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The Concurrent Effect of Lead and Noise on Noise-Induced Hearing Loss at 4 kHz Frequency: An Experimental Study

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Abstract

Background: Hearing loss is an occupational chronic disabling and is due to complex processes of numerous risk factors such as excessive noise, ototoxic agents and aging. We aimed to examine the concurrent effect of lead and noise on rats' noise hearing function as a sub-acute exposure.

Materials and Methods: In this experimental study, 40 male rats were divided into 4 groups as 1) control group 2) exposed to 105 dB noise at 4 kHz frequency 8h/day, 6day/week for 4 weeks 3) exposed to 4 mg/kg lead acetate by gavage 6day/week for 4 weeks 4) exposed to both lead and noise. Blood lead levels were evaluated by Graphite Furnace Atomic Absorption Spectrometry. In addition, before and after exposure, the auditory brainstem response (ABR) was performed to examine the hearing loss in rats.

Results: The hearing threshold at noise exposure (105 dB at 4 kHz frequency) group indicated a significant increase (10 dB and 11.5 dB with click and tone burst stimuli, respectively) compared to the control group ($p<0.0001$). Moreover, there was a significant difference between the lead concurrent noise-exposed group and the control group regarding the latency of waveform II with both stimuli ($p<0.0001$). There was a positive correlation between Blood Lead Level in lead-treated rats with Auditory Brainstem Response threshold by tone burst stimulus ($r = 0739$, $p = 0.015$).

Conclusion: we concluded that lead and noise have a synergistic effect and can exacerbate hearing loss. However, additional studies at various doses are needed to confirm this finding.

Keywords: Hearing Loss, Lead, Heavy Metals, Noise, Blood, Auditory Brainstem Response

Introduction

Hearing impairment is an occupational chronic disability all over the world [1, 2]. Accordingly, hearing disorders have been recommended as one of the high priority research areas of the 21st century by the National Institute for Occupational Safety and Health (NIOSH) [3]. Hearing loss is a

serious defect that affects communication, leads to a decrement in quality of life and speech recognition, and decreases understanding of audible warnings [2, 4]. Furthermore, it is correlated with social isolation, cognition, functional reduction as well as poor psychosocial health such as depression and loneliness [5, 6]. Long-term exposure to noise affects the sensory hair cells of the inner ear and causes hearing loss especially in the range of 3–6 kHz [7, 8].

Even though noise exposure above 85 dB is regarded as the main risk factor in the progression of hearing loss, recent documents have indicated that heavy metals are additional significant contributors to occupational hearing impairment both alone and when combined with noise exposure. Any material, like drugs or chemical materials that can affect the hearing and/or balance and are poisonous to the auditory or vestibular system, is known as “ototoxic” and “vestibulotoxic” substances [9, 10]. Ototoxic substances, including solvents (example: toluene, styrene, and so on), heavy metals (such as lead, cadmium, arsenic, and so on), and drugs (aminoglycoside antibiotics; some chemotherapeutic drugs) are widely used in industries [11-16].

Lead is certainly one of the common heavy metals used in industries. Occupational lead exposure includes glass factories, mining, steel plants, agriculture, and other industries using lead-based products [17, 18]. Non-occupational exposure includes the use of gasoline with lead added ingredients, incineration of waste containing lead, paints comprising lead, lead-acid batteries, and so on [19-22]. Lead is accumulated in water and soil and is absorbed into the human body principally via inhalation and ingestion [23, 24]. Recent evidence indicates that acute and chronic exposure to lead may have harmful effects on the kidneys, blood, brain, vitamin D metabolism, and central nervous system [25, 26, 15, 27]. Moreover, the Centers for Disease Control and Prevention (CDC advisory committee report, 2012), suggested that blood lead levels ≥2 µg/dl (under the action level (5 µg/dl)) were associated with hearing disorders and disrupting the structure and the function of the auditory system [26].

Although the ototoxic effect of lead is yet to be fully understood, experimental research indicates that it can injure the cochlea or central auditory system via reactive oxygen species (ROS) production and apoptosis, causing impairment in the auditory nerve and hearing loss [28, 29]. Furthermore, it seems that exposure to high-intensity noise may damage the stereocilia of hair cells and also can produce hair cell loss and even lead to the collapse of Hensen cells that are associated with temporary and permanent hearing loss [30, 9].

Although research on both animals and humans have investigated the effects of noise and lead alone, few studies have concentrated on the effects of co-exposure to lead and noise on hearing ability. Therefore, this study aimed to investigate the effects of concomitant exposure to noise and lead on male rats’ hearing function as a subacute exposure.

Materials and Methods

In this experimental study, 40 male rats weighing 250-300g were chosen for the experiment and purchased from the Experimental and Comparative studies center, Iran University of Medical Science, Tehran, Iran. In addition, all the experiments were performed according to ethical standards in the university guideline considerations. The work has been approved by the Ethical committee of the Iran University (IR.IUMS.REC 1395.9413139004) (The code of ethics was taken in 2016 but after getting the budget for several months, we were able to design and build an animal exposure chamber. Next we started the practical steps. Therefore, this process was very long.).

They were kept in a place with an ambient temperature (22˚c ±2), alterable ventilation, and relative humidity (40-50%) in a 12:12 light-dark cycle; they were fed by libitum and water. The rats were randomly categorized into 4 groups, 10 rats per each [31]: control group (I), noise exposure group (II), lead exposure group (III) [31], and lead plus noise exposure group (IV). The rats in the control group did not receive anything except standard diet and water. The rats in the noise exposure group were exposed to 105 dB in a frequency 4 kHz for 8h/day, 6 days/week for 4 weeks. The animals in the lead exposure group received 4 mg/Kg body weight (BW) lead acetate (prepared with distilled water) by gavage 6 days/week for 4 weeks (the half-life of lead in rat’s blood is a few weeks (28-36 days)) [31] and the last group exposed to lead (4 mg/Kg BW, by gavage) plus noise (105 dB at 4 kHz frequency) 6 days/week for 4 weeks. The animals of control and noise groups received distilled water via gavage instead.

According to the prior research on rats, ABR thresholds were noted at 4, 8, 16, and 32 kHz following the 100 and 110 dB SPL noise exposures. The reason for choosing an octave band noise of 4 kHz was that considering the physiology of cochlea, the synaptopathic noise would result in a threshold shift mostly in half an octave or an octave above the offending

frequency. As our electrophysiological device could assess up to 16 kHz, the synaptopathic effect of 4 kHz noise could be fully monitored. Also, it seems that exposure to noise at levels of 100–110 dB SPL is needed to cause measurable hearing loss in the rat model, therefore we selected 105 dB in our study [32]. Lead acetate (Pb(C2H3O2)2) powder (CAS: 107375) was purchased from MERCK.

A small box was fabricated in dimensions of 80*80*95 cm as noise exposure. Two speakers were above the chamber and the animals were housed alone in an individual cage (10 animals can be placed in the box). 4 holes were designed on the center of sidewalls to measure and monitor the room circumstances [33]. Conforming to the recommended situation for maintenance rats during noise exposure, chamber temperature was regulated at 22˚C using a temperature control device. Noise was generated by signal software played by the Cool Edit software on a computer and the animals were exposed to octave band noise centered at 4 kHz, 105dB, for 8h/day, 6 days/week, for 4 continuous weeks. Moreover, 4 points were chosen in the center of the walls to measure noise intensity. Measurements were carried out using sound level meter model CEL-400. The result of measurements indicated that in different parts of the chamber the level of noise was 105 ± 1dB; it confirmed that noise variation in different points of the box was negligible and alteration of noise was very low at the chamber. The animal's exposure chamber was cleaned daily. The ventilation in the room was used and the chamber had open pores for air exchange.

In this study, Auditory Brainstem Response (ABR) threshold test was evaluated as an auditory function. The ABR is one of the common electrophysiological measures of hearing and is an auditory evoked potential that occurs in the first 10 milliseconds after the onset of an auditory stimulus and provides a noninvasive measure of the health of the auditory system from the cochlea up to the brainstem. Wave II, in rats is well known as being the most dominant wave and latency in waveform II indicates the effect of the studied variables on the auditory threshold [32]. Click and Tone burst stimuli are the most suitable stimuli for recording ABR waves. It seems that click stimulus provides a quick overall estimate of hearing ability, whereas tone burst provides frequency-specific information [32]. Before each ABR test, animals were anesthetized with a blend of 10 mg/kg xylazine and 40 mg/kg ketamine by intraperitoneal injection, and a heating pad was used to maintain the animal's normal body temperature. Performing the ABR procedure has been described previously [34]. Briefly, the electrodes were inserted at the vertex, at the back of the ears and in the animal's leg. The signal of stimulation was conducted by plastic tubes into the rat's ear canals. Sub-dermal needles were used to record brainstem-evoked responses. Alternating click and tone burst stimuli (duration 5 msec, the rate of 23.1 or 39.1 signal/sec for both tests) generated by Interacoustics EP25 (Denmark) were regularly provided to the left ear of the animal. The intensity of the stimulus was started at 70 dB and 20-dB decreased in each step. Near the threshold, it declined 5dB until physiological hearing thresholds were detectable. The ABR latency of wave II was measured. In this research, we considered the difference between the primary ABR threshold and ABR threshold at each frequency on the 30th day as the Temporary threshold shift (TTS).

The assessment of Lead Levels of Blood in Rats (BLL) was performed using Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Currently, GFAAS is one of the most generally used methods to characterize lead concentrations in blood [35]. In brief, 0.8 ml of APDC-TX100 was added to 2 ml of blood and mixed for 10 sec; next 2 ml of MIBK was added and mixed for 2 min; then it was centrifuged at 2000 rpm for 10 min and analyzed by GFAAS (GF5000, GBC, Australia) [36].

In this work, SPSS.V.22 was used to analyze the data. Comparisons between variables among the groups were performed with ANOVA (Bonferroni post-hoc test) and paired t-test. In addition, the Kolmogorov-Smirnov test was carried out to investigate the normal distribution of data (shown in Fig. 1). Association between hearing threshold and latency of wave II with blood lead level were measured by Pearson correlation. All of the data were expressed as mean ± SD. Outcomes with p-values< 0.05 were considered statistically significant.

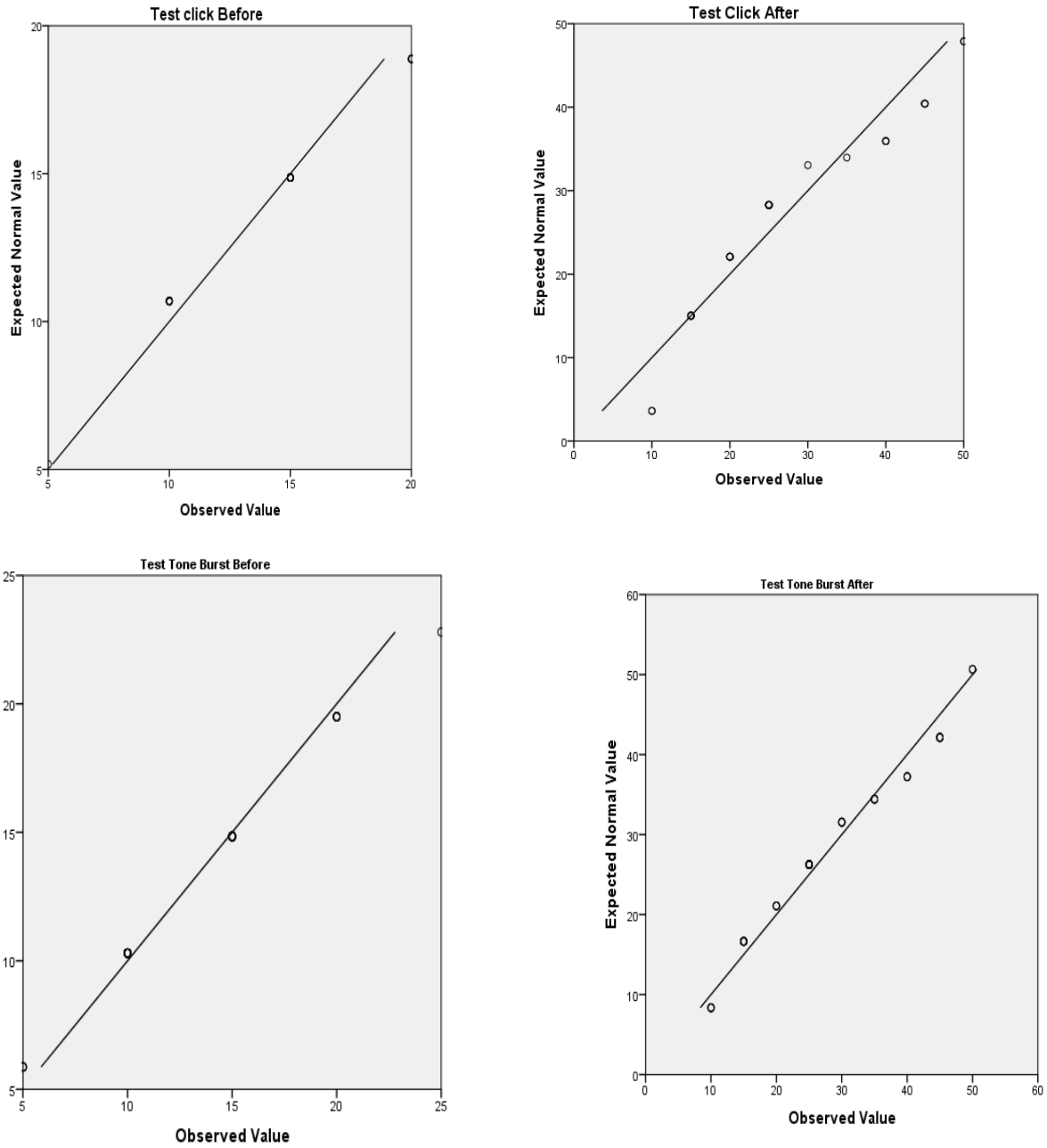

Fig.1. Normal Q-Q plot for click and tone burst test before and after exposure.

Results

The blood lead levels of four groups of rats were analyzed using Graphite Furnace Atomic Absorption spectrometric method, and the results are shown in Table 1. The average BLL of the lead exposure group and lead + noise group were appreciably higher compared with the control group ($p<0.0001$).

Table 1. The mean value and standard deviation (SD) of blood lead levels at the control and exposure groups (N=10)

Groups	Lead level of blood(µg/dl)
Control	0.27 ± 0.01
Noise	0.26 ± 0.008*
Lead	2.89 ± 0.2**
Lead + Noise	2.95 ± 0.3**

P-Value of each variable is represented compared to the control group. *p> 0.05, **p< 0.000. One-way ANOVA (post hoc Bonferroni)

Table 2. Average ABR thresholds determined with click and Tone burst stimuli before and after exposure in each group (N=10)

Groups	Click test		Tone burst test at 4 KHz	
	Before exposure	**After exposure**	**Before exposure**	**After exposure**
Control	13 ± 3.4	14 ± 2.1	12 ± 4.8	13 ± 2.5
Noise	14.5 ± 3.6	24 ± 3.1	11 ± 3.9	24.5 ± 3.6
lead	14 ± 3.9	21 ± 4.1*	16.5 ± 4.1	29 ± 4.5*
Lead + Noise	13 ± 4.8	43 ± 4.7	13 ± 3.4	44.5 ± 3.6

Values are indicated as mean ± SD. P<0.0001 compared to the control group. One-Way ANOVA (Bonferroni post hoc) and paired t-test were applied. Examination of data normality was performed with Kolmogorov-Smirnov test (p>0.05).
*p>0.05 vs noise group

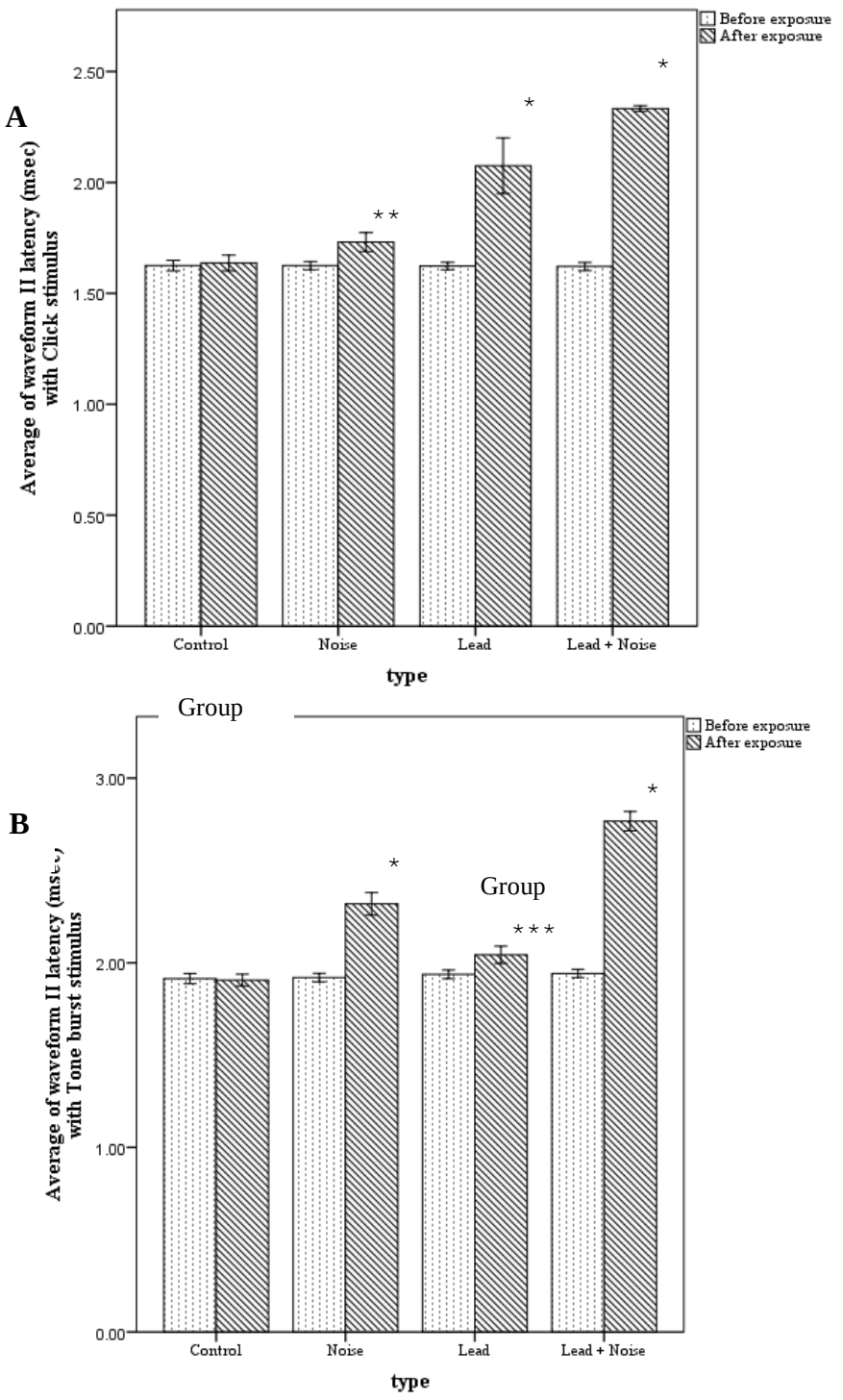

Fig. 2. Comparison of waveform II latency with click stimulus (A) and tone burst stimulus (B) before and after exposure in groups exposed to noise (105 dB at 4 KHz frequency), lead (4 mg/kg lead acetate by gavage) and lead + noise (4 mg/kg lead acetate by gavage + 105 dB at 4 KHz frequency) and the control group. *p<0.0001, **p<0.37, ***p<0.002 compared to the control group. Paired t-test and one-way ANOVA (Bonferroni post hoc) was performed. Kolmogorov-Smirnov test was performed to examine data normality (p>0.05).

In the present study, we used click and tone burst stimuli to investigate the hearing threshold and latency of waveform II (the dominant wave in rats). The ABR threshold of the groups is presented in Table 2 and the latency of waveform II is shown in Fig. 2. As explained earlier, The ABRs test was performed for all rats before and after exposure. No remarkable difference was identified between the study groups in the hearing threshold before exposure ($p<1.000$). Investigation of the hearing threshold at noise exposure (105 dB at 4 kHz frequency) group indicated a significant increase (10 dB and 11.5 dB with click and tone burst stimuli, respectively) compared to the control group ($p<0.0001$).

Furthermore, exposure to lead acetate (4 mg/kg per day) once a day for a period of 4 continuous weeks, revealed a considerable increase in hearing threshold 7 dB with click and 16 dB with tone burst 4 kHz compared to the control group ($p<0.0001$). Nevertheless, there was no considerable difference between hearing threshold in lead and noise groups ($p>0.05$).

In addition, exposure for 4 successive weeks with lead acetate and noise (105 dB at 4 KHz frequency & 4 mg/kg per day) notably raised the hearing threshold and showed a greater hearing impairment than exposure to lead or noise alone ($p<0.0001$). The results of the hearing test in the noise group and lead group showed an increase of about 1.5 times in the hearing threshold, while the results of the group of exposure to both noise and lead showed an increase of about 3.5 times, which might be due to the synergistic effect of noise exposure and lead on hearing loss.

Furthermore, the results of the present research showed latency in waveform II in exposure groups. At the end of the experiment (30th day), the latency of waveform II with tone burst stimulus for the noise group was significantly ($p<0.0001$) more than that in the control group. Nevertheless, the latency of waveform II with click stimulus in this group showed no difference from the control group ($p>0.05$).

Moreover, for lead (4 mg/kg per day) treated rats, as compared with the control group, significant latency of wave II was observed 4 weeks later ($p<0.0001$). The mean value of latencies of waveform II were 1.63 ± 0.05 and 2.07 ± 0.19 ms with click stimulus and 1.90 ± 0.05 and 2.04 ± 0.07 ms with tone burst stimulus for control and lead groups, respectively.

Additionally, the findings of the present study demonstrated a significant difference between lead plus noise exposed group and the control group regarding the latency of waveform II with both stimuli ($p<0.0001$). The average of latencies of waveform II were 1.63 ± 0.05 and 2.33 ± 0.02 ms with click stimulus and 1.90 ± 0.05 and 2.76 ± 0.08 ms with tone burst stimulus for control and lead plus noise group, respectively.

Likewise, the latency of waveform II of animals exposed to lead plus noise was remarkably higher than that of the animals which were exposed to noise or lead alone ($p<0.0001$).

Clinically, for rats, the latency of wave II is used as a dominant wave to distinguish the brainstem center lesions [37, 38].

The correlation between blood lead levels (BLL) and the results of all tests is represented in Table 3. The Pearson correlation represented that there is a positive correlation between BLL in lead-treated rats with ABR threshold by tone burst stimulus ($r = 0739$, $p = 0.015$). Nonetheless, there was no correlation between blood lead levels and ABR threshold with click stimulus and the latency of waveform II with both stimuli.

Also, there was no significant relationship between BLL in lead plus noise group and the outcomes of all experiments.

Table 3. Correlation between ABR threshold and latency waveform II (click and tone burst stimuli) with blood lead concentration in study groups (N= 10)

		ABR Threshold with click	ABR Threshold with tone burst	Latency waveform II with click	Latency waveform II with tone burst
		r (p)	r (p)	r (p)	r (p)
Blood lead levels	Control	0.005 (0.989)	-0.293 (0.411)	-0.171 (0.637)	-0.433 (0.211)
	Noise	0.44 (0.203)	-0.40 (0.912)	0.041 (0.911)	-0.459 (0.182)
	Lead	0.18 (0.618)	**0.739* (0.015)**	0.061 (0.867)	0.448 (0.194)
	Lead + Noise	0.323 (0.362)	0.342 (0.333)	-0.247 (0.492)	-0.378 (0.282)

Pearson correlation = r, sig. (2-tailed) = p,*p<0.05. Pearson correlation was applied. Examination of data normality was performed with Kolmogorov-Smirnov test (p>0.05).

Discussion

In the workplace, there are numerous sources of occupational exposure and, as a consequence, workers may be simultaneously exposed to many of them [39]. While exposure to very extreme noise is certainly the main cause of job-related hearing loss, new evidence refers to the additional role of heavy metals in occupational hearing impairment [40, 41]. Few studies have been conducted to identify the co-exposure effects of lead and noise on hearing function [42]. Therefore, this study was carried out to determine the effect of coinciding exposure to lead and noise on hearing function in male rats. From the ABR test of control groups, it can be discerned that there was no difference between hearing threshold before and after exposure. Moreover, the waveform of the ABR was normal for this group. For the lead-exposed group, the latency of wave II was prolonged and their hearing was damaged by lead exposure. Similarly, in the noise-exposed group, there was a significant difference between the hearing threshold and latency of wave II before and after exposure. Moreover, the results of our study indicated that the hearing threshold was considerably enhanced at the time of simultaneous exposure to noise and lead compared to noise or lead alone. However, there was no significant difference between hearing threshold in lead and noise groups. In the same way, Johnson and Morata indicated that the risk of hearing loss with simultaneous exposure to lead and noise is greater than exposure to ototoxic agents alone [9]. Likewise, Park et al. in their research found that hearing threshold changed owing to lead exposure, especially in the frequency of 4 KHz [43].

Furthermore, the results of the current study are in line with previous reports on lead exposure in the presence of noise. A study performed by Counter et al. showed a sensory-neural hearing loss in men, exposed to a combination of lead (long-term) and noise [44]. A study by Choi and Kim showed that the probability of hearing loss in the 2000-4000 Hz range was 1.64 times greater for a group of persons exposed to metals (lead) than in unexposed individuals [3].

According to the previous evidence, there are two mechanisms for noise-induced hearing loss. The mechanical injury occurs at the excessive noise levels (damage to the organ of Corti); however, metabolism mechanism is correlated with long-term noise exposure and happens at low levels of noise (damage through oxidative stress) [45]. On the other hand, ototoxic substances may damage hair cells and might have a negative effect on conduction in the auditory nerve [46, 47]. Outer hair cell is affected by enhanced blood lead and may lose their protective damping effect for inner hair cells. Thus, inner hair cells might be damaged with exposure to the lower levels of sound [17]. It seems that in case of simultaneous exposures, hearing disorder can occur through cochlear destruction and damage the central and peripheral auditory system [46, 48].

However, animal studies have revealed conflicting results about the effect of lead on hearing function. According to Alimohammadi et al., lead exposure in rats can cause hearing loss [49], but some studies have demonstrated that this does not happen [50-52].

Overall, based on these results, it could be concluded that occupational deafness may be a result of not only noise but also occupational lead exposure. In the current study, the effects of lead on hearing were observed at a frequency of 4000 Hz.

In addition, the results of the current study also showed that there was an association between blood lead level and hearing threshold with tone burst stimulus. In line with this, Hwang et al. demonstrated that there is a notable relationship between blood lead level (7 µg/dl) and noise-induced hearing loss [17]. Also, another study showed that hearing loss at high-frequency was associated with blood lead levels lower than the threshold limit [26]. By contrast, Carlson et al. were unable to prove the relationship between blood lead level and hearing loss [52]. There were some limitations in our study including sound generator device failure, animal death, Lack of funds to examine animals as sub-chronic study, and so on.

Conclusion

The results of the present study indicated that in addition to noise, ototoxic agents could damage hearing function. Moreover, concurrent exposure to lead and noise plays an important role in increasing hearing loss compared to noise and lead exposure alone. Also, we showed that there is a positive relationship between blood lead levels and hearing impairment. Therefore, co-exposure to lead and noise have synergistic effect and can exacerbate hearing loss. Future studies need to be done to specify the effect of various concentrations of ototoxic substances and different levels of noise on hearing system.

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