BLOOD ZINC PROTOPORPHYRIN, SERUM TOTAL PROTEIN, AND TOTAL CHOLESTEROL LEVELS IN AUTOMOBILE WORKSHOP WORKERS IN RELATION TO LEAD TOXICITY: OUR EXPERIENCE

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ABSTRACT

Blood zinc protoporphyrin (ZPP), serum total protein (TP), and total cholesterol (TC) levels in automobile workshop workers in relation to lead toxicity were analysed. In the present study, automobile workshop workers (healthy male workers at an age between 28 and 35 from four major automobile workshops in Kottayam, Kerala State, India) and the control (male healthy adults at an age between 28 and 35 residing at Aymanam, a distant village at Kottayam District, Kerala having reduced or no chance of lead exposure) displayed significant difference in blood lead (BPb) and blood ZPP (BZPP) level. The mean value of BPb in automobile workshop workers was $15.76 \pm 0.33 \mu\text{g/dl}$, while in the control it was $8.20 \pm 0.15 \mu\text{g/dl}$. In automobile workshop workers, the mean value of BZPP was $34.2 \pm 0.62 \mu\text{g/dl}$. The control group exhibited a mean of $11.5 \pm 0.22 \mu\text{g/dl}$. Automobile workshop workers exhibited significant increase in BZPP was corresponding to the increase in BPb level. The total protein levels estimated in automobile workshop workers showed significant decrease compared to control individuals, but was within the reference range of healthy individuals. The mean value of TP level in automobile workshop workers and control was $6.9 \pm 0.13 \text{ g/dl}$ and $7.71 \pm 0.18 \text{ g/dl}$, respectively. There was no significant difference in blood haemoglobin (BHb) level among the automobile workshop workers and control. The serum TC level in automobile workshop workers showed significant decrease compared to the control individuals, but was within the reference range of healthy individuals. The mean level of serum TC in automobile workshop workers was $162.00 \pm 3.44 \text{ mg/dl}$ and the same in control was $172.86 \pm 4.32 \text{ mg/dl}$. The present study affirms occupational lead toxicity in automobile workshop workers and its effect on serum protein and cholesterol levels.

KEY WORDS

Lead toxicity, occupational exposure, serum total protein and total cholesterol

INTRODUCTION

Environment contains both toxic and non-toxic atoms, ions and molecules. Industries are the major sources of toxic ions and its entry to biological systems disturb the biochemical pathways, in some cases leads to death of the organism. Lead, the most abundant toxic heavy metal in Earth's crust, has been used from prehistoric times and its distribution, mobilization in the environment and human exposure

consequently increased. Leaded petrol contributed substantial increase of environmental lead in the last century (1).

Lead, though affects every systems in the body, is detrimental to developing brain and numerous systems of children and fetus (2). Chronic toxicity manifestations such as hypertension, kidney impairment, (3) and cognitive disturbances have also been documented because of long-term asymptomatic lead exposure (4). Biochemical and sub-clinical expressions of toxicity starts at blood concentrations around $10 \mu\text{g/dl}$ and leads to coma and death at levels over $100 \mu\text{g/dl}$ (5). Lead is a competitor of calcium mediated cellular and sub-cellular process. Inhibitory effect of lead on certain steps in haem metabolism has also been documented. Lead inhibits haem biosynthesis and causes a decline in red cell δ -aminolevulinic

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acid dehydrogenase activity and an increase in urinary δ -aminolevulinic acid, urinary coproporphyrin and red cell zinc protoporphyrin (6). The measures imposed by the Government and other agencies in the last decades brought down the exposure to lead and related toxic effects in developed countries (7,8). According to Shilu *et al.* (9), lead toxicity still pertains as a menace due to the uncontrolled use of leaded petrol in developing countries.

Automobile workshop workers are a class of labors prone to long-term lead toxicity due to their routine works such as motor vehicle assembly, spray painting, burning of petrol, welding, brazing, and repairing of radiators. The outfits of automobile workshop workers serve as a source of lead exposure to their family members, young children in particular, arise the occupational toxicity to a community problem. Drastic increase in the number of automobile vehicles in last two decades incremented the exposure of this labor class to lead. The present study attempts to analyze lead toxicity in relation to occupation in automobile workshop workers. Blood zinc protoporphyrin (BZPP) levels may be a better measure of chronic lead toxicity because of its longer half-life (10), so we included BZPP estimation among these workers. A significantly decreased serum protein and total cholesterol levels has been reported in animal experimental lead toxicity studies (11,12). As the two important parameters of health of an individual and its various role in metabolic activities we have decided to study serum total protein and total cholesterol levels in automobile workshop workers in comparison with their blood lead level, if any.

MATERIALS AND METHODS

Twenty healthy male workers ranging in age between 28 and 35 (31 ± 2.2 ; 31) from four major automobile workshops in Kottayam town, Kerala State, India, who voluntarily donated blood, were randomly selected to study the lead toxicity. Twenty male healthy volunteers of the same age group (31.2 ± 2.2 ; 31) residing at Aymanam, a distant village in Kottayam District, Kerala State having reduced or no chance of lead exposure, were treated as control.

The blood for lead estimation was collected by finger puncture method. The hands of individuals were thoroughly washed with soap and then dried with clear lint towel. The first drop of blood was discarded and subsequent drops were drawn in to a sterile lead free sample tubes containing anticoagulant. The blood for estimation of ZPP, haemoglobin, total cholesterol, and total protein from each individual was collected by antecubital venipuncture. The samples from different

individuals collected in separate tubes were kept at 2-8°C and transported into the laboratory at the earliest.

The estimation of lead in blood sample was carried out by Anodic Stripping Voltametry (ASV) using the ESA Model 3010 B lead analyzer (13). The operating range of the machine is 1 to 100 $\mu\text{g/dl}$. The instrument performance has been validated by regularly checking precision using known standards (8-14 $\mu\text{g/dl}$, ESA Inc.) The fundamental principle of operation is, when sufficiently large negative potential is placed on an electrode, lead ions in solution are plated on a mercury electrode. After fixed plating time, the potential is made less negative to strip the metal of the electrode. The observed current during stripping process is integrated and is directly proportional to the concentration of the lead in the sample.

ZPP in blood was estimated by hematofluorometric method (14). The hematofluorometer (Helena Laboratories, Beaumont, TX), a simple filter type fluorometer, measures the front surface fluorescence of whole blood and washed erythrocytes. The instrumental parameters were set such that the fluorescence signal is presumed to be due to the presence of ZPP (this is a first good approximation). The fluorescence of the blood specimen is proportional to the molar ratio of ZPP to heme. The values were expressed as $\mu\text{g/dl}$ of blood. Total cholesterol and total protein in serum were estimated by the enzymatic method and biuret method respectively. Cyanmethaemoglobin method was used for the estimation of Haemoglobin. All the reagents used for the above estimations were commercial kits (Glaxo India Ltd., Mumbai).

Statistical Analysis

Statistical analysis was performed using Student's 't' test.

RESULTS & DISCUSSION

Moderate level of lead toxicity among automobile workshop workers from Kottayam town of Kerala when compared to control individuals from Aymanam village in the same district showing occupational route of exposure to lead still pertain as a problem. Lead is a known inhibitor of ferrochelatase enzyme of the haem biosynthesis. Incorporation of Zn^{2+} , instead of Fe^{2+} in protoporphyrin IX result in the formation of zinc protoporphyrin (ZPP), which can serve as a marker of chronic lead toxicity because of longer half life (14). The significant increase in ZPP level in the automobile workers compared to the control in the present study, with normal haemoglobin range was in concurrence with the studies on battery workers by Froom *et al.* (15). Among the workers

Table 1
Correlation between BPb and other parameters.

Group	BPb ($\mu\text{g/dl}$)	BZPP ($\mu\text{g/dl}$)	BHb (g/dl)	TC (mg/dl)	TP (g/dl)
Control (n=20)	8.20 $\pm$ 0.15	11.5 $\pm$ 0.22	14.61 $\pm$ 0.31	172.86 $\pm$ 4.32	7.71 $\pm$ 0.18
Workshop Workers (n=20)	15.76 $\pm$ 0.33*	34.2 $\pm$ 0.62*	14.69 $\pm$ 0.34	162.00 $\pm$ 3.44*	6.9 $\pm$ 0.13*

Values are expressed as Mean $\pm$ SEM and range comparison was done between control & workshop workers; *P<0.001; rest not significant.

group BPb and BZPP concentrations were in the range of 10.7 $\mu\text{g/dl}$ to 17.8 $\mu\text{g/dl}$ and 27.4 $\mu\text{g/dl}$ to 39.2 $\mu\text{g/dl}$ respectively. The haemoglobin concentrations in workers group were in tune of minimum level as 14.2g/dl and maximum level as 15.7g/dl. All these showing workers group had exhibited inhibitory effect on haem biosynthesis at this moderate level of lead toxicity and thereby increased BZPP level, but not in way to manifest changes in haemoglobin concentration. Further exposure to lead may cause anaemia and related problems in these workers due to its cumulative effect on haem metabolism and needs to be controlled.

Analyses of serum total protein and total cholesterol among these workers have strengthened the earlier findings of lowered levels of these two important parameters in health due to lead toxicity in experimental animal studies. The serum total protein (TP) and total cholesterol (TC) levels in automobile workshop workers in the present study exhibited a significant decrease in comparison to control group. TP levels were 6.6g/dl or below in eight workers and the maximum level at 7.9g/dl, whereas only one person in the control group exhibited TP level below 6.6g/dl and maximum level at 8.79g/dl. This significant decrease in TP level among workers group having almost double value of mean BPb levels than control group is an important observation in this study. A significant decrease in serum globulin level has also been reported in *Hymenolepis nana* mouse model treated with lead prior infection (11). Serum total protein levels is a rough measure of protein status but reflects major functional changes in kidney and liver functions. One of the main targets of lead poisoning is the kidney. Chronic poisoning can lead to kidney failure, and acute poisoning to tubulopathy with Toni-Debre-Fanconi syndrome. β -2-microglobulinuria and enzymuria were reported in lead toxicity in children (17). Proteinuria due to kidney impairment in lead toxicity may be a cause of protein loss among these workers because inhibitory role of lead in protein synthesis is not yet reported. Protein loss in lead toxicity might decrease the level of specific proteins such as albumin, hormones, hormone and metal binding proteins, drug binding proteins, enzymes etc.

and thereby disturb the homeostasis and rate of metabolic activities. The serum total cholesterol level was significantly decreased among this group when compared to control group, but the decrease was well within the reference range. TC levels were 160mg/dl or below in twelve workers and six control individuals. The maximum TC level exhibited was 247mg/dl and 173mg/dl in control and workers groups respectively. The lowered serum total cholesterol level reported earlier in Buffalo rats on experimental lead toxicity (12) along with our observation in this study strengthens the inhibitory role of lead on cholesterol metabolism. But the actual mechanism of decrease in serum total cholesterol is not clear in this study apart from earlier report on low level synthesis of 1,25(OH)₂ cholecalciferol from cholesterol, a precursor of vitamin D, in moderate levels of lead toxicity (16). Cholesterol in reasonable quantities is critical to good health as it is a component of cell membranes, is found in all body tissues, and is converted into various hormones. The above results showed the effect of moderate level of lead toxicity in decreasing serum total protein and total cholesterol levels among the automobile workshop workers and these findings necessitate further analysis on cholesterol and protein metabolism in lead toxicity.

As per WHO's report (18), atmospheric lead deposited in soil contaminates the water and food sources, the baseline sources of lead exposure. Leaded petrol serves as an important source of lead in the environment. The developed countries during the last decades witnessed curtailment of lead toxicity by the implementation of rigorous measures by Government and other agencies, and improvement in industrial methods. In the United States of America, during the period of 1976-1991, the mean blood lead level of persons aged 1-74 years exhibited 78% reduction *i.e.* from 12.8 $\mu\text{g/dl}$ to 2.8 $\mu\text{g/dl}$ (Pirkle *et al.* 1994; Edwards-Bert *et al.* 1994). Based on the observations of our studies, we can say occupational lead exposure still pertains as a concern in developing countries like India and chronic lead exposure in workers such as automobile workshop workers and their low economic status may have cumulative effect on deteriorating their health. Thus,

the present study emphasizing the need for regular monitoring of blood lead levels among general population and also to build up awareness on lead toxicity in relation to occupation particularly among automobile workshop workers and abstain from job for short periods to reduce load.

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