Evaluation of Lead and Copper content in hair of workers from oil product distribution companies in Iraq

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Serious health consequences can occur not only from acute exposure to environmental toxicants, but also from chronic exposures to minute quantities. Petroleum substances are widely distributed and used in huge amount, mainly as fuels. A total number of 45 males aged between 20 and 45 years participated in this study. The participants were divided in to two groups: The first group of 25 subjects worked at least 8 hours/day in different oil product distribution companies in both Basrah and Thiqar Governorates-Iraq. The second group (control group) of 20 healthy individuals with age and weight matched with the test group and lived far away from hazardous exposure. Heavy metals levels in hair were measured by Atomic Absorption Spectrophotometer. Statistical analysis was performed using GraphPad Prism (version 6.0). Higher levels of lead and cupper were observed in the hair of subjects working in different oil product distribution companies compared to control group. Heavy metals evaluation in hair samples could be used as a good bioindicator of heavy metal exposure in occupational and environmental surveys for various populations for screening for heavy metals toxicity. In the present study high levels of heavy metals were observed in workers at different oil product distribution companies in Iraq.

Keywords: Fuel. Heavy metals/effects. Hair. Pollutions.

INTRODUCTION

Nowadays, it is clearly known that serious health consequences can occur not only from acute exposure to environmental toxicants, but also from chronic exposures to diminutive quantities. Such exposures and their adverse outcomes are often insidious, in particular at an individual level (Reis et al., 2007). Heavy metals pollution is one of the most serious hazard to the natural environment due to their potent toxicity, persistence, absorption and accumulation which is faster than detoxification (Wang et al., 2009). Petroleum substances are widely distributed and used in bulky quantities, mainly as fuels. These fuels are complex mixtures of numerous ingredients. According to PETROTOX and PETRORISK spreadsheet models that designed to facilitate the performance of hazard and risk assessments of petroleum substances, the primary ingredients that are responsible for danger health consequences are hydrocarbons, in addition to heavy metals. Humans are exposed to lead (Pb) through dust and vapor via several routes from different sources (Reis et al., 2007; Wang et al., 2009). Anthropogenic sources like fossil fuel combustion (petroleum, coal and natural gas), smelting industries and waste incineration have been considered the major source of environmental bioaccumulation and persistence of Pb and copper (Cu) (Pan, Wang, 2012). Air and water are the major sources for lead exposure while soil surface and plants are the most sink for airborne lead through processing industry that in turn through air dusting contaminates drinking water, surface water, ground waters and rivers. (Kennedy et al., 2016; Jaleel, Noreen, Baseer, 2001).

Hair had been used as biological indicator as well as nail and blood for environmental and occupational health assessment and for evaluating extent of previous exposure (Cho, 1997). In addition, chemical analysis of hair samples can be utilized to establish a record of the past exposure of people to contaminants for monitoring environmental changes that affect people (Rasmussen et al., 2008). Lead is a toxic element that have various unfavorable physiologic effects on human, like hemoglobin synthesis inhibition, hypertension, kidney and brain damage plus impact on reproduction in male and female (Castellino, Castellino, Sannolo, 1997).
Copper is a vital dietary nutrient like an essential trace minerals that act as a cofactor for many important enzymes for physical and mental health. But due to wide spread occurrence of copper this increases chances of overexposure leading to neurotic symptoms like affective disorder (Ashish, Neeti, Himanshu, 2013; Nastoulis et al., 2017). Both copper and lead are harmful in highly exposed individuals, but copper is much less toxic, it has been shown that elevated levels of copper for 14 days or more can cause nausea, vomiting, diarrhea and permanent organs damage (Andrade, Aschner, Marreilha, 2017).

Continuous exposure of workers to vapor of an oil product in different distribution companies caused hundreds of workers to be hospitalized seeking for medical treatment for acute and chronic diseases. Therefore, the present study was designed to evaluate the levels of heavy metals in hair of workers as a possible bioindicator of the primary causes of progressive deterioration of workers.

**MATERIAL AND METHODS**

**Subjects**

A total number of 45 males aged 20 between and 45 years participated in this study. The study was conducted from January 2015 to June 2015 at Department of pharmacology and toxicology, College of Pharmacy, University of Basrah, Basrah, Iraq.

The participants were divided in to two groups: The first group was test group that consisted of 25 subjects each worked at least 8h/day in different oil product distribution companies in both Basra and Thiqar Governorates-Iraq. The second group was the control which consisted of 20 healthy individuals living far from hazardous exposure and with age and weight matching the test group. The purpose of the study was explained to the participants all signed the consent form. The protocol was approved by Local Ethical Committee of college of pharmacy, Basrah University. Exclusion criteria were shown in Table I.

<table>
<thead>
<tr>
<th>Exclusion criteria</th>
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<tbody>
<tr>
<td>Patients with history of chronic diseases</td>
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<tr>
<td>Subjects who had undergone significant physiologic stress</td>
</tr>
<tr>
<td>(surgery, fever, etc)</td>
</tr>
<tr>
<td>Individuals who received treatment for chronic diseases</td>
</tr>
<tr>
<td>Individuals who received tonics and minerals</td>
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<tr>
<td>Smoking individuals</td>
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**Preparation of hair samples**

Approximately 0.5g of hair sample was cut with thin-blade stainless steel scissors. Hair samples range between 1 to 2 cm lengths near scalp area were collected, washed with liquid soap, rinsed with de ionized water to remove dirt’s, and then dried. Subsequently samples were accurately weighed to 0.2 g. Then hair samples were prepared for the wet digestion procedure.

The Cu and Pb in hair samples must be released firstly from the binding protein matrix using wet digestion method and then measured by Atomic Absorption Spectrophotometer (Buck Model 211-VGP).

**Digestion method**

In a clean vessel, 0.2 g of hair was mixed with 1.5 mL HNO₃:0.5 mL HClO₄. The vessel was heated on hot plate at 100 °C for 20 min and then diluted with distilled water to a final volume of 5 mL, and stored for later analysis.

**Estimation of Cu and Pb levels**

Heavy metals levels were estimated by Atomic Absorption Spectrophotometer. According to the manufacturer, the flame conditions were fixed and standard solutions for both Cu and Pb were prepared and aspirated to calibrate the instrument. Three concentrations of standards for each element (depending on samples levels of both elements overriding upper and lower limits) were prepared using 1000 ppm STD supplied by Buck company, and their absorbency’s were measured and calibration curves were auto executed by the software of the instrument. The analytical method was evaluated by adding known concentrations of standard solutions to initial measured concentrations of Cu and Pb. It showed excellent correlation between major/minor values ranging from 0.7% to 3.2% exemplifying the high precision of the instrument and accuracy of the experiment. To obtain more accurate data, the regression coefficient of the prepared standard calibration curves for Cu and Pb were made to be greater than 0.99 to obtain more sensitive results and linear check.

The method used for the analysis of Pb and Cu were based on operator’s manual (February 2005 VER 3.94 C by Analyst: Gerald J. De Menna), the detection limits were identified as the lowest concentration given a detectable absorbance above the noise range with values of 0.005 ppm for Cu at wave length 324.8 nm, and 0.4 ppm for Pb at wave length 217 nm. From the prepared standard curves the unknown concentrations of elements were calculated using the following equation:
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[Sample concentration = Read concentration × dilution factor]

Statistical analysis

Values were expressed as mean ± SD. Level of significant difference between the control and tested groups was determined using the unpaired t-test. Correlation analysis were achieved using GraphPad Prism software for windows (version 6.0).

RESULTS

Determination of heavy metals concentrations in hair samples by Atomic Absorption gave an idea about the potential previous long-term heavy metal exposure and accumulation. A higher level of lead (31.64±3.8 µg/g) was observed in hair of workers in different oil product distribution companies compared to control group (19.1±1.09 µg/g) as shown in Figure 1.

Figure 2 shows a 2 fold significant increase in hair copper concentration between occupationally exposed oil product distribution workers (69.6±15.7 µg/g) and non-exposed individuals (36.8±1.5 µg/g).

Table II illustrates the median levels of copper and lead in hair of normal individuals in different countries and in Iraq. The highest mean levels of copper and lead was observed in workers from southern Iraq and also in the control group compared to the rest of the countries.

Figure 3 and 4 illustrate the correlation analysis of heavy metals of copper and lead in both worker and control groups. There was no correlation observed (R=0.1386) between copper and lead in workers group. Regarding correlation analysis between Cu and Lead in normal control group, the same finding was obtained(R=0.1076).

TABLE II - Median levels of Pb and Cu in hair of peoples living in different countries compared to median levels in Iraq

<table>
<thead>
<tr>
<th>Country</th>
<th>Median metals level (µg/g)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pb</td>
<td>Cu</td>
</tr>
<tr>
<td>China</td>
<td>3.9</td>
<td>23.9</td>
</tr>
<tr>
<td>India</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Italy</td>
<td>1.03</td>
<td>18.5</td>
</tr>
<tr>
<td>South Soudan</td>
<td>9.3</td>
<td>18.5</td>
</tr>
<tr>
<td>Russia</td>
<td>1.04</td>
<td>5.8</td>
</tr>
<tr>
<td>Pakistan</td>
<td>8.18</td>
<td>12.8</td>
</tr>
<tr>
<td>Spain</td>
<td>0.5</td>
<td>28.6</td>
</tr>
<tr>
<td>Poland</td>
<td>1.15</td>
<td>5.8</td>
</tr>
<tr>
<td>South Iraq (worker)</td>
<td>31.6</td>
<td>69.6</td>
</tr>
<tr>
<td>South Iraq (control)</td>
<td>9.1</td>
<td>36.8</td>
</tr>
</tbody>
</table>
DISCUSSION

The mean level of determined elements (Pb and Cu) in hair samples collected from individuals working in oil product distribution companies was higher than those from normal individuals living far from oil product companies. This reflects a possible high concentrations of pollutants in the environment (air, water, and soil), thus resulting in occupational exposure. The fuel combustion (petroleum, coal and natural gas), smelting industries and waste incineration are considered the major source of heavy metals persistence especially Pb and Cu that cause serious problems to ecosystem (Singh et al., 2011). Air, Water, plant and soil surface play a role in human heavy metal toxicity through several routes including ingestion, inhalation and direct skin exposure (Kennedy et al., 2015; Farsam, Zand, 1991). Cu and Pb exist in high concentrations in oil product that lead to toxicity. This disturbing levels of heavy metals found in the petroleum products possess significant pollution and health risk problems to the local population and environment. The fuel combustion cause liberation and spillage of metals into the environment and water that could threaten the existence of the local population and could be deleterious to the ecosystem and aquatic life (Pulles et al., 2012).

Various biological samples such as blood, urine, hair and nails had been used for heavy metals analysis. In the present study hair selection as an accurate biological sample was evidence based and have many attractive advantages compared with other biological samples. Hair analysis considered as a non-invasive monitoring method used to measure occupational and environmental exposure for toxicity in the population. Hair is composed mainly of keratin, which is a protein that can bind to various metals and is considered as a storage part. In addition melanin pigments can bind to cations by ionic interaction at physiological pH (Chlopicka et al., 1998; McLean et al., 2009). Hair is highly stable simple to transport and metals concentrations are higher in hair compared to other tissues and fluids. Furthermore, human hair allows long-term monitoring due to slow growth rate of approximately 1cm per month (Moreda-Piñeiro et al., 2007). Hair analysis as an indicator of the area of residence and levels of toxicity of metals in hair are generally accepted as positive parameters of health risk and environmental pollution (Gil, Hernández, 2015).

Therefore, the exposure of the local population to heavy metals deserves future attention.

Chronic exposure to heavy metals can affect health and may lead to development of chronic and acute diseases. Lead for example can inhibit enzymes that involve in various physiologic functions including hemoglobin synthesis and erythroblast growth resulting in damage of kidneys, reproductive system, brain, nervous system and red blood cells (White, 1975; Castellino, Castellino, Sannolo, 1997). On the other hand copper is an essential element that is vitally important for mental and physical health mainly as co factor, but due to widespread occurrence, the chances of copper toxicity is high (Ashish, Neeti, Himanshu, 2013). All these factors may explain frequent hospitalization and endocrine disorder of individual working in contaminated locations.

The present study revealed that, there were significant levels of both lead and copper in hair of workers at different oil product distribution companies compared to control group. This reflects higher rate of exposure to toxic metals and higher incidence of pollutions of oil product distribution workers. Workers might be exposed to toxic metals through different routes. Khudzari et al reported that high level of heavy metals in hair of sanitation workers.
or people living nearby compared to people living far from hazardous waste sites (Khudzari et al., 2013). Our findings correlate with Khudzari’s group findings.

The results are consistent with that previously reported by Al-Rudainy (2010) from Basrah city and by Al-Shamri et al. (2010) from Najaf city in Iraq. They found significant elevation in blood lead levels in workers from different factories. In addition, Bahrami et al. (2002) found a significant elevation of blood lead in the fuel stations worker in Hamadan City of Iran nearby Basra city. The highest blood lead concentration was found in the fuel stations workers with more than 14 years of exposure. This is consistent with the fact that duration of exposure to leaded fuel was significantly correlated with the blood lead level reported previously (Schafer et al., 2005). In another study conducted in Karachi city of Pakistan, blood lead in petrol-pump workers was found to be not significantly higher compared to normal control (Yakub et al., 2009).

CONCLUSION

1. Our data showed that Cu and Pb in hair could be used as an indicator of heavy metal exposure in occupational and environmental surveys for various populations
2. Lead and copper concentrations in hair were significantly increased in oil station workers compared to normal individuals living in Basrah city far away from hazardous contaminants
3. The workers in oil stations or petrol stations and refiner were exposed frequently to high levels of heavy metals that may be the cause of frequent illness.

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REFERENCES


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