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Biochemical and Haematological Parameters Among Gas Station Employees

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Abstract
Human exposure to heavy metals has significant harmful health effects and might be associated with the risk of blood abnormalities and biological parameters. The wasteful in the use of these metals was reached a high level of risk, leaving a huge burden on the environment. Therefore, the current study was designed to investigate any changes in the hematological and biochemical profile with the blood lead level among employees occupationally exposed to gasoline in different gas stations in Riyadh City. The exposed group included 35 gas station employees. They were compared to a corresponding group of healthy 35 male at the Department of Forensic Science, College of Criminal Justice, Naif Arab University for Security Sciences. The number of white blood cells, platelets, lymph, mean corpuscular volume, liver enzymes, Alanine transaminase, Aspartate Aminotransferase, and creatinine mean corpuscular hemoglobin were significantly higher with relatively small decreased in red blood cells, Haemoglobin, Mean corpuscular hemoglobin concentration and Hematocrit with increasing in the level of lead in gas stations employees compared to the control group.
Keywords: Lead, CBC, ALT, AST, creatinine.
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1. Introduction

Gasoline (petrol) consists mostly of organic compounds obtained by the fractional distillation of petroleum enhanced with different additives, whilst diesel is a specific fractional distillate of petroleum fuel oil which is a mixture of hydrocarbons (Donaldson *et al*., 2005). At the gas stations, workers have many hazards for their life because of their daily exposure to petrol, diesel and the inhalation of the fuel vapor for a long time, this lead too many respiratory system problems. It was reported that exposure to petroleum products and their vapors have been a highly toxic effect. The danger lies in the presence of some chemicals as benzene, lead (Pb), and oxygenates which affect different body systems and the human respiratory system. The major exposure pathways of Pb through the inhalation and the ingestion of dust and vapors through Pb-bearing. After absorption, Pb transferred to the blood, then accumulate in soft tissue, kidneys, bone marrow, liver, and brain, and deposited in bones (Herman *et al*., 2006).

The inhalation symptoms of gasoline include effects on the central nervous system, nose and throat irritation, hematological, headaches, hepatic, dizziness, renal, nausea, vomiting, confusion, lung functions, and breathing difficulties. The main effect of long exposure to benzene is on the blood, causing anemia, bleeding and increase the chance of infection, while The Department of Health and Human Services (DHHS) demonstrated that benzene has a role in causing human leukemia (Patel *et al*., 2004). Petroleum products and Pb content in the fuel having a negative impact on the organs of the body, there will be an evident impact on hematological parameters which we will investigate (Christian *et al*., 2016). New research has confirmed that the level of Pb below 50 µg/dl in the blood lead level (BLL) has toxic effects (Tak *et al*., 2008). The World Health Organization (WHO) Allowed 40 µg/dl as the highest BLL for adults (World Health Organization 1995), while the Centers for Disease Control (CDC) decreased the limit for BLL to 35 mcg/d-l, as the highest of BLL for adults (CDC, 2008).

Different researchers showed that exposure to gasoline fume causes a reduction in hemoglobin (Hb) level, hematocrit (Ht), and leukocytes in fuel attendants, which worsens with prolonged exposure (Okoro *et al*., 2006). Other researchers studied the hematological parameters in the workers who had been working in the gas stations for more than five years, Peripheral smear revealed basophilic punctuated erythrocyte and reticulocytosis. Hematological disturbance included mild white blood cells (WPCs) (7 of 37), lymphocytosis (an increase in the number of lymphocytes in the blood) (20 of 37), mild lymphopenia (3 of 37) and the decrease of erythrocytes count (11 of 37). These results significantly differed from those of controls (Pranjić *et al*., 2003).

Some researchers reported that there was a linear increase in red blood cells (RBCs) count and (WPCs) count with increasing BLL (Ovuru and Ekweozor 2004). Lately, some researchers observed when studying the chronic inhalation of petroleum products on hematological parameters a decrease in Hb level, erythrocytes, and total leucocyte counts and they proved that the chronic exposure to gasoline vapors has reverse effects on the human hematopoietic system (Aleemuddin et al., 2015). The effect of Pb on some liver enzymes has been studied by Kapaki, who proved that the level of Aspartate Aminotransferase (AST) and Alanine transaminase (ALT) were high in the gasoline stations employees, significantly lower activities were observed for ALP in occupationally exposed subjects were higher compared with controls.

The activities of ALT and AST in occupationally exposed subjects were higher compared with controls (Kales *et al*., 2001). Therefore, the current study was designed to investigate any changes in the hematological and biochemical profile in correlation to the BLL among employees occupationally exposed to gasoline.

2. Material and Methods

A comparative study was carried out on gas station employees at Riyadh City during the period from March 1, 2019, to June 31, 2019.

2.1 Subjects

The study included 35 out of 42 gas station employees; their age ranged between (20 and above 50 years) working in the gas stations between 20 and 30 years (57.14 %) and between 6 and 10 years (48.57%) , with a response rate of 93.33 %. Recruited from five gas stations located in Riyadh City, Saudi Arabia, were used as locations for this study and they represent the test subjects, all employees are males working in fueling service, with the average daily working hours ranged from 8 to 10 hours/day with an estimated average of 63 hours/week (**Table 1**) (**Figure 1**), who agreed to participate in the study. While 35 employees from Naïf Arab University for Security Science, were recruited as a control group. The questionnaires were distributed and accurately filled by the participants with age, duration of work, safety measures. The gas station employees were met, and blood samples were taken at the managerial office at each station at 8 a.m.

Table 1. Mean values of duration working hours with age

Age year	Frequency	Percent
20-30	20	57.14
31-50	13	37.15
above 50	2	5.71
Total	35	100.0
Duration working years	**Frequency**	**Percent**
(6 month- 5 years)	9	25.72
(6 years-10 years)	17	48.57
(above 10)	9	25.71
Total	35	100.0

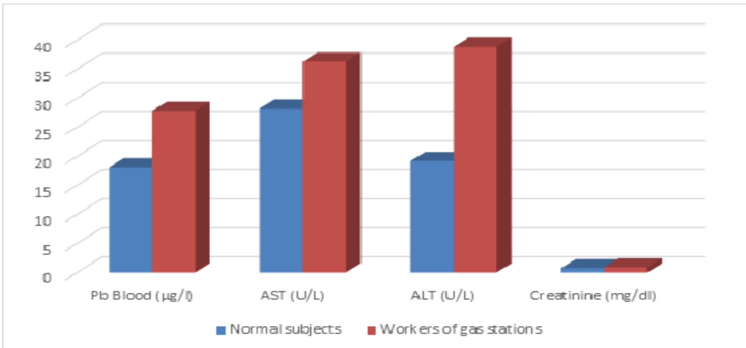

Figure 1. Duration working years with age.

2.2 Ethical Consideration

Endorsements of the gas stations, College of Criminal Justice, and the research ethics council were gotten. Educated verbal assent regarding study subjects to participate voluntarily in the study with a full right to withdraw, as they were eager to make a health check for free, was gotten with the confirmation of secrecy and namelessness of the information.

2.3 Samples

Nine ml of venous blood samples were from a peripheral vein on the arm of each subject. The blood divided into three portions, each portion has 3ml blood. The first portion collected in tubes with (anticoagulant EDTA) for complete blood picture, the second and third portions collected in a dry plastic tube for liver and kidney function tests (including urea, uric acid, and creatinine), liver function tests including (ALT, AST and creatinine activities) and lead analysis. The specimens were allowed to coagulate normally to isolate the serum for samples and were stored at room temperature until transporting to the laboratory for analysis. In the lab, each sample was centrifuged and put away in the freezer at −70◦C until being prepared.

2.4 Instrumentation

The determination of Pb in blood was carried out using an ICP-OES (model: 5110 VDV, Agilent Technologies, and the USA), while all hematological parameters were measured using a fully automated Hematology analyzer Sysmax K-4500. Liver and kidney activities were determined using a UV test technique. ETHOS One™ Microwave Digestion System (Milestone Inc., USA) for blood digestion to determine Pb.

2.5 Hematological tests

Hematological tests were performed using cell counter Sysmex KX21 to measure (Hb), (RBC) and (WBC) count. The values were then used for calculating Mean Corpuscular Volume (MCV) Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

2.6 Biochemical analysis

(ALT), (AST) and creatinine were analyzed by using KENZA 240 TX/ISE chemical auto analyzer, BioLabo, France.

2.7 Methods

2.7.1 Microwave digestion

For the determination of lead in blood samples, the following was added in sequence in digestion vessels of the microwave oven; 1 ml blood and 6 ml distilled water. The contents were mixed by the vortex for 2 minutes before adding 5 ml HNO_3 (69%) and 2 ml H_2O_2 (35%), and digested according to the procedure described by Piccinini (Piccinini *et al*., 2013). The blank followed the same extraction method. After preparing the samples, vessels were closed firmly and placed inside the microwave digestion oven. The ETHOS equipped with a temperature control was used for the sample digestion. The temperature program composed of 60 min at 200°C (power 1500 W). The resulting solutions were cooled, filled up to 25 ml with distilled water, filtered and analyzed by ICP-OES, where the samples were introduced in triplicates with the coupled Agilent SPS4 injector with a pump speed of 12 rpm. The instrument was operated under suitable conditions including choosing the suitable wavelength for Pb (220.353 nm) with calibration correlation coefficient limit of 0.998, plasma argon flow rate of 12 l/min, the auxiliary argon flow rate of 1 l/min, nebulizer argon flow rate of 0.7 l/min.

2.7.2 Hematological tests

The method was studied in smears prepared according to the procedure described by Sharma *et al*. (Sharma *et al*., 2012).

2.7.3 Biochemical analysis

Components such as creatinine, (AST) and (ALT) were determined according to Holding and Collee (Holding *et al*., 1971).

2.8 Statistical analysis

The statistical analysis used was an independent-test to compare means by SPSS Version 20.0 statistical software (SPSS Inc., Chicago, IL). The P-values of less than 0.05 were considered significant.

3. Results

The (**Table 2**) summarizes the t-test of blood lead and biochemical parameters (AST, ALT, and creatinine) between employees of gas stations and the control group, while the (**Table 3**) demonstrates the t-test of hematological parameters between gas stations employees and control group. The duration working with the age of the employees illustrated in the (**Figure 1**), while the values of BLL and biochemical parameters in the employees of gas stations with the control group displayed in the (**Figure 2**).

Table 2. T-test for BLL and biochemical parameters of both studies groups

Parameters	Control (N = 35)	Employees of gas stations (N = 35)	Levene's Test for Equality of Variances		t-test for Equality of means	
			F	Sig.	t	Sig. (2-tailed)
Pb Blood (µg/l)	18.15 ±2.75	27.86 ±7.59*	32.97	0.000	7.11	0.000
AST (U/L)	28.26±6.67	36.34±7.57*	0.698	0.410	4.74	0.000
ALT (U/L)	19.23±4.32	38.86±7.96*	10.81	0.002	12.83	0.000
Creatinine (mg/dl)	0.78±0.27	0.92±0.29*	0.006	0.936	2.196	0.031

* P < 0.05

Table 3. T-test for of haematological parameters of both studies groups Independent samples test

Parameters	Control (N = 35)	Employees of gas stations (N = 35)	Levene's Test for Equality of Variances		t-test for Equality of means	
			F	Sig.	t	Sig. (2-tailed)
WBCs (4-10 x10^3) u/L	8.58±1.92	8.63±2.28	1.21	0.275	0.132	0.895
RBCs (4 – 5.5 x10^6) u/L	5.30±0.19	4.50±0.26*	3.80	0.054	13.70	0.000
Hb (12-16) g/dl	15.15±1.17	12.45±1.13*	0.07	0.800	9.76	0.000
Lymph 20-40%	34.72±4.56	38.35±6.61*	2.25	0.138	2.68	0.009
Platelets uL x10^3 (100-300)	246.80±5.69	302.09±12.42*	23.97	0.000	23.94	0.000
MCHC g/dL (32-36)	35.47±0.68	35.33±0.83	0.17	0.682	0.744	0.459
MCH Pg (27-34)	27.47±2.36	23.99±2.60*	4.16	0.045	5.8	0.000
Mcv fL (80-100)	83.82±4.63	85.57±1.9*	5.5	0.022	2.07	0.004
Hematocrit % (40-54)	42.19±7.5	44.10±6.08	0.51	0.477	1.17	0.247

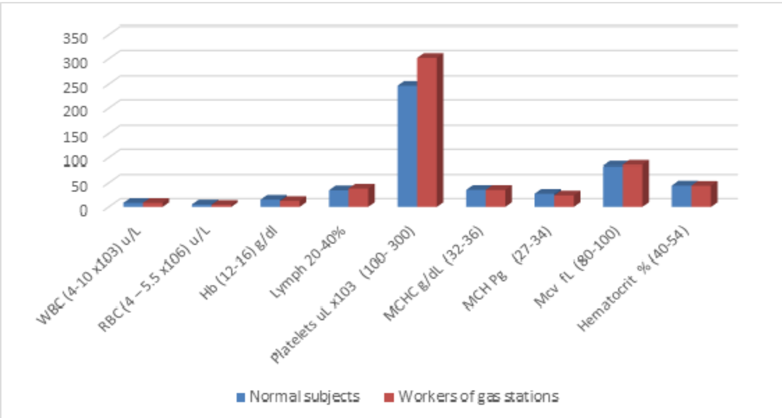

Figure 2. Values of BLL and biochemical parameters of both studies groups

Discussion

A relation between exposure to mixtures containing gasoline and certain types of blood disorders have been shown in many countries through epidemiological researches (Lan *et al.*, 2005). Employees who worked in gasoline stations are regularly exposed to many harmful toxic heavy metals as Pb which causes abnormal changes in the functioning of many vital organs, and they are associated with increased risk of heme-biosynthesis related parameters (Ali *et al.*, 2013). Unfortunately, the majority of the employees are neglecting or lacking protective safety measures such as; face masks, protective cloths, gloves and washing after finishing their working shift. In turn, this carelessness makes them more susceptible to those toxic fumes.

In the United States, Pb has been completely eliminated from all engine fuel, since 1990, and most Western European countries have reduced the Pb content in gasoline to (0.4g/l). Since 1986, further reduction of Pb has been planned to (0.15 g/l), as in Japan 96% of the gasoline is unleaded. Riyadh refinery in Saudi Arabia produces about (30%) of the total domestic gasoline and the average Pb level in the manufacture gasoline is approaching (0.30 g/l). The lead content of benzene in Riyadh (0.84 g/l) is higher than the international level and is now gradually reduced to (0.42 g/l) which is still higher than the international level (Ibrahim *et al.*, 2012). It was reported that Pb toxicity probably leads to malaise, anorexia, vomiting, abdominal pain, constipation, colic, lethargy, apathy, irritability, weakness, generalized fatigue and the next were prickle and anesthesia of leg or arm, feeling agitated at the slightest disorder, and weakness of wrist or ankle joint are the most symptoms which were found among Pb gas stations employees (Gordon *et al.*, 2002). The symptoms of depression and decreased and motor abilities were identified in employees of the Croatian gas station who had exposed to leaded gasoline for more than five years (Pranjić *et al.*, 2003).

Several studies have reported that Pb exposure has toxic effects on humans, and most of these studies have observed a link between signs and symptoms of the disease and levels of Pb in the blood, in addition, the concentration of Pb in the blood has an important relationship with pellets white and red blood, where high BLL causes cracking of (RBCs) (Toplan *et al.*, 2004).

The BLL is generally vastly used for the measure of Pb exposure and consider the best biomarker for determination and distinguishing Pb contamination, and generally, reflects acute (current) exposure (Sommar *et al*., 2014). In this study, the mean BLL of gas stations employees (27.86 ± 7.59 µg/l) was significantly higher than that of the control group (18.15 ± 2.75.75), (t-test=7.11), (P-value=0.00) (table 2). The mean duration exposure was (7.58 ± 4.39.39), this means that the higher the exposure period, the higher Pb level in the blood. These results were agreed with the results reported in a study of employees exposed to professional work at gas stations (Nuwayhid *et al*., 2001). Our results are also much higher than the results reported in other countries such as Denmark and Ghana (3.5 and 8.6 µg/dl) respectively (Kales *et al*., 2001).

Blood parameters like Hct, Hb, RBCs, WBCs, Mcv, MCHC, MCH, PLTs, enzymes activities as AST, ALT, and creatinine, are taken as an indicator of heavy metal contamination in polluted environments (Shah and Altindag 2004). The data available in this study are consistent with the previous study, where the mean of (RBCs) counts was decreased in employees (5.17±0.353) with the increase in BLL, and statistically significant (t-test=13.75) (P-value =0.00) (Table 3) , which agree with Sahb (Sahb, 2011). In the case of (WBCs), no statistically significant differences in the mean number of (WBCs) between the control group and gas station employees, where the value of the (t-test and p-value) was (0.132, 0.895), respectively. The results of the current study were consistent with Ragini, who indicated that the number of (WBCs) did not change with increasing BLL of gas stations employees (8.63 ± 2.28) compared with the control group (8.58 ± 1.92) (table 3) (**Figure 2**) (Ragini *et al*., 2012).

Hb, Ht and MCH levels were significantly decreased with increasing of BLL. Some studies have suggested that high BLL causes anemia by inhibiting Hb production and shortening the life of (RBCs) (Shah and Altindag 2004). This was revealed by this study that, there was a significant difference between Hb and MCH in tested samples and control group (12.45 ± 1.13 and 23.99 ± 2.6.6) and (15.15 ± 1.17and 27.47 ± 2.36.36) , respectively, while no significant differences in Ht between control subjects and tested samples (44.49 ± 6.49 and 44.10 ± 6.08.08) , where the value of (t-test and P-value) are (9.76, 0.00), (0.261, 0.795) and (5.8, 0.00), respectively (**Table 3**) (**Figure 3**). This is also consistent with Krishna and Ramachandran who showed that exposure of Pb causes anemia in rats (Krishna and Ramachandran 2009). The reason for the anemia due to exposure to Pb has been associated with its ability to decrease serum iron concentration thereby interfering with hemoglobin synthesis (Goel *et al*., 2006). BLL is bounded with negative health effects when reaching (100 to 200 µg/l) and can lead to damage to the hematopoietic, kidney, endocrine, and reproductive system (Assi *et al*., 2016).

Previous studies have also revealed that a relatively high Mcv and lower MCHC ratio indicates the presence of anemia with increasing exposure to Pb (Ambali *et al*., 2010), this consistent with present study, where it was observed that a relatively significant increase of Mcv (85.57 ± 1.9.9) and a small decrease in MCHC (35.33 ± 0.83.83) between the control group and gas stations employees, where (t-test and p-value) (2.07, 0.744) and (0.459, 0.04) for Mcv and MCHC respectively (table 3). PLTs or Thrombocytosis (PLTs count means that the presence of high platelet counts in the blood) is fragments of cytoplasm; they derived from large bone marrow with another type of blood cells; it consider a diagnostic tool for assessing platelet diseases and its analysis and evaluation allows the determination of several parameters, especially in bleeding patients or in the presence of thrombocytopenia or extreme thrombocytosis (Stokol, 2010). Seriki et al. indicate in his research that gasoline may have an important role in stimulating the efficiency of the bone marrow to produce more PLTs (Seriki *et al*., 2016). The increase of platelet (thrombocyte) count in the blood leads to Thrombocythemia (is a condition of high platelet count in the blood) (Altomare and Kessler 2019), which leads to megakaryocyte proliferation and acute hemorrhage (Stokol, 2010; Levine *et al*., 2005). The previous study agrees with the current study, where PLTs were higher in gas station employees than the control group (302.09 ± 12.42 and 246.80 ± 5.69.69) respectively (**Table 3**), and statistically significant (t-test=23.94, P-value = 0.00) (**Table 3**).

Pb may cause immune modification and effects in humans, although the mechanisms inside the body are not well understood. Pb is considered as an important environmental and occupational poison (John *et al*., 2006). This study revealed that the ratio of lymphocytes in gas stations employees higher than the control group (38.35 ± 6.61 and 34.72 ± 4.56.56) respectively and statistically significant (t-test=2.68, P-value = 0.009) (**Table 3**). Pb present in gasoline leads to liver cell damage because having a hepatotoxic effect, which leads to increase serum levels of AST and ALT (Abdou *et al*., 2007).

High levels of AST and ALT plasma is related to high alteration in the liver cells and production of liver microsomal membrane fluidity and free radicals when animals were exposed to lead level (Ibrahim *et al*., 2012). One study found that fuel station employees suffered from liver disorders: chronic fatty liver degeneration, functional liver damage, and liver cirrhosis (Pranjić *et al*., 2003). The results of this study showed that AST and ALT enzymes in gas stations employees were higher than the control group (36.34 ± 7.57 and 38.86 ± 7.96.96) and (28.26 ± 6.67 and 19.23 ± 4.32.32) respectively, and statistically significant (t-test=4.74 and 12.83) (P-value=0.00 and 0.00), respectively (**Table 3**). The enzyme's activities were higher with the elevation of lead exposure. This can happen because of increased cell membrane damage or cell membrane permeability of hepatocytes under the influence of exposure to lead elements (Ovuru and Ekweozor 2004). The results of this study are consistent with some studies, which proved an increase in ALT and AST levels after exposure to lead

(Patil *et al*., 2007).

On the other hand, the concentration of creatinine was examined to check how kidney work. An increase in blood creatinine level was detected in gas stations employees (0.92 ± 0.29.29) compared with the control group (0.78 ± 0.27.27) and statistically significant (t-test = 2.2, P-value = 0.03) (**Table 3**). This height may be due to kidney dysfunction and considered functional evidence of lead-induced nephrotoxicity (Odigie *et al*., 2004).

4. Conclusion

The levels of blood lead are slightly elevated among gasoline stations employees in Riyadh City. As well as, hemoglobin level and red blood cells count had lower in the employees. While other hematological parameters were significantly elevated. The level of serum ALP and creatinine were significantly higher among the employees. A clear educational and protection policy are required for those who are occupationally exposed to benzene and other noxious gasoline vapors.

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Biography of Dr. Hatem Ahmed

Associate Professor of Applied Chemistry - Dean of the College of Criminal Justice - Naif Arab University for Security Sciences - Saudi Arabia. I have 24 scientific research in the field of applied chemistry (toxic elements and pesticides). Supervising 22 master's theses and discussing 17 master theses. Participation in about 38 participation between conferences, seminars, workshops, forums, scholarships, and training courses in the field of toxicology and drug analysis.