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Analysis of Chromosomal Aberrations in PBL of Chrome Tanning Industrial Workers

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Abstract

In order to monitor the geno-toxic effects of chromium compound, 160 chrome tanning industrial workers (80 each of smoker and non-smoker) were selected from a leather tanning industry to perform the cytogenetic protocol of chromosomal aberrations in the peripheral blood lymphocytes. The workers were further classified into two groups on the basis of their personnel habit (Smoker and Non-Smokers) and on the duration of their employment (1-5, 6-10 and 11-15 years). Even 120 individuals (62 smokers and 58 non-smokers) were also selected as controls for the purpose of comparison. A statistically significant increased frequency of total chromosomal aberrations was observed in the chrome tannery workers (6.20 ± 0.12) as against the control (1.61 ± 0.09). The increased frequencies of aberrations were duration dependent. Even higher incidence of chromosome aberrations was reported among the smoker group of both control and exposed workers. The results clearly establish the mutagenic nature of the chromium compound in human beings.

Keywords: Chromium; Chromosomal aberration; Leather tanning; Industrial workers; Peripheral blood lymphocyte; Smokers and non-smokers

Introduction

Metals are the ubiquitous chemical entities, known to cause mutations in a variety of test systems. Based on the epidemiological studies, some of them are classified as human carcinogen [1] as well as mutagens and the human exposure to these metallic compounds was found among the workers in a large number of professional groups. The occupational exposure to chromium compound was typically found in stainless steel welders, chrome platters [2], leather tanning and chromate production workers [3].

Leather tanning is an age-old practice in India, which is recently recognized as one of the potential polluting industry of considerable importance. The wastes from tannery contain an excess amount of chromium ranging from 100-200 mg/l and the spent chrome liquor contains 2900-4500 mg/l [4]. Chronic exposure, poor working conditions, lack of civic sense and awareness of the potential hazard leads to an occupational health hazardous situations at an industrial set up.

Chromium compounds were known to be potent carcinogens and mutagens [5], which can induce a spectrum of DNA damage [6], gene mutation [7], sister chromatid exchanges [8] and chromosomal aberrations [9]. Several workers already reported the mutagenic and carcinogenic potentialities of chromium compound in bacterial and mammalian cell-based mutagenicity assays [10-13]. The investigations carried out to examine the geno-toxicity in workers occupationally exposed to chromium are meagre [14-18]. However, there are reports on positive geno-toxic effects in populations exposed to chromium also a negative findings [19-24]. The studies on the evaluation of genetic damage in chrome tanning workers exposed to chromium are scanty and rather conflicting. Hence an attempt was made during this study to evaluate the mutagenic potential of chromium compound in occupationally exposed leather tanning industrial workers in a tanning industry situated at Bakaram industrial area, Hyderabad, Andhra Pradesh, India by using the standard cytogenetic protocol of chromosome aberrations.

Materials and Methods

Air and blood chromium analysis

Ambient air samples at different locations of the industry were collected to evaluate chromium levels. The samples were collected on membrane filter (37 mm, 0.8 µm pore size) and in 1M HNO_3 using KIMOTO air samples at a flow rate of 1-2 LPM for 8hrs in day shift. The collected samples were wet digested with concentrated HNO_3 and analysed for chromium by using atomic absorption spectrophotometer (Double Beam 3100 model, Perkin Elmer, USA) [25].

Blood samples were collected in heparinized vials and were wet digested in microwave digestive system using conc. HNO_3 and were analyzed for the metal by atomic absorption spectrophotometer (Double Beam 3100 model, Perkin Elmer, USA) [26].

Study population

160 tannery industrial workers (80 each of smoker and non-smoker group) were selected from a leather tanning industry situated at Bakaram industrial area, Hyderabad, Andhra Pradesh, India, to evaluate the genotoxic potential of Chromium compound by using the standard cytogenetic protocol of chromosomal aberrations in peripheral blood lymphocytes . The selected workers belong to the age group of 25-50 years and belong to the same socio-economic status.

Simultaneously 120 individuals (62 smokers and 58 non-smoker group) who didn't have any history of exposure were also selected for comparison (control). The data was further analyzed on the basis of their personnel habitat into smokers and non-smokers and also on their duration of service into 1-5, 6-10 and 11-15 years, respectively. All participants were informed about the objectives of the investigation and written consent was obtained from each person. Personnel data and family histories were collected by the interviews and questionnaires. This study was approved by institutional ethics committee.

Lymphocyte isolation and cell culture

Blood samples were collected by vein puncture in Heparinized centrifuge test tubes and transported to the laboratory within 2-4 hrs. Lymphocytes were isolated by gradient centrifugation and washed three times in phosphate buffer saline. Lymphocytes from each individual sample were divided into several aliquots. One part was used to prepare 2 ml lymphocyte cultures, with a cell density of 1 × 106/ ml in RPMI – 1640 culture media, supplemented with 15% fetal calf serum , antibiotic and 1% phytohaemagglutinin. The lymphocyte cultures were incubated at 37°C in a humid atmosphere with 5 % CO_2 for 48 hrs. Cell harvesting and slide preparation

Cell harvesting was done followed by colchicine treatment (0.1 µg/ml culture medium) for the last 4 hours of the 48 hours incubation, hypotonic treatment in prewarmed 0.075M KCl solution for 15 minutes at 37°C and three fixations in chilled methanol – acetic acid mixture (v/v, 3:1). The cells were dropped on to cool wet slides. The slides were air dried and stained in 2% Giemsa stain (pH 6.8) mounted with DPX and scored for chromosomal aberrations by adopting the method of [27].

Aberration scoring and statistical analysis

Chromosomal aberrations were scored on coded slides by two independent observers using research microscope under 10× and 100× oil immersion. One hundred metaphases per subject were scored in the heavy metal exposure study. Only well spread metaphases with 46 centromeres were selected. Gaps (A chromatic lesions) were counted separately but not included in the frequency of the cells with aberrations. The frequencies of the cells with aberrations were tested statistically by using a Chi-square (2×2 contingency) test to find out the significant levels between the groups tested.

Results

The result on chromium concentrations in the ambient air and in the blood was depicted in Table 1. The levels of chromium were found in higher concentrations at tanning unit (43.26 ± 20.20 µg/m^3). Spray dyer (22.14 ± 10.60 µg/m^3) and in administrative office (3.86 ± 0.61 µg/m^3). The chromium levels observed are much lower than threshold limit value of 500 µg/m^3. The mean blood chromium levels in the exposed workers was 6.83 ± 1.32 µg/100 ml which was considered higher when compared to the control value of 2.67 ± 0.34 µg/100 ml.

Chromium	Ambient air µg/m^3 Blood(µg/100ml)			Blood(µg/100ml)	
	Tanning unit	Spray dyer	Admn. office	Control	Exposed
	43.26 ± 0.20	22.14 ± 0.60	3.86 ± 0.61	2.67 ± 0.34	6.83 ± 1.32
Values in the ambient air at different work stations were less than the threshold values of 500 µg/m^3.					

Table 1: Chromium concentration in different study areas.

The overall result on the incidence of chromosomal aberrations in leather tanning industrial workers is presented in Tables 2 and 3.

Group	No. of Examinees	% of Aberrant cells	P value
Control	120	1.61 ± 0.09	-
1-5 yrs	75	4.82 ± 0.33	0.01
6-10 yrs	50	6.84 ± 0.73	0.05
11-15 yrs	35	8.22 ± 1.38	0.05
Total exposure	160	6.20 ± 0.12	0.05
100 metaphases were scored for each sample. Gaps and polyploids are not included in aberrant cells. Values in parenthesis are percentages ± S.E.			

Table 2: Chromosomal aberration frequencies in occupational tannery workers (smokers and non-smokers) exposed to chromium.

An increased pattern in the frequency of chromosomal aberration was observed in the chromium exposed group when compared to the control. As a result of this the percentage of total chromosomal aberrations got increased from 6.20 ± 0.12 in the exposed workers as against 1.61 ± 0.09 in the control subjects. In order to monitor the longitudinal variations of chromosomal aberrations, further analysis was carried out on the basis of duration of employment and on the basis of their smoking habit. A gradual increase in the frequency of total chromosomal aberrations of 4.82 ± 0.33, 6.84 ± 0.73 and 8.22 ± 1.38 were observed with the increase in the duration of exposure of 1-5, 6-10, 11-15 years respectively (Table 2). Similar trend was observed among the smoker and non-smoker subjects. A 5.35 ± 0.36, 7.76 ± 0.55 and 10.80 ± 0.84 increased percentage of chromosomal aberrations were observed among the smoker exposed group as the increase in the duration of exposure at the industry as against the control smoker group of 3.70 ± 0.30, 5.92 ± 0.48 and 8,40 ± 0.74 respectively (Table 3).

Group		Smokers		Non-Smokers	
		No. of Examinees	% Aberrant cells ± S.E	No. of Examinees	% Aberrant cells ± S.E
Control	-	62	134 (2.16 ± 0.18)	58	60 (1.03 ± 0.13)

Duration of exposure	1-5	40	214 (5.35 ± .36)**	40	148(3.70 ± .30)**
	6-10	25	194 (7.76 ± .55)*	25	148(5.92 ± .48)*
	11-15	15	162 (10.80 ± .84)*	15	126 (8.40 ± .74)*
Total experience	1-15	80	570 (7.12 ± .29)*	80	422(5.27 ± .25)*

100 metaphases were scored for each sample.

Values in parenthesis are percentages ± S.E.

Gaps and polyploidy are not included in aberrant cells.

* P< 0.05 and **P<0.01.

Table 3: Chromosomal aberration frequencies in Smoker and Non-Smoker groups exposed to chromium.

Discussion

Large number of industries releases the chromium compound into the air, water and soil. In the air this compound is mainly present in the form of fine dust [28]. The permissible exposure limits for chromium in work place during an 8 hr, 40 hrs work weak is 100 µg/m3 and the recommended exposure limit is 1 µg/m^3. The investigated result is supported by the observations made by [29] in a chemical based industry.

There is clear evidence that some metals represent a carcinogenic hazard to man and several metallic compounds has been identified as human carcinogens [30]. Evaluation of mutagenic hazards has become an integral part in the toxicological assessment with a number of environmental chemical [31]. The cytogenetic methods were routinely employed in monitoring the populations, exposed to industrial chemicals [32]. A significant increase in the frequency of chromosomal aberrations among the workers of leather tanning industry is once again establishing the mutagenic nature of the chromium compound, as the tannery effluents have the potential to damage the DNA of test organisms [33].

Induction of chromosomal aberrations in human peripheral blood lymphocytes by chromate compounds were reported earlier [34]. However the present results were attributed to the observations made in peripheral blood lymphocytes of chromate workers, exposed to chromium containing fumes [35-39]. Further the results were comparable with that of the observations among the workers exposed to benzene pyrene-epoxides [40-42].

The chromosomal aberrations observed in the study was mainly of chromatid type, which can be capable to induce more number of aberrations in late S1 phase or early G2 phase of the cell cycle [43]. The presence of less iso-chromatid aberrations may reflect a direct effect of the compound on G1 phase. Further, the aberrations recorded even after 11-15 yrs of exposure may be due to the phenomenon of aging of the cells in circulating blood lymphocytes.

A significant increase in chromosomal aberrations in smoker and non-smoker exposed group to chromium compound than their respective controls was due to the effect of cigarette smoke on the genetic material. Even the synergistic interaction would also be possible, but the actual mechanism is not yet to be documented. Similar findings were reported by several workers among the smoker groups, occupationally exposed to rubber, heavy metals and plastic workers which will support the present investigation [44-49].

The chromate compounds are well known as human and animal carcinogens and the workers occupationally exposed to chromium compound are prone towards the increased risk of cancer. The exact mechanism for chromium mutagenicity has not been so far reported, but it has been suggested that the chromium compound give off hydroxyl, cysteinyl and thionyl radicals during cellular reductions [50-53]. These radicals can interact directly with DNA – chromatin to form DNA single strand breaks, DNA-protein cross links, chromium-DNA adducts and DNA-DNA cross links, ultimately leads to chromosomal breakage and mutations. In conclusion, the study reveals that there is a significant increase in the frequency of chromosomal aberrations in the peripheral blood lymphocytes of leather tanning industrial workers. This increase may be due to chromosomal instability and also the fact that chromium being used in tannery industry is a potent mutagen. Further this study needs an elaborate and exhaust study in order to interpret the data in a conceptual frame work. The present study facilitates to make a decision on issue of pollution control, mutagenesis and risk analysis. It is also helpful to have a solution for the damage caused by the polluting industries.

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