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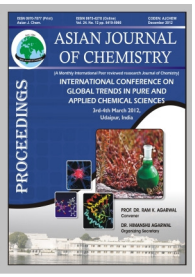


# A Biochemical Study on Lead Effect Upon Oil Refinery Workers†

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This study dealt on 40 males workers at Doura refinery oil in Baghdad city of Iraq in order to check the effect of using tetra ethyl lead on human health. The study involved as well (30) age matched healthy individuals to be used as control group. The measured parameters were:- blood lead level, Hb concentration, total serum protein's concentration and ceruloplasmin (ferroxidase; iron(II):$O_2$ oxidoreductase, EC 1.16.3.1) ferroxidase activity. The workers group was divided into two subgroups according to their blood lead level: subgroup A (10-19 µg/dL) and subgroup B (19-42 µg/dL). The results indicated presence of an increase in blood lead level and a decrease in hemoglobin concentration in the workers subgroups in comparison with that of control group ($p < 0.05$). While a non significant differences were observed in the concentration of total serum protein among the studied groups ($p < 0.05$). Moreover upon the comparison of cp ferroxidase activity and specific activity between the workers groups and control groups, a non significant decrease in the workers subgroup A ($p < 0.05$), but a significant decrease was noticed in the workers subgroup B ($p < 0.05$).

**Key Words: Refinery workers, Lead pollution, Ceruloplasmin ferroxidase activity.**

## INTRODUCTION

Lead has been mined and used by mankind for 6000 years[1]. It is one of the first discovered and most widely used metal in human history[2,3] and therefore is one of the metal most commonly of widespread industrial use and household products for centuries[4-6]. Although lead is one of the most useful metals, it is associated with a variety of health effects[7]. In the first half of the 20th century, tetraethyl lead was introduced as an additive in gasoline. The toxic effects of tetraethyl lead were evident shortly after industrial production started. This was in part due to general motors' interest in launching leaded gasoline onto the market, causing an occupational lead poisoning problem which lead to several deaths and also to many illnesses among the workers[8]. In Iraq, lead is being used in several industries, among which are battery manufacturing, leaded gasoline and printing type. The uses of leaded gasoline in Iraq started long time ago[9]. In the developed countries uses of leaded gasoline now is prohibited, but in Iraq its use increase significantly due to the rapid increase in the number of vehicles.

The aim of the present study is to study the effect of lead on human health at the molecular level.

## EXPERIMENTAL

All laboratory chemicals and reagents throughout this study were of highly purified grade.

**Determination of lead in blood samples:** Blood lead level was determined using graphite furnace atomic absorption spectrophotometer (GFAAS)[10].

**Determination of hemoglobin concentration:** Hemoglobin concentration was determined by using Drabkin's reagent[11].

**Determination of total serum protein concentration:** The total serum protein concentration was determined by Lowry's method, using bovine serum albumin as a standard protein[12].

**Determination of ceruloplasmin ferroxidase activity:** Sera ferroxidase activity of ceruloplasmin was calculated in term of the decrease in the concentration of the substrate (ferrous ion) upon its incubation with the enzyme as described by Erel[13].

## RESULTS AND DISCUSSION

Lead's concentration was measured in the blood of the workers and control groups by flameless atomic absorption spectrophotometer and the results are presented in Table-1.

It is obvious from these results that the lead's concentration in the blood of the workers group is about (4) fold more than it's concentration in non exposed individuals. According to lead's level in their blood, the workers group was divided into two subgroups, (A and B) and as shown in Table- 2. It clear

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TABLE-1
MEAN VALUES OF AGE, BLOOD LEAD LEVEL AND DURATION OF EXPOSURE IN BOTH THE WORKERS GROUP AND THE CONTROL GROUP

| Group | Sample number | Age (year) | | Blood lead level (µg/dL) | | Duration of exposure to lead (year) | |
|---|---|---|---|---|---|---|---|
| | | mean ± SD | Range | mean ± SD | Range | mean ± SD | Range |
| Workers group | 40 | 40.25± 8.78 | 24-53 | 21.89 ± 9.35 | 10-42 | 13.87± 7.83 | 2-32 |
| Control group | 30 | 37.60± 10.97 | 23-52 | 5.35 ± 1.69 | 3-8.2 | - | - |

TABLE-2
MEAN VALUES OF AGE, BLOOD LEAD LEVEL AND DURATION OF EXPOSURE IN BOTH THE WORKERS GROUP AND THE CONTROL GROUP

| Group | Sample number | Age (year) | | Blood lead level (µg/dL) | | Duration of exposure (year) | |
|---|---|---|---|---|---|---|---|
| | | mean ± SD | Range | mean ± SD | Range | mean ± SD | Range |
| Subgroup A | 19 | 38.26 ± 9.98 | 24-53 | 13.98 ± 2.34 | 10-19 | 13.26 ± 8.75 | 2-32 |
| Subgroup B | 21 | 41.29 ± 6.91 | 25-52 | 29.25 ± 7.25 | 20-42 | 14.43 ± 7.08 | 3-29 |
| Control group | 30 | 37.60 ± 10.97 | 23-52 | 5.35 ± 1.69 | 3-8.2 | - | - |

from the results that lead concentration in the blood of group B is about (2) fold more than it's concentration in group A.

The correlation is significant at the 0.05 level (two-tailed) between the blood leads concentration in the workers group and the period of their exposure to lead as it is shown in (Fig. 1). While none significant correlation was observed between the blood lead's concentration in the workers group and their age (Fig. 2).

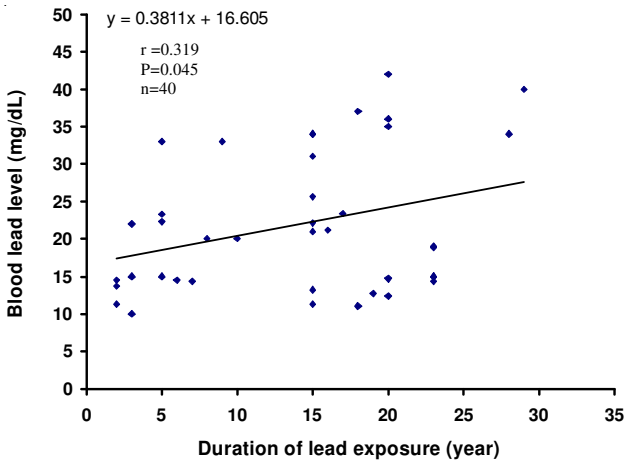


Fig. 1. Correlation between blood lead level in the workers group and duration of their exposure to lead

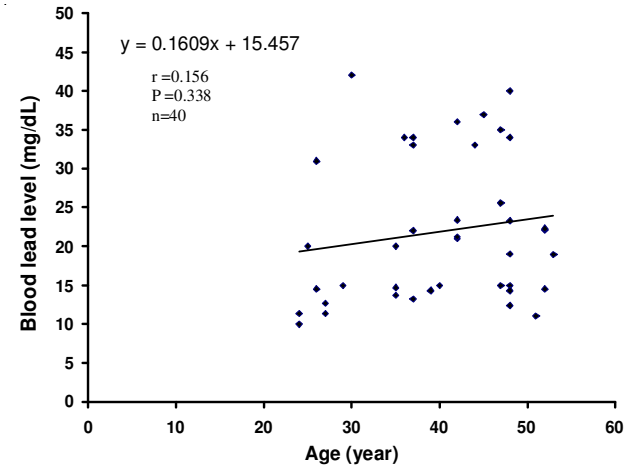


Fig. 2. Correlation between blood lead level and age of workers group

Hemoglobin (Hb) concentration in both the control and the workers groups, was determined using Drabkin's method[11]. The result is shown in Table-3. As the results in Table-3 show, there is a significant variation ($P > 0.05$), in the mean value of erythrocytes hemoglobin level between the workers group and the control group. The hemoglobin concentration non significant difference was found between subgroup A and control group ($P < 0.05$), but asignificantly decrease ($P > 0.05$) was observed in this concentration in subgroup B when they were compared with that of control group.

TABLE-3
MEAN VALUES OF HEMOGLOBIN CONCENTRATION (gm/dL) IN BOTH THE CONTROL GROUP AND THE WORKERS GROUP

| Group | Sample size (n) | Mean ± S.D (gm/dL) | Range (gm/dL) |
|---|---|---|---|
| Control group | 30 | 14.92 ± 1.12 | 13.2-17.5 |
| Workers group | 40 | 14.037 ± 1.058 | 12.0-17.1 |
| P-value | | P < 0.05 | |

When total serum protein of both the control group and the workers group was determined by lowry's method[12]. Table-4 shows that there is no significant variation in this concentration in sera of control group and the workers group.

TABLE-4
MEAN VALUES OF TOTAL SERUM PROTEIN CONCENTRATION (gm/dL) IN BOTH THE CONTROL GROUP AND THE WORKERS GROUP

| Group | Sample number | Mean ± SD (gm/dL) | Range (gm/dL) |
|---|---|---|---|
| Workers group | 40 | 7.45 ± 0.635 | 6.1 - 9.3 |
| Control group | 30 | 7.09 ± 0.65 | 6.0 - 8.3 |
| P-value | | P < 0.05 | |

Neither a significant variation was found between subgroup A and control group and between subgroup B and control group, as well as between subgroup A and subgroup B ($P< 0.05$).

Ceruloplasmin has oxidase activity towards polymines, polyphenols, as well as towared inorganic ferrous ions, which is reported the only biological substrate for ceruloplasmin. This oxidase activity reported to have the highest affinity for Fe(II) among other substrates[13]. The ferroxidase activity was

TABLE-5
MEAN VALUES OF CERULOPLASMIN FERROXIDASE ACTIVITY (U/L) AND SPECIFIC IN SUBGROUP A, SUBGROUP B AND CONTROL GROUP

| Group | Sample number | Ferroxidase activity (U/L) | | Specific ferroxidase activity (U/mg) × $10^3$ | |
|---|---|---|---|---|---|
| | | Mean ± SD | Range | Mean ± SD | Range |
| Workers group | 40 | 562.75 ± 103.78 | 410.9 - 860.3 | 7.70 ± 1.66 | 12.6 - 4.4 |
| Control group | 30 | 633.11 ± 108.03 | 450.3 - 870.3 | 8.87 ± 1.83 | 13.9 - 5.7 |
| P-value | | 0.05 < P | | 0.05 < P | |

measured photometrically in both the workers groups and the control group. Then the specific activity was calculated in sera of the studied groups.

The result shown in Table-5, indicated the presence of significant decrease in both ferroxidase activity and specific activity in the workers group when compared with that of the control group ($p > 0.05$).

The results presence of a significant decrease in both ferroxidase activity and specific activity when they were measured and the compared between group B and control group, as well as between group A and group B ($p > 0.05$). While non significant difference was found when the comparison was between the sera activity and specific activity of group A and control group ($P < 0.05$).

Flameless atomic absorption analysis method was recommended to be used for determination of lead in blood[14]. Therefore throughout this study, Lead was measured in venous blood using graphite furnace atomic absorption spectrometer. The results of the present study agree with Saito *et al.*, who reported the blood lead level increased along with increasing employment duration[15], as well as with Lormphongs *et al*., who reported that the correlation between age with blood lead level was not significant[16]. While disagree with AL-Chabban, 1986, who reported that duration service in the factory of manufacture of batteries and printing factory had no significant effect on blood lead level. He interpreted these results on the ground that duration of exposure to lead increases total body burden of lead rather than blood lead level[17]. Lead has been known to alter the hematological system. The anemia induced by lead is microcytic and hypochromic and results primarily from both inhibition of heme and globin synthesis[18]. The obtained decrease in hameoglobin concentration upon exposure to lead in this study, agrees with the above mentioned fact that lead disturbs heme biosynthesis, at the molecular level[19]. Lead interferes with heme biosynthesis by altering the activity of three enzymes involved in heme production, δ-aminolevulinic acid synthetase, δ amino levulinic acid dehydratase and ferrochelatase, resulting in the dose dependent diminished production of heme and in the accumulation of precursor molecule[20]. The obtained results of protein concentration agree's with that of AL-Awsey, who recorded non significant variation in total plasma protein concentration when compared that of the workers in factory manufacturing lead storage batteries with that of the control group[21]. The significant decrease in both ferroxidase activity and specific activity in the subgroup B, may be explained by what Leelakunakorn *et al.*, concluded that ceruloplasmin from Pb-intoxicated serum possessed Pb in its molecule, such binding of Pb to ceruloplasmin cause a rduction in ceruloplasmin oxidase activity when using PPD as a substrate, with a strong correlation between the blood lead level and Cp oxidase activity[22]. Ceruloplasmin is unique since it has a total copper stoichiometry ranging between 5 and 6 atoms/molecule, depending on the species, of the isolated protein[23]. Messerschmidt *et al*., reported that one of these copper atoms plus the three-copper complex may be responsible for Cp ferroxidase activity So the decrease in the Cp ferroxidase activity in the present work may be due to that Pb replace copper on the ceruloplasmin molecule, since both Pb and cu have approximately equal radius, therefore when the concentration of pb was high, a significant decrease in Cp ferroxidase activity was observed[24].

## Conclusion

On the basis of the study, a conclusion can be reached that among all studied biochemical parameters, Ceruloplasmin, which is one of the important extracellular antioxidants, plays a role in the oxidative stress that reported in Pb- intoxicated patients through its ferroxidase activity. This activity was found to be decreased in the sera of these patients. Such decrease in this enzymatic activity leads to increase the concentration of the ferrous ions in the serum and thus increase the oxidative stress.

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