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RESEARCH ARTICLE

Study of Heavy Metals and their effects on Oxidant / Antioxidant Status in Workers of fuel Station in Hilla city-Iraq

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ABSTRACT:
Heavy metals are chemical elements that have a specific gravity at least five times that of water . Some heavy metals, such as lead, mercury and cadmium have hazardous effects on human health. These metals become toxic when an increase from the normal level allowed. Many workers are exposed to heavy metals in the fuel stations. The present study was aimed to evaluate the effects of some heavy metals (pb & Cd) on oxidant (MDA)and antioxidants(TAC, SOD, Zn, Cu, and Mg , Vit. C and Vit .K) in workers of fuel station compared with healthy control group in Hilla city- Iraq. The study was conducted on different area of Hilla city fuel stations . The study included 60 workers who have been working for at least 6 months, all of them were males . Sixty healthy volunteers serve as healthy control group, also all of them were males. Aged between (18) and (50) years for workers and control group. The results revealed that Pb and Cd and MDA levels significantly higher in the blood of workers than healthy controls ($p < 0.01$) respectively. This study also found a significant decrease in the levels of TAC, SOD, Zn, Cu, and Mg ,Vit. C and Vit .K in the blood of workers than healthy controls at ($p < 0.05$) respectively. The present study suggests the exposure to heavy metal pollution in the work place (fuel stations) led to increase in the oxidative stress in workers which decreased the antioxidant levels.

KEYWORDS: fuel station workers, Heavy metals, Trace elements, Antioxidants, Oxidative stress.

1. INTRODUCTION:
Many workers are exposed to heavy metal pollution at fuel stations. Heavy metals such as lead, mercury and cadmium have serious effects on human health as a result of their diffusion into the environment and their ability to accumulate inside the human body. Heavy metals are chemical elements that have a specific gravity at least five times that of water (1). These metals become poisonous and harmful when they are higher than normal levels. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic (2).

These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO (3). The toxicity of heavy metals has been well documented and has been recognized as major environmental health risks worldwide. The heavy metals affect humans, animals and all ages, but in young children lead traces are the most dangerous. Their poisoning results from the interaction of the metal with biological electron-donor groups and with essential cations, particularly calcium, iron, and zinc (1) or with anions (i.e., carbonate, hydroxide and oxalate) negatively charged moieties on macromolecules such as proteins (4,5). It can absorb up to 50% of the heavy inorganic minerals inhaled in the lungs. (6).

Heavy metals linked to red blood cells, and are eliminated mainly slowly by the urine but some of them like lead accumulated in the skeleton, and are released only slowly from this body compartment.(7).When not digest the heavy metals accumulated in the human body become very toxic and cause many problems to human health, including damage to nerves, blood composition and many organs such as liver, lungs and kidneys (8).Gasoline is a highly volatile substance found in fuel stations, with many organic and inorganic organisms, which when activated, leads to the production of reactive oxygen species (ROS) and the consumption of antioxidants in the body. This leads to damage to DNA, RNA, and proteins and this leads to genetic modification and change in important lipid functions, enzymes and other proteins(9,10). To prevent this injury, the body has a powerful mechanism using substances known as antioxidants. Antioxidants can be either an enzyme such as Superoxide Dismutases (SOD), Catalases (CAT), Glutathione Peroxidases (GPx), Glutatione Reductases (GRx) and Glutathione Transferases (GST)or a vitamin like beta-carotene, vitamin E (vit. E) and vitamin C (vit.C). These enzymes utilizeantio xidant trace metals (selenium, manganese, zinc and copper) as cofactors to help protect against free radical-induced cell damage (11) .As a general rule, severe poisoning of heavy metals is likely to result from inhalation or contact with the skin from dust, vapors or substances in the workplace. The enhanced generation of ROS dominates the intrinsic antioxidant defenses resulting in the cells' status known as oxidative stress.

Oxidative stress is defined as an impaired balance between free radicals production and antioxidant capacity and this leads to an increase in oxidative products(12). Malondialdehyde (MDA), which is the final product of the oxidation of polyunsaturated fatty acids, is used as an indicator to estimate oxidative stress(13). Damage to cells is produced from free radicals and is believed to play a major role in the aging process and in the development of the disease.(14). Antioxidants are the first line of defense against damage from free radicals and maintain optimal health and well-being.

The need for antioxidants is becoming more important with increased exposure to free radicals. Total Antioxidant capacity(TAC) is a dynamic balance that is influenced by interactions between each antioxidant component of the serum. The cooperation of antioxidants in the human serum is believed to provide greater protection against free radical attacks than any antioxidant alone .

The purpose of this study is to study the effects of heavy metals(Pb, and Cd)and oxidants(MDA) on antioxidants trace metals (Zn, Cu, and Mg) and Vit. C, Vit. K, TAC and SOD in workers of fuel station and control group in Hilla city.

MATERIALAND METHOD:

The study was conducted on different area of Hilla city fuelstations. The study included 60 workers in fuel station who have been working for at least 6 months, all of them were males . Sixty healthy volunteers serve as healthy control group, all of them were males. Aged between (19) and (50) years for workers and control group. All workers and control groups were non-smokers and free of deseases. Blood samples were taken from the capital vein between 6.00 and 8.00 hours with fasting. About 10ml of the blood was drawn from vein of each fasting worker in fuel station and control subjects. Five milliliters were added into EDTA containing polypropylene tubes and shacked gently to be used for measurement the concentration Pb and Cd. The rest5ml of whole blood samples were allowed to clot, and then centrifuged in (402 Xg for 10 min.) to be used serum samples immediately for measuring. The obtained sera immediately use in determining Mg, Zn, Cu and estimate other parameters included Vitamin E ,Vitamin C, Total Antioxidant capacity(TAC), SOD and Malondialdehyde (MDA).Sera were removed, frozen at -20 C° until analysis .

The concentrations of Mg, Zn and pb samples were measured by flame atomic absorption spectrometry (AAS) (GBC 933 Plus)(15)while the concentrations of Cu and Cd were measured by flameless atomic absorption spectrometry (1TAA500-PG)(16). MDA,TAC and SOD were measured according to the methods of Burtis and Ashwood (11) Koracevic et al., (17) and Winterboun et al (18) respectively. Vitamin E was measured according to Emmerie-Engel reaction(19) and Vitamin C was mesured according to 2,4-dinitrophenylhydrazinederivatization method(20).

Biostatistical analysis:

Statistical data analysis was done using Statistical Package for Social Sciences (SPSS version 15). All data were presented as mean ± Standard Deviation (SD) and the comparison was made with respective control groups. The level of significance was fixed at level $P < 0.05$ and as a highly significant at $p < 0.01$.

RESULT AND DISCUSSION:

The results in the Table (1) showed the average of age and the duration of exposure among the exposed workers in fuel stations.

Table 1. Average of age and duration of exposure amongthe workers in fuel stations.

	M±SD
Average of age(years)	30.3±10.31
Duration of exposure (years)	6.4±2.4
Average work hours per day	7.5±0.61

Mean ± standard deviation (M ± SD)

The results in table (2) showed the biochemical parameters in fuel station workers and control group. The present study shows the comparison of various trace elements and heavy metals of blood in workers with healthy control group. A significant decrease in Zn, Cu and Mg with ($p < 0.05$) was recorded in fuel station workers, whereas heavy metals (Pb, and Cd) were found to be elevated ($p < 0.01$).

The results also showed that the level of MDA in serum was increased in workers compared to healthy controls ($p<0.01$) and the results found there is a significant decrease of TAC, SOD, vit. E and vit. C with the workers of fuel station when compared with the control group at ($P<0.05$).

Table 2: The biochemical parameters of petrol station workers and control group.

parameters	Workers group M±SD	control group M±SD
Zn (µg/ml)	0.532*±0.0301	0.882±0.042
Mg (µg/ml)	12.04*±0.6401	15.6±1.02
Cu (µg/ml)	0.261*±0.022	0.733±0.04
Pb (µg/ml)	0.69**±0.018	0.267±0.022
Cd (µg/ml)	0.035**±0.0023	0.017±0.002
MDA (µmol/L)	5.45**±0.18	2.251±0.578
TAC (mmol/L)	1.217*±0.042	2.541±0.031
SOD (U/ml)	1.161*±0.025	1.83±0.191
Vit.E(mg/dl)	0.72*±0.15	1.1± 0.01
Vit.C(mg/dl)	0.268*± 0.01	0.86± 0.16

N= 40, Mean ± standard deviation(M ± SD), * p<0.05; ** p<0.01.

Trace elements play a vital and important role in the human body to perform its functions in a neutral and true manner . In this study there was found a significantly lower level of antioxidant trace metals in the fuel workers station when compared with the control groups . This may be because the trace elements (Zn, Mg and copper) were make as cofactors of the antioxidant enzymes and they are continuously utilized to produce the antioxidant enzymes that participate in the detoxification of the ROS (21). The Zn, Mg and Cu are as components of SOD and are Linked with antioxidant functions, therefore, that their deficiency maylead to impaired free-radical scavenging mechanisms thereby increasing oxidative stress in the workers group .As well as low levels of Cu concentration in the serum of the fuel workers station may be due to hydroquinone interaction (the one metabolites of benzene) , with copper and zinc components of the SOD enzyme and release Cu of the enzyme. Then the reaction between the released Cu and H_2O_2 generates ROS and initiate lipid oxidation chain reactions and Cu deficiency leads to increase in the peroxide processes.

There are significant differences between the levels of heavy metals(Pb and Cd) in the serum of fuel station workers and control group. This elevation was higher in the serum of the workers with exposure period . Fuel stations are classified as a very dangerous source of pollution for human population, due the toxicity of emissions from evaporated vehicle fuels. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Their toxicity can result in damage central nervous, blood composition and many organs (5,8). Heavy metal poisoning can be caused by inhalation or skin contact with dust, vapors or materials in the workplace. The increased level of heavy metals causes oxidative damage by the increase production of free radicals ROS, reducing the antioxidant defense system of cells via inhibiting sulfhydryl dependent enzymes or antioxidant enzyme activities or increasing lipid peroxidation and increased *MDA*as the final products and reducing of antioxidant enzyme (*SOD*) in fuel stations workers compared to healthy controls.

Increased accumulation of these elements (Pb and Cd) within the body leads to increased ROS. This study showed there were high-significant increase in Pb and Cd at ($p<0.01$) and these results may be due to non-use of facial masks during work that increases the level of heavy metals in blood due to inhalation of lead and Cd present in air and neglect use protective clothing and body wash after work , also this increase may be due to dermal absorption.

The present study is one of few studies carried out recently in Hillato determine heavy metals in blood for the workers in fuel station to see possible effect of environmental and long term working on the worker health. A number of heavy metals, as well as dust, fumes and gases, are found in these working environments. The exposure may have both acute and long term health effect. Many studies suggested that blood concentrations of some heavy metals was a useful indicator of recent exposure(9,10) **.** Lead and its compounds are potentially toxic; the toxicity of lead can cause aberrant function to multiple human organs. It inhibits many enzymes, including pyruvate dehydrogenase, and enzymes of the heam synthetic pathway. The data from the present study show significant increase in the levels of lead in the blood of fuel station workers when compared to healthy men with P value ≤ 0.01. These findings are consistent with that previously reported by Al-Rudainy 2010(22) from Basrah city in Iraq, AlShamri et al 2010 (23) from Najaf city in Iraq too, Bahrami et al 2002 (24) from Hamadan City of Iran, and Freije and colleagues 2009 (25) from Bahrain Kingdom concluded significant elevationof blood lead in the benzene stations worker. Also these findings are agreements with study that reported byAdnan J.M. AL-Fartosy and et al. 2014(26) in increased of (Pb and Cd) , while in disagreement with that reported by Yakub et al. 2009 (27) from Karachi city of Pakistan and Schafer el al. 2005 (28) from USA in petrol exposed workers.

MDA is one of the end products of the lipid peroxidation produced by ROS formation and it is considered a key marker of oxidative stress (29). This study showed high statistical significant difference of MDA at ($P<0.01$) and significant decrease of TAC, SOD, vit. E and vit. C with the workers of fuel station when compared with the control group at ($P<0.05$).

This is in agreement with other studies which illustrated that Benzene exposure has been associated with increases in the overall formation of MDA (30,31), also this study is consistent with other studies which found a significant increase in the level of MDA in petrol station workers compared to their control group (32).The findings in our study showed high significant levels (MDA) while the level of TAC significantly decreased in the fuel station workers. This increase in MDA level with the increase in the heavy metals were cause increase generation of ROS and decrease TAC , therefore the enzymatic and non-enzymatic antioxidants levels decreased to reduce the oxidative stress that might be produced from the ROS. This is in agreement with other studies (33,34,35, 36).SOD requires copper and zinc for its activity. Both the metal ions are replaced by lead, which decreases the activity of SOD and this explains a decrease in SOD with Zn and Cu and increase of Pb.

The decrease of antioxidant refers to its utilization which prevents the cellular damage from free radicals, therefore a decrease in vitamin E and vitamin C(as antioxidants)were observed. There is significantly lower level of antioxidant vitamins in the fuel station workers than the control groups. Since antioxidant vitamins (C and E) play a major role in protecting individuals from oxidative stress by neutralizing free radicals through donating one of their own electrons (37).The reduced levels of antioxidants in worker subjects may indicate increased consumption as a result of increased free radicals. Vitamin E is a powerful antioxidant chain breaker, essentially works to inhibit lipid peroxidation. Significant decrease of vitamin E observed in fuel filling workers as it is utilized in preventing lipid peroxidation. This observation is similar to the findings of Basso et al(38) who reported lower concentrations of vitamin E among gasoline station workers than control subjects. Other studies have also shown significantly elevated levels of ROS, and significantly decreased antioxidant enzymes in the gasoline exposed workers compared with the controls.(39)

Vitamin C is a water soluble free radical scavenger, can directly scavenge hydroxyl radicals and super oxide and helps to neutralize the oxidative stress burden created by both exogenous and endogenous sources.(40)Decrease levels of vitamin C and increase in levels of MDA may be due to the depletion of vitamin C when the oxidant burden is increased during exposure to heavy metals. This results is similar to the findings of Luay A. Al-Helaly who reported lower concentrations of vitamin E and C among petroleum station workers than control subjects(41).

CONCLUSION:

From the results of this study, we concluded that occupational exposure to heavy metals (Pb and Cd) levels among many fuel stations workers in Hilla cityinduce oxidative stress through excessive produced of ROS and MDA compared to control group. This is due to decrease in the antioxidant Levels of TAC, SOD , vitamin E , C and trace elements (Cu , Zn and Mg).This behaviors might suggest that workers subject to fuel stations must supply with antioxidants and vitamins dose especially with increasing period of exposure for its importance in strengthening the immune system and must supply with protective clothing such as face mask and gloves and other protective clothing.

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