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Impact of Pb and Cd exposure on oxidative stress and inflammatory markers in Baghdad fuel station workers: A case-control study

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Abstract

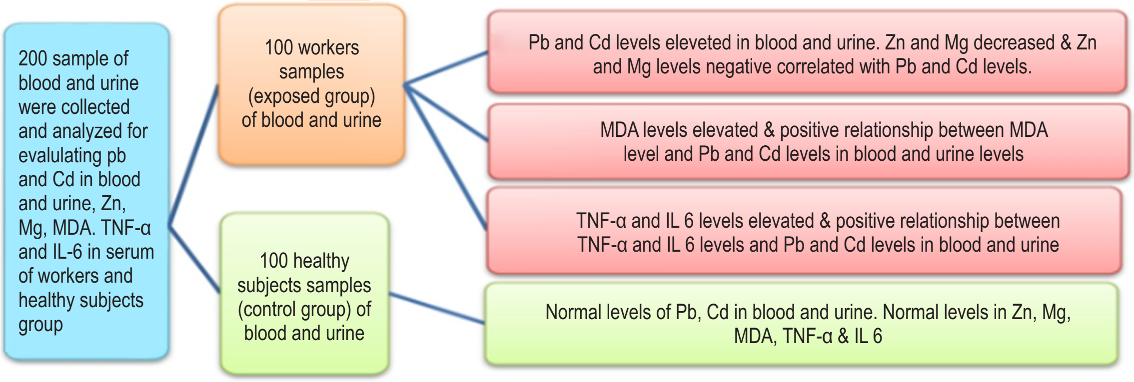
Aim: This study assessed early alterations in oxidative stress and inflammatory markers in fuel station workers in Baghdad who were exposed to toxic heavy metals, specifically lead and cadmium.

Methodology: A case-control design was employed, involving 200 male participants: 100 fuel station workers (exposed group) and 100 non-exposed individuals (control group). Blood and urine samples were collected and analyzed to quantify levels of toxic metals, oxidative stress biomarkers, pro-inflammatory cytokines, and antioxidant elements.

Results: The exposed group showed significantly higher levels of lead and cadmium in their blood and urine compared to the control group ($P < 0.0001$). Additionally, oxidative stress biomarkers and pro-inflammatory cytokines were significantly elevated, while antioxidant elements were significantly lower in the exposed group ($P < 0.0001$).

Interpretation: These findings demonstrate that occupational exposure to lead and cadmium increases oxidative stress and triggers inflammatory responses among fuel station workers. Further research is needed to develop effective protective measures and explore the long-term health effects of such exposure.

Key words: Fuel station workers, Heavy metals, Inflammatory markers, Oxidative stress



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Introduction

Heavy metals are elements with high atomic numbers and densities that persist in the environment due to their non-biodegradable nature, posing significant risks to human health and ecosystems globally. Certain heavy metals, including magnesium, zinc and selenium are essential and vital trace elements that contribute to essential physiological processes such as enzymatic activity and immune system regulation. Conversely, others, metals like lead, mercury and cadmium are highly toxic, non-essential for biological functions, and can pose serious health risks when accumulated in the body. These toxic metals bioaccumulate in tissues, causing oxidative damage, inflammatory responses, and contributing to chronic diseases such as cancer, cardiovascular disorders and neurodegenerative conditions (Anka *et al.,* 2022). Pb and Cd are particularly concerning from a public health perspective. Both are classified by the World Health Organization (WHO) as high-priority hazardous substances and are listed among the top ten chemicals of major public health concern due to their widespread use and severe health impacts (Balali-Mood *et al.,* 2021).

These metals are released into the environment from sources such as fossil fuel combustion, industrial discharges, paint residues, battery waste and pigment production. Human exposure occurs primarily through inhalation of contaminated air, dermal contact, and ingestion of polluted food and water (Fu and Xi, 2020; Kim *et al.,* 2021). Workers at fuel stations are at especially high risk due to their direct and continuous exposure to these metals (Verma *et al.,* 2024). Biological monitoring studies conducted in petrol station workers across different regions have provided valuable insights into lead exposure and its associated health risks. For instance, in India, research revealed significantly elevated blood lead levels among fuel station workers, emphasizing the urgent need for regulatory measures and better protective practices (Rana, 2018). In Italy, a study highlighted a significant reduction in blood lead levels among petrol station workers following the removal of tetraethyl lead from gasoline, underscoring the importance of biological monitoring in evaluating exposure and the effectiveness of policy interventions (Ghittori *et al.,* 2005).Conversely, in Japan, stricter environmental regulations have resulted in lower lead levels among petrol station workers compared to other regions, demonstrating the positive impact of effective policy interventions (Ogata, 1993).

Chronic exposure to lead and cadmium has been associated with various adverse health effects, including cardiovascular diseases, hypertension, neurotoxicity, immunotoxicity, renal dysfunction, and increased cancer risk (Etsuyankpa *et al.,* 2022; Kim *et al.,* 2021). The toxic effects of lead and cadmium are mainly mediated through oxidative stress mechanisms. These metals stimulate the production of reactive oxygen species (ROS) and free radicals, disrupting the balance between oxidants and antioxidants. Additionally, lead and cadmium can induce oxidative stress and inflammation by activating specific signaling pathways, such as Nuclear Factor-kappa B (NF-κB) and Nuclear factor erythroid 2-related factor 2 (Nrf2). The NF-κB pathway is triggered by increased ROS production within cells, which activates kinases like IKK (IκB kinase), leading to the degradation of IκB and the release of NF-κB from its cytoplasmic complex. The free NF-κB then moves to the nucleus, where it promotes the expression of pro-inflammatory genes, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Ma, 2013; Valko *et al.,* 2006). Oxidative stress, resulting from an imbalance between oxidants and antioxidants, damages lipids, proteins and DNA, ultimately impairing cellular functions and contributing to various disease (Balali-Mood *et al.,* 2021). Studies show that aging and higher body mass index (BMI) exacerbate oxidative stress by increasing ROS production and reducing antioxidant capacity, accelerating health deterioration (Liguori *et al.,* 2018).

Smilarly, research conducted in Hilla city, Iraq, demonstrated that fuel station workers exposed to heavy metals such as lead and cadmium experienced significant oxidative stress, as indicated by elevated levels of MDA and reduced antioxidant defenses, including SOD, TAC, and trace elements like Zn and Mg (Azize, 2018). Moreover, deficiencies in essential trace elements such as Mg, Zn, Cu, and Se—key cofactors for antioxidant enzymes—reduce the body's ability to counteract oxidative stress, elevating vulnerability to diseases (Prashanth *et al.,* 2015). Further, the deficiency of essential trace elements like zinc and magnesium may exacerbate the harmful effects of heavy metals. These elements are crucial for the function of antioxidant enzymes, maintaining the oxidative balance in the body, and protecting cells from oxidative damage (Prashanth *et al.,* 2015). Exposure to multiple heavy metals simultaneously can cause synergistic or additive toxic effects. For instance, the combined presence of lead and cadmium in the body can worsen oxidative damage, further disrupt redox balance, and weaken the immune system. Understanding these interactions is essential to evaluate their cumulative impact on human health(Jomova and Valko, 2011).

This is particularly relevant in regions like Iraq, where the quality of fuel remains a concern. Iraqi gasoline contains high lead levels due to the use of tetraethyl lead as an octane enhancer, contributing significantly to environmental pollution and occupational exposure. Lead concentrations in Baghdad's air have reached 3426 $\mu g\ m^{-3}$, far exceeding the WHO's recommended limit for urban areas with heavy traffic (1.30–1.37 $\mu g\ m^{-3}$). Moreover, Iraqi petroleum products contain other toxic metals, including cadmium, nickel, chromium, and arsenic, which pose serious health risks (Joint and Organization, 2007; Needleman, 2004). While leaded gasoline is banned in many countries due to its harmful health effects, it is still used in some regions, including Iraq. This presents a significant public health challenge, as the use of leaded fuels can have transboundary environmental and health implications. For example, lead particles dispersed through air currents can contaminate distant areas, increasing the global disease burden (Joint and Organization, 2007). Consequently, the health risks associated with lead and cadmium are not limited to Iraq but represent a global concern, particularly in regions with weak environmental regulations and continued reliance on fossil fuels. Research indicates that exposure to lead and cadmium reduces systemic antioxidant levels, including total antioxidant capacity (TAC) and superoxide dismutase (SOD), while also lowering essential trace elements such as Zn, Cu, Se and Mg. Concurrently, oxidative damage markers like malondialdehyde (MDA) increase, indicating lipid peroxidation and cell membrane damage(Genchi et al., 2020) . These metals also disrupt the production and activity of pro-inflammatory cytokines, including interleukins (IL-2, IL-1b, IL-6, IL-4, IL-8) andTNF-α, exacerbating inflammatory responses and potentially leading to chronic inflammation, autoimmune disorders, and increased susceptibility to infections.

Heavy metals toxicity is influenced by factors such as the type of metal, exposure dose, route of entry, duration, age, genetic predisposition, and the overall health status of the exposed individuals (Balali-Mood *et al.,* 2021; Harshitha *et al.,* 2024). Fuel station workers, especially pump attendants, face significant health risks due to continuous exposure to toxic metals like lead and cadmium. Despite these risks, few comprehensive studies have examined specific inflammatory responses and oxidative stress levels among these workers in Baghdad. The high levels of toxic metals in Iraqi fuel and inadequate regulatory measures highlight the need for focused research to understand the health impacts of such exposure and to guide policy interventions. This study aims to fill this gap by evaluating the effects of occupational exposure to lead and cadmium on oxidative stress and pro-inflammatory cytokines among Iraqi fuel station workers, using healthy Iraqi volunteers as a control group. Conducted between 2023 and 2024, this research provides crucial insights into the occupational health risks faced by fuel station workers in Baghdad and supports the development of protective measures at both local and global levels.

Materials and Methods

Study Participants: This study involved 200 male participants: 100 gasoline station workers and 100 healthy controls. Participants were recruited from multiple gasoline stations in Baghdad, Iraq, between June and August 2023. Demographic data, including age, weight, duration of exposure to fuel vapors (years), daily working hours, smoking and alcohol consumption habits, history of chronic diseases (e.g., infections, diabetes mellitus, liver diseases), and use of medications or supplements, were collected through structured interviews. The workers' age range was 22–60 years, and the control group was 20–58 years. Control participants were confirmed healthy based on their medical history and physical examination.

Anthropometric and Physiological Measurements: BMI was calculated using each participant's height and weight. Systolic (SBP) and diastolic blood pressure (DBP) were measured with a standard sphygmomanometer after a 5-minute rest, recording the average of three consecutive readings. Hypertension was defined as SBP ≥140 mmHg or DBP ≥90 mmHg. Heart rate was measured in a seated position and recorded within the normal range of 60–100 beats per minute (BPM).

Blood and Urine Collection: Bood samples were collected after a minimum 10-hour fasting period. Approximately 8 ml of venous blood was drawn from each participant; 2 ml was transferred to an EDTA tube for Pb and Cd analysis, and the remaining 6 ml to a gel tube. After centrifugation, the serum was separated for further analysis. Concurrently, participants collected all their urine over a 24-hour period while strictly adhering to standard guidelines for sample collection. The urine samples were appropriately stored in a cold place to ensure integrity.

Biochemical Analysis

Determination of Toxic Metal Levels (Pb and Cd): Blood and urine lead levels (B-Pb and U-Pb) were measured using a flame atomic absorption spectrophotometer (Analytic Jena, Germany). Cadmium levels in blood and urine (B-Cd and U-Cd) were determined using a flameless atomic absorption spectrophotometer (Buck Scientific, USA), following the method described by Haswell (Haswell, 1991). The flameless approach relies on a non-flammable source of atomization (a graphite tube). The tube is electrically heated, first drying the sample at 100-200 °C to degrade the organic content. During this process, smoke produced is flushed away using a nitrogen gas flow. The method also requires a radiation source, which in this case is a hollow cathode lamp (HCl) specific to each element being measured.

Determination of Antioxidant Elements (Zn and Mg): Serum Zn and Mg concentrations were estimated using a flame atomic absorption spectrophotometer (Analytic jena, Germany) following the method described by Haswell (1991). In this method, Zn and Mg were atomized using a flame. Initially, the analytes were aspirated through a small tube and transported to nebulizer, where they were converted into a fine aerosol. This aerosol was then carried to the flame by a carrier gas, where the heat disintegrated it into atoms, enabling their measurement.

Determination of Oxidative Stress Marker (MDA): Serum MDA levels were quantified using the Enzyme-Linked Immunosorbent Assay (ELISA) with kits procured from ELK Biotechnology (China), having a detection range of 15.63–1000 pg ml^{-1}.

Determination of Inflammatory Markers (IL-6 and TNF-α): Serum levels of IL-6 and TNF-α were measured using ELISA kits procured from ELK Biotechnology (China), with detection range of 7.82–500 pg ml^{-1} for IL-6 and 15.63–1000 pg ml^{-1} for TNF-α. To ensure accuracy and reproducibility, the ELISA assays for inflammatory markers were calibrated against standard curves created using known concentrations, and intra- and inter-assay coefficients of variation were kept below 10% (Moore and Roberts, 1998).

Exclusion Criteria: Participants were excluded if they met any of the following criteria: female gender, history of alcoholism or drug abuse, diabetes mellitus, use of antioxidant or vitamin supplements, obesity, arthritis, or any other conditions that could affect the study outcomes.

Statistical Analyses: Statistical analyses were conducted using SPSS software (version 26). Data normality was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Independent t-tests compared group means, Pearson's correlation coefficient evaluated relationships between variables, and the Chi-square test examined associations between categorical variables.

Results and Discussion

This study involved 200 male participants, equally divided into two groups: 100 gasoline station workers (exposed group) and 100 healthy individuals (control group). Demographic analysis confirmed that variables in both groups were normally distributed. A t-test at a 95% confidence level revealed no significant differences between the groups in age andBMI ($p = 0.44$ and $p = 0.34$, respectively). However, waist circumference (WC) was significantly larger in the exposed group, with a 33.21% increase ($p < 0.0001$), which was associated with higher levels of Pb and Cd in the blood and urine of exposed workers (29.94 vs. 14.01 μg dl^{-1} for Pb and 0.33 vs. 0.15 μg dl^{-1} for Cd; Table 1).

This increase in waist circumference, an indicator of visceral fat accumulation, likely results from disruptions in lipid metabolism and heightened inflammation due to heavy metal exposure. Metals like lead and cadmium can promote the production of ROS, leading to oxidative damage of endothelial cells and increased inflammation. Studies indicate that exposure to toxic metals disrupts normal lipid regulation, contributing to metabolic disorders such as insulin resistance, dyslipidemia, and type 2 diabetes. These findings highlight the role of heavy metals in metabolic disruption, through both direct effects on lipid metabolism and secondary effects from oxidative stress and inflammation (Jomova and Valko, 2011; Tinkov *et al.,* 2017). Systolic blood pressure in the exposed group was significantly higher than controls (5.67% increase, $p < 0.05$), likely due to excessive ROS production and oxidative stress, leading to damage to vascular endothelial cells and vasoconstriction. Although differences in diastolic blood pressure and heart rate between groups were not statistically significant ($p = 0.08$ and $p = 0.95$, respectively), the controlled measurement conditions (early morning and fasting) likely minimized confounding factors such as physical activity and stress, which may explain the lack of significant variation in the heart rate.

Additionally, heavy metals may activate inflammatory pathways, increasing vascular resistance and the risk of hypertension, suggesting potential long-term cardiovascular effects from chronic exposure (Navas-Acien *et al.,* 2007 and Tellez-Plaza *et al.,* 2008). Chronic exposure to lead and cadmium is linked to adverse effects on cardiovascular, renal and metabolic health. These metals can increase the risks of hypertension, atherosclerosis and myocardial infarction through pro-inflammatory and oxidative mechanisms. Lead exposure contributes to endothelial dysfunction and arterial stiffness, while cadmium is associated with direct tubular damage, nephrotoxicity and renal failure. Both metals also disrupt glucose metabolism, elevating the risk of insulin resistance, metabolic syndrome and type 2 diabetes (Johri *et al.,* 2010; Lanphear *et al.,* 2018; Tinkov *et al.,* 2017).

Educational Level and Health Risks Associated with Heavy Metal Exposure: Further analysis revealed that the education level was significantly higher in the control group compared to the exposed group (70% vs. 10%; $p < 0.0001$). This disparity may reflect a lack of awareness and limited access to preventive education among gasoline station workers, increasing their vulnerability to health risks associated with heavy metal exposure. These findings underscore the need for targeted educational programs and preventive interventions to reduce these risks. There were no significant differences in smoking status or fish consumption between the two groups ($p = 0.09$ and $p = 0.88$, respectively), suggesting these behaviors may have a less direct impact on the observed effects of heavy metal exposure. A Chi-square test was used to compare smoking status, education level, and fish consumption between the groups (Table 2). In this study, significant differences and positive correlations were observed between age, BMI, and WC with blood and urinary levels of lead (B-Pb, U-Pb) and cadmium (B-Cd, U-Cd), except for

Table 1: Demographic characteristics of subjects in the control and exposed groups

Variables		Control n= 100	Exposed (case) n= 100	Test	Value	P-value
Age (year)	mean ±SD	43.02±8.83	43.99±9.08	T	0.76	0.445 NS
BMI (Kg m^{-2})		37.11±1.20	36.78±3.21	T	-0.94	0.34 NS
WC (cm)		106.07±11.31	141.93±10.74	T	22.98	<0.0001*
Systolic blood pressure (mmHg)		128.84±14.34	136.15±2212	T	2.77	0.006*
Diastolic blood pressure (mmHg)		76.74±10.55	79.58±12.62	T	1.72	0.08 NS
Heart rate (p min^{-1})		78.97±8.45	79.04±10.85	T	0.051	0.95 NS

NS: non-significant difference, * Significant difference, n: number of samples

Table 2: Descriptive statistics of subjects in the control and exposed groups

Variables			Control n= 100	Exposed (case) n= 100	Test	Value	p-value
Smoking status	Yes	n (%)	43(43)	55(55)	Chi-Square	2.88	0.09 NS
	No		57(57)	45(45)			
Education level	≤high school		30 (300)	90 (90)	Chi-Square	75.00	<0.0001*
	>high school		70 (70)	10 (10)			
Fish consumption	Yes		51 (51)	52 (52)	Chi-Square	0.02	0.88 NS
	No		49(49)	48 (48)			

NS: non-significant difference, * Significant difference, n: number of samples

Table 3: Correlation of age, WC and BMI with blood toxic metal levels in the exposed group

Correlation test		B-Pb	U-Pb	B-Cd	U-Cd
Age	R	o.394	0.272	0.151	0.254
	p-value	<0.001*	0.006*	0.134	0.011*
WC		0.38	0.24	0.21	0.27
		<0.001*	0.016*	0.031*	0.005*
BMI		0.39	0.26	0.26	0.28
		0.001*	0.009*	0.008*	0.004*

* Significant Correlation

Table 4: Correlation between duration of work and blood ($\mu g\ d\ l^{-1}$) and urine ($\mu g\ l^{-1}$) toxic heavy metal levels in the exposed group

Correlation test		B-Pb	U-Pb	B-Cd	U-Cd
Duration of work	R	0.71	0.40	0.45	0.33
	p-value	<0.001*	<0.001*	<0.001*	0.001*

*Significant Correlation

age with B-Cd, which showed a non-significant difference and a weak positive correlation (Table 3). Participants in the exposed group had an average work experience of 16.96 ± 8.06 years and worked 12 hours per day. The study found a significant positive correlation between the levels of lead and cadmium in blood and urine and both the duration of work experience and daily working hours among the exposed workers ($p < 0.001$; Table 4). These results suggest that longer occupational exposure leads to greater accumulation of these heavy metals in the body, impairing physiological functions and increasing the risk of cardiovascular, renal and metabolic disorders. This finding underscores the need for effective occupational health policies and monitoring strategies to minimize exposure and protect workers in high-risk environments (Nordberg and Costa, 2021).

Analysis confirmed significantly higher concentrations of lead and cadmium in the blood and urine of the exposed group compared to the control group ($p < 0.0001$). These findings suggest that occupational exposure to these heavy metals increases oxidative stress and inflammatory responses by affecting specific signaling pathways (Sharma *et al.,* 2005). Lead and cadmium enhance ROS production, activating the NF-κB pathway and promoting the release of pro-inflammatory cytokines, exacerbating inflammation. Furthermore, these metals inhibit the Nrf2 pathway, reducing the body's antioxidant capacity and leading to greater oxidative damage (Flora *et al.,* 2008). Trace elements such as zinc and magnesium may play a protective role by enhancing the activity of antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx), thus reducing the oxidative damage induced by heavy metals (Tchounwou *et al.,* 2012). The presence of these elements might help in stabilizing cellular membranes and preventing lipid peroxidation, which is commonly triggered by reactive oxygen species generated from heavy metal exposure (Engwa *et al.,* 2019).

The study revealed substantial increase in heavy metal concentrations among the exposed workers: blood lead levels increased by 113.7%, urine lead levels by 30.4%, blood cadmium levels by 120%, and urine cadmium levels by 180%, respectively (Table 5). These levels indicate significant exposure to heavy metals, far exceeding global safety standards. The blood lead level (BLL) in this study was 29.94 $\mu g\ d\ l^{-1}$, which is notably higher

Table 5: Comparison of Pb and Cd levels in blood (µg d l^{-1}) and urine (µg l^{-1}) and serum antioxidant elements Zn and Mg (µg d l^{-1})

Variables		Control n= 100	Exposed (case) n= 100	t. test value	p. Value
B. Pb	Mean ±SD	14.01±2.18	29.94±4.62	31.18	<0.0001*
U. Pb		60.07±10.01	78.39±5.26	16.19	<0.0001*
B. Cd		0.15±0.1	0.33±0.06	14.85	<0.0001*
U. Cd		0.05±0.02	0.14±0.06	13.33	<0.0001*
S. Zn		100.72±16.08	75.36±7.98	- 14.12	<0.0001*
S. Mg		1.62±0.33	1.13±0.13	- 13.45	<0.0001*

*Significant difference, n: number of samples

than permissible levels reported in other countries. A comparison with global studies high (Nriagu, 1996). For instance, the lead levels found in this study are substantially greater than those reported for gas station workers in the United States, China, and Brazil. These variations may be attributed to differences in environmental regulations, industrial practices, and fuel composition across regions (Kessler, 2014). In countries with stricter environmental policies and better fuel quality standards, such as the United States and many parts of Europe, lead exposure levels are typically lower, reducing health risks (Goyer and Clarkson, 1996). Conversely, regions where leaded gasoline is still widely used, such as Iraq, show significantly higher exposure levels (Needleman, 2004). These findings underscore the importance of considering local and regional conditions when interpreting study results and developing strategies to mitigate the health risks associated with heavy metal exposure (Landrigan and Etzel, 2013).

The high concentrations of lead and cadmium observed in this study are likely due to factors such as the extensive use of leaded gasoline and increased levels of airborne particulate matter containing cadmium, particularly in Baghdad since 2003. These fine particles can penetrate the respiratory system, accumulate in the body, and contribute to oxidative stress and cardiovascular problems (Harrison and Yin, 2000; Hassoon, 2015).A significant reduction in the serum levels of antioxidant elements such as Zn and Mg was observed in the exposed group compared to the control group ($p < 0.0001$), with 25.17% and 18.67% decreases, respectively.

This reduction likely reflects an increased consumption of these elements in response to oxidative stress caused by heavy metal exposure. Lead and cadmium can replace essential ions like zinc and magnesium in metalloproteins, disrupting antioxidant enzyme activity and cellular metabolism. These disruptions promote excessive ROS production, leading to lipid peroxidation and cellular damage. While the reduction in antioxidant elements may represent a compensatory response, it can also weaken the body's defenses, increasing vulnerability to further oxidative damage (Flora *et al.,* 2008; Jaishankar *et al.,* 2014). This observation is consistent with findings reported in a study conducted in Iraq, which highlighted similar oxidative stress patterns, including increased MDA levels and depleted antioxidant defenses, in fuel station workers exposed to lead and cadmium (Azize, 2018). The study found a significant positive correlation between U-Cd and B-Pb levels, as well as between U-Pb and B-Cd levels. A non-significant weak positive correlation was observed between B-Cd and U-Pb. There was also a negative correlation between serum antioxidant elements (zinc and magnesium) and blood levels of lead and cadmium: ($r = -0.63$, $P < 0.001$) for zinc and ($r = -0.33$, $P < 0.001$) for magnesium. A strong association was found between serum zinc concentration and urinary lead, while no significant relationship existed between serum zinc and urinary cadmium or between serum magnesium and both urinary lead and cadmium (Table 6).

This may be due to magnesium's interaction with other chemicals in tissues and the body's regulation of trace elements through urination, which affects the accuracy of urinary magnesium measurements (Workinger et al., 2018).The Pearson correlation coefficient was used to assess these correlations at a 95% confidence level (Nemmiche, 2017). These results align with global research showing that prolonged exposure to heavy metals, such as lead and cadmium, significantly impacts human health by increasing the risks of cardiovascular diseases, diabetes, and other metabolic disorders. The findings reinforce the importance of monitoring heavy metal exposure in high-risk populations to prevent long-term health complication.

Exposure to Pband Cd increases oxidative stress by significantly elevating MDA levels, a biomarker of oxidative damage, by 109.6% in the exposed group compared to the control group ($p < 0.0001$; Table 7). This rise is due to enhanced production of ROS and reduced antioxidant elements, such as Zn and Mg. Heavy metals interact with lipids and proteins, promoting lipid peroxidation and further increasing MDA levels, leading to persistent tissue damage and impaired organ function (Gutteridge and Halliwell, 2010; Jomova and Valko, 2011).The study demonstrates that chronic exposure to Pb and Cd among fuel station workers significantly induces oxidative stress by depleting cellular antioxidants like Mg and Zn. This stress results in lipid peroxidation, elevated MDA levels, and the activation of inflammatory pathways, leading to increased TNF-α and IL-6 secretion, which may contribute to cardiovascular diseases,

Table 6: Correlation between blood ($\mu g\ dl^{-1}$) and urine ($\mu g\ l^{-1}$) toxic metals and serum antioxidant elements ($\mu g\ dl^{-1}$) in the exposed group

Correlation test		B-Pb	U-Pb	B-Cd	U-Cd
U-pb	R	0.511	---	---	---
	p-value	<0.001*	---	---	---
B-Cd		0.47	0.17	---	---
		<0.001*	0.07 NS	---	---
U-Cd		0.22	0.25	0.27	---
		0.026*	0.01*	0.006*	---
S-Zn		-0.63	-0.35	-0.33	-0.14
		<0.001*	<0.001*	0.001*	0.14 NS
S-Mg		-0.32	-0.61	-0.22	-0.08
		0.001*	0.11 NS	0.02*	0.41 NS

NS: non-significant Correlation, *Significant Correlation

Table 7: Comparison of MDA levels ($pg\ ml^{-1}$) between control and exposed groups

Variables		Control n= 100	Exposed (case) n= 100	t.test	P-Value
MDA	mean±SD	2.59±1.49	5.43±1.35	14.06	<0.0001*

*Significant difference, n: number of samples

Table 8: Correlation of MDA levels ($pg\ ml^{-1}$) and age with blood toxic metal levels in the exposed group

Correlation test		B-Pb	U-Pb	B-Cd	U-Cd
MDA	R	0.45	0.31	0.36	0.27
	p-value	<0.001*	0.002*	<0.001*	0.006*

* Significant correlation

kidney failure, and metabolic disorders. Regular health check-ups, antioxidant supplementation, and stricter PPE usage are recommended to mitigate these risks. This study also highlights a significant positive association between Pb/Cd exposure and inflammatory responses, emphasizing the need for further research on metal interactions with trace elements to develop effective interventions (Tchounwou *et al.,* 2012).

Global studies confirm that exposure to heavy metals like lead and cadmium elevates oxidative stress and reduces antioxidant capacity. Research in Europe and Asia has shown that workers in heavily contaminated environments have increased MDA levels and diminished antioxidant defenses, leading to serious health issues such as cardiovascular diseases, kidney failure, and metabolic disorders (Genchi *et al.,* 2020; Nordberg and Costa, 2021). This study found strong positive correlations between serum MDA levels and concentrations of Pband Cd in both blood ($r = 0.45$, $p < 0.001$ for Pb; $r = 0.36$, $p = 0.001$ for Cd) and urine ($r = 0.31$, $p < 0.002$ for Pb; $r = 0.27$, $p < 0.006$ for Cd) (Table 8). These findings suggest that higher MDA levels, a marker of oxidative stress, are closely associated with elevated heavy metal concentrations in the body, likely due to continuous peroxidation of cellular molecules and disruption of the antioxidant balance, leading to persistent tissue and organ damag (Engwa *et al.,* 2019; Jomova and Valko, 2011).

Occupational exposure to Pb and Cd can cause substantial damage to the immune system. Cytokines such as IL-6 and TNF-α are key signaling molecules produced by immune cells, particularly helper T cells and macrophages and play a crucial role in regulating inflammatory responses. Exposure to Pb and Cd has been shown to stimulate the production of these cytokines, leading to inflammatory responses (Silbergeld *et al.,* 2000). In this study, TNF-α and IL-6 levels were significantly higher in the exposed group compared to the control group, with 92.7% and 133.4% increase, respectively ($p < 0.0001$) (Table 9).

These elevated cytokine levels are likely due to the higher concentrations of Pb and Cd in the serum of gasoline station workers, which promote oxidative stress by generating ROS. However, despite the elevated heavy metal levels, some individuals in the exposed group did not show significant increase

Table 9: Comparison of TNF-α (pg m l^{-1}) and IL-6 (pg m l^{-1}) levels between control and exposed groups

Variables		Control n= 100	Exposed (case) n= 100	t.test	P-Value
TNF-α	Mean ±SD	3.03±0.73	5.84±0.83	25.19	<0.0001*
IL-6		4.61±1.58	10.76±1.29	30.02	<0.0001*

*Significant difference, n: number of samples+

Table 10: Correlation of IL-6 (pg m l^{-1}) and TNF-α (pg m l^{-1}) levels with blood toxic metal levels in the exposed group

Correlation test		B-Pb	U-Pb	B-Cd	U-Cd
IL-6	R	0.60	0.32	0.25	0.21
	p-value	<0.001*	0.001*	0.01*	0.036*
TNF-α		0.98	0.53	0.47	0.24
		<0.001*	<0.001*	<0.001*	0.036*

* Significant Correlation

in TNF-α or IL-6 levels, suggesting potential individual variations in inflammatory responses or the presence of compensatory anti-inflammatory mechanisms (Engwa *et al.,* 2019). This oxidative stress reduces serum levels of antioxidant elements, such as Zn and Mg, and damages cellular structures, further activating inflammatory signaling pathways and increasing cytokine secretion in immune responses (Flora *et al.,* 2012; Genchi *et al.,* 2020).

Numerous studies have shown that heavy metals like lead and cadmium worsen inflammatory processes by reducing antioxidant levels and causing oxidative stress, which activates intracellular pathways producing pro-inflammatory cytokines such as IL-6 and TNF-α (Bayraktar *et al*., 2006; Moro *et al*., 2019; Machon-Grecka *et al*., 2017). These changes increase the risk of cardiovascular diseases, kidney failure, and metabolic disorders (Tchounwou *et al.,* 2012). Additionally, prolonged exposure to these metals can cause epigenetic changes in immune cells, increasing susceptibility to chronic inflammation and altering immune gene regulation, underscoring the importance of preventive measures to reduce exposure(Hou et al., 2012).This study demonstrated a significant positive correlation between IL-6 levels and exposure to Pb and Cd in both blood ($r = 0.60$, $p < 0.001$ for Pb; $r = 0.25$, $p < 0.01$ for Cd) and urine ($r = 0.32$, $p < 0.001$ for Pb; $r = 0.21$, $p < 0.036$ for Cd) (Table 10). Similarly, TNF-α levels showed strong correlations with Pb and Cd exposure in blood ($r = 0.98$, $p < 0.001$; $r = 0.47$, $p < 0.001$) and urine ($r = 0.53$, $p < 0.001$ for Pb; $r = 0.24$, $p < 0.036$ for Cd).

These findings suggest that even low levels of Pb and Cd in the body can trigger significant inflammatory responses by enhancing the secretion of pro-inflammatory cytokines (Li *et al.,* 2021). The disruption of cellular redox states by these metals likely promotes the release of inflammatory mediators, leading to ongoing oxidative stress, cellular damage, and increased risk of chronic diseases such as cardiovascular disorders and metabolic syndromes (Alruhaimi *et al.,* 2024; Wu *et al.,* 2016). Continuous exposure to heavy metals like lead and cadmium can elevate pro-inflammatory cytokine levels and potentially cause long-term epigenetic changes in the immune system. These findings underscore the need to minimize exposure to prevent chronic inflammation and protect cellular health (Wu *et al.,* 2016).

Given the significant health risks associated with chronic heavy metal exposure, comprehensive monitoring for at-risk populations is essential. Regular medical check-ups, including testing for heavy metals, oxidative stress markers and organ function are crucial for early detection. Preventive measures should focus on reducing exposure through personal protective equipment (PPE), adherence to safety standards, and educational programs. Dietary interventions, such as antioxidant-rich foods and supplements (vitamins C and E, zinc, selenium), can help protect against oxidative damage. In cases of high-level exposure, chelating agents (EDTA, dimercaprol) may reduce heavy metal burden and mitigate toxic effects (Jaishankar *et al.,* 2014).

This study offers a comprehensive analysis of the effects of occupational exposure to heavy metals, such as lead and cadmium, on oxidative stress and inflammatory responses among fuel station workers in Baghdad, providing valuable data to the field. However, it has certain limitations that future studies could address. Longitudinal research could explore the long-term effects of chronic exposure, while studies on genetic susceptibility could clarify individual risk factors. Additionally, future investigations might examine the combined effects of multiple metal exposures and the effectiveness of protective interventions, like nutritional supplements or chelation therapy, in reducing health risks. Such research could help develop better strategies for managing occupational hazards and improving health outcomes for similar worker populations.

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