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Delta-Aminolevulinic Acid Dehydratase Inhibition and RBC Abnormalities in Relation to Blood Lead among Selected Jordanian Workers.

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Abstract

Lead toxicity is a public health hazard particularly to the occupationally exposed workers. Evidence is mounting successively regarding the adverse health effects of lead at low levels. This study is conducted to assess lead cytotoxicity and the antioxidant status of selected groups of Jordanian workers occupationally exposed to lead poisoning or automobile buffs. A total of ninety male workers were selected for the study. The workers studied included: Radiator welders, exhaust workers, automobile electronics and mechanics, metal workers, and car painters in Amman city. The control group included 20 subjects of the same age group without any occupational exposure to lead. A number of biological parameters have been studied in order to estimate the degree of lead intoxication in the selected worker groups. The groups did not significantly differ among each other in the average of age (34± 0.9) and work years (17.1±1.8**)**. The researchers studied the effect of exposure to lead on the activity of δ-ALAD (a key enzyme in heme biosynthesis). A significant decrease and a negative correlation between δ-ALAD and blood lead were observed in all the studied worker groups. The effect of exposure to lead on the morphology of RBCs was investigated. Fibrillation and particulate structures on the surface of RBCs and irregular plasma membrane evaginations were seen under scanning electron microscope.Determination of blood-lead concentration, δ-ALAD activity as well as morphological investigations of RBCs could be bebenificial in the evaluation of lead toxicity.

KeyWords: Blood lead, Lead toxicity, δ-ALAD, RBC morphology, SEM.

1. Introduction

The exposure of humans to lead and its adverse health effects is of a global concern because of its ubiquity in the environment. Studies from different countries have revealed that lead poisoning is a problem for urban populations, and more so in developing countries, where there are no (or few) environmental regulations (Ahamed *et al*., 2006; Ambrogi *et al.,* 1996). Although car emissions from the consumption of leaded gasoline has been the chief source of wide-spread environmental lead contamination in urban areas (Bakalli *et al*., 1995 and Baghurst *et al*., 1992). Other sources, such as leaded pipes for water supply, lead-based paints, the use of leaded ceramics and canned food, lead in cosmetics and folk remedies, are good sources of lead exposure, in addition to lead smelters and industrial processes (Baghurst *et al*., 1992; Bilto, 1992) following the phasing out of leaded gasoline in 2000 in India (Boyd *et al*., 1981).

Several antioxidant molecules, such as glutathione (GSH) and glutathione disulfide (GSSG) levels, and enzymes, glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) activities are the most commonly used parameters to evaluate lead-induced oxidative damage in animals and occupationally-exposed lead workers (Burns and Godwin, 1991; Burns *et al*., 1996 and Bustos and Battle, 1989). The delta-aminolevulinic acid dehydratase (δ-ALAD) is the second enzyme in the heme biosynthesis pathway and catalyses the condensation of two molecules of delta-aminolevulinic acid (δ-ALA) to porphobilinogen (PBG), with the thiol (SH) group essential for its activity. Because of its affinity with the SH group, lead is known to inhibit δ-ALAD activity (Dacie and Levis, 1984), resulting in the accumulation of d-ALA. The latter has been shown to undergo metal catalyzed autooxidation and give rise to the formation of reactive oxygen species (ROS) as superoxide ion (O_2^+), hydroxyl radical (OH^+) and hydrogen peroxide (H_2O_2). This possibility implies that δ-ALAD inhibition, in addition to being a biochemical indicator of lead toxicity, might also be a promising indicator of lead-induced oxidative stress.

2. Material and Methods

2.1. Worker Groups

Blood samples were obtained from ninety male occupational workers in Amman city. The Jordanian workers were divided into six categories (fifteen workers each) including: Radiator welders, exhaust workers, automobile electronics and mechanics, metal workers and car painters. Blood samples of the control group were obtained from twenty males of non-exposed persons.

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2.2. Determination of Blood Lead

Blood lead levels were determined according to the method described by Shraideh *et al.* (2018). Using a graphite furnace, atomic absorption spectrophotometer and blood lead contents were expressed as µg/dL.

2.3. Determination of δ -ALAD Activity

2.3.1. Principle

The enzyme δ-ALAD which is found in RBC's converts two units of δ-ALA to one unit of PBG in peripheral blood; the reaction stops at this monopyrrole stage rather than proceeding to porphyrin. Therefore, δ-ALAD activity in erythrocyte can be determined simply by incubating a sample of peripheral blood with ALA and measuring the PBG spectrophotometrically. The formed PBG (aromatic amine) condenses with *p*- dimethyl-aminobenzaldehyde to produce a cherry red- color.

2.3.2. Preparation of Reagent for Enzymatic Measurements

1. 0.2 g of Triton X-100 reagent were dissolved in 100 ml double deionized distilled water.
2. 16.8 mg of δ-ALA hydrochloride substrate were dissolved in 10 mL of 0.25 M citrate-phosphate buffer of pH 6.6 using a pH meter.
3. 0.25 g of *N*-ethylmalemide were dissolved in 40 mL of warm double deionized distilled water, then 10 g of trichloroacetic acid were added, and the final volume was completed to 100 mL in a volumetric flask to prepare the protein precipitant reagent.
4. On the day of the test, 9.33 mL of 60 % perchloric acid were completed to 50 mL with *p*-dimethylbenzalde-hyde in a volumetric flask to prepare Erlich'sh reagent.

2.3.3. Procedure

Heparinized blood samples were drawn on the day of test and the hematocrit value was determined on aliquot. Enzyme assay was performed on freshly drawn blood samples. The procedure of Tietz (1987) was followed. It consists of the following steps:

0.2 mL of blood was added to 1.3 mL of Triton X-100 reagent causing immediate hemolysis. 1 mL of the buffered δ-ALAD substrate was added to the mixture, and mixed well. Before incubation, 1 mL of the mixture was removed immediately for the blank, and was added to a tube containing 1 mL of protein precipitant. The remainder of the reaction mixture was covered, and incubated at 38ºc for one hour. 1.5 mL of protein precipitate were added immediately at the end of the incubation period. After centrifugation, 1.5 mL of the supernatant solution (Blank and test) were removed, then 1.5 mL of the modified Ehrlich's reagent was added. Color was measured at 555 nm after fourteen minutes using PYE UNICAM SP-6-350 spectrophotometer (PYE UNICAM, Ltd-Cambridge/ England). The activity of δ-ALAD was calculated according to the formula:

Units δ-ALAD / mL erythrocyte=

$(A_{test} - A_{blank})$ x 100 x12.5 x 1.0/0.1 x hematocrit

Where:100\hematocrit = correction for erythrocyte fraction of whole blood 12.5= dilution factor of the blood

0.1= correction for the definition of enzyme activity one unit being defined as an increase in A_{555} of 1.100 (1.0 cm light path) per h at 38ºC

2.4. Erythrocyte Morphology (Scanning Electron Microscopy (SEM) Preparation):

Nine grams of NaCl were added to 1 L of 0.15 M isosmotic phosphate buffer solution of pH 7.4 to have phosphate-buffer saline (PBS) (Dacie and Levis, 1984).

Erythrocytes were fixed with 1.25 % (w/v) glutaraldehyde in PBS, and were kept at 4ºC as described by Bilto (1992). After two washes with PBS, cells were dehydrated in a series of alcohol [from 30 to 100 % (v/v) ethanol]. First, the pellets were suspended in 30 % ethanol, mixed and kept for ten minutes, then were centrifuged for two minutes at 2000 rpm. The same procedure was repeated with 50, 70, 90 and 100 % ethanol. Finally, pellets were suspended in 100 % ethanol, and gently dropped onto a glass coverslip that had been cleaned with ethanol. The samples mounted on glass slide were gold-coated by the sputtering method at 1200v, 20 mA for 165 seconds using polarcon E6100 coater. Specimens were viewed and photographed by Vega 3 TESCAN SEM, 19 Kv.

2.5. Statistical Tests

In all statistical analysis, 'students' " t-test was used to compare the means of two groups. It determines whether the two means are significantly different or not at $P<0.05$. In addition, analysis of variance (ANOVA) was used to determine if mean values are significantly different among different worker groups or not at $P<0.05$.

Correlation coefficients (r values) were also calculated to determine whether the two variables are related positively (up to +1), negatively (down to-1), or not at all (0). It was used to determine the relation between Blood-Pb level and δ-ALAD activity.

3. Results

3.1. Worker Groups

For this study, blood was collected from ninety Jordanian workers distributed over six different work groups as shown in table 1.

Table 1. Distribution of workers in worker groups (TRT).

TRT#	Number	Worker
1	15	Radiator Welders
2	15	Exhaust Workers
3	15	Automobile Electronics
4	15	Car painters
5	15	Mechanics
6	15	Metal workers

3.2. Blood- Pb Concentration in Occupational Worker

A comparison between Blood –Pb levels in different types of work groups is shown in Table 2. The blood samples were obtained from ninety males of occupational workers in Amman city. The Jordanian workers were divided into six categories (fifteen workers each) including: Radiator welders, exhaust workers, automobile electronics and mechanics, metal workers and car painters. The blood samples of the control group were obtained from twenty males of non-exposed persons.

Blood-Pb concentrations for all individuals were measured, and Table 2 shows the results obtained according to the occupation. The results indicated that there is a significant increase in the mean values of blood-

Pb concentration in the studied groups compared to the control group. Also, there was a significant difference in blood lead among the different worker groups (Group 3 showed the highest increase).

Table 2. δ-ALAD activity (U/ mL) and B: blood [pb] among TRT (µg/dl) among workers group

TRT#	A: Mean ± S E	B: Mean ± S E
1.	170 ± 7	14.5±1.4
2.	160 ± 6	16.4±1.2
3.	140 ± 6	21.0±1.6
4.	165 ± 6	15.0±1.3
5.	160 ± 6	15.0±1.7
6.	165± 7	15.0±1.6
7. (control)	220 ± 9	4.3±o.5

SE= Standard Error

3.3. δ-ALAD activity in occupational workers:

The researchers studied the effect of exposure to lead on the activity of δ-ALAD (key enzyme in heme biosynthesis). A significant decrease and a negative correlation between δ-ALAD and blood lead (Table2) were observed in all the studied worker groups (Table 2).

δ-ALAD activity was determined for all the blood samples obtained and was calculated as shown in Table 2. The control group has the maximum δ-ALAD activity (220 ± 9 U/ mL). In the worker groups that are occupationally exposed to lead, the δ-ALAD activity ranged from 140 U/mL to 160 U/mL with a mean value of ± SD of 160 ± 6.3U/mL. Automobile electronics, with the highest blood lead concentration, show the lowest δ-ALAD activity (140 U/ mL). On the other hand, the control group had a maximum value of (220 ± 9 U/ mL).

When the δ-ALAD activities of the workers were compared with the control group, significant differences were observed between the mean values of the control group and each of the other study's groups. In addition, there were significant differences ($P < 0.05$) in the means of δ-ALAD activity among the groups using ANOVA test.

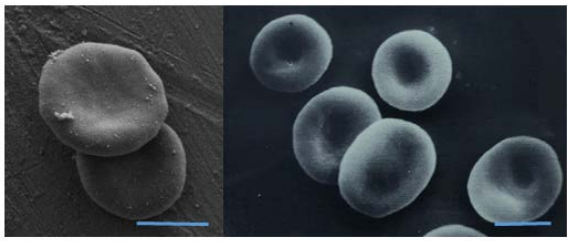
When the correlation between δ-ALAD activity and blood-Pb level was studied, a significant negative correlation (r = -0.44) was observed between the blood-Pb level and δ -ALAD activity in exhaust workers at ($P < 0.05$). No correlations were observed in radiator welders, automobile electronics and car painters. However, when the whole samples were studied together, there was a negative correlation (r = -0.41) between blood-Pb level and δ-ALAD activity at ($P < 0.05$).

3.4. Erythrocyte Morphology:

The examination of the blood samples using scanning electron microscopy (SEM) revealed shape abnormalities and morphological changes in erythrocytes. In every worker group, one or more of the following erythrocyte abnormalities was observed: fibrillar and particulate matter on the surface of RBCs, acanthocytes with irregular RBC boundary, target cell with a central membrane evagination and irregular cell shape with many membrane evaginations. Sometimes more than one abnormality was observed in the same individual.

Morphological changes in erythrocytes can be seen in SEM micrographs of representative worker cases (Figures 2-9).

Figure 1 shows SEM of RBCs of control individuals not exposed to occupational lead contamination. Figure 2 shows RBC of radiator welders with fibrillar structures on the surface of RBCs. Figure 3 shows RBC of exhaust workers with acanthocytes, fibrillar structures and membrane evaginations. Figure 4 shows RBC of Automobile electronics workers with acanthocyte and target cell abnormalities. Figure 5 shows RBC of car painters with target cell and surface fibrillar abnormalities. Figure 6 shows RBCs of mechanics with surface fibrillar abnormalities. Figures 7 and 8 show RBC of metal workers with target cell and surface fibrillar abnormalities. These abnormalities will badly affect the normal functions of RBCs.



A B

Figure 1 . SEM of RBCs of two control individuals, not exposed to occupational lead contamination. Scale bar = 5 microns.

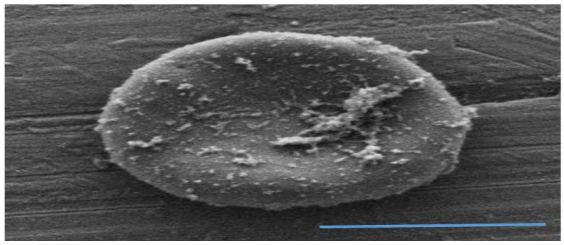


Figure 2. RBC of radiator welder with fibrillar structures on the surface of RBCs. Scale bar = 5 microns.

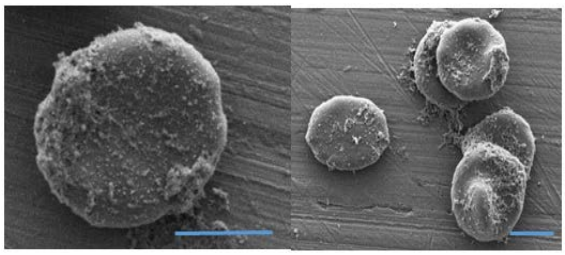
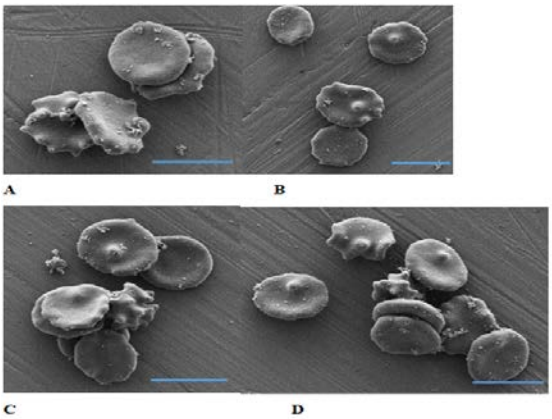


Figure 3. RBC of exhaust workers with acanthocytes, fibrillar structures, and membrane evaginations. Scale bar = 4 microns.



A B C D

Figure 4. Shows RBC electronics workers with acanthocyte and target cell abnormalities. Scale bar = 7 microns.

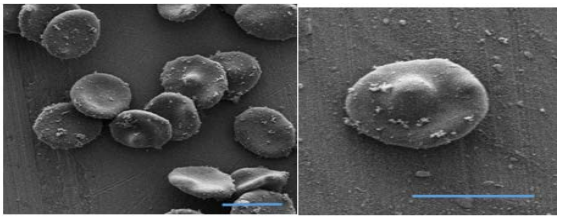


Figure 5. RBC of car painters with target cell and surface fibrillar abnormalities. Scale bar = 7 microns.

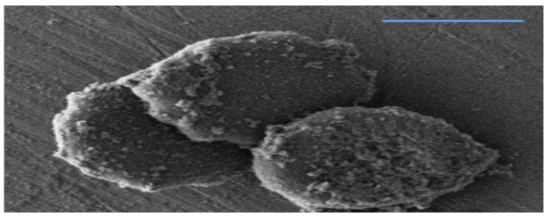
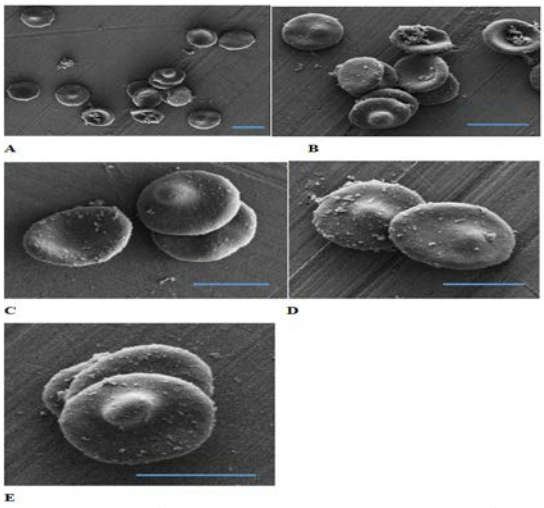


Figure 6. RBCs of mechanics with surface fibrillar abnormalities. Scale bar = 5 microns.



Figures 7. RBC of metal workers with target cell and surface fibrillar abnormalities. Scale bar (A and B)= 7 microns, (C, D, E)= 5 microns.

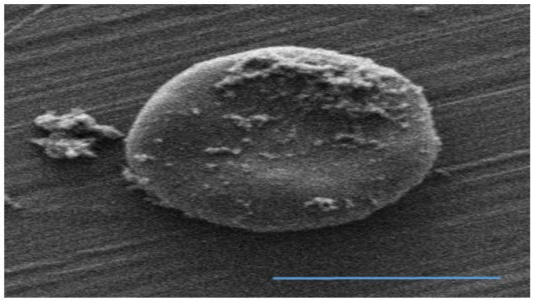


Figure 8. RBC of metal worker with surface fibrillar abnormalities. Scale bar = 5 microns.

4. Discussion

4.1. δ-ALAD Activity in Occupational Workers

In the present study, significant differences ($P < 0.05$) were observed between the control group and each of the studied groups, as well as among groups. Automobile electronics have the lowest mean value (140 U/ml), followed by exhaust workers and mechanics (160 U/mL). Car painters and metal workers have equal mean values (165 U/ml). Radiator welders have the highest mean value (170 U/mL), whereas the control group has a mean value of 220 U/mL. On the other hand, a significant negative correlation (r=-0.41) was observed between blood-Pb level and δ-ALAD activity in occupational workers at $P < 0.05$.

La-Llave-Leon *et al*. (2017) described an association between blood lead levels and Delta-Aminolevulinic Acid Dehydratase in pregnant women.

The inhibition of enzymes in the heme biosynthesis pathway serves as potentiality important biological indicators of chemical exposure and cell injury (Goering and Rehm, 1990). Lead plays a dual role as both pseudo substrate and inhibitor of enzyme δ-ALAD, hence Pb^{+2} acts as a parabolic competitive inhibitor of the enzyme.

The enzyme results of the present study are consistent with what have been reported in previous studies. Inhibitors of δ-ALAD is well-documented and the accumulation of the substrate of this enzyme (i.e. δ-ALAD) is reported in individuals exposed to lead (Dacie and Levis, 1984). In north Australia, Burns *et al*. (1996) found significant differences between blood-Pb level and δ-ALAD activity in the sniffers of leaded and unleaded petrol. In addition, the results could be explained in the light of molecular biology. Polymorphism in δ-ALAD may be associated with differenced in blood-Pb levels. Individuals who inherited ALAD2 allele are more susceptible to lead poisoning than individuals who inherited ALAD1 allele (Smith *et al*., 1995).

Stansley and Resco (1996) found that elevated tissue lead concentrations and depression of δ-ALAD activity in blood of small mammals was significant at the 0.05 level. Lead supplementation to broiler chickens caused a linear decrease in δ-ALAD activity (Bakalli *et al*., 1995). Chunping *et al*. (2011) demonstrated that lead exposure suppressed *ALAD* transcription by increasing methylation level of the promoter CpG islands. Furthermore, Baghurst *et al*. (1992), found that the activity of δ-ALAD was negatively correlated with blood-Pb level in Granite City where a former secondary lead smelter was in operation, and the surrounding area was heavily contaminated with lead. On the other hand, Ahamed *et al*. (2006) demonstrated the inhibition of δ-ALAD activity in relation to blood lead.

4.2 Erythrocyte Morphology

In lead toxicity erythropoietic cells of the bone marrow undergo morphological changes resulting in increased production of abnormal erythrocytes (i.e stippled erythroblasts have nuclear abnormalities, variation in cell size and inadequate hemoglobin).

The inhibition of P5N brings about the intracellular accumulation of pyrimidine nucleotides with consequent metabolic changes causing an accelerated destruction of erythrocytes. The hemolytic component of the anemia of lead poisoning may be explained therefore by the basis that the excess nucleotides diverts ATP activity from important metabolic functions consequently resulting in red-cell membrane damage (Tietz, 1987).

Acanthocytosis can be attributed to alterations in the structure, contours and surface properties of the red cell-membrane, resulting from the abnormal phospholipids metabolism (Dacie and Levis, 1984).

5. Conclusion

- Occupational workers dealing with radiators, exhausts, electronics and car painters are at risk of developing lead poisoning; they should, therefore, take protection

steps against lead poisoning. These steps may include medical monitoring, educating the workers, and following the safety rules.

- Subclinical cases of lead poising may develop abnormal hematological parameters and RBC abnormalities.

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