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Lead Exposure Effects on Batteries Manufacturing Factory Workers in Baghdad

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Abstract

Background Lead is an environmentally persistent toxin that causes pathologies and induced oxidative stress by reactive oxygen species (ROS) causing reduction of antioxidants and a weakening of defense system of the cell.

Objective To evaluate the occupational lead level and its impact on workers in Batteries Manufacturing Factory / Baghdad.

Method Blood, hair and urine samples were taken from 45 occupational lead exposed workers in Batteries Manufacturing Factory in Baghdad with age ranged (25-63) years during the period from October 2010 to the end of January 2011. Flame and flameless Atomic Absorption spectrophotometer were used in the measurements of blood lead and hair lead concentrations, HPLC was used in the measurement of vitamin E concentration, and ELISA was used for the determination of 8-Hydroxydeoxyguansin concentration.

Results The results in this study showed a high concentration of lead in blood and hair for exposed workers in comparison with the normal corresponding values for the control. The results also showed that there was a significant decrease in δ –Aminolevulinic acid dehydratase activity, a low level of vitamin E in the serum and an increase in the level of 8-Hydroxydeoxyguansin in urine of exposed workers.

Conclusion The correlation between oxidative stress parameters and clinical indices implies that there is a disrupted antioxidant balance which might contribute to lead induced toxicity in erythrocytes.

Keywords Lead exposure, δ–Aminolevulinic acid dehydratase, 8- Hydroxydeoxyguansin, Antioxidants, Lead battery.

Introduction

Lead is a dangerous heavy metal which is widely spread in the environment. Lead content in the air, food and tap water has increased several folds during recent years due to extensive use of this metal in petrol, paints, battery and other industries [(1)]. The high lead levels in the blood of exposed workers are expected to produce clinical symptoms of lead intoxication such as anorexia, muscular pain and headache. Despite of many attempts to reduce the exposure to this metal, there are still some reports of cases with severe lead toxicity [(2-4)].

The pathogenesis of lead toxicity is multifactorial as lead directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body [(5)].

Lead affects directly the hair because lead is an electrophile that forms covalent bonds with sulfhydryl group of cysteine in proteins. Keratin in hair contains a high fraction of cysteine relative to the other amino acids and strongly binds to lead [(6)]. Lead toxicity leads to free radical damage via two separate, although

related, pathways (A) The generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxide. (B) The direct depletion of antioxidant reserves [5].

In any biological system where ROS production increases, antioxidant reserves are depleted. In this situation, the negative effects on the human systems ability to deal with increased oxidative stress occur via independent pathways, the body can limit and repair the damage of these species by some enzymes like ceruloplasmin (Cp), superoxide dismutase (SOD), glutathione peroxidase (GPx), Catalase (CAT), as well as by some vitamins such as vitamin A, vitamin E and vitamin C [7].

In a study of 137 lead exposed workers, those with high blood lead levels (over 40 µg/dl) had significant reductions in blood GPx that correlated with elevated erythrocyte malondialdehyde (MDA) level (a clinical marker of oxidative stress). Those with lower lead exposure (25-40 µg/dl) had elevated levels of GPx, a suggested compensatory reaction for increased lipid peroxidation [8].

On the other hand there are some studies which suggest that the supplement of vitamin E reduce the effect of oxidative stress which is induced by lead levels increasing SOD and catalase activity [9-11].

Nakao *et al* found that the erythrocyte δ–Aminolevulinic acid dehydratase (δ–ALA-D) activities of exposed workers ranged from 10 to 58 mmol/ml of ertherocytes/hr which is lower than normal [12].

Urinary δ–AminolevulinicAcid (δ–ALA) and (δ–ALA-D) activity in blood were convenient indicators with a better specificity if compared with other indicators such as porphobilinogen (PBG), and zinc protoporphyrinogen (ZPP), for screening occupational lead exposure [13]. δ–ALA-D concentration is currently regarded as the most reliable index of exposure to lead [12,14,15].

8-Hydroxydeoxyguanosine (8OHdG) is a modified base that occurs in DNA due to attack by hydroxyl radicals that are formed as by-products and intermediates of aerobic metabolism and during oxidative stress. 8OHdG has become increasingly popular as a sensitive, stable and integral marker of oxidative damage in cellular DNA.

A significant increase in the concentration of 8-OHdG is caused by exposure to tobacco smoke, heavy metals and ionizing radiation. 8-OHdG is correlated with oxidative stress and damage to DNA, which leads to the development of an antibody [16], this antibody can be used in the measurement of (8-OHdG) in urine using immunoassays technique, so (8-OHdG) can serve as a biomarker of oxidative stress [17].

Methods

Chemicals and Instruments

During the work of this study a large number of chemicals has been used, and many instruments, therefore it has been decided to mention these chemicals and instruments with their origin in the text of procedure instead of having a list of these chemicals and instruments.

Methods

This study was carried out in Batteries Manufacturing Factory in Baghdad and the laboratory investigations were conducted in the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University within 6 months period starting from the first of October 2010 till the end of January 2011. The occupational lead exposed workers were 45 total, 30 were males while 15 were females; their age between (25-63) years and mean of 43.2±8.9 years. They were in contact with lead in different parts of the factory.

Forty healthy volunteers with matching age and Body Mass Index (BMI) were chosen as control. Prior to biological specimens' collection, occupational and clinical information were collected from the exposed subjects and control group using questionnaire and interviews. Dietary intake and food habits of subjects were also recorded. Workers suffering from major chronic diseases such as diabetic mellitus, heart or kidney diseases were excluded from this study. Random urine samples were collected to

avoid the errors from the inadequate collection of 24hr urine samples.

Determination of lead concentration

● In whole blood

After the dilution of samples and standards (1:10) with distilled water, the blood was homogenized by (25ml) of 10% triton X-100 (Riedel-deHaen). This step was followed by the addition of 5ml of 20% ammonium dihydrogen phosphate (Merk-Darmstdt), with one ml of concentrated nitric acid (BDH) then, the volume was completed to 500ml with distilled water, and the measurements were carried out at 283 nm, using flameless atomic absorption spectrophotometer (Graphite furnace) (GFAS), (GFA-EX7i –Shimadzu).

●In hair

After the digestion of the hair by concentrated nitric acid (BDH) then the solution was diluted (1:50) with deionized water. The calibration curve was plotted automatically by the instrument itself. Flame atomic absorption spectrophotometer (Shimadzu-6200) was used in this determination.

Determination of urinary δ-ALA concentration and the activity of δ-ALA dehydratase

A spectrophotometer (UV/VIS), (biotech-UV-2601-UK), was used in the measurement of urinary ALA concentration by method of Wada *et al.* 1969 [12]. The elevated urinary ALA concentration was indicated by reddish color in chloroform, while normal concentration usually gives faint yellow or faint red color at 556nm.

The activity of δ-Aminolevuilinic acid dehydratase in erythrocytes was estimated using Helen's method [18]. The principle of method to determine δ-Aminolevulinic acid dehydratase activity in blood is to adequately control the pH of the enzyme substrate solution at the optimum throughout the incubation period. This control of pH was to improve the sensitivity and reliability of the assay. N-ethylmaleimide (Fluka Company) has been substituted for a potentially hazardous mercury salt, used to remove sulfhydryl groups before color development with a modified Ehrlich's reagent (Merk-Darmstdt).

Evaluation of vitamin E level in serum

High Performance Liquid Chromatography (HPLC), (shimadazu–Japan), was used for this purpose. The serum was deproteinized by mixing with 15% sulphosalicylic acid (Fisher Scientfic UK limit). The mixture was then centrifuged, diluted, and analyzed at 285 nm.

Estimation of the 8-Hydroxydeoxyguansin level in urine

Urinary 8-hydroxydeoxyguansin level was measured using, Enzyme Linked Immunosorbent Assay (ELISA), (Biotech-ELx800/England), according to the procedure of the kit cayman USA (Item No. 58920).

Results

Results in table 1 show the lead levels in the blood and hair of exposed workers which reflects the different duration of exposure.

Table 1 shows also a high level of lead in the hair of exposed workers when compared with that of control. This high level of lead in hair becomes even higher with longer duration of exposure.

Figure 1 show the correlation between the lead level in the blood for exposed workers and lead level in their hair (r=0.4302).

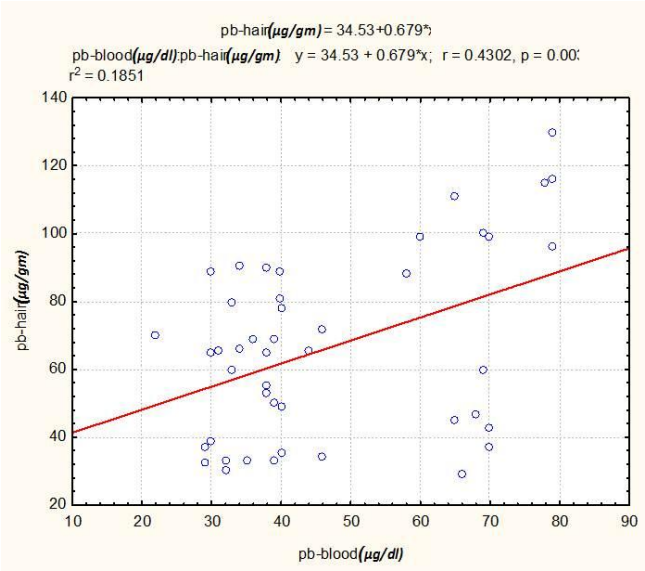

Figure 1. Correlation between pb-hair and pb-blood in EW

The results in table 2 show the activities of δ-Aminolevulinic acid dehydratase in the blood of exposed workers and the control group. The results in table 2 show the concentrations of δ-Aminolevulinic acid (ALA) in the urine of the exposed workers and the control group. The results in table 3 show the concentrations of 8-OHdG in the urine of exposed workers and the control group.

Table 1. The effect of exposure duration on the concentrations of lead in blood and lead in hair for exposed workers (EW)

Groups		Sample size	Pb-blood (µg/dl) Mean±SD	Pb-hair (µg/gm) Mean±SD
NEC		40	13.15±5.64	16.3±9.15
EW	0-10 years	8	34.3±7.28^{a*}	59.6±22.89^{a*}
	11-20 years	23	44.5±14.52^{a*,b}	56.9±20.5^{a*,b}
	21-30 years	9	52.97±18.79^{a*,b*,c}	74.4±26.0^{a*,b,c,}
	31-40 years	5	69.0±15.18^{a*,b*,c*,d}	107.5±24.46^{a*,b*c*,d†}

NEC: non exposed control, EW: exposed workers, †= P<0.01, * = P<0.0001, a = comparison between EW exposure duration and control group, b = comparison between exposure duration for 0-10 years period and other subgroups, c = comparison between exposure duration for 11-20 years, 21-30 years period and 31-40 years period, d = comparison between exposure duration for 21-30 years period with 31-40 period.

Table 2. The effect of lead exposure on the U δ-ALA concentration and δ-ALAD activity in the blood for exposed workers

Groups		No.	Urineδ- ALA Mean±SD (µmol/L)	δ-ALA dehydratase Mean values (mmol/ml of erythrocytes/hr)	p-value
NEC		40	3.6±o.65	97.4±10.5	
EW	0-10 years	8	4.6±1.64a*	60.7±14.8a*	t^a -p<0.0001
	11-20 years	23	7.3±1.2a*,b*	47.1±9.9a*,b	t^b - p<0.0001
	21-30 years	9	9.2±1.4a*,b*,c*,	33.9±12.0a*,b†,c*,	t^c - p<0.0001
	31-40 years	5	13.2±0.69a*,b*c*,d* (ns)	31.1±7.2a*,b†,c*,d	t^d -p<0.0001

NEC: non exposed control, EW: exposed worker, U-δ-ALA: urinary δ-Aminolevulinic acid, δ-ALA-D: δ-Aminolevulinic acid dehydratase, † = P <0.01, * = P<0.0001, a = comparison between EW exposure duration and control group, b = comparison between exposure duration for 0-10 years period and other subgroups, c = comparison between exposure duration for 11-20 years, 21-30 years period and 31-40 years period, d = comparison between exposure duration for 21-30 years period with 31-40 period.

Table 3. The effect of exposure duration on the mean values of the urine 8-OHdG concentrations of the exposed workers (EW)

Groups		Sample size	Urine level of 8-OHdG Mean±SD (ng/ml)
NEC		40	100.1±16.33
EW	0-10 years	8	131.4±6.0^{a*}
	11-20 years	23	160.7±17.4^{a*,b*}
	21-30 years	9	178.0±15.6^{a*,b*,c*,}
	31-40 years	5	186.0±10.3^{a*,b*c**,d}

NEC: non exposed control, EW: exposed workers, 8-OHdG: 8-hydroxydeoxyguansin, † = P<0.01, * = P<0.0001, a = comparison between 8-OHdG concentration for EW and control group, b = comparison between exposure duration for 0-10 years period and other subgroups, c = comparison between exposure duration for 11-20 years, 21-30 years period and 31-40 years period, d = comparison between exposure duration for 21-30 years period with 31-40 period.

Table 4. The mean value of vitamin E concentrations (mg/dl) in serum of exposed workers (EW) according to exposure duration

Groups		Sample size	Serum level of vitamin E Mean±SD (mg/dl)
NEC		40	1.0±0.36
EW	0-10 years	8	$0.47\pm0.11^{a*}$
	11-20 years	23	$0.28\pm0.13^{a*,b*}$
	21-30 years	9	$0.23\pm0.09^{a*,b,c,}$
	31-40 years	5	$0.15\pm0.07^{a*,b*,c*,d}$

NEC: non exposed control, W: exposed worker, * = P<0.0001, a = comparison between EW and control group, b = comparison between exposure duration for 0-10 years period and other subgroups, c = comparison between exposure duration for 11-20 years, 21-30 years period and 31-40 years period, d = comparison between exposure duration for 21-30 years period with 31-40 period.

The results in table 4 show the concentrations of vitamin E for exposed and control group. Figure 2 shows the correlation between the concentrations of lead in blood and urinary δ – ALA for the exposed workers. Figure 3 shows the correlation between the concentrations of lead in the blood and the activities of δ-ALA-D in the blood for the exposed workers. Figure 4 gives the correlation between vitamin E values in serum and 8-OHdG in urine for the exposed worker.

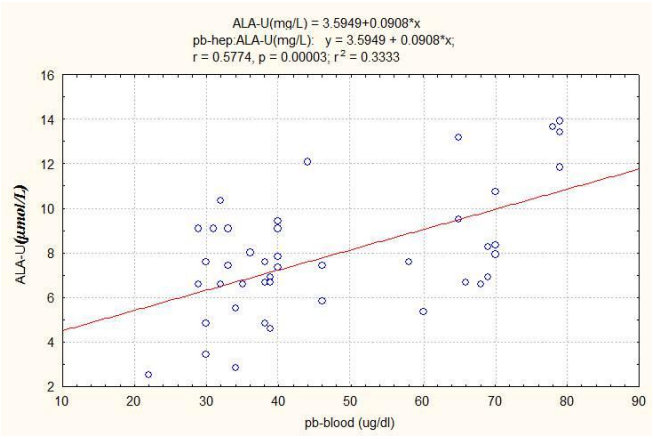

Figure 2. Correlation between ALA-U and pb-blood in EW.

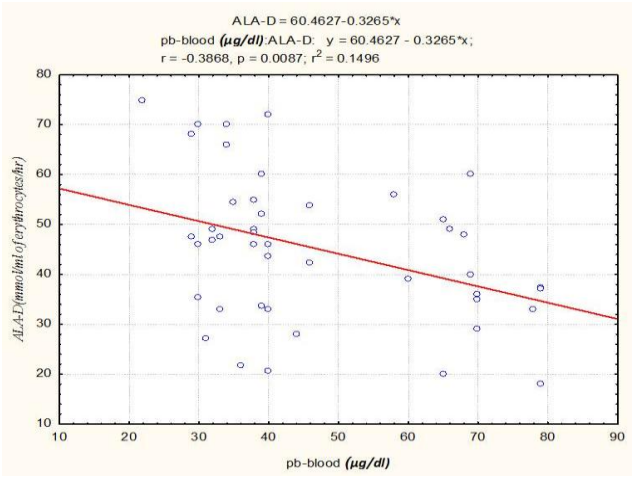

Figure 3. Correlation between ALA-D and pb-blood in EW

Discussion

The results in table 1 prove the expectation of pollution in this factory and show clearly that this group of exposed workers having a high concentration of lead in their body represented by their blood and hair. The measured values of the lead blood levels depend on an equilibrium between the lead that is stored in the different parts of the body and that which is excreted outside the body [19].

The longer duration of lead exposure caused an increase in the concentration of lead in the blood and hair, and the correlation between blood lead levels and that of hair lead was positive for exposed workers, (r=0.4302, p=0.0032).

Results in table 2 revealed that there is a decrease in the activity of δ-Aminolevulinic acid dehydratase (δ-ALA-D) and this decrease become even more with the increased duration when compared with that of the control group. The reason behind this decrease in the activity of

δ-Aminolevulinic acid dehydratase enzyme is the inhibition by lead. The enzyme of δ-ALA-D is the most sensitive enzyme to the toxic effects of lead [20], therefore it has been selected for this purpose.

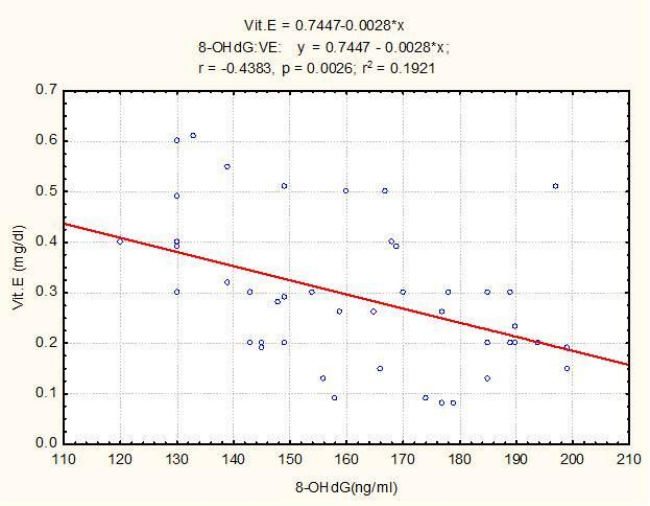

Figure 4. Correlation between vitamin E and 8-OHdG in EW groups

Table 2 shows also that the concentration of δ-Aminolevulinic acid (δ-ALA) in urine was higher than that of the control group and increases with increased duration. This increase in δ-Aminolevulinic acid concentration in urine can be explained as follows: Under normal physiological conditions, 90-95% of the ALA that is filtered is reabsorbed in the tubules. The reabsorption rate of ALA decreased with increasing serum ALA concentration [21]. The toxic damage to the renal tubules caused by the high concentration of lead may contribute to the decreased reabsorption rate [22]. The urinary δ-ALA and δ-ALA-D activity in the blood could be considered as biomarkers for the lead toxicity.

There is a positive correlation between the concentration of lead in blood and urinary δ-ALA for exposed workers with (r=0.5774,p=0.00003) as shown in figure 2, while figure 3 show a negative correlation between the concentration of blood lead and the activity of δ-ALA-D in the blood for the exposed workers where (r= -0.3868, p= 0.0087).

The results that have been obtained in table 3 show an increase in the concentration of 8-HydroxyDeoxyguanosine (8-OHdG) in urine and this increase become even more with the increase in the duration of exposure. The increase of 8-OHdG in urine could be explained depending on the fact that lead is inducing the oxidative stress which has been identified as the primary contributory agent in the pathogenesis of lead poisoning[23].

Results obtained in table 4 show a reduction in the level of vitamin E and this reduction increased even more with increasing duration of exposure. Vitamin E (Alpha-tocopherol, active form) protects the membrane lipoproteins of RBC from oxidative damage that is caused by lead toxicity through the lowering of lipid peroxide levels and increasing the activity of Superoxide dismutase (SOD) and catalase [24]. Therefore, this decrease in vitamin E enhances the susceptibility of RBC to the hemolytic effect of lead poisoning [25].

Figure 4 indicated a negative correlation between vitamin E in the serum and 8-OHdG in the urine for exposed workers (r= - 0.438, p= 0.00026).

The results of this study indicated that there is a definite oxidative stress due to lead pollution. This increased concentration of 8-OHdG in urine and impaired antioxidant such as vitamin E could be attributed to the increased blood lead concentration which induce oxidative stress.

Also, blood lead levels were considerably elevated in battery manufacturing factory workers, and the degree of elevation was increased with increasing their duration to lead exposure.

The determination of lead in hair could be considered as a useful screening test in estimating occupational lead exposure and it is a short term, inexpensive and non – invasive method.

The biomarkers urine ALA and erythrocyte δ-ALA-D activities levels are most suitable and convenient markers to screen and confirm occupational lead exposure.

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