

THERAPEUTIC POTENTIAL OF THIAMINE IN LEAD TOXICITY -A CLINICAL STUDY

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S u m m a r y Occupational exposure to lead is hazardous and hence requires to be prevented and treated. The use of sensitive biochemical markers such as erythrocytic ALAD and renal tubular lysosomal enzyme NAG are gaining importance as early parameters to detect exposure to lead and sub-clinical toxicity. The monocasters, occupationally exposed to lead continuously for 10-15 Years had significant high blood lead levels ($44.1 \pm 1.3 \mu\text{g/dl}$), impaired ALAD activity ($227 \pm 57 \text{ nmole/hr/ml}$)-coupled with increased % stimulation in vitro (239 ± 72) and elevated enzymuria (NAG activity $11.9 \pm 0.8 \mu\text{mol/g creatinine}$) as compared to normal subjects indicating sub-clinical toxicity of soft tissues. Thiamine, a vitamin which has sulphhydryl group was assessed as chelator of lead from tissues. With the administration of thiamine there was a significant reduction in blood lead levels ($33.2 \pm 1.8 \mu\text{g/dl}$), and reversion in basal ALAD activity ($281 \pm 33 \text{ nmol/hr/ml}$) and in vitro % stimulation (154 ± 7.0) in a span of 12 months. The results indicate the beneficial effect of thiamine in the treatment of chronic subclinical lead toxicity.

Key words Thiamine subclinical lead toxicity occupational lead toxicity

Lead intoxication remains a public health problem. Millions of workers throughout the world and the common man are exposed to lead through the occupation and environment. Occupational or environmental exposure to lead is hazardous and its toxic effects are well documented^{1,2}. Low levels of lead exposure often do not result in the manifestation of toxic symptoms, but sub-clinical toxicity of haematopoietic and renal system are reported^{3,4}.

The decreased erythrocytic delta amino levulinic acid dehydratase activity (ALAD) and increased urinary excretion of renal tubular lysosomal enzyme N-acetyl-B-D-glucosaminidase (NAG) are reported to be sensitive indicators of early haematopoietic and renal toxicity respectively^{5,6}.

Until now the treatment of acute and severe lead poisoning involves use of well known potent chelators like ethylene diamine tetra acetic acid (EDTA), Dimercaprol (BAL) etc. But use of these existing potent chelators have its own disadvantages like renal toxicity, pain at the injection site, low absorption, etc.^{8,9} The development of antagonists which are safe in treating lead intoxication is of considerable significance. Efforts are therefore on to identify the safest compounds for preventing/treating chronic lead poisoning cases. In the last decade attempts have been made to identify therapeutic potentials of various vitamins and naturally occurring substances in treating/preventing lead toxicity^{10,11}.

The therapeutic potentials of thiamine in preventing lead toxicity in rats, calves and sheep have been reported to be promising¹¹⁻¹³.

Therefore the present study has been envisaged to evaluate the sub-clinical toxicity of lead (Pb) on haemopoietic and renal system using sensitive indices and to evaluate therapeutic potential of thiamine in treating the lead toxicity in monocasters who are occupationally exposed to lead fumes.

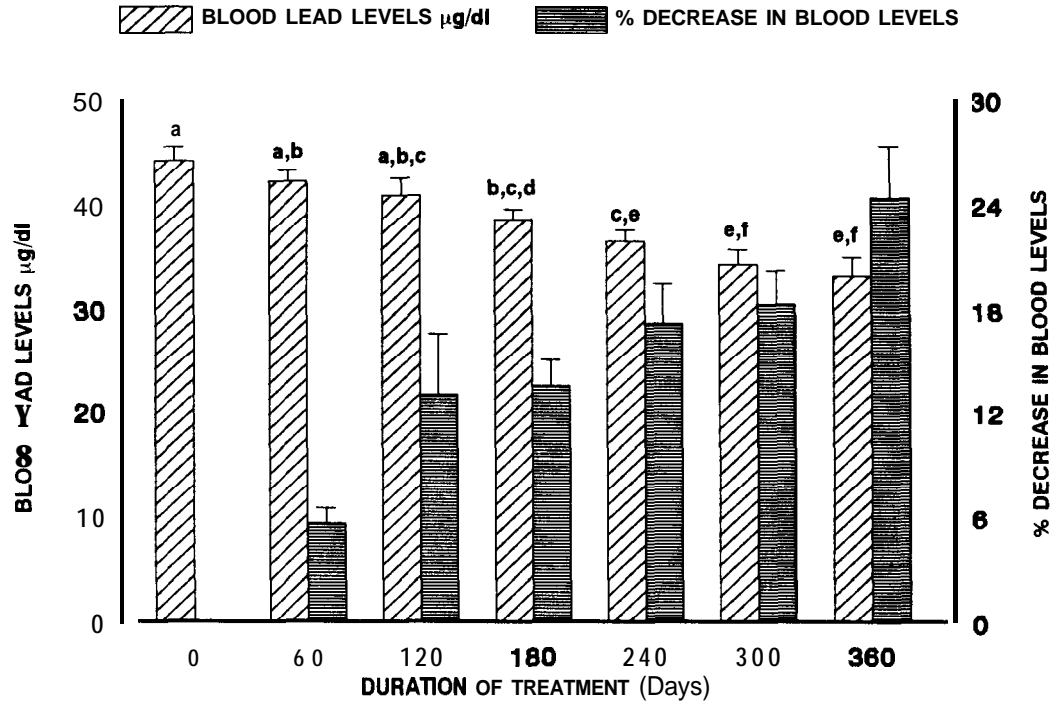
MATERIALS AND METHODS

Thirteen male individuals aged between 22-44 years, who were occupational monocasters since 10-15 years employed in a printing press, volunteered for the study. Twenty seven normal male individuals, aged between 32-45 years with no history of anaemia/renal disorder/hypertension/diabetes and occupational exposure were selected from the institute staff to serve as controls. All the subjects were clinically examined for various lead toxicity signs and symptoms. The study was undertaken after taking approval of ethics committee.

The blood lead levels were estimated in whole blood by a graphite furnace atomic absorption spectrophotometry¹⁴, using diammonium hydrogen phosphate as matrix modifier and triton-X-100 as a solubilising agent. Five μl sample aliquots were injected into the graphite furnace, dried, ashed and

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Figure 1. Effect of thiamine on blood lead levels. Values are expressed as Mean \pm SE. Values bearing different superscripts are significant $P < 0.05$.



atomised. The absorbance of lead was read and compared with known concentration of lead at 283.3 nm. It was observed that method was sensitive enough to detect 0.02 $\mu\text{g/dl}$ of lead in blood with 99% of recovery.

Urinary NAG activity was measured by spectrophotometric method¹⁵. The enzyme was separated from urine by filtration using sephadex G-25. The activity was assayed in a reaction mixture using the substrate P-nitrophenyl N-acetyl B-D-glucosaminide. The released amount of P-nitrophenol was measured after arresting the reaction with 2-amino 2-methyl 1-propanol buffer (AMP).

The basal ALAD activity was determined by a modified method of Granick et al¹⁶ where in the presence of low ALAD activity and the complete restoration of same (% stimulation) in presence of 20 mmol of dithiotheritol (DTT) was also measured.

Thirteen monocasters participated in the trial. The treatment schedule initially was 50 mg thiamine in the form of tablet twice a day for the first three months, 100 mg twice a day for the next nine months with an addition of 10 mg/ml/day intramuscular injection twice a week from six months onwards till the end of the study. Our earlier studies⁷ on absorption and retention of thiamine by oral and parenteral route has indicated that the maximum absorption

is 14 mg and half maximum absorption on a oral dose is 20 mg, while retention of thiamine by both routes is not more than 15 mg even after administration of mega doses of thiamine from 50 to 200 mg. Hence the dosage schedule was to begin with 50 mg (112 tablet) twice a day. As patients were not satisfied with a 1/2 tablet the dose was increased to 100 mg and intramuscular injections were given only to satisfy the patients. The blood samples were drawn at 2,4,6,8,10 and 12 months. The results of the study were computed and analysed by analysis of variance (ANOVA) value. P value less than 0.05 was considered significant.

RESULTS

The basal mean blood lead level in the monocaster was almost double as compared to that in control subjects (Table 1). The blood lead levels decreased significantly from fourth month onwards after initiating thiamine therapy and there was approximately a 20% decrease at the end of 12 months treatment (Figure 1).

A significant decrease in blood ALAD activity and a greater % stimulation of same with DTT was seen in monocasters (Table 1). The basal ALAD activity increased significantly in the first two months after which, it remained static till the end of the study.

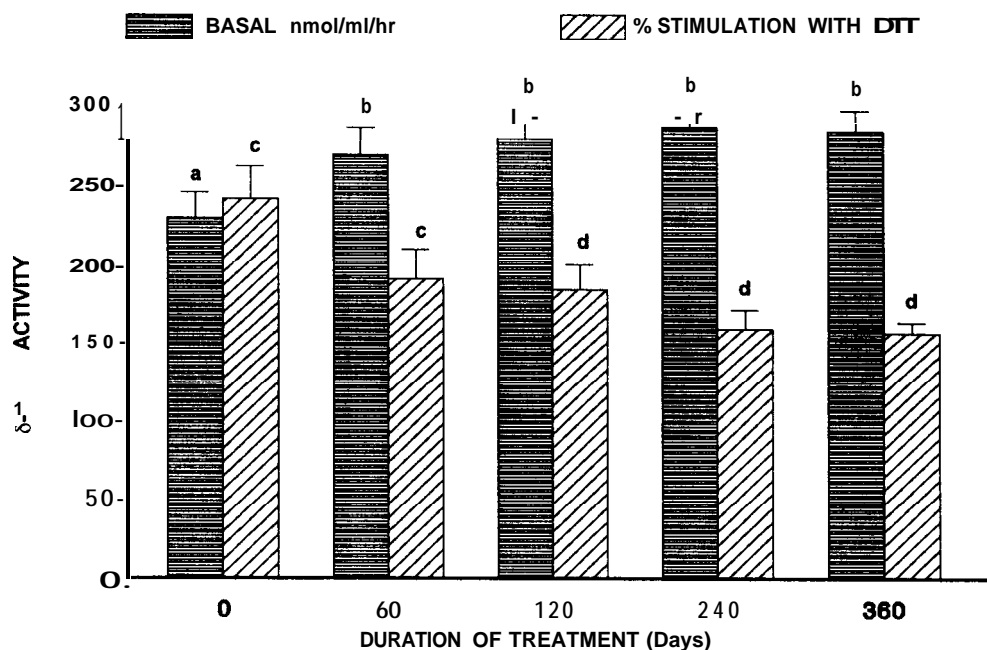
Figure 2. Effect of thiamine on blood δ -ALAD activity.Values are expressed as Mean \pm SE. Values bearing different superscripts are significant $P < 0.05$.

Table 1. Blood lead, blood ALAD and urinary NAG levels on control subjects and monocasters.

Biochemical parameters	Control subjects n (27)	Monocasters n (13)
Blood lead $\mu\text{g/dl}$	23.2 \pm 0.8 (18.9 - 30.9)	44.1 \pm 1.3' (31.9 - 50.1)
Blood ALAD activity		
Basal $\mu\text{mol/hr/ml}$	355.0 \pm 14.9 (250.2 - 431.0)	227.2 \pm 15.8' (122.5 - 326.4)
<i>In vitro</i> % stimulation	157.53 \pm 6.5 (126.2 - 199.4)	239.2 \pm 19.9* (127.4 - 394.8)
Uninar NAG $\mu\text{mol/gm creatinine}$	4.27 \pm 0.5 (0.97 - 6.9)	11.9 \pm 0.77' (8.3 - 17.4)

Values are expressed in Mean \pm SE.

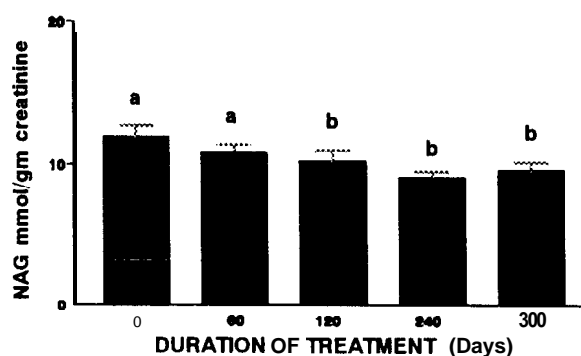
Figures in the parenthesis indicate the range of values.

n = number of observation. * $P < 0.05$

On the other hand, % stimulation decreased further from 3 to 6 months and reached levels comparable to that of controls by six months and there was no further change till the end of the study (Figure 2).

There was a three fold elevation in the urinary NAG activity in monocasters as compared to normal subjects (Table 1). After the treatment, there was no significant decrease in the urinary NAG activity till three months. A significant decrease in the urinary

Figure 3. Effect of thiamine on urinary NAG activity.

Values are expressed as Mean \pm SE. Values bearing different superscripts are significant $P < 0.05$.

NAG activity was observed from fourth month onwards and it remained at the same level till the end of the study (Figure 3).

DISCUSSION

Mild or moderate lead poisoning produces a variety of symptoms, many of which are not typical of the classical symptoms of lead poisoning. But the advancement in the techniques of estimating micro enzymes etc. has made it possible to detect sub-clinical toxicity caused by lead. In the present study, the decreased ALAD activity coupled with increased

percentage stimulation with DTT is indicative of haemopoietic toxicity even at the levels of lead which are below the toxic levels prescribed by WHO¹⁸. Granick et al¹⁶ and Somashekaraiah et al¹⁹ also reported that the ALAD activity is reduced to approximately 50% if circulating blood lead levels are between 40-60 $\mu\text{g/dl}$. Our results are in line with their reports.

Occupational lead nephropathy is very well documented from various countries. Weeden et al³ and Crammer et al²⁰ reported histopathological and ultramicroscopic renal tubular changes, without any renal function changes in the subjects having blood lead levels between 40-60 $\mu\text{g/dl}$. In our subjects whose blood lead levels were high, a three to four fold increase in enzymuria (excretion of enzyme in urine) was observed indicating early renal tubular toxicity. Meyer et al⁷ have observed similar effects. Sub-clinical nephrotoxicity caused by such pollutants can be detected by using these non-invasive techniques.

The prevention/treatment of lead toxicity in occupationally exposed populations is of primary importance especially in countries like India where there are no provisions to control lead fumes, dust etc. There are sufficient evidences to indicate that dietary factors have a vital role in absorption/prevention of lead toxicity^{11,21}. However, only few reports documented a role of thiamine in treatment/prevention of lead toxicity in animals^{12,13}. Our pilot study in monocasters, positively indicate that thiamine can reduce blood lead levels and further reverse early biochemical markers of lead toxicity.

It is documented that ALAD contains sub-units of sulph-hydryl functional units which can be stimulated by the action of thiol and amino chelators^{22,23}. Therefore most of the heavy metals, specifically lead bind to these sulph-hydryl groups and thus inhibiting the enzyme activity. Since thiamine also contains a pyrozole ring and thiazole nucleus, it is more likely to form a complex with lead due to its affinity to SH and -OH groups. This can result in the dissociation of lead from ALAD. It is one of the reasons for increased basal ALAD activity in monocasters supplemented with thiamine.

The excretion of urinary NAG also registered a significant decrease in the fourth month. This activity did not reduce further probably because of mobilisation lead from bone and soft tissue excretion through kidney.

The present study suggests that thiamine may be an useful agent in the individuals who are continuously exposed to low levels of lead. Moreover, use of nutrients for prolonged periods of chronic exposure to lead may be more useful as compared to chelating agents such as EDTA which may have deleterious side effects.

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