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RESEARCH PAPER

The Effects of Occupational Noise Pollution and Shift Work on Oxidative Stress Markers in Cement Workers, Iran

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ABSTRACT

Both noise and shift work generate oxidative stress, independently; however, in some work places workers are exposed to both at the same time, where their combined effect might increase the oxidative damage. This research is based on the question whether noise and shift work have a synergistic effect on oxidative stress or not. It tries to investigate the effects of these two factors simultaneously, at the biggest cement factory of Iran. For so doing, it enrols 88 male workers, equally in four groups, with one group serving as the control (i.e., Group 1 with 8 hours of fixed shift, exposed to less than 85 dB sound level) and three groups as the cases (Group 2 with 12 hours of rotational shifts, exposed to less than 85 dB sound level; Group 3 with 8 hours of fixed shift, exposed to more than 85 dB sound level; and Group 4 with 12 hours of rotational shifts, exposed to more than 85 dB sound level). Stress oxidative is evaluated by Malondialdehyde (MDA) and Superoxide dismutase (SOD). Finally, the results show that SOD levels ($p<0.001$) are significantly decreased in Group 4 and Group 3, compared to the control. Also, MDA levels are significantly increased in Group 4 (in which, the workers are exposed to noise and shift work simultaneously) compared to the control ($p < 0.001$). The current study shows that co-exposure to noise and shift work has a combined effect (a synergistic role) in MDA. Thereore, more attention should be paid to shift workers, who are exposure to noise simultaneously.

Keywords: Occupational hazard; Superoxide Dismutase; Malondialdehyde, Workplace

INTRODUCTION

Daily exposure to hazardous factors in workers (i.e., noise, sleep deprivation, mental stress, bad dietary pattern) can disrupt the reactive oxygen species (ROS) balance or antioxidant defense, which can lead to oxidative stress and inflammation (Gowda et al., 2019; Khajehnasiri et al., 2013; Daiber et al., 2020; khorasaniha et al., 2020). Severe life stress, including sleep deprivation as induced by noise exposure during sleep, leads to cerebral oxidative stress. (Hahad et al,2019). With industrialization, more people are exposed to noise and shift working (Noorpoor et al., 2016). Noise exposure and shift working are serious environmental stressors leading to numerous disturbances in human and non-human species. occupational noise is mentioned as a significant environmental health risk factor in the Global Burden of Disease (GBD) Study (GBD 2016 Risk Factors Collaborators , 2017).

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Oxidative stress is a topic of interest as harmful effects of noise. Free radicals were produced by noise exposure. Evidence has shown that noise causes HPA axis activation and instantaneous secretion of corticosteroids (Said & El-Gohary, 2016). The increased Corticosterone level accelerates the production of free radicals, which in turn suppresses the immune system. Due to the highest percentage of unsaturated fats in the neurons, cerebral tissues are highly sensitive and vulnerable to oxidative stress damage caused by lipid peroxidation. It is reported the levels of MDA were significantly higher in the staff exposed to noise than in the controls (Hosseinabadi,et al 2019). In addition to noise, shift work is also a factor to cause stress in employees (Khajehnasiri, et al., 2013; Zare, et al., 2019). 15 to 25 percent of the workforce does not work according to routine-working hours (from 7 or 8 AM until 4 or 5 PM), in other words their work schedule is called shift work. Shift work often causes abnormal lifestyles, that can cause workers sleep disorders, problems with circadian rhythm, and even different kinds of stress (Filip, et al., 2017). In the shift work program, the quality and quantity of sleep is reduced. In fact, the circadian rhythm is disturbed, and this disorder affects the behavior of the immune system and oxidative stress. The circadian cycle is essential for maintaining the balance of many vital functions. Disturbance in this cycle is a risk factor for metabolic disorders and an increase in the metabolites in blood, which can be the cause of non-communicable diseases, and a change in the levels of antioxidant enzymes (Nserat, et al., 2017; Kröller-Schön, et al., 2018).

Malondialdehyde (MDA) levels, a marker of lipid peroxidation (Sousa, et al., 2017; Cipak Gasparovic, et al., 2017) represents the most investigated end product of lipid oxidation. Also, superoxide dismutase (SOD), is the best-known defense system against enzymatic scavengers of ROS (Wang, et al., 2018). Superoxide Dismutase (SOD) as a biomarker of oxidative stress, is an enzyme catalyst that converts superoxide radicals into harmless hydrogen peroxide and oxygen molecules.

Simultaneous exposure to highly-used chemical and physical agents is expected in many occupations and has a synergistic effect on worker health Also, the effect of noise and dust exposure and noise and carbon monoxide had this synergistic effect (Mirmohammadi, et al., 2020; Bagheri et al., 2020).However, based on the search of the research team, the simultaneous impact of noise and shift working on oxidative stress was not recognized in literature.

In previous studies, the results of the effects of sound and shift work on oxidative stress have been reported independently of each other (Hermansson, et al., 2019; Bhatti, et al., 2017). And most of those studies have been in medical and hospital settings, and few studies have been conducted in industry, few studies analyzing the combined effect of sound and circadian rhythm, have been done on animals (Teixeira, et al., 2019; Nadri, et al., 2018).

A synergistic impact on oxidative stress indices of subjects exposed to noise and shift working schedule is expected. So, in this study we amid to determine the impact of simultaneous exposure to noise and shift working. By comparing the indicators of oxidative stress (MDA) and antioxidant defense (SOD) between shift workers exposed to noise (sound pressure level above 85 decibels) and the control group, we can determine the effect of these harmful occupational factors on the state of oxidative stress. The additive effects of noise and shift work might have increased the oxidative damage.

Therefore, to determine the combined effects of noise and shift work on oxidative stress in a human study, we studied cement workers, in one of the most important industries in Iran, for the effects of sound pressure level above 85 decibels and shift work, on MDA levels, SOD enzyme as stress oxidative indicators.

MATERIAL AND METHODS

This case-control study was conducted with the aim of determining the combined effects of sound and shift work on MDA and SOD oxidative stress parameters, in the blood serum of employees in one of the cement factories in Tehran.

This study was conducted according to the guidelines in the Declaration of Helsinki and approved by the Ethical Committee of Tarbiat Modarse University with code IR.MODARES.REC.1397.089. Informed consent was obtained from all study participants. The study was conducted at Tarbiat Modares University, Faculty of Medicine, and Department of occupational health.

We recruited male volunteers, who had been employed for at least one year in the cement company in Tehran Iran. Subjects, who decided not to continue the study, were excluded from the study.

Based on previous studies, the sample size was determined to be 22 people in each group (Yildirim, et al., 2007). 88 workers were globally divided into four groups: The case groups (three groups) of this study were the workers (totally n=66, in each group n= 22), who work in a high-noise workplace (higher than 85 dB) or work in rotational shift work program (from 7AM by 7PM or invers, after 12 hours of work, they had 24 hours of rest.). The control group (n= 22) were the workers, who work in a low-noise workplace (lower than 85 dB) and in fixed work program (from 7AM by 3 PM).

Criteria for entering the study included at least one year of work experience, lack of chronic diseases, a specific drug use, history of tobacco use and alcohol consumption. These data were extracted from the medical records of the employees in the factory clinic. Subjects, who decided not to continue the study or changed their work schedule or work locations were excluded from the study.

Environmental noise was measured to determine people's exposure to noise. All measurements were performed in the morning shift and in sunny weather (average ambient temperature 22. C, humidity 38%, and pressure of 208 mm Hg and very low wind speed). Environmental sound measurement was performed in different units of the factory according to the standard method (1997) ISO 9612, regular network measurement method. Here, the unit under study was divided into equal squares (10×10 square meters) and the centers of these stations were determined as measuring points. In cases where the designated station was located on a device or location that could not be measured, that point was removed as a blind spot from the sum of the measuring points. Because of this industrial complex, the ambient sound was continuous and sound changes were insignificant, three sound measurements were performed at each point, and the logarithmic average of these three readings was registered (Golmohammadi, et al., 2010). Sound measurement was performed on network A and Slow response speed using Casella-Cell sound-level meter 6X0 made in England. To obtain more accurate and reliable results, the sound-level meter was calibrated before each measurement by the Cel 1/110 calibrator. The microphone of this device was a capacitor condenser microphone. All blood samples were collected in tubes containing k-EDTA and for separation of the serum was centrifuged for 10 minutes at 2,000 rpm. The plasma samples were stored at –20℃.

The assessments, which were performed during one regular day of work, to measure MDA and SOD from each worker, 4 cc of blood sample were taken by the nurse in a fasting state of 11 hours from 7 AM to 8AM. It should be noted that people were sitting for sampling. The blood samples were centrifuged at 2000 rpm for 10 min. After separating blood serum, the samples were transferred to a medical diagnostic laboratory and stored at -20^0C until analysis.

To analyze MDA, the German-made ZellBio GmbH kit was used for measuring. In this method production of Malondialdehyde (MDA) and thiobarbituric acid (TBA) in 90-100° C is measured at 535 (530-540 nm) colorimetrically. According to the manuscript of the kit, serial dilutions of standard solution were prepared and used for drawing a standard curve. Using this curve and its equation, the MDA rate of the samples was calculated. The method has been written in the brochure of ZellBio GmbH assay kit in detail, to analyze SOD, the German-made ZellBio GmbH kit was used for measurement. In this kit, the protocol for measuring SOD has been mentioned, superoxide anion converts to hydrogen peroxide and oxygen under SOD activity. The final product is measured at 420 nm colorimetrically.

Data analysis was performed by SPSS software (statistical package for the social sciences), version 22. One-way analysis of variance (ANOVA) and t-test was used to compare the groups; for determining the correlation between MDA and SOD and independent variables. A critical alpha of 0.05 and lower were assumed in the analysis of this research.

RESULT AND DISCUSSION

In this case-control study, to determine the combined effect of sound and shift work on MDA (µmoL) and SOD (U/moL) blood serum of cement factory workers as oxidative stress parameters, a study was conducted on 4 groups of 22 people. The cement workers, were globally divided into four groups, in each group n=22: group1 (control)- who work in low-noise work place (lower than 85 dB) and in a fixed work schedule (from 7AM by 3 PM). group 2-who work in low-noise work place (lower than 85 dB) and in a rotational shift work schedule (from 7AM by 7PM or invers, after 12 hours of work, they had 24 hours of rest). group 3-who work in high-noise workplace (higher than 85 dB) and in fixed work schedule (from 7AM by 3 PM). Group 4-who works in high-noise workplace (higher than 85 dB) and in rotational shift work schedule (from 7AM by 7PM or invers, after 12 hours of work, they had 24 hours of rest). group2, 3 and 4 were case groups.

In the subjects, the minimum and maximum of age were 25 and 50 years, with a mean and standard deviation of 36.84 ± 4.84, the range of work experience was from 3 to 25 years with an average and standard deviation of 11.40 ± 4.48 years, the BMI of the study population varied from 20.8 to 36.1 kg/m2 with a mean and standard deviation of 27.36 ± 3.36, 33% of the subjects had regular physical activity and 49% consumed the required number of fruits and vegetables.

The results of one-way (ANOVA) showed there was no significant difference in the mean age, work experience, height, weight and body mass index in different study groups ($p>0.05$). Also, there was no significant difference between marital status, physical activity and adequate fruit and vegetable consumption categories in 4 study groups (Chi-Square test $p>0.05$) (Table 1).

Table 1. Characteristics of independent quantitative variables in groups.

Variables	**Group1 low noise+no shift work**	**Group2 low noise+rotational shift work**	**Group3 high noise+no shift work**	**Group4 high noise+rotational shift work**	***P value**
Age	37.00±5.89	36.18±4.70	36.70±4.37	37.50±4.65	ns
Work experience	12.68±6.62	10.95±3.96	10.59±3.49	11.41±3.03	ns
Height	176.54±7.57	176.18±6.13	176.59±6.91	179.68±6.78	ns
Weight	83.41±13.05	84.41±11.93	86.64±10.03	88.50±11.69	ns
Body mass index	26.71±3.40	27.14±3.32	27.91±3.36	27.69±3.48	ns

*ANOVA test (Tukey test).

* ($p<0.05$) is a significant n=22 in each group (Mean ± SD)

a Group1= lower than 85 dB, work schedule from 7AM to 3 PM.

bGroup2= lower than 85 Db, rotational shift work schedule from 7AM to 7PM or inverse, after 12 hours of work, they had 24 hours of rest.

cGroup3= higher than 85 dB, work schedule from 7AM to 3 PM.

dGroup4= higher than 85 dB, rotational shift work schedule from 7AM by 7PM or inverse, after 12 hours of work, they had 24 hours of rest

The average exposed sound level in the 4 groups in comparison is shown in figure 1.

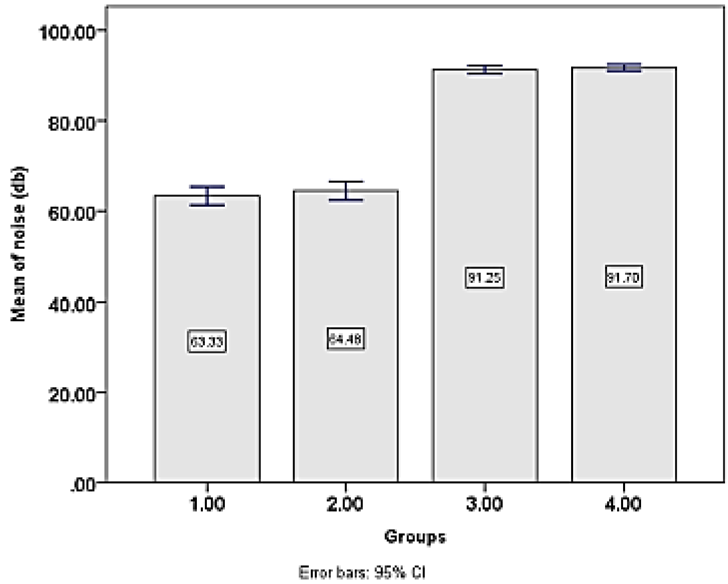


Fig 1. The sound level (dB) encountered in the 4 groups examined

1= lower than 85 dB, work schedule from 7AM to 3 PM.

2= lower than 85 Db, rotational shift work schedule from 7AM to 7PM or inverse, after 12 hours of work, they had 24 hours of rest.

3= higher than 85 dB, work schedule from 7AM to 3 PM.

4= higher than 85 dB, rotational shift work schedule from 7AM by 7PM or inverse, after 12 hours of work, they had 24 hours of rest.

Malondialdehyde is a byproduct of lipid peroxidation and a good indicator of oxidative stress. The results of our study showed that malondialdehyde concentrations of groups 2,3 and 4 increased significantly when compared to the control. This indicates that the noise stress at that sound pressure level (higher than 85 dB) caused an increased oxidation stress. One way analyze variance (ANOVA) showed, there were significant differences in serum MDA between all groups, except between groups 2 and 3. However, the amount of MDA in group 3 was higher than in group 2. Differences between groups 1 and 2, groups 1 and 3, groups 1 and 4, groups 3 and 4 were significant. ($P<0.05$, $P<0.001$, $P<0.001$, $P<0.05$), respectively (Tukey's). In other words, having a work schedule or being faced with a higher sound pressure level could lead to a significant increase in MDA. However, the findings of this study suggest that exposure to higher sound pressure levels has a more severe effect on increasing MDA than exposure to shift work. This result agrees with the result from many studies done on noise effects on lipid peroxidation. (Mirmohammadi, et al 2020; Nwuke, 2021). Also, the results of research conducted on Hosseinabadi et al study that was reported,

The MDA levels were significantly higher in the staff exposed to noise than in controls and based on the linear regression model, noise exposure level was the most important predictor variable for levels of MDA (β = 0.48, CI 95% = 0.04–0.93) (Hosseinabadi, et al 2019).

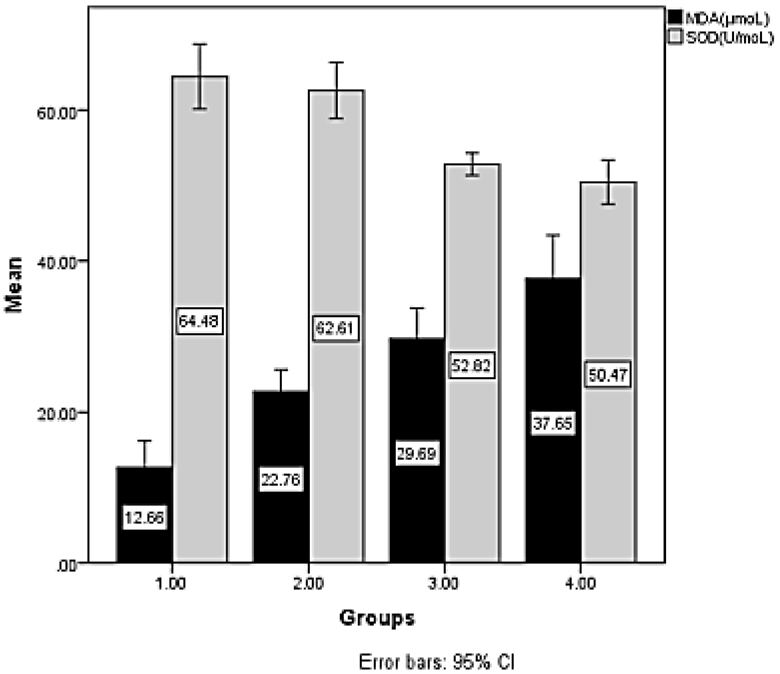
Regarding to the One way analyze variance (ANOVA) on SOD values, it was found that the SOD values were significantly different Between group 1 with groups 3 and 4 and between group 2 with groups 3 and 4 (P<0.001, P<0.001, P<0.001, P<0.001, respectively) but between groups 1 and 2, groups 3 and 4, the differences were not significant (Tukey's). Although SOD was lower in group 4 (high noise + rotational shift work) than group 3(high noise + no shift work), but the difference was not significant. So, we cannot say that high noise and shift work had a synergistic effect on SOD in this study (Table 2).

Table 2. Mean and standard deviation of Biomarkers Concentration in Different Groups among cement Workers

variables	**groups**				***P value**
	Group1 low noise+no shift work (n=22)	**Group2 low noise+rotational shift work (n=22)**	**Group3 high noise+no shift work (n=22)**	**Group4 high noise+rotational shift work (n=22)**	
MDA (µmoL)	12.66±7.85	22.76±6.41	29.69±9.17	37.65±13.02	0.000
SOD (U/moL)	64.48±9.73	62.61±8.35	52.82±3.30	50.47±6.63	0.000

n: Number of subjects.
Data are expressed as mean ± standard deviation.
p ≤ 0.05 is significant
*ANOVA test (Tukey test).
a: p<0.05, p<0.001, p<0.001, compared with MDA in group 1 with group 2 and group 3 and group 4, respectively.
b: p<0.001, compared with MDA in group 2 with group 4.
c: p<0.05, compared with MDA in group 3 with group 4.
d: p<0.001, compared with SOD in group 1 with group 3 and group 4, respectively.
e: p<0.001, compared with SOD in group 2 with group 3 and group 4, respectively.

As shown in Figure 2, moving across groups 1 to 4 the mean of MDA level increased and the mean of SOD enzyme activity level decreased.



1= no shift work & dB<85 2= shift work & dB<85 3= no shift work & dB>85 4= shift work & dB>85

Fig 2. mean of MDA (µmoL) level and SOD (U/mol) enzyme activity level in the 4 groups examined

The results obtained in this study are also in accordance with the results of research conducted on Reastuty et al that a decrease in SOD expression as well as an increase in MDA expression in rats exposed to noise happened. The decrease was found in the SOD expression in groups of rats that received noise exposure in both groups with 100 dB noise exposure and 110 dB compared to the control group (Reastuty, et al, 2021).

The results of Said research reported, that sound increases oxidation and reduces antioxidants (Said, et al., 2016). Our findings showed that between quantify independent variables, only age was directly related to MDA (p value <0.001), also it was inversely related to SOD activity level (p value <0.05) (in a stepwise regression, work experience and BMI excluded).

The study results of Bakhtiari *et al* are consistent with this finding. The result showed the mean age increased with MDA from the lowest to the highest quartile (Bakhtiari, et al., 2017).

Also, the results of this study were in accordance with the results of Koile et al study that found the SOD activity showed a negative correlation with age ($r = -0.375$, $P < 0.05$) Koile, et al., 2018). And Sciskalska et al study, the influence of age on decreased intracellular total SOD activity was shown(Sciskalska, et al.,2020).

World Health Organization (WHO) declared noise and shiftwork as an international health problem being the widest spread sources of environmental stress in human life. Also, MDA, caused by oxidative stress, is known as one of the best markers for lipid oxidation.

SOD as an antioxidant enzyme plays the role of transferring free oxygen radicals produced by oxidative stress. In some experimental studies have been mentioned that these enzymes have protective effects (Yildirim, et al., 2007).

Also, case study of Ozguner and et al. in the field of sound effects on SOD, MDA showed that the activity of SOD, GSH in 93 workers of Yarn factory who were exposed to sound higher than allowed compared to 43 people outside the factory as a control group were significantly lower (p-value <0.05) and MDA was significantly higher in the exposed group than in the control group (p-value <0.05) (Özgüner et al., 1999).

In the Yildrim study, a comparison of MDA, and SOD levels of plasma from 30 textile factory workers with exposure to 105 dB level of sound was reported with 30 volunteers as a control group. MDA levels in the group exposed to noise increased significantly. The amount of SOD in the group exposed to sound was reduced but not significantly (Yildirim, et al., 2007). This study is consistent with our study and confirms a significant increase in MDA, but unlike the above study, SOD has had a significant decrease in our study.

The reason for this difference could be the difference in the average age of the population. The population was younger in the Yildrim study (mean age of 27.67 ± 4.8 yr).

In an animal study, 144 mice were randomly divided into two control groups (expose to 80 dB in A) and the intervention group (100 dB in A). The results of the study showed that a significant decrease in SOD and an increase in MDA in mice with 100 dB sound compared to the 80 dB was observed and it was concluded that sound exposure could reduce enzyme activity and increase MDA levels in the brain (Tang, et al., 2009).

Also, under oxidative stress, superoxide ions are produced. These byproducts are toxic and may accumulate in the cells. SOD as a protective enzyme, scavenges these byproducts of oxidative stress. Protective effects of SOD as antioxidant defense systems fail when ROS generation increases. So, decreased SOD may indicate the presence of active oxygen species [Haghighat, , et al., 2017).

Shift working by disrupting the circadian cycle, puts a strain on the person, thereby altering the blood factors that indicate oxidative stress (Khajehnasiri, et al., 2013). The proper functioning of the antioxidant defense depends on the function of the circadian cycle, so a disturbance in the circadian cycle interferes with the antioxidant defense [Gowda, et al., 2019).

Our findings are in agreement with the information presented by Hossein Mohammad, who conducted a study on the effects of nocturnal effects on cortisol and MDA levels among male nurses. 96 male nurses participated in this study. The first group consisted of 67 male nurses with 3 to 12 years of work experience, who had 8 to 10 night shifts in a month. The second group consisted of 29 male nurses who had worked for 3 to 12 years in a routine work schedule. The results showed that MDA levels in the blood serum of the first group, have significant differences with the second group (Pvalue < 0.01) (Muhammad, et al., 2017).

In our study, the MDA mean of the shift work group was significantly higher than the MDA mean of the non-shift work group (exposed to noise below 85 decibels or higher than 85 decibels) (Pvalue < 0.05). Result of Jolantana's study showed that in 359 female nurses, shift work increased MDA and GSH, but SOD did not show a significant difference [Gromadzińska, et al., 2013).

The results of this study were in accordance with the results of our study. In our study, the mean of SOD in the shift working group exposed to noise lower than 85 decibels, was lower than the mean of SOD in the routine work schedule exposed to noise lower than 85 decibels, but there was not a significant difference (P value> 0.05). Also, SOD in the shift working group that was exposed to noise higher than 85 decibels was lower than workers with the non-shift work schedule that were exposed to noise higher than 85, but not a significant difference (p-value> 0.05).

Based on the results of MDA in group 4 (high noise + rotational shift work) was significantly higher than groups 2 and 3(low noise + rotational shift work and high noise + no shift work respectively). So, we can say that high noise and shift work have a synergistic effect on MDA in this study (Table 2). Also, the result showed although SOD in group 4 (high noise + rotational shift work) was significantly lower than groups 2 (low noise + rotational shift work), SOD in group 4 (high noise + rotational shift work) was not significantly lower than groups 3 (high noise + no shift work). So, in this study, we cannot say that high noise and shift work have a synergistic effect on SOD (Table 2).

Also, this study shows a positive correlation between age and MDA and negative correlation between age and SOD level. The reason for this relationship might be explained in terms of the accumulation of ROS in the aging body.

The study strength may be defined in terms of the use of occupational noise exposure and shift working schedule, which remains stable throughout the whole working day. The selection of such working site grants maximum possibility of researching the workers exposed with defined sound levels and shift working schedules.

As limitations of the study, we didn't have MDA and SOD parameters in pre-employment clinical exam. Also, data on inflammation diseases were not present in employee medical records at the factory clinic. Furthermore, we did immeasure the sound level of noise at their living place and the groups were not involved in a similar diet, however their lunch at the factory was similar.

CONCLUSION

After this preliminary investigation, we can say that there is oxidative stress in the groups exposed to noise and shift work. We can say that having a shift work schedule or being faced with a higher sound pressure level could lead to a significant increase in MDA. However, the findings of this study suggest that exposure to higher sound pressure levels has a more severe effect on increasing MDA than shift work. Noise and shift work are synergistic factors of MDA level. These results support that the effect of noise and shift work together can have more significant effects on oxidative stress markers than each alone. Also, in the significant reduction of SOD, exposure to higher sound pressure levels is more effective than the type of work program (shift or no-shift).

It is recommended that further studies be conducted in the area of simultaneous exposure of noise and shift work schedule to confirm the results of this study and to promote the health of the workers

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interest regarding the publication of this manuscript. Additionally, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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