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Original Article

Impact of Heavy Metal Pollution on Oxidized Low-density Lipoprotein Levels among Car Maintenance Workshop Workers

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Abstract
This study aimed to evaluate the relationship between heavy metal pollution and oxidized low-density lipoprotein (OxLDL) among workers in car maintenance workshops in Baghdad, Iraq. Blood samples were collected from 70 male workers, including car maintenance and vehicle painting workers, along with a control group of healthy males. The levels of heavy metals, specifically cadmium (Cd) and lead (Pb), as well as OxLDL and the marker of lipid peroxidation, malondialdehyde (MDA), were measured. The results revealed a significant increase in the level of Pb in the blood of workers compared to the control group, whereas Cd levels did not exhibit a significant difference. Furthermore, both groups of workers exhibited significantly elevated levels of OxLDL compared to the control group. Additionally, the MDA level was significantly increased across all the study groups. Correlation analysis demonstrated positive associations between OxLDL levels and the extent of Pb and Cd pollution in the car maintenance worker group. These findings indicate an increased risk of developing cardiovascular diseases (CVDs) among workers in car maintenance workshops due to exposure to heavy metals. The observed elevation in the levels of OxLDL and lipid peroxidation markers underscores the potential health issues associated with occupational exposure to these polluted environments.

Keywords: Pollution, Heavy metal, OxLDL, Atherosclerosis, Car workshops

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1. Introduction

As a form of LDL cholesterol, oxidized low-density lipoprotein (OxLDL) undergoes oxidative modifications, increasing its likelihood of contributing to the development of cardiovascular disease (CVD) and atherosclerosis. Heavy metals, which are toxic elements commonly found in the environment, have been linked to various health problems, including CVD (1, 2). Numerous studies have provided evidence supporting the notion that exposure to heavy metals, such as cadmium (Cd) and lead (Pb), can indeed lead to an increase in OxLDL level in the blood (3). This is believed to occur because heavy metals can induce oxidative stress and inflammation, leading to the oxidation of LDL cholesterol (4, 5). Workers in car maintenance workshops, including mechanics, car welders, tire specialists, and vehicle painters, are regularly exposed to toxic elements in their occupational settings (6). In addition to vehicle emissions, workers may also encounter heavy metals through contact with oil spills from storage tanks or gas leaks (7). Workers employed in car maintenance workshops are susceptible to potential exposure to a diverse array of hazardous substances, including diesel and gasoline fuels, lead-based products, and oil spills. These exposures can result in the dissemination of heavy metals, especially Pb and Cd, thereby posing health risks to workers (8). Substantive prior investigations have established a direct and discernible relationship between exposure to heavy metals and a multitude of deleterious health outcomes. The pervasiveness of heavy metals in the environment has emerged as a prominent public health concern, predominantly because of the perilous potential of exposure and subsequent adverse health ramifications (9). Within the context of Iraq, several empirical studies have documented a progressive increase in heavy metal concentrations in the soil and water matrices across diverse geographical locales (10).

Workers in car maintenance workshops are particularly susceptible to heavy metal exposure due to the presence of these elements in automotive parts and fluids (11). It was revealed that exposure to heavy metals can result in lipid peroxidation, DNA damage, protein modification, and the development of chronic kidney disease, neurological disorders, CVD, neuronal damage, diabetes, and cancer (12, 13). Furthermore, high levels of OxLDL have been identified in individuals exposed to heavy metals through occupational exposure. Given the non-biodegradable nature of heavy metals, exposure to these toxic pollutants tends to accumulate in tissues of living organisms, posing significant health risks (14). Previous studies have indicated that heavy metals can cause damage to cell membranes, disrupt biochemical processes, and inactivate enzyme systems or affect protein structure. These effects can lead to cellular dysfunction or cell death (15, 16). Heavy metals can also generate radicals that cause the oxidation of biological molecules (17, 18). Several in vitro studies on lead-induced oxidative stress have primarily been conducted, demonstrating the ability of lead to bind directly to cell membranes and increase their susceptibility to lipid peroxidation (19, 20). Malondialdehyde (MDA), an end product of lipid peroxidation, is a useful biomarker for assessing membrane lipid peroxidation and diagnosing lead-induced oxidative stress (21, 22). Implementing appropriate surveillance systems and utilizing oxidative stress biomarkers are crucial for the protection of lead-exposed workers from lead poisoning (23, 24). Cd, even at trace levels, is a toxic heavy metal that is associated with bone defects such as osteomalacia, high blood pressure, and heart muscle dysfunction (25). Its effects on oxidative stress and inflammation in the body have been linked to CVD (26). The present study aimed to investigate the effect of heavy metals on workers in car maintenance workshops and their role in increasing the risk of heart disease.

2. Materials and Methods

2.1. Design of the Study

This comparative study which involved automobile workers as test subjects and non-automobile workers as controls was conducted in Baghdad. The study participants were apparently healthy automobile workers aged 18–50 years and age-matched controls. They were spray painters, automobile mechanics, welders, panel beaters, radiator repair workers, and battery repair workers who were routinely engaged in auto repairing activities in automobile workshops. The control group included individuals who had never worked in an automobile workshop, resided in the vicinity of an automobile workshop, or were exposed to paint in their environment. The aims and scope of the study were explained to participants, and written consent was obtained before enrollment in the study. The study protocol, the subject information, and the consent form were reviewed and approved by the Research Ethics Committee of the College of Science (Ref.: CSEC/1123/0111 on 8/11/2023). Moreover, the study was conducted in compliance with the Helsinki Declaration of 1975.

2.2. Study Population

From September 2022 to January 2023, a study was conducted in Baghdad involving a population of auto workers. A total of 70 male Iraqi individuals participated in the study. The duration of their exposure or years of work ranged from 1 to 20 years. The sample size of the study was determined by the availability of participants meeting the inclusion criteria and consenting to take part. Participants were categorized into two groups according to their specific job roles: car mechanics (Group WI, n = 35) and vehicle painters (Group WII, n = 35). These two groups were then matched with a control group (C, n = 25) consisting of healthy individuals. Only individuals who were assessed as healthy and showed no symptoms of chronic diseases or disorders were included in the study. None of the participants had occupational exposure to Pb and Cd. Individuals who had consumed vitamin supplements in the past year, used steroids or medications that affected lipid metabolism, or had CVD, kidney disease, liver disease, or inflammatory disease were not included in the study.

All participants completed a questionnaire to provide pertinent information. A demographic information questionnaire was utilized to gather data on socio-demographic factors (such as age and marital status), work experience in the automobile workshop (including number of working hours and years employed), adherence to safety precautions (such as usage of personal protective equipment like hand gloves and face masks), family and medical history of previous illnesses, and lifestyle habits (including smoking, alcohol consumption, drug use, and substance abuse) of the individuals involved. Participants with chronic illnesses, undergoing long-term medication, or with a history of smoking, alcohol consumption, or drug addiction were excluded from the study. The anthropometric measurements for all participants were conducted, with weight and height measurements taken to calculate the body mass index (BMI) by dividing body weight by height (27).

3. Clinical Measurements

3.1. Blood Sample Collection

A total of 70 blood samples were obtained from workers representing various occupations in Baghdad. The participants were requested to fast prior to sample collection. Gel tubes were utilized to collect the fasting blood samples. Subsequently, the tubes were subjected to centrifugation at 3000 rpm for 10 minutes at room temperature. This centrifugation process facilitated the separation of the serum from the red blood cells. The resulting serum samples were then utilized for the estimation of parameters including OxLDL, lipid profile, lipid peroxidation (MDA), as well as Cd and Pb levels. Each participant's serum was carefully divided into 200 µL portions and stored in Eppendorf tubes. These Eppendorf tubes containing the serum samples were appropriately stored in a freezer at -20 °C until they were ready to be analyzed or utilized for further investigations.

3.2. Biochemical Analysis

The concentration of serum oxidized LDL was measured using the Human OxLDL Lipoprotein ELISA Kit (MyBioSource, USA). To measure MDA, the Human MDA ELISA Kit (MyBioSource, USA) was used. This involved the formation of a colored substance when MDA reacted with thiobarbituric acid (28). The concentrations of heavy metals, specifically Cd and Pb, in the collected blood samples were determined using a flame atomic absorption spectrophotometer (FAAS). Additionally, the BMI of the participants was calculated as a measure of body composition. The BMI was determined by dividing the body weight of each individual in kilograms by the square of their height (29).

3.3. Analysis of Lead and Cadmium

The analysis of Cd and Pb was conducted using a Perkin Elmer 2380 atomic absorption spectrophotometer (AAS) (Norwalk, CT, USA). AAS operates by directing a beam of electromagnetic radiation emitted from a light source through a vaporized sample. In this process, the atoms within the sample absorb a portion of the radiation, with the quantity of light absorbed being directly linked to the concentration of the specific element being targeted in the sample (30).

Total serum Cd and Pb concentrations were measured using the digestion method with flame atomic absorption technique (31).

3.4. Statistical Analysis

Statistical analysis is a fundamental component in data analysis, as it aids in drawing meaningful insights and facilitating data interpretation. In this study, the means (±SD) of the collected samples was calculated using the SPSS version 22.0. To compare the means of variables between different groups, a one-way analysis of variance (ANOVA) was performed. In determining statistical significance, a P value of less than 0.05 was employed.

4. Results and Discussion

The present study involved 70 Iraqi auto workers (W) who were matched with 25 healthy males serving as a control group (C). The age range of all participants was 18-50 years. The study group (W) was further divided into two groups: car mechanics (WI, n = 35) and vehicle painters (WII, n = 35). The study results are summarized in Table 1, indicating no statistically significant differences between the groups in terms of age and BMI ($P > 0.05$). This implies that the age and BMI distributions were similar across the various groups studied, and the observed variations were not statistically significant. The lack of significant differences suggests that age and BMI were not influential factors in the specific outcomes or variables investigated in this study.

Table 2 displays the mean values of OxLDL (ng/mL) in different groups. The study findings revealed that the mean OxLDL levels were significantly higher ($P = 0.0001$) in the car mechanics (WI) group (1042.55 ± 206.69 ng/mL) and the vehicle painters (WII) group (1054.19 ± 208.64 ng/mL) compared to the control group (632.57 ± 138.86 ng/mL). OxLDL refers to LDL cholesterol that has undergone chemical modifications, increasing its propensity to contribute to the development of atherosclerosis. Atherosclerosis is a medical condition in which plaque accumulates within the arteries. This plaque is composed of cholesterol, fat, calcium, and other substances that form on the inner walls of the arteries. As time progresses, this plaque builds up inside the arteries, diminishing blood flow to important organs and tissues (32, 33). OxLDL, resulting from the oxidative modification of LDL under oxidative stress, leads to vascular endothelial injury, triggers the release of inflammatory factors, promotes the formation of foam cells, compromises plaque stability, facilitates thrombosis, and plays a critical role in initiating and accelerating the progression of atherosclerosis (34, 35).

Table 3 displays the impact of different occupations on the mean concentration of heavy metals in the blood of workers exposed to varying levels of these pollutants. Considering Cd concentrations in the workers' blood, the mean Cd levels were recorded as (0.06 ± 0.12 µg/g) for car mechanics and (0.05 ± 0.13 µg/g) for vehicle painters. These results indicate that car mechanics and vehicle painters had relatively similar mean Cd concentrations in their blood, with car mechanics exhibiting a slightly higher level. The results of ANOVA indicated that the differences in mean Cd concentration among all occupations were not significant ($P > 0.05$) compared to the mean Cd concentration of the control group. Considering Pb concentrations, car mechanics had a mean Pb concentration of 0.31 ± 0.03 µg/g in their blood,

Table 1. The Mean (±SD) Values of Age and BMI in the Studied Groups

Parameters	C(n=25) Mean±SD	WI(n=35) Mean±SD	WII(n=35) Mean±SD	*P* value	
Age (Years)	29.96±6.48	34.06±8.68	32.66±6.53	C&WI	0.348
				C&WII	0.092
				WI&WII	0.708
BMI(Kg/m^2)	23.03±1.61	23.56±3.74	24.79±2.52	C&WI	0.270
				C&WII	0.290
				WI&WII	0.999

WI, Car mechanics; WII, Vehicle painting; C, Control
Significant *$P < 0.05$; highly significant**$P < 0.001$; no significant $P > 0.05$

Table 2. The mean (±SD) Level of the OxLDL in the Studied Groups

Parameters	C (n=25) Mean±SD	WI (n=35) Mean±SD	WII (n=35) Mean±SD	*P* Value	
OxLDL (ng/mL)	632.57±138.86	1042.55±206.69**	1054.19±208.64**	C&WI	0.0001
				C&WII	0.0001
				WI&WII	0.965

WI: car mechanics, WII: vehicle painters, and C: control.
Significant: *$P<0.05$; highly significant: **$P<0.001$; not significant: $P>0.05$.

Table 3. Mean (±SD) Concentration of Heavy Metals in the Blood of Workers

Parameters	C (n=25) Mean±SD	WI (n=35) Mean±SD	WII (n=35) Mean±SD	*P* Value	
Cd (μg/g)	0.00±0.01	0.06±0.12	0.05±0.13	C&WI	0.193
				C&WII	0.085
				WI&WII	0.899
Pb (μg/g)	0.05±0.02	0.31±0.03**	0.27±0.08**	C&WI	0.0001
				C&WII	0.0001
				WI&WII	0.026

WI: car mechanics, WII: vehicle painters, and C: control.
Highly significant: **$P<0.001$; significant: *$P<0.05$; not significant: $P>0.05$.

while vehicle painters had a mean Pb concentration of 0.27±0.08 μg/g. ANOVA analysis revealed significant differences ($P=0.0001$) in the mean Pb concentration between workers and controls (0.05±0.02 μg/g). Additionally, the WI group showed a significant increase ($P=0.026$) when compared to the WII group. Previous studies have consistently reported that auto mechanics often exhibit higher concentrations of heavy metals in their blood, which can be attributed to the nature of their work (18,36). The work of auto mechanics involves various activities, such as disassembling and assembling car engines and other vehicle parts. These tasks expose them to a range of hazardous substances, including heavy metals like Pb, Cd, and chromium, as well as other potentially toxic compounds such as polyaromatic hydrocarbons, resins, and solvents. Exposure to heavy metals occurs through direct contact with contaminated components, such as engine parts or exhaust systems, as well as through inhalation of airborne particles and fumes generated during maintenance and repair activities. These substances can enter the body through the respiratory system or by skin absorption, leading to increased levels of heavy metals in the blood. The presence of heavy metals in the blood of auto mechanics is a concern due to their potential health effects. Heavy metals are known to pose risks to various organ systems, including the nervous, cardiovascular, renal, and respiratory systems. Prolonged or repeated exposure to these substances can lead to adverse health outcomes, including neurological disorders, respiratory problems, kidney damage, and certain cancers. The risk associated with exposure depends on the source, duration, and type of exposure (37,38). Instances of lead poisoning and fatalities due to exposure to Pb have been documented among car mechanics due to inhalation and ingestion of gasoline used for handwashing, leading to the absorption of tetraethyl lead through mucous membranes and high blood levels of Pb (22,39). Heavy metals are known to be toxic to the body, and exposure to them has been linked to various health issues, including CVD (40). Table 4 summarizes the mean concentration of MDA across all the groups studied. MDA serves as a useful biomarker for lipid peroxidation in chronic human diseases (41).

The results displayed in Table 4 show a significant increase in the mean value of MDA in the car mechanics group (WI) (224.03±58.34 nmol/mL) and the vehicle painters group (WII) (179.07±52.89 nmoL/mL) compared to the control group (C) (93.99±14.13 nmol/mL) ($P=0.0001$). Furthermore, there was a significant increase between the WI and WII groups ($P=0.001$). The elevated levels of MDA observed in auto workers can indeed be attributed to the presence of heavy metals in their work environment. Heavy metals, such as Cd, Pb, and chromium, have been shown to induce oxidative stress in the body. Exposure to heavy metals can overwhelm the antioxidant defense mechanisms of the body, leading to an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity. This process weakens the cellular antioxidant defense mechanism and raises the concentration of MDA (42, 43). Notably, free radicals are highly reactive chemical species that can attack various biomolecules, with lipids being particularly susceptible (44). Oxidative radicals tend to target cell membranes rich in polyunsaturated fatty acids, often resulting in lipid peroxidation (45). Certainly, heightened oxidative stress significantly contributes to the development and advancement of coronary artery disease (CAD). CAD involves the constriction or obstruction of the coronary arteries, crucial for the delivery of oxygenated blood to the heart muscle (46). These findings align with the results of other studies (47,48). Table 5 presents the results of the present study, revealing a strong

Table 4. The Mean (±SD) Level of Malondialdehyde (MDA) in the Studied Groups

Parameter	C (n=25) Mean±SD	WI (n=35) Mean±SD	WII (n=35) Mean±SD	*P* value	
MDA (nmol/mL)	93.99±14.13	224.03±58.34**	179.07±52.89**	C&WI	0.0001
				C&WII	0.0001
				WI&WII	0.001

WI: car mechanics, WII: vehicle painters, and C: control.
Highly significant: ***P*<0.001; significant: **P*<0.05; not significant: *P*>0.05.

Table 5. Correlation of OxLDL Level With Parameters in Studied Groups

Parameters	OxLDL Level ng/mL					
	WI (n=35)			WII (n=35)		
	r	*P* Value	Sig.	*r*	*P* Value	Sig.
BMI (kg/m²)	0.292	0.089	NS	-0.131	0.455	NS
Age (year)	0.217	0.212	NS	0.249	0.149	NS
WHR	-0.075	0.671	NS	0.139	0.427	NS
MDA (nmol/mL)	0.218	0.207	NS	0.008	0.966	NS
LDL (mg/dL)	0.232	0.181	NS	-0.155	0.375	NS
VLDL (mg/dL)	0.185	0.287	NS	-0.223	0.197	NS
Cd (µg/g)	0.438**	0.008	HS	-0.053	0.760	NS
Pb (µg/g)	0.607**	0.0001	HS	-0.085	0.629	NS

positive correlation between heavy metal exposure and OxLDL levels in car mechanics (Cd: r=0.438, *P*=0.008; Pb: r=0.607, *P*=0.0001). Another study reported a positive association between Cd and Pb levels and OxLDL levels in the blood of workers (15). Based on the results of this study, it can be concluded that exposure to heavy metals has the potential to induce oxidative stress and inflammation, which can contribute to the development of atherosclerotic disease (49).

Auto repair shop workers are indeed exposed to heavy metals through various means, including inhaling welding fumes, car exhaust, gasoline fumes, paints, and lubricants. The risks associated with exposure to these pollutants can be influenced by several factors, including the location and type of work, as well as the duration of exposure (50). In the specific context of auto repair shops in Baghdad, the situation is further compounded by the fact that many of these shops are situated on roadsides. This continuous proximity to busy roads leads to constant exposure of workers to heavy metal pollution from multiple sources, including vehicle emissions and other environmental pollutants. As a result, the accumulation of heavy metals in the body tissues of these workers can be accelerated (51). Prolonged exposure to heavy metals in the workplace can induce changes in physiological processes in the body of workers (52).

5. Limitations

The study has limitations that should be acknowledged as the small sample size is due to the limited research time.

6. Conclusion

In conclusion, this study provides insight into the significant relationship between elevated levels of heavy metals, such as Cd and Pb, and OxLDL levels. The findings suggest that dermal exposure to heavy metals in automobile workers is associated with increased OxLDL levels, which serve as an early marker for the development of heart disease. This highlights the potential contribution of heavy metal pollution to the progression of CVDs. Based on the results of this study, it is crucial to prioritize the implementation of occupational safety procedures and guidelines in automobile workshops to protect workers from the adverse health effects of heavy metal exposure. This includes ensuring the use of requisite personal protective equipment by automobile workers to prevent heavy metal-induced organ and systemic toxicity. Adequate hygiene measures should also be implemented to minimize dermal exposure to heavy metals. Regular check-ups are recommended for automobile workers to monitor their health status, including the assessment of heavy metal levels and cardiovascular risk factors. This proactive approach can help identify early signs of heavy metal toxicity and allow for timely intervention and preventive measures.

Authors' Contribution

Conceptualization: Mustafa Fuad.
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Writing–original draft: Nada A. Kadhim, Mustafa Fuad.

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Competing Interests
The authors of the study declare that there is no conflict of interests.

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