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ASSESSMENT OF NEW PREDICTING BIOMARKERS OF POLLUTION TOXICITY IN WORKERS OR INDIVIDUALS LIVING CLOSE TO OIL REFINERY AREA IN AL-MUTHANNA CITY, IRAQAS ALARM INDICATORS FOR CLINICAL DISORDERS

Mariam Ryadh Alghara[1], Ausama Ayob Jaccob[2*] and Azza Sajid Jabbar[3]

[1]*BSC in pharmacy, AL Muthanna Health Department, Planning Department, al Muthanna, Iraq*
[2]*Department of Pharmacology and Toxicology, College of Pharmacy, Basra University, Basra, Iraq*
[3]*Department of Physiology, College of pharmacy, Basra University, Basra, Iraq*

Objective, This study aims to assess the impact of petroleum exposure on certain biochemical parameters; to find out the sensitive predicting biomarkers which may be considered of a clinical role in detecting the toxic effect of exposure as well as to conclude the possible correlation among the parameters.Methods, 150 individuals were enrolled in this study. The participants were separated into three groups: Group 1 is a control group of 50 people; Group2 is 50 people (who were oil well workers); and Group3 is 50 people (the individuals who live close to oil wells), working in the Middle Refinery Company unit for more than two years. The hematological parameters, serum interleukin 6, serum vitamin D3, serum homocysteine, and serum glutathione were all measured. Results showed significant increases in white blood cells,and platelets in Group2 and Group3 compared to Group1as well as a significant decrease of hemoglobin, hematocrit, and glutathione in workers compared to control groups. Interestingly, we found significant increases in serum interleukin 6 and homocysteine in workers. Conclusions, The individuals who work or live close to oil wells showed significant alterations in some hematological, immunological, and inflammatory parameters,as well as they, revealed more production of reactive oxygen species referring to an increase in oxidative stress.

Keywords: *Oil wells, air pollution toxicity, predicting biomarkers.*

INTRODUCTION

Air is a homogeneous mixture of gases and suspended particles that exist in various compositions and sizes. This chemical composition is in a state of continuous change according to place and time as a result of many chemical reactions and physical transformations that affect air quality[1].The main source of gaseous emissions that damage the environment is human activity, particularly in cities where the concentration of polluting gasessuch as CO, CO2, NOx, SOx, O3, and others in the air is directly correlated with the surrounding sources of pollution[2].

suspended particles are one of the major atmospheric pollutants that have an impact on the energy balance, climate, health of humans and animals, and other environmental components. because include high levels of toxic substances such as heavy metals and polycyclic aromatic hydrocarbons, which play an important role in the pollution of land and aquatic ecosystems when deposited, and contribute to atmospheric pollution[3]. Animals exposed to gaseous contaminants cause endothelial dysfunction and oxidative stress[4]. Heavy metals are frequently present in natural waters, some of them are necessary for the survival of living things, and others may be

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*Corresponding author: Ausama Ayob Jaccob, E-mail: Ausama1979@yahoo.com

harmful in excessive doses. Metals like (iron, copper, and zinc) are essential metals because they play a crucial role in biological systems, whether functional or structural, whereas metals (mercury, lead, and cadmium) are non-essential metals and they are toxic. Heavy metals acquired through the food such as seafood as a result of pollution pose potential chemical hazards, threatening consumers[5].

Iraq is a major oil producer in both Asia and the rest of the globe. Oil is important to the expansion and development of the global economy and serves as an indicator of a nation's economic performance[6].Chemical substances found in these gases such as benzene, toluene, lead, oxygenates, ethyl benzene, and 3 isomers of xylene[7]. While the main elements that are present in gases are carbon and hydrogen, sulfur, nitrogen, and oxygen usually occur in subordinate quantities and have potential occupational health hazards[8].

Filling station personnel, service station attendants, gasoline truck drivers, and refinery workers are all at higher risk of being exposed to gasoline vapors[9]. The actual chemical hazards depend on toxicity and route of exposure. Inhaling small amounts of gasoline vapors may cause either early acute toxicity (reversible) hematotoxicity or chronic toxicity due to high-dose exposure (irreversible) bone marrow damage, acute toxicity exhibited a variety of hematologic side effects, such as anemia, leukopenia, and thrombocytopenia[10].Sometimes, the number of different cell types is reduced,It has one found that Pancytopenia is typically linked to irreversible bone marrow damage(chronic toxicity)[10].In some cases, occupational exposure to fumes, such as petroleum, has been linked to higher oxidative stress biomarkers., A wide range of clinical disorders has been linked to oxidative stress, including diabetes, cancer, aging, liver problems, gastrointestinal disturbancehypertension, atherosclerosis, and neuronal problems[11].

At the kinetic level, the metabolism of hydrocarbon can cause cell damage due to the production of Reactive oxygen species (ROS) and hydroxyl radicals, if they are produced at elevated levels, the balance between formation and removal is disrupted, shifting the balance toward oxidative stress and subsequent metabolic disorders and lipid peroxidation[12]. Antioxidant systems, which include enzymes like superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and glutathione peroxidase (GPx), protect against oxidative stress. Furthermore, they may act as indicators of the impact of hazardous pollutants. This idea has recently been put to the test via bioassays on samples that were gathered for a short period, which revealed the quick toxic effect to detect contamination and assure environmental safety. Sometimes they are considered indicators of pathogenic processes, or pharmacologic responses to treatment[11]. Several predictive biomarkers reflect the possible future organ toxicity and are considered disease-specific.[14].

Environmental exposure to crude oil can lead to inflammatory-related cell injury, which is mediated by cytokines. Cytokines are small molecular polypeptides or glycoproteins synthesized and secreted by activated immune cells and non-immune cells. They have either pro-inflammatory or anti-inflammatory effects[15]. Elevated plasma homocysteine concentrations due to Crude oil may inhibit the vasodilation of nitric oxide and promote vascular smooth muscle growth, both of which may lead to an increased risk of cardiovascular disorders. Additionally, elevated levels of pro-inflammatory markers like interleukin-6, fibrin, and C-reactive protein are linked to homocysteine[16]. Furthermore, it has been reported that vitamin D deficiency could be a biomarker for particular matter exposure[17].

The number of medical cases and occupational diseases is increasing in both industrialized and developing countries[9]. This study aims to assess the impact of petroleum exposure on certain biochemical parameters; to find out the sensitive predicting biomarkers which may be considered of a clinical role in detecting the toxic effect of exposure as well as to conclude the possible correlation among these parameters.

MATERIAL AND METHOD

Study design and ethical approval

This observational study was done between October 2021 and January 2022at Middle Refineries Company (MRC) in Al-Muthanna City, in the south of Iraq, followingSTROB guidelines. The study was authorized by the University of Basrah's, College of Pharmacy's ethics committee. After completing the institutional ethical agreement (3/5/293 in 21/10/2021) and obtaining informed consent by the tenets of the Helsinki Declaration

Participants

This study included 150 participants in total. They were classified into 3 groups: a control group of 50 participants, the second group of 50 participants (who were oil well workers), and the third group of 50 participants (where individuals live close to oil wells). Working in the MRC unit for more than two years, the workers ranged in age from 25 to 60, working every day and working three days a week. The study excluded participants with cardiovascular disease, endocrine disorders, respiratory problems, obesity, pregnancy, and breastfeeding as additional exclusion criteria.

Gather information about each person by inquiring about their age, gender, place of employment, address, weight, height, and body mass index (BMI); personal lifestyle choices (such as smoking, drinking, or coffee use); and medical, surgical, and drug histories.

Measurements

Five milliliters of blood were taken and stored in a cold setting to prevent any effects from the environment on the samples.

Hematology Profile

One ml of blood was obtained into a tube containing EDTA (ethylene diamine tetra acetate). The tube was placed in a roll shaking mixer for a few minutes to prevent blood clotting and then placed below the needle of auto-analyzer hematology to test hematological parameters. White blood cell count (WBC10^9/L), hemoglobin (HGB g/dl), hematocrit (HCT %),; platelet (PLT (10^9/L)[18]by (Ruby Hematology Analyzer, Germany)[18].

Immunofluorescence assay

The remaining 4 milliliters of blood were placed in a centrifuge to obtain clear serum. Interleukin-6 (IL-6) was measured in serum by Getein Biotech, Inc. in the United Kingdom. An immunofluorescence assay was used to achieve this (IL-6 Fast Test Kit). The test was carried out as directed by the manufacturer[19].

Measuring serum vitamin D3 was by Cobas E411, a fast immunofluorescence assay intended for the detection of serum VitD3[20].

ELISA assay

Thiskitusesenzymelinkedimmunesorbentassay (ELISA)basedontheBiotindoubleantibodysandwichtechnologytoassaythehumanhomocysteine (Hcy), and human glutathione (GSH)[21].

RESULTS AND DISCUSSION

Results

150 participants responded to answer questions using a pre-prepared questionnaire with demographic data documented. More than 80% of the participants were males in all studied groups as seen in figure1A. Smoking and coffee habits patterns were also documented. The majority of participants among groups don't prefer smoking or coffee drinking as shown in Figure1 B and C. Body mass index (BMI) among individuals of all groups is measured as illustrated in figure 1D. The majority of the participants are of a healthy weight.

Hematological parameters results among all groups, as seen in Figure 2A showed that WBC, B/platelet(PLT), C (HGB), and D (HCT) revealed significant variation. Figure A, shows a significant increase in WBC in Group 2 and Group 3 compared to Group 1 (control). Figure B shows a significant increase in platelets in Group1 and Group3 compared to Group2 (workers). Figure C shows that there is a significant decrease in HGB in Group2 compared to Groups 1 and 3. figure D significant decrease of HCT in Group2 compared to other groups. This result might be attributed to that continuous exposure to petroleum products lead to common hematological abnormalities.

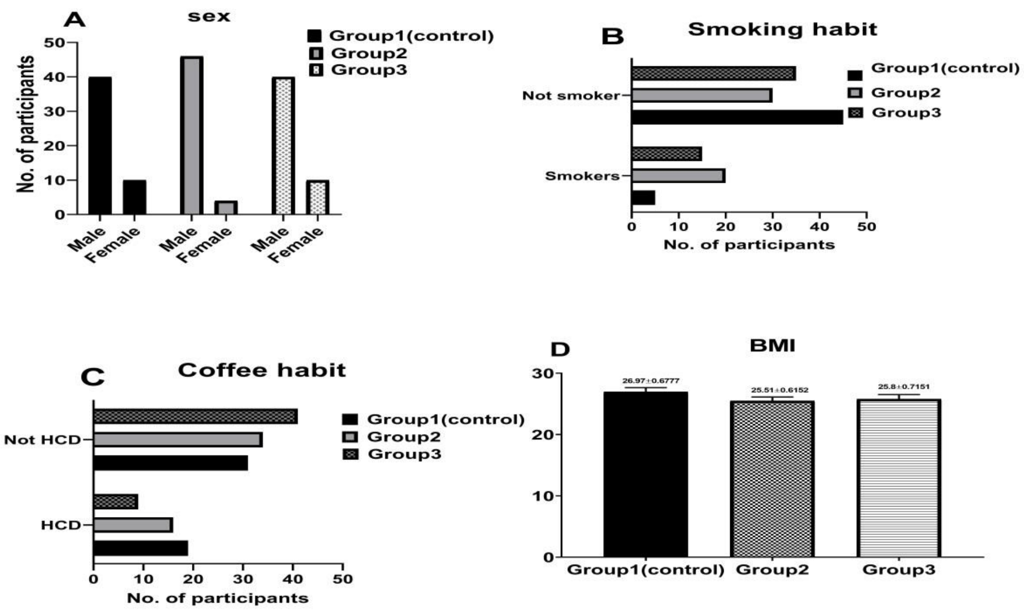

Fig.1: Demographic information of the participants(n=150). A/sex distribution, B/smoking habit, C/coffee habit, and D/BMI of the participants.

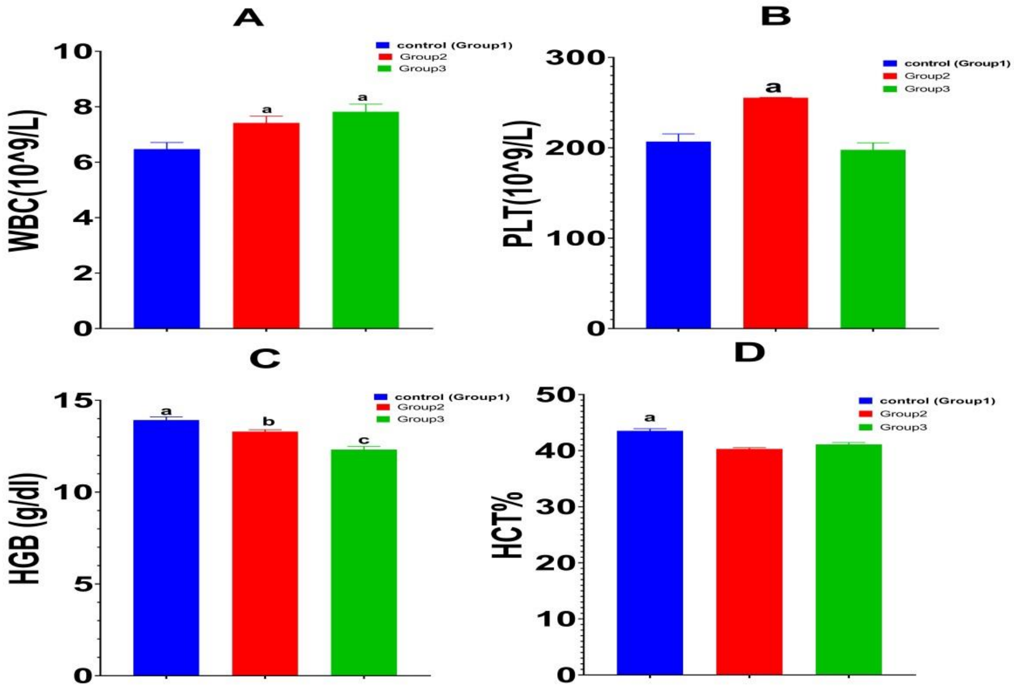

Fig. 2: Hematological parameters among groups, A/WBC, B/Platelet, C/Hemoglobin, D/Hematocrit. These letters represent significant differences P<0.05 between group.

Figure 3 demonstrates a significant rise in serum IL-6 concentration (14.56±2.907 pg/ml) in persons of group 2 who work closer to a petroleum refinery unit than the other groups. A high serum concentration is thought to be a clear biomarker for prognosis or a risk factor for occupational toxicity that could occur before cardiovascular or metabolic disorders. When comparing the persons who live in the city center or a remote location as the control group, (Group 3) members (who live nearby demonstrated a non-significant elevation in serum IL-6.

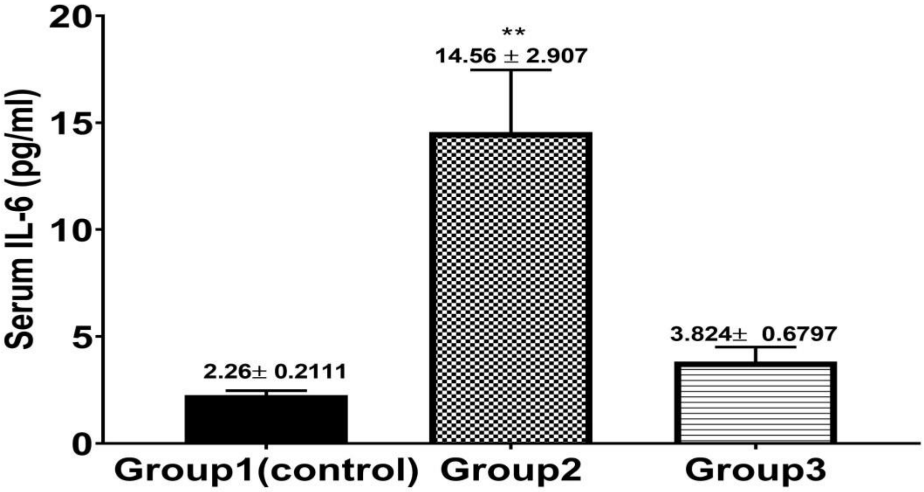

Fig. 3: Serum interleukin-6 levels of the participants in the study (n=150). Values are expressed as Mean ± SEM; *represents a significant difference P< 0.01among groups.

Moreover, the level of homocysteine was found to be significantly higher 4.569± 0.4107 (P< 0.001) in workers (Groups 2) compared to the controls (Group 1) and (Group 3) (figure 4). High serum homocysteine concentrations are considered predictive biomarkers for early deterioration of physiological function. It can be seen that participants in group3 showed a significant increase in homocysteine compared to the control.

Low serum vitamin D concentration (18.39± 0.664) ng/ml has been documented in persons working at the petroleum refinery unit (Group2) compared to the control (Group1) and participants in Group3. Actually, in this study serum, and vitamin D in all participants even in the control group below the approved normal range as summarized in figure5.

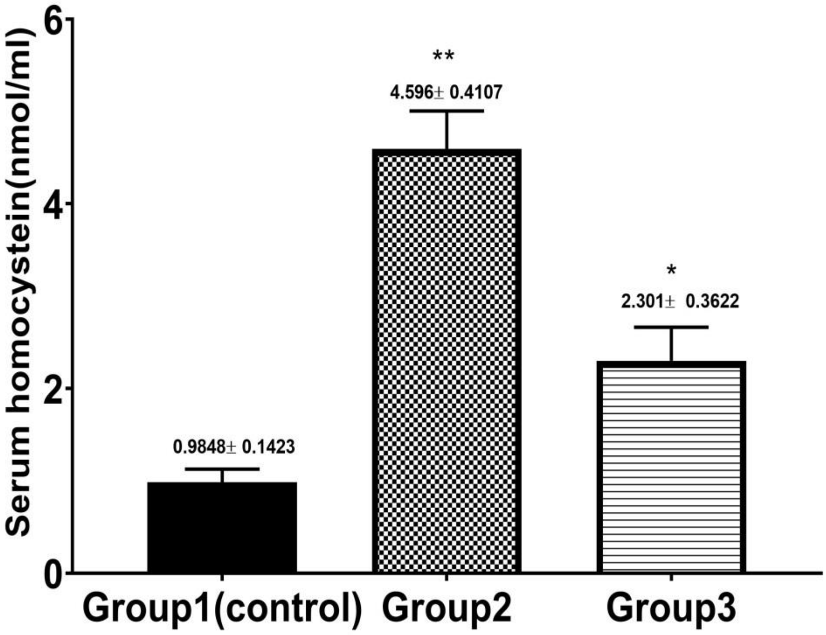

Fig. 4: Serum homocysteine concentrations of the participants in the studied groups (n=150). Values are expressed as Mean± SEM. ** represents a significant difference (P<0.001). * represents a significant difference (P<0.05).

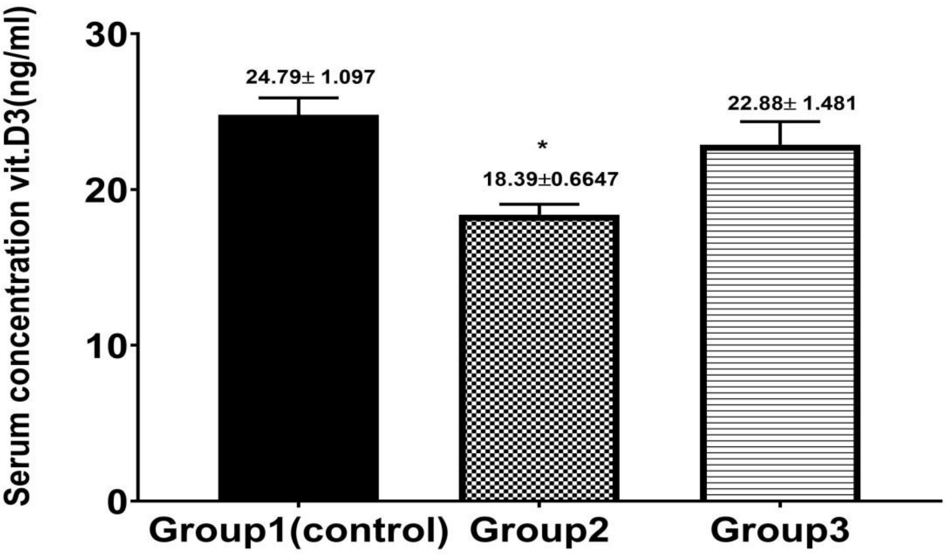

Fig. 5: Serum Vitamin D concentrations of the participants in the studied groups (n=150). Values are expressed as Mean± SEM. * represents a significant difference (P<0.05).

Since free radical generation and oxidative stress are implicated as causative factors in different clinical disorders related to occupational toxicity, measurement of glutathione levels is important to correlate and explain our results as it is currently the most widely studied antioxidant. Serum glutathione concentrations were significantly reduced in workers (Group2)(1.067± 0.0624) and (Group3)(1.231± 0.093) compared to the normal control (Group1) as seen in figure 6.

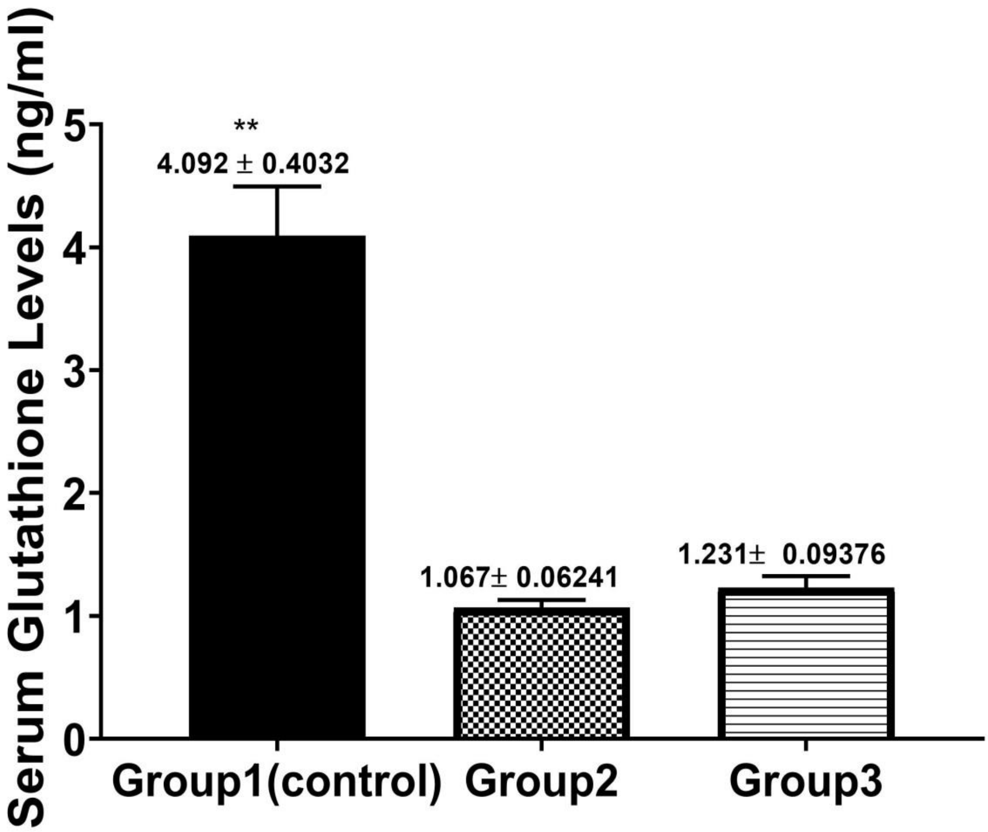

Fig. 6: Serum glutathione levels of the participants in the studied groups (n=150). Values are expressed as Mean± SEM. ** represents a highly significant difference (P<0.001).

Discussion

This study investigated the impact of benzene-containing gasoline vapor on human hematological, inflammatory and immunological markers. In Iraq, women are typically underrepresented among the primary workers at gas stations. Due to the difficult conditions and nature of the work, men made up the majority of the participants in the current study who had direct contact with the Oil Wells Refinery (80–90%). There were significant differences ***(P<0.05)*** among the groups in smoking habits. Regarding coffee consumption, the participants in both Group2 workers and Group3 drank a lot of coffee. As well as no significant differences were found in BMI among the individuals of all groups, in contrast to another study that discovered that exposure to several polycyclic aromatic hydrocarbons(PAHs) and volatile organic chemicals (VOCs) was directly correlated to an elevated risk of obesity[22]. The results of hematological parameters showed a significant increase in WBC in (Group 2 and Group 3) compared to (Group 1) (control). Furthermore, hemoglobin and HCT were negatively related to air pollution in Group2 and Group3. As well as there was a significant rise in platelet count in Group 2 workers in comparison with Group1 and Group3. This difference could be attributed to the fact that long-term exposure to petroleum compounds affects bone marrow functions to produce blood cells[23]. In similarity, other previous studies found that workers who were exposed to vapors of oil for 2 years or longer had significantly increased WBC and PLT levels, and decreased HGB, and HCT levels.[18,9]. Additionally, another study presents the number of harmful consequences resulting from gasoline and its compounds may explain the significant decline in the measured parameters of exposed individuals, such as red blood cell (RBC), hemoglobin (HGB) concentration, hematocrit (HCT), and platelet (PLT) count, as compared to controls. One of the main components of gasoline, benzene, is a known systemic toxin to people and a contributor to aplastic anemia. It is hemotoxic and suppresses the bone marrow, causing pancytopenia[24].

Our results show a significant increase in serum concentration of IL-6 in (Group2) individuals who work more closely in the petroleum refinery unit compared to the remaining groups. It has been discovered that low-level petroleum exposure may change how pro-and anti-inflammatory cytokines are expressed, as well as affected how the cytokines and early inflammatory response are released by peripheral blood mononuclear cells (PBMCs)[15]. Another study that indicated that benzene might increase the synthesis of pro-inflammatory IL-6 while decreasing the synthesis of anti-inflammatory cytokines like IL-10 is also consistent with this conclusion[18].The release of pro-inflammatory mediators into the bloodstream may be further triggered by the exposure of pulmonary macrophages and epithelial cells to oxidative stress. These mediators can damage endothelial cells and have an impact on the entire body. it has been proposed that breathing in diesel exhaust may activate receptors in the respiratory tract, and autonomic nervous system and affect cardiac regulation[25].

According to the current study, workers' homocysteine levels were found to be significantly higher in Group2 than those of controls (Group 1) and (Group 3).Published research has demonstrated that elevated plasma levels of total homocysteine (tHcy) are important predictors of cardiovascular events, including coronary artery, cerebral, and peripheral occlusive disorders[26]. This result was in agreement with other previous results that reported that exposure to particular matters and crude oil is associated with elevated plasma homocysteine[16].

Additionally, low serum vitamin D concentrations have been reported in individuals working in the petroleum refinery unit (Group2) compared to the control (Group1) and participants in (Group3). A prior study has concluded that long-term exposure even with low concentrations of air pollutants, such as those produced by burning gases in industrial sites, heating buildings, and automobile exhaust, affects human health on a worldwide scale such as the effect on serum vitamin D[27]. Our result was also consistent with other studies' evidence that smoking, environmental pollutants, and air pollution exposure cause vitamin D deficiency(VDD)[28]. In similarity with another study VDD and endocrine-disrupting substances develop as a result of exposure to environmental pollutants. Both endocrine disturbing chemicals exposure and VDD lead to observing human deleterious developmental, neurological, cardiovascular, metabolic, and immunological consequences[28].

Another interesting finding revealed by the present study was that serum glutathione concentration was significantly reduced in workers (Group2 and Group3) compared to normal control (Group1). Reactive oxygen species (ROS), can be produced by air pollutants such as fine and ultrafine particles, ozone, nitrogen oxides, polycyclic aromatic hydrocarbons, and transition metals. Thepollutantsmay also act as free radical initiators, and oxidative stress,results from an imbalance between the productions of reactive oxygen species (ROS) and antioxidant enzymes, leading to tissue, lipid, protein, and nucleic acid damage. As a result, ROS may be considered a key factor in many environmental diseases that affect humans, such as cancer, asthma, respiratory conditions, and arteriosclerosis[29]. Since free radical generation and oxidative stress are implicated as causative factors in different clinical disorders related to occupational toxicity, the measurement of glutathione levels is important to correlate and explain these effects.Antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and glutathione peroxidase (GPx) are the first line of defense against ROS[30]. Glutathione peroxidase plays a crucial role in antioxidant defense which is reduced due to toxic free radical scavenger[31&32].

Conclusion

Workers who are exposed to oil products and their metabolites increase the risk of hematological disorders including anemia, an inflammation disorder, and metabolic disorders which lead to alteration of serum IL-6 and increase the risk of cardiovascular diseases by raising serum homocysteine levels and lowering serum vitamin D3. Exposure to oil refinery metabolites in the air causes the production of ROS, which changes serum antioxidant enzymes like glutathione peroxidase, which is thought of as a defense enzyme against air pollution. Because of this, employees at these places of employment must be aware of any dangers associated with their work and submit to routine clinical evaluations to prevent and treat any health issues.

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نشرة العلوم الصيدليـــة
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تقييم المؤشرات الحيوية الجديدة للتنبؤ بسمية التلوث لدى العمال أو الأفراد الذين يعيشون بالقرب من منطقة مصفاة النفط في مدينة المثنى، العراق كمؤشرات إنذار للاضطرابات السريرية

مريم رياض الغرة[١] – أسامة أيوب يعقوب[٢*] – عزة ساجد جبار[٣]

[١] بكالوريوس صيدلة ، دائرة صحة المثنى ، دائرة التخطيط ، المثنى ، العراق

[٢] قسم علم الأدوية والسموم ، كلية الصيدلة ، جامعة البصرة ، البصرة ، العراق

[٣] قسم الفسيولوجيا ، كلية الصيدلة ، جامعة البصرة ، البصرة ، العراق

الهدف: تهدف هذه الدراسة إلى تقييم تأثير التعرض للبترول علـى بعـض التحاليـل البيوكيميائيـة. لايجادالتحاليلالاكثرتأثرا والتي يمكن اعتبارها ذات دور سريري في الكشف عن التأثير السام للتعرض وكذلك لاستنتاج العلاقة المتوقعة بين هذه التحاليل. طريقة العمل، تم اخذ ١٥٠ شخصا في هذه الدراسة. تم تقسيم المشاركين إلى ثلاث مجموعات: المجموعة ١ عبارة عن مجموعة control تتكـون مـن ٥٠ شخصًا ؛ المجموعة ٢ هي ٥٠ شخصًا (كانوا من عمال آبار النفط) ؛ والمجموعة ٣ هم ٥٠ شخصـاً (الأفراد الذين يعيشون بالقرب من آبار النفط) ، يعملون في شركة المصافي الوسطى منذ أكثـر مـن عامين. تم قياس جميع التحاليل الدموية ، انترلـوكين ٦ ، فيتـامين D3 ، مصـل الهوموسيسـتين ، وجلوتاثيون المصل. أظهرت النتائج زيادة في خلايا الدم البيضاء والصفائح الدموية في المجموعـة ٢ والمجموعة ٣ مقارنة بالمجموعة ١ بالإضافة إلى انخفاض في الهيموجلوبين والهيماتوكريتوالجلوتاثيون في العاملين مقارنة بمجموعةcontrol. ومن المثير للاهتمام ، وجدنا زيادات كبيرة فـي انترلـوكين ٦ والهوموسيستين في الدم في العمال. الاستنتاجات ، الأفراد الذين يعملون أو يعيشون بالقرب من آبـار النفط أظهروا تغيرات كبيرة في بعض العوامل الدموية والمناعة والالتهابات ، وكذلك كشفوا عن المزيد من إنتاج أنواع الأكسجين التفاعلية مما يشير إلى زيادة الإجهاد التأكسدي.