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Original Research Paper

Evaluation of Lead Genotoxicity by Micronuclei Assay in Occupationally Lead Exposed Population

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ABSTRACT

Lead, a ubiquitous element in the environment as a result of mining and industrialization, is found as a contaminant in humans. Lead is one of the heavy metals increasingly used for the petrol additives, and for various industrial purposes such as manufacture of storage batteries, solder manufacture, printing, smelting, painting, etc. In this study the lead genotoxicity was studied by using one of the recommended biomarker to assess environmental lead exposure is micronucleus assay. The blood samples were collected from 50 workers and 40 aged matched controls (5mL of venous blood from each lead exposed individual in heparinized sterile bottles). The battery manufacturers showed a significant increase in the frequency of micronuclei in comparison to that people who were not exposed to any toxicants. The results of present investigation indicate the clastogenic nature of lead in *in vivo* human lymphocytes of occupationally exposed population.

INTRODUCTION

Lead is commonly found in home and industrial surroundings and causes a variety of adverse health effects (Zou et al. 2003). Lead has been a known toxicant for thousands of years, and it remains a persistent environmental health threat. Exposure to lead can result in significant adverse health effects to multiple organ systems. Toxic effects to the nervous, haematologic, renal and reproductive systems have been studied extensively and are well documented (Goyer 1996, Mahaffey 2000). Additionally, living in close proximity to lead-emitting industrial facilities can present a significant source of cumulative exposure to lead via air, water and soil. Occupational exposure to lead is most often encountered at lead smelters and battery manufacturing facilities, as well as in housing renovations projects in which workers inhale and ingest lead-contaminated fumes and dust from lead-based paint. Children's hand-to-mouth activity, increased respiratory rates, and increased intestinal absorption of lead make them more susceptible than adults to lead exposure (Lin-Fu 1973, Ziegler 1978). Poor nutrition, particularly inadequate intake of calcium and iron, is probably an important risk factor for children as well (Mahaffey 1995). Blood lead level (µg/dL) is the biologic index most often used by healthcare providers as an indicator of recent lead exposure (NIOSH 1997). Two analytical techniques, anodic stripping voltametry and atomic absorption spectroscopy, are used to measure blood lead level and have detection limits less than 1 µg/dL (Osterlow 1990). In addition to blood lead level, other lead exposure indices include free erythrocyte protoporphyrin and zinc protoporphyrin; both are precursors of heme whose levels elevate upon moderate to high exposure to lead. However, free erythrocyte protoporphyrin and zinc protoporphyrin are neither sensitive enough nor

specific enough to be used as primary indicators of lead exposure (McElvaine 1991, Zou 2003). Lead levels in plasma, urine, bone and teeth (dentin lead) are less commonly used measures of exposure and body burden. The studies on genotoxic investigation were controversial, hence in the present investigation evaluation of cytogenetic damage was carried out in human lymphocytes in occupationally exposed population.

MATERIALS AND METHODS

Study population: Human biomonitoring study was carried out on workers working at Hyderabad Batteries Industries located at Nandigam, Mahaboobnagar district. During the study 50 male workers were selected of which 28 were non-smokers and 22 were smokers. The control group consisting of 40 who were not exposed to any toxicant belonging to same socio-economic group was selected, among which 22 were non-smokers, and 18 smokers. The personal data, duration of exposure and habits were collected using a standard questionnaire. The range of age of selected workers was from 36.8 ± SD 1.2 to 45.2 ± 2.3 years in control, and 38.8±2.1 to 42.6±2.3 years in the exposed group.

Sample collection: 5 mL of blood samples were collected aseptically in sterile heparinized vaccutainers and brought to the laboratory in an ice bath. The samples were brought to the room temperature prior to setting up the culture and coded.

Micronucleus assay: Lymphocyte cultures were started according to the method of Fenech et al. (2000). By adding 0.5mL of blood to 5 mL of RPMI-1640 medium, supplemented with phytohaemoagglutinin, 15% fetal calf serum (Sigma) and 1% pencillin and streptomycin. The cultures were incubated at 37°C for 72 hrs. Cytokinesis was blocked with 6 µg/mL Cytochalasin-B (sigma), added 44 hrs after PHA stimulation. Cells were harvested by centrifugation and after a mild hypotonic treatment with 3 mL 0.075M KCl and another centrifugation, were fixed by adding 5mL of fixative solution (methanol: glacial acetic acid, 3:1). Then 50µL formaldehyde was added during the next hour. Two steps of centrifugation with consequent fixation of the material as already described, without adding any more formaldehyde was performed. Air dried preparation was stained with 5% GIemsa for 15 mins.

RESULTS AND DISCUSSION

Table 1 shows the characteristics of the control and exposed groups with respect to age of individuals, years in their employment and smoking habits. Table 2 shows the results of MN scoring indicating both, the average total number of MN scored for 1000 binucleated cells and average of binucleated cells presently one or more MN. The frequency of micronucleus in non-smokers group was 1.90 and 4.10 in smokers of control group whereas the frequency of micronucleus increased to 10.20 (non-smokers) and 21.00 (smokers) in workers exposed to lead. The differences were found to be significant ($p<0.001$), indicating a clear genotoxic effect of lead exposure.

The workers occupationally exposed to lead, who were monitored in the investigation, showed a clear evidence of genetic damage in peripheral blood lymphocytes when evaluated by MN assay. Among the different cytogenetic protocols the MN assay in human lymphocytes using cytokinesis block method (Fenech 1985) has increasingly been accepted as a reliable biomarker of cytogenetic damage induced by genotoxic agents, both physical and chemical agents (Fenech 1993, Bauchinger 1990) positive findings using this biomarker indicate evidence of exposure to clastogenic/aneugenic compounds in particular MN assay has proved very reliable in assessing the genotoxic effects of metal ions in occupational exposures (Bercés et al. 1993, Vaglenov 1998, Vagelnov 1999).

Table 1: Characteristics of the control and exposed population.

	No. of Sample	Age in years	Duration of exposure
Control (non-smokers and smokers)	40	36.8 yrs ± SD 1.2 to 45.2 yrs ± SD2.3 to	-
Exposed (non-smokers and smokers)	50	38.8 yrs ± SD2.1 42.6 yrs ± SD2.3	20 + 1.2 yrs

Table 2: Frequencies of BNMN in lymphocytes of lead exposed population.

	Control		Exposed population	
	Non-smokers	Smokers	Non-smokers	Smokers
No. of Samples	22	18	28	22
BNMN/1000Cells	1.90 ± 1.2	4.10 ± 2.2	10.20 ± 2.1	21.00 ± 2.4

$P < 0.01$

Earlier, studies on biomonitoring have performed in humans occupationally exposed to lead although relatively few studies have examined the genotoxic potential of lead and they offer some equivoral results, showed increased in chromosomal aberrations (Forni et al. 1976, Kentner 1994). The high degree of variability in the available data represents perhaps, different levels of exposure and makes the explanation of biomonitoring results quite complex. The data from highly exposed to lead seem to support the hypothesis of an association between exposure to lead and stomach and lung cancer (Fu et al. 1995). In mammalian cells, induction of mutation rates has been reported in the hp rt locus in Chinese hamster v79 cells by lead compounds (Zelikoff 1988), although studies on the induction of chromosomal aberrations both *in vivo* and *in vitro* showed ambiguous because the genotoxic compounds appear to depend on factors such as cell type, duration and route of exposure and can be influenced by synergistic effects.

The MN assay detects both clastogenic and aneugenic effects covering wide spectrum of damage than chromosome aberration test. (Norppod et al. 1992) thus it could be possible that lead induces genotoxicity via induction of chromosome loss. The binucleated cells analysed in the cytokinesis MN assay might have completed their first in vitro mitosis and were observed in culture. Then in vivo MN inducer would have initiated its effects in Go stage lymphocytes (Maria Pitenque et al. 2002).

The present results are comparable with our earlier studies such as increased frequencies of micronuclei in industrial painters (Madhavi 2008) and in shoe factory workers (Jitender Naik et al. 2005). In summary, this study shows a clear genotoxic effect associated with occupational exposure to lead. These data are relevant and permit an estimate of genetic risk of lead by using biomarkers of exposure.

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