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Assessment of Oxidative Stress Parameters for some of Baghdad City Fuel Stations Workers

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Abstract

Because of inhalation of the volatile substances and unintended ingestion, the risk of health problems for fuelling employees exposed to gasoline vapours in gas stations is increased. Therefore, the goal of the study was to compare the oxidative stress state of healthy volunteers, who served as the control group, to Iraqi male fuelling staff, who served as the exposed groups. The levels of total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI), malondialdehyde (MDA), Uric acid (UA), and total peroxidase activity were measured. A total of 102 subjects divided into three groups according to the duration of fuel vapours exposure: 25 exposed for 1-10 years (G1), 50 exposed for 11-19 years (G2) and 27 exposed for ≥ 20 years (G3), as well as 50 healthy people as a control group (C). The results exhibited that the levels of TOS, OSI, MDA, and total peroxidase activity were highly significant increased in all exposed groups (G1, G2 & G3) as compared to the C. A highly significant decrease was observed in TAS levels in all exposed groups in comparison with the C, while there was no significant difference in UA levels in all studied groups. The exposure to petrol associated with significant increase of TOS, OSI, MDA and total peroxidase activity with a significant decrease of TAS among workers in fuel stations and they increased oxidative stress, which likely resulted in negative impacts on human health later on as a result of prolonged labour at gas stations.

Keywords: Fuel, Oxidative stress, Lipid peroxidation, Peroxidase activity, Uric acid.

تقييم معايير الإجهاد التأكسدي لبعض العاملين في محطات الوقود في مدينة بغداد

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الخلاصة

تزداد مخاطر المشاكل الصحية بسبب استنشاق المواد المتطايرة والابتلاع الغير مقصود لعمال التعبئة المعرضين لابخرة الوقود في محطات الوقود.لذلك، هدفت الدراسة إلى مقارنة حالة الإجهاد التأكسدي للمتطوعين الأصحاء كمجموعة سيطرة مع عمال تعبئة الوقود من الذكور العراقيين في كمجموعات معرضة. تم قياس مستويات حالة الأكسدة الكلية (TOS) ، حالة مضادات الأكسدة الكلية (TAS) ومؤشر الإجهاد التأكسدي (OSI)،المالونديالديهايد (MDA) ، حمض اليوريك (UA) ، والفعالية الكلية للبيروكسيداز . مجموع 102 فردا" قسمت إلى ثلاث مجموعات وفقًا لمدة التعرض لابخرة الوقود: 25 متعرض لمدة 1–10 سنوات (G1) ، 50

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متعرض لمدة 11–19 عامًا (G2) و 27 متعرض لمدة 20 عامًا (G3) ، بالاضافة الى 50 شخصا" سليما" كمجموعة ضابطة (C). اظهرت النتائج ان مستويات TOS و OSI و MDA والفعالية الكلية للبيروكسيداز ارتفعت بشكل ملحوظ لدى جميع المجموعات المعرضة (G1 و G2 و G3) مقارنة بالمجموعة C . لوحظ انخفاض معنوي كبير في مستويات TAS في جميع المجموعات المعرضة مقارنة بالمجموعة C ، بينما لم يكن هناك فرق معنوي في مستويات حمض اليوريك في جميع المجموعات المدروسة. التعرض للبترول مرتبط بزيادة كبيرة في TOS و OSI و MDA و فعالية البيروكسيداز الكلية مع انخفاض كبير في TAS بين العاملين في محطات الوقود مما تسبب في زيادة الإجهاد التأكسدي ، والذي قد يؤدي إلى آثار سلبية على صحة الإنسان في المستقبل بسبب العمل لفترة طويلة في محطات الوقود.

1. Introduction

In contemporary culture, petroleum may be the most extensively used material. In addition to providing raw materials for making plastics and other goods, it also supports industry, heating, transportation, and energy. Petroleum, which is derived from the Latin words Petra and oleum, literally translates as "rock oil" and describes hydrocarbons that can be in the form of gases, liquids, semisolids, or solids that are found in sedimentary rocks [1]. According to science, petroleum is a carbon-based resource that is made up of a complex combination of hydrocarbon molecules that also contains trace amounts of compounds including metals, oxygen, nitrogen, and sulfur [2]. Paraffin, naphthenic, olefin, and aromatic hydrocarbons are all included in the complex combination of hydrocarbons [3]. Petrol contains approximately 54% paraffin and isoparaffins (alkanes from C4 to C12), 36% aromatics (primarily benzene, toluene, ethylbenzene, and xylene), 6% olefins (or alkenes), 5% naphthenic hydrocarbons (or saturated cyclic hydrocarbons), and 1% other compounds [4]. The most dangerous aromatic petrol components are BTEX (Benzene, Toluene, Ethylene, and Xylene) [5]. As a proxy for exposure to the total fuel, BTEX has been the accepted approach for evaluating the health effects of exposure to gasoline [4].

The International Agency for Research on Cancer (IARC) has categorized petrol as a potential carcinogen based on data from experimental research, human studies, and supporting evidence from the known carcinogenicity of certain of its constituents, such as benzene and probably 1, 3-butadiene [6, 7]. The workers may be exposed to relatively high levels of petrol vapour (benzene emission of 0.91mg/m^3) in petrol service stations, or to relatively low levels of petrol vapour in the general population (benzene emission of 0.06mg/m^3) [8]. Nephrotoxicity and hepatotoxicity are side effects of long-term exposure to benzene, and the severity of benzene poisoning depends on the dose, route, and duration of exposure as well as the age and health status of the exposed individual [9]. While brief exposure to gasoline has been associated with skin and sensory irritation, the central nervous system (CNS) can also be affected, which can result in symptoms like fatigue, headache, loss of coordination, respiratory system irritation (throat and nose irritation), and eye irritation [10].

The pollutants known as volatile organic compounds (VOCs), which are connected to gasoline vapour emissions and vehicle exhaust, are present in a variety of commercial, industrial, and household items [11]. However, direct health concerns to humans have been connected to several hazardous VOC species [12]. The VOCs can cause an oxidant-antioxidant imbalance by increasing intracellular reactive oxygen species (ROS) levels and decreasing antioxidant capacity which is known as oxidative stress (OS) [13]. When triggered, the various organic and inorganic organisms contained in gasoline, a highly volatile chemical found at gas stations, induce the body to produce ROS and deplete antioxidants. This results in genetic

modifications, damage to DNA, RNA, and proteins, as well as adjustments to crucial lipid activities, enzymes, and other proteins [14, 15].

In polyunsaturated fatty acids (PUFAs), lipid peroxidation happens when oxidants such free radicals or non-radical species remove one of the labile hydrogen atoms from the bis-allylic position methylene groups. This causes the generation of lipid peroxyl radicals and hydroperoxides [16]. It is used as a marker of OS in cells. Thus, increased lipid peroxidation as a result of a disruption in the oxidant-antioxidant balance [17]. Malondialdehyde (MDA) is a lipid peroxidation biomarker and one of the oxidative damage biomarkers in the human body [18]. It is toxic, causing OS to damage the cell membrane. Therefore, MDA should be reduced if it has reached its maximum level [19].

Peroxidases are a large group of enzymes (EC 1.11.1.X). Heme with a histidine residue as a proximal ligand is frequently used in the prosthetic group of peroxidases [20]. Peroxidases are divided into two categories: heme and non-heme peroxidases, which are distinguished by the absence or presence of heme [21]. Human peroxidases are also expressed in a number of organs, and the tissues in which they are expressed determine the functions they serve. This multigenic enzyme family has similarities in both structure and function, notably with regard to the catalytic domains. This illustrates how closely connected they are to one another in terms of evolution. Active site residues are conserved in all heme peroxidases [22, 23]. The end product of purine metabolism in humans is uric acid (UA). The enzyme xanthine oxidoreductase catalyses the final two reactions in the biochemical chain that leads to the formation of UA, namely the conversion of hypoxanthine to xanthine and xanthine to UA [24]. Internal sources (liver, muscle, intestine) account for about two-thirds of UA load, with dietary sources accounting for the remaining one-third. The kidneys excrete the majority of UA (65%–75%), with the gastrointestinal tract excreting the remaining (25–35%) [25]. The purpose of the current study was to ascertain the effects of petroleum exposure on several oxidative stress indicators, lipid peroxidation, and peroxidase enzyme activity among filling personnel at gasoline stations in Baghdad City.

2. Materials and Methods

2.1 Chemicals

All of the chemicals used in this study were of extremely high purity.

2.2 Subjects

One hundred and two male Iraqi filling station employees from various parts of the Baghdad governorate participated in the present study. The employees were chosen from the filling stations in Baghdad's Al-Kilani, Al-Mustansiriya, Al-Khalisa, Al-Muthanna, Palestine, Al-Idrisi, and Bab Al-Moadham. Depending on how long they were exposed to fuel, the subjects were split into three groups. As the control group, fifty (50) healthy volunteers in the same age range were chosen. The information about the groups was shown in Table 1.

Table 1: Details of individuals groups

Characteristics	Exposed groups			Non-exposed group
	Group 1 G1 (n=25)	**Group 2 G2 (n=50)**	**Group 3 G3 (n=27)**	**Control C (n=50)**
Age (Years)	25-50	30-55	37-60	24-60
Duration of exposure (Years)	1-10	11-19	≥ 20	-
No. of working hours/day	10	10	10	-

Alcoholism, drug misuse, diabetes mellitus, the use of antioxidant and vitamin supplements, being fat, arthritis, and liver conditions that may interfere with the study were the exclusion criteria. The management of the Iraqi gas stations and the College of Science at the University of Baghdad gave their clearance for this study to be carried out. All subjects gave their agreement after being informed that the samples will be utilized for study.

2.3 Body Mass Index (BMI) calculation

The BMI was calculated for all subjects by using the following equation based on weight and height [26].

$$\boldsymbol{BMI}\left(\frac{\boldsymbol{Kg}}{\boldsymbol{m^2}}\right) = \boldsymbol{Weight}\frac{\boldsymbol{Kg}}{\boldsymbol{Height(m^2)}} \qquad -----(1)$$

The BMI ≤18 was classified as underweight while the BMI ≥ 30 was classified as obesity according to the World Health Organization.

2.4 Blood samples

The venous blood around 5 ml was collected from all volunteers. Each blood sample was allowed to clot in room temperature and centrifuged at (1500 x g) for 10 min to collect serum. Then, the serum was stored at (-20 °C) until used for analysis.

2.5 Determination of oxidative stress status

The Erel assays were used to measure the total oxidant status (TOS) (μmol H_2O_2 Eq./L) [27] and the total antioxidant status (TAS) (μmol glutathione Eq. /L) [28]. While, the following formula was used to calculate the oxidative stress index (OSI) in serum samples [29]:

$$\boldsymbol{OSI} = \frac{\boldsymbol{TOS\ (\mu mol\ H_2O_2\ Eq./\ L)}}{\boldsymbol{TAS\ (\mu mol\ glutathione\ Eq./\ L)}} \qquad -----(2)$$

2.6 Determination of lipid peroxidation level in serum

The most widely used method for assessing lipid peroxidation is the measurement of malondialdehyde (MDA) via thiobarbituric acid (TBA) reactivity. A colored substance is formed when MDA, a lipid peroxidation end product, reacts with thiobarbituric [30]. The MDA was measured in (nmol/ml).

2.7 Determination of Uric acid level in serum

The uric acid (UA) level in serum was determined by using an enzymatic colorimetric method [31]. The level of UA in serum represented as (mg/dl).

2.8 Determination of serum total peroxidases activity

The colorimetric method was used to measure peroxidase activity [32]. The dissociation of H_2O_2 per time was used to determine peroxidase activity. The peroxidase activity was expressed as (U/L).

2.9 Statistical Analysis

The results were expressed in the form of mean value ± the standard deviation (mean ± SD). The data were compared using SPSS version 26 and Excel 2013 by One-Way ANOVA and Pearson correlation; where the difference is considered as highly significant when (p<0.01), significant when (p<0.05), and non-significant when (p>0.05).

3. Results

The mean ages of the exposed groups included in the current study were 42.20±8.99, 44.46±7.15 and 50.22±5.91 years for G1, G2 and G3 respectively; while 42.44±9.43 year for the control (C) group.

Table 2 displayed the average BMI for each group under study and indicated no noticeable variations between the exposed groups and the control group. The results demonstrate the presence of a highly significant increase in TOS in all exposed groups (G1, G2, and G3) when compared with the control. Additionally, a highly significant increase in this parameter is evident in G3 with significant increase in G2 when compared to C.

Table 2: The comparison of biochemical parameters in all studied groups

Dependent Variables	Groups			
	C	G1	G2	G3
BMI (Kg/m^2)	26.92±2.48	26.48±2.16	26.64±2.51	27.02±1.94
TOS (µmol/L)	10.85±2.04	43.03±16.90 a**	49.27±14.59 a**b*	53.30±16.64 a**b**
TAS x10^3 (µmol/L)	0.949±0.15	0.582±0.19 a**	0.563±0.23 a**	0.516±0.19 a**
OSI	0.012±0.003	0.089±0.065 a**	0.102±0.055 a**	0.118±0.054 a**b*
MDA (nmol/ml)	2.58±0.32	5.09±1.16 a**	5.23±1.25 a**	5.82±2.27 a**b*
UA (mg/dl)	5.36±1.18	5.74±1.34	5.59±1.37	5.69±1.24
Total peroxidase activity (U/L)	9.08±1.38	27.35±6.62 a**	27.73±7.99 a**	29.82±10.31 a**

a: significant difference between C and (G1,G2,G3)
b: significant difference between G1 and (G2,G3)
***p<0.05; **p<0.01**

The findings in Table 2 also demonstrate the presence of a highly significant decline (p<0.01) in TAS in the exposed groups when compared to the control group, although no significant difference in TAS is observed when this parameter is compared between exposed groups (G1, G2 & G3). The aforementioned findings also show a statistically significant rise in OSI in all exposed groups compared to the control group. Additionally, a significant shift in this parameter when its level is compared between the G1 and G3 groups is seen (p<0.05).
The results presented in Table 2 show that all exposed groups had significantly higher mean serum MDA concentrations than the control group. Comparing the MDA concentration in the G3 group to the G1 group, a substantial rise was seen. In all of the tested groups, the serum UA levels did not differ significantly (p>0.05).

It is obvious from these results there are a highly significant increase (p<0.01) in serum total peroxidase activity (U/L) in all exposed groups compared to that of control group, while no significant difference in its activity is found between exposed groups (G1, G2 & G3)

The correlation of all parameters in the exposed groups was done as illustrated in Tables 3, 4 and 5. The results in Table 3 indicated a highly significant positive correlation between (OSI and TOS) and (UA and TAS) as well as a highly significant negative correlation between OSI and TAS. While the results showed a significant negative correlation between Total peroxidase activity with TAS, as well as a significant positive correlation with OSI (in G1 group).

Table 3: Correlation analysis between studied parameters of exposed group (G1)

Parameters		BMI (Kg/m^2)	TOS (µmol/L)	TAS (mmol/L)	OSI	MDA (nmol/ml)	UA (mg/dl)	Total peroxidase (U/L)
BMI (Kg/m^2)	r	1						
	p							
TOS (µmol/L)	r	0.225	1					
	p	0.280						
TAS (µmol/L)	r	-0.106	-0.107	1				
	p	0.616	0.612					
OSI	r	0.300	0.637**	-0.753**	1			
	p	0.145	0.001	0.000				
MDA (nmol/ml)	r	0.094	-0.064	-0.029	-0.080	1		
	p	0.656	0.763	0.889	0.702			
UA (mg/dl)	r	-0.052	0.126	0.546**	-0.303	0.057	1	
	p	0.806	0.548	0.005	0.140	0.786		
Total peroxidase (U/L)	r	0.191	0.232	-0.474*	0.433*	-0.005	-0.124	1
	p	0.631	0.264	0.017	0.031	0.982	0.554	

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

In addition, the results in Table 4 showed a highly significant positive correlation between (OSI and TOS) as well as a highly significant negative correlation between OSI and TAS. While the results showed a highly significant positive correlation between Total peroxidase activity with OSI, as well as a significant positive correlation with TOS (in G2 group).

Table 4: Correlation analysis between studied parameters of exposed group (G2)

Parameters		BMI (Kg/m^2)	TOS (µmol/L)	TAS (mmol/L)	OSI	MDA (nmol/ml)	UA (mg/dl)	Total peroxidase (U/L)
BMI (Kg/m^2)	r	1						
	p							
TOS (µmol/L)	r	0.261	1					
	p	0.067						
TAS (µmol/L)	r	-0.045	-0.022	1				
	p	0.754	0.880					
OSI	r	0.181	0.665**	-0.632**	1			
	p	0.209	0.000	0.000				
MDA (nmol/ml)	r	-0.222	0.222	-0.105	0.179	1		
	p	0.120	0.120	0.467	0.214			
UA (mg/dl)	r	0.104	-0.058	-0.077	-0.052	-0.206	1	
	p	0.474	0.689	0.597	0.722	0.152		
Total peroxidase (U/L)	r	0.127	0.314*	-0.204	0.369**	-0.213	-0.067	1
	p	0.379	0.026	0.156	0.008	0.137	0.644	

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

Meanwhile, Table 5 presented a highly significant positive correlation between (OSI and TOS) as well as a highly significant negative correlation between OSI and TAS (in G3 group).

Table 5: Correlation analysis between studied parameters of exposed group (G3)

Parameters		BMI (Kg/m^2)	TOS (µmol/L)	TAS (mmol/L)	OSI	MDA (nmol/ml)	UA (mg/dl)	Total peroxidase (U/L)
BMI (Kg/m^2)	r	1						
	p							
TOS (µmol/L)	r	-0.100	1					
	p	0.618						
TAS (µmol/L)	r	0.186	-0.008	1				
	p	0.354	0.968					
OSI	r	-0.210	0.560**	-0.766**	1			
	p	0.292	0.002	0.000				
MDA (nmol/ml)	r	0.058	0.047	-0.051	0.271	1		
	p	0.775	0.817	0.800	0.172			
UA (mg/dl)	r	-0.146	-0.103	-0.088	-0.041	-0.260	1	
	p	0.467	0.608	0.662	0.840	0.191		
Total peroxidase (U/L)	r	-0.185	0.267	-0.221	0.336	0.022	0.165	1
	p	0.356	0.178	0.267	0.087	0.913	0.411	

4. Discussion

Antioxidants naturally control the production of free radicals. Antioxidants have the ability to stabilize or inactivate free radicals before they cause harm to the various cell components [33]. OS is a condition characterized by an imbalance between the production of too many oxidants and the defence mechanism of having too few antioxidants [34]. The intracellular antioxidant defense network can be overpowered by significant ROS generation, which can activate neutrophils, cause lipid peroxidation, modify proteins, and damage DNA [35]. The end products of the lipid peroxidation process, MDA or TOS, can be utilized to measure the amounts of lipid peroxidation [36]. TAS is composed of antioxidant properties such as total protein (albumin accounts for 85 %), bilirubin, ascorbic acid, uric acid, carotenoids, and tocopherol, as well as enzymes such as glutathione peroxidase, catalase, and superoxide dismutase [36, 37]. To assess the state of OS, it is generally necessary to estimate antioxidant parameters and oxidant markers such as TAS, TOS, and OSI, as well as MDA, which should be done in both normal and pathological conditions [38]. The TOS can be defined as an *in vivo* marker of an oxidative/antioxidative ratio shift favouring the oxidative side [39]. While TAS is a biomarker for evaluating the antioxidant capacity of body fluids and is defined as the number of oxidants that one litter of solution neutralizes in terms of moles [40].

Malondialdehyde (MDA) is a helpful biomarker of lipid peroxidation and OS. As a result, monitoring MDA levels in various biological systems can be used as a crucial indicator of lipid peroxidation both *in vitro* and *in vivo* for a variety of chronic diseases in humans [41]. Additionally, since MDA is produced by the oxidative breakdown of lipids in cell membranes, changes in MDA concentration may be a sign of lipid peroxidation and oxidative cell damage [42-44].

In this study, the OS parameters in a group of fuel station workers in Baghdad city divided into three groups based on the duration of petrol exposure and compared them to the healthy control group (C) was measured. The results indicated a highly significant lower level of TAS in all exposed groups (G1, G2 & G3) compared to group C, along with highly significant higher levels of TOS, OSI, MDA, and total peroxidase. As well as, no differences observed in UA level in all studied groups. The current result is in line with other research [15, 45] that indicated benzene exposure was associated with an increase in the levels of MDA production in general. When compared to the control group, fuel station employees had significantly higher MDA levels according to different research by Moro *et al*. [46]. The rise in MDA level was associated with an increase in ROS generation and a decrease in TAS, according to other research [47, 48]. Additionally, other study has revealed that the gasoline-exposed workers had noticeably higher levels of MDA than the controls [49]. It could be non-statistical differences in MDA between workers in fuel stations and healthy subjects under the study of Adeniji *et al*. [50], which does not consistent with our findings. A study by Toson *et al.* which was proved that there are no statistically significant differences in TAS between gas station workers and healthy people that do not agree with our study [51].

It should be noted that the results of this study showed no significant differences in serum UA levels ($p>0.05$) in all studied groups which is in the line with study by Abou-ElWafa *et al*. [52], while not in agreement with the other studies by Al-Helaly *et al*. [53] and Mohammed [54].

The study carried out by Malini *et al*. confirmed that there are no statistically significant differences in BMI between gas station workers and healthy people, which agree with our study, while also in the same study proved that there are no statistically significant differences in MDA and TAS, which do not agree with our study [55]. Moreover, the results of the current study showed that there was a higher increase level of oxidant and OSI in the fuel station workers groups than the control group, which may be caused by the continuous production of ROS and lipid peroxidation with continuous exposure, which was agrees with the study of Soytürk *et al*. [56].

However, to our knowledge, there are no studies in Iraq with few worldwide found in the literature dealing with TOS, TAS and OSI on workers in fuel stations. Meantime, there are several studies that spotlight total peroxidase activity by studying antioxidants and oxidants parameters in a single form as the activity of SOD, CAT, and GPx. The present study is one of a few that have recently been conducted in assessment of some OS parameters amongst fuel stations attendants at Baghdad City to examine any potential environmental and long-term work-related effects on workers' health.

It's interesting to note that antioxidants defend the body from free radicals. The serum contains various antioxidants, including glutathione, UA, and numerous enzymes, such as: catalase, superoxide dismutase, peroxidase, and glutathione peroxidase [57]. Instead of examining individual antioxidants separately, serum was measured for (TAS), which represents the sum of the effects of all antioxidants present in a body fluid and was used to assess the antioxidant status in the various study groups. Because this test measures the antioxidant capacity of all the substances present in a biological specimen rather than just one particular compound, it has the major advantage of being more accurate [58]. The most accurate way to determine the degree of OS is through the OSI [59]. Therefore, increased levels of TOS, OSI and decreased levels of TAS as well as increased the total peroxidase activity was indicated in petrol station workers in Baghdad city, which strongly indicates the imbalance between

oxidants and antioxidants leading to OS. Thus, increased OS and lipid oxidation may play a potent role in pathogenesis of several diseases.

5. Conclusion

The changes in the OS parameters point to an increase in OS in the sera of Iraqi fuelling workers as a result of ongoing exposure to the vapours of petroleum products, which is the cause of the markedly harmful increase in MDA level and supports the idea that OS persistence plays a significant role in the progression of health issues.

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