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ORIGINAL ARTICLE

Effect of Supplementation with Vitamin E and Omega-3 Fatty Acids on Oxidative Stress Parameters of Workers Exposed to High-Level Noise

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ABSTRACT

Background: The present study aimed to assess the effects of vitamin E and Omega-3 fatty acid supplementation on oxidative stress parameters among workers exposed to high noise levels in an automobile parts manufacturing plant. Additionally, the effects of noise exposure on superoxide dismutase (SOD), total antioxidant capacity (TAC), and malondialdehyde (MDA) were investigated.

Method: Participants were divided into four groups (vitamin E [100 mg], Omega-3 fatty acids [180 mg EPA and 120 mg DHA], vitamin E + Omega-3, and Placebo) using a double-blind block randomization method. Oxidative stress parameters were analyzed before and after three months of supplementation using the enzyme-linked immunosorbent assay method. Workers' noise exposure levels were measured according to ISO 9612.

Results: Before the intervention, mean MDA, SOD, and TAC levels were 27.52 (7.46) nmol/ml, 58.84 (10.44) U/ml, and 2.57 (0.67) mM, respectively. After the intervention, mean MDA, SOD, and TAC levels were 24.57 (7.58) nmol/ml, 63.46 (11.02) U/ml, and 2.70 (0.84) mM, respectively. Omega-3 fatty acid supplementation significantly decreased MDA levels. Moreover, vitamin E combined with Omega-3 fatty acids significantly increased SOD activity. Noise exposure decreased TAC and SOD levels and increased MDA levels, but the changes were only statistically significant for TAC.

Conclusion: The simultaneous use of vitamin E and Omega-3 fatty acids for three months positively affected the antioxidant performance of workers exposed to noise.

KEYWORDS: *Occupational exposure, Noise, Oxidative stress, Vitamin E, Omega-3*

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INTRODUCTION

Employees in occupational environments are at risk of various hazardous physical and chemical agents which, depending on the nature of the stress and exposure conditions, can have detrimental effects on the health of those exposed [1]. These environmental stressors can disrupt the balance of Reactive Oxygen Species (ROS) inside the body, causing oxidative stress [2]. Oxidative stress is the prognosis of oxidative damage and occurs when the body is unable to outnumber the free radicals by producing enough antioxidants to neutralize them [3]. Increased oxidative stress is involved in the onset of disorders and results from either high levels of ROS or the weakening of the capacity to neutralize them, which can lead to tissue damage [3]. Environmental stressors are known to affect the endocrine system and the functioning mechanism of hormones [4]. Research has shown that the damaging effects on the endocrine glands are due to oxidative stress caused by exposure to environmental agents [5].

Oxidative stress increases the production of free radicals, which are atoms or molecules that are extremely reactive due to having free outer electrons and can cause significant harm to macromolecules in the body, including DNA, proteins, fats, and hydrocarbons. ROS are free radicals created through various metabolic processes such as aerobic respiration in mitochondria. They play a central role in tissue damage caused by metabolic stress. Oxidative stress is also involved in the pathogenesis and progression of various disorders. During oxidative stress, many macromolecules are damaged, and oxidation of lipids, DNA, and proteins, inactivation of certain enzymes, and disruptions in the functioning of various membranes have been reported [6, 7].

Noise is recognized as an environmental stressor and is of interest in occupational environments due to its adverse auditory and non-auditory effects [8]. The mechanism of the biological and physiological effects of noise is still not fully understood, but research has shown that oxidative stress, vascular issues, and mechanical damage can be caused by exposure to noise. The role of oxidative stress in the onset of disorders associated with noise has been confirmed in the studies [9-11]. Literature suggests that chronic or acute exposure to noise can cause excessive amounts of free radicals with subsequent oxidative stress resulting in irreversible effects on the auditory system. This can also result in neurological, endocrine, and cardiovascular disorders [12].

Research shows that sound waves create mechanical vibrations in tissues, activating cellular responses and signaling pathways that produce ROS. Prolonged loud sound exposure triggers an inflammatory response, activating immune cells that generate ROS, contributing to oxidative stress. It has been indicated that intense sound exposure can impair mitochondrial function, increasing electron leakage during oxidative phosphorylation and leading to higher ROS levels. The resultant oxidative stress can damage cellular components like lipids, proteins, and DNA, perpetuating a cycle of further oxidative damage [13-15].

The antioxidant response within the body is either enzymatic or non-enzymatic, both of which suppress free radicals. Enzymatic responses include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase. Non-enzymatic responses include vitamin C, vitamin E, melatonin, coenzyme Q10, pyruvic acid, hypo taurine, and thiol antioxidants [16]. Omega-3 fatty acids are known to have anti-coagulant properties and can improve antioxidant performance by raising catalase levels in the peroxisome and cytoplasm. The mechanism of Omega-3 fatty acids' effect in the removal of ROS has already been established [17]. There are two major groups of Omega-3 fatty acids: docosahexaenoic acids (DHA) and eicosapentaenoic acids (EPA). Omega-3 fatty acids function as anti-inflammatory agents by distancing the arachidonic acid metabolism from PGF2α and increasing PGE1 levels, which are less inflammatory [18]. It has been demonstrated that vitamin E can reduce oxidative stress and inflammation by reducing the release of pre-inflammatory cytokines, monocytes adhesion to the endothelium, plasma C-reactive protein (CRP), and plasma MDA, and by increasing the ROS-neutralizing function of red blood cells [19]. Furthermore, antioxidants with different chemical properties may reinforce each other in the antioxidant network and improve internal antioxidant defense by including antioxidant enzymes [20].

The present study aimed to assess the effects of vitamin E and Omega-3 fatty acid supplementation on oxidative stress parameters among workers exposed to high noise levels in an automobile parts manufacturing plant. Additionally, the effects of noise exposure on SOD, total antioxidant capacity (TAC), and MDA were investigated. MDA is the final product of lipid peroxidation and is often used to describe oxidative stress. SOD is an enzyme responsible for converting anion superoxide radicals (O2•–) to hydrogen peroxide

and molecular oxygen and is important in controlling cellular ROS levels. TAC is related to a group of compounds, including enzymatic systems such as SOD, small molecules such as vitamin E, and proteins such as albumin [21]. A literature review shows that very few field studies have been conducted on the effects of exposure to high levels of noise on oxidative stress parameters among workers. Almost no study has evaluated the effects of vitamin E and Omega-3 fatty acid supplementation on oxidative stress parameters in workers exposed to high-level noise.

MATERIALS AND METHODS

Participants

The present study was conducted on workers of an automobile parts manufacturing plant. After calculating the sample size, considering entry criteria [22, 23], and obtaining consent forms, a number of consenting participants were enrolled in the clinical trial (Figure 1). The adequacy of the sample was calculated using Equation 1 and the results of a previous study [24].

$$N = \frac{\left(Z_{1-\alpha/2} + Z_{1-\beta}\right)^2 \left(S_1^2 + S_1^2\right)^2}{\left(\bar{X}_1 - \bar{X}_2\right)^2} \quad (1)$$

$Z_{(1-\alpha/2)} = 1.96$
$Z_{(1-\beta)} = 0.84$
$S_1 = 1.1$
$S_2 = 1.3$
$\bar{X}_1 = 3.2$
$\bar{X}_2 = 3.4$

As per the formula for calculating sample adequacy, a group sample size of 23 was determined, with the total number of participants being 92 (23×4). Using the double-blind block randomized method, the participants were divided into four groups:

• Group 1 was given vitamin E (100 mg) plus a placebo of Omega-3 fatty acids.

• Group 2 was given Omega-3 fatty acids (180 mg EPA and 120 mg DHA) plus a placebo of vitamin E.

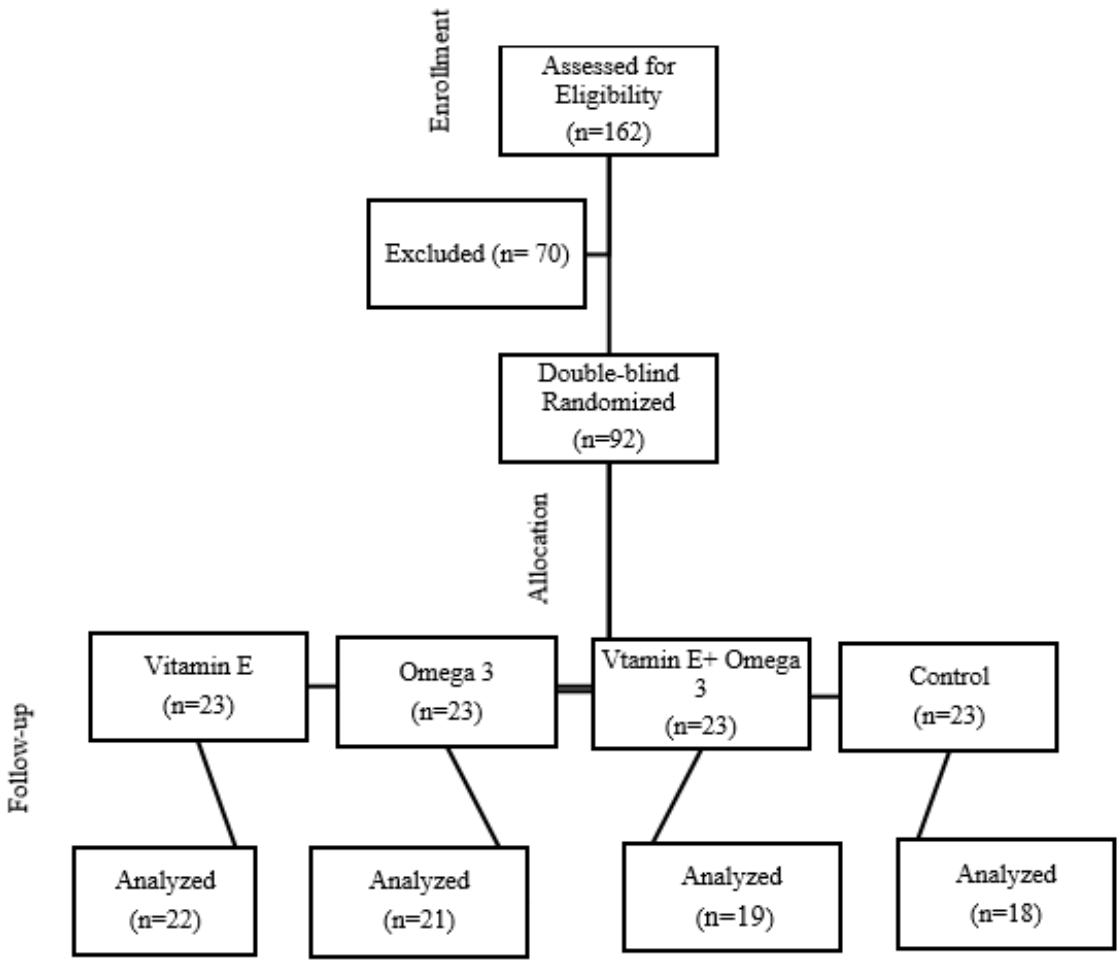

Figure 1. Flow diagram of participants included and excluded at each stage

• Group 3 was given vitamin E as well as Omega-3 fatty acids.
• Group 4 received placebos (containing light liquid paraffin) of both vitamin E and Omega-3 fatty acids.
Serum levels of oxidative stress, including SOD, TAC, and MDA, were analyzed before and after three months of supplement use. During follow-up, some participants left the study due to job changes, sickness (Covid-19), or an unwillingness to continue (Figure 1).

The vitamin E, Omega-3 fatty acids, and their placebos were obtained from Daana Pharma Co. (Tabriz, Iran) and Karen Pharma and Food Supplement Co. (Tehran, Iran), respectively. This clinical trial was registered and approved by the Iranian Registry of Clinical Trials (IRCT) with the registration number ID: IRCT20210207050290N1.

Noise Measurement
In order to measure individual exposure to occupational noise, a calibrated noise dosimeter (TES-1354; Taiwan) was used. Since the exposure pattern of the participants had a similar alternation, short-term dosimetry was employed. This involved measuring exposure during two separate sampling periods: one hour during the work shift (10 hours daily) and another during the rest period (2 hours daily). The microphone of the dosimeter was attached to the collar, about 10 to 30 cm away from the participant's ear. The dose equivalent levels were measured according to Equation 2, while 12-hour (usual shift duration) equivalent levels were calculated using Equation 3 [25].

Dose equivalent level= TLV +10log (exposure duration ×daily shift/100 × noise dose) (Eq.1)

$$leq_{12h} = 10\log\left[\frac{1}{12}\sum_{i=1}^{n} 10^{lp_i/10} \times t_i\right] \quad (3)$$

Where daily shift is equal to 10 hours of work and 2 hours of rest, exposure duration is 1 hour, and the threshold limit value (TLV) is 85 dBA for 8 work hours (considering the 3 dBA rule). Additionally, Lpi is the measured equivalent level (calculated from dose equivalent level) and ti is the relevant exposure duration. The TLV of exposure to noise based on 12-hour equivalent continuous sound pressure level equals 83 dBA [26]. It must be noted that the nature of the noise was combined (continuous, impact), and all workers were using hearing protection (Uvex Earmuffs 3V, Germany; -31 dBA reduction).

Nutritional supplementation
Before any intervention, the participants were instructed to balance their daily lifestyle in terms of physical activity, diet, and work situation to prepare for the study (run-in period). A daily supplements reminder checklist was provided to the participants along with the supplements, allowing them to more easily track and remember their supplement regimen. As mentioned, the participants were designated into four groups via the double-blind block randomized method. The placebos used were identical to their supplement counterparts, with similar containers, except they contained light paraffin oil instead. Per the double-blind criteria (i.e., both research participants and researchers were unaware of the treatment type), a third party was responsible for coding and providing the containers to the researchers.

Participants were instructed to maintain their regular diet and exercise routines throughout the intervention and refrain from changing any dosage without informing the researchers. They were informed through various methods regarding dosage, consumption, when to stop consumption, potential side effects, cautionary advice, and storage conditions both before and during the intervention. During the study, researchers stayed in contact with the participants at least once every two weeks to ensure protocols were followed. It must be stated that neither the participants nor the researchers were aware of the contents of each dose until the end of the study.

Oxidative Stress Measurements
The levels of SOD, MDA, and TAC were measured as the target oxidative stress parameters. Laboratory professionals took blood samples (10 cc) from each participant between 7 and 9 in the morning, requiring participants to abstain from eating for 8 to 10 hours beforehand. The blood samples were placed in an ice box and transferred to the lab, where they were kept at -20°C until analysis. The target parameters were measured using an ELISA reader (StatFax 2100, Awareness CO., USA) in a biochemistry lab according to the guidelines of the manufacturer (Kiazist CO., Iran).

All stages in the preparation of work solutions, standards, and samples (serum) were performed according to the catalogue provided by the manufacturer of the TAC measurement kit (Kiazist CO., Iran). In this test, Cupric (Cu^2+) is converted to Cuprous (Cu^1+) in the presence

Table 1. Demographic characteristics of participants

Parameter		Statistical measure (N = 80)			Supplementation Group				P-value*
		Mean (SD)	Median (IQR)	Max-Min	Vitamin E (N=22)	Omega-3 (N=21)	E+ Omega-3 (N=19)	Placebo (N=18)	
					Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
Age (years)		33.57(5.91)	32.50(11.75)	46.25-22.00	34.27(6.27)	31.85(6.33)	35.52(5.53)	32.66(4.99)	0.144
Employment duration (years)		8.30(5.95)	6.50(7.75)	19.2-02.00	10.31(5.96)	6.33(4.79)	10.63(5.93)	5.66(3.72)	0.005
Manufacturing Section N (%)	Aluminum		17(22)		9(5.9)	0(0)	4(3.5)	4(3.5)	0.004
	Cast iron		14(17)		3(21.4)	2(14.2)	7(50)	2(14.3)	
	Grinding		49(61)		10(20.4)	19(38.8)	8(16.3)	12(24.5)	
Education N (%)	Illiterate		2(2)		0(0)	0(0)	1(50)	1(50)	0.838
	Primary school		7(9)		2(28.6)	1(14.3)	2(28.6)	2(28.6)	
	Junior high school		20(25)		5(25)	6(30)	3(15)	6(30)	
	High school Diploma		44(55)		13(29.5)	12(27.3)	13(29.5)	6(13.6)	
	Associate Degree		4(5)		1(25)	1(25)	0(0)	2(50)	
	Bachelor's degree		3(4)		1(33.3)	1(33.3)	0(0)	1(33.3)	
BMI (kg/m^2)		25.06(4.49)	24.79(6.23)	33.90-16.33	27.98(3.49)	2388(4.65)	25.93(4.32)	21.95(3.13)	**0.001
N (%)	Underweight (<18.5)		6(7)		0(0)	2(33.3)	1(16.7)	3(50)	0.009
	Normal (18.5-24.9)		34(43)		3(8.8)	10(29.4)	9(26.5)	12(35.3)	
	Overweight (25-29.9)		27(34)		13(48.1)	6(22.2)	5(18.5)	3(11.1)	
	Obese (>30)		13(16)		6(46.2)	3(23.1)	4(30.8)	0(0)	
WHR		0.93(0.21)	0.93(0.12)	1.95-0.10	0.96(0.06)	0.89(0.06)	0.97(0.04)	0.89(0.05)	0.002
N (%)	Healthy (≤0.9)		30(37)		3(10)	10(33.3)	5(16.7)	12(40)	0.003
	Unhealthy (>0.9)		50(63)		19(38)	11(22)	14(28)	6(12)	
Smoker- N (%)	Yes		25(31)		7(28)	8(32)	6(24)	4(16)	0.594
	No		55(69)		15(27)	13(24)	13(24)	14(25)	
Regular exercise N(%)	Yes		44(55)		13(29.5)	15(34.1)	7(15.9)	9(20.5)	0.161
	No		36(45)		9(25)	6(16.7)	12(33.3)	9(25)	
Physical activity (IPAQ score)	Walking		-		-	-	-	-	-
	Moderate		-		-	-	-	-	
	Vigorous		80 (100)		22 (100)	21 (100)	19 (100)	18 (100)	

* Differences of variables between different manufacturing units (Kruskal-Wallis Test / Chi-squared Test)

** Mean differences of BMI between different manufacturing units (One-way analysis of variance (ANOVA))

SD= Standard Deviation IQR=Interquartile Range BMI= Body Mass Index WHR=Waist to Hip Ratio IPAQ = International Physical Activity Questionnaire

of antioxidants and produces color due to the existence of Chromogen. This color has adsorption at the 450 nm range and can be read. Adsorption has a direct correlation with the amount of antioxidants. The dynamic measurement range of this method is 40-400 nmol/ml and its Trolox Equivalent sensitivity is 20 nmol/ml.

For SOD monitoring, all stages in the preparation of work solutions, standard samples, and unknown samples (serum) were performed according to the catalogue provided by the manufacturer of the SOD activity measurement kit (Kiazist CO., Iran) and per the colorimetry method (wavelength = 570 nm). MDA was monitored in the same way. In this test, MDA is combined with Thiobarbituric acid to form a TBA-MDA complex, which is adsorbent at 532 nm. The dynamic measurement range of this method is 20-100 µM with a sensitivity of 10 µM.

Statistical Analysis

The SPSS v.22 (Chicago IL, US) software suite was used for statistical data analysis. Descriptive statistics including mean, percentage, minimum, maximum, and standard deviation were used to show blood analysis before and after the use of the supplements. The Shapiro test was applied to determine whether the data followed a normal distribution. Median differences between pre- and post-intervention values of variables were assessed by the Wilcoxon Signed Ranks Test. Medians of variables between different studied groups were compared using the Kruskal-Wallis test or Chi-squared test. The effect size of the dietary supplements on the target parameters, as well as the predictive model for the effect mechanism, were estimated using univariate analysis of variance (ANOVA). A significance level of 0.05 was considered for the present study.

RESULTS

Table 1 contains descriptive-analytic statistics regarding the participants' demographic properties based on the supplement group. The Shapiro test shows that demographic data had a mostly non-normal distribution except for the Body Mass Index (BMI) ($P<0.05$). The participants had a mean age and employment duration of 33.57 (±5.19) and 8.30 (±5.59) years, respectively. Among the various participants, 61% worked in the grinding hall; 64% of them had either a high school diploma or higher education. BMI was normal in 43% of the participants, while 37% had a waist-hip ratio (WHR) in the normal range. As for exercise, 55% had regular exercise and 69% of the participants were not former or current smokers.

The participants were separated into various exposure groups based on the amount of their exposure to noise. The cut-off point for this was the 33rd and 66th percentiles. Table 2 presents descriptive-analytic statistics regarding the level of exposure for both the exposure groups and the supplement groups. As per the Shapiro test, exposure data distribution was non-normal ($P<0.05$). Mean exposure to noise was higher than the Threshold Limit Value (TLV) (83 dBA for 12 hours of work) in all three exposure groups. The differences between exposure levels and TLVs were statistically significant ($P=0.001$). Results indicate that differences in exposure to noise among the supplement groups were statistically significant when considering overall exposure ($P=0.002$) but not significant when considering categorized exposure levels ($P=0.053$).

Table 3 presents descriptive-analytic statistics regarding the participants' oxidative stress parameters before and after the intervention. No statistically significant differences in MDA levels before and after the intervention were found in any of the supplement groups (except for the Vitamin E group). However, differences in MDA levels were statistically significant between the supplement groups after the intervention ($P=0.045$). Overall, after the intervention, mean serum MDA was lower in the supplement groups. The differences in SOD levels before and after the intervention were not significant in any of the supplement groups (except in the Vitamin E + Omega-3 group). Differences in SOD levels between the supplement groups were statistically significant after the intervention ($P=0.018$). Overall, mean SOD levels were higher among the supplement groups after the intervention. The differences in TAC levels before and after the intervention were not statistically significant in any of the supplement groups. The differences in TAC levels between the participants after the intervention were also insignificant. Overall, mean serum TAC levels were higher among the supplement groups after the intervention.

Table 4 shows the results of the univariate analysis of variance, which was used to model the effect of supplement use, noise exposure, and demographic factors on oxidative stress parameters. To determine the effect of each variable and its role within the regression model, the standard regression coefficient (Beta/B) is considered. A variable with a higher Beta has a more impactful role in determining the dependent variable (oxidative stress).

Table 2. Exposure levels of participants to noise

Categorized Values of Noise	Total (N=80)				Supplementation Group								P-value*
	N	Mean (SD)	Median (IQR)	Min-Max	Vitamin E (N=22)		Omega-3 fatty acids (N=21)		E+ Omega-3 (N=19)		Placebo (N=18)		
					N (%)	Mean (SD)	N (%)	Mean (SD)	N (%)	Mean (SD)	N (%)	Mean (SD)	
Overall	80	93.71(7.37)	94.04(12.86)	78.14-104.68	22	92.03(7.19)	21	97.85(6.15)	19	90.05(6.49)	18	94.79(7.63)	0.002
<90.44	26	84.88(3.63)	86.09(6.24)	78.14-90.01	11(42.3)	85.72(2.97)	2(7.7)	84.68(7.53)	9(34.6)	84.34(2.81)	4(15.4)	83.91(5.99)	
90.44-97.74	28	94.51(2.48)	94.04(5.39)	90.60-97.74	6(21.40)	95.75(2.22)	8(28.6)	95.22(2.35)	6(21.4)	92.26(0.89)	8(28.6)	94.58(2.82)	0.056
>97.74	26	101.67(4.16)	101.05(3.13)	97.78-104.68	5(19.20)	101.46(1.50)	11(42.3)	102.17(2.33)	4(15.4)	99.60(1.23)	6(23.1)	102.33(1.62)	

* Differences of variables between different studied groups (Kruskal-Wallis Test / Chi-squared Test)
SD= Standard Deviation IQR=Interquartile Range

Table 3. Descriptive-analytical statistics regarding stress oxidative parameters of participants before and after intervention

Parameter		Supplementation Group									
		Vitamin E (N=22)		Omega-3 fatty acids (N=21)		E+ Omega-3 (N=19)		Placebo (N=18)			
		Before	After	Before	After	Before	After	Before	After		
MDA (nmol/ml)	Median (IQR	28.07(6.54)	20.85(9.98)	28.17(5.61)	21.10(13.20)	23.95(7.82)	23.90(4.40)	26.60(8.50)	28.40(7.43)		
	Mean (SD)	27.57(4.64)	23.26(8.99)	28.08(5.89)	24.40(8.60)	26.95(10.14)	23.02(6.20)	27.41(9.03)	28.00(4.75)		
	P-value*		0.033		0.079		0.171		0.349		
SOD (U/ml)	Median (IQR	62.35(10.99)	60.79(9.07)	64.33(13.88)	62.75(27.45)	57.21(16.08)	69.79(13.54)	59.16(12.03)	57.29(10.75)		
	Mean (SD)	57.87(12.34)	60.01(5.36)	61.87(8.28)	65.56(15.62)	58.15(12.55)	69.11(11.06)	57.21(7.44)	59.26(6.09)		
	P-value*		0.884		0.454		0.009		0.372		
TAC (nmol/ml)	Median (IQR	1.21(0.95)	1.66(1.01)	1.66(0.50)	1.81(1.10)	1.54(1.00)	1.53(0.82)	1.55(1.21)	1.41(0.73)		
	Mean (SD)	1.44(0.76)	1.68(0.85)	1.56(0.44)	1.73(0.80)	1.58(0.72)	1.80(0.96)	1.75(0.75)	1.56(0.78)		
	P-value*		0.284		0.520		0.629		0.231		
	P-value**								0.390		
	P-value***								0.869		

*Median difference between pre and post intervention values of variables (Wilcoxon Signed Ranks Test)
** Median differences of variables between different studied group before intervention (Kruskal-Wallis Test)
*** Median differences of variables between different studied group after intervention (Kruskal-Wallis Test)
SD= Standard Deviation MDA= Malondialdehyde SOD= Superoxide dismutase TAC= Total antioxidant capacity

Table 4. Results of univariate analysis of variance

Variable	MDA		SOD		TAC	
	P-value	**B**	**P-value**	**B**	**P-value**	**B**
	0.031	-20.561	0.052	25.106	0.317	1.056
Supplementation Group	0.147	-14.029	0.951	0.850	0.416	0.938
	0.680	-3.012	0.007	27.763	0.059	1.577
Noise exposure	0.938	0.010	0.387	-0.156	0.001	-0.051
Age	0.069	0.330	0.552	-0.145	0.153	-0.003
Employment Duration	0.631	0.092	0.205	-0.033	0.130	-0.033
BMI	0.243	0.261	0.073	-0.550	0.552	-0.015
Smoking	0.136	12.797	0.784	-3.166	0.952	-0.057
Physical Activity	0.092	-18.484	0.583	5.246	0.868	0.132

BMI= Body Mass Index
MDA= Malondialdehyde
SOD= Superoxide dismutase
TAC= Total antioxidant capacity

As per Table 4, the use of ω-3 fatty acids can have a significant decremental effect on MDA levels (B=-20.561; P=0.031), with a 20-fold increase in the probability of reduced MDA levels compared to the placebo group. The use of Vitamin E + ω-3 had a decremental effect on MDA, but this was not statistically significant. Supplement use increased SOD activity in all supplement groups, though this was only significant in the case of Vitamin E + ω−3 (B=-27.763; P=0.007), with a 27-fold increase in the probability of higher SOD levels compared with the placebo.

Supplement use had an incremental effect on TAC levels in all supplement groups, but this was not statistically significant. The highest effect on TAC was observed in the Vitamin E + ω−3 group (B=-1.577; P=0.059), with a 1.5-fold increase in the probability of higher TAC levels compared to the placebo. Exposure to noise only had a significant effect on TAC (P=0.001), with a single unit increase in noise level corresponding to a mean TAC reduction of 0.05 units. Noise had a decremental effect on SOD levels and an incremental effect on MDA levels. None of the demographic variables had a statistically significant effect on oxidative stress parameters.

DISCUSSION

Before the intervention, mean MDA, SOD, and TAC were 27.52 (7.46) nmol/ml, 58.84 (10.44) U/ml, and 2.57 (0.67) mM, respectively. After the intervention, mean MDA, SOD, and TAC were 24.57 (7.58) nmol/ml, 63.46 (11.02) U/ml, and 2.70 (0.84) mM, respectively. Since there is no commonly acknowledged normal range for oxidative stress parameters, the levels observed among the control groups are usually considered the basis for comparison. It must be noted that due to budget limitations, regulations of the manufacturing plant, and moral considerations regarding sampling, it was impossible to include employees from office environments as controls (no noise exposure), which raises the issue of exposure levels.

In a previous study by the authors, potential biomarkers involved in exposure to crystalline silica were investigated in an insulation manufacturing company. In that study, mean serum MDA was 8.26 (4.65) nmol/ml in the control group (office employees) [27], while this was significantly higher at 22.48 nmol/ml among the participants of the present study. Joshaghani and Shafe'i conducted a study to determine if serum superoxide dismutase levels and red blood cells had any relationship with serum homocysteine among patients with myocardial infarction. They reported the mean serum SOD among the control group (selected among healthy candidates) to be 8.44 (6.24) U/ml [28], while this was significantly higher at 61.28 U/ml among the participants of the present study. Prohan et al. conducted a study to determine dietary and serum TAC levels and their relationship with depression among men. Mean TAC levels in their control group were 1.92 (0.34) mM [29], which is higher than that measured among the participants of the present study at 1.64 nmol/ml.

The Effects of Noise Exposure on Stress Oxidative Parameters

Oxidant and antioxidant levels may differ depending on exposure duration, type of exposure, and intensity. However, in general, increased oxidative stress during exposure is usually accompanied by a steady rise in antioxidant mechanisms [21]. The results of the partial correlation test (while controlling for demographic variables) showed no significant correlation between exposure to noise and oxidative stress parameters, except for SOD activity, which was statistically significant (R=-0.242, P=0.042). A weak inverse relationship was observed between exposure levels and TAC and SOD activity, while MDA levels had a weak but direct relationship with exposure levels. As per the univariate analysis of variance, noise exposure had only a significant relationship with TAC (P=0.001), with a single unit increase in noise levels resulting in a 0.05 unit decrease in TAC. Noise exposure had a decremental effect on SOD activity and an incremental effect on MDA levels.

Elsayed and Gorbunov (2003) stated that oxidative stress caused by exposure to high levels of noise can reduce TAC, which is followed by lipid peroxidation [30]. Haghighat et al. showed that acute exposure to noise increases 8-hydroxy-2-deoxyguanosine and MDA levels while decreasing Glutathione (GSH), catalase (CAT), and SOD activity [31]. Hosseinabadi et al. reported that noise exposure among workers in the food industry increased the number of free radicals released while also increasing MDA levels depending on the increase in noise exposure. Their results show that MDA was higher in the exposure group at 19.96 (2.55) nmol/ml compared to the control group at 18.04 (2.41) nmol/ml, with the difference being statistically significant (P>0.001) [32]. Additionally, SOD activity was higher in the exposure group at 15.68 (2.01) U/ml compared to the control group at 13.57 (1.81) U/ml, but this difference was not statistically significant.

According to their regression model, among the demographic and noise variables, the noise level was the most important predictor of MDA level (B=0.48, P=0.033), SOD activity (B=-0.34, P=0.068), and TAC (B=0.11, P=0.001).

Mean MDA level and SOD activity among the participants of the present study were higher than those reported by Bagheri et al. in their study. Also, the effect of noise on oxidative stress parameters was only significant in the case of TAC, with the effect of noise on MDA being incremental while its effect on SOD and TAC was decremental. The reason for the differences between these two studies may be due to the fact that the participants of the present study were simultaneously exposed to various physical and chemical stressors at varying intensities.

Yildirim et al. also reported that MDA levels among textile workers exposed to 105 dBA noise were 2.17 (1.09) nmol/ml compared to the control group at 1.37 (0.50) nmol/ml, with the difference being statistically significant [33]. Demirel et al. (2019) investigated the effects of noise on oxidative stress parameters in rats. Their results showed that MDA levels and Glutathione were significantly higher after the experiment. This suggests that the effects of noise exposure are not limited to the auditory system and may affect the whole body, leading to oxidative stress [34].

The inverse relationship between the level of exposure to noise and SOD, as well as TAC, means that with increased exposure, antioxidant capacity is reduced since these two parameters determine the antioxidant defense system of the body. Under normal conditions, the formation of free radicals is usually the result of cellular processes such as the mitochondrial respiratory chain, which is controlled by the body's enzymatic and non-enzymatic defense mechanisms. When the body is exposed to an oxidative agent, the formation of free radicals in the body is increased, stimulating the antioxidant defense system. To control the chain reactions of these free radicals, antioxidants step in with various mechanisms and combat the free radicals. By giving a hydrogen atom to the free radicals, the antioxidant itself is used up, mitigating the oxidative chain reactions and preventing oxidative tissue damage.

Prolonged exposure to high-intensity stressors can lead to uncontrolled lipid peroxidation beyond the capacity of the immune system. This reduces enzymatic activity due to its sensitivity to damage from the oxidative system, which can result in reduced TAC. The increased oxidative stress observed in the present study may be due to various issues such as increases in general oxidations, reduction in the creation of antioxidants, the inability of the cell to recover from oxidative damage, as well as the damage caused to the cell from ROS [35]. During exposure to oxidative agents, the antioxidant defense system attempts to maintain the balance between oxidants and antioxidants. Initially, the antioxidant situation in the body changes, and when the antioxidant system is unable to maintain redox balance, damage to macromolecules and the onset of lipid peroxidation occur. Evaluating oxidative parameters in the present study reveals that among the various exposure groups, the amount of exposure was so high as to stimulate an antioxidant response within the body. Several studies have evaluated oxidative stress parameters and enzymatic activity such as glutathione peroxidase, superoxide dismutase, and catalase in response to exposure to physical agents (such as noise).

However, most studies in this regard look at oxidative stress in response to exposure to chemical agents or various disorders such as diabetes, Alzheimer's disease, high blood pressure, cardiovascular disorders, and cognitive function among human subjects, as well as laboratory-scale studies on animals. Evaluating these studies is outside the scope and aim of the present paper, thus there were limitations regarding the comparison and discussion of results obtained regarding changes in TAC. Still, in various disorders and under physiologically stressful situations, research suggests changes in TAC, which are usually decremental. Keshvari et al. looked at oxidative stress biomarkers in workers of a ceramics manufacturing plant. Their results showed a significant reduction in TAC and total serum thiol groups among workers compared to the control group [36].

The Effects of Nutritional supplementation on Stress Oxidative Parameters

Regarding supplement use and its effect on oxidative stress parameters, results showed that the difference in MDA levels before and after the intervention was only significant in the Vitamin E group and not significant in any other supplement group. After the intervention, mean serum MDA levels had decreased in all supplement groups. Differences in mean serum SOD levels before and after the intervention were only significant in the Vitamin E + Omega-3 supplement group and not significant in any other supplement group. After the intervention, mean serum SOD levels

had increased in all supplement groups. The differences in TAC levels before and after the intervention were not significant in any supplement group, but overall, serum TAC had increased among the participants.

Based on univariate analysis of variance, the use of Omega-3 can have a significant decremental effect on MDA levels. The use of Vitamin E + Omega-3 on MDA levels was decremental but not statistically significant. Supplement use had an incremental effect on SOD levels in all supplement groups, but this was only significant in the Vitamin E + Omega-3 group. Supplement use in all groups had an incremental effect on TAC, with the largest effect observed in the Vitamin E + Omega-3 group, but none were statistically significant. Similar to the present study, a significant reduction in MDA levels after daily Omega-3 supplement use among those suffering from atherosclerosis [37] and hemodialysis [38] has been observed. Fazlian et al. showed in their systematic meta-analysis review that the use of Omega-3 fatty acids can cause a significant reduction in MDA levels [39].

One of the important targets of oxidative stress is lipid profiles. Oxidation of lipid profiles leads to increased production of MDA as a secondary by-product. The positive effects of Omega-3 consumption on MDA levels may be due to its effect on improved lipid profiles and reduced lipid peroxidation. Lipid peroxidation is mediated by free radical compounds, and thus the reduction in MDA production resulting from Omega-3 use may be due to its anti-inflammatory properties [40]. Several studies agree with the findings of the present study regarding the positive effects of Omega-3 and vitamin E supplement use on the antioxidant system. Rahmani et al. (2017) have shown that a 12-week Omega-3 and vitamin E supplement regimen in women suffering from polycystic ovary syndrome resulted in a significant increase in plasma TAC (+89.4 ±108.9 vs. +5.9 ±116.2 mmol/L, P=0.003) as well as a significant decrease in MDA levels compared with the placebo (-0.3 ±0.4 vs. -0.008 ±0.6 µmol/L, P=0.01) [41]. Liu et al. (2015) investigated the effects of Omega-3, Omega-6, and vitamin E consumption on the antioxidant performance of wild boars [42]. Their results show that using 400 mg/kg of vitamin E (compared to 200 mg/kg) increased SOD and TAC antioxidant parameters while reducing MDA levels. Liu et al. (2015) state that using Omega-3 and Omega-6 at a ratio of 6/6, as well as 400 mg/kg of vitamin E, can improve antioxidant performance [42]. Similarly, another study conducted on pregnant women shows that a 6-week consumption of vitamin E (400 units) and Omega-3 (1000 mg) resulted in a significant increase in plasma TAC (224.9 mmol/L vs. 136.1 mmol/L) as well as a significant reduction in MDA (0.9 µmol/L vs. 6.4 mmol/L) compared to the placebo [42].

Vitamin E is fat-soluble and can be found in various foods such as wheat, meat, plant-based oils, eggs, and leafy vegetables. Vitamin E is also useful in curing many disorders. In humans and mice, it is converted to alpha-Tocopherol metabolite. Vitamin E (Tocopherol) plays a role in preventing cell membrane destruction due to free radicals [43]. The benefits of Omega-3 fatty acids in preventing cardiovascular disorders are also well-documented. Evidence has been mounting in recent years regarding the positive effects of using Omega-3 fatty acids, including studies on animals. However, these studies have not been conclusive, and further investigation is required. It is important to maintain a suitable daily intake of fatty acids since these acids (such as DHA and EPA) are not naturally created in the human body. Omega-3 fatty acid supplements are widely available, safe, and affordable, making them a highly valuable solution [44].

It is not easy to arrive at a definitive conclusion in the present study regarding the effects of exposure to high-level noise on oxidative stress parameters, as well as the role of supplementation in improving antioxidant performance. This is mainly because, even though the calculated sample size and the requirements for entry were all determined with confounding factors in mind, it is not feasible to control all factors that may be influential in this regard. This is usually the case when it comes to human trials and field studies. This does not mean that the testing of theories in field studies is not without merit, as these studies better reflect real-world conditions [45]. Despite the valuable insights gained from this study, several limitations should be acknowledged:

- **Lack of Control Groups:** Due to budget constraints, suitable control groups from office environments with no noise exposure were not established, which limits the capacity to draw definitive conclusions.
- **Natural Variability:** Daily fluctuations in oxidative stress parameters may influence the results, adding variability that is difficult to control.
- **Budget Constraints on Monitoring:** Financial limitations hindered the ability to conduct repeated self-monitoring and to biologically assess the supplements used, which may affect the reliability of the findings.

• **Influential External Factors:** Various external factors, including exposure to harmful chemical agents (such as polycyclic aromatic hydrocarbons or heavy metals), physical agents (like electromagnetic fields and vibration), and psychological stressors, could have confounded the results.
• **Impact of the Covid-19 Pandemic:** Limitations and sample drop due to the Covid-19 pandemic may have affected the study's comprehensiveness.
• **Participant Homogeneity:** The inability to homogenize participants based on demographic characteristics (age, employment duration, BMI) may have introduced variability in the results.

CONCLUSION

The present study aimed to determine the role of vitamin E and Omega-3 fatty acid supplement use in improving antioxidant performance. Furthermore, the effects of high-level noise exposure on oxidative stress parameters were evaluated. The use of supplemental Omega-3 fatty acids had a significant decremental effect on MDA levels. The use of vitamin E alongside Omega-3 fatty acids had a significant incremental effect on SOD activity. Noise exposure had a decremental effect on TAC and SOD, as well as an incremental effect on MDA, but this was only statistically significant for TAC. It appears that the simultaneous use of vitamin E and Omega-3 fatty acids for three months had a positive effect on the antioxidant performance of workers exposed to noise.

A follow-up study is highly recommended, considering the limitations of the present study noted above. Such a follow-up study should focus on workers exposed to noise and should monitor oxidative stress parameters from the beginning of their employment, include a larger sample size, and have a more suitable control group. Despite its limitations, the present study still has merits since it is rare to find field studies that evaluate not only the effects of noise exposure on oxidative stress parameters but also the role of supplementation in improving antioxidant performance, while considering the amount of occupational exposure.

Ethical approval for this study was obtained from the School of Public Health & Allied Medical Sciences, Tehran University of Medical Science (IR.TUMS.SPH.REC.1398.297), and the School of Public Health & Neuroscience Research Center, Shahid Beheshti University of Medical Sciences (IR.SBMU.PHNS.REC.1399.157). All participants filled out consent forms and participated voluntarily in the study.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Data are available from the corresponding author upon reasonable request.

CONFLICT OF INTERESTS

The authors have no competing interests to declare.

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AUTHORS' CONTRIBUTIONS

HM carried out experiments and analyzed data. FG and SFD supervised the research, contributed to the study design, managed and planned the project, and provided critical revision of the article. MAR contributed to the environmental assessment. MCH supervised the nutrition intervention and NM supervised the serum analysis. All authors reviewed and provided final approval of the version to publish.

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ABBREVIATION LIST

ANOVA	Analysis of Variance
BMI	Body mass index
CAT	Catalase
CRP	C-reactive protein
DHA	Docosahexaenoic Acid
DNA	Deoxyribonucleic acid
EPA	Eicosatetraenoic Acid
GSH	Glutathione
IPAQ	International Physical Activity Questionnaires
ISO	International Organization for Standardization
MDA	Malondialdehyde
PGE1	Prostaglandin E1

PGF2α	Prostaglandin F2alpha
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
TAC	Total Antioxidant Capacity
TLV	Threshold Limit Values
WHR	Waist-Hip Ratio

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