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Detection of Sub-Clinical Lead Toxicity in Monocasters

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Lead poisoning has been documented since antiquity but occupational lead intoxication still continues to occur (Rampel 1989). Now there is a growing consensus that low levels of lead exposure often do not result in the manifestation of toxic symptoms, but may have sub-clinical toxicity on haemopoitic and renal system (Marks (1985; Hunter 1986; Goyer 1990). Such toxicities are reported even at blood lead concentrations which were thought to be safe (60-80 μg/dl) a decade ago (Who 1986; Staessen et al, 1992).

One of the several effects of lead, is inhibition of erythrocyte delta-aminolevulinic acid dehydratase (d-ALAD), rate limiting enzyme of the heme synthesis (Gibsson et al 1968; Sassa et al 1973; Marks 1985). Similar effect on d-ALAD has been reported even at the blood lead concentrations of 20-30 μg/dl which are much below the toxic limits of 60 μg/dl (WHO 1986; Somashekhariah et al, 1990).

Occupational lead nephropathy has been reported from several countries (Emmerson 1973; Cramer et al 1974; WHO 1980). Ultrastructural alteration in renal tubules, due to chronic exposure of lead, are seen in both animals and human renal biopsy samples (Goyer, 1971; Weeden et al 1975). However, detecting early renal damage is a difficult task, since the routine renal function tests like creatine clearance, Insulin clearance (GFR) etc. are altered only after severe kidney damage. Recently increased urinary excretion of lysosomal enzyme N-acetyl-B-D-glucosaminidase, a marker of early nephrotoxicity has been reported in the workers exposed to various chemicals including lead (Meyer et al 1984).

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The present study has therefore been undertaken to evaluate the subclinical lead toxicity on haemopoetic and renal system using non invassive techniques in monocasters, who are occupationally exposed to lead fumes while preparing the type set letter blocks.

MATERIALS AND METHODS

Twenty three male Monocasters aged between 20 and 50 years, who were occupationally monocasters since 15-30 years, employed in a printing press, voluntered for the study. Twenty seven normal males aged between 22-45 years with no history of anaemia/renal disorder/ hypertension/diabetes or occupational exposure to lead were selected from the Institute staff and served as controls. All the subjects were clinically examined for various signs like loss of appetite, headache, abdominal discomfort, vomiting, metalic taste etc. and symptoms such as lead line, fine tremors, sensory and motor disturbances etc. of lead toxicity in a pre-tested schedule.

The following biochemical parameters were estimated.

The blood d-ALAD activity was determined by a modified method of Granick et al (1972). The enzyme was assayed by estimating the amount of porphobilinogen liberated from known amount of ALA utilising modified Ehrlich reagent. The extent of lead poisoning was indicated by (a) low activity of enzyme and (b) the complete restoration of the same in the presence of Dithiotheirtol (DTT) (% stimulation).

Urinary NAG activity was measured by spectrophotometric method (Horak et al 1981). The enzyme was separated from urine by gel filteration using sephadex G-25. The activity was assayed in a reaction mixture using the substrate para nitrophenyl N-acetyl B-D-glucosaminide. The released amount of P-nitrophenol was measured after arresting the reaction using 2-Amino 2Methyl 1 Propanol buffer (AMP) by spectrophotometry at 406 nm.

The blood lead levels were determined by a graphite furnace atomic absorption spectrophotometer (Subramanian and Meranger 1981). The results of the study were analysed and tested statistically using analysis of variance (ANOVA) and correlation between various parameters were evaluated.

RESULTS AND DISCUSSION

Mild or moderate lead poisoning produces a variety of symptoms, many of which are not classical symptoms of lead poisoning. The mean blood lead levels in the

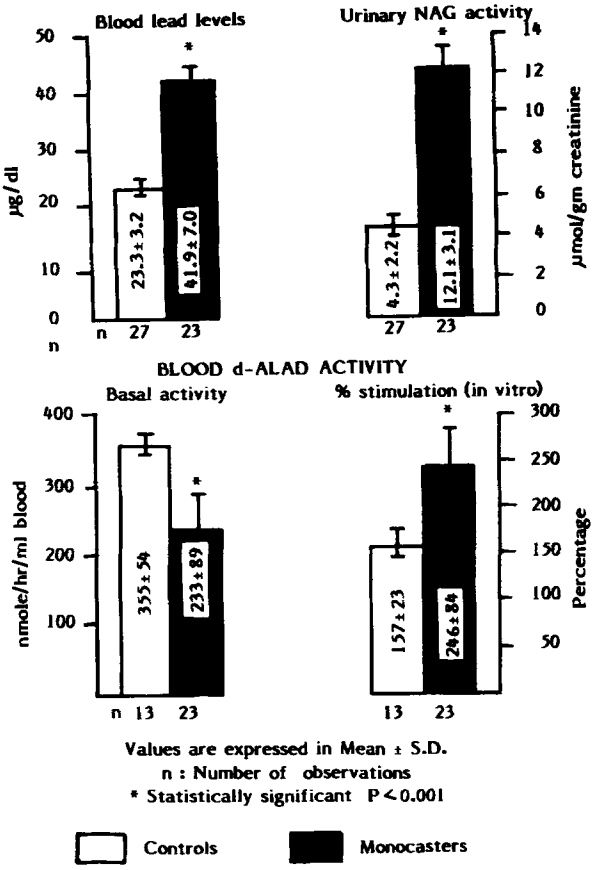

Figure 1. Blood lead levels, urinary NAG activity and blood ALAD activity in controls and monocasters

monocasters were twice (41.9±7.0 µg/dl) as compared to that in the control individuals (23.3±3.3 µg/dl) (Fig. 1). Even at these blood lead levels in monocasters, we have observed that higher proportions (70%) of subjects with blood lead levels of more than 30 µg/dl had clinical symptoms of lead poisoning such as, loss of appetite, vomiting, insomnia etc., than those with lead levels below 30 µg/dl. In more than three fourth of the monocasters investigated, specific signs of lead poisoning such as lead line, fine tremors and sensory disturbances in the extremeties were noted. However, there was no correlation between the years of exposure and blood lead levels.

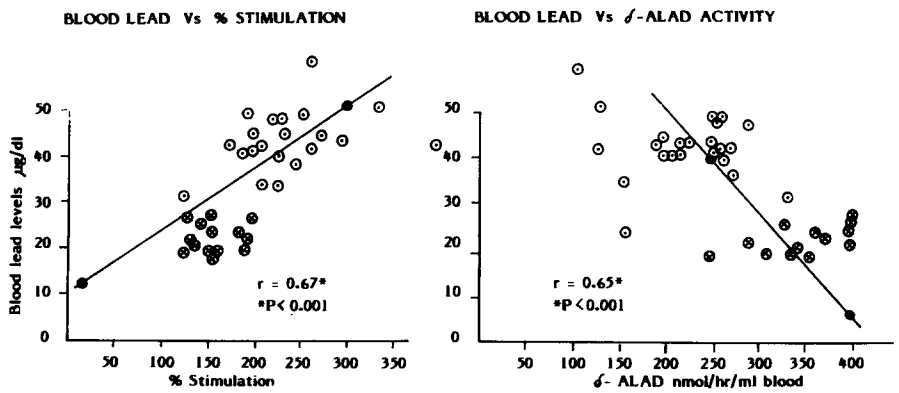

Figure 2. Correlations: between blood lead level and ALAD activity

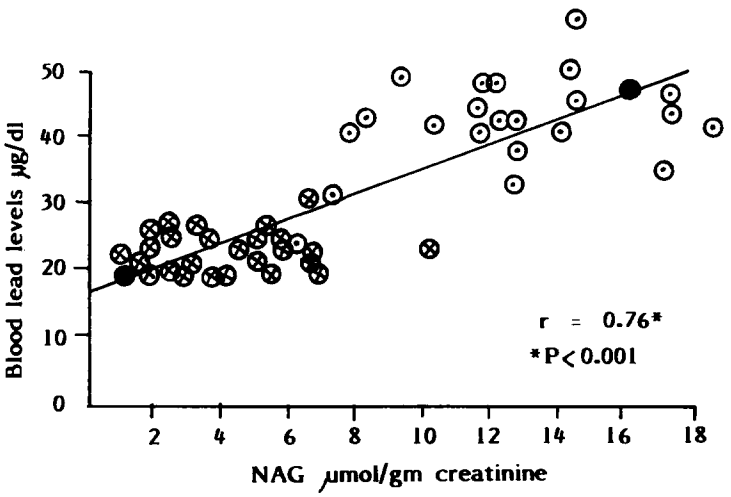

Figure 3. Correlations between blood lead level and urinary NAG activity

⊗ Controls ⊙ Monocasters

Estimation of erythrocyte ALAD activity is commonly used as sensitive indicator of chronic low level lead toxicity. In the present study we have observed approximately 40-50% inhibition in the basal ALAD activity even at the blood lead levels which were very much with in the normal range recommended by various international agencies (Hunter 1986; WHO 1986; Graff 1991). Mitochondrial enzyme ALAD from most species appear to have same molecular weight and number of subunits of sulphhydryl groups. It is allosteric in nature and requires a metal ions for maximum activity. It is estimated that a concentration of 1.9x10 M lead is sufficient to inhibit 50% of d-ALAD activity in vitro (Davis and Auvam 1978). We have a specific method of Granick et al (1972) to find out the extent of inhibition of d-ALAD activity by lead which can be subsequently restored completely when incubated in the presence of sulphhydryl groups (DTT) in vitro. The percentage stimulation of basal d-ALAD activity on addition of DTT reflects the level of inhibition. In monocasters, a two fold increase in stimulation of d-ALAD was observed when compared to that controls, suggesting the presence of haemopoitic toxicity at a sub-clinical level (Fig. 1). Somashekaraiah et al (1990) also reported that the basal d-ALAD activity is reduced to approximately 50% at blood lead levels of 40-60 µg/dl. Inverse correlation ($r = -0.69$) was observed between blood lead level and d-ALAD activity, with a positive correlation ($r = 0.67$) between blood lead levels and percentage stimulaton (Fig. 2). These results are in line with the observations of Granick et al (1972) and Someshakaraiah et al (1990).

It has been suggested that the earliest severe effect of lead is on the cells of the proximal convoluted tubule, and which is difficult to detect in the early stages of renal dysfunction (Goyer 1990). The altered ultrastructural changes of proximal tubules produced by lead has been also suggested to be one of the reason for increased enzymuria and aminoaciduria (Chisolm 1962). In the present study, the urinary NAG levels, the enzyme which is present in the lysosomes of the cells of proximal tubules, was elevated by 3-4 fold in monocasters having blood lead levels around 40-60 µg/dl (Fig. 1). This elevated enzymuria indicates incipient but progressive renal damage and can be used as a sensitive diagnostic tool to detect early lead nephropathy. Meyer et al (1984) have also used urinary NAG activity to detect the extent of nephrotoxicity in workers exposed to various chemicals including lead. Interestingly we also observed a direct correlation ($r = 0.76$) between urinary NAG activity and blood lead levels and inverse correlation ($r = 0.56$) with basal ALAD activity (Fig. 3).

Current recommendation by WHO (1987) regional office Europe is that 98% of the normal population should have blood lead levels below 20 µg/dl. In the present study, the monocasters had blood lead levels above 20 µg/dl. In addition, 66% of monocasters had more than 2 SD from the mean for normal subjects (23.3±3.27 µg/dl) thereby indicating that high proportion of monocasters have high lead levels and sub-clinical toxicity.

The study highlights the fact that even mild chronic lead exposures can significantly increase the blood lead levels and it will have adverse effects on haemopoitic and renal systems. Further, these adverse effects can easily be detected by using simple, sensitive, non invassive biochemical techniques so that suitable remedial measures could be taken to prevent further toxicity.

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