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Evaluation of heavy metals concentrations(Pb and Cu) in hair of individuals working in oil station places

بحث التخرج للمرحلة الخامسة

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<u>اعداد الطالبات   باشراف</u>

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Abstract

Heavy metals are one of the most serious pollutants in the natural environment due to their toxicity, persistence and ability toaccumulate in soft tissues and enter the body via inhalation,ingestion, and skin absorption,The aim of study was to determine of heavy metals concentrations(Pb and Cu) in hair of individuals working in oil station places,

The purpose of the study was explained to the participants and all of them agree for study purposes.During interview with the participants we collected information on questionnaire . They provided an informations on their ages, weight, sex, disease history, drug history, .

conducted from jan 2015 to Mar 2015 at Department of pharmacology and toxicology, college of pharmacy, Basrah, Iraq.hair samples were prepared for the wet digestion procedure.The participants were divided in to two groups: The first group consist of 11 subject works in alhabobi station while the second group is the control group consist of 11 healthy individuals,The Cu and Pb in hair samples must be released firstly from the protein matrix by wet digestion method and then determined by Atomic Absorption Spectrophotometer (AAS)using a Buck Model 211-VGP spectrophotometer according to operator's manual, the resultsThere was a significant increase in hair leadconcentration of heavy metals from male subject working in oil station of petrol extraction units ranging from 31.64± 3.8 for worker compare to control group 19.1±1.09.

Introduction

Nowadays, it is known that serious health consequences can occur not only from acute exposure to environmental toxicants,but also from low-level chronic exposures. Although these exposureand their adverse consequences are often insidious, inparticular for children and at an individual level[1]. Heavy metals are one of the most serious pollutants in the natural environment due to their toxicity, persistence and ability toaccumulate in soft tissues and enter the body via inhalation,ingestion, and skin absorption that whenever they accumulate in body tissue faster than the body's detoxification pathways,a gradual buildup of these toxins will occur[2]. Hair is one of bio-indicator apart fromnail and blood. The research of heavy metals in hair sample hasbeen continually a subject of interest in the biomedical and environmentalsciences. In recent years, the determination of metals levels in hair has become popular for monitoringenvironmentalexposure, evaluating heavy metal poisoning, assessingnutritional status and diagnosing diseases. The analytical result ofhair gives a permanent record of the trace element rather thanblood and urine analysis. It is well known that trace elementconcentrations of the hair are possibly related to the trace elementconcentrations in the body[3].Chemical analysis of hair samples is an analytical technique that may be utilized by archaeologists for establishing a record of the past exposure of peoples to contaminants, for evaluating past dietary habits, and as a monitor of environmental changes that may have impacted different peoples.[4].Hair analysis is a non-invasive investigation method and has been used for many years to calculate exposure to toxic metals in occupational and environmental health studies in both individuals and population groups, hair is amaterial ideally suited for the biological monitoringof arsenic, cadmium, chromium, lead (Pb) and mercury.Some authors have also found Pb in hair to be agood indicator of environmental pollution [5] however, somecritical aspects have been reviewed by several authors(6)]. It is understood that Pb content in hair does not

reflect the total amount of Pb in the body but it hasbeen reported that hair Pb correlates with blood Pb

Among the biological materials, hair is an attractive choice for occupational and environmental health surveys and has the following advantages over other biological samples: Firstly, hair is mainly composed of keratin, a protein rich in cysteine sulfhydryl(thiol) groups that can bind various elements, Therefore, elements circulating in the blood can accumulate in the hair as hair shaft continuously contact the bloodstream at the hair follicle during growth. As a consequence, hairmay reflect tissue metal concentrations. Additionally, melanin pig-ments can bind cations by ionic interaction at physiological pH because melanin are polyanionic polymers containing negativelycharged carboxyl groups and semiquinones[7].All of these chemical interactionsallow trace elements to be persistent in human hair for a significant amount of time. Therefore, hair can serve as a useful sample for non-invasive environmental health surveys.

Secondly, hair is highly stable with a high capacity to accumulate metals dur-ing extended periods and its collection and transportation is simple.It has been reported that the metal concentrations found in hair areusually ten times higher than those in blood and urine[8].Thirdly, human hair growsat a rate of approximately 1 cm per month, allows for long-term monitoring of past and recent exposure and does not show changesin storage; thus, the level of an element in hair reflects the level inthe body from which the hair was formed and provides a histori-cal record of the assimilation of elements from the environment[9]. These three advantages resulted in the widespread use of trace element analyses in hair as a tool for biological investigation of nutrition, growth and development, and disease processes. Furthermore, trace element analyses are conducive not only to present evaluations and estimations, but also to the reconstruction of past biological events [10].From this the use ofhair as a noninvasive and cost-effective method could provide pre-liminary information on the exposure of heavy metals and toxicelements to residents in areas that were heavily contaminated

The symptoms relating to copper toxicity syndrome can often be described as an affective disorder of neurotic rather than psychotic nature. They can be the "bread and butter" of general practice, treated often with anxiolytics, anti-depressants, lithium or psychological counselling. The symptoms are related to the biochemical syndrome of histapenic schizophrenia, which is characterized by high serum and tissue copper levels and low serum histamine levels terns, insomnia, depression, memory loss, butalso psychotic symptoms of hallucinations,paranoia, and to a lesser extent, obsession –compulsion[11].Lead is used as the metal in alloys and in compounds in paint, glazesand petrol. Lead can affect every organ and systemi n the body. Long-term exposure of adults can result in decreased performance in some tests that measure functions of the nervous system; weakness in fingers wrists, or ankles; small increases in blood pressure; and anemia. Exposure to high lead levels can severely damage the brain and kidneys and ultimately cause death In pregnant women, high levels of exposure to lead may cause miscarriage High level exposure in men can damage the organs responsible for sperm

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production(12). Air can be consider as source of lead exposure even Humans can be exposed to heavy metals such as lead in dust through several routes including inhalation ingestion and skin. Centers for(13). Water also consider as a source of lead exposure Heavy metals processing industry has always been a major of concern which affect surface water, drinking waters ground waters and rivers contamination. There are several sources of water around us which make concern about our future life.(14).Also Plant and soil surface are the major sink for airborne lead in the environment and may potential of soil in industrial areas, vehicular traffics, and near shore areas take a contribution to dietary lead intake(15)Lead is one of the toxins that can inhibit hemoglobin synthesis as a result of its influence on erythroblast growth and interference with hemoglobin production.(16).

Many studies have shown that lead can inhibit enzymes that take part in hemoglobin synthesis. Lead and its related compounds are toxic materials that have various physiologic unfavorable effects on human including hematopoietic system.(17). Copper (Cu) is an essential trace minerals that is vitally important for physical and mental health. But due to wide spread occurrence of copper in our food, hot water pipe, nutritional deficiencies tablet and birth control pills increases chances of copper toxicity. Copper is not poisonous in its metallic state but some of its salts are poisonous. Copper is a powerful inhibitor of enzymes. It is needed by the body for a number of functions, predominantly as a cofactor for a number of enzymes such as ceruloplasmin, cytochrome oxidase, dopamine β-hydroxylase, superoxide dismutase and tyrosinase(18). Copper can be found in numerous electronics and in wiring. It is also used to make cooking pots. This metal is also relatively corrosion resistant. For this reason, it'soften mixed with other metals to form alloys such as bronze and brass. The metal is closely related with silver and gold, with many properties being shared among these metals. Modern life has a number of applications for copper, ranging from coins to pigments, and demand for the metal remains high, especially in industrialized nations . Many consumers interact with it in various forms on a daily basis In addition to being useful in manufacturing, copper is also a vital dietary nutrient(19).

Both lead and copper are harmful when too much is ingested, but lead is more toxic because it builds up in the body until it reaches toxic levels. Lead damages the brain, nervous system, kidneys, reproductive system, and red blood cells. It is more toxic to children than to adults, and it can harm their mental and physical development. Copper is much less toxic than lead;

however, elevated levels of copper for 14 days or more can cause permanent kidney and liver damage in infants under the age of one year and it can cause nausea, vomiting, and diarrhea in people of all ages. Persons with Wilson's disease (one in 30,000 people worldwide) cannot excrete excess copper and it can accumulate to poisonous levels. If not detected and treated, this disease can be fatal.[20].Heavy metals likePb and Cu accumulated in terrestrial andmarineenvironments can cause serious problems to ecosystemdue to theirtoxicity, persistence and bioaccumulation. There are two major sources of heavy metals natural inputs and anthropogenic contaminations,anthropogenic sources include biomass and fossil fuel combustion(coal, petroleumand natural gas), waste incineration, aswell as miningand smelting industries[21].

At relatively high levels of lead exposure, anemia may occur due to the interference with heme synthesis and also to red cell destruction. Also lead inhibits the insertion of iron into protoporphyrin by the mitochondrial enzyme ferrochelatase, possibly through binding of lead to the sulfhydryl groups of the active site or indirectly through disruption of mitochondrial structure., which does not appear to occur at the zinc allosteric site and is relatively insensitive Mechanisms by which lead might affect blood pressure include effects on several hormonal and neural regulatory systems, changes in vascular smooth muscle reactivity, cardiac muscle contractility, changes in cell membrane cation transport systems, and possible effects on vascular endothelial cells.Copper (Cu) is an essential element to most life forms. However, at high bioavailable concentrations, Cu becomes toxic. With its application in industry and agriculture (e.g., Cu containing fungicides and herbicides), Cu release from these sources into the environment is substantia(22)

Patients and methods

Subjects

A total number of 22 males aged 20 – 45 years were participated in this study . The present study was conducted from jan 2015 to Mar 2015 at Department of pharmacology and toxicology, college of pharmacy, Basrah, Iraq.The participants were divided in to two groups: The first group consist of 11 subject works in ------------ while the second group is the control group consist of 11 healthy individuals. The purpose of the study was explained to the participants and

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all of them agree for study purposes.During interview with the participants we collected information on questionnaire . They provided an informations on their ages, weight, sex, disease history, drug history,

Exclusion criteria include :

1- patients with chronic diseases history

2-subjects who had undergone significant physiologic stress (like surgery ,high grade fever) prior to samples collection

3- Individuals who had history of received treatment for diseases such as (thyroid diseases, hepatic failure, chronic renal failure, epilepsy, etc) .

4-Individuals who had history of received nutrient supplement

Preparation of hair samples

Hair samples were cut near the scalp area with thin-blade stainless steel scissors, 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 protein matrix by wet digestion method as mentioned below and then determined by Atomic Absorption Spectrophotometer (AAS)using a Buck Model 211-VGP spectrophotometer according to operator’s manual.

Digestion method

0.2 gram of hair was taken in a clean test tube and a mixture of 3.0 ml HNO_3:1.0 ml $HClO_4$ were added to the hair. The tube was heated at 100°C on a hot plate for 20 min. Then the tube content was diluted with distilled water to a final volume of 10 ml, and stored in a test tube for later analysis by Atomic Absorption Spectrophotometer (AAS).

Estimation of Cu and Pb levels in hair and serum samples

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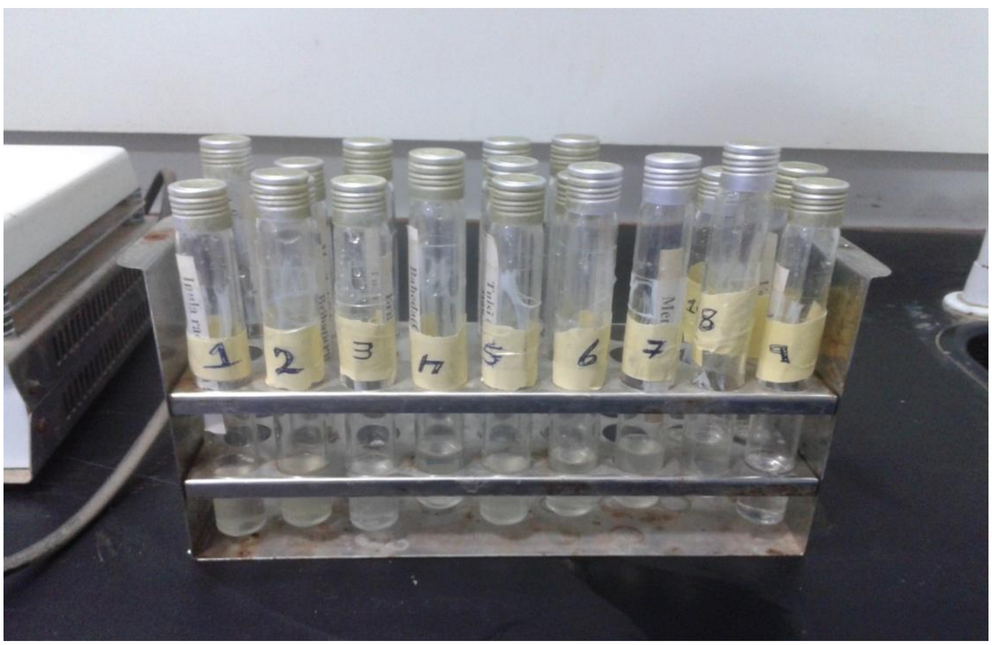
Estimation of heavy metals levels is done by Atomic Absorption Spectrophotometer, in this method the flame conditions were fixed as recommended by the instrument manufacturer for both Cu and Pb. Standard solutions of those elements were first aspirated to calibrate the atomic absorption spectrophotometer(AAS) before the aspiration of the samples. 3 concentrations of standards for each element are prepared using 1000 ppm STD supplied by Buck company, and their absorpance by the instrument was constructed in preparation of the calibration curve was autoexcuted by the software of the instrument.

From the prepared standard curves the concentration of each element was calculated using the following formula:

[Sample concentration= Read concentration × dilution factor]

All metals concentrations were presented as (ppm).



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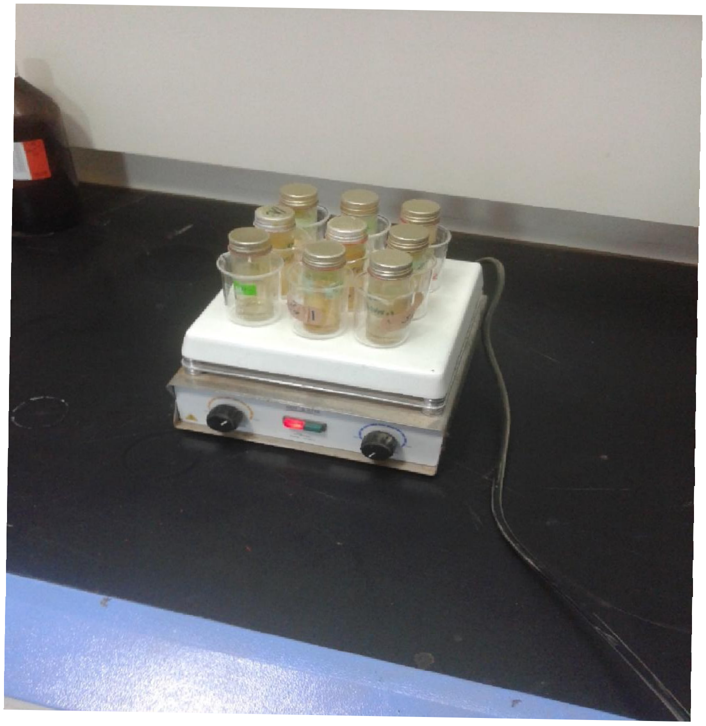

Results

There was a 2-3-fold difference in hair lead and copper concentration between occupationally exposed and non-exposed individuals: There was a significant increase in hair leadconcentration of heavy metals from male subject working in oil station of petrol extraction units ranging from 31.64± 3.8 for worker compare to control group 19.1±1.09as shown in figure1.

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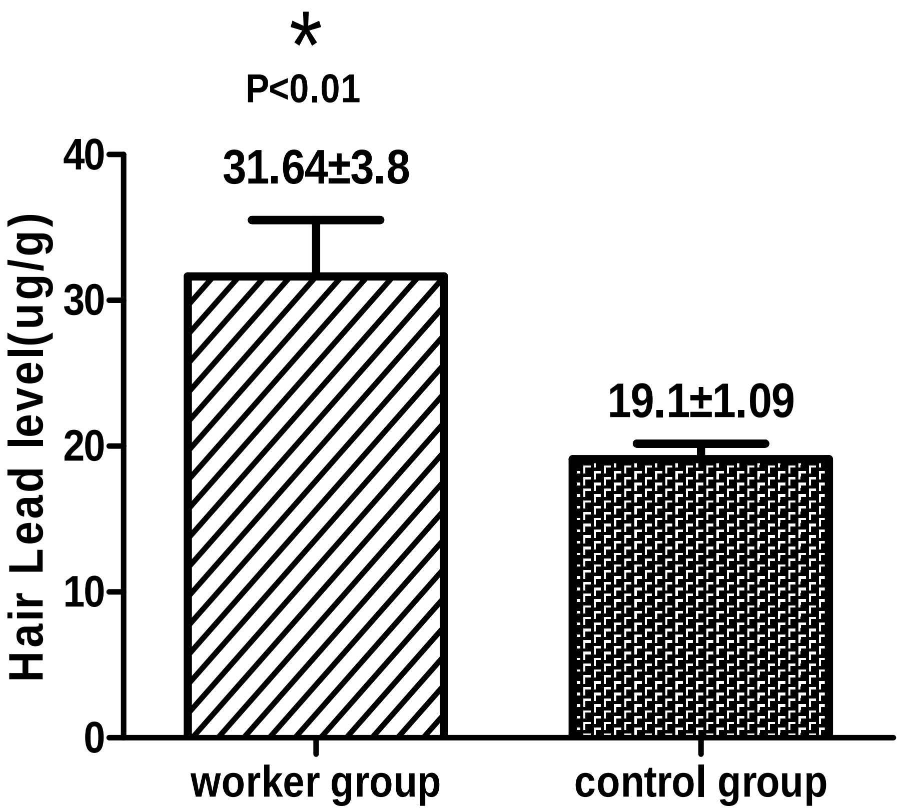

Figure1: Hair lead concentration in oil station worker compared with control group.

*** represent significant difference with P<0.01.**

Regarding copper concentration it has been found thatsignificantly increased (69.6±4.7)in worker in oil station compared to (36.8 ± 1.5)in normal control group., as show in figures 2 .

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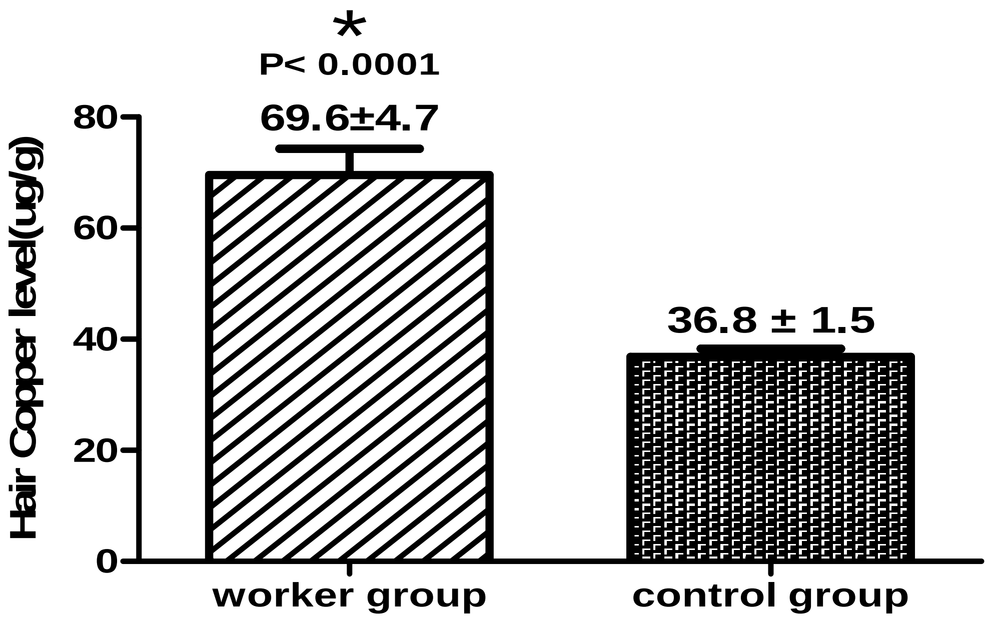
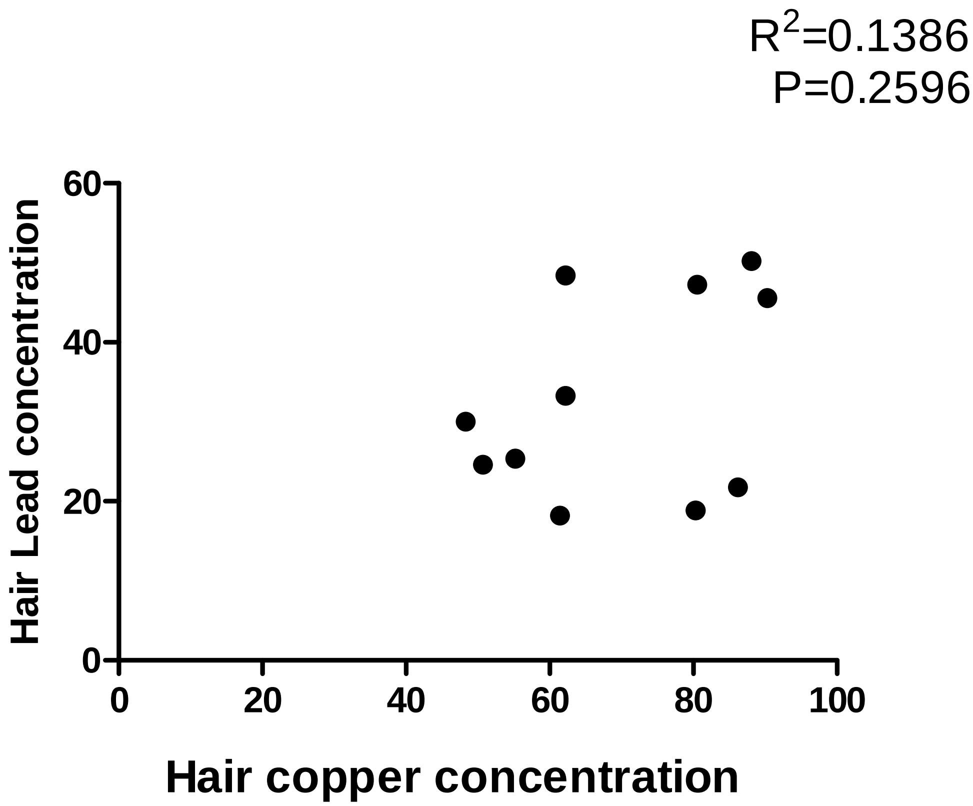


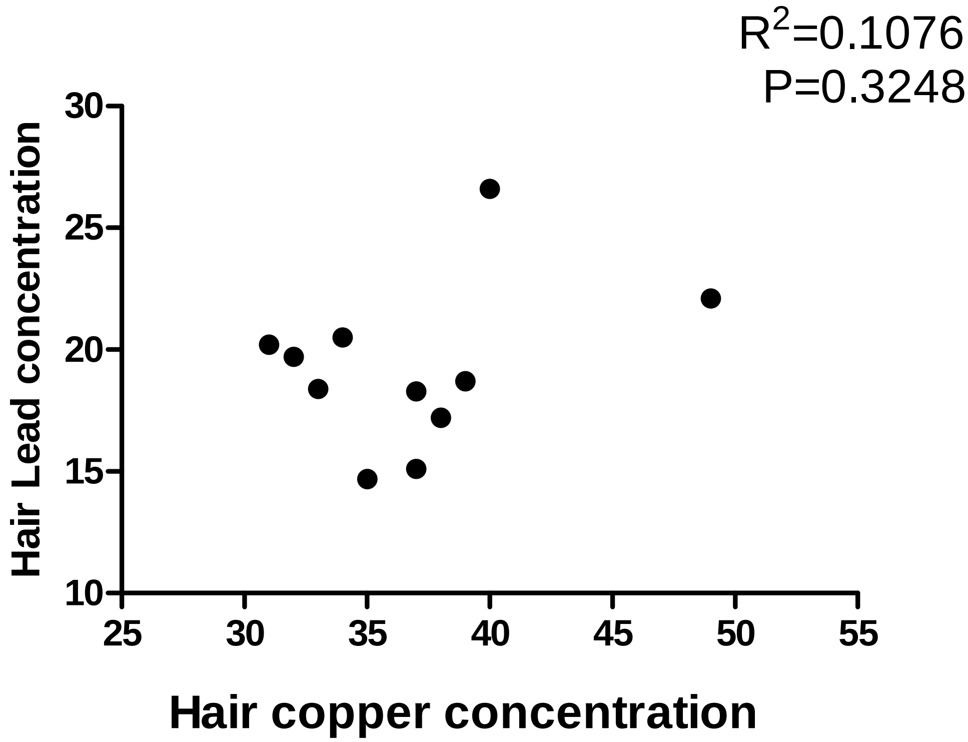
Figure2: Hair copper concentrations in oil station worker compared with control groups.

*** represent significant difference with P<0.0001.**

No correlations analysis were observed between heavy metals levels with respect to worker and control group as shown in figures 3,4



Figure(3):correlation analysis of copper and lead levels in worker group



Figure(4):correlation analysis of copper and lead levels in control group

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The data obtained in the present study was compared to that observed in normal individual in different countries depending on literatures search as summarized in table(1).

Element	lower	upper	country	reference
Cu	9.1	59.7	Italy	1
	8	36	Korea	2
	10.1	46.6	Belgium	3
	31	49	Iraq(control)	Present study
	48	90	Iraq(worker)	Present study
Pb	0.28	3.03	Italy	1
	0	Less than3	Korea	2
	0.008	0.34	Brazil	4
	14.6	26.6	Iraq(control)	Present study
	18.2	50.2	Iraq(worker)	Present study

Table1: comparison of Cu and Pb levels obtained in the present study from control and worker groups to that in different countries

Discussion

Occupational exposure to heavy metals sources, particularly working in petrol refining and gasoline stations, was the most strongly correlated with lead exposure. The results of this study shows that heavy metals in hair samples from different subject accumulate differently based on exposure,from workers in oil station It was observed that the highest concentration of lead was obtained inSubjects of Workers of oil station.Thisindicates that the concentrations of metals in the body is a function of metal in the work environment, incorporation of elements into the keratin structure of hair which takes placeby binding to the sulfhydryl groups that are present in the follicular protein. In this regard, the detergents such as soap, and shampoos, hair pomades, lotions, hair bleaches and dyes actually compete with the complexing ability of these reactive sites, thus leading to a significant leaching of elements from the shaft bulk(27), The suitability of hair analysis in assessing environmental exposure to Pb is still discussed among scientists. Some support the hypothesis that Pb in hair can be used to assess environmental exposure(28) although others do not agree Our data revealed that the employees who were occupationally exposed to Pb showed higher Pb concentrations in their hair than persons not exposed.A positive significant association of Pb in hair on different levels of exposure with the coefficients Increased Pb in hair was found in persons with chronic Pbpoisoning ,Somestudies have found that hair Pb levels were higher in persons from polluted areas such as cities then from rural districts or areas with lower pollution(29). Hair is included in reticulo-endothelial system of tissues, Elements are irreversibly incorporated and this is a part of excretory mechanism for metabolic elimination(30). Consequently, the elements can be either adsorbed or desorbed by the hair. Therefore washing techniques and sample preparation procedures do not necessarily should aim to remove only elements bound on the surface of hair(31). Generally, the reference ranges for hair toxic trace element in male, female, and general samples have been estimated. Consistency of the obtained data with previously published studies, a large number of samples examined, and the use of Dynamic Reaction Cell technology during analysis indicate high quality of the data obtained The estimated refer ence ranges may be used in environmental risk assessment(32) Studies have demonstrated that anemia is one of the earliest manifestations of lead intoxication, after its effect on hemoglobin synthesis(33) The results of different study shows that heavy metals in nails and hair samples from different subject accumulate differently based on exposure. (34,35)

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Small amounts of these elements are common in our environment and diet and are actually necessary for good health, but large amounts of any of them maycause acute or chronic toxicity (poisoning). 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 organ(36).Ourresult came in consistent with thatpreviously reported by Al-Rudainy 2010 (37) and from Basrah city and AlShamri et al 20 10 (38) from Najaf city in Iraq, they find significant elevationin blood lead levels in industerial worker. Bahrami et al 2002 (39) from Hamadan City of Iran who concluded significant elevation of blood lead in the fuel stations worker. while in disagreement with that reported by Yakub et al. 2009 (40) from Karachi city of Pakistan who found the blood lead in petrol- pump workers not significantly changed (47)Depending on the duration of the working; the highest blood lead concentration (43.9µg/dl) was found in the fuel stations workers with more than 14 years of exposure , consisting with the fact that duration of exposure to leaded fuel was significantly correlated with the blood lead level (41,42).

Conclusion

1- Our data show that hair Cu and Pb could be used as an indicator of heavy metal exposure in occupational and environmental surveys for various populations

2-hair lead and copper concentrations were significantly increased in oil station workers compared to normal individuals lives in Basrah

3-the workers in oil stations or petrol stations and refiner exposed frequently to high levels of heavy metals

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