

Chromosomal Study on Lead Exposed Population

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ABSTRACT Exposure to extremely toxic substance like lead might lead to a broad range of undesirable health consequences. As a result of lead poisoning both adults and children experience adverse physical conditions. The objective of the present study is to estimate the level of lead in the blood of the exposed population and analyze the haematological parameters along with chromosomal damage. Lead level from blood was estimated by atomic absorption spectrophotometry. Microcytic anaemia was present in the individuals with high lead level in the blood. Leucocyte culture revealed higher percentage of chromosomal aberrations in the individuals with high lead content in the blood. Further studies will help to define the role of genes in lead intoxication to prevent irreversible damage.

INTRODUCTION

During past decades, due to rapid industrialization, the evaluation of potential toxic effect of industrial emissions on residential populations has assumed considerable importance. India is a multiracial population with a wide variety of life styles and microclimates. The effect of various heavy metals on the human population is a widely discussed topic. Lead is a heavy metal, which has many adverse effects on human physiology.

Lead is everywhere in the environment as a result of natural occurrence and its industrial use. The primary sources of environmental exposure to lead are paint and drinking water. Most of the overt toxicity from lead results from environmental and industrial exposure (Dregus and Klaassen 2001). Elevated levels of lead in the air are associated with elevated blood lead level in humans (Thomas et al. 1999). Lead is found in storage batteries, lead solders, pipes, construction materials, dyes and wood preservatives (Farjami et al. 2004). Lead is also present in paint, leaded gasoline, cable sheathing and many building materials. It may also be found at firing ranges (from bullet dust) (UNICEF

2000) and in some contaminated herbal remedies and candies (Hu 2005).

Though organic lead compounds (tetraethyl lead) have been used as gasoline additives; inorganic lead compounds are the species of current concern. Lead is ubiquitous in the environment and is a major environmental pollutant of air, water and soil. Lead toxicity depends on the site of entry of lead into the body (ingested or absorbed through skin), nature of lead (organic or inorganic), period of exposure, age and sex of the individual and the socio-economic condition. The occurrence of lead poisoning depends on the developmental stage, behavior, nutritional status, and metabolism of an exposed individual. Generally, males have higher blood lead levels than females, even when the exposures to both groups are controlled (Walter et al. 1980).

The objective of the present study is to observe the effect of lead on the haematopoietic system.

MATERIAL AND METHODS

A total of one hundred and fifty patients (among which one hundred patients had high blood lead content and fifty had low blood lead content) were considered for the present study. An elevated blood lead level (BPb) is defined as $>10 \mu\text{g/dL}$ according to the Center for Diseases Control and Prevention guidelines (CDC 1991). The World Health Organization has adopted a critical level of $10\text{--}15 \mu\text{g/dL}$ (WHO 2005). Age and sex matched control population ($n=50$, 27 males and 23 females) and the exposed individuals ($n=100$, 62 males

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and 38 females) were the subjects of the present study. The age ranges for both the groups were 19-45 years.

Each subject was given a standardized questionnaire interview to collect the information on demographics, life-style factors, occupation, diet, source of daily water intake, medical history and residential history. Physicians examined the study participants.

Estimation of Lead

Blood lead was estimated by an atomic absorption spectrophotometer (Perkin-Elmer AAS-100) by forming a complex of the lead with 2% ammonium pyrrolidone dithiocarbamate and extraction of it into methyl isobutyl ketone. Organic phase was measured at 283.3 nm. Background correction and calibration were made by standard additions (Zinternofer et al. 1971). A 0.71 nm slit aperture was used, lamp current being at 15 mAmp. The detection limit was 2.8 µg/dl with a standard deviation of 3.5%. Precautions were taken to avoid contamination by ambient lead from reagents and other materials (that is, heparinized collection tubes). Quality control sera of Technician were used for quality control.

Chromosomal Study

A short-term leucocyte culture was conducted to study chromosomal aberration and mitotic index by the method modified from Moorhead et al. 1960 (Preston et al. 1981; Sharma and Talukder 1974; Sharma et al. 1983).

Five ml of peripheral blood samples were taken from each subject in heparinised vials by venipuncture. The blood samples were coded, kept at 4° C in a cooling device, and brought to the laboratory within two hours of collection to study the complete hemogram like Hb, MCV, MCH, MCHC etc. and to carry out leucocyte culture.

Leucocyte culture was carried out for CA analysis by the method of Sharma and Talukder (1974). For each subjects duplicate cultures were maintained. Leucocyte rich plasma (0.5ml) was added to five ml culture media supplemented with 20% fetal bovine serum and phytohaemagglutinin M (0.04ml/ml of culture media). The cultures were incubated at 37° C. The harvesting was done 72 hours after initiation of the

culture. At 70 hours of culture colchicine was added. Two hours later cells were centrifuged at 1000rpm for 10 min, treated with pre-warmed KCl (0.075M) for 15 minutes, centrifuged at 1000rpm for 10 minutes and fixed in methanol: acetic acid (3:1). Fixatives were removed by centrifugation and two more changes of fixative were performed. Fixed cell suspension was laid on a clean grease-free glass slide and air-dried. The preparation was stained with aqueous Giemsa. All slides were coded and 100 metaphase plates were scored randomly for chromosomal aberrations (chromatid and chromosome types) per individual.

The same person scored 1000 cells blindly in each case to determine mitotic index percentage. Chromosomal aberrations such as break and gaps were also detected but not included in the statistical analysis.

The significance of the differences between lead contents obtained in the control and exposed groups was calculated by Fisher's *t*-test. Different haematological parameters, chromosomal aberrations (CA) and mitotic index (MI) assays were also analyzed using Fisher's *t*-test and the level of significance is presented in the respective tables (both sided *p* value).

RESULTS

By using 11 gm/dL of the haemoglobin (Hb) as the cutoff point (the WHO standard for developing countries) a comparative data distribution of lead content (mean±SE) in blood is represented in Table 1. The mean age of the control population was 29±1.04 years and mean age of the exposed group was 30.39±0.716 years. The mean (±) blood lead content in the control group was 6.04±1.69 µg/dL and mean blood lead content of the exposed group was 241.8±19.36 µg/dL. The mean hemoglobin concentration of the control group was 14±1.4 g/dL and the hemoglobin concentration of the exposed group was 9.7±0.016 g/dL. The table clearly indicates that the lead content was significantly higher in the patients with low hemoglobin content, thus it can be assumed that elevated lead content in blood lead may cause anemia.

A comparative study was done using different haematological parameters (MCV, MCH, and MCHC) among the control and exposed population (Table 2). The mean MCV, MCH and

Table 1: History and the mean values of lead content in blood of the control and the exposed group

Type	No. of Individuals	Hb (g/dL) Mean±S.E	No. of Individuals		Age(yrs) Mean± S.E	Lead level (µg/dL) Mean±S.E
			Male	Female		
Control	50	14±1.49	27	23	30±1.04	6.04±1.69
Exposed	100	9.7±0.016**	62	38	30.39±0.716***	241.80±19.36***

** Significant at $p \leq 0.01$ *** Significant at $p \leq 0.001$

MCHC of the control group were 80.20 ± 1.32 fL, 28.40 ± 0.6 pg and 35.51 ± 0.27 g/dL, respectively. The mean MCV, MCH and MCHC of the exposed group were 70 ± 1.02 fL, 23.34 ± 0.44 pg and 33.42 ± 0.21 g/dL respectively. The table indicates that the hemoglobin concentration was low among patients with elevated lead content. The MCV and MCH were also low in the exposed population indicating microcytic anemia.

The present study indicates that there was a significant change in CA and MI frequencies of exposed individuals when compared to the control group as indicated in Table 3. The mean mitotic index of the exposed group was 1.78 ± 0.12 and the chromosomal aberration was 5.2 ± 0.10 . The mean mitotic index of the control group was 5.57 ± 0.13 and the chromosomal aberration was 0.83 ± 0.10 . The mitotic index was higher in the control group than in the exposed population and the chromosomal aberration was high in the exposed group. These results indicated that there was a significant increase in cytogenetic damage in leucocytes due to lead exposure. Chromatid breaks were the main CA observed. Chromosome type aberrations, such as chromosomal breaks and dicentrics were also observed. In this study we only present the av-

erage results. For calculation of CA, chromatid breaks were taken as one change and chromosome breaks, dicentrics as two changes. In this study a decrease in the mean MI value of the exposed population was noted.

DISCUSSION

The changes in haematological parameters due to environmental lead exposure may have severe impact on the health system. Lead toxicity leads to anemia which may result from two basic defects, shortened erythrocyte life span and impairment of heme synthesis (Timbrell 1995). Young children are more susceptible to lead exposure (Kaiser et al. 2001). The shortened life span of RBC is probably the consequence of increased mechanical fragility of cell membrane (WHO 1996). Also, many steps in heme biosynthesis are inhibited by lead by interfering with the function of mitochondrial enzyme "ferrochelatase" leading to the accumulation of the substance "protoporphyrine" in erythrocyte during the maturation of bone marrow (Tandon et al. 2001). Interference with heme synthesis causes hematological damage. The overall effect is the disruption of the synthesis of hemo-

Table 2: The haemoglobin, MCV, MCH and MCHC of the studied cases

Set Number	No. of Cases	Concentration of Lead	Hb (g/dL) Mean±S.E	MCV (fL) Mean±S.E	MCH (pg) Mean±S.E	MCHC (g/dL) Mean±S.E
1	100	Lead level above 10µg/L	9.7±0.016	70±1.02	23.34±0.44	33.42±0.21
2	50	Lead level below 10µg/L	14±1.4**	80.20±1.32***	28.40±0.6***	35.51±0.27***

** Significant at $p \leq 0.01$ *** Significant at $p \leq 0.001$ **Table 3: Chromosomal aberration and mitotic index of the studied cases**

Set number	No. of cases	Concentration of lead	Total chromosomal aberrations			Chromosomal aberrations (%) Mean±S.E	Damaged cell (%) Mean±S.E	Mitotic index (%) Mean±S.E
			B'	B''	RR			
1	100	Lead level above 10µg/L	311	87	15	5.2±0.10	5±0.10	1.78±0.12
2	50	Lead level below 10µg/L	27	6	-	0.83±0.10***	0.67±0.10***	5.57±0.13***

B'=Chromatid break; B''=chromosome/isochromatid break; RR=rearrangements;

*** Significant at $p \leq 0.001$

globin as well as, other respiratory pigments, such as cytochromes (CDC 1997). Study found that foetal hemoglobin has a higher affinity for lead than adult hemoglobin (ATSDR 1998). Consumption of paint chips as a result of acute exposures to leaded paints will make a child seriously ill. The child may develop anemia (Schwartz et al. 1990). Acute exposures to lead with blood lead level (BPb) of $> 62\text{--}80\text{ }\mu\text{g/dL}$ can cause impaired hematopoiesis. Subclinical exposures in children (BPb $25\text{--}60\text{ }\mu\text{g/dL}$) are associated with anemia. In adults, chronic subclinical exposures (BPb $> 40\text{ }\mu\text{g/dL}$) are associated with an increased risk of anemia (Hu 2005). The anemia of lead poisoning is usually thought to be normochromic and normocytic. Punctuate basophilia is the constant feature of the well-developed case (Hutchinson and Stark 1961). In a study it was found that a high blood lead concentration is associated with an increased urinary lead concentration, and an increased urinary excretion of delta-aminolaevulinic acid (Spriewald et al. 1999). From a study on lead exposed workers it was found that there is a change in chemical physical state in erythrocytes, with a decrease of membrane fluidity. Changes in the membrane structural organization could be the molecular basis of some pathological alterations induced by lead (Valentino et al. 1982). Iron deficiency anaemia is correlated with high blood lead level in children, as well as, in adults (Kapil and Suri 2004).

Screening of a population in a high-risk area for lead exposure showed that there is prevalence of lead poisoning, in an anemic population (Gawarammana et al. 2006). This study indicates that the hemoglobin concentration was low in patients with high BPb in comparison with the patients with low BPb. MCV and MCH levels were also low in the patients with high BPb. It was evident from a study that blood lead level (BPb) is significantly related to Hb, RBC, and hematocrit, among the lead exposed workers, when controlling for age and working status. Average BPb levels were significantly higher in the workers with anemia (1.85 mmol/L), based on the WHO criteria, than in those without anemia (1.26 mmol/L). These findings correlates with the studies of Kavita et al. (2005) which stated that reduction in hematopoietic indicators may be initiated with high BPbs concentrations.

Lead is a known clastogen (Wise et al. 1994) and thus has effects on the chromosomal aber-

rations. Our studies also revealed that the mitotic index percentage was low in patients with high blood lead levels in comparison with cases with low blood lead levels. This suggested that a slower progression of lymphocytes from S to M phase of the cell cycle (Gonsebatt et al. 1994) as every known clastogen disturbs the orderly progression of cells towards division. As all the cells are harvested at similar time period so this effect may be due to chronic exposure to lead.

Occupational exposure to lead has been reported to cause an increase in sister chromatid exchange and chromosomal aberrations (ATSDR 1999). Chromosomal analysis of the workers with high blood lead level, from a smelting plant for storage batteries showed aberration-rings and dicentrics. Increased numbers of chromosomal fragments were seen in those with high level of urinary delta-aminolevulinic acid (Deknadt et al. 1977). In this study chromosomal aberration percentage was higher in the cases with elevated blood lead levels than in patients with low blood lead concentrations. So it can be said that lead induces chromosomal aberrations in human (Table 2).

This similar result was observed from a study of lead-exposed workers. The frequencies of CA and sister chromatid exchange (SCE) in the peripheral blood lymphocytes of 21 lead-exposed workers from a battery factory were studied, and their blood lead levels determined. The results demonstrated that when the mean BPb level reached $50\text{ }\mu\text{g/dl}$, the CA percentage increased significantly compared with that of the non-exposed controls. The CA percentage increased as the mean Pb-B level increased, showing a dose-effect relationship. When the mean Pb-B level was as high as $80\text{ }\mu\text{g/dl}$, the rate of SCE in the lymphocyte also increased significantly, but no correlation was observed between these two parameters. On the basis of such results, lead should be considered an agent harmful to the human chromosome (Biomed Environmental Science 1988). At least three polymorphic genes have been identified that potentially may influence the bioaccumulation and toxicokinetics of lead in humans. One of these genes is the one coding for delta-aminolevulinic acid dehydratase (ALAD), an enzyme of heme biosynthesis that exists in two polymorphic forms. The resulting isozymes have been shown to affect the blood and bone lead levels in human populations (Onaja and Claudio 2000). Lead's ability to inhibit production of this enzyme varies

between individuals. This variance is transmitted as an autosomal genetic trait (Vainio and Sorsa 1981).

From a recent study it was reported that chronic exposure to lead chromate cause centrosome abnormalities and aneuploidy in human lung cells (Holmes et al. 2006)

Considerable steps have been adopted to facilitate the decrease of lead intoxication among the common population, but research work employing genetic markers to detect susceptibility to environmental toxicants, are often doubtful. The vulnerability of certain individuals towards environmental toxicants such as lead by the presence or absence of certain genes is thus yet to be proved by further research in this field.

CONCLUSION

The heavy metal lead is associated with a medical condition called plumbism, which causes various health hazards. Elevated concentrations of lead in the present population pose an alarming situation. Major steps should be taken to eliminate the source of lead exposure, thereby inhibiting further poisoning. Biological markers for toxic substances such as lead are being validated by new research. In the general population there are significant differences in the advancement of toxicity as a result of exposure to lead. There are genetic factors that modify the absorption, metabolism or excretion of lead. It is therefore necessary to determine the genetic variants that predispose individuals to accumulate lead, so as to prevent the transmission of chromosomal damage to the future generations.

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