δ-amino Levulinic Acid Dehydratase in Relation to Blood Lead Level in Occupational Lead Exposure

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ABSTRACT

The lead level in blood and the activity of δ-amino levulinic acid dehydratase (ALAD) were determined in blood samples taken from lead smelter workers and unexposed lead workers. The lead concentrations were higher in the blood of lead workers (17 subjects) than controls (47 subjects) with mean values of 50 ± 16.86 and 28.98 ± 6.35, respectively. Close negative correlation between PbB and ALAD was found in all subjects. The ALAD activities were lower in smelter's blood than in the control group by about 42.5%.

Determination of ALAD activity in erythrocytes is considered a suitable method for demographic studies of exposure to lead. The test is too sensitive for routine follow-up of the degree of lead intoxication in occupationally exposed workers.

INTRODUCTION

Lead is a poisonous element which tends to accumulate in the tissues and bones of exposed individuals. It can be absorbed by inhalation from air or by ingestion of solids or liquids, especially by occupational lead workers. Most lead is excreted through the kidneys, but the process of elimination is much slower than the rate at which it is absorbed[1]. Prolonged exposure to lead is known to exert toxic effects on the renal, haemopaetic, embryonic and central nervous systems[2-5].

The measurement of lead in whole blood has been, for many years, the principal biological index of dosage because it is an accessible estimate of the biologically active part of the body burden of lead. This was because of the effects of lead on the hematological system and the fact that there is a gradation of these effects through the (normal) range of exposure[6]. Moreover, there is a close correlation with blood lead levels and certain indices of haematotoxicity, particularly ALAD and erythrocyte protophorin which have been increasingly considered as alternatives or co-indicators of exposure, as well as being indices of effect[7-9].

ALAD is the sulphydryl enzyme responsible for the condensation of two molecules of δ-amino levulinic acid (ALA) to form the monoprolle prophobilinogen needed for haemoglobin sysnthesis which has been inhibited both *in vitro* and *in vivo* in animals and humans[8,10-13]. Lead affects ALAD in peripheral blood to the same extent as in the erythroid cells in the bone marrow, so that the measurement of ALAD activity in blood samples is representative of the inhibition of this enzyme in erythopoiesis[14].

Smelter workers are exposed to a relatively high level of lead fumes or finely divided lead dust. It must be known if they reach the critical blood lead (PbB) level "triggers" when a worker should be removed from the job or known ALAD activities as a sensitive response tool of lead exposure[15].

The aim of this study is to investigate the

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inter-relationship between indices of lead exposure in lead smelter workers and lead effects on the hematopoietic system. It provides additional proofs of ALAD activities estimation as a reliable and sensitive technique for analysis of the blood of humans exposed to lead.

MATERIALS AND METHODS

Subjects

The subjects under study were heavily lead exposed. They were seventeen male workers from a fertilizer plant (at Kafr El-Zayat, Egypt), working there for two to thirty years. They melted and used molten lead in repairing tubes and acid dilution containers at the plant. In this place the lead fumes are liberated and the atmosphere of the place is contaminated with dust containing lead oxide and particles. A control group was formed of forty-seven male manual workers from the same fertilizer plant. None of the members of this group had any known occupational contact with lead.

Lead Analysis and ALAD Activities Determination

Blood samples (10 ml) were drawn from each subject's (cubital) arm vein into heparinized test tubes. All samples were stored at +4°C in the dark until analyzed. Blood lead levels were determined using a method of Zinterhofer, et al. (1971)[16]. Lead ammonium pyrrolidine dithiocarbamate complex was extracted into methylisobutyl ketone, then aspirated into flame atomic absorption (Shimadzu type 630-11).

The activity of ALAD in red blood cells was determined and calculated by the method of Burch and Siegel (1971)[21]. Units of enzyme activity were expressed as an increase in absorbance (at 555 nm) of 0.100 (with a 1.0 cm light path) per ml of erythrocytes per hour assayed at 38°C and calculated by the following formula:

$$\text{Unit of ALAD activity} = \frac{\text{Corrected absorbance} \times 100 \times 12.5 \times 10}{\text{hematocrit}}$$

where 12.5 is the dilution factor of the blood. The hematocrit values were measured by the micro-hematocrit method using an international micro-capillary centrifuge at 7500 rpm for five minutes.

Table 1
Comparison of Values Given by Various Authors as Normal Subjects for ALAD Activity in RBC*.

Author	Nation	No.of subj.	ALAD activity in RBC(units)+
Lichtman & Feldman (1963)[11]	American	16	28-327 mean 178
Bonsignore, et al., (1965)[17]	Italian	18	80-120 mean 100
Bonsignore (1966)[18]	Italian	50	60-120 mean 90
Nakao, et al., (1968)[19]	Japanese	?	758-1082 mean 920
deBruin & deJong-Heisterkamp(1968)[20]	Dutch	10	29-111++ mean 86
Hernberg & Nikkanen (1970)[8]	Finish	16 10	50-190++ mean 86
Haeger-Aronsen, et al., (1971)[10]	Swedish	50	mean 121 SD = 32
Present study	Egyptian	47	mean 148.9 SD=23.1

ble 1:
he method of Granick and Mauzerall was used by
chtman and Feldman and by Nakao, et al., while
l other authors used method of Bonsignore, et
.

ctivity of ALAD in RBC expressed as $x10^{-3}$ µmol
PBG synthesized per milliliter of packed RBC
r hour of incubation.

Evaluated from a given figure.

RESULTS AND DISCUSSION

Table 1 illustrates the comparison of values
ven by previous authors as normal for ALAD
tivity in RBC with the present study. The mean
AD activity in blood of the unexposed workers
148.9 units ±23.1 (n=47). This agrees with
ose on record (Table 1) except for a Japanese
eries in which the values fell far to the out-
de ranges of all the others (Nakao, et al.,
68)[19]. In order to depend on the ALAD activity
a sensitive tool for lead exposure it should
set up using the values of normal people for
AD activity to follow the changes in this index.

Table 2 shows that lead concentrations were
gher (50 µg PB/dL) in lead smelter workers,
ile the lead level of the control group for
is study was 27 µg Pb/dL.

Table 2
ead Blood Levels (PbB) and δ-amino Luvinulinic
id Dehydratase ALAD in Lead Exposed and Non-
xposed Workers.

roup	No.	Pb (µg/dL)	ALAD activity in RBC (unit)
ead workers	17	50 ±16.8	85.5 ±31.5
ontrol	47	27 ±6.3	148.9 ±23.1

Although low-level expsoure is tolerated, lead acts as a poison when taken into the body in sufficient quantity. Recognizable symptoms of poisoning are known to occur in some individuals when levels of lead inthe blood stream exceed 70-80 µg (Pb) per 100 ml of whole blood. It was found that the concentration of lead in the smelter of this study is high but below this threshold value with a mean of 50 µg/Pb/dL ±16.8. In comparison, typical levels of lead in the blood of the general population lie between 10 and 30 µg (Pb) per 100 ml for adults. The lead levels of the control group for this study are within this range (27 µg/dL ±6.3).

Figure 1 presents a scatter plot of ALAD activity in RBC and blood lead concentrations in µg Pb/dL for seventeen lead workers and forty-seven occupationally unexposed subjects. The correlation between ALAD activity and blood lead level was found to be curvilinear in the control group and the lead workers. The high blood lead level combined with a relatively low ALAD activity in RBC, however, fitted well with the results obtained in previous studies[22-24].

Inhibition of red blood cell ALAD has become accepted as a standard bioassay to detect acute and chronic lead exposure in himans[8]. Our investigation has shown that activity of ALAD in RBC accurately reflects the amount of lead in the blood. Activity of ALAD diminishes in lead poisoning. The variation of ALAD activity with the concentration of lead in blood are exponential and the correlations inverse.

In conclusion, it is recommended to depend on ALAD activity as a sensitive, accurate and easy tool for the monitoring of lead toxic effects after high or low exposure. This biochemical parameter can be done by a low volume of blood sample using easy spectrophotmetric techniques.

Figure 1

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Fig.1: Correlation Between ALAD Activity in RBCs and Blood lead Level in 47 Normal (Dots) and 17 lead Exposed (Triangles) Workers.