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University of Basrah
College of Science
Department of Chemistry

"Determination of Benzo[a]pyrene, Cadmium, and Lead Levels in the Blood of Petroleum Workers and Study of their Effects on Cardiovascular Disease in Basrah Province"

A Thesis

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By

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

قُلْ هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ وَالَّذِينَ لَا يَعْلَمُونَ إِنَّمَا يَتَذَكَّرُ أُولُوا الْأَلْبَابِ

صدق الله العلي العظيم

﴿الزمر: 9﴾

DEDICATION

I dedicate this work to my lady and mistress

﴿ Fatimah Al-Zahra عليها السلام ﴾

the lady of the women of the worlds.

Nagham Jawad

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Abstract

Nowadays, air pollution has become an independent risk factor for cardiovascular morbidity. This study was conducted on two groups. The first group consisted of ninety individuals working at the Nahran Bin Omar crude oil site in Basrah City. The workers' ages ranged from 23 to 55. The second group included 90 non-exposed individuals (students and faculty members). Their ages ranged from 21 to 54. This study evaluates the concentrations of fine particles in the participants' blood, including the polycyclic aromatic hydrocarbon metabolite benzo[a]pyrene diol epoxide (BPDE), and heavy metals, including Cadmium and Lead.

The results revealed a significant increase in the mean value of **BPDE (Pg/mL)** level of the exposed group (203.3 ± 9.42) compared to the control group (60.1 ± 1.64), with statistical significance ($P \leq 0.001$). The workers also had elevated levels of **Cadmium (ng/mL)** in their blood with a mean value of (85.8 ± 0.83) compared to the control group, with a mean value of (43.7 ± 3.07), and these differences were highly statistically significant ($P \leq 0.001$). The high **Lead (ng/mL)** levels in the workers' blood with a mean value of (347.65 ± 14.24) were higher than that of the control group, with a mean value of (165.0 ± 11.49), with statistical significance ($P \leq 0.001$).

The study also included the evaluation of the levels of Malondialdehyde (MDA), a derivative of polyunsaturated fatty acid peroxidation as a potential indicator of oxidative stress, and Superoxide dismutase (SOD), an antioxidant enzyme, in the serum of the study participants. The results showed a significant increase in workers **MDA(µmol/L)** with a mean value of (1219.8 ± 27.53) compared with the control group, with a mean value of (710.3 ± 52.47), with statistical significance ($P \leq 0.001$). Moreover, **SOD (U/L)** decreased significantly in workers with a mean value of (8.90 ± 0.22 versus a mean value of 11.65 ± 0.15 in the control group, with statistical significance ($P \leq 0.001$).

The study also addressed the antagonistic relationship between occupational exposure to fine particles and the levels of essential trace elements (selenium and zinc) in workers compared to the control group. **Selenium (ng/mL)** levels decreased significantly in workers with a mean value of (146.2± 20.00) versus a mean value of (165.0 ± 19.25) in the control group, with statistical significance ($P \leq 0.001$). Additionally, **Zinc (ng/mL)** levels also decreased significantly in workers with a mean value of (661.2 ± 82.52) versus a mean value of (977.83 ± 122.28) in the control group, with statistical significance ($P \leq 0.001$).

Lactate dehydrogenase (LDH) enzyme was also evaluated in both groups. The results showed a significant increase in **LDH (µmol/L)** values in exposed workers, with a mean value of (432.70 ± 26.717) compared to the control group, with a mean value of (300.60 ± 10.16), with statistical significance ($P \leq 0.001$).

Exposure to particles can stimulate inflammatory signaling pathways, reducing disease resistance. This study investigated the risks of occupational exposure to particles and its effect on increased C-reactive protein **(CRP)** and high-sensitivity C-reactive protein **(hs-CRP)**. The results documented the presence of C-reactive protein in the workers' blood at higher concentrations than in the unexposed control group (5.26 ± 0.38 vs. 3.01 ± 0.22), respectively. This is a general indicator of inflammation in the body, with statistical significance $P \leq 0.001$. The data revealed a significant difference in the mean value of high-sensitivity C-reactive protein between the two groups (2.14 ± 0.26 vs. 0.49 ± 0.07), respectively, with statistical significance $P \leq 0.001$, which indicates an increased risk of cardiovascular disease.

The blood **lipid profile** revealed differences in mean values between the exposed workers and the unexposed control group. Total cholesterol was (189.7 ± 3.70 vs. 170.2 ± 1.24) with ($P \leq 0.001$), LDL (115.8 ± 3.75 vs. 103.6 ± 3.17) with ($P \leq 0.01$), and there was a significant increase in triglycerides (205.3 ±

11.90 vs. 94.9 ± 2.12) with ($P \leq 0.001$) and VLDL (41.04 ± 2.09 vs. 19.98 ± 0.75), with ($P \leq 0.001$), the mean HDL value decreased (35.78 ± 0.64 vs. 38.76 ± 0.40) with ($P \leq 0.001$).

List of Abbreviations

Abbreviations	*Descriptions*
Ahr	Aryı hydrocarbon receptor
B[a]P	Benzo [a] pyrene
BPDE	Benzo[a]pyrene diol epoxide
CRP	C-Reactive Protein
CVD	Cardiovascular Disease
CYP P450	Cytochrome P450
ELISA	Enzyme-linked Immuno-sorbent assay
GSH	Glutathione peroxidase
HDL	High-Density Lipoprotein
HOCl	Hypochlorous acid
HS-CRP	High- Sensitive C-Reactive Protein
ICP-OES	Inductively coupled plasma-optical Emission spectroscopy
LDL	Low-Density Lipoprotein
PAHs	Polycyclic aromatic hydrocarbons
PM	Particles matter

RCS	Reactive Chloride Species
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RSS	Reactive Sulfur Species
S. D	Standard Deviation
SOD	Super oxide Dismutase
SPSS	Statistical Package for the Social Sciences
TC	Total cholesterol
TG	Triglyceride (Triacylglycerol)
UFP	Ultrafine particles
VLDL	Very-low-density lipoprotein
VSMCs	vascular smooth muscle cells
WHO	World Health Organization

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ONE

Introduction

1. Introduction

The rapid growth of oil production in Iraq over the past two decades has increased the number of wells and oil extraction operations in Basrah City [1]. The production of crude oil, refining, and burning are major sources of increased gaseous pollutants, hydrocarbons, and heavy metals [2–4]. Workers are exposed to the effects of chemicals associated with oil and related industries, including toxins, carcinogens, and irritants, through skin contact or inhaling crude oil fumes [5, 6]. These chemicals can enter the bloodstream and stimulate inflammatory pathways, affecting the cardiovascular system through oxidative stress [6–8]. These pollutants contribute to the high incidence of cancer, cardiovascular, and respiratory diseases in southern Iraq [9, 10].

1.1 Challenges associated with processing heavy crude oil and flare gas

Over the past two decades, oil production and refining in Iraq have increased rapidly. Crude oil production and refining are major sources of increased gaseous pollutants such as hydrocarbons, and heavy metals. Flaring of excess natural gas from crude oil is common in the oil and gas industry, and is often called “associated gas.” This associated gas is valuable when it can be separated from the oil and transported appropriately. However, practically, the facility to support such processing is often lacking. Iraq ranked second among the top five countries (17 billion cubic meters) in gas-flaring **(Figure 1-2)**. Flaring is a method to safely dispose of unwanted gas that could otherwise be a safety hazard or interfere with oil production. Venting associated gas from oil and gas pressure or processing equipment due to system upset conditions, or releasing pressure during an emergency, is also common in oil and gas production and processing [11]. Natural gas flaring generates significant

particulate and gaseous emissions due to the rapid burning of large quantities of gas. Gas flaring creates a visible glow, noise, and heat, which can cause various impacts on climate, air quality, and human health [12]. Data from some studies have indicated that these pollutants exceed national and international standards, which are often responsible for the high incidence of cancer, cardiovascular disease, and respiratory disease in this region of southern Iraq [13–16]

Fig. (1-2): Flaring of natural gas in oil wells in Basrah City.

Emissions from natural gas combustion contain a variety of pollutants, including inorganic substances [2, 17], methane (CH_4), black carbon (Soot)[18], Carbon monoxide (CO), hydrogen sulfide (H_2S), sulfur dioxide (SO_2), nitrogen oxides (NOx), and several volatile organic compounds (VOCs) [4, 19], polycyclic aromatic hydrocarbons (PAHs) [20, 21], free radicals and ozone [22]. This results in ambient air pollution with a complex and heterogeneous mixture of fine particulate matter (PM) and other gaseous components. In addition, aerosols and global warming gases are created when particles emitted from burning natural gas interact with atmospheric gases [22,

23]. **Figure 1-3** displays the various particles produced during the oxidation of crude oil components at low temperatures:

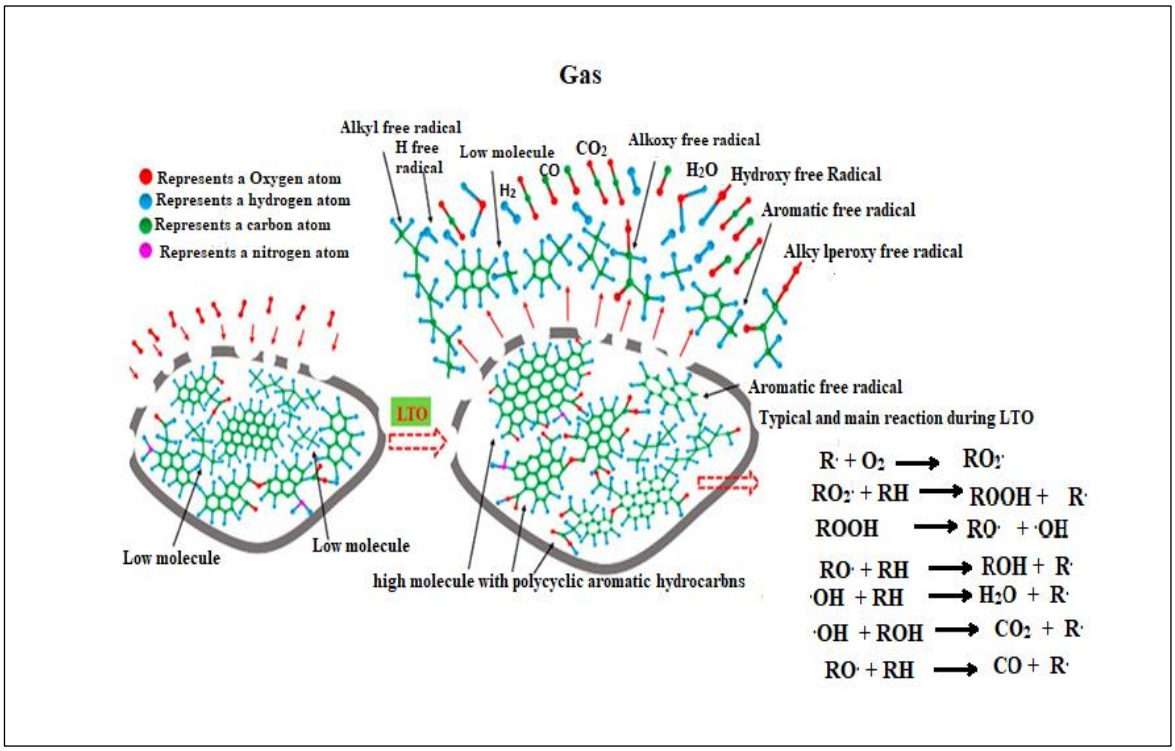


Fig. (1-3): Types of particles emitted from the oxidation of crude oil components at low temperatures [24].

1.2 Particulate Matter (PM)

Airborne particulate matter (PM) is a heterogeneous mixture of hundreds of different airborne chemicals that form fine solid particles or liquid droplets that vary in size and shape and are a major contributor to air pollution [25–27]. The aerodynamic diameter of PM is critical; they are classified into subgroups according to their consistency in air quality regulation and toxic effects: PM_{10} is a course, inhalable particle that settles in the upper airways of the human respiratory system [28]. Fine particles are less than 2.5 micrometers in diameter $PM_{2.5}$, and Ultra-fine particles (UFP) PM0.1 are smaller than 100 nm **Figure**

1-4. PM2.5 and PM0.1 can easily enter narrow airways, penetrate the alveoli and alveolar-capillary membrane, and diffuse into the circulation [29, 30]. Consistent exposure to particulate emissions in the workplace puts workers at risk for various diseases affecting different body systems [31]. There is also a strong association between PM2.5, PM0.1, and cardiovascular diseases [32, 33]. Inhaled fine particles can rapidly reach the smallest airways and alveoli, penetrate the alveolar-capillary membrane, and eventually enter the systemic circulation directly [34]. Oxidative stress and systemic inflammation are the two primary mechanisms by which fine particles increase the risk of cardiovascular disease. Inhaled air pollutants cause oxidative stress in various respiratory cells and can travel throughout the body through several mechanisms of action, including inflammation. Inhaled fine particles induce a variety of inflammatory mediators in the lungs, including pro-oxidants (ROS) and pro-inflammatory stimuli [35–38].

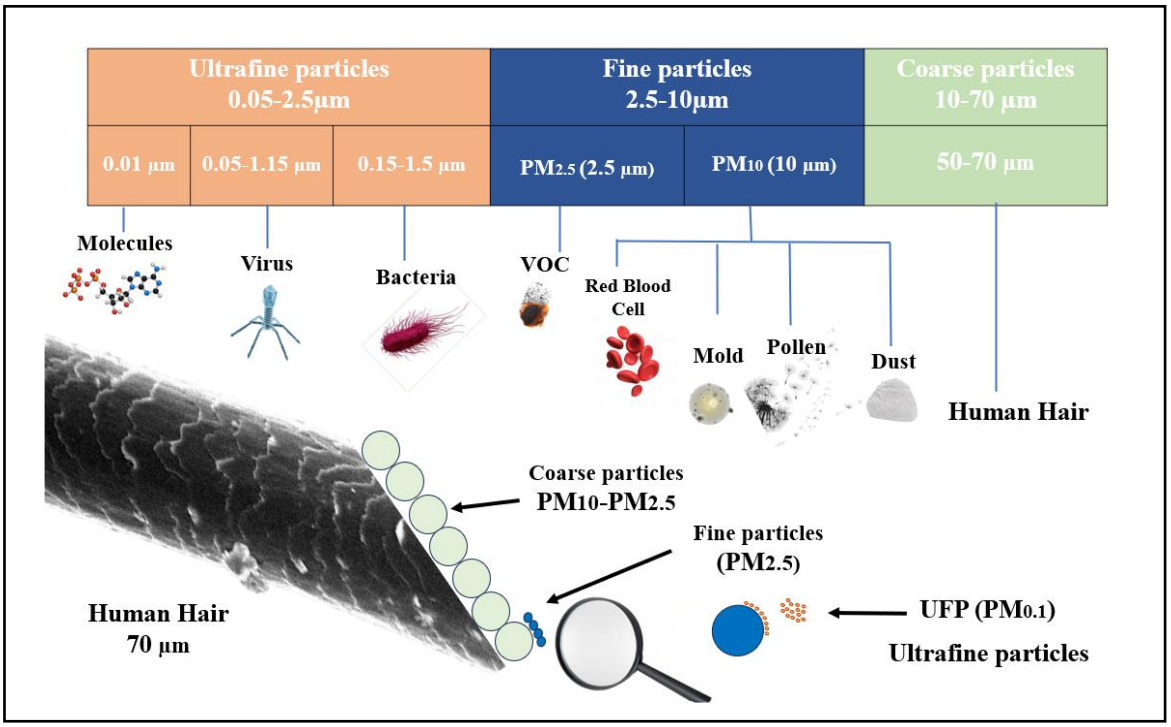


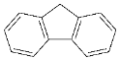
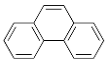
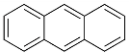
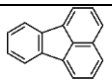
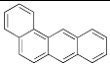
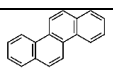
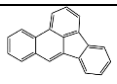
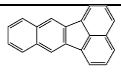
Fig. (1-4): Comparison of the sizes of different air pollutants compared to the size of a human hair (Drawing is derived from the source [39]**).**

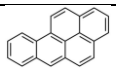
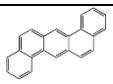
1.2.1 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are chemicals naturally produced from burning coal, crude oil, gasoline, and other organic materials. These PAHs can form small molecules in the air [40]. There are two main ways that incomplete combustion of organic matter generates particulate PAHs in the atmosphere: the release of residues or parts of aromatic compounds present in fuels and raw materials, and chemical synthesis during combustion, such as thermal decomposition of hydrocarbons, cyclization of chained alkanes or monocyclic aromatics, or condensation and polymerization of low-carbon PAHs [41]. PAHs are important environmental pollutants consisting of fused aromatic carbon rings and hydrogen. The U.S. Environmental Protection Agency has identified over 100 PAHs, 16 of which are priority pollutants due to their toxicity [42]. Polycyclic aromatic hydrocarbons (PAHs) have strong and long-lasting environmental resistance due to their intrinsic properties, such as heterogeneous aromatic ring structures, hydrophobicity, and thermal stability [43]. Low molecular weight PAHs (two or three rings) are mostly found in the atmosphere in the vapor phase. Medium molecular weight PAHs (four rings) are distributed between the vapor and particulate phases, depending on the atmospheric temperature. PAHs with five or more rings are generally associated with particulate matter [44]. PAHs pose a serious concern to human health since they are well-recognized to cause cancer, mutagenesis, and birth abnormalities [45, 46]. Long-term exposure to PAHs may increase the risk of developing tumors in various organs, including the skin, lungs, pancreas, esophagus, bladder, colon, and breast in women. Exposure to PAHs may increase the risk of cardiovascular disease, stroke, hypertension, and myocardial infarction. Preliminary clinical studies have shown a link between exposure to PAHs and oxidative stress and atherosclerosis [40]. Benzopyrene

(B[a]P) is often used as a marker for overall exposure to PAHs. The vapor pressure and solubility of PAHs in water generally decrease with increasing molecular weight, while homologs of the same molecular weight exhibit similar physical properties [41]. **Table 1.1** shows the physicochemical properties of 16 PAHs.

Table (1.1): Physicochemical properties of 16 polycyclic aromatic hydrocarbons[42].

Name	Formula	Structure	Molecular weight (g/mole)	Phase Distribution
Naphthalene	$C_{10}H_8$		128.17	Gas
Acenaphthylene	$C_{12}H_8$		152.20	Gas
Acenaphthene	$C_{12}H_{10}$		154.21	Gas
Fluorene	$C_{13}H_{10}$		166.22	Gas
Phenanthrene	$C_{14}H_{10}$		178.23	Particle gas
Anthracene	$C_{14}H_{10}$		178.23	Particle gas
Fluoranthene	$C_{16}H_{10}$		202.26	Particle gas
Pyrene	$C_{16}H_{10}$		202.26	Particle gas
Benzo(a)anthracene	$C_{20}H_{12}$		228.29	Particle
Chrysene	$C_{18}H_{12}$		228.29	Particle
Benzo(b) fluoranthene	$C_{20}H_{12}$		252.32	Particle
Benzo(k) fluoranthene	$C_{20}H_{12}$		252.32	Particle

Benzo(a)pyrene	**$C_{20}H_{12}$**		**252.32**	**Particle**
Benzo (g, h,i) perylene	**$C_{22}H_{12}$**		**276.34**	**Particle**
Dibenzo(a,h) anthracene	**$C_{22}H_{14}$**		**278.35**	**Particle**
Indenol [1,2,3 cd] pyrene	**$C_{22}H_{12}$**		**276.34**	**Particle**

1.2.1.1 Benzo [a] pyrene

Benzopyrene B[a]P consists of five benzene rings and two isomers can be recognized based on where the fifth ring is attached: B[a]P (one benzene molecule linked by an (a) bond) or benzopyrene (one benzene molecule linked by an (e) bond). A single benzene molecule is a liquid, while benzopyrene is a solid [47]. B[a]P is highly lipophilic and is rapidly absorbed into cells across the plasma membrane. B[a]P can be metabolized to dozens of metabolites by Aryl hydrocarbon receptor (AhR) and aromatic hydrocarbon metabolizing enzymes to form a more hydrophilic product called 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a] pyrene (BPDE) [47]. The detoxification event that results from the conversion to hydroxyl compounds or ketones is regrettably an activation response since the resultant BPDE has a significant oxidizing potential that can impact DNA replication and induce oxidative DNA damage [48, 49]. Oxidative stress from B[a]P and its metabolites can lead to increased reactive oxygen species (ROS), damaging proteins, lipids, DNA, and other cellular components and fuel inflammation and cell dysfunction. The carcinogenic polycyclic aromatic hydrocarbon B[a]P has been shown to damage cells lining blood vessels [50, 51]. B[a]P and Cardiovascular Disease (CVD) are associated with DNA damage, including DNA adducts and oxidative

DNA damage, in both proliferating cells and cells of the vascular wall. Carcinogenic chemicals have been identified as a risk factor for cardiovascular disease [52]. Polycyclic aromatic hydrocarbons are also associated with cardiotoxicity such as atherosclerosis, cardiac hypertrophy, arrhythmia, and contractile dysfunction [53]. Benzopyrene has been shown to metabolize and generate reactive oxygen species (ROS) in the liver [54]. Studies have also shown that the aryl hydrocarbon receptor (AhR) and its signal transduction pathway play an important role in oxidative stress, inflammatory response, and genotoxicity of vascular wall cells [50, 55]. **Figure 1-5** explains the pathway of metabolic activation benzo[a]pyrene.

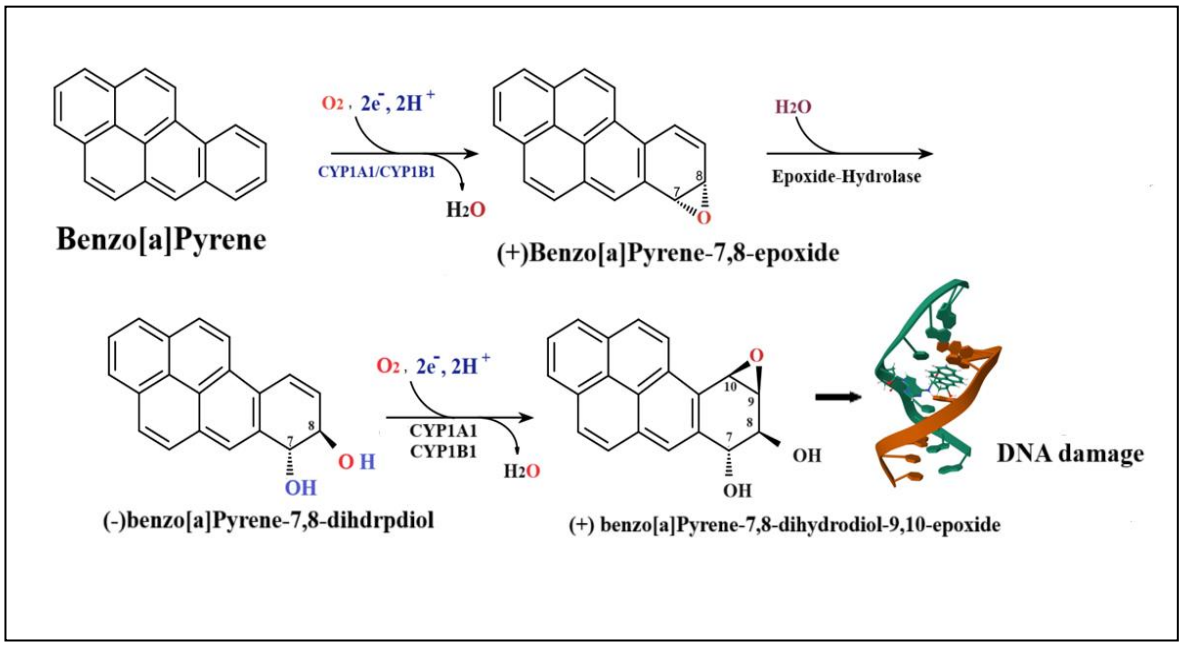


Fig. (1-5): The metabolic pathways of benzo[a]pyrene [56].

1.2.2 Heavy Metals

The most comprehensive definition of a "Heavy metal" is an element with an atomic number (Z) greater than 20 and a density greater than 5 g/cm^3 [57]. According to this definition, 51 elements in the periodic table fall under the heading of “heavy metals.” Heavy metals are toxic even at low concentrations and pose significant health risks. Some common heavy metals include Lead (Pb), Nickel (Ni), Chromium (Cr), Cadmium (Cd), Mercury (Hg), Cobalt (Co), and Copper (Cu). Metalloids such as Arsenic (As), are often classified as heavy metals because they exhibit similar chemical properties and environmental behaviors. Metals cannot be degraded or biodegraded [58]. Organisms detoxify metal ions by sequestering them in proteins or depositing them in intracellular granules for excretion or storage. When ingested or inhaled, these heavy metals bioaccumulate in the body, causing biological and physiological complications [59]. Recent studies have provided strong evidence that exposure to heavy metals is linked to an increased risk of diabetes and hypertension, both of which are significant risk factors for cardiovascular disease [60, 61].

1.2.2.1 Cadmium

Crude oil metals released during natural gas combustion can cause inflammation, hypertension, nephrotoxicity, lipid metabolism, myocardial electrical disturbances, cardiotoxic effects, and genetic effects [17, 62]. Cadmium Ranks 7^{th} in the US (ATSDR 2022) Priority Hazardous Substances List [63]. There is strong evidence linking cadmium to cardiovascular disease. At low concentrations, cadmium, with a half-life of 10 to 30 years, can interfere with a biological process [60, 64, 65]. It can cause vascular tissue damage, dysfunction, and atherosclerosis through oxidative mechanisms and increased

reactive oxygen species (ROS). Cadmium is a divalent cation and can participate in Fenton reactions in mitochondria by indirectly displacing endogenous Fenton minerals (e.g., Fe^{2+}) [66]. Cadmium primarily accumulates in smooth muscle cells after being transported through endothelial cells. This accumulation increases lipid buildup and alters lipid profiles towards a more atherogenic state. Consequently, cadmium can damage the integrity of endothelial cells and promote their death [67]. Because of their similar +2 valence, intestinal absorption of cadmium is greater in iron, calcium, or zinc deficiencies. Cadmium displaces essential biominerals, altering their balance and interfering with biological processes. For example, cadmium and zinc compete for binding sites in antioxidant enzymes and metal-absorbing proteins, reducing their ability to scavenge free radicals [68].

Cadmium can target the thiol (-SH) groups in cysteine proteins. Disruption of these sulfhydryl groups leads to various functional defects in the nucleus, endoplasmic reticulum, and mitochondria [69]. Chronic exposure to Cd leads to direct DNA damage or affects gene expression through interactions with essential metals and various proteins, thus increasing the risk of multiple tumors [68]. Long-term exposure to cadmium significantly contributes to cardiovascular diseases. The disease's processes may include inflammation and oxidative stress, which when paired with changes in lipoproteins lead to vascular health issues [70].

1.2.2.2 Lead

Oil sites contain nanoparticles abundant in heavy metals and organic compounds in aerosols, polluting the workplace air and environment, including Lead (Pb) and its compounds [71]. Lead is a dangerous heavy metal due to its harmful effects at low concentrations and accumulation in the human body [72–

74]. The International Agency for Research on Cancer has classified lead and its inorganic compounds as carcinogenic to humans [75, 76]. The Centers for Disease Control and Prevention guidelines state that adults with blood lead levels greater than 5 μg/dL are at high risk. The World Health Organization (WHO) advises against considering any blood lead level safe [77]. Exposure to small amounts of Lead in the human body can damage various cells and organs. It can damage the kidneys, brain, reproductive organs, central nervous system, gastrointestinal tract, vitamin D deficiency, and cardiovascular system, resulting in acute and chronic symptoms [78, 79]. Lead exposure can break down cell components, induce lipid peroxidation, trigger inflammatory signaling pathways, and produce reactive oxygen species (ROS), which leads to cellular oxidative stress [80]. Lead can also interfere with the activity of antioxidant enzymes such as glutathione, catalase, and superoxide dismutase [81, 82]. In addition, it can inhibit enzymes important in heme synthesis, and oxidize hemoglobin, causing damage to red blood cells through free radicals [83–86]. Lead can interact with nuclear proteins, such as transcription factors and DNA repair enzymes. Both cytosolic and endoplasmic reticulum proteins can chelate lead and weaken the balance for dozens of final goals [87, 88]. Lead can disrupt the endocrine system in humans by affecting steroid hormones and the thyroid gland [69, 89]. Lead exposure contributes significantly to heart failure by increasing vasopressor levels and decreasing vasodilators and by causing nephropathy and autonomic nervous system dysfunction. This can increase peripheral resistance and stress on the heart. Moreover, lead exposure elevates cardiovascular risk factors, including hypertension, hyperlipidemia, and diabetes. Additionally, lead disrupts the electrical conduction of the heart, impairing the physiological relationship between heart rate and myocardial performance and impairing heart function [90].

1.3 Oxidative Stress

The reactive oxygen species (ROS) scavenger's inability to control the overproduction of oxidants leads to oxidative stress in vertebrates. Most use oxygen oxidation for energy production, with mitochondria producing water at the end of the respiratory chain. However, partial oxygen reduction can produce reactive oxidant intermediates like superoxide anion and hydroxyl radicals, triggering further oxidation [91]. Environmental stresses such as particulate matter, organic pollutants, heavy metals, UV radiation, and ionizing radiation increase the production of reactive species [92]. This upsets the delicate biochemical balance between oxidants and antioxidants, favoring the oxidants and creating a state of "oxidative stress." In this state, reactive species can lead to oxidative damage to key biomolecules, including proteins, carbohydrates, lipids, and DNA [93, 94]. Reactive oxygen species (ROS) are a group of compounds that include reactive oxygen, such as non-radical species like hydrogen peroxide (H_2O_2) and free radicals like hydroxyl ($^{\bullet}OH$) and superoxide ($O_2^{\bullet-}$). Other than oxygen, certain reactive species also contain nitrogen (RNS), sulfur (RSS), and Hypochlorous acid (HOCl), a prototype for reactive chlorine species (RCS), which are reactive species that contain chlorine [95, 96]. Instead of directly measuring reactive species, researchers often use oxidative damage products from proteins, lipids, and DNA to assess the level of oxidative damage, these products are commonly referred to as biomarkers [91].

1.3.1 Malondialdehyde (MDA)

Exposure to microparticles triggers irreversible lipid peroxidation reactions and gives reactive carbonyl species (RCS) that mediate signaling to proteins, DNA, and lipids. RCS are molecules with a highly reactive carbonyl (aldehyde and ketone) group, which can attack the side chains of neighboring fatty acids and start producing other fatty radicals [94, 97]. The lipid radicals generated

during this chain reaction accumulate in the cell membrane and can have a wide range of effects on cellular function, including plasma leakage and dysfunction of membrane-bound receptors. It is important to note that the end products of lipid peroxidation, such as unsaturated aldehydes such as malondialdehyde (MDA) and other metabolites, have cytotoxic properties. MDA can bind covalently to nuclear clusters, causing dysfunction and DNA damage [98, 99]. RCS induced by oxidative stress may contribute to disease states such as cancer and atherosclerosis by promoting endothelial cell activation, inflammation, and foam cell formation [100, 101]. Lipid peroxidation can be measured in biological fluids (e.g., blood, serum, plasma, and urine) through the concentration of lipid peroxides themselves and products of lipid peroxidation, such as malondialdehyde. Representing MDA synthesis and metabolism in **Figure 1-6:**

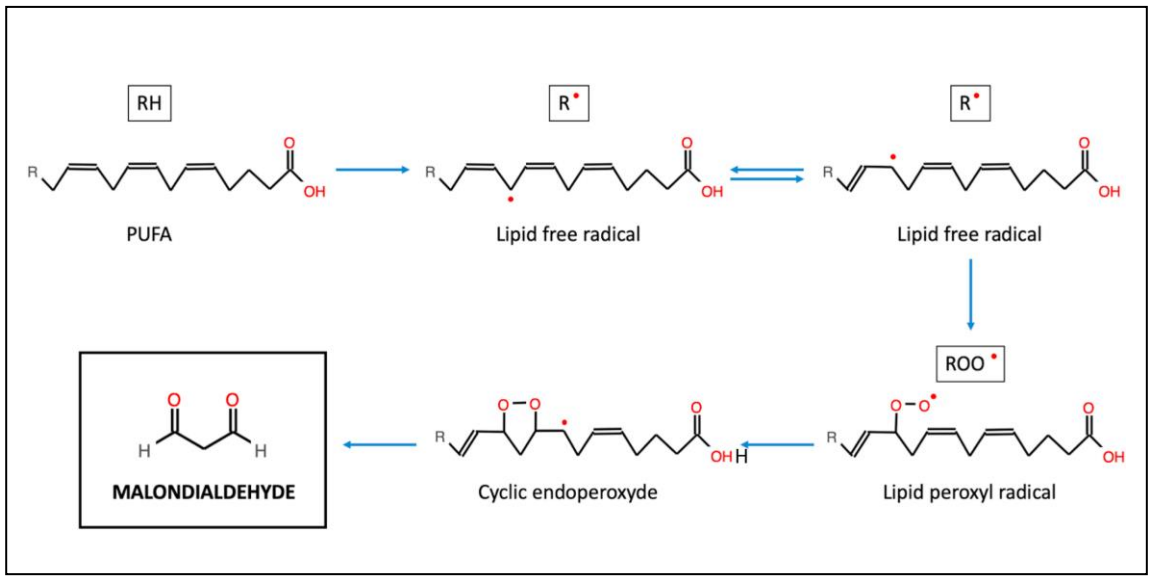


Fig. (1-6): MDA formation through lipid peroxidation [102].

1.3.2 Antioxidant Systems

The human body has a robust endogenous enzymatic antioxidant system and various small molecular-mass non-enzymatic antioxidants [91]. Antioxidant enzymes are a group of proteins and metalloproteins that help convert reactive oxygen species (ROS) and their byproducts into more stable and less harmful substances. These enzymes are essential for defending against oxidative stress caused by ROS, which can damage all cellular components. Key antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase, and catalase, work together effectively to combat reactive oxygen species. In addition to enzymes, important low-molecular-weight antioxidants include vitamins C and E, carotenoids, flavonoids, glutathione, and other similar compounds [94]. Antioxidants also play a role in improving inflammatory disorders by counteracting excessive inflammatory responses caused by cytokines [103].

1.3.2.1 Superoxide Dismutase (SOD)

The superoxide dismutase (SOD) family (enzyme code EC 1.15.1.1) consists of metalloenzymes that capture superoxide radicals [104]. These enzymes have antioxidant effects, converting superoxide radicals to oxygen (O_2) and hydrogen peroxide (H_2O_2)[105]. Enzymes such as catalase (CAT), glutathione peroxidases (GPXs), and peroxiredoxins (PRXs) then convert the hydrogen peroxide to water (Figure 3). This process helps protect cells from oxidative stress damage. In eukaryotes, SODs are classified into three types based on their mineral cofactors and their cellular localization [83]:

Copper-zinc superoxide dismutase (Cu/Zn SOD or SOD1), which is located in the cytoplasm or secreted into the extracellular fluid. Manganese superoxide

dismutase (Mn-SOD or SOD2) is in the mitochondrial matrix. Extracellular superoxide dismutase (EC-SOD or SOD3), is outside the cells.

An increase in superoxide anions causes inactivation of NO, leading to the generation of ONOO—a powerful oxidant. ONOO- inhibits endothelium-dependent vasorelaxation, reducing the beneficial effects of NO on platelet aggregation and vascular smooth muscle cell proliferation and causing DNA and lipid oxidation; In general, these factors are involved in the development of atherosclerosis [95, 106]. **Figure 1-7** Reactions of SOD enzymes with the superoxide anion generating hydrogen peroxide (H_2O_2).

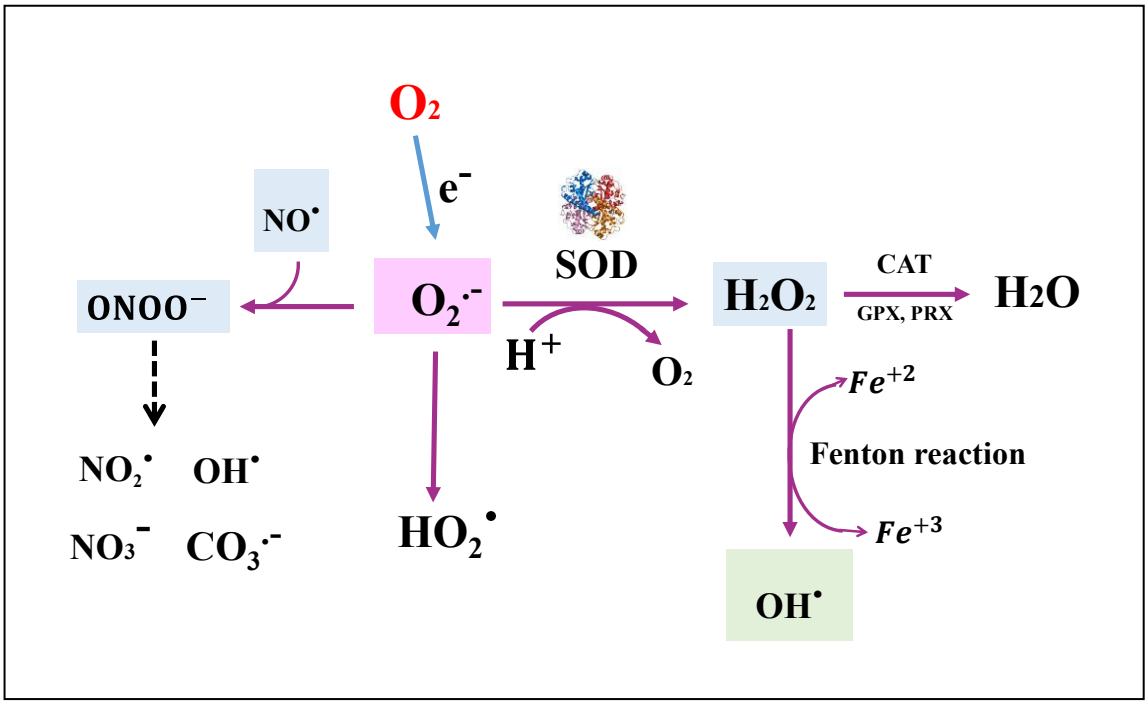


Fig. (1-7): Reactions of SOD enzymes with the superoxide anion generating hydrogen peroxide (H_2O_2) [107].

1.3.2.2 Essential Trace Elements

Essential minerals are required in minute concentrations for metabolic activities in organisms such as (selenium, zinc, iron, and magnesium) [108]. Essential minerals are important for innate immune system function, adaptive

immune defense, and inflammation regulation. These defense mechanisms against pathogens and the long-term pro- and anti-inflammatory regulation balance are essential. Deficits can temporarily reduce immune competence or disrupt the regulation of systemic inflammation in the long term [109].

1.3.2.2.1 Selenium (Se)

Selenium is a naturally occurring element essential for normal human physiological processes but in trace amounts. It plays a crucial role in cardiovascular health and helps protect against oxidative stress. Additionally, selenium is significantly associated with cardiovascular well-being [110]. Keshan disease is an endemic condition marked by cardiomyopathy and heart failure, initially linked to selenium deficiency. Since then, various cardiovascular conditions including myocardial infarction, heart failure, coronary heart disease, and atherosclerosis have also been associated with a lack of selenium [111, 112]. Selenium is incorporated into selenocysteine, an amino acid essential for the function of selenium-containing proteins. Twenty-five genes that encode selenium proteins have been found in humans. Many of these proteins protect the body against oxidative stress by managing the damaging effects of reactive oxygen molecules [113, 114]. Although the functions of most selenium proteins are unclear, selenium protein P has been identified as a selenium transporter, which transports and delivers selenium to peripheral tissues; thyroid hormone deiodinase as a regulator of thyroid hormone metabolism; and thioredoxin reductase and glutathione peroxidase (GPx) as antioxidants [115].

Experimental studies have shown that redox-active selenium proteins, including GPx1, GPx3, GPx4, and Txnrd2, play against cardiovascular disease by reducing reactive oxygen species (ROS) [113]. These proteins help maintain healthy endothelial function by regulating vascular reactivity, preventing

harmful remodeling, and facilitating new blood vessel formation after ischemic injury [114]. Endothelial dysfunction arises from the accumulation of reactive oxygen species and the loss of essential nitric oxide (NO), which can contribute to conditions like atherosclerosis, stroke, and heart injury [115]. Recent research indicates that GPx4 plays a role in regulating cell death, autophagy, and various diseases. A decrease in GPx4 may contribute to the progression of atherosclerosis and specific forms of cardiomyopathy [116, 117].

1.3.2.2.2 Zinc (Zn)

Zinc is a cofactor in metalloenzymes and metalloproteinases is involved in diverse biological functions, including translation and replication, phagocytosis, immunoglobulin, cytokine production, cell transport, endocrine and central nervous system function, reproduction, gene expression, and homeostasis [118, 119]. Zinc is necessary for over 300 enzymes and 2000 transcription factors in the human body [120]. It plays a role in insulin metabolism and pancreatic beta-cell function, influencing insulin release and glucose transporter protein regulation. Inadequate zinc levels can lead to insulin resistance and impaired glucose tolerance [121]. Zn is also required for protein and collagen synthesis and thus contributes to wound healing [122]. Zinc is an important biomarker for cardiovascular health, as sufficient levels of bioavailable zinc are critical for proper cardiovascular function. Specific events, such as ischemia and infarction, can trigger the release of zinc from proteins, which may result in myocardial damage [123, 124]. Zinc has antioxidant and anti-inflammatory properties that may help protect the vascular system. Oxidative stress and chronic inflammation are major factors in the development of atherosclerosis. Zinc enhances the production of glutathione (GSH) and metallothionein and enhances the activity of the enzyme superoxide dismutase (SOD) [123].

Zinc, a reduced metal, exhibits antioxidant properties through three mechanisms: binding to -SH groups in proteins and enzymes, protecting them from reactive oxygen species (ROS) attacks; competing with oxidizing metals such as iron and copper; and stimulating the synthesis of antioxidant enzymes through transcription factors such as MTF-1. By displacing oxidized and reduced metals from their binding sites, zinc reduces their bioavailability for catalytic reactions and reduces the formation of harmful hydroxyl radicals [124].

1.4 Inflammation

The body's reaction to illness, trauma, or exposure to harmful substances is inflammation. To preserve immunological homeostasis, cytokines control immune responses. Inflammatory diseases that cause tissue damage are among the negative effects of dysregulated cytokine signaling. Chronic inflammation contributes to cardiovascular disease, affecting endothelial dysfunction, foam cell formation, plaque development, and cytokine-mediated plaque rupture [125, 126]. Research suggests that lead and cadmium can negatively affect the immune system and may lead to an increased inflammatory response [127–129]. In addition, $PM_{2.5}$ can cause inflammation at other vascular sites, including adipose tissue and the brain.

1.4.1 C-Reactive Protein (CRP) & High-Sensitive C-Reactive Protein

C-reactive protein (CRP) is an indicator of inflammation. CRP is produced primarily by the liver, which releases it into the bloodstream in response to inflammation. Its levels remain stable for 24 hours [130]. One test that is frequently used to identify inflammation in the body, whether it be from

rheumatoid arthritis, bacterial infections, inflammatory bowel disease, or any inflammatory disorder, is C-reactive protein. Less than 5 mg/L is the usual range. A high-sensitivity C-reactive protein test, which typically ranges from 0.5 to 3 mg/L, can identify inflammation at low levels in individuals with risk factors and predict heart disease and stroke in healthy individuals with risk factors [131, 132].

1.5 Lactate Dehydrogenase (LDH)

One essential enzyme in the anaerobic metabolic cycle is lactate dehydrogenase (LDH). It has the enzyme number EC 1.1.1.27 and is a member of the oxidoreductase class. The enzyme is in charge of catalyzing the reduction of NAD^+ to NADH and the reversible conversion of lactate to pyruvate. One of the H transfers (oxidoreductase) enzymes, lactate dehydrogenase, uses NADH to catalyze the reversible conversion of pyruvate to lactate. When oxygen is scarce or nonexistent, the enzyme plays a role in the anaerobic metabolism of glucose [133]. Lactate dehydrogenase is present in practically every bodily tissue and is a normal metabolic process. Lactate, a signaling molecule, can influence the immune system and act as a "danger" signal for life. Serum lactate is a chemical indicator of disease severity under inflammatory conditions [134]. Liver disease, anemia, heart attacks, bone fractures, muscle damage, malignant tumors, and infections like HIV, encephalitis, and meningitis can all increase LDH levels in the blood [135, 136]. When organs are damaged by massive cell death, which results in loss of cytoplasm, LDH levels in the blood rise. The enzyme can remain elevated in the bloodstream for up to 7 days, depending on the type of tissue damage. Acute myocardial infarction, anemia, pulmonary embolism, hepatitis, acute renal failure, cancer, and infections are among the conditions that can cause tissue damage. LDH can be used as a disease marker to stage the disease and monitor prognosis or response to treatment [133]. LDH-

1 (H4), LDH-2 (H3M), LDH-3 (H2M2), LDH-4 (HM3), and LDH-5 (M4) are the five isoenzymes of LDH. The M and H subunits make up its homogeneous or heterogeneous tetrameric structure. Because the M subunit is so effective at catalyzing this reaction, LDH-5 (LDH-A) has the greatest capability to convert pyruvate to lactate. On the other hand, in the opposite process, the H subunit may change lactate into pyruvate **Figure 1.8**. Although it has recently been proposed that LDH may exist in mitochondria, it is mostly active in the cytoplasm [137].

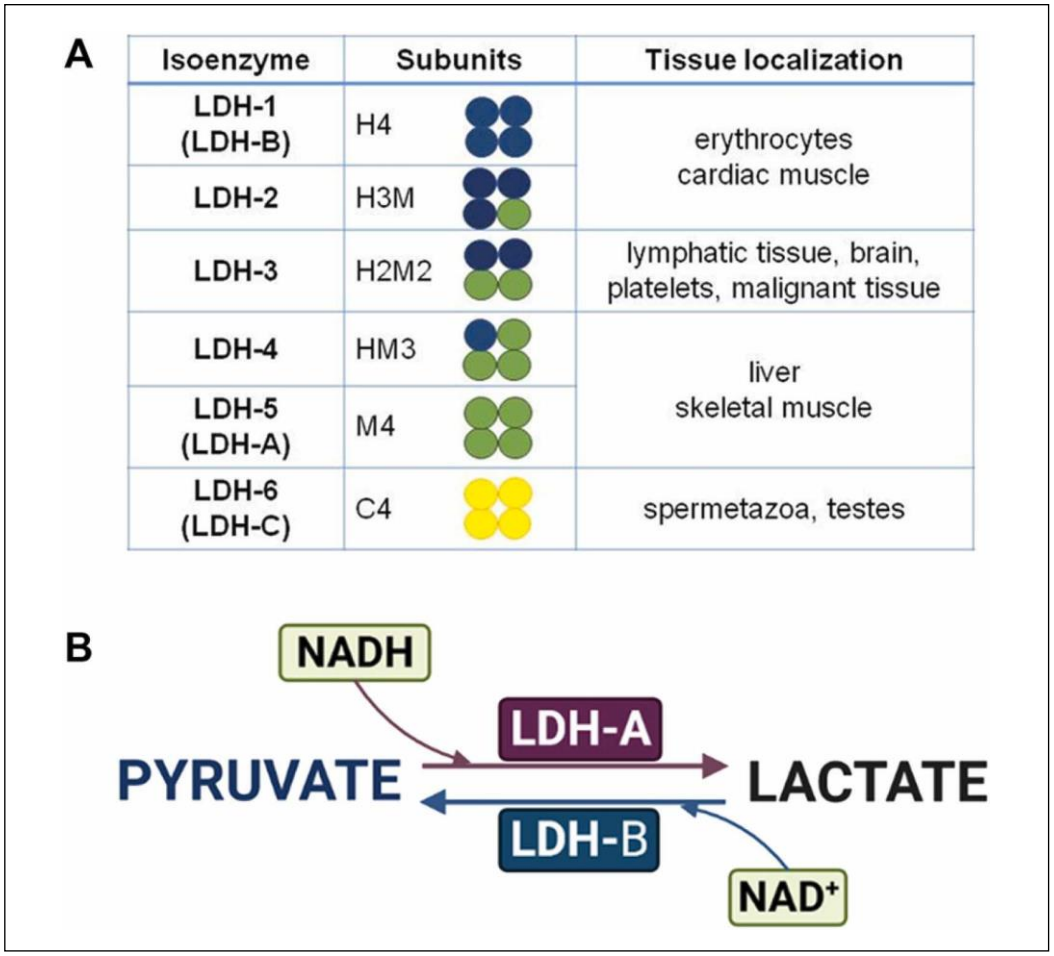


Fig. (1-8): Localization of LDH isoenzymes and reaction catalyzed by LDH.
(A) Tissue localization of LDH isoenzymes.
(B) Schematic representation of the reverse reaction of converting pyruvate to lactate, catalyzed by LDH-A or LDH-B [138]

The five forms of LDH are:

The primary enzyme present in heart tissue is LDH-1

The primary enzyme in red blood cells and the reticuloendothelial system is LDH-2.

The main enzyme in the lungs is LDH-3.

The main enzyme in the kidneys is LDH-4.

The liver and skeletal muscle both have high expression levels of the protein LDH-5. These five forms catalyze the same overall reaction and differ in substrate affinity, inhibition concentration, isoelectric point, and electrokinetic [137].

1.6 Lipid Profile

The subject of Clinical Lipidology provides lipid analysis for clinical applications, enabling prediction, diagnosis, management, and health [139]. Cholesterol and triglycerides are insoluble in water, so these lipids must be transported in association with proteins. Lipoproteins are complex molecules with a central core containing cholesterol esters and triglycerides surrounded by free cholesterol, phospholipids, and lipoproteins, which facilitate the formation and function of lipoproteins. There are five main types of lipoproteins in the blood: chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Each of these lipoproteins is responsible for transporting triglycerides and cholesterol to their intended destinations in the body. Cholesterol levels play an important role in the development of cardiovascular disease. Elevated blood lipid levels, known as hyperlipidemia, increase the risk of atherosclerotic cardiovascular disease. In clinical practice, obtaining a lipid profile is essential for screening, diagnosing, and treating these conditions [140].

1.7 Epidemiology: Fine Particles Matter and CVDs

An estimated 17.9 million people die from cardiovascular disease each year, making it the world's leading cause of death. Cardiovascular disease includes conditions that affect the heart and blood vessels, such as rheumatic heart disease, coronary heart disease, and cerebrovascular disease [141, 142]. Several adverse cardiovascular effects have been linked to air pollution, with fine particulate matter ($PM_{2.5}$) being an invisible killer that puts heart health at risk. While long-term exposure increases the risk of cardiovascular death and shortens life expectancy, short-term exposure to $PM_{2.5}$ causes acute cardiovascular death and non-fatal events [38]. The entry of inhaled particles into the alveoli increases reactive oxygen species and stimulates an inflammatory response, which is one way inhaled particles affect cardiovascular health. Alveolar macrophages release pro-inflammatory cytokines, which likely have secondary effects on vascular control and contractility. On the other hand, trace amounts of insoluble fine particles (like metals) may enter the bloodstream straight from the lung and impact cardiovascular and central nervous system functions [143]. Several studies of short-term exposure to fine particles have found positive associations between ultra-fine particles and cardiovascular hospitalization. Researchers have found increased ischemic heart disease mortality associated with chronic exposure to ultra-fine particles [144–146]. The clinical significance of inhaled particles in cardiovascular effects includes acute coronary syndrome, myocardial infarction, unstable angina; cardiac arrhythmias; worsening of chronic heart failure; stroke; and sudden cardiac death. Such effects can be measured after acute exposure; there is evidence that chronic exposure accelerates atherosclerosis and reduces life expectancy [38].

1.8 Aims of Study:

This clinical study aimed to evaluate the effects of occupational exposure to particulate emissions from crude oil well sites on the cardiovascular system, focusing on oxidative stress and inflammatory mechanisms. The study included the following:

1. Determination of benzo[a]pyrene diol epoxide (BPDE) levels in the workers' blood and a control group.
2. Estimating the concentration of certain heavy metals, such as cadmium and lead, in the workers' blood and a control group.
3. Estimation of the concentration of some essential trace elements, such as zinc and selenium, in the blood of exposed workers and the control group.
4. Evaluation of markers of oxidative stress, including assessment of levels of malondialdehyde (MDA), and superoxide dismutase (SOD), an antioxidant enzyme, in the serum of study participants.
5. Estimating C-reactive protein (CRP) and high-sensitivity C-reactive protein (hs-CRP) levels as inflammation biomarkers.
6. Evaluation of Lactate dehydrogenase (LDH) levels in the serum of study participants.
7. Evaluation of the lipid profile (TC, TG LDL, HDL, and VLDL,) in the serum of exposed workers and an unexposed control group.
8. Studying the relationship between the studied biomarkers and elevated (Cadmium, Lead, and BPDE) levels and the relationship in causing cardiovascular diseases.

CHAPTER

TWO

Material

&

Methods

2.1 Material

2.2 Chemical material

Table 2.1 lists the compounds used, their purity, the manufacturer, and their place of origin:

Table (2.1): Chemical used and manufacturing purities

Chemicals material	Purity %	Company	Origin
Nitric acid	70	Fisher	Germany
Hydrochloric acid	36.49	RDH	UK
Perchloric acid	70	Fluka	Switzerland
Sulfuric acid	95	BDH	UK
Selenium stock solution 1000 mg/L	-----	HORIBA	France
Zinc stock solution1000 mg/L	-----	HORIBA	France
Cadmium stock solution1000 mg/L	-----	HORIBA	France
Lead stock solution1000 mg/L	-----	HORIBA	France

2.3 Diagnostic Kits

The diagnostic kits purchased from different companies are listed in **Table 2.2** below:

Table (2.2): kits used in the study

Diagnostic Kits	Company	Origin
BPDE DNA Adduct ELISA Kit	ELLBIOLABS, INC.	USA
MDA (Malondialdehyde) ELISA Kit	ELK Biotechnology	China
SOD (Super Oxidase Dimutase) ELISA Kit	ELK Biotechnology	China
CRP-Rapid Quantitative Test Kit	Wondfo	China
LDH Kit	Biolabo	France
Total Cholesterol Kit	Biolabo	France
Triglyceride Kit	Biolabo	France
HDL- Cholesterol Kit	Biolabo	France

2.4 Instruments

Table 2.3 lists the study's instruments along with their model, firm, and place of origin.

Table (2.3): Study instruments utilized

Instruments	Model	Company	Origin
Inductively coupled plasma-optical Emission spectroscopy	JY2000-2	HORIBA Scientifical	France
Finecare Plus	FS112/FS-113/FS-205	Wondfo	China
UV visible Spectrophotometer	7200	Cecil	France
Elisa Microplate Reader	MR-96A	Mindray	China
Centrifuge	2002	Hettich	Germany
Water bath	WNB10	Memmert	Germany
Oil bath	F3	Hake	Germany

2.5 Study Design

This study was conducted in Basrah, southern Iraq, and involved two groups of participants. The first group of ninety workers was exposed to polluted working conditions at the Nahran Bin Omar crude oil field. Each worker had at least two years of experience and worked eight hours a day, five days a week. The second group included ninety individuals not exposed to these conditions (the control group).

2.6 Ethical Committee Approval

The Health and Safety Ethics Committee of Basrah Oil Company approved all studies. All participants provided written informed consent**.** The demographic information of the participants (age and gender), personal lifestyle (drinking and smoking), work history, duration of exposure (number of years of work and working hours per day), and health status (genetic disorders, medications consumed, and other blood disorders such as diabetes, kidney disease, and hypertension) was requested using a questionnaire. At 9:00 am, data collection began. Each participant had to fill out this form to be included as a selected sample in this study. Each participant signed a written consent form, as shown in **Appendix (2.1)**, and participants were interviewed using a detailed questionnaire as shown in **Appendix (2.2)**.

2.7 Sample Collection:

A 3 mL blood sample was taken from each participant using an evacuated tube containing the anticoagulant EDTA K3 FV01003. For trace element measurements. 3 mL was also taken from an evacuated tube model G1326331 with a gel activator and a clot tube and separated by centrifugation at the Nahran Bin Omar Health Care Department (2000 rpm for 10 min), where serum and lysates required for the study were isolated. To avoid any effects of ambient temperatures on the samples, the collected blood samples were always stored in cold conditions in an icebox until measurement.

2.8 Determination of Elements

2.8.1 Digestion Procedure:

The samples were digested by transferring (1 mL) of whole blood into a Pyrex test tube, adding (1 mL) of conc. HNO_3 and (1 mL) of conc. H_2SO_4, placed in an oil bath at 130°C for 45 minutes, until nitric acid (brown fumes) evaporates. Then the tubes were removed from the bath and cooled to room temperature, followed by the addition (1 mL) of 5M $HClO_4$, they were placed again in an oil bath at 130°C for 45 minutes. The tubes were removed again, from the bath and cooled to room temperature, then (1 mL) of 6M HCl was added and the tubes were placed again in the bath at 95°C for 30 minutes then cooled to room temperature and diluted to (10 mL) by 6M HCl [147–149].

Appendix (2.3) Picture showing blood samples after digestion

2.8.2 Measurement of Elements:

This study used inductively coupled plasma optical emission spectroscopy (ICP-OES) to target elements [150]. Heavy metals (Cadmium and Lead) and essential elements (Selenium and Zinc) were measured by a device (HORIBA Scientifical, JY2000-2, France) located at the Ministry of Science and Technology/Department of Environment and Water/Food Contamination Research Center. ICP-OES is an advanced analytical technique with excellent detection properties and has been widely used to analyze various chemical elements. It offers shorter detection time, lower detection limits, wider linear dynamic range, greater matrix tolerance, and no chemical interferences. It can handle different samples, including liquids, organic and inorganic solids, and liquids. ICP-OES can detect up to 70 elements simultaneously with high accuracy. ICP-OES is used in various analytical determinations, including food analysis, agricultural investigations, geological studies, drug/metabolite

analysis, environmental science, and forensics[151]. **Appendix (2.4)**, shows the method's parameters, and **Appendix (2.5)**, shows the plasma parameters.

2.8.2.1 Heavy Metals

2.8.2.1.1 Determination of Cadmium (Cd) [152]

Calibration curve (0.5-100) ng/mL was prepared using dilution method by deionized water. Cadmium was measured at the wavelength 226.502 nm. The levels of Cd were obtained by extrapolation of the standard curve, followed by multiplication with the dilution factor. Parameters of Curve in **Table 2.4** and Standard curve of Cd in **Figure 2-1**:

Table (2.4): Parameters of calibration standard of Cadmium

Wavelength (*nm*)	Linearity range (*ng/mL*)	Right position (*nm*)	Left position (*nm*)	Integration time (*s*)	Sigma	BEC (*µg/L*)	R^2
226.502	0.5-100	0.0177	0.01806	4.0	0.234	67.4	0.999

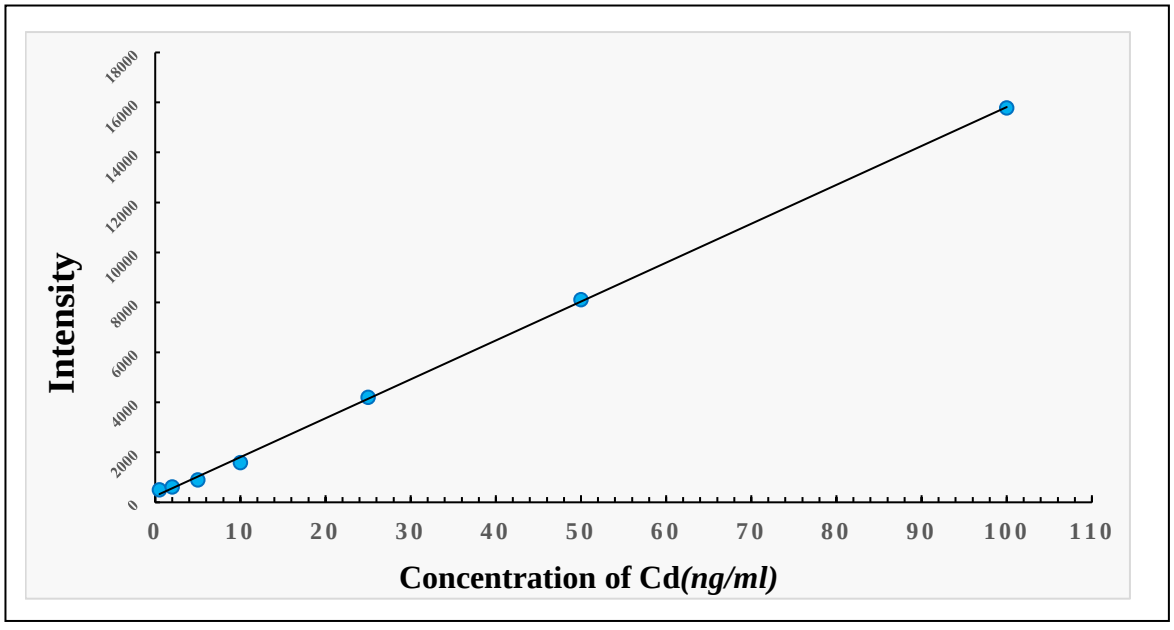


Fig. (2-1): Cadmium standard curve

2.8.2.1.2 Determination of Lead (Pb) [152]

Calibration curve (5 – 500) ng/mL was prepared using dilution method by deionized water. Lead was measured at the wavelength 220.353 *nm*. The levels of Pb were obtained by extrapolation of the standard curve, followed by multiplication with the dilution factor. The curve parameters are in **Table 2.5**, and the standard curve of Pb is in **Figure 2-2**.

Table (2.5): Parameters of calibration standard of Lead

Wavelength (*nm*)	Linearity range (*ng/mL*)	Right position *nm*	Left position *nm*	Integration time (*s*)	sigma	BEC (*µg/L*)	R^2
220.353	5-500	0.0177	0.01806	4.0	0.288	102	0.999

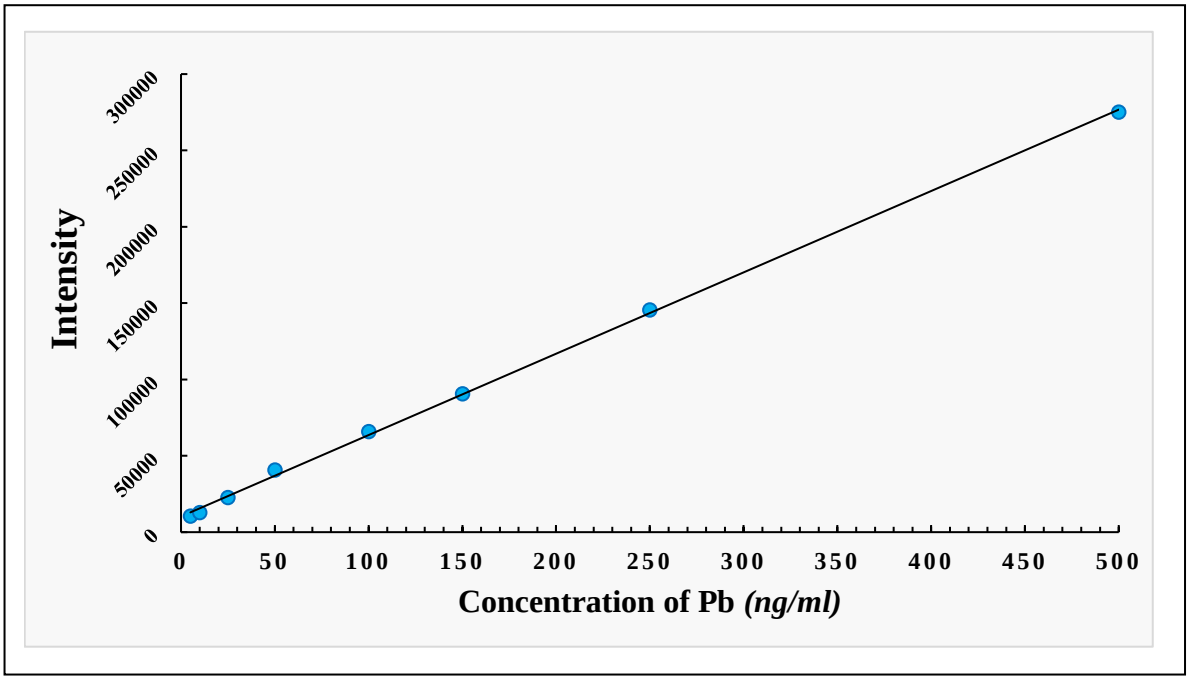


Fig. (2-2): Lead standard curve

2.8.1.1 Essential Element

2.8.1.1.1 Determination of Selenium (Se) [153]

Calibration curve (25 – 200) ng/mL was prepared using dilution method by deionized water. Selenium was measured at the wavelength 196.025 *nm*. Se levels were obtained by extrapolating the standard curve and multiplication with the dilution factor. Parameters of Curve in **Table 2.6** and Standard curve of Se in **Figure 2-3**:

Table (2.6): Parameters of calibration standard of Selenium

Wavelength (*nm*)	Linearity range (*ng/mL*)	Right position (*nm*)	Left position (*nm*)	Integration time (*s*)	sigma	BEC (*µg/L*)	R^2
196.025	25-200	0.0177	0.01806	4.0	5.322	102	0.998

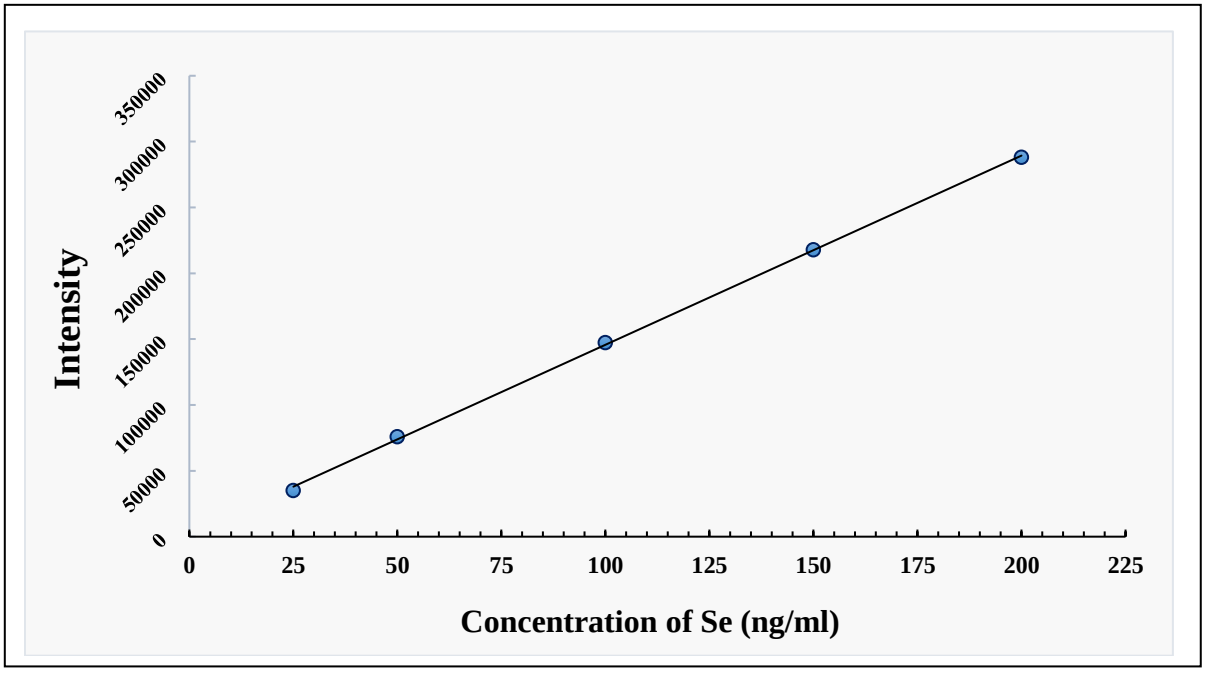


Fig. (2-3): Selenium Standard curve

2.8.1.1.2 Determination of Zinc (Zn) [154]

Calibration curve (50 – 1000) ng/mL was prepared using dilution method by deionized water. Zinc was measured at the wavelength 213.856 nm. Zn levels were obtained by extrapolating the standard curve and multiplication with the dilution factor. Parameters of Curve in **Table 2.7** and Standard curve of Zn in **Figure 2-4:**

Table (2.7): Parameters of calibration standard of Zinc

Wavelength (*nm*)	Linearity range (*ng/mL*)	Right position (*nm*)	Left position (*nm*)	Integration time (*s*)	Sigma	BEC (*µg/L*)	R^2
213.856	50-1000	0.0177	0.01806	4.0	5.405	8.77	0.998

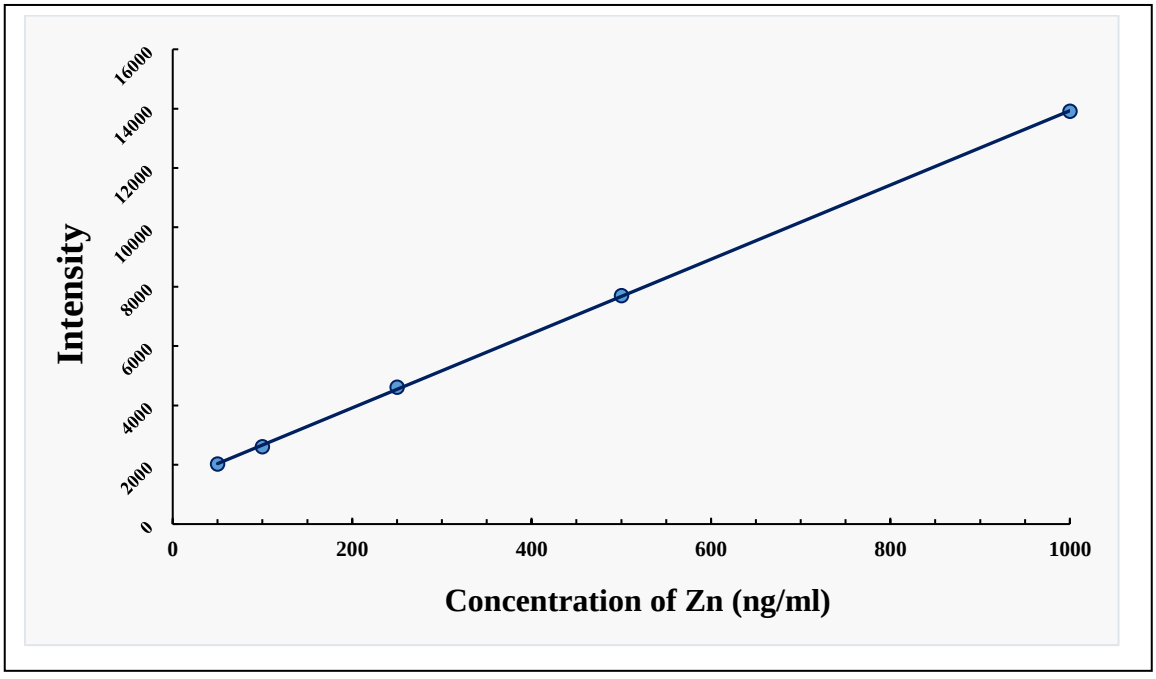


Fig. (2-4): Zinc Standard curve

2.9 Analysis of B[a]pyrene metabolite Biomarkers [155, 156]

2.9.1 Principle of Assay:

Cell Biolabs, Inc.'s (USA) BPDE DNA derivatives ELISA kit, is used. According to the manufacturer's instructions, BPDE-DNA standard or unknown samples were absorbed plate. The BPDE-DNA adducts in the sample or standard were examined using an anti-BPDE-I antibody followed by an HRP-conjugated secondary antibody. The levels of BPDE-DNA adduct in the unknown sample were compared to a standard curve created from previously established BPDE-DNA standards. **Table 2.8** shows the contents of the BPDE kit:

Table (2.8): Materials available in the (BPDE) Kit

Reagents	Quantity
DNA Binding Solution	6 Ml
Anti-BPDE antibody (1000X)	20 µL of anti-BPDE-I antibody
Secondary antibody, conjugated to HRP (1000X)	50 µL
Assay Diluent	50 mL
10X Wash Buffer	100 mL
Substrate Solution	12 mL
Stop Solution	12 mL
Reduced DNA Standard	200 µL of 0.2 mg/mL reduced DNA in TE Buffer.
BPDE-DNA	30 µL of 0.1 mg/mL BPDE-DNA in TE Buffer

2.9.2 Assay Procedure of Analysis of BPDE:

1. Samples were diluted to 4 µg/mL in 1X TE Buffer. 50 µL of unknown samples or BPDE-DNA standards were added to the wells of the high-binding DNA plate.
2. 50 µL of DNA binding solution was added to each well. The mixture was mixed well with a pipette and incubated overnight on an orbital shaker. Each DNA sample, including unknown and standard samples, was analyzed.
3. The DNA solution was removed and washed twice with PBS. (Wipe the dish with a paper towel to remove excess liquid) 200 µL of analysis solution was added to each well and left for 1 h at room temperature.
4. The buffer used in the analysis was removed. The plate was then dried on paper towels to remove excess liquid.
5. 100 µL of diluted anti-BPDE-I antibody was added to all wells and incubated for 1 h with stirring on an orbital shaker.
6. The wells were washed several times with 250 µL of 1X Wash Buffer, with thorough aspiration between each wash. After the last wash, the wells were drained, and the microwell strips were pressed onto an absorbent pad or paper towel to remove excess 1X Wash Buffer.
7. 100 µl of the diluted secondary antibody-HRP complex was added to all wells and incubated for 1 h at room temperature on an orbital shaker. The wells were then washed several times according to the step 6 above.
8. The substrate solution was warmed to room temperature. Then 100 µL of the substrate solution, including the blank wells, was added to each well. They were incubated at room temperature on an orbital shaker. The actual incubation time may vary from 2 to 30 min. Note: If the color changes rapidly, the reaction can be stopped early to prevent saturation.

9. The enzyme process is stopped by adding 100 µL of stop solution to each well. The color gradually fades, but the results are read immediately.
10. The absorbance of each well was read on a microplate reader using 450 nm as the base wavelength. **Appendix (2.6)** shows the color change in the BPDE-DNA Kit.

The concentration of BPDE-DNA in the samples was determined by comparing the OD to a standard curve, as shown in **Figure 2-5**.

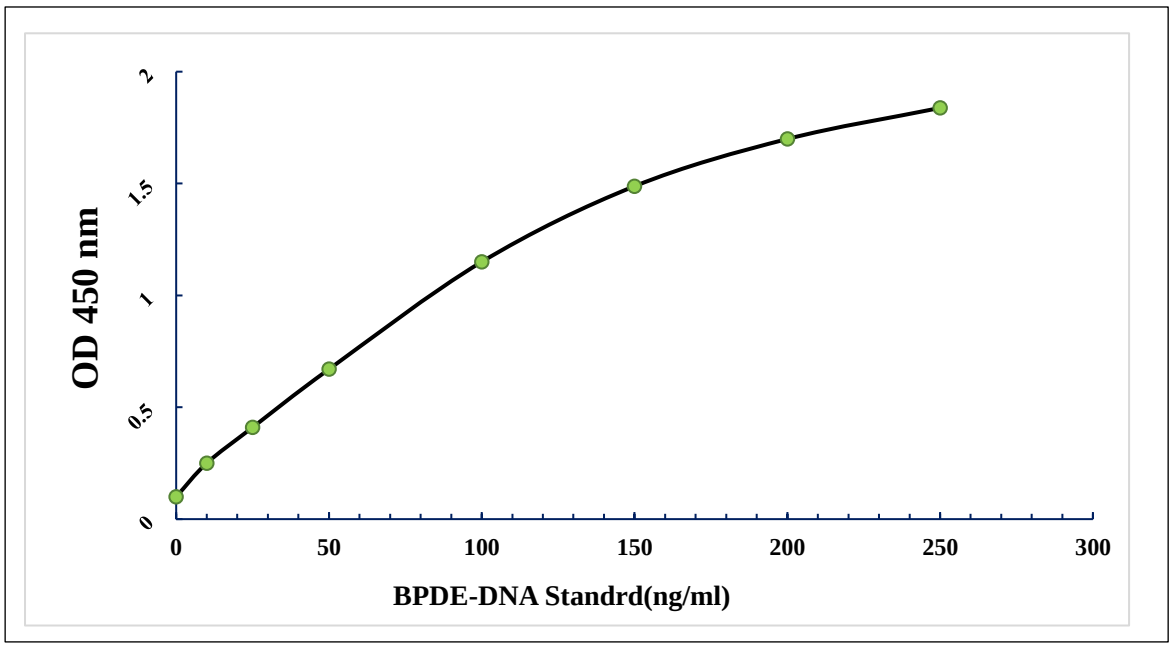


Fig (2-5): BPDE-DNA standard curve

2.10 Oxidative Stress System

2.10.1 Biomarker Analysis of Malondialdehyde (MDA) [157–159]:

2.10.1.1 Principle of Assay:

The Human MDA (Malondialdehyde) ELISA kit is used. According to the manufacturer's instructions, standard or unknown MDA samples were adsorbed onto the high-binding plate. The competitive enzyme immunoassay method is

used in this test. Malondialdehyde (MDA) protein has already been applied to the microtiter plates included in this kit. Using a biotin-conjugated antibody specific for malondialdehyde (MDA), standards or samples are introduced to the corresponding microtiter plate wells. Each microtiter plate is thoroughly coated with Avidin conjugated to Horseradish Peroxidase (HRP) and allowed to incubate before being supplemented with TMB substrate solution. The enzyme-substrate reaction is stopped by adding sulfuric acid solution, and the color shift is monitored using a spectrophotometer set at 450 nm ± 10 nm. The MDA kit's ingredients are listed in **Table 2.9**:

Table (2.9): Material provided in (MDA) Kit.

Reagents	Quantity
Standard Diluent Buffer	**20 ml**
Biotinylated Conjugate (100x)	**60 Ml**
Diluent of Biotinylated Conjugate	**10 Ml**
Streptavidin-HRP (100×)	**120 µL**
Diluent HRP	**12 mL**
Wash Buffer Solution (25×)	**20 mL**
Substrate Solution (TMB)	**9 mL**
Stop reagent	**6 mL**

2.10.1.2 Assay Procedure:

1. 50 µL of the sample was added to each well after the kit had been equilibrated at room temperature. Immediately after, 50 µL of Biotinylated-Conjugate (1x) (Biotin Antigen Working Solution) was added to each well, thoroughly mixed, and incubated for one hour at 37°C.
2. The liquid in the plate was discarded, and 200 µL of wash solution was added to each well. The plate was washed several times. After washing, the plate was dried by inverting it and wiping it with a clean paper towel. Then, 100 µL of Streptavidin-HRP working solution (1x) was added to each well, and the plate was incubated at 37°C for 1 h.
3. The liquid in the plate was discarded and 200 µL of wash solution was added to each well; the plate was washed several times. After the last wash, any remaining wash solution was removed by vacuum. The plate was inverted and wiped with a clean paper towel. 90 µL of TMB substrate solution was added to each well and incubated at 37°C for 20 min. **Appendix (2.7)** Pictures showing the color change to blue upon adding the TMB substrate solution to the MDA Kit.
4. 50 µL of stop solution was added to each well, the plate was read at 450 nm immediately, and the results were calculated. The concentration of malondialdehyde (MDA) in the samples was then determined by comparing the OD of the samples to the standard logarithmic curve, as shown in **Figure 2.6**.

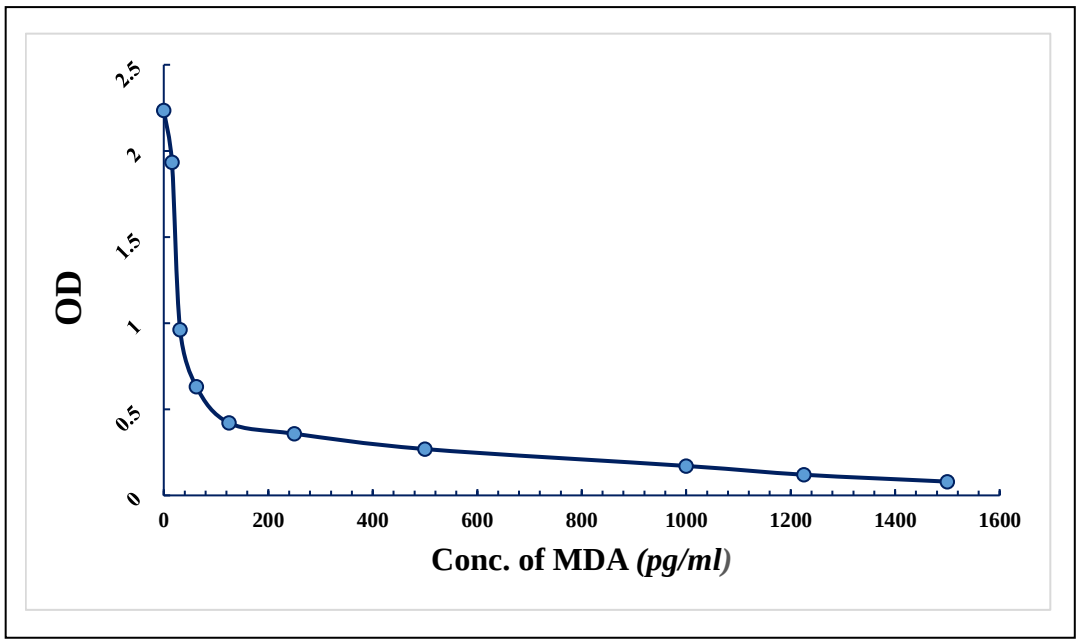


Fig. (2-6): MDA Standard curve

2.10.2 Analysis of Super Oxidase Dimutase (SOD) Biomarkers

2.10.2.1 Principle of Assay [157, 159]:

The human superoxide dismutase (SOD) ELISA kit is used. According to the manufacturer's instructions, standard or unknown SOD samples were adsorbed onto a 96-well high-binding plate. The test principle applied in this kit is sandwich enzyme immunoassay. A superoxide dismutase-specific antibody was pre-coated on the microtiter plate included in this kit. After the standards or samples were added to the appropriate microtiter plate wells, a biotin-conjugated antibody to superoxide dismutase (SOD) was applied. Each microtiter plate was then completely coated with avidin conjugated to horseradish peroxidase (HRP) and incubated. Only the wells containing superoxide dismutase, biotin-conjugated antibodies, and enzyme-conjugated avidin showed a color shift after adding TMB substrate solution. The enzyme-

substrate reaction is stopped by adding sulfuric acid solution, and a spectrophotometer was used to measure the color change at 450 nm ± 10 nm. The content of the SOD group is shown in **Table 2.10**:

Table (2.10): Material provided in (SOD) Kit.

Reagents	Quantity
Diluent Buffer	**20 Ml**
Antibody of Biotinylated (100×)	**120 mL**
Diluent of Biotinylated Antibody	**12 mL**
Streptavidin-HRP (100×)	**120 µL**
Diluent HRP	**12 mL**
Wash Buffer solution (25×)	**20 mL**
Substrate Solution (TMB)	**9 mL**
Stop reagent	**6 mL**

2.10.2.2 Assay Procedure:

1. After equilibration at room temperature, 100 µL of the sample was placed in each well, and the set was then incubated for 80 min at 37°C.
2. After the liquid in the plate was discarded, 200 µL of wash solution was added to each well, and the plate was washed several times. The plate was inverted and dried on absorbent paper after vacuum drying. Each well received 100 µL of anti-biotin antibody working solution, which was then incubated for 50 min at 37°C.
3. After discarding the liquid in the plate, 200 µL of wash solution was added to each well, and the plate underwent three rounds of washing. Following drying, each well received 100 µL of Streptavidin-HRP working solution. The wells were then sealed with a disk and incubated for 50 minutes at 37°C.
4. The liquid in the plate was discarded. 200 µL of wash solution was added to each well, and the plate was washed several times. After drying, 90 µL of TMB substrate solution was added to each well, and the well was covered with a new plate sealant.
5. The plate was incubated for 20 min at 37 °C (not to exceed 30 min), protected from light. The liquid turned blue after adding the TMB substrate solution, as shown in **Appendix (2.8).**
6. 50 µL of stop solution was added to each well, the plate was read at 450 nm immediately, and the results were calculated. The OD of the samples is then compared to the standard curve, as illustrated in **Figure 2-7**, to ascertain the concentration of superoxidase dismutase (SOD) in the samples.

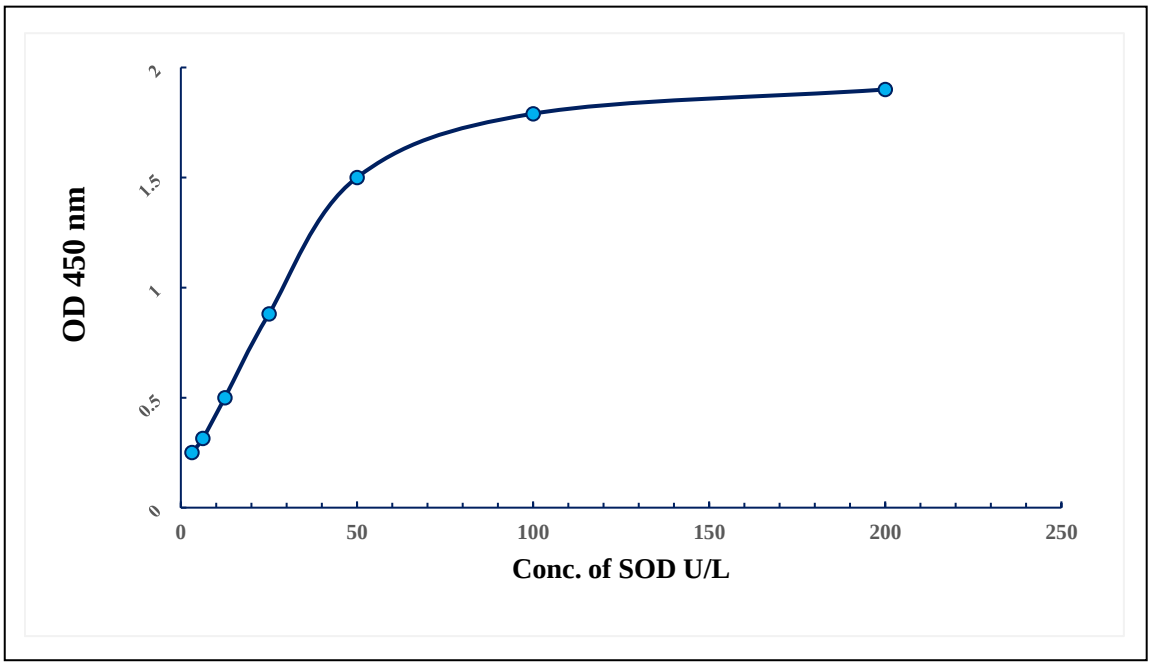


Fig. (2-7): SOD Standard curve

2.11 Inflammation

2.11.1 CRP and hs- CRP Rapid Quantitative Test [160, 161]

2.11.1.1 Principle of Assay:

The Finecare™ CRP Rapid Quantitative Test is a fluorescent immunoassay used with the Finecare™ FIA System for C-reactive protein (CRP) in human serum or plasma. The high-sensitive CRP test predicts future cardiovascular disease (CVD) by detecting inflammation. The Finecare™ CRP test uses a sandwich immunodetection method; when a sample is added to the sample well of the test cartridge, a fluorescently colored anti-CRP antibody binds to the CRP antigen in the blood sample. Through capillary action, the sample mixture migrates onto the nitrocellulose matrix of the test strip, capturing and transferring the detector antibody and CRP complexes to the anti-

CRP antibody immobilized on the test strip. Therefore, the more CRP antigens in the blood sample, the more complexes accumulate on the test strip. The intensity of the fluorescent signal of the detector antibody reflects the amount of CRP captured, and the Finecare™ FIA Meter displays the CRP concentrations in the blood samples. The default result unit of the Finecare™ CRP Rapid Quantitative Test is 3X mg/L of Finecare™ FIA Meter. The detection limit of the CRP test system is 0.5 mg/L, with a working range of 0.5 to 200 mg/L.

2.11.1.2 Assay Procedure:

The test was used according to the Finecare FIA Operating Manual.

1. The ID chip was placed within the apparatus.
2. 5 µL of serum was withdrawn from a transfer pipette and added to the buffer tube.
3. The sample was mixed well with the buffer solution for 1 minute by tapping or inverting the tube.
4. 75 µL the sample mixture was taken and loaded into the sample well of the test cartridge.
5. A standard test performed with the Finecare FIA: After inserting the test cartridge into the test cartridge holder and clicking "Test," after 3 minutes, the result will appear on the screen and be printed when clicking "Print."

2.12 Lactate dehydrogenase (LDH) [133]

2.12.1 Principle:

UV Kinetic Method (SFBC):

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^+$$

The decrease in absorbance due to the conversion of NADH to NAD+ is directly proportional to the activity of LDH in the sample and is measured at 340 nm.

2.12.2 Reagents

Table (2.11): Content Kit of LDH

R1 (LDH-P) Substrate- Buffer	
Tris Buffer (pH 7.2)	**80 mmole /L**
Pyruvate	**1.6 mmole/L**
Preservative	
R2 (LDH-P) Coenzyme	
NADH	**≥ 0.02**
NaCl	**200mmole/L**

2.12.3 Assay Procedure [162]:

Wave length: 340 nm

Temperature: 37°C

Reagent Preparation

3 ml of R1 were immediately added to flask R2.

Then the container was covered and the components were gently mixed until completely dissolved. Reagents and samples were left at room temperature. All samples, blanks, and standards were prepared as follows:

The micropipette was inserted into a sealed container with a path length of 1 cm:	
Reagent	**1000 µL**
The temperature was raised to 37 degrees Celsius and added:	
Calibrator, control, Specimen	**20 µL**
Mix phase with the timer running. **After 30 seconds, the initial absorbance at 340 nm (or 334 nm) was recorded.** **The absorbance was re-recorded after 1 and 2 minutes.** **The change in absorbance per minute (Abs/min.) was calculated.**	

2.12.4 Calculation:

$$\text{LDH activity (IU/L)} = \frac{(\Delta Abs\backslash min)Assay}{(\Delta Abs\backslash min)\ Calibrator} \text{ x Calibrator Activity}$$

With theoretical factors:

Activity (U/L) = Δ Abs/min x Factor

$$\textbf{Factor} = \frac{\text{VR X 1000}}{\text{6.3 X VE X P}}$$

With:

VR = Total reaction volume (mL), VE = Specimen volume (mL)

6.3 = Molar extinction coefficient for NADH at 340 nm, P = Pathlength (cm)

2.13 Determination of Lipid Profile [140, 163, 164]

The lipid profile includes examining all types of lipids in the blood, including the following:

1. Total Cholesterol
2. Triglycerides
3. Bad cholesterol, which is low-density lipoprotein (LDL).
4. Good cholesterol, and high-density lipoprotein (HDL).

2.13.1 Total Cholesterol [165]

2.13.1.1 Principle

Using the enzymatic method (Allen) as follows:

$$\textbf{Cholesterol esters} \xrightarrow{\text{CE}} \textbf{Cholesterol + Free Fatty Acids}$$

$$\textbf{Cholesterol + } O_2 \xrightarrow{\text{CO}} \textbf{Cholesten 4 one 3 + } H_2O_2$$

$$2H_2O_2 \textbf{ + Phenol + PAP} \xrightarrow{\text{POD}} \textbf{Quinoneimine (Pink) + } 4H_2O$$

2.13.1.2 Reagents

Table (2.12): Content of Total Cholesterol Group

R1 Cholesterol CHOD PAP Buffer	
Phosphate buffer	100 mmole /L
Chloro-4-phenol	5 mmole /L
Sodium Cholate	2.3 mmole /L
Triton x	100 mmole /L
R2 Cholesterol CHOD PAP Enzymes	
Cholesterol oxidase (CO)	> 100 IU/L
Cholesterol esterase (CE)	> 170 IU/L
Peroxidase (POD)	> 1200 IU/L
4-Amino-antipyrine (PAP)	0.25 mmole /L
PEG 6000	167 µmol/L
R3 Cholesterol CHOD PAP Standard	
Cholesterol	200 mg/dL (5.17 mmole /L)

2.13.1.3 Procedure

Wavelength: 500 nm

Temperature: 37°C

Samples, blanks, and standards were all prepared as follows:

Reagent	Blank	Standard	Sample
Working reagent	1 Ml	1 mL	1 mL
Standard	-	10 µL	-
Serum	-	-	10 µL
Distilled water	10 µL	-	-

Mix and wait 5 minutes at 37°C or 10 minutes at room temperature. Absorbance at 500 nm was recorded against the reagent sample.

2.13.1.4 Calculation

$$\text{Result} = \frac{\text{Abs(Assay)}}{\text{Abs(Standard)}} \text{ x Standard concentration}$$

2.13.2 Triglyceride [166]

2.13.2.1 Principle

Wavelength: 505 nm

Temperature: 37°C

The Fossati and Principe method is associated with the Trinder reaction. The reaction scheme is as follows:

The absorbance of the colored quinones was measured at 505 nm, proportional to the concentration of triglycerides.

2.13.2.2 Reagent

Table (2.13): Content of Triglycerides kit

R1 Reagent	
PIPES	100 mmole /L
Magnesium chloride	9.8 mmole /L
Chloro-4-phenol	3.5 mmole /L
Lipase	> 1000 IU/L
Peroxidase (POD)	> 1700 IU/L
Glycerol 3 phosphate oxidase (GPO)	> 2000 IU/L
Glycerol Kinase (GK)	> 1000 IU/L
4-Amino-antipyrine (PAP)	0.5 mmole /L
Adenosine triphosphate (ATP)	1.3 mmole /L
R2 ETALON Standard	
Triglycerides	1 g/L

2.13.2.3 Procedure

Wave length: 505 nm

Temperature: 37°C

Reagent	Blank	Standard	Sample
Working reagent	1 mL	1 mL	1 mL
Standard		10 µL	
Serum			10 µL
Distilled water	10 µL		

Stir and let stand at room temperature for 10 minutes. At 505 nm, absorbance was measured compared to a blank reagent sample.

2.13.2.4 Calculation

$$\textbf{Result} = \frac{\textbf{Abs(Assay)}}{\textbf{Abs(Standard)}} \textbf{ x Standard concentration}$$

2.13.3 High-Density Lipoprotein (HDL) [167]

2.13.3.1 The Principle

This method precipitates chylomicrons, low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) from samples using Phosphotungstic acid (PTA) and magnesium chloride. The solution is centrifuged, and the upper fluid containing HDL is collected and quantified using a total cholesterol reagent.

2.13.3.2 Reagent

Table (2.14): Content of HDL Kit

Vial R1: Precipitant	
Phosphotungstic acid (PTA)	**13.9 mmole /L**
Magnesium Chloride	**570 mmole /L**
Vial R2: Standard	
Cholesterol	**100/dL (2.58 mmole /L)**

2.13.3.3 Procedure

The upper sediment and the reagents were kept at room temperature. The pre-treated serial calibrator or the standard in the package can be used for calibration. The total cholesterol kit was used.

Pipette in centrifuge tube	Macro-method
Specimen, Calibrator, Controls	**1 mL**
Precipitant	**100 µL**
The ingredients were mixed well left at room temperature for 10 minutes and centrifuged for 15 minutes at 3500-4000 rpm.	

After mixing, the solution is incubated at 37°C for 5 minutes or at room temperature for 10 minutes.

Read absorbance at wavelength 500 nm against the reagent blank. The color remains stable for 1 h.

2.13.3.4 Calculation:

$$\text{Result} = \frac{\text{Abs(Assay)}}{\text{Abs(Standard)}} \text{ x Standard concentration x1.1}$$

Standard remaining undiluted, 1.1 factor takes into account dilution of the specimen during the precipitation step.

2.13.4 Low-Density Lipoprotein (LDL) [168]

2.13.4.1 The Principle

The direct method was used, which uses selective detergents without pretreatment of the sample:

- In the first stage, detergent 1 solubilizes HDL and VLDL and this resulting cholesterol, cholesterol oxidase (CO), and cholesterol esterase (CE), produces a colorless compound.
- In the second stage, detergent 2 is used to solubilize LDL-cholesterol. Chromogenic coloration is Proportional to the concentration of LDL-Cholesterol. The absorbance was measured at a wavelength of 546 nm.

2.13.4.2 Reagent

Table (2.15): LDL Content Kit

R1 LDL Reagent enzymes	
MES Buffer pH 6.3	**Cholesterol oxidase**
Ascorbic acid oxidase	**Cholesterol esterase**
4-Amino -antipyrine	**Detergent 1**
Peroxidase	**Preservative**
R2 LDL Specific detergent	
MES Buffer pH 6.3	**DSBmT**
Detergent 2	**Preservative**

MES:morpholino-ethane-sulfonic acid
DSBmT: N, N bis(4-sulphobutyl)-m-toluidine-disodium

2.13.4.3 Procedure

All samples, blanks, and standards were prepared as follows:

Set up the instrument to Read micro-volumes	Blank	Calibrator	Assay
Reagent R1	300 µL	300 µL	300 µL
Calibrator		3 µL	
Specimen			3 µL
Mix vigorously, let stand for 5 minutes 37°C. Record absorbance A1 at 546nm against reagent blank			
Add:	Blank	Calibrator	Assay
Reagent R2	100 µL	100 µL	100 µL
Mix well and leave for 5 min at 37°C. The absorbance of A2 was recorded at 546 nm against the blank reagent sample.			

2.13.4.4 Calculation

Abs= Δ (A2- 0.75A1) for assay and calibration

The result Will be:

$$\text{LDL–C} = \frac{\Delta\ \text{Abs(Assay)}}{\Delta\ \text{Abs(Standard)}} \text{ x Calibrator concentration}$$

2.14 Statistical Analysis

Data analysis was carried out using the available statistical package of IBM SPSS-22 (IBM Statistical Packages for Social Sciences, version 22, Chicago, IL, USA). Current study data were analyzed using the Chi-square test or Fisher exact probability (F.E.P.) test to compare percentages (qualitative data). The T-test was used to compare two numeric variables. Pearson Correlation: to examine the degree of relation between variables. The correlation coefficient value (r) is either positive (direct correlation) or negative (inverse correlation). The sensitivity and specificity of the parameters studied were evaluated using a receiver operating characteristic (ROC) curve.

The comparison of significance (p-value) in any test was considered as follows:

- P-value greater than 0.05 ($P>0.05$) was non-statistically significant (NS).
- P-value of less than or equal to 0.05 ($P\leq0.05$) was statistically significant (S).
- P-value of less than 0.01 ($P<0.01$) was highly statistically significant (HS).

CHAPTER

THREE

Results

3. Results

3.1 Study Design

The study was conducted in Basrah Governorate, southern Iraq, and female workers in the oil production department were excluded, so a male-only control group was selected to make the comparison valid. The participants were divided into two groups: the first group included 90 people exposed to $PM_{2.5}$ particles in the Nahran Bin Omar field. All study participants had been working for at least two years. The exposure was five days a week for eight hours each day. The second group, which included 90 people, was a group of healthy people (control group). The study examines the effect of chronic occupational exposure to petroleum emissions on cardiovascular diseases.

3.2 Demographics of the Study

3.2.1 Population distribution by age group

Table 3.1 shows the demographic information of the workers exposed to the study who worked at crude oil well sites in Basrah Province, nonexposed (control group). The workers' ages ranged from 23 to 55 years; the mean value was 41.44 ± 1.07. The second group consisted of 90 non-exposed individuals aged 21-54, with a mean value of 41.93 ± 1.05.

Table (3.1): Distribution of the studied group according to age range group

Age range group (Years)	Studied group (N, %)		Total	P-value
	Exposed Workers (n=90)	Non-exposed Control (n=90)		
Age (M±SE)	41.44±1.07	41.93±1.05	-	T-test=0.32 P-value =0.74
(20-29)	11 (47.8%)	12 (52.2%)	23 (100.0%)	Chi-square =5.16 P-value= 0.27
(30-39)	32 (59.3%)	22 (40.7%)	54 (100.0%)	
(40-49)	26 (44.1%)	33 (55.9%)	59 (100.0%)	
(50-59)	17 (43.6%)	22 (56.4%)	39 (100.0%)	
59>	4 (80.0%)	1 (20.0%)	5 (100.0%)	
Total	90 (50.0%)	90 (50.0%)	180(100.0%)	

3.2.2 Smoking

This study included smoking subjects, **Table 3.2** shows the number of smoking participants in case and control.

Table (3.2): Smoking Participants in This Study

Demographical Picture	Status	Group		Total	P-value
		Exposed Workers (n=90)	Non-exposed Control (n=90)		
Smoking habit	Yes	42 (46.6 %)	40 (44.4 %)	82 (100.0%)	Chi-square= 6.70 *P* =0.2
	No	48 (53%)	50 (55.5 %)	98(100.0%)	

3.2.3 Chronic Diseases

Table 3.3 shows the chronic diseases in this study's exposed workers and nonexposed control groups. The workers showed higher rates of diabetes, hypertension, allergies, and heart disease compared to the control group with a significant difference (P<0.001).

Table (3.3): Chronic diseases in both groups studied

Demographic Picture	Status	Exposed Workers	Non-exposed Control	
Chronic Diseases	None	22	85	Chi-square =31.7 $P < 0.001$
	DM	26	2	
	Hypertension	17	3	
	Heart disease	13	None	
	Allergy	12	None	

3.3 Blood biomarkers parameters:

3.3.1 Toxic particles

In this study, two components of $PM_{2.5}$ particles were evaluated in the blood of the study group: levels of toxic heavy metals (cadmium and lead) and the metabolite benzo[a]pyrene diol epoxide (BPDE).

3.3.1.1 Heavy Metals

3.3.1.1.1 Determination of Cadmium and Lead

Table 3.4 compares the blood concentrations of cadmium **(Figure 3-1)** and lead **(Figure 3-2)** between exposed workers and an unexposed control group.

Table (3.4): Comparison between exposed workers and unexposed control in the concentration of Cd(*ng/mL*) & Pb(*ng/mL*)

Toxic heavy metals	Group	Mean ± SD	T-test	P-value
Cd(*ng/mL*)	Exposed workers	85.8 ± 0.83	13.21	≤ 0.001
	Non-exposed control	43.7± 3.07		
Pb(*ng/mL*)	Exposed workers	347.6± 14.24	4.51	≤ 0.001
	Non-exposed control	165.0± 11.49		

P is significant statistics at level < 0.05.

Data are reported as mean ± standard deviation

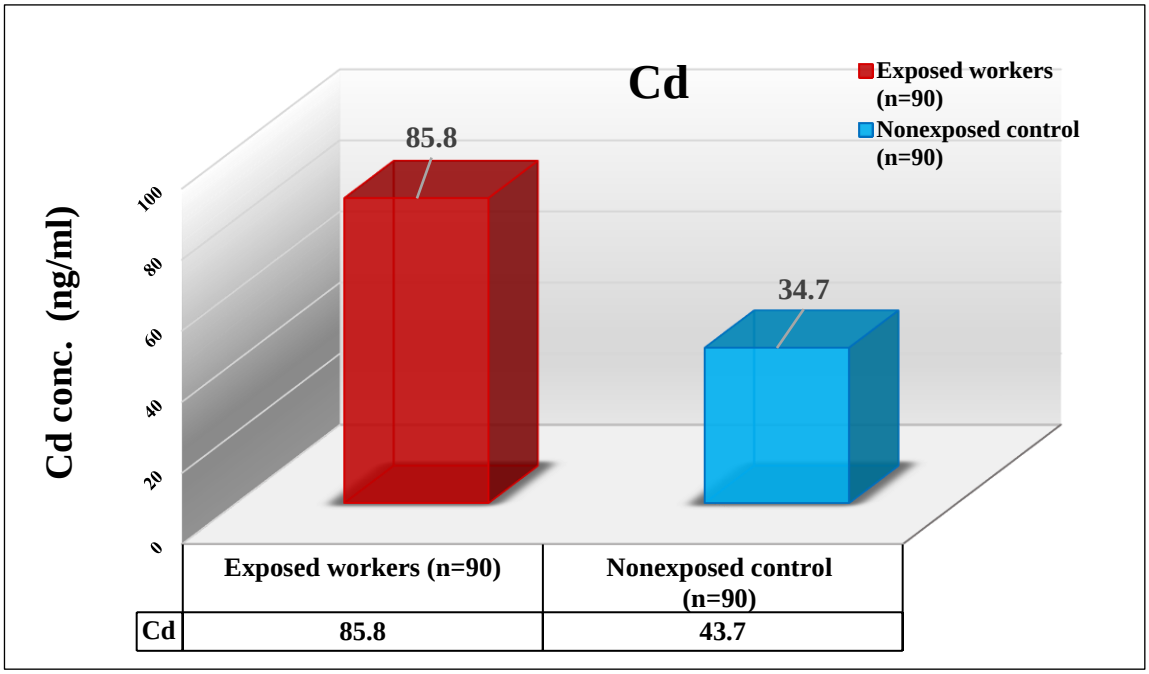


Fig. (3-1): Concentration of Cadmium in exposed workers and unexposed control groups.

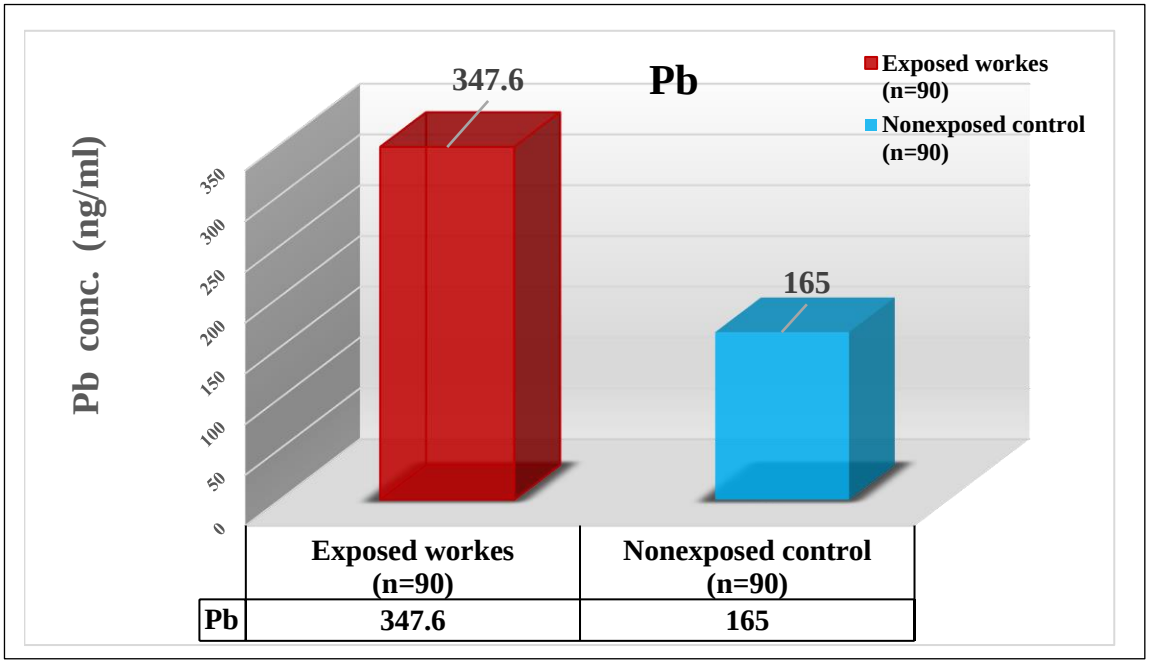


Fig. (3-2): Concentration of Lead in exposed workers and unexposed control groups.

3.3.1.2 Polycyclic Aromatic Compounds (PAH)

3.3.1.2.1 Determination of Benzo [a] pyrene/ BPDE

Table 3.5 and (Figure 3-3) show BPDE levels in exposed workers and unexposed control groups.

Table (3.5): Comparison of BPDE concentrations (Pg/ml) between exposed workers and control

Biomarker	Group	Mean ± SD	T-test	P-value
BPDE (Pg/mL)	Exposed workers	203.3 ± 9.42	14.97	≤ 0.001
	Nonexposed control	60.1 ± 1.64		

P is significant statistics at level < 0.05.
Data are reported as mean ± standard deviation

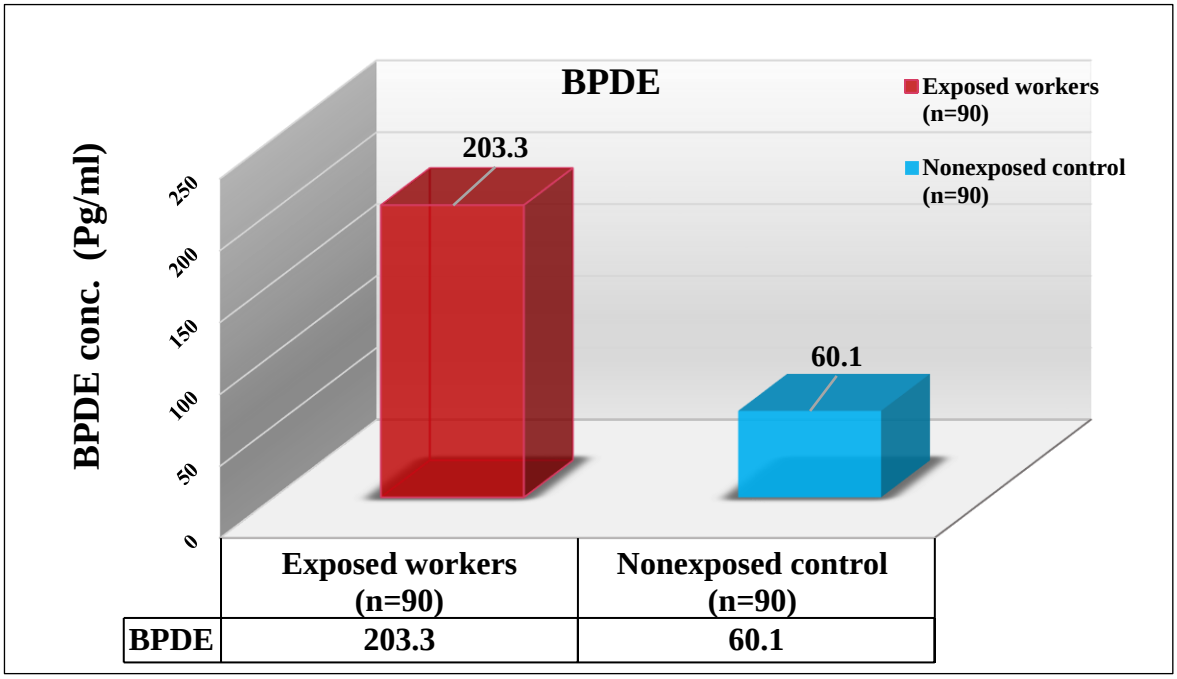


Fig. (3-3): Concentration of Benzo [a] pyridyl Epoxide (BPDE) in exposed workers and unexposed control groups.

3.3.2 Essential Trace Element

3.3.2.1 Determination of Selenium and Zinc:

Table 3.6 compares the blood concentrations of selenium **(Figure 3-4)** and Zinc **(Figure 3-5)** between exposed workers and an unexposed control group.

Table (3.6): Comparison between exposed workers and unexposed control in the concentration of Se(ng/mL) & Zn(ng/mL).

Essential Trace element	Group	Mean ± SD	T-test	P-value
Se(*ng/mL*)	Exposed workers	146.2± 20.00	9.07	≤ 0.001
	Nonexposed control	165.0± 19.25		
Zn(*ng/mL*)	Exposed workers	661.2 ± 82.52	3.58	≤ 0.001
	Nonexposed control	977.83 ± 122.28		

P is significant statistics at level < 0.05.
Data are reported as mean ± standard deviation

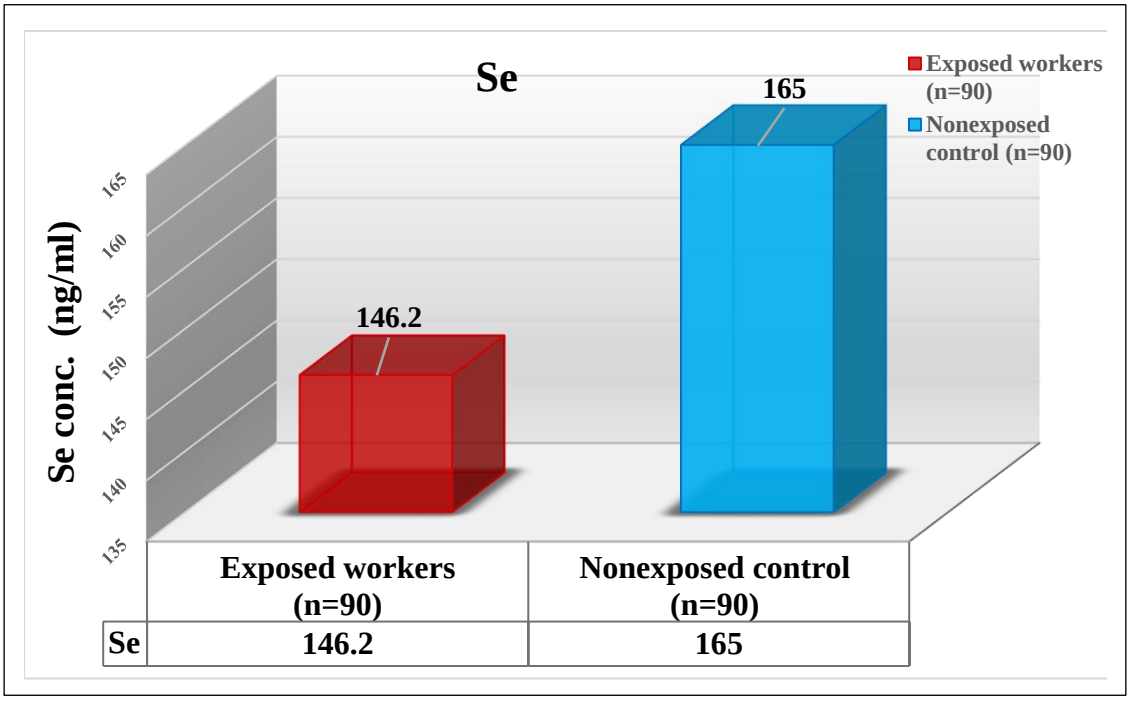


Fig. (3-4): Concentration of Selenium in exposed workers and unexposed control groups.

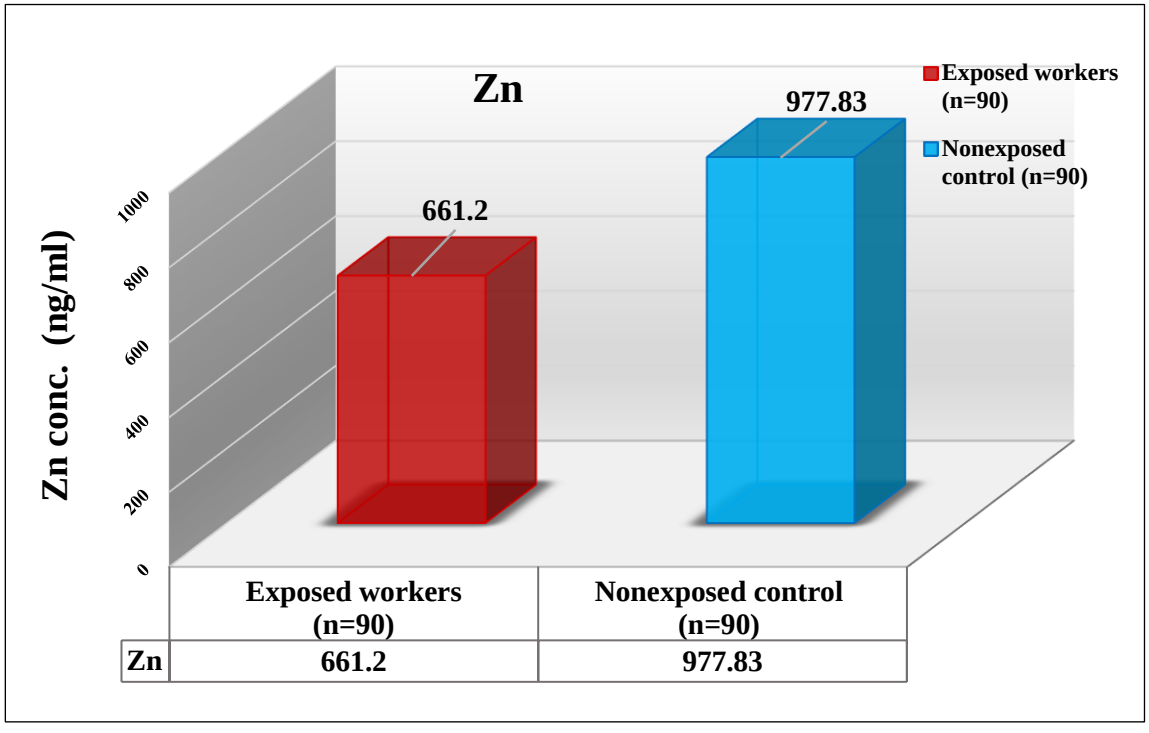


Fig. (3-5): Concentration of Zinc in exposed workers and unexposed control groups.

3.3.2.2 Evaluation of the correlation between Se levels and (Cd, Pb, and BPDE) levels:

Table 3.7 illustrates the correlation between the levels of Se (ng/mL) and those of Cd (ng/mL), Pb (ng/mL), and BPDE (Pg/mL) in the two groups studied.

Table (3.7): Correlation between the levels of Se (ng/mL) and the levels of Cd (ng/mL), Pb (ng/mL), and BPDE (Pg/mL) between the two groups studied.

		Cd	Pb	BPDE
Se	Pearson Correlation	-0.417**	-0.351**	-0.568**
	P-value	0.000	0.000	0.000
	Total number	180	180	180

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

3.3.2.3 Evaluation of the correlation between Zn levels and (Cd, Pb, and BPDE) levels:

Table 3.8 illustrates the correlation between the levels of Zn (ng/mL) and those of Cd (ng/mL), Pb (ng/mL), and BPDE (Pg/mL) in the two groups studied.

Table (3.8): Correlation between the levels of Zn (ng/mL) with Cd(ng/mL), Pb (ng/mL), and BPDE (Pg/mL) between the two groups studied.

		Cd (ng/mL)	Pb (ng/mL)	BPDE (Pg/mL)
Zn (ng/mL)	Pearson Correlation	- 0.254**	- 0.271*	- 0.156*
	P-value	0.001	0.01	0.037
	Total number	180	180	180

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

3.4 Oxidative Strasse Biomarker

The study assessed oxidative stress markers in the blood of workers and a control group, including the evaluation of the levels of malondialdehyde (MDA), a derivative of polyunsaturated fatty acid peroxidation as a potential indicator of oxidative stress, and superoxide dismutase (SOD), an antioxidant enzyme, in the serum of the study participants.

3.4.1 Malondialdehyde (MDA) and Super Oxidase Dismutase (SOD) Determination:

Table 3.9 compares serum MDA **(Figure 3-6)** and SOD **(Figure 3-7)** levels between exposed workers and unexposed controls.

Table (3.9): Comparison between exposed workers and unexposed control (n=90) in the MDA(µmol/L) and SOD(U/L)

Biomarker	Group	Mean ± SD	T-test	P-value
MDA(µmol/L)	Exposed workers	1219.8 ± 27.53	8.59	≤ 0.001
	Nonexposed control	710.3 ± 52.47		
SOD(U/L)	Exposed workers	8.90 ± 0.22	9.98	≤ 0.001
	Nonexposed control	11.65 ± 0.15		

P is significant statistics at level < 0.05.
Data are reported as mean ± standard deviation

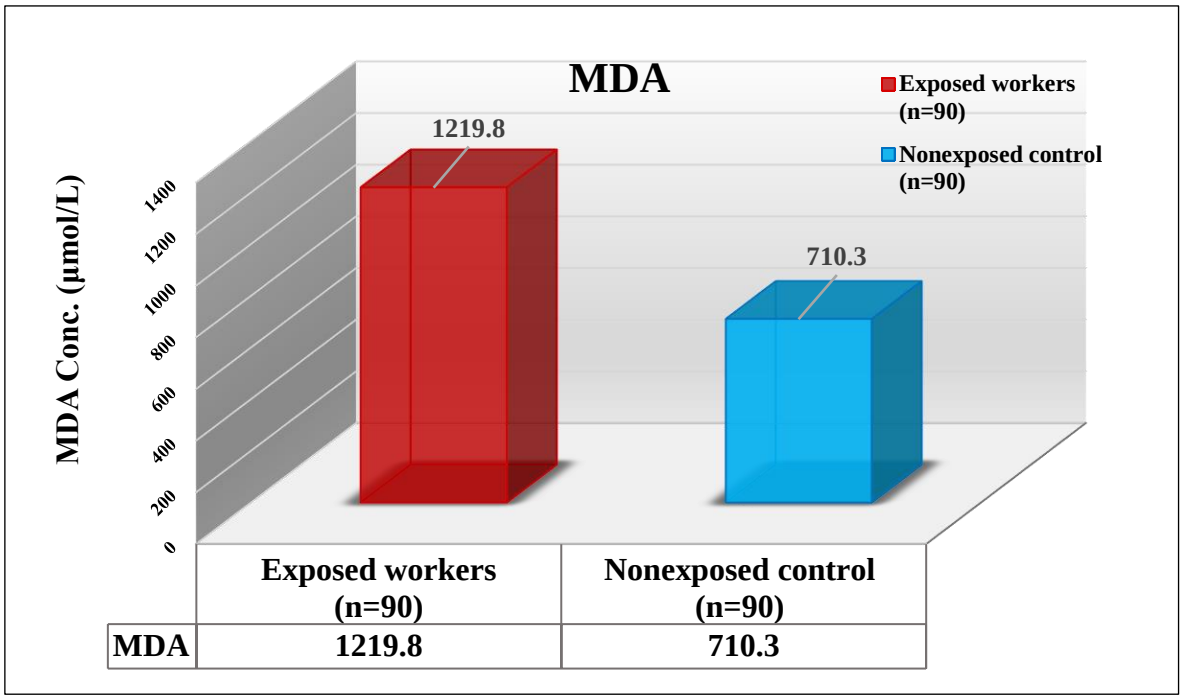


Fig. (3-6): Concentration of Malondialdehyde (MDA) in exposed workers and unexposed control groups.

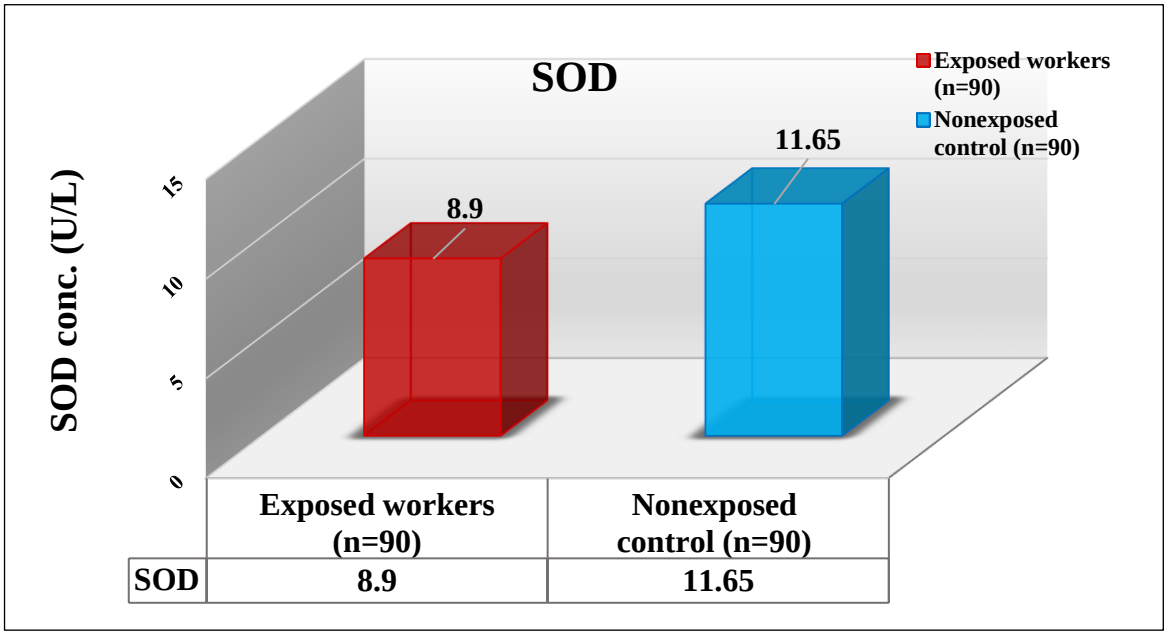


Fig. (3-7): Concentration of Super Oxidase Dismutase (SOD) in exposed workers and unexposed control groups.

3.4.2 Evaluation of the correlation between MDA levels and (Cd, Pb, and BPDE) levels:

Table 3.10 displays the correlation between MDA and Cd, Pb, and BPDE levels in the two groups under study.

Table (3.10): Correlation between the levels of MDA(µmol/L) with Cd(ng/ml), Pb (ng/mL), and BPDE (Pg/mL) between the two groups studied.

		Cd(ng/mL)	Pb(ng/mL)	BPDE(Pg/mL)
MDA(µmol/L)	Pearson Correlation	0.551**	0.368**	0.416**
	P-value	0.000	0.000	0.000
	Total number	180	180	180

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

3.4.3 Evaluation of the correlation between MDA levels and (Se and Zn) levels:

The relationship between MDA and (Se and Zn) in the two groups under study is shown in **Table 3.11**.

Table (3.11): Correlation between the levels of MDA(µmol/L) with Se(ng/mL) and Zn(ng/mL) between the two groups studied.

		Se(ng/mL)	Zn(ng/mL)
MDA(µmol/L)	Pearson Correlation	- 0.293**	- 0.232**
	P-value	0.000	0.002
	Total number	180	180

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

3.4.4 Evaluation of the correlation between MDA levels and SOD levels:

The correlation between the MDA and SOD levels in the two groups under study is displayed in **Table 3.12**.

Table (3.12): Correlation between the levels of MDA(µmol/L) and SOD(U/L) between the two groups studied.

		SOD (U/L)
MDA(µmol/L)	Pearson Correlation	-0.395**
	P-value	0.000
	Total number	180

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

3.4.5 Evaluation of the correlation between SOD levels and (Cd, Pb, and BPDE) levels:

Table 3.13 illustrates the correlation between the levels of SOD and those of (Cd, Pb, and BPDE) in the two groups studied.

Table (3.13): Correlation between the levels of SOD (U/L) with Cd(ng/mL), Pb (ng/mL), and BPDE (Pg/mL) between the two groups studied.

		Cd(ng/mL)	Pb(ng/mL)	BPDE(Pg/mL)
SOD (U/L)	Pearson Correlation	- 0.450**	-0.250**	-0.395**
	P-value	0.000	0.001	0.000
	Total number	180	180	180

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

3.5 Inflammation Biomarker:

3.5.1 Determination of C-Reactive Protein (CRP), and High-Sensitivity C-Reactive Protein (hs-CRP)

C-reactive protein (CRP) and high-sensitivity C-reactive protein (hs-CRP), two markers of inflammation, were compared between the two groups in **Table 3.14** and **Figures (3-8)** and **(3-9)**.

Table (3.14): Comparison between exposed workers and unexposed control in CRP (mg/L) and hs-CRP (mg/L)

Biomarker	Group	Mean ± SD	T-test	P-value
CRP (mg/L)	Exposed workers	5.26 ± 0.38	5.04	≤ 0.001
	Nonexposed control	3.01± 0.22		
hs-CRP (mg/L)	Exposed workers	2.14 ± 0.26	6.06	≤ 0.001
	Nonexposed control	0.49 ± 0.07		

P is significant statistics at level < 0.05.
Data are reported as mean ± standard deviation

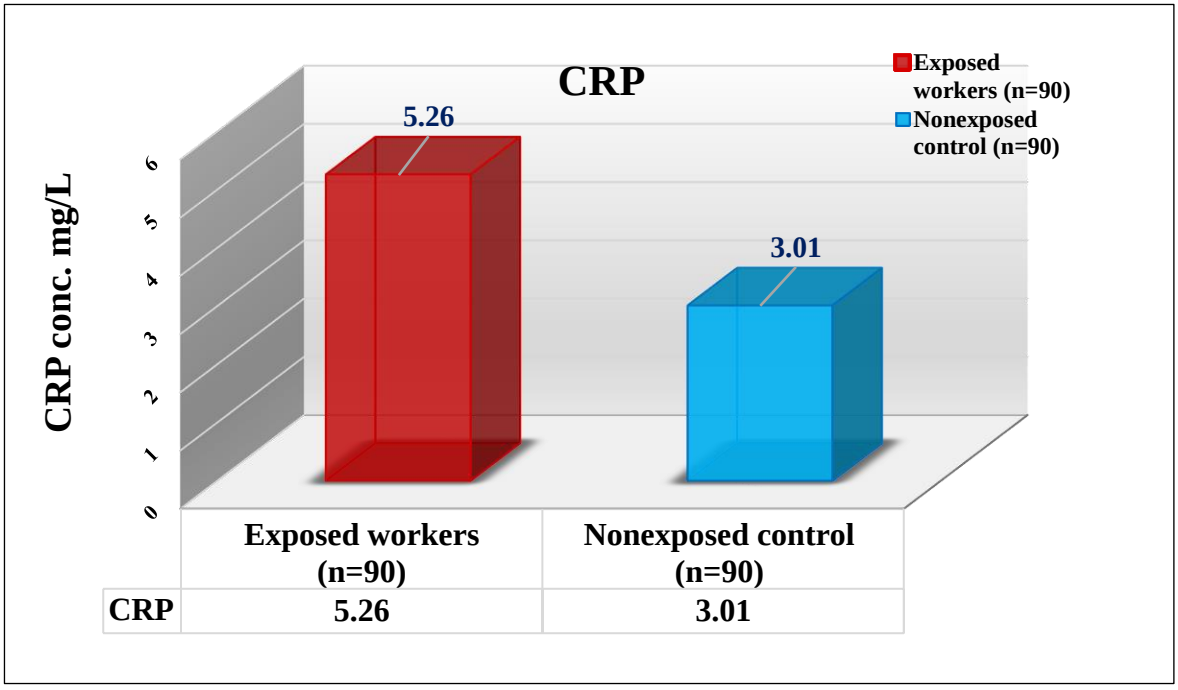


Fig. (3-8): Concentration of C-Reactive Protein (CRP) in exposed workers and unexposed control groups.

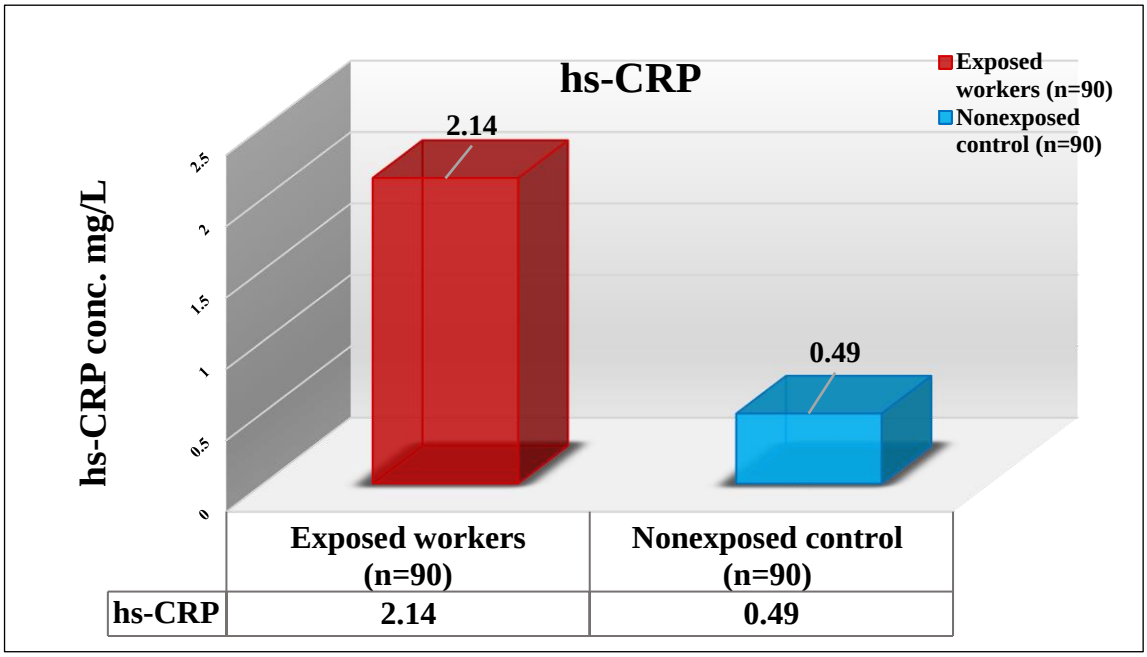


Fig. (3-9): Concentration of hs-C-Reactive Protein (hs-CRP) in exposed workers and unexposed control groups.

3.5.2 Evaluation of the correlation between hs-CRP levels and (Cd, Pb, and BPDE) levels:

Table 3.15 shows the correlation between hs-CRP levels and (Cd, Pb, and BPDE) levels.

Table (3.15): Correlation between the levels of hs-CRP (mg/L) with Cd(ng/mL), Pb (ng/mL), and BPDE (Pg/mL) between the two groups studied.

		Cd(ng/mL)	Pb(ng/mL)	BPDE(Pg/mL)
hs-CRP (mg/L)	Pearson Correlation	0.332**	0.26**	0.250**
	P-value	0.000	0.000	0.001
	Total number	180	180	180

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

3.6 Lactate Dehydrogenase (LDH)

3.6.1 Determination of Lactate Dehydrogenase (LDH):

The levels of (LDH) were compared between the two studied groups in **Table 3.16** and (**Figure 3.10**).

Table (3.16): Comparison between exposed workers (n=90) and unexposed control (n=90) in LDH (µmol/L)

Biomarker	Group	Mean ± SD	T-test	P-value
LDH (µmol/L)	Exposed Workers	432.70 ± 26.717	4.62	≤ 0.001
	Nonexposed control	300.60 ± 10.16		

P is significant statistics at level < 0.05.
Data are reported as mean ± standard deviation

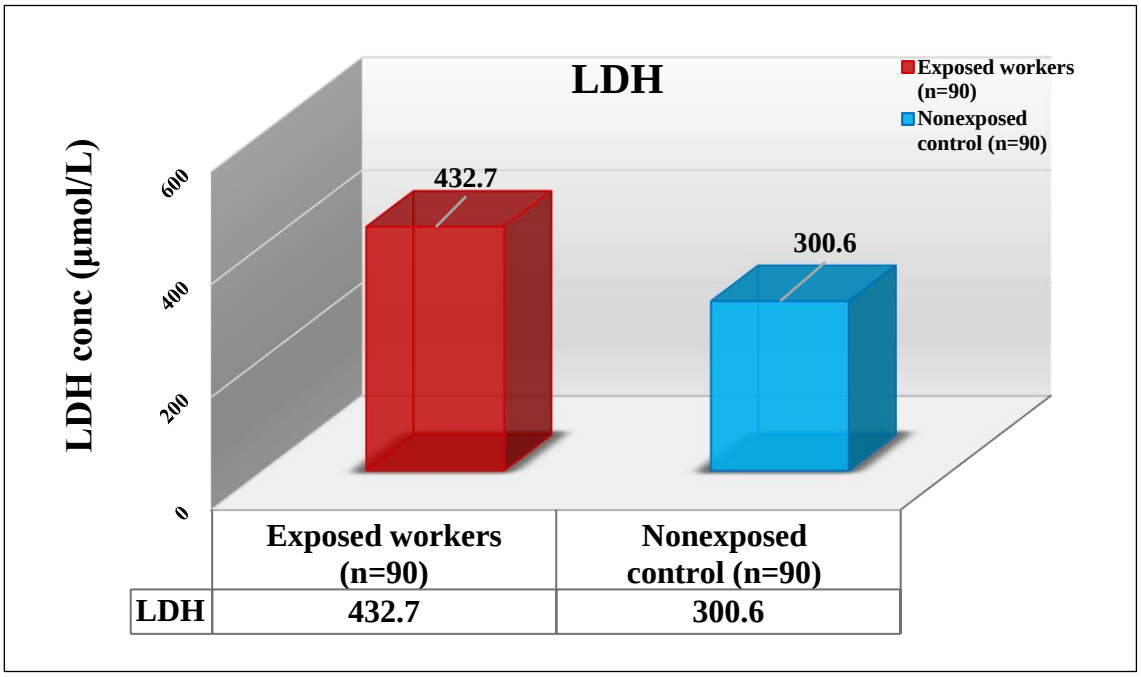


Fig. (3-10): Concentration of Lactate Dehydrogenase (LDH) in exposed workers and unexposed control groups.

3.6.2 Evaluation of the correlation between LDH levels and (Cd, Pb, and BPDE) levels:

Table 3.17 shows the correlation between LDH levels and (Cd, Pb, and BPDE) levels.

Table (3.17): Correlation between the levels of LDH (µmol/L) with Cd(ng/mL), Pb (ng/mL), and BPDE (Pg/mL) between the two groups studied.

		Cd(ng/mL)	Pb(ng/mL)	BPDE(Pg/mL)
LDH (µmol/L)	Pearson Correlation	0.231**	0.145*	0.323**
	P-value	0.002	0.01	0.000
	Total number	180	180	180

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

3.7 Lipid Profile

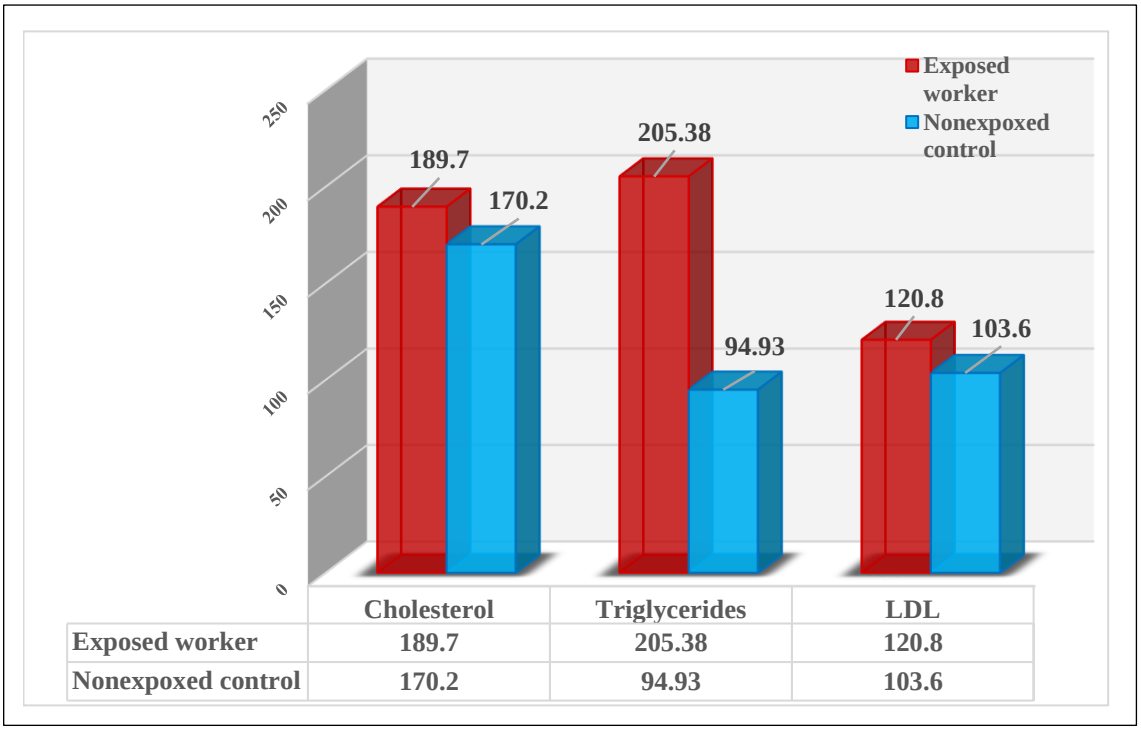
3.7.1 The Blood Lipid Profile Determination:

As indicated in **Table 3.18** and **Figures 3.11, 3.12,** and **3.13**, the blood lipid profile revealed substantial variations in mean values between the exposed workers and the unexposed control group.

Table (3.18): Comparison between exposed workers in petroleum stations and unexposed control in blood Lipid profile

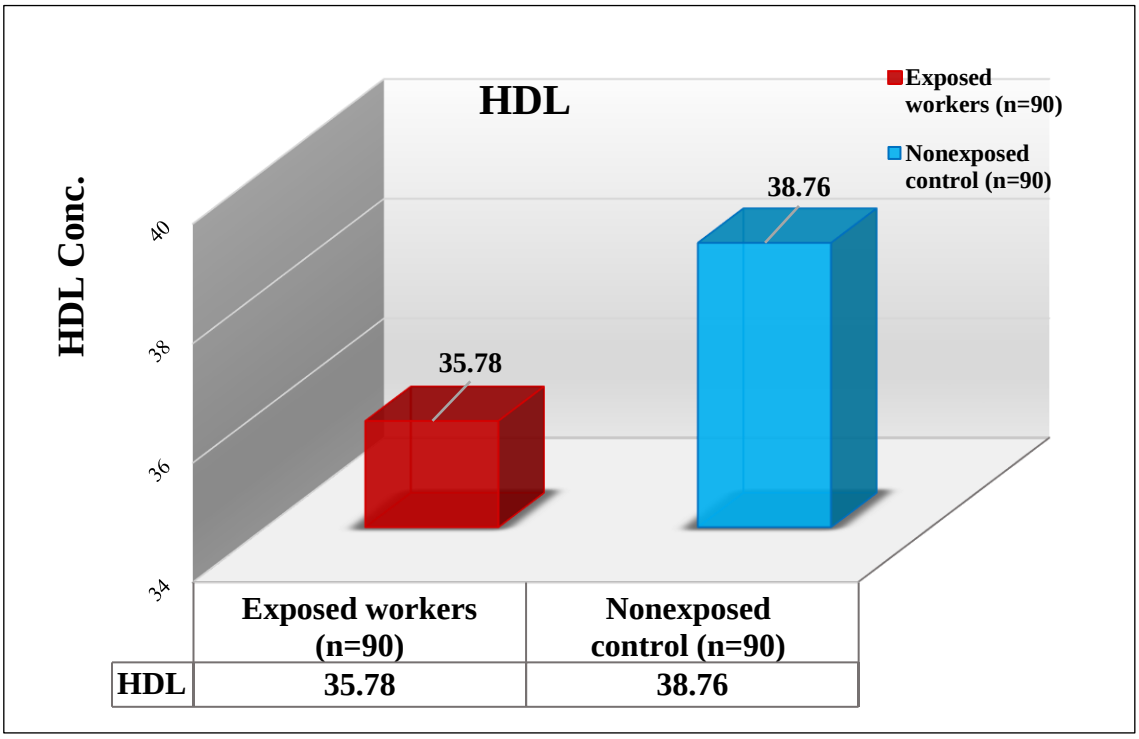
Test / Group	Cholesterol < 200 mg/dL	Triglycerides < 150 mg/Dl	LDL < 100 mg/dL	HDL > 60 mg/dL	VLDL < 30 mg/dL
Exposed Workers	189.7± 3.70	205.3 ± 11.90	120.8 ±3.75	35.7 ± 0.64	41.04 ±2.09
Nonexposed Control	170.2± 1.24	94.9 ± 2.12	103.6 ± 3.17	38.7 ± 0.40	19.98 ±0.75
T-test	4.98	9.13	2.48	3.93	4.07
P-value	≤ 0.001	≤ 0.001	≤ 0.01	≤ 0.001	≤0.001

P is significant statistics at level < 0.05.
Data are reported as mean ± standard deviation



	Cholesterol	Triglycerides	LDL
Exposed worker	189.7	205.38	120.8
Nonexpoxed control	170.2	94.93	103.6

Fig. (3-11): Concentration of Cholesterol, Triglycerides, and LDL in exposed workers and unexposed control groups.



	Exposed workers (n=90)	Nonexposed control (n=90)
HDL	35.78	38.76

Fig. (3-12): Concentration of HDL in exposed workers and unexposed control groups.

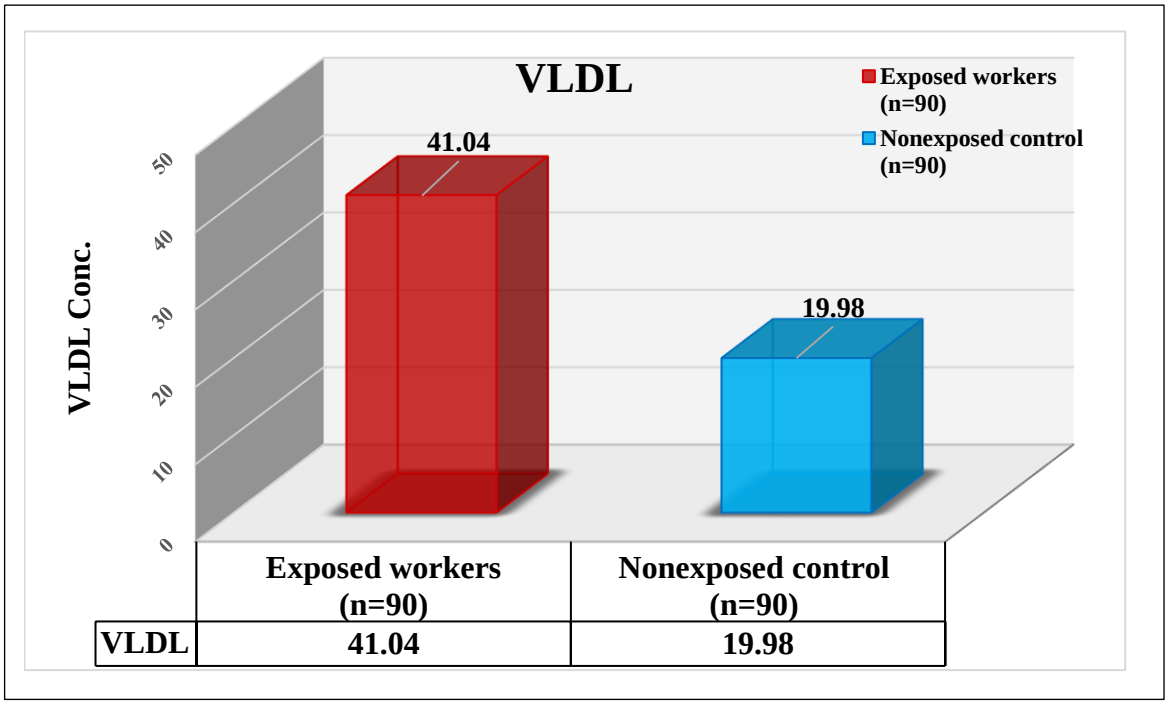


Fig. (3-13): Concentration of HDL in exposed workers and unexposed control groups.

3.7.2 Evaluation of the correlation between Lipid Profile levels and (Cd, Pb, and BPDE) levels:

Table 3.19 shows the correlation between the Lipid profile levels and (Cd, Pb, and BPDE) levels in exposed workers and unexposed control groups.

Table (3.19): Correlation between the levels of Lipid profile and the levels of Cd(ng/ml), Pb (ng/ml), and BPDE (Pg/ml) between the two groups studied

Lipid profile Test		Cd	Pb	BPDE
Cholesterol	Pearson Correlation	0.216**	0.174*	0.037
	P-value	0.004	0.029	0.618
Triglycerides	Pearson Correlation	0.421**	0.236**	0.142
	P-value	0.000	0.001	0.057
HDL	Pearson Correlation	-0.162*	-0.188*	-0.127
	P-value	0.029	0.01	0.090
LDL	Pearson Correlation	0.223**	0.211**	0.185*
	P-value	0.003	0.0017	0.025
VLDL	Pearson Correlation	0.262**	0.251**	0.174*
	P-value	0.000	0.002	0.020

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

CHAPTER

FOUR

Discussion

4. Discussion

4.1 Demographic Characteristic According to Age

The ages of study sample were divided into five age groups, as shown in **Table (3.1)**. Statistical analysis showed no significant difference between the patients and control groups (P =0.74) due to the close age of the two groups. The first age group (≤ 29) years includes workers with the least length of employment during this period.

In the second age group (30- 39) years, there was a clear increase in the incidence of allergic diseases and respiratory diseases in the number of workers registered in the study. With the advancement of age in the third, fourth, and fifth age groups (40-49) and (50-59) and more than 59 years respectively, and with the increase in the number of years of service in work, the incidence of chronic diseases such as diabetes, high blood pressure, heart disease, and even spleen diseases and cancer increased.

4.2 Smoking

Table (3.2) shows the number of smokers in the worker and control groups. Pollutants were significantly higher among workers, both smokers and nonsmokers, so comparisons between smokers and nonsmokers were not focused on. However, the chemicals smokers inhale damage their hearts and blood vessels, making them more susceptible to atherosclerosis and plaque accumulation in the arteries [169]. In addition to increased exposure to particulate matter and other risk factors for heart disease, such as unhealthy blood cholesterol levels and high blood pressure, smoking further increases the risk of heart disease [170, 171].

4.3 Cadmium and Lead as Cardiovascular Risk Factors:

Heavy metals, particularly lead and cadmium, are among the particulate matter components that may harm heart health.

Table (3.4) and **Figures (3.1** and **3.2)**, compare blood cadmium and lead concentrations between exposed workers at crude oil well sites and the control group. Cadmium levels were higher in exposed workers than in the control group, with a mean value of (85.8 ± 0.83 vs. 43.7± 3.07) ng/mL, respectively with differences statistically significant ($P \leq 0.001$).

Cadmium exposure is associated with increased mortality from coronary heart disease, heart failure, and dilated cardiomyopathy. Low doses can cause significant increases in cardiac levels, which may play a role in the pathogenesis of these conditions. Cadmium exposure also increases the risk of ischemic stroke and carotid artery plaque proliferation. Cadmium is independently associated with peripheral arterial disease and can predict mortality in patients with heart failure and myocardial infarction [67]. Cadmium, bound to albumin and proteins, is transported to soft tissues such as the liver and kidney. Free and protein-bound cadmium can cause adverse effects such as mitochondrial damage, cell death, inflammation, and fibrosis. Cadmium disrupts the normal tone of organs by reducing nitric oxide synthase phosphorylation in endothelial cells, impairing nitric oxide function and signaling [172]. Recent studies have provided evidence to support a strong positive association between increased cadmium levels and the risk of cardiovascular disease, and all dose-response curves assessing cardiovascular outcomes from whole blood biomarkers showed a linear positive association [173]. The mean blood lead levels were significantly higher in exposed workers, at (347.6± 14.24) ng/mL, compared to (165.0± 11.49) ng/mL in

unexposed individuals, this difference was highly statistically significant ($P \leq 0.001$).

Lead's health effects stem from its strong bonding with proteins and interference with zinc and calcium-dependent functions, particularly antioxidant functions and cellular signaling. Lead inhibits glutathione synthesis and depresses superoxide dismutase activity, affecting human antioxidant effects. Exposure to lead replaces calcium in intracellular signaling reactions, potentially causing hypertension. It also results in oxidative stress, epigenetic changes, and oxidative damage. Long-term exposure to lead can lead to protein binding inhibition and DNA methyltransferase alteration [174]. Previous studies have shown a positive association between high blood lead levels and an increased risk of death from cardiovascular disease [175–177]. The observed blood levels of cadmium and lead in this study's occupationally exposed and non-exposed subjects were above the permissible range and could be a potential health hazard. According to the Centers for Disease Control and Prevention (CDC), the average concentration of blood Pb among healthy unexposed adults was estimated at 0.9 µg/dL. The calculated reference values of permissible Pb blood levels are < 5µg/dL in adults, while the critical/ hazardous levels are ≥ 70 µg/dL in adults. The average range of Cd blood level of healthy unexposed adults is 0.1–4 µg/L. The estimated reference value of the permissible Cd blood level is ≤ 0.4 µg/L, While the critical value is >5 µg/dL [178]. In addition to exposure to cadmium in the work environment, cigarette smoking is an additional source of cadmium and lead in the blood [179]. The results of our study coincide with previous studies in which the Pb and Cd concentrations were in the blood samples of people living near polluted areas [178].

4.4 High Levels of B[a]pyrene/ BPDE

During the first stage of exposure to aromatic compounds, the biotransformation of polycyclic aromatic hydrocarbons, including B[a]P, requires the activity of cytochrome P450 (CYP1 family) [180]. PAHs are converted to some phenols (hydroxyl derivatives), bi phenols, dihydro diols, quinones, and reactive bicyclic epoxides. Reactive oxygen species are also produced as by-products of these reactions. The oxidation of B[a]P by P450 at positions 7 and 8 is one of the most dangerous reactions, leading to the formation of toxic metabolites of B[a]P, which forms the final carcinogenic metabolite: benzo [a]pyrene diol epoxide (BPDE)[47].

In this study, the results in **Table (3.5)** and **Figure (3.3)** indicated higher levels of BPDE in workers' blood compared to the control group (203.3±9.42 vs. 60.1±1.64). The data showed a significant difference in the mean BPDE values between the two groups ($P \leq 0.001$).

B[a]P exposure significantly contributes to atherosclerosis development, leading to endothelial dysfunction, cell death, and local inflammatory response in the vascular endothelium, which is inevitably exposed to systemically absorbed genotoxic substances through direct blood contact [50]. A recent study has shown that analysis of volatile organic compounds in the blood can reflect the internal exposure dose; they are stable biomarkers of this exposure[181]. Previous studies have also shown a positive association between exposure to PAHs and the risk of cardiovascular disease in both occupational and general populations [182, 183].

In a Greek study, environmental pollutants in the serum of heart failure patients were associated with high concentrations of PAHs, heavy metals, smoking, and atmospheric degradation [184].

4.5 Elevated MDA Levels and Decreased SOD

Table (3.9) shows a comparison between the levels of MDA and SOD between the two groups. According to **Figure (3.6)**, there was a significant increase in the levels of MDA (1219.8 ± 27.53 vs. 710.3 ± 52.47) in the worker's blood was found in the study compared to the control group ($P < 0.001$). **Figure (3.7)** shows a significant decrease in SOD levels (8.90 ± 0.22 vs. 11.65 ± 0.15) among workers compared to the control group ($P < 0.001$).

The oxidative stress status of oilfield workers, assessed by MDA determination, appears to be aggravated by elevated levels of lead, cadmium, and BPDE. The reason is the imbalance between antioxidants and pro-oxidants. **Table (3.10)** shows the correlation between MDA and cadmium, lead, and BPDE levels in the two studied groups. MDA shows a positive correlation with cadmium ($r = 0.551^{**}$, $P = 0.000$), lead (0.368^{**}, $P = 0.000$), and BPDE ($r = 0.416^{**}$, $P = 0.000$).

Malondialdehyde, which is produced by oxidative stress, can be metabolized by enzymes, especially mitochondrial aldehyde dehydrogenase, or covalently react with proteins and nucleic acids, causing DNA-protein cross-links and added products that damage biomolecules. These effects are associated with the expression of pro-inflammatory genes and the activation of inflammatory signaling pathways [98].

The correlation between MDA and SOD levels in the two groups is shown in **Table (3.12)**. An inverse relationship between MDA and SOD was found by data analysis ($r = -0.395^{**}$, $P = 0.000$). The results also showed that superoxide dismutase analysis showed an inverse relationship with cadmium ($r = -0.450^{**}$, $P = 0.000$), with an inverse relationship with lead ($r = -0.250^{**}$, $P = 0.001$), and with BPDE ($r = -0.395^{**}$, $P = 0.000$), as shown in **Table (3.13)**. This

antagonistic relationship suggests that the antioxidant system of workers is weakened by exposure to particulate pollutants, as superoxide dismutase is a metalloenzyme that acts as a first line of defense against reactive oxygen species (ROS). It captures the free radical superoxide anion (O_2^-) into molecular oxygen and hydrogen peroxide, reducing the level of oxygen harmful to cells at high concentrations [185]. When the extent of oxidative damage exceeds the ability to repair, cell damage. Redox imbalance has been associated with many diseases, including heart disease, the aging process, and cancer [186].

Numerous studies have examined the impact of heavy metal exposure in the oil sector on malondialdehyde levels and antioxidants [187, 188].

4.6 Selenium and Zinc Levels

Table (3.6) shows Selenium and Zinc blood levels in the blood of the two participating groups. **Figure (3.4)** shows that exposed workers had lower selenium levels than the control group, with an average value of (146.2± 20.00 vs. 165.0± 19.25) ng/mL, respectively, and a high statistical significance ($p \leq 0.001$). Selenium is involved in oxidative stress, thyroid hormone metabolism, and calcium influx. Selenium deficiency can disrupt these processes and cause cardiovascular diseases such as myocardial infarction, heart failure, coronary heart disease, and atherosclerosis [111, 112]. Selenium-dependent enzymes protect cells from oxidative damage caused by ROS and reactive nitrogen species (RNS)[113]. The results also showed an antagonistic relationship between selenium levels and increased levels of the studied particulate pollutants. Data analysis in **Table (3.7)** showed a moderate negative correlation between selenium and BPDE (r = -0.568**, P = 0.001), and with cadmium (r = -0.417**, P = 0.00) and lead (r = -0.351**, P = 0.00).

Additionally, the average blood zinc levels of the exposed workers were lower than those of the unexposed individuals (661.2 ± 82.52 vs. 977.83 ± 122.28) ng/mL respectively, and these differences were highly statistically significant ($p < 0.001$), as shown in **Figure (3.5).**

Data analysis showed a negative correlation between zinc and BPDE (r = -0.156*, P = 0.037), with cadmium (r = -0.254**, P = 0.001), and with lead (r = -0.351**, P = 0.00), as shown in **Table (3.8)**. The relationship between MDA and (Se and Zn) in the two groups under study is shown in **Table (3.11)**. Data analysis also revealed an inverse relationship between MDA and Se (r = -0.293**, P = 0.000) and between MDA and Zn (r = -0.232**, P = 0.002).

Numerous studies have emphasized the role of zinc in cardiovascular disease, where oxidative stress with ROS is linked to inflammation as one of the molecular processes driving the condition. Consequently, inflammatory cytokines and enzymes related to inflammation are induced, by generating Metallothionein MT, zinc functions as a cofactor for the enzyme superoxide dismutase (SOD), and preventing the generation of ROS. Moreover, zinc reduces inflammation [189]. A previous study indicated a negative relationship between selenium levels of workers exposed to bullets in Rajstistan [190], another study also showed that zinc and selenium levels decreased due to the exposure to cadmium between cadmium exposure and control groups [191].

4.7 Inflammation and Cardiovascular Disease

C-reactive protein (CRP) and high-sensitivity C-reactive protein (hs-CRP), two markers of inflammation, were compared between the two groups in **Table (3.14) and Figures (3-8) and (3-9)**. The levels of CRP in the workers' blood were higher than those in the control group (5.26 ± 0.38 vs. 3.01 ± 0.22,

respectively), with $P < 0.001$, according to the data, this is a common marker of inflammation in the body. Additionally, there was a statistically significant difference between the two groups in the mean value of hs-CRP, a specific marker of cardiovascular disease (2.14 ± 0.26 vs. 0.49 ± 0.07, respectively), with a statistical significance of $P < 0.001$. There was a strong relationship between exposure to particulate matter and hs-CRP levels. Data analysis showed that hs- CRP had a positive correlation with lead (0.26**, $P = 0.000$), a significant positive correlation with cadmium ($r = 0.332$**, $P = 0.000$), and a positive correlation with BPDE ($r = 0.250$**, $P = 0.001$), as shown in **Table (3.15)**.

High-sensitivity C-reactive protein (CRP) is associated with the formation of atherosclerotic plaques. Along with low-density lipoprotein (LDL) cholesterol, it is a real risk factor for cardiovascular disease [192, 193].

Inflammation may lead to the release of inflammatory cytokines that cause atherosclerosis. Atherosclerosis is a chronic inflammatory disease influenced by the immune system and characterized by increased permeability of lipoproteins, subendothelial accumulation, leukocyte recruitment, and platelet activation due to endothelial dysfunction caused by inflammation [194]. Macrophages and smooth muscle cells secrete inflammatory cytokines, including interleukins, and oxidize low-density lipoprotein cholesterol within the arterial wall. Triglyceride-rich lipoproteins and residual lipoproteins also contribute to inflammation. Macrophages degrade these oxidized lipoproteins, forming a lipid-rich necrotic core surrounded by a fibrous collagen cap [195]. In chronic inflammation, macrophages break down the fibrous cap, creating a thin, fragile plaque. Over time, these plaques may transform into stable calcified plaques [196]. In several studies, hs-CRP is a straightforward test utilized in primary care to identify patients at risk for metabolic disorders,

cardiovascular disease, and hypertension, as elevated levels can affect systemic inflammation [197]. Various studies have proven the presence of disorders in multiple body systems and the role of inflammation resulting from exposure to lead [198], exposure to cadmium causes inflammation of blood vessels and promotes atherosclerosis[60].

4.8 Serum Level of Lactate Dehydrogenase is Associated with Cardiovascular Disease Risk

The levels of (LDH) were compared between the two studied groups in **Table (3.16)** and **Figure (3.10)**. According to the results, there was a significant increase in the exposed workers compared to the control group (1219.8 ± 27.53 vs. 710.3 ± 52.47) respectively with statistical significance ($P < 0.001$).

There was a relationship between exposure to particulate matter and lactate dehydrogenase (LDH) levels. A positive correlation was observed between lactate dehydrogenase (LDH) and cadmium (Cd), with a correlation coefficient of $r = 0.232^{**}$ ($P = 0.002$), lead (Pb), with $r = 0.145^{*}$ ($P = 0.01$), and BPDE (benzo[a]pyrene diol epoxide), with $r = 0.323^{**}$ ($P = 0.000$), These findings are summarized in **Table (3.17)**.

Serum LDH levels are associated with cardiovascular diseases and events. LDH, which consists of cardiac and muscle subunits, has much higher activity in cardiac and muscle tissues than in normal serum. Damage to these tissues leads to a significant increase in serum LDH. Also, the coronary arteries have different degrees of sclerosis, affecting the blood supply to the heart muscle. This scenario will not cause myocardial infarction but will cause distinct degrees of myocardial ischemia and damage to myocardial cells. At the same time, a certain concentration of LDH will be released into the blood [199].

4.9 Lipid Profile

As shown in **Table (3.18)** and **Figures (3.11), (3.12),** and **(3.13)**, the blood lipid profile revealed differences in mean values between the exposed workers and the unexposed control group. Total cholesterol was (189.7 ± 3.70 vs. 170.2 ± 1.24) with ($P \leq 0.001$), LDL (115.8 ± 3.75 vs. 103.6 ± 3.17) with ($P \leq 0.01$), and there was a significant increase in triglycerides (205.3 ± 11.90 vs. 94.9 ± 2.12) with ($P \leq 0.001$) and VLDL (41.04 ± 2.09 vs. 19.98 ± 0.75), with ($P \leq 0.001$), the mean HDL value decreased (35.78 ± 0.64 vs. 38.76 ± 0.40) with ($P \leq 0.001$).

High serum lipid profile levels are associated with increased cardiovascular mortality and are important risk factors for cardiovascular disease and atherosclerosis [139, 200]. HDL cholesterol level is inversely related to cardiovascular mortality [201]. High triglycerides are a significant indicator of thickening artery walls (atherosclerosis), which raises the risk of stroke, heart attack, and heart disease. Extremely high triglycerides can also lead to severe inflammation of the pancreas (pancreatitis). Additionally, high triglycerides may indicate type 2 diabetes or prediabetes [202]. A recent study aimed at determining the role of triglyceride levels in various outcomes for patients with heart failure found that high triglyceride levels were linked to hospitalization for atherosclerotic cardiovascular disease or death. Conversely, low triglyceride levels were associated with a heightened risk of hospitalization for heart failure or death [203].

Table (3.19) shows the relationship between lipid profile levels and (Cadmium, Lead, and BPDE) levels in exposed workers and control groups:

Cholesterol: According to the data analysis, there was a slight correlation between cholesterol and Cadmium ($r = 0.216^{**}$, $P = 0.002$), Lead ($r = -0.174^{**}$, $P = 0.029$), and BPDE ($r = 0.037$, $P = 0.010$).

Triglycerides: There was a positive correlation between triglycerides and Cadmium (r = 0.421**, P = 0.000), Lead (0.268**, P = 0.001) and BPDE (r = 0.142, P = 0.057).

HDL: Data analysis revealed inverse associations between HDL and Cadmium (r = -0.162*, P = 0.029), Lead (r = -0.188*, P = 0.01), and BPDE (r = -0.127, P = 0.090),

LDL: Data analysis results showed that LDL and Cadmium were associated (r = 0.223**, P = 0.003), as were LDL and Lead (r = 0.211**, P = 0.017) and BPDE (r = 0.185*, P = 0.025).

VLDL: Data analysis results showed an association between VLDL and cadmium (r = 0.262**, P = 0.000), Lead (r = 0.251**, P = 0.002), and BPDE (r = 0.174**, P = 0.020).

A growing body of studies has documented significant associations between short-term exposure to $PM_{2.5}$ and adverse changes in human blood lipids or their metabolites [204]. Korean research indicates a connection between heavy metal exposure and dyslipidemia, suggesting abnormal lipid metabolism and prompting further investigation into this relationship[205].

A study of 215 adult residents of Wuhan and Zhuhai found that increased four- and five-ring PAHs can negatively impact lipid metabolism, highlighting the need for pollution reduction strategies for cardiovascular health[206].

4.10 Other Factors

Environmental factors, such as high temperatures in Basrah, southern Iraq, significantly affect the concentration of particulate matter pollutants at oil work sites and thus negatively impact the health of workers at those sites and the population of Basrah in general [10, 207].

Various other factors also affect the development of cardiovascular diseases, including lifestyle and diet being two of the most important factors, along with smoking, lack of physical activity, and sleep deprivation [208].

Finally, the controversial results in studies are due to factors including sample size, duration of contamination, worker safety protocols, and regulations at crude oil well sites in oil-producing countries, which may limit the ability to draw similar conclusions [209, 210].

▪ PERSPECTIVE

The adverse health effects of air pollution have been repeatedly demonstrated. As discussed above, extensive, convincing, and strong evidence supports the idea that exposure to air pollution in highly polluted areas results in higher mortality rates and increased risk of chronic disease. In addition, excessive associated gas flaring in the petroleum industry and expanded oil drilling are negatively impacting the population of Basrah and increased incidences of cardiovascular disease and cancer. This high, irrelevant, and preventable burden of disease is a paradigm case in environmental cardiology, as it reinforces the view that much of cardiovascular disease does not arise from an intrinsic biological defect of the individual but rather from adverse exposures or a mismatch between individual biology or behavior and prevailing environmental conditions. Exposure to fine particles is a modifiable risk factor,

attributable to the environment rather than the individual. Thus, to understand the effects of fine particles, we must look beyond biological explanations to more distant environmental "causes of causes," as solutions may lie beyond the scope of current medical science. This broad-based search for viable solutions will require experts—engineers, sociologists, economists, atmospheric scientists, and policymakers—working in partnership with affected communities. Only through this broad collaboration, based on understanding the distal environmental causes of cardiovascular disease, can we develop lasting, applicable, and sustainable ways to manage and prevent cardiovascular risk. This task is increasingly urgent. Cardiovascular disease is the leading cause of death worldwide, and even in high-income countries, the decline in cardiovascular disease prevalence has stalled. This increase in cardiovascular disease and other noncommunicable diseases is particularly ominous in the context of climate change. Air pollution has been shown to have adverse health effects, with occupational exposures of workers in highly polluted areas leading to higher mortality rates and a higher risk of chronic disease. This high disease burden represents a model in environmental cardiology, as it suggests that many cardiovascular diseases are caused by environmental exposures rather than intrinsic biological defects, rather than a mismatch between individual behavior and environmental conditions.

Conclusions

&

Recommendations

▪ Conclusions:

In this study conducted in Basrah, the largest oil-producing Province in Iraq, long-term exposure of workers in these areas to fine, heterogeneous particles was associated with increased incidence of cardiovascular disease.

1. The examinations showed that oil workers are exposed to high levels of toxic chemical fine particles ($PM_{2.5}$), and the particulate matter content was:

   i. Mineral Particulate Content:

One of the main particulate matter components we identified is Cadmium and Lead. We observed significantly higher levels of Cadmium and Lead in workers compared to the control group.

ii. B[a]pyrene/ BPDE

Another important contributor we identified was BPDE (a metabolite of B[a]P. The results indicated higher levels of BPDE in the blood of oilfield workers compared to the control group.

2. The blood levels of selenium and zinc in the control group were higher than those in the workers' group.
3. The level of antioxidants superoxidase dismutase (SOD) in the control group was higher than that in the workers' group, and the level of malondialdehyde (MDA) was the opposite.
4. The decrease in antioxidants in the serum is a sign of high levels of free radical formation including hydroxyl radicals and superoxide within the worker's body and causing oxidative stress due to exposure to fine particles.
5. Increased C-reactive protein (CRP) levels compared to the control group indicate inflammation caused by daily exposure to numerous toxins, and elevated high-sensitivity CRP (hs-CRP) is a predictor of cardiovascular disease and atherosclerosis indicators.

6. The correlation between antioxidant elements (Se and Zn) was in a negative significant direction with (BPDE, Cd, and Pb), which led to the conclusion that the antioxidant element has an important function in getting rid of harmful particulate matter.
7. The correlation between (SOD) levels and (Se, and Zn) levels was in a positive significant direction, and (MDA) level was in the opposite direction, which led to the conclusion of the important role of (Se, and Zn) as an antioxidant element in reducing oxidative stress.
8. This continuous and excessive exposure to these substances poses a great risk to the health of workers and makes them susceptible to various diseases, including cardiovascular diseases.

▪ Recommended:

For further work, the following investigations should be recommended

1. Studying other heavy elements such as Arsenic, Mercury, Chromium, and Nickel.
2. Future study on the negative impact of occupational exposure to particulate matter on the immune system and liver and kidney functions.
3. Supporting these studies by the relevant authorities in the Ministry of Oil.
4. Considering the implications of these results on occupational safety regulations and policies that protect workers from harmful exposures in industrial environments such as crude oil well sites.
5. Also, exploring the feasibility of implementing regular health checks and monitoring workers to detect early signs of cardiovascular diseases.
6. It is important to evaluate the effectiveness of current safety protocols by wearing PPE and consider updating them to ensure the safety of workers in high-risk environments.

7. The results of this research can help policymakers and industry stakeholders realize the importance of prioritizing worker safety and health in industrial environments to prevent long-term health consequences.
8. Implementing preventive measures can mitigate the health risks linked to particulate matter pollution in Basrah by treating and utilizing the associated gas.
9. Finally, the increase in well drilling in Basrah, causing significant particulate pollution and carcinogenic effects on the local population, should be limited, and stricter taxes, legislation, and standards should be imposed.

❖ **Workers should take into account the following recommendations:**

- Reducing exposure: Avoid areas with high pollution, and use masks in polluted places.
- Maintaining a healthy lifestyle involves regular exercise, a balanced diet, and a healthy weight.
- Controlling risk factors: Control blood pressure, diabetes, and high cholesterol.
- Visit your doctor regularly

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Appendix (2.1)

الموافقة على سحب الدم من المشاركين في الدراسة

أني الباحث: ..... / طالبة دكتوراه في قسم الكيمياء / كلية العلوم / جامعة البصرة.

أود الحصول على موافقتك على أخذ بعض المعلومات وسحب الدم منك وذلك لغرض اكمال المتطلبات الخاصة بالبحث الذي أقوم به بعنوان:

" تحديد مستويات بنزو[أ] بيرين والكادميوم والرصاص في دم العاملين في قطاع البترول ودراسة تأثيرها على امراض القلب والأوعية الدموية في محافظة البصرة "

على ان يكون سحب الدم عن طريق الوريد. الأمر متروك لك أذا كنت توافق على ذلك أو لا توافق، لا أحد سوف يقدم لك أو يتقاضى منك أي مبلغ من المال مقابل مشاركتك أن وافقت أو لم توافق.

أذا كنت لا ترغب في المشاركة فشكرا لك على وقتك.

الاسم ..... نعم أوافق على سحب الدم.

الاسم..... لا أوافق على سحب الدم.

التوقيع.....

التاريخ.....

Appendix (2.2)

Questionnaire form

Confidential information

[PROTOCOL NAME]

Number:

Date:

Name: **Gender:** ❑ male ❑ Female

Phone: **Age:**

Address:

Place work:

Duration of work:

Number of daily working hours:

Number of working days per week:

Personal medical information:
Do you have problems with any of these systems? If yes, please check the box.

❑ Cardiovascular	❑ High blood pressure
❑ Diabetes	❑ Allergies
❑ Fainting spells	❑ Kidney problems

➢ Please check Yes or No

Are you in good health?	❑ Yes	❑ No	
Do you have a family history of the disease?	❑ Yes	❑ No	How much?
Do you smoke?	❑ Yes	❑ No	How much?
Do you drink alcohol?	❑ Yes	❑ No	How much?
Are you currently taking any medications?	❑ Yes	❑ No	

If yes, please list all medications on the back.

Do you have any other restrictions that affect your activity? ❑ Yes ❑ No
If yes, please explain on the back.

* **I have agreed to provide information and donate blood samples for study purposes.**

❑ Yes ❑ No

All information relating to this study will be kept strictly confidential, and these confidentiality requirements apply to all staff at the study site or participants. The procedures required to conduct this study will be maintained under the principles of good clinical practice.

Appendix (2.3) Picture showing blood samples after digestion

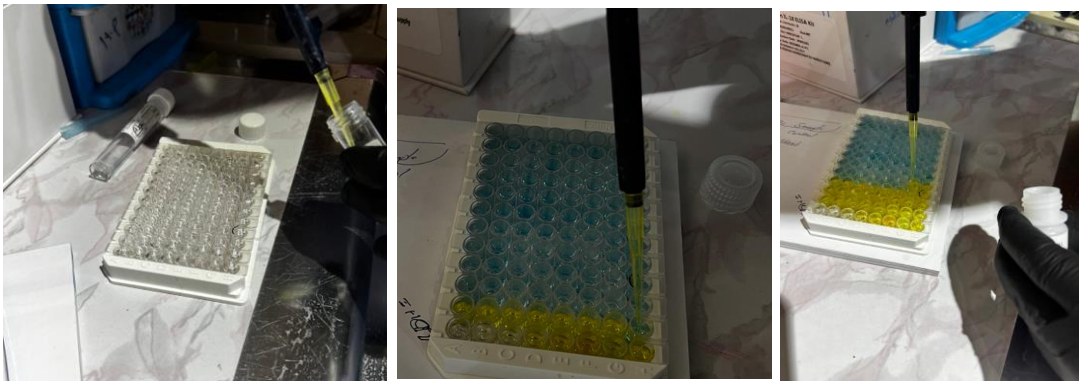
Appendix (2.4): The Parameters of the ICP-OES method

Analysis mode	**Normal**
Rinsing time	**Fixed (30.0 s)**
Rinsing pump speed	**High speed**
Transfer time	**15.0 s**
Stabilization time	**15.0 s**
Transfer pump speed	**High speed**
Delay of synchronization	**0 s**
Stop the pump during the replacement	**No**
Integration time	**Fixed 4.0 s**
Fast Mode	**Yes**
Int. time	**Fast**
Window	**Small**
Int time normal	**1.0**
Int time fast	**0.2**
Small window	**25**
Large window	**51**
Incr.(nm)	**0.003**

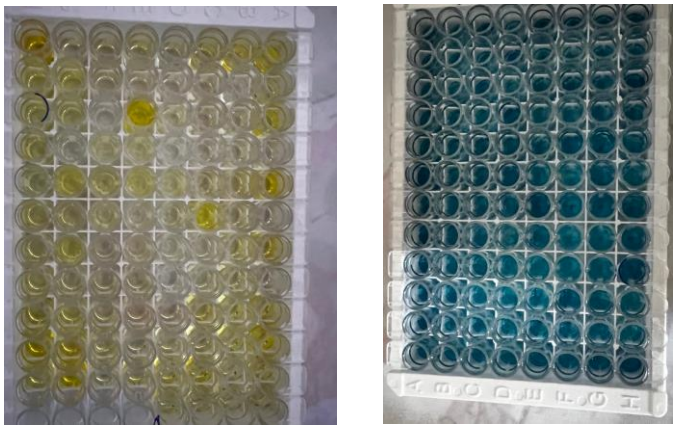
Appendix (2.5): The plasma parameters ICP-OES method

Power	**1100**
Normal Speed of the pump	**15**
Plasma gas flowrate	**PL2**
Sheath gas flowrate	**G1**
Auxilary flowrate	**0.0**
Sheath gas stability time	**15.0 s**
Nebulisation flowrate	**0.02**
Nebulisation pressure	**1.0 bar**
Use argon humidification	**Yes**

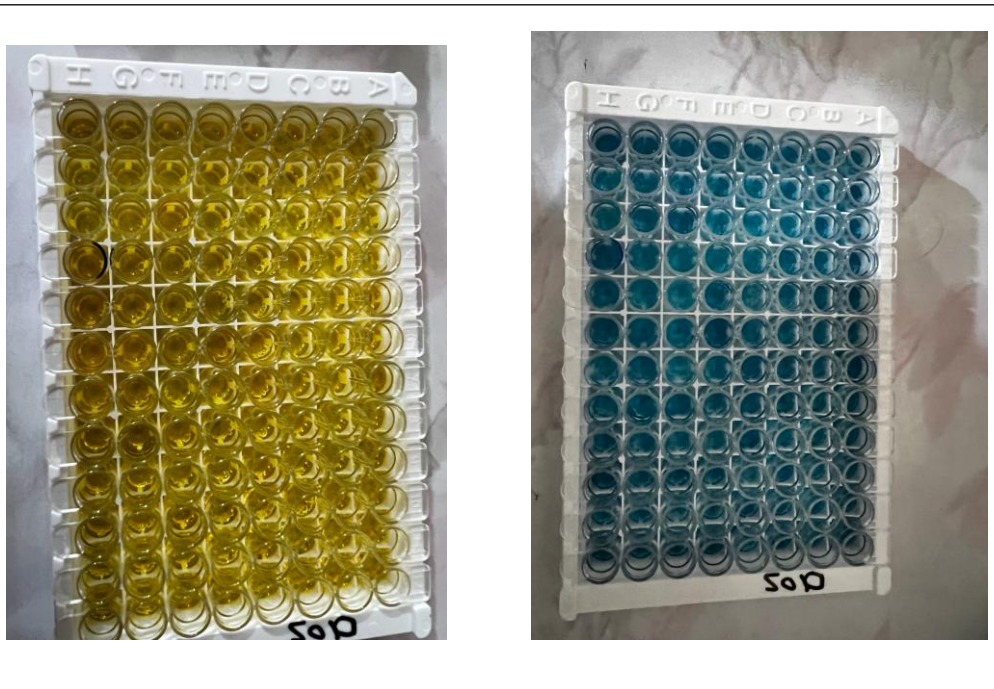
Appendix (2.6): Pictures showing the color change in the BPDE Kit.



Appendix (2.7) Pictures showing the color change to blue upon adding the TMB substrate solution to the MDA Kit.



Appendix (2.8) Pictures showing the color change to blue upon adding the TMB substrate solution to the SOD Kit.



الخلاصة

في الوقت الحاضر، أصبح تلوث الهواء عامل خطر مستقل للإصابة بأمراض القلب والأوعية الدموية .أجريت هذه الدراسة على مجموعتين تتكون المجموعة الأولى من تسعين فردًا يعملون في موقع نهران بن عمر للنفط الخام في مدينة البصرة .تراوحت أعمار العمال من 23 إلى 55 عامًا . وتضمنت المجموعة الثانية 90 فردًا غير معرضين (طلاب وأعضاء هيئة تدريس) تراوحت أعمارهم من 21 إلى 54 عامًا. تقيم هذه الدراسة تركيزات الجسيمات الدقيقة في دم المشاركين، بما في ذلك مستقلب الهيدروكربون العطري متعدد الحلقات بنزوبيرين ديول إيبوكسيد(BPDE) ، والمعادن الثقيلة، بما في ذلك الكادميوم والرصاص.

أظهرت النتائج ارتفاعاً معنوياً في متوسط قيمة مستوى BPDE (Pg/mL) للمجموعة المعرضة (203.3 ± 9.42) مقارنة بمجموعة الضبط (60.1 ± 1.64) مع دلالة إحصائية .($P \leq 0.001$) كما كان لدى العمال مستويات مرتفعة من الكادميوم (ng/mL) في دمائهم بمتوسط قيمة (85.8 ± 0.83) مقارنة بمجموعة الضبط بمتوسط قيمة (43.7 ± 3.07) وكانت هذه الفروقات عالية الدلالة إحصائياً ($P\leq0.001$)وكانت مستويات الرصاص المرتفعة (ng/mL) في دم العمال بمتوسط قيمة (347.65 ± 14.24) أعلى من تلك الموجودة في مجموعة الضبط بمتوسط قيمة (165.0 ± 11.49) مع دلالة إحصائية .($P \leq 0.001$) كما تضمنت الدراسة تقييم مستويات المالونديالدهيد (MDA) وهو مشتق من بيروكسيد الأحماض الدهنية المتعددة غير المشبعة كمؤشر محتمل للإجهاد التأكسدي، وفائق أكسيد ديسميوتاز (SOD) وهو إنزيم مضاد للأكسدة، في مصل المشاركين في الدراسة، وأظهرت النتائج ارتفاعاً معنوياً في MDA (µmol/L) لدى العمال بمتوسط قيمة (1219.8 ± 27.53) مقارنة بمجموعة الضبط بمتوسط قيمة (710.3 ± 52.47) مع دلالة إحصائية ($P\leq 0.001$). انخفضت قيمة SOD (U/L) بشكل ملحوظ لدى العمال بمتوسط قيمة (8.90 ± 0.22 مقابل متوسط قيمة 11.65 ±

0.15 في المجموعة الضابطة، مع دلالة إحصائية ($P \leq 0.001$) كما تناولت الدراسة العلاقة العدائية بين التعرض المهني للجسيمات الدقيقة ومستويات العناصر النزرة الأساسية (السيلينيوم والزنك) لدى العمال مقارنة بالمجموعة الضابطة. انخفضت مستويات السيلينيوم (ng/mL) بشكل ملحوظ لدى العمال بمتوسط قيمة (146.2 ± 20.00) مقابل متوسط قيمة (165.0 ± 19.25) في المجموعة الضابطة، مع دلالة إحصائية ($P \leq 0.001$) بالإضافة إلى ذلك، انخفضت أيضًا مستويات الزنك (ng/mL) بشكل ملحوظ لدى العمال بمتوسط قيمة (661.2 ± 82.52) مقابل متوسط قيمة (977.83 ±122.28) في المجموعة الضابطة، مع دلالة إحصائية ($P \leq 0.001$) تم أيضًا تقييم إنزيم لاكتات ديهيدروجينيز (LDH)في كلتا المجموعتين .أظهرت النتائج زيادة كبيرة في قيم LDH (µmol/L) لدى العمال المعرضين، بمتوسط قيمة (432.70 ± 26.717) مقارنة بالمجموعة الضابطة، بمتوسط قيمة (300.60 ± 10.16)، مع دلالة إحصائية ($P \leq 0.001$) . يمكن أن يؤدي التعرض للجسيمات إلى تحفيز مسارات الإشارات الالتهابية، مما يقلل من مقاومة الأمراض والالتهابات. بحثت هذه الدراسة في مخاطر التعرض المهني للجسيمات وتأثيرها على زيادة بروتين سي التفاعلي (CRP) وبروتين سي التفاعلي عالي الحساسية (hs-CRP) ، وقد وثقت النتائج وجود بروتين سي التفاعلي في دم العمال بتركيزات أعلى من تلك الموجودة في مجموعة التحكم غير المعرضة (5.26 ± 0.38 مقابل 3.01 ± 0.22) على التوالي . وهذا مؤشر عام على الالتهاب في الجسم، مع دلالة إحصائية $P \leq 0.001$. وكشفت البيانات عن وجود فرق كبير في القيمة المتوسطة لبروتين سي التفاعلي عالي الحساسية (hs-CRP) بين المجموعتين (2.14 ± 0.26 مقابل 0.49 ± 0.07) على التوالي، مع دلالة إحصائية$P \leq 0.001$ ، مما يشير إلى زيادة خطر الإصابة بأمراض القلب والأوعية الدموية .

أظهر ملف الدهون في الدم اختلافات في القيم المتوسطة بين العمال المعرضين ومجموعة التحكم غير المعرضة . كان إجمالي الكوليسترول (189.7 ± 3.70 مقابل 170.2 ± 1.24) مع ($P \leq 0.001$)، و LDL (115.8 ± 3.75 مقابل 103.6 ± 3.17) مع ($P \leq 0.01$) ، وكان هناك زيادة

كبيرة في الدهون الثلاثية (205.3 ± 11.90 مقابل 94.9 ± 2.12) مع ($P \leq 0.001$) و VLDL (41.04 ± 2.09 مقابل 19.98 ± 0.75) مع ($P \leq 0.001$) ، وانخفض متوسط قيمة HDL (35.78 ± 0.64 مقابل 38.76 ± 0.40) مع ($P \leq 0.001$) .

وفي الختام، فإن التعرض للملوثات الكيميائية في مكان العمل، مثل بنزو[أ]بيرين والكادميوم والرصاص، يمكن أن يزيد من إنتاج أنواع الأكسجين التفاعلية. ويمكن أن تؤدي هذه الزيادة إلى ارتفاع مستويات مالونديالدهيد (MDA) وتثبيط وظيفة إنزيم أكسيداز الفائق ديسميوتاز (SOD)، ونقص العناصر النزرة المهمة لوظيفة الإنزيمات داخل الجسم، مثل السيلينيوم والزنك، مما يسبب الإجهاد التأكسدي. وتبحث هذه الدراسة في تأثير الالتهاب على تطور أمراض القلب والأوعية الدموية من خلال القيمة التنبؤية للعلامات الحيوية المرتبطة بالالتهاب، مثل المستويات المرتفعة من بروتين سي التفاعلي عالي الحساسية. كما يشير ارتفاع مستوى LDH واختلال مستوى الدهون إلى أمراض القلب والأوعية الدموية.

وزارة التعليم العالي والبحث العلمي
جامعة البصرة
كلية العلوم
قسم الكيمياء

" تحديد مستويات بنزو[أ] بيرين والكادميوم والرصاص في دم العاملين في قطاع البترول ودراسة تأثيرها على امراض القلب والأوعية الدموية في محافظة البصرة "

أطروحة مقدمة الى

كلية العلوم - جامعة البصرة وهي جزء من متطلبات

نيل شهادة الدكتوراه

في الكيمياء التحليلية

من قبل

نغم جواد كاظم

ماجستير علوم كيمياء 2018

بإشراف

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