 Hz measurement gave a mean result of 31.52 and standard deviation of 13.4.

The analysis of the correlation between MDA level and hearing threshold level at frequency 4000 Hz post gunshot exposure of East Java SPN students had used Pearson correlation test, because the data spread was normal based on distribution test. The Pearson correlation test gave a significant correlation between MDA level and the hearing threshold level at frequency 4000 Hz (p=0.00) with strong positive coefficient value (r=0.74).

The result confirmed that the higher MDA level post gunshot exposure of East Java SPN students, the higher also the hearing threshold level at frequency 4000 Hz post gunshot exposure of East Java SPN students.

DISCUSSION

The basic data of this study covered age, gender, and complaints. The majority students were 20 years old. Sasongko[18] in his reseach mentioned that the youngest age in his study was 19 years old, while the oldest was 22 years old with mean age was 20.8 years old and standard deviation of 0.90. Hidayati[19] stated that SPN students were graduates of High School or the same level at an age range of 17 to 22 years old.

Based on gender, samples of this study were all male (100%). A prior study by Mahardana et al.[5] all samples were male, by Sasongko[18] all samples were male, and by Cetin et al.[1] also all samples were male. The samples in this study and in prior studies were all male due to the fact that the population of East Java SPN students were all male.

Hearing loss in this study was found in one student (2%), there was no tinnitus nor vertigo reported (table 2). Moon et al.[20] in his research revealed that the highest complaint post gunshot exposure was tinnitus in 63 samples (94.2%). Ghasemi et al.[21] in his study had evaluated the complaints of 40 military soldiers right after post gunshot exposure and one week post gunshot exposure, with result of 53% tinnitus, 32% vertigo, and 20% hearing loss complaints. One week evaluation post exposure obtained decreased complaints, i.e. 7.5% tinnitus, 7.5% hearing loss, while vertigo was 0%. Rezaee et al.[22] in his study concluded that discomfort due to tinnitus was more complained than discomfort due to hearing loss or vertigo.

Noise pollution had caused increased basilar membrane movements resulting in the rise of intracellular cytoplasma tension, and outer hair cells became susceptible triggering a temporary threshold shift (TTS) or permanent threshold shift (PTS). The effect of noise pollution could cause damage on the tip of stereocilia, damage on stereocilia actine filament, and the decrease of hair cells stiffness. The tip of stereocilia will regenerate in 24-120 hours, while the actine filament of protein nucleus will regenerate in 48 hours. The outer cell stiffness will be restored in 2 weeks. The complaints were different than in previous studies, it could be caused by different sample taking times. Samples of this study was taken 2 weeks post gunshot exposure, therefore, it was allegedly that only TTS occurred in some students, whereas during that time, a regeneration of stereocilia took place, also the restoration of outer hair cell stiffness had made complaints of tinnitus, hearing loss, and vertigo decreased. PTS occurred in 1 student (2%).

Correlation analysis between MDA level and hearing threshold level at frequency 4000 Hz in this study had used Pearson correlation test based on the rational scale of attained data.

Normality test using scatter plot diagram showed a spreading data around the line, confirming a normal distribution.

Statistical result of Pearson correlation test showed a significant correlation between MDA level and hearing threshold level ($p=0.00$) with strong positive correlation coefficient ($r=0.74$).

Correlation analysis is a statistical method used to evaluate the strength of linear correlation between two variables or more. The value of correlation coefficient is in a range of -1 and 1. Positive correlation value means that the correlation of two variables is in the same direction. Negative correlation value means that the correlation of two variables is in opposite direction. The size of correlation coefficient (r) exhibits the strength

of inter variables correlation. The value of r was divided into 4 levels i.e. 0-0.09 was no correlation, 0.10-0.29 was weak correlation, 0.30-0.70 was moderate correlation, and 0.71-1 was strong correlation.[23] Ourstudy showed positive correlation coefficient with strong significant correlation. This means that the higher MDA level, the higher also the hearing threshold level at frequency 4000 Hz post gunshot exposure of East Java SPN students.

The minimum MDA level in this study was 29.60 ng/mL, and the maximum level was 2234 ng/mL with mean 958.86 ng/mL and standard deviation (559.2). Cetin et al.[1] stated that MDA level post gunshot exposure was 462.7 mg/dl (normal level 80-284 mg/dl). The result of MDA level measurement in this study was different with previous studies, due to different applied technique. TBA method was utilized in previous studies, while ELISA method was used in this study. A research by Kashani et al.[7] stated that free radical level and oxidative stress process as a result of high intensity noise pollution had the highest level in day 7th until day 10th post exposure. A previous study still exhibited increased MDA level in day 12th and day 14th, where the hearing had regained recovery.[24] Our study was conducted 2 weeks post gunshot exposure and still showed an increased MDA level.

In our study, the minimal hearing threshold level at frequency 4000 Hz was 5 dB and the maximal was 65 dB, with mean (SD) 31.52 dB. The injury of basal part of hair cells was caused by high intensity noise exposure in high frequency, while the injury of apex part of hair cells was caused by high intensity noise exposure in low frequency. The difference of outer basal cells and apex part injury could be caused by tonotopic difference in the basal and apex cochlea, such as outer hair cell viability condition, vascularization, and intrinsic vulnerability of basal hair cells towards free radicals. These conditions had made basal receptors more sensitive for damage. The level of antioxidants in the basal part was lower than the apex part, thus, the deterioration of high frequency tended to happen in the basal part.[2,12] The mean result of hearing threshold in this study showed an increase, which was similar with previous researches. This study and the previous researches had shown an increase in hearing threshold level at high frequency, particularly in frequency 4000 Hz. This study showed a decrease in hearing threshold level at frequency 4000 Hz, which was indicated as acoustic trauma in 28 students (56%).

A study by Mahardana et al.[5] on 100 Singaraja SPN students showed acoustic trauma of 11%. A research in Makassar, in 2001 directed towards Mobile Brigades, SPN students, and the Municipal Police members, obtained an incidence of 16.67% trauma acoustic.[25] A previous study towards 100 Mojokerto SPN students showed 15% acoustic trauma. The result of acoustic trauma in this study was higher than other studies, could be as the result of many exposures.[4] A study in Bali reported that the examinations were performed after one time post gunshot exposure, and another previous study in East Java SPN reported after 12 times shooting exercise.[4,5] This study was performed after 20 times shooting exercise. The result of this study revealed a significant correlation between MDA level and hearing threshold level at frequency 4000 Hz with $p<0.05$ ($p=0.00$). A study by Cetin et al.[1] showed a significant correlation between acoustic trauma and MDA level post gunshot exposure with $p<0.05$. Ohinata et al.[26] on his animal experimentation gave the subjects noise pollution 115 dB for 5 hours, had exhibited that lipid peroxidation process was in direct proportion with the length of noise exposure. Acoustic trauma caused an increased MDA level. Several studies supported the theory that free radicals had a certain role towards hearing impairment as a result of acoustic trauma.[24] Gunshot exposure to ears could stimulate free radicals raise, apoptosis, and hair cell necrosis.[8]

A study by Sasongko[16] demonstrated a significant correlation between erythrocyte glutathione level and plasma MDA level with positive statistic value ($p<0.001$), meaning the higher the erythrocyte glutathione level, the lower plasma MDA level.

Nowadays, free radicals become a new target in preventing acoustic trauma.[27] Further researches are being conducted to prevent acoustic trauma. Several items had been examined, among others were antioxidants. The increased of free radicals was caused by imbalance between oxidants and antioxidants. This study contributes scientific additional information supporting previous studies regarding the role of free radicals towards trauma acoustic.

This study has several limitations. MDA level could be influenced by other unknown conditions such as inflammation process, and history of antioxidants consumption prior to this study. Time limitation for examination, causing data collection was taken only once which was post gunshot exposure without examination of pre gunshot exposure.

Conclusion of this study showed a significant correlation between MDA level and hearing threshold level at frequency 4000 Hz with strong positive coefficient value.

Intake of antioxidants could be considered as an additional therapy to prevent acoustic trauma in SPN students.

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