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Journal of Environmental and Occupational Science

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Original Research

Relationship between exposure to petrol products and the trace metal status, liver toxicity and hematological markers in gasoline filling workers in Sulaimani city

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Received: March 28, 2012

Accepted: April 19, 2012

Published: May 3, 2012

DOI : 10.5455/jeos.20120419103934

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Key words:

Petrol workers, liver toxicity, trace
elements, hematotoxicity

Abstract

Objective: Long-term occupational exposure to gasoline fumes have been reported to have toxic effects on various organs and systems. The present study was designed to evaluate the expected toxic effects of long-term exposure to petrol products in gasoline filling workers within Sulaimani city area.

Method: This study was carried out on 48 adult subjects, with age range between 27 to 65 years, who work in gasoline filling stations and exposed to different fuel derivatives for not less than 5 years. Twenty seven healthy subjects, matched with age with workers, with no access to such type of occupational exposure were included as controls. Venous blood (10 ml) was taken from each subject and immediately transferred into 2 tubes, 3 ml in a tube containing potassium EDTA anticoagulant and 7ml in a plain tube for preparing serum. The blood samples obtained were analyzed for hematologic markers, while the serum was utilized for estimation of liver enzymes (GOT, GPT), bilirubin and the levels of copper, zinc and iron.

Result: The results showed that liver enzymes and serum levels of Cu, and Zn were significantly increased, while serum Fe and platelets count were significantly decreased.

Conclusion: Long-term exposure to petroleum derivatives increases the risk of liver and hematopoietic toxicity.

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INTRODUCTION

Fractional distillation of crude petroleum yields different fractions of petroleum of which petrol, kerosene and gasoline are constituent parts. These fractions of crude oil contain aliphatic, aromatic and a variety of branched saturated and unsaturated hydrocarbons [1,2]. Occupational exposure to petroleum fumes have been reported to have toxic effects on various organs and systems, and these include respiratory, immune and nervous systems. Organs such as the heart, lungs, skin and kidneys are affected by these toxic effects resulting in various diseases and different forms of genotoxic, mutagenic, immunotoxic, carcinogenic and neurotoxic manifestations [3,4]. The daily use of petrol products both in and outside petroleum industries may have effects on users, and those who work directly in

petroleum industries (those occupationally exposed) are likely to be more affected than their counterparts who do not work in these industries [5]. Previous research studies carried out were on composite fumes evaporating from kerosene, petrol and gasoline and such studies were carried out on experimental animals. Hydrocarbons like benzene, metals like lead and volatile nitrates have all been shown to produce harmful effects on the bone marrow, spleen, and lymph nodes [6]. Most often they add up to other environmental and physiological factors already known, to affect blood parameters and the resultant effect is stress in the animals exposed. These toxic compounds destroy or inhibit the hematopoietic component in the red marrow [7]. Benzene, which is an aromatic hydrocarbon contained in gasoline, is known to induce leukaemia during occupational exposure [8].

Although petroleum derivatives are known to have toxic effects on the hematopoietic system (hematotoxicity) at high occupational doses for over a century [9], the degree of hematotoxicity at low levels of exposure was largely unknown. Overall, these hematologic effects could reflect events in the bone marrow that may be associated with adverse health effects in the future [10]. Although many types of petrol derivatives have delivered daily in Sulaimani petrol stations, Gasoline and Kerosene automobile fuels are the major derivatives of great concern regarding daily exposure. The present study was designed to evaluate the expected toxic effects of long-term exposure to petroleum products on the liver function, hematopoietic system and trace elements homeostasis in gasoline filling workers within Sulaimani city area.

MATERIALS AND METHODS

Subjects

This study was carried out on 48 adult subjects, with age range between 27 to 65 years (45.0±10.8 years), who work in petrol filling stations and exposed to different fuel derivatives for not less than 5 years (20.2±11.6 years), and gave informed consent to participate in the study. Questionnaires were distributed and accurately filled; candidates who met the criteria for participation in this study were enrolled in the study. Several fuel stations located in Sulaimani region were used as sites for this study. Twenty seven healthy subjects, matched with age with workers, with no access to such type of occupational exposure were included as controls. Venous blood (10 ml) was taken from a peripheral vein on the arm of each subject and immediately transferred into 2 tubes, 3 ml in a tube containing potassium EDTA anticoagulant and 7ml in a plain tube for preparing serum. The blood samples obtained were analyzed for hematology markers using automated hematology analyzer (Coulter Swelab, Switzerland) [11]. The separated serum was utilized for estimation of liver enzymes, GOT, GPT and bilirubin using

autoanalyzer based method (Kenza 120, Biolabo, France) [12], and measurement of the levels of copper, zinc and iron using ICP atomic absorption spectrophotometer (Perkin Elmer, USA) [13].

Statistical analysis

Values are expressed as the mean ± SD. Two-tailed unpaired Student's *t*-test was used to compare means. *P*<0.05 was considered statistically significant. Analyses were processed using GraphPad Prism software for Windows (version 5.0, GraphPad Software, Inc., San Diego, CA).

RESULTS

Table 1 showed that activity of liver transaminases were significantly increase in the serum of gasoline filling workers, where GOT and GPT activities significantly increased by 44.6% and 64% respectively compared to controls; serum bilirubin did not significantly changed. In table 2, most of the hematological markers are not significantly affected due to exposure to petroleum products; only the number of platelets showed significant decrease (13%) compared to controls. Concerning the effects on trace elements homeostasis, exposure to petroleum products produced significant (*P*<0.05) elevation in serum levels of copper and zinc (15% and 15.6%, respectively) compared to control subjects; meanwhile, serum iron levels were significantly decreased (29.3%) compared to controls (Figure 1). When the ratio of Cu/Zn in petrol station workers compared with that in control subjects, no significant differences were reported between the two groups in this respect (Figure 2). Correlation between serum levels of Zn and Cu revealed a decrease in the correlation coefficient (*r*) and level of significance in workers compared to those reported in controls (Figure 3). However, correlation between serum levels of copper, zinc and iron and the Cu/Zn ratio with the duration of exposure did not show any significant level of correlation in this respect (Figures 3-5).

Table 1. Effect of long-term exposure of gasoline filling workers to gasoline products on the liver function markers.

Groups	Liver Function Markers		
	Serum GOT IU/L	Serum GPT IU/L	Serum total Bilirubin mg/dl
Control n=27	29.8 ± 10.9	24.1 ± 12.2	0.88 ± 0.3
Workers n=48	43.1 ± 12.9*	39.5 ± 22.2*	0.80 ± 0.2

Values are presented as mean±S.D; n=number of subjects; * significantly different compared to control group (*P*<0.05).

Table 2. Effect of long-term exposure of gasoline workers to gasoline products on the hematological markers.

Groups	Hematological Parameters				
	RBC x 10 ¹² /L	Hct%	Hb g/dl	WBC x 10 ⁹ /L	Plt x 10 ⁹ /L
Control n=27	5.04 ± 0.4	41.7 ± 3.5	14.8 ± 1.2	7.1 ± 2.1	218 ± 38.8
Workers n=48	5.12 ± 0.4	42.0 ± 3.7	14.2 ± 1.4	7.4 ± 1.7	190 ± 42.4 *

Values are presented as mean±S.D; n=number of subjects; * significantly different compared to control group (P<0.05).

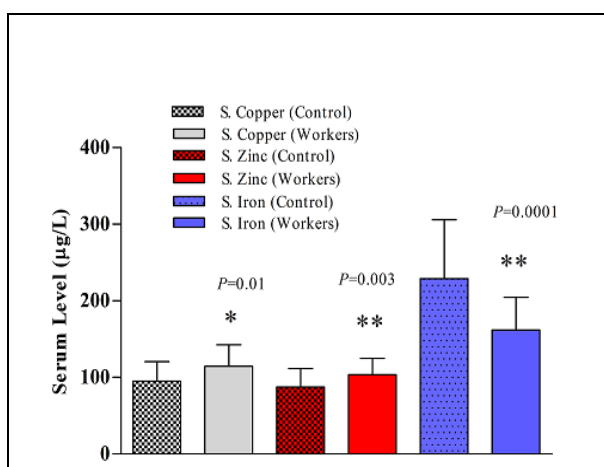


Figure 1. Effect of long-term exposure of workers to gasoline products on the serum levels of free copper, zinc and iron. * Significantly different compared to control group.

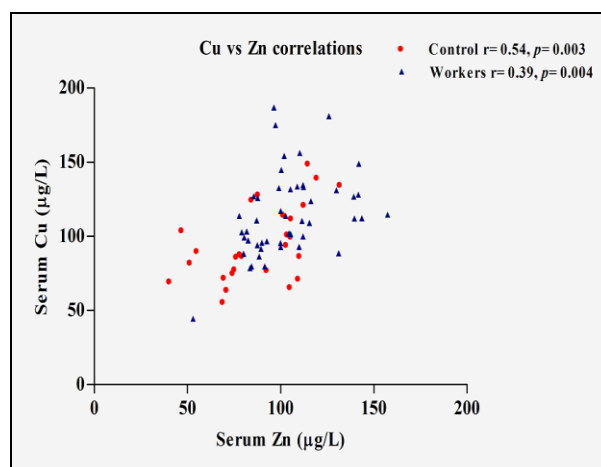


Figure 3. Correlation between serum levels of Zn and Cu in controls and gasoline filling workers; P<0.05 is considered significant.

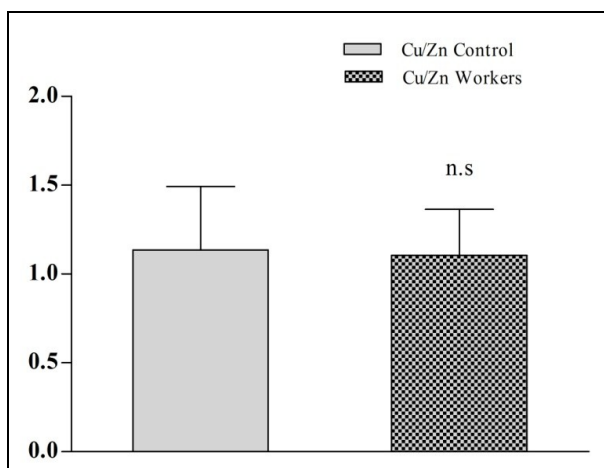


Figure 2. Effect of long-term exposure of workers to gasoline products on the serum Copper/Zinc ratio. n.s: non-significantly different compared to control group.

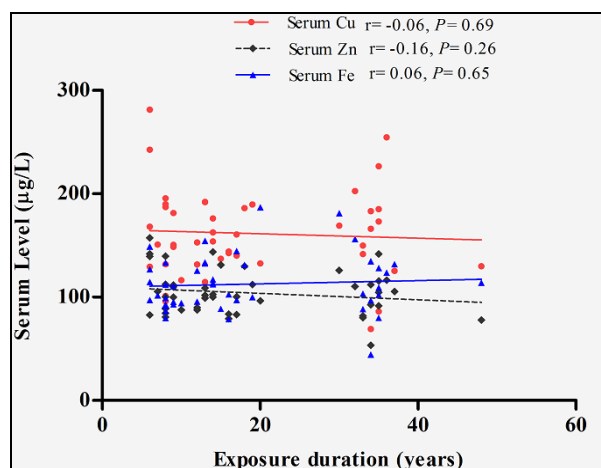


Figure 4. Correlation between serum levels of copper, zinc and iron in gasoline filling workers with the exposure duration (years) in Sulaimani city.

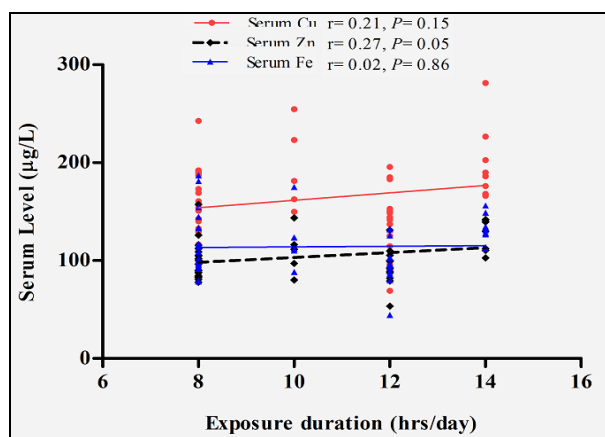


Figure 5. Correlation between serum levels of copper, zinc and iron in gasoline filling workers with the exposure duration (hours/day) in Sulaimani city.

DISCUSSION

Repeated or prolonged exposure to fuel products is more reflective of the occupational norm. Studies have shown that repeated dermal exposures to fuels result in skin irritation [14]. However, the specific causative fuel components have not been identified. It has been reported that many types of fuel release pro-inflammatory cytokines such as IL-8 from normal human epidermal keratinocytes [15]. However, these studies also revealed that there were no significant differences among fuel types with respect to IL-8 release. Therefore, one may propose that the components responsible for this response must exist in all types, eliminating performance additives as the causative mechanism [16]. The present study shows that the fuel-filling workers with daily exposure to different fuel products had a higher serum transaminases activity compared to controls (Table 1), but they are still within the normal accepted range for clinical diagnosis of hepatic injury. However, it is difficult to imagine that the fuel-filling workers would reduce their intake of certain types of food rich in contents with relatively high hepato-protecting properties during their daily life. Fuel products are mixtures of aliphatic and aromatic hydrocarbons mostly related to gasoline, most of them are inducers of microsomal enzyme activity in rats [17], this will increase the possibility of liberating toxic metabolites including reactive oxygen species. While experiments with rats indicate that exposure by inhalation to the aromatic hydrocarbons toluene, styrene, and xylene are inducers of microsomal enzyme activity [18], this effect has not been confirmed in man [19]. Previous studies have shown that exposure to petrol derivatives may have detrimental effects on liver, kidney, pulmonary and nervous systems [20]. Moreover, Petroleum middle distillate steams, which are similar to

kerosene, have been shown to increase the incidence of skin cancer in mice that were treated for 24 months to a lifetime. Skin sensitization potential of various fuels has been reported in murine local lymph node assay, and kerosene was reported to be a mild skin sensitizer [21]. Although the present study did not report significant changes in the conventional clinical chemistry indices of toxic liver injury (serum transaminases and bilirubin) with respect to the accepted normal reference values, they are in agreement with most previous studies, in which occupational chemicals that induce clearance through accelerating biotransformation, leave the transaminase and alkaline phosphatase unchanged [17]. Meanwhile, clinically important therapeutic failure or toxicity of drugs associated with occupational exposure to chemicals has hitherto only been reported in a few case histories [22]. However, the possible impact of such type of exposure to automobile fuel in Sulaimani on health during long-term scale is not very well established. The toxic components, especially those in petrol fumes, have been reported to change blood chemistry and induce anemia by causing bone marrow hypoplasia in experimental animals [23]. Toxic constituents of petrol fuel such as benzene and lead are reported to be activated in the bone marrow and the cytotoxic effects observed are mediated through disturbance in DNA function. The resultant bone marrow depression is characterized by inadequate production of red cell and other formed elements [24]. This is in line with part of the findings in this study related to platelet count. The decrease in platelets count observed in this study is possibly as a result of pancytopenia, which may predispose to impaired blood clotting and increase in bleeding tendency. The observation in this study is similar to previous findings attributed to toxicity from constituents of petrol derivatives, combined with stress imposed by gasoline hydrocarbons [6,4]. From the results of this study, it is thus concluded that exposure to petrol fumes causes depression of platelets count. Petrol fumes are therefore environmental pollutants that could have serious consequences on hematological parameters in exposed humans. Although benzene was known to have toxic effects on the hematopoietic system (Hematotoxicity) at high, occupational doses for over a century [9], the degree of hematotoxicity at low levels of exposure was largely unknown. Recently, a study of workers exposed to varying levels of gasoline revealed a significant decrease in almost all blood cell counts, such as white blood cells (WBC), and platelets, even at exposures below 1 ppm. These findings, based on the differentiated blood cell counts, provide evidence of bone marrow toxicity in workers exposed to benzene at or below 1 ppm [25]. In the present study, because not all types of cells counts were suppressed, it was

suspected that the number or functionality of hematopoietic stem and/or progenitor cells generated in the bone marrow had been only partially affected by exposure to petrol derivatives. Overall, these hematologic effects could reflect events in the bone marrow that may be associated with adverse health effects in the future [10]. Having established that hematotoxicity, specifically effects myeloid progenitor cells, as a phenotypic anchor of petrol derivatives toxicity, we recommend further evaluations to examine the mechanisms underlying these effects, through the comprehensive screening approach.

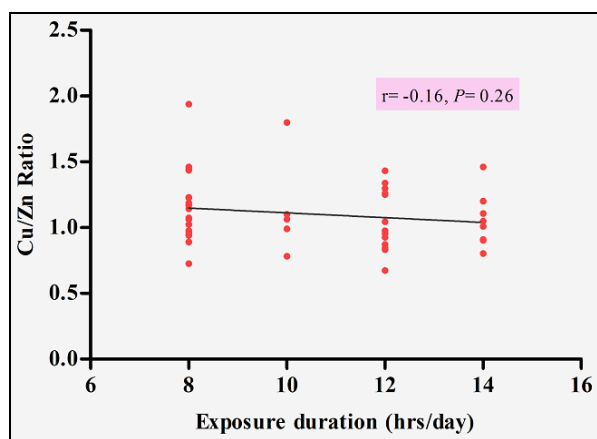


Figure 6. Correlation copper/zinc ratio in the serum of gasoline filling workers with the exposure duration (hours/day) in Sulaimani city.

There is increasing evidence of the important role of essential trace elements zinc and copper in many pathological conditions including inflammation [26]. The reported increase of serum Cu in workers is compatible with that reported by others [27], where high Cu concentration may be induced by chronic inflammation of the respiratory tract due to exposure to hydrocarbons in cigarette smokers. Meanwhile, the present study failed to report significant decrease in serum Zn as appeared in literatures elsewhere [28]; this might be attributed to availability of a compensatory source of Zn in the food or drinking water. A correlation between two trace elements in blood, or serum, indicates that interactions can be suspected. For example, the concentrations of Cu and Zn are known to correlate [29] and to interact [30] in the body. In the present study, figure 3 shows positive and highly significant correlation between serum Zn and Cu, which is compatible with observation previously reported by others [31]. However, long-term exposure to gasoline fumes in workers results in a decrease in the level of correlation revealed as decrease in both r and p values (Figure 3). Both Zn and Cu bind to Cu, Zn superoxide dismutase in erythrocytes and to

metallothionein (MT) in erythrocytes and serum [32]; during exposure to stressful conditions, a suggested mechanism for inhibition of Cu absorption by Zn-induced MT in the intestinal cells may be the cause for the change in correlation pattern [33]. Moreover MT sequesters Cu and retards its transport across the serosal membrane [34]. The reported increase in serum Cu and Zn might be attributed to the increase in release of free forms after protein oxidation resulted from long-term exposure to gasoline fumes [35]. In the present study, the reported positive correlation between the two elements could be due to exposure from the same source or metabolic interactions such as binding to the same proteins; however, the non-significant correlation between exposure time and Cu/Zn ratio may be due to an effective compensatory homeostatic mechanism still functioning in workers group. In conclusion, long-term exposure to gasoline in filling stations within Sulaimani city represents a risk factor for myelosuppression, hepatotoxicity and impairment of trace metals homeostasis.

ACKNOWLEDGMENT

The author thanks University of Sulaimani for supporting the project.

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