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A Study on Chromosomal Aberrations and Sister Chromatid Exchanges in Lead Industry Workers

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ABSTRACT

Lead is a widely used heavy metal with known toxicological effects, including severe health problems such as neurological damage, cardiovascular issues, and kidney damage. This study aimed to evaluate the genotoxic effects of lead exposure in lead factory workers. Methodology: 173 lead factory workers and 179 control subjects were included in the study. Blood samples were analysed for lead levels using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Cytogenetic damage was assessed by examining chromosomal aberrations (CA) and sister chromatid exchanges (SCE) in peripheral blood lymphocytes. Results: Blood lead levels in lead factory workers significantly increased compared to controls. Chromosomal aberration analysis, which included gaps, breaks, dicentrics, and polyploidy, showed no significant differences between the two groups. Sister chromatid exchange frequencies were higher in lead factory workers (SCE/cell: 3.47) than in controls (SCE/cell: 3.17), although this difference was not statistically significant. Conclusion: The study showed no cytogenetic damage in lead factory workers although there was an increase in the BLLs in the workers.

Keywords: *Lead Genotoxicity, Chromosomal Aberrations, Sister Chromatid Exchanges*

1. INTRODUCTION

Lead is the ubiquitous heavy metal widely used in various industries and domestic appliances, such as batteries, paints, and plumbing. Higher levels of lead contribute less to biological activity but result in more significant toxicological damage. Lead typically enters the body through inhalation, ingestion, and dermal contact (Ashraph et al., 2012). Once absorbed, lead enters the bloodstream and accumulates in mineralizing tissues such as bones and teeth, as well as in soft tissues including the kidneys, muscles, and liver. In adults, lead poisoning is predominantly due to occupational exposure.

Elevated lead levels can lead to significant health problems, including neurological damage, developmental delays in children, cardiovascular issues, and kidney damage. Lead poisoning also negatively impacts reproductive health in both males and females(Pant et al., 2014, Lee et al., 2020). Chronic lead exposure has been associated with cognitive decline and behavioural issues in adults (Reuben et al., 2019). The International Agency for Research on Cancer (IARC) classifies lead as a Group II carcinogen, and the Centres for Disease Control and Prevention (CDC) has set acceptable blood lead levels at 10 µg/dL for adults and 5 µg/dL for children.

Both endogenous and exogenous factors continually induce DNA damage, but the DNA repair mechanisms work to maintain genetic stability. Recent studies have revealed that lead interferes with these repair mechanisms by disrupting DNA repair pathways and downregulating repair genes (Singh et al., 2021). This disruption can lead to increased genomic instability and a higher risk of mutations. The genotoxic effects of lead can be evaluated using various biomarkers, including chromosomal aberrations (CA), sister chromatid exchanges (SCE), and the comet assay. Earlier studies have shown contradictory results on the genotoxicity of lead in man. While some studies showed evidence of cytogenetic damage(Das et al., 2013, Shaik et al., 2006), some other studies did not

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indicate increased chromosomal anomalies (Hasani et al., 2016, Grandjean et al., 1987, Maki et al., 1981). In this context, the present study attempted to understand the genotoxic effects on lead factory workers.

2. MATERIALS AND METHODS

The present study consists of 173 Lead factory workers (subjects) and 179 control subjects who were not exposed to any chemical. A questionnaire was prepared to collect information such as age, gender, occupation, and health problems. Signed consent forms were collected and the purpose of the study was explained to the participants before starting the questionnaire and blood collection. The present study was accepted by the Institutional Ethical Committee, Mahavir Hospital and Research Centre, Hyderabad.

a) Inclusion criteria:

Male employees over 18 years of age who had not been exposed to other chemicals were included.

b) Exclusion criteria:

Employees who did not provide their consent or answered "I don't know" to any question on the questionnaire were excluded.

c) Sample Collection:

5 mL of peripheral blood was drawn into EDTA-coated vacutainers and stored at -20°C until use.

3. METHODOLOGY

Analysis of Blood Lead Levels (BLLs):

Blood samples were thawed and analysed for lead content using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Analysis of Cytological Damage:

Analysis of chromosomal aberrations and sister chromatid exchanges were used to investigate cytological damage.

(i) Analysis of Chromosomal Aberrations:

0.5mL of blood out of 5mL blood drawn from each individual was added to 5mL medium and each sample was maintained in duplicate. A control batch of cultures was set up simultaneously and all the cultures were incubated at 37°C for 72 hours.

2 hours before the termination of the cultures, at the 70th hour, colchicine (0.3 µL/mL) was added to inhibit spindle formation. At the end of the 72 hours, each culture was transferred to a 15 mL centrifuge tube and centrifuged at 1000 rpm for 10 minutes, after which the supernatant was discarded. 5mL of pre-warmed hypotonic solution (0.75 M KCl) was then added to the pellet, and the cells were incubated at 37°C for 20 minutes. Following the hypotonic treatment, the cell suspension was centrifuged again at 1000 rpm for 10 minutes. The supernatant was discarded, and pre-chilled fixative (3:1 methanol and acetic acid) was added carefully along the tube walls to prevent cell clumping. The cells were allowed to stand for 10 minutes, after which the suspension was centrifuged at 1000 rpm for 10 minutes once more. The supernatant was removed, and a fresh fixative was added to the pellet. This fixation process was repeated 3 to 4 times, with the final cell suspension made in 1 mL of fresh fixative.

Preparation of the slides:

The micro slides were cleaned and kept in chilled distilled water before use. Two or three drops of cell suspension were dropped on each micro slide using a Pasteur pipette. The slides were air-dried. All the slides were coded and stained in 2% Giemsa (2 ml of Giemsa, 2 ml of Sorenson's buffer and 46 ml of distilled water) for 5 minutes. After staining, the slides were rinsed in distilled water and dried. The slides were coded and then observed for chromosomal aberrations under the microscope.

Scoring of chromosomal aberrations:

For each 100 well-spread metaphases were screened for structural (gaps, breaks, fragments., exchanges, dicentrics) and numerical (polyploidy) aberrations. However, gaps and polyploids were not included in total number of aberrations. Well-spread metaphases were micro-photographed.

(ii) Analysis of Sister-chromatid exchange:

Culturing of peripheral blood lymphocytes was carried out similarly to the analysis of chromosomal aberration except that BrdU (3 ug/ml) was added after 24 hours of culturing. The culture vials were then wrapped in dark black paper to avoid photolysis of BrdU substituted DNA and incubated at 37'C. Colchicine was added to the culture vials at the 70th hour of incubation to arrest the spindle formation and the cultures were harvested for 72 hours. The slides were prepared and dried by the conventional flame drying method and were stained by using the FPG (Fluorescence Plus Giemsa) technique of Perry and Wolf (1974).

Staining procedure:

Three-day-old slides were kept in Hoechst — 33258 (2 ug/ml) working solution for 20 minutes. After Hoechst treatment, the slides were rinsed in distilled water and placed in a petri dish containing Sorenson's phosphate buffer until they were immersed and exposed to tube light for 18 hours, at a distance of 4 inches. After 18 hours the slides were rinsed in distilled water and incubated in 2 x SSC solution at 60 C for 2 hours. After this treatment, the slides were thoroughly rinsed in distilled water 2 to 3 times. The slides were allowed to dry and were stained in 4% Giemsa (2m1 Giemsa and 48 mL Sorenson's buffer) for 3 to 5 minutes.

Scoring of SCEs:

50 well-spread metaphases, showing clear differential staining between chromatids, were scored per sample for sister chromatid exchanges under a microscope.

4. STATISTICAL ANALYSIS

We analysed the following variables: age, blood lead levels (BLLs), and years of service. These variables were summarized using Mean ± SD (standard deviation) to describe the central tendency and variability within our study groups. To determine whether there were significant differences in genotoxic markers between the lead-exposed subjects and the control group, we employed a 2x2 contingency Chi-square (χ^2) test.

5. RESULTS

The results on chromosomal aberrations and sister chromatid exchanges in lead factory workers are shown in Table 1-2. The frequency of CA was comparable between the control and subjects. The incidence of CA was 1.94% in the control subjects it was 2.02% in lead factory workers. A similar observation was made about SCEs the frequency of SCEs was 3.17 and 3.47 in the control subjects and factory workers respectively. The statistical analysis did not indicate any significant differences between the two groups.

Table 1: Frequency of chromosomal aberrations in peripheral blood lymphocytes

Study group		**Lead factory workers(n=173)**	**Control subjects (n=179)**
No. of samples cultured		157	170
No. of metaphases screened		15700	17000
Chromosomal aberrations	**Gap**	155(0.98)	148(0.87)
	Breaks	208(1.32)	212(1.24)
	Dicentrics	110(0.7)	118(0.69)
	Polyploidy	86(0.57)	80(0.47)
Total		*318(2.02)	330(1.94)

*Gaps and Polyploidy are not included in total chromosomal aberrations.

Table 2: Frequency of Sister chromatid exchanges in peripheral blood lymphocytes

Study group	**Lead factory workers (n=173)**	**Control subjects (n=179)**
No. of samples cultured	157	170
No. of metaphases screened	7850	8500
Total no. of SCEs	27240	26915
SCE/Cell	3.47	3.17

6. DISCUSSION

This study examined the genotoxic effects on lead factory workers, revealing that blood lead levels (BLLs) were significantly elevated and exceeded permissible limits in this group. Investigating chromosomal aberrations and sister chromatid exchanges is crucial for detecting the cytogenetic damage of lead exposure in the workers of the lead industry.

In our analysis, we evaluated 100 metaphases per sample to identify chromosomal aberrations, including gaps, breaks, dicentrics, and polyploidy. However, no significant differences were found between the subjects and the control group. This aligns with the findings of Hasani et al., (2016), and Karakaya et al. (2005), who reported no increase in chromosomal aberrations among battery manufacturing workers and did not link lead exposure to DNA repair inhibition. However, Grover et al. (2010) and Balasubramanian et al. (2020) observed elevated chromosomal aberrations in exposed workers.

We also assessed sister chromatid exchanges (SCEs) in both groups and noted no significant increase in SCEs among subjects compared to the controls. This observation is similar to that of Maki et al. (1981), who also reported no significant increase in SCEs in exposed workers compared to controls. Huang et al. (1988) found a higher frequency of SCEs in exposed workers but could not establish a correlation between BLLs, chromosomal aberrations, and SCEs. We have estimated the BLLs in the factory workers and reported a mean of 29µg/ml (report in press). However, no cytogenetic damage in these workers was observed. Similarly, Hasani et al., (2016) reported increased BLLs and no difference in CA in lead exposed workers.

In conclusion, our study did not find significant chromosomal aberrations, and SCEs linked to lead exposure. However, Further studies with higher sample sizes and longer exposure are worthwhile.

7. CONCLUSION

This study provides insights into the genotoxic effects of lead exposure among workers in a lead factory. Elevated blood lead levels in the exposed group confirm the occupational exposure, aligning with known health risks associated with lead toxicity. Although our analysis did not show significant differences in chromosomal aberrations between lead-exposed workers and controls, we observed a trend towards increased sister chromatid exchanges in the exposed group. This suggests that lead exposure may influence certain genotoxic parameters, although the changes were not statistically significant in this study. The lack of significant findings

in chromosomal aberrations could be due to various factors, including sample size or the sensitivity of the methods used. Further research with larger cohorts and more refined techniques is warranted to better understand the full spectrum of lead-induced genotoxicity and its potential implications for worker health.

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