ORIGINAL ARTICLE

Relationship between Acoustic Trauma and Serum Level of Vitamin B12, Folic Acid, Zinc, Magnesium and Malondialdehyde

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INTRODUCTION: In our study, relationship between hearing loss due to acoustic trauma and serum levels of vitamin B12, folic acid, zinc, magnesium and malondialdehyde were investigated.

MATERIAL AND METHODS: The study was carried out on 135 healthy male individuals who were subjected to 138 dB sound intensity level. The mean age was 22 years. Pure-tone audiometry tests including high frequencies were performed to all individuals at one day before, same day and one month later with reference to the time of acoustic trauma. One day and four days after acoustic trauma, serum levels of vitamin B12, folic acid, zinc, magnesium and malondialdehyde were measured.

RESULTS: After acoustic trauma, notch type hearing loss at 4000 Hz frequency was observed in 83 of the participants. However there was no significant difference among high frequency audiometry thresholds ($p>0.05$). There was a statistically significant difference between serum levels of malondialdehyde before and after acoustic trauma in all subjects ($p<0.05$). There was no significant relationship between hearing loss due to acoustic trauma and serum levels of vitamin B12, folic acid, zinc and magnesium ($p>0.05$).

CONCLUSION: These results indicate that free oxygen radicals play a role in the pathophysiology of acoustic trauma even though it is not directly related to the serum levels of vitamin B12, folic acid, zinc and magnesium.

Noise-induced hearing loss (NIHL) is one of the most common ten occupational diseases. It causes both manpower and financial loss [1].

The most common reason for NIHL is loss of outer hairy cells in organ of Corti. At first stereocilia of outer hairy cells become stiff and dysfunction. After cessation of noise, stereocilias renew themselves. This condition is defined as temporary threshold shift. If noise continues, stereocilias adhere to each other and it may lead to permanent hearing loss. At further period of time inner hairy cells injury and secondary neural degeneration may develop. Pathology occurring at basal turn of cochlea leads to hearing loss of high frequencies at first. In due course, injury happens at apical part, and low frequencies are affected. At noise levels of more than 85 decibels (dB), hearing loss begins and it affects first high frequencies rather than low frequencies [2]. An intense and short term sound stimulation may cause permanent threshold shift without a period of temporary threshold shift and if this occurs it is called as "acoustic trauma". This trauma may lead to damage of organ of Corti, rupture of the membranes and flow of the perilymph and endolymph to each other. As a result, acoustic trauma may cause much more hearing loss compared to chronic noise. There is no single degenerative mechanism taking part in noise-induced cell injury. As a result of more than one stimulus such as free oxygen radicals (FORs) and calcium increase may play role in noise-induced cell injury. The most important injury caused by FORs is mutations in DNA component of nucleus and mitochondria. The greatest resource of toxic oxygen radicals is electron transport chain. That is why mitochondrial DNA is injured much more by FORs and that leads to mutations affecting energy metabolism. As a consequence of mitochondrial damage that exhibits electromechanical differential within other cells, ATP production is broken down. Finally cellular necrosis and apoptosis occur then hearing loss becomes [3,4].

In our study, we tried to explain role of oxidative stress in pathogenesis of hearing loss in NIHL and we aimed to investigate relationship between serum electrolyte levels and hearing loss if it is present. For this purpose we investigated hearing loss and serum levels of vitamin B12, folic acid, zinc, magnesium and malondialdehyde (MDA) in individuals subjected to high intensity noise.

MATERIAL AND METHODS

This study is performed on 135 healthy male military personnel with normal hearing levels. Their ages were 21 to 24 years old (mean age 22). All individuals were subjected to acoustic trauma during Habitable Place Battle Training (HPBT). This training is a routine for Infantry School Commandership training program. A G3 A3 automatic infantry rifle with 7.62 mm caliber was used in training, and shoots were sequentially repeated for 21 times. This process takes about 3 minutes at most. All subjects were exposed to acoustic trauma with intensity between 134 to 142 dB Sound Pressure Level (average 138 dB) according to our measurements. They were all informed for the risk of the blast injury by trainers but because of training standards, protection against the sound was not an obligation. Almost all of the participants did not used ear plug or goggle during training. The volunteers among individuals were accepted to the study. Before being drafted, all the participants without protection against sound were examined and informed. Sound intensity was measured by "Radio Shack Sound level meter (Texas, USA)" device. Examination and laboratory study of participants were made in GATA Haydarpasa Training Hospital, Biochemistry and Audiology Laboratories. The study program was approved by the ethics committee of our hospital, and each participant provided signed informed consent after receiving and extensive explanation of the examination.

Complete blood count, blood and urine biochemical analyses (fasting blood glucose, AST, ALT, alkaline phosphatase, lipid profile, urea, creatinine, T3, T4, TSH) were done before acoustic trauma. Individuals with abnormal results are excluded from study.

Neuro-otological examination and pure tone audiometry including high frequencies were performed to all participants one day before acoustic trauma. Audiometry was done by the same audiometrist with "Interacoustic AC-40 (Denmark)" model audiometer. Participants with abnormal results, those with ear and/or mastoid surgery history and those with hearing loss were excluded from study. Those with hearing loss more than 24 dB at high frequencies and those using ototoxic medications were also excluded from study. Pure tone audiometry was repeated 24 hours and one month after acoustic trauma in order to avoid temporary threshold shift.

All participants' serum level of vitamin B12, folic acid, zinc, magnesium and MDA were measured before and 4 days after acoustic trauma.

Measurement of serum vitamin B12, folic acid, zinc, and magnesium were performed by means of microparticle enzyme immunoassay method with "Abbott Axsym System (Germany)" automatized analysator. MDA levels were measured by Shinmadzu RF 5000 spectroflorophotometer (Spain). In the existence of antioxidant, MDA hydrolyzing itself in acid environment at 95 0C reacts with 1,3-diethyl-2-thiobarbituric acid (DETBA). After this reaction recent formed complex can be measured with synchronized florophotometer and MDA level can be detected.

Statistical test were made using SPSS (Statistical Package for the Social Sciences for Windows 11.0). Descriptive statistics were calculated for individuals demographics. Mann-Whitney U Test was used to analyze the non-parametric data. Student t-test was used to analyze the parametric data. Statistical significance was considered at $P < 0.05$.

RESULTS

Taking into consideration that American Academy of Otolaryngology and Head & Neck Surgery Foundation's suggestion; "It should be accepted as acoustic trauma depending on hearing loss when a change of 15 dB or more at 500, 1000, or 2000 Hz; or 20 dB or more at 3000,4000 or 6000 Hz frequencies in consecutive audiograms". These values were accepted as standard in our study [5].

We have detected permanent hearing loss more than 20 dB (37.5±17.5) at 2000-6000 Hz in 83 participants (62 %) (Paired Sample T Test $p<0.05$). No significant difference was found between high frequency audiometer thresholds (8000-20,000 Hz) (Mann-Whitney U Test, $p<0.05$).

There was no significant relationship between hearing loss and serum levels of vitamin B12, folic acid, zinc and magnesium measured before and after acoustic trauma (Mann-Whitney U Testi, $p<0.05$). After acoustic trauma, serum levels of MDA have increased, and this increment was statistically significant (Paired Samples T Test, $p<0.05$). However there was no statistically significant difference in serum MDA levels between those with hearing loss and those with no hearing loss (Mann-Whitney U Test, $p<0.05$) (Table 1).

Table-1: Average values of biochemical tests (n=patients'number)

	Group with hearing loss (n: 83)			Group without hearing loss (n: 52)			Normal Laboratory value (mg/dl)
	Before trauma (mg/dl)	After trauma (mg/dl)	P	Before trauma (mg/dl)	After trauma (mg/dl)	P	
B12	257,3	269,6	p>0.05	189,7	242,7	p>0.05	220-914
Folic Acid	4,6	4,9	p>0.05	4,6	4,9	p>0.05	3,1-17,5
Zinc	106,5	101,9	p>0.05	105,9	105,1	p>0.05	73-140
Magnesium	2,6	2,5	p>0.05	2,6	2,5	p>0.05	1,9-2,5
MDA	112,9	451,2	p<0.05	111,9	462,7	p<0.05	80-284

DISCUSSION

While cochlea transforms acoustic energy into neural signals, some FORs appear and intracellular defense mechanisms against FORs protect inner ear. However at acoustic trauma these mechanisms fail and hearing loss happens irreversibly. Reinforcement of natural defense mechanisms prevent or lessens hearing loss [6].

FORs are products of routine aerobic cellular metabolism and they disrupt structure of lipids, proteins and nucleic acids [7]. Lipid peroxidation products caused by FOR can be measured. Measurement of serum MDA level can be used in order to indicate oxidative stress [8].

After exposing 4 kHz octave 115 dB SPL noise for 5 hours to guinea pigs, Ohinata et al demonstrated that lipid peroxidation increases directly proportional to duration of noise exposure. In the wake of lipid peroxidation, apoptosis become stimulated then apoptotic cell death occur [7].

Liu showed that after acoustic trauma in cochlea, MDA level elevates in first hours, reaches peak level at third to sixth day, returns to normal level at eighth day, then makes a second peak at twelfth day. They concluded that MDA level increases as a result of NIHL [9].

Derekoy applied acoustic trauma at 100 dB SPL intensity for one hour to guinea pigs, then showed that serum MDA level increased and glutathione level decreased. They concluded that there is a relationship between noise and body antioxidant system, and noise induces oxidative stress not only in cochlea but also in whole body [10].

In our study, serum MDA level is measured for the purpose of indicating oxidative stress resulting from acoustic trauma. After acoustic trauma, serum levels of MDA have increased and this was statistically significant when compared to the levels of MDA before acoustic trauma ($p<0.05$). However there was no statistically significant difference in MDA level between those with hearing loss and those with no hearing loss ($p<0.05$). Since high oxidative parameters have been found in all participants subjected to acoustic trauma and hearing loss has been observed in only 83 participants (%62), there should be some other searches delving into factors causing hearing loss or limiting hazardous effects of oxidative stress (antioxidant enzyme levels, trophic factor levels).

Magnesium plays an important role in energy turnover of hair cell. In magnesium deficiency, noise has more destructive effect. Attias et al. exposed 90 dB sensational level noise to 20 healthy individuals with mean age 21 and administered 122 mg magnesium per day during 10 days. They have shown that magnesium administration protects cochlear function and accelerates recovery of hearing [4]. In another study, 300 individuals with normal hearing have been administered 167 mg magnesium per day during 2 month- period of military training. Individuals not having magnesium intake have experienced noise induced hearing loss at the end of training [11].

Experimental studies have shown that NIHL increased in case of magnesium deficiency. Protective mechanism of magnesium in NIHL has not been proven. Magnesium is physiologic antagonist of calcium. Its protective effect attributed to this antagonism. In conclusion it is thought that magnesium shows protective effects by decreasing oxidative stress, vasospasm, neurotransmitter release, vasoconstriction and calcium intoxication [12].

After increasing mononuclear magnesium level, hearing thresholds due to acoustic trauma decreased [12]. Scheibe et al. claimed that taking more magnesium in diet may increase blood flow to inner ear, and thus provides more oxygenation and hinders NIHL [13]. However in our study there was no significant relationship between serum level of magnesium and hearing loss ($p<0.005$).

Gok et al. investigated serum Vitamin B12 and folic acid levels in patients working in hydroelectric plant and found that level of vitamin B12 in patients with acoustic trauma was 199,87±75,25 pmol/L and was 323,62±121,91 pmol/L in control group

(p<0.001). Level of folic acid in patients with acoustic trauma was 10,71±4,16 µmol/L and was 12,69±3,61 µmol/L in control group (p<0.05). In the view of these findings they recommended that people working in noisy place should be checked regularly with measurement of serum level of Vitamin B12 and folic acid [14].

Harada et al. treated 52 patients with acute acoustic trauma. 60 mg prednisolone, low molecular weight %10 dextran and 1,5 mg vitamin B12 have administered in 42 patients and 10 patients after seven and ten days respectively. It has been found that early beginning of treatment have good affect for hearing recovery but no significant relationship between age, ear plug usage and medication of prednisolone, low molecular weight dextran and vitamin B12 (p<0.05) [15].

Shemesh et al. found vitamin B12 deficiency (<250pg/ml) in 47% of 113 soldiers with mean age 39. After vitamin B12 treatment, 12 soldiers recovered from their tinnitus. Authors recommend measurement of serum B12 level for patient with chronic tinnitus in a routine fashion [16].

Quaranta revealed protective effect of vitamin B12 in guinea pigs to which he exposed 112 dB SPL noise for 10 minutes [17]. Patients with low serum level of vitamin B12 and folic acid were more prone to hearing loss due to acoustic trauma [18,19]. However in our study there was no significant relationship between hearing loss and vitamin B12 -folic acid level (p< 0.05).

Zinc has protective effect against oxidative stress and prevents free radical formation [20]. Since oxidative stress has a role in pathogenesis of acoustic trauma [21,22] there were no significant difference in serum zinc level of patients with hearing loss between before and after acoustic trauma (p< 0.05).

CONCLUSION

There was no significant relationship between hearing loss due to acoustic trauma and serum level of vitamin B12, folic acid, zinc, magnesium (p>0.05). However there was a statistically significant difference in serum levels of malondialdehyde between before and after acoustic trauma in all subjects (p<0.05). These results indicate that free oxygen radicals play an important role in the pathophysiology of acoustic trauma, and acoustic trauma causes stress by increasing free oxygen radicals in whole body. However every participant with increased MDA level did not suffer from hearing loss. This finding tells us that more potent antioxidant defense mechanisms may be playing roles in oxidative stress.

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