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ORIGINAL ARTICLE

The relationship between blood lead level with iron status and hemopoietic parameters in smoker and non-smoker workers at lead battery factory

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Abstract The present study aimed at evaluating the effect of lead exposure on hemopoietic system (through the index delta-aminolevulinic acid dehydratase [δ-ALAD] activity, hemoglobin concentration [Hb], hematocrit concentration [Hct]) and on iron status (levels of serum iron [Fe], ferritin [Fr], total iron binding capacity [TIBC], percentage of transferrin saturation [TS%]) in Iranian workers at lead batteries factory. Forty-four workers were divided into two groups: smokers (n=21) and non-smokers (n=23). Also, 45 healthy subjects were studied as control group. δ-ALAD, iron status, and hematological parameters were evaluated. Activity of δ-ALAD ratio showed significant decrease (p<0.05), while Hb and Hct were non-significant (p>0.05) in smoker workers compared to non-smoker and control groups. There was no significant decrease (p>0.05) in the sera levels of Fe, TS%, and Fr in all workers as compared to control. There was a significant increase in the level of TIBC in workers as compared to control; this elevation is more in smoker than in non-smoker workers. In smoker workers, there was a significant positive correlation between the blood lead levels (BLLs) and Hb, Hct, and TIBC whereas a significant negative correlation was observed between BLLs and Fe and TS%. The results revealed the importance of monitoring the level of iron status in smokers who deal with lead and therefore anemia will develop for them. δ-ALAD ratio was considered to predict the efficiency of heme synthesis as a new marker for the diagnosis of early stage of anemia.

Keywords Blood lead levels (BLLs) · δ-ALAD · Iron status · Workers · Smoker

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Introduction

Lead is a heavy toxic metal for humans. It does not have any useful performance in human bodies, so it causes harmful effects when it enters into human body either by ingestion, inhalation, or dermal contact (organic lead) (Mohammad et al. 2008). Industry is the major source of lead pollution, particularly in battery factories (Mañay et al. 2008). The battery-manufacturing factories are one of the most important places for occupational lead poisoning, and workers in these factories are easily exposed to lead (Ghiasvand et al. 2013; Lormphongs et al. 2003).

Lead is related to a broad range of physiological, biochemical, and behavioral changes (Luch 2009). Lead toxicity is not only for hematopoietic system but also for gastrointestinal tract, central and peripheral nervous systems, and kidneys (Navas-Acien et al. 2007). The main target of lead toxicity is erythrocytes, and inhibition of a key enzyme, cytoplasmic δ-aminolevulinic acid dehydratase

(δ-ALAD), in heme biosynthesis is one of the major manifestations of acute lead poisoning (Flora and Pachauri 2010). δ-ALAD is the second enzyme in the heme biosynthetic pathway which catalyzes the condensation of two molecules of ALA to form one molecule of porphobilinogen (Hrehova 2012). δ-ALAD is one of the most sensitive indicators of blood lead accumulation due to exposure (Elezaj 2012).

The iron status, of industrial workers occupationally exposed to lead, is particularly important because substantial evidence supports that iron deficiency not only impairs worker performance but also may increase the absorption and biotoxicity of lead in animals and humans (Mason et al. 2014). Although metals such as copper, zinc, and iron are essential for human beings, chronic metabolic disturbances may result from an excess or lack of these metals (Josko 2011; Ozmen et al. 2013). The level of a metal in blood is considered as an index of biologically active metals in the body, which reflects the environmental exposure of population (Faris Mohammed 2013).

The aim of this study was to investigate and evaluate the iron status and δ-aminolevulinic acid dehydratase in blood of Iranian workers in battery-manufacturing factories so as finding the type of correlation between all iron status index and δ-ALAD activity with blood lead levels.

Material and methods

This study was approved by the ethics committees at the Shahrekord University of Medical Sciences. A total of 44 males with mean age 39.13±6.76 years (range 29–57 years) who were occupationally exposed to lead in battery manufacture for the period of 10–20 years (mean 13.68±1.99 years) was enrolled in this study. The workers were divided into two groups: smokers (n=21) and non-smokers (n=23). A total of 45 healthy male volunteers with mean age 33.97±5.08 years (range 24–45 years) served as controls.

Blood samples (about 10 ml) were taken from each individual. The blood sample was divided into three tubes, 2 ml (EDTA tube) for estimation of Hb and Hct, 4 ml (EDTA tube) for lead estimation, and 4 ml of blood was left for short time to allow blood to clot, then clear serum sample was obtained by centrifugation at 1500g for 5 min to measure iron (Fe), total iron binding capacity (TIBC), and ferritin (Fr). Measurement of blood Pb level was carried out by flame atomic absorption spectroscopy (Buck 210 VGP, USA). Hb was assayed by the cyanomethemoglobin method. Hct concentration was measured following centrifugation in a micro hematocrit centrifuge (H-1200F, Kokusan Denki, Japan). Serum iron and TIBC were measured by colorimetric assays using kit supplied by Randox, UK. Serum Fr was measured by the ELISA method. Percentage of transferring saturation (TS%) was calculated by dividing Fe by TIBC and multiplying by 100. Erythrocyte δ-ALAD activity was estimated by a new modified colorimetric method. In this method, erythrocyte ALAD acts as an ALA to form porphobilinogen (PBG) which further reacts with regular Erilch's reagent to form a pink-colored compound which can be measured using a spectrophotometer at 555 nm. Hg-TCA solution stops the reaction by precipitating the proteins. The erythrocyte ALAD activity activated by zinc chloride and dithiothreitol (DTT) was also measured, and the ratio of activated versus non-activated ALAD was determined.

The data was analyzed using SPSS statistical package version 16. For comparison of hematological and other parameters between workers and control groups, Student's t test was used. Subsequently, correlation analysis was determined. p value <0.05 was considered significant.

Results

As shown in Table 1, the differences of exposure duration were non-significant for smokers (12.206±2.10 years) and

Table 1 Hematological findings for both workers and control group

	Smoker (n=21)		Non-smoker (n=23)		Control (n=45)
	Mean±SD	p value	Mean±SD	p value	Mean±SD
Age (year)	37.97±4.82	NS	40.78±7.89	NS	33.97±5.08
Duration of exposure (year)	12.20±2.10	NS	14.16±1.32	NS	–
Blood lead level (BLL) (μg/dl)	26.57±5.24	0.0001	24.73±4.49	0.0001	16.64±1.88
δ-ALAD ratio	0.262±0.067	0.05	0.310±0.089	NS	0.316±0.116
Hemoglobin (g/dl)	14.78±1.37	NS	14.11±2.04	NS	14.29±1.59
Hematocrit (%)	44.30±4.10	NS	42.78±7.24	NS	42.86±4.77

NS non-significant

non-smokers (14.16±4.32 years). The mean±SD BLLs in smokers (26.57±5.24 μg/dl) and non-smokers (24.73±4.49 μg/dl) significantly ($p<0.000$) increased as compared to the control group. The ratio of δ-ALAD activity was significantly decreased in smoker (0.262±0.067) while the decrease in non-smoker group (0.310±0.089) in comparison to the control group was not significant. Non-significant differences were observed in Hb and Hct for lead-exposed workers, but a slight increase in the smoker group and decrease in the non-smoker group were observed.

The mean±SD serum iron levels of smokers (73.23±23.80 μg/dl) and non-smokers (76.43±29.36 μg/dl) showed non-significant decrease as compared to the control group (Table 2). The mean±SD of serum TIBC for non-smokers (478.61±196.73) significantly increased while smokers (542.24±161.62) showed a non-significant increase compared to the control group. In comparison with the control group, TS% was significantly decreased in non-smokers (18.95±13.88) but the decrease for the smoker group was not significant (16.54±11.90). Non-significant differences of serum ferritin were found for smokers (102.10±76.42) and non-smokers (92.60±55.67) as compared to the control group.

Table 3 shows the results of the analysis of correlation between BLLs and iron status index (represented by Fe, TIBC, TS, and Fr) of workers. A significant positive correlation was noticed with Hb ($r=0.441$, $p=0.045$), Hct ($r=0.440$, $p=0.046$), and TIBC ($r=0.458$, $p=0.037$), while a highly significant negative correlation was observed with Fe ($r=-0.545$, $p=0.011$) and TS% ($r=-0.663$, $p=0.001$) for smoker workers. δ-ALAD ratio for non-smokers showed no correlation or non-significant correlation between BLLs and all other parameters that were studied in this survey.

Table 3 Correlation coefficients and the significant levels of BLLs with different parameters, hemoglobin, and components of iron status in lead workers

	Smoker ($n=21$)		Non-smoker ($n=23$)	
	r	p value	r	p value
Hb	0.441	0.045	−0.267	0.267
Hct	0.440	0.046	−0.291	0.178
Fe	−0.545	0.011	0.267	0.218
TIBC	0.458	0.037	0.076	0.981
TS%	−0.663	0.001	−0.005	0.981
Fr	0.174	0.453	0.079	0.720

Discussion

The results of this study emphasize a significant increase in mean of BLLs for the worker group as compared to control group. The smoker workers have more BLLs than non-smokers, while the duration of exposure of the smoker workers is less than that of non-smoker workers. This finding is consistent with reports from other regions (Faridah 2013; Gottesfeld and Pokhrel 2011). Effect of smoking on the BLL in workers is due to increase of hand to mouth movements especially in workers that do not use protective masks. Also, tobacco plants may invariably contain certain amounts of lead absorbed from the soil (Annan et al. 2010; Kalicanin et al. 2014).

The ratio of δ-ALAD activity, which is highly sensitive and specific for lead exposure, can be used to diagnose a lead-exposed individual (Gültepe et al. 2009). In the present study, the mean ratio of δ-ALAD non-significantly decreased in workers as compared to the control group. This result indicates that lead inhibits the activity of δ-ALAD for these workers. δ-ALAD is usually reduced to 50 % or less from its normal activity when blood lead values are in the range of 30–50 μg/dl (Dongre et al. 2011). At the molecular level, lead displaces zinc ion at the metal binding site, not the active site, producing inhibition through changing the enzyme's quaternary structure. Decreased δ-ALAD activity caused by lead can be reversed by adding Zn or DTT or by heating (Rocha et al. 2012).

Table 2 Iron status findings for both workers and control group

	Smoker ($n=21$)		Non-smoker ($n=23$)		Control ($n=45$)
	Mean±SD	p value	Mean±SD	p value	Mean±SD
Fe (μg/dl)	75.23±23.80	NS	76.43±29.76	NS	88.22±30.67
TIBC (μg/dl)	542.2±161.6	NS	478.6±196.7	>0.0001	421.8±117.6
TS (%)	16.54±11.90	NS	18.95±13.68	>0.05	22.52±9.59
Fr (ng/dl)	102.1±76.4	NS	92.60±55.67	NS	114.0±88.03

NS non-significant

Lead affects the hematopoietic system through reduction of hemoglobin synthesis, but this occurs only when high levels of exposure is presented. Hb and Hct for non-smoker were slightly decreased. It might be due to decreased heme and globin synthesis or erythrocyte formation and function. Erythrocyte survival is also decreased by lead due to inhibition of membrane Na^+/K^+ ATPase activity (Malfatti et al. 2012). Smoker Hb and Hct levels were slightly increased. It may be due to smoking effects whereas CO binds hemoglobin 250 times more than O_2 producing carboxyhemoglobin which produces hypoxia, and this will accelerate the erythropoiesis (Tomei et al. 2008).

Iron is an essential element which plays a critical role in the heme synthesis pathway. Fe, TIBC, TS%, and Fr levels were used clinically to evaluate the iron status. The lower iron status of workers exposed to lead could be attributable to higher BLL or lower dietary iron intake (Keramati et al. 2013). Many studies reported that low iron status causes a higher absorption of lead in the gastrointestinal tract resulting in higher BLLs (Hegazy et al. 2010; Khan et al. 2011), and the theoretical basis for this observation is that iron extraction from the diet is small and limited, as humans have no physiological pathway for Fe excretion. Duodenal enterocytes are responsible for iron absorption. Iron is transferred across the apical membrane of the enterocyte into the cell using a protein named divalent metal transporter 1 (DMT1). DMT1 is not specific for iron; it can transport a wide variety of divalent metal ions, including Cu, Zn, and Pb. Therefore, if the iron content of the diet is low, the other divalent metal ions may be absorbed instead including trace quantities of lead (Keramati et al. 2013). In a previous study (Gültepe et al. 2009), δ-ALAD ratio was examined as a new diagnostic marker for heme synthesis by using the ratio instead of activated values.

According to our knowledge, this is the first study on this enzyme by using a new modified method. To predict whether the value of BLLs can be used to avoid any hematopoietic damage in people exposed to the lead, iron status parameters may be used from Table 3. We found a good positive correlation between BLLs and Hb, Hct, and TIBC while a negative correlation between BLLs and Fe and TS%. These correlations represent the direct effect of lead on iron status and heme synthesis. Our findings indicate that the increased levels of lead are associated with a reduced iron concentration in smoker peoples, which will impair their heme synthesis pathway.

Conclusion

It can be concluded that the decreasing in iron status for the smoker workers exposed to lead may be a beginning for iron deficiency especially their blood lead levels show a highly significant increase and the activity of δ-ALAD ratio was significantly decreased.

Conflict of interest None declared.

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