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Research Article

Cytomorphometric analysis of buccal cells of steel workers exposed to chromium in south indian population

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ABSTRACT

Steel workers are exposed to a mixture of chemicals and chromium (IV) and (III), which is suspected to cause genetic damage. And to evaluate the MN assay along with the cellular diameter (CD) and nuclear diameter (ND) in the buccal epithelial cells of workers residing in Coimbatore District, South India, using cytomorphometric analysis. Methods: 40 samples from steel workers and 20 samples were exposed, 20 as standardized control group were examined for frequencies of micronucleus (MN) in buccal epithelial cells. PAP staining techniques were used to examine the nucleus and micronucleus. The frequency of micronuclei is more in smokers group when compared to alcohol consumers. Results: The genetic damage observed in the buccal cells of steel workers was significantly higher than in controls with cigarettes smoking in exposed group (12.28 ± 0.61) and alcohols in exposed group (12.61 ± 0.46) while compare to that of non-smoking and non-alcohols workers (6.94 ± 0.60 and 6.47 ± 0.44). Conclusions: Occupational exposure of chromium from steel workers has been associated with increased genetic damage in smoking and alcohol habits represent an additional risk factor. Exposure of chromium may be related to increased risk of cancer in steel workers.

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Introduction

Chromium is a steely-gray, lustrous, hard metal that makes a high polish and having high melting point, also it is silvery white, hard, and bright metal plating on steel and other materials. Commonly known as chrome, it is one of the most important materials that uses mainly in industrial metals because of its hardness and resistance to corrosion. It is used for the production of stainless steel and nonferrous alloys. In the past century's, chromium became widely used in steel production. Today, the main uses of chromium are in alloys production. Also, in the production

of various chemical forms like chromium used in pigments, metal surface treatments and corrosion control etc. The compounds present in the chromium protect against rust, provide color, conserve energy as components of catalysts, prevent decay, and resist soiling. In fact, the uses of chromium are so extensive that today's world would be almost unrecognizable without it [1]. Trivalent chromium is not regarded as having the same toxicity as Cr (VI), apparently owing to its relative difficulty in crossing cell membranes [2]. Nevertheless, evidence obtained from in vitro cell-free systems shows that, once inside the cell, Cr(III) readily complexes several intracellular macromolecules,

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whereas Cr(VI) is relatively inert towards the genetic material in the absence of reducing systems [3,4].

During the reduction of Cr(VI) more reactive forms of chromium are generated, namely the short-lived Cr(V) and Cr(IV) and possibly Cr(II), and the stable Cr(III), as well as other reactive species, including singlet oxygen or hydroxyl radicals [5], capable of inflicting oxidative damage to the cell. The deleterious effects known to chromium may result from the reaction and binding of the reduced forms of the metal to intracellular macromolecules including DNA or from oxidative damage initiated by the side products of chromium reduction. The redox biochemistry of chromium is rich, involving oxidation states from (2 to 16), from which by far the most stable are the elemental Cr (0), Cr(III) and Cr(VI) valences [6]. Hexavalent chromium compounds are classified by IARC as known human carcinogens, after substantial epidemiological and experimental data proved their mutagenic and carcinogenic properties [7]. Nevertheless, chromium is still a widely used industrial metal to which millions of workers are exposed worldwide in industries, such as pigment production, chrome plating, leather tanning, stainless steel production and welding. Welders are estimated to receive some of the highest acute exposures to hexavalent chromium in welding fumes [7].

The presence of micronuclei (MN) and nuclear abnormalities (NA) are biomarkers broadly used; the detection of MN and NA offers a great opportunity to monitor individuals or populations exposed to mutagenic, genotoxic, or teratogenic events, mainly the evaluation of micronucleogenic cells presence in epithelial tissues. A micronucleus test is a test used in toxicological screening for potential genotoxic compounds. The assay is now recognized as one of the most successful and reliable assays for genotoxic carcinogens. Micronuclei form during anaphase from lagging acentric chromosome or chromatid fragments caused by incorrectly repaired or unrepaired DNA breaks or by nondisjunction of chromosomes. This incorrect segregation of chromosomes may result from hypomethylation of repeat sequences present in pericentromeric DNA, irregularities in kinetochore proteins or their assembly, dysfunctional spindle apparatus, or flawed anaphase checkpoint genes [8].

Materials and Methods

A total number of 40 workers consisted in the study were (20 steel workers and 20 controls) with their habits of smoking and alcohols consumption. Steel workers were employed in casting of molten metal, mold operator, welders, gas cutters, fitters etc. around Coimbatore city, South India. At the time of sample collection, the subjects signed a term of informed consent. The subjects were selected based on questionnaire which included items about age, sex, marital status, medical history, life style) and also the details of occupational related questions (days and years of exposure). Where the experimental subjects smoked more than 15cigarettes/per day for at least one year and more were considered as smokers and those who consume more than 200gm of alcohol/day were considered as alcohol consumers. The study was conducted according to the principles for human experiences as defined by the Helsinki declaration.

Buccal Cell Collection

The buccal cells from the experimental and control subjects were collected from the shift workers. Before taking samples from workers, were asked to rinse their mouth with distilled water thoroughly. The buccal cells were collected by scraping inside of booth cheeks of the mouth for one minute with the tooth picks. The toothpicks were dipped into the eppendroff tubes consisting of 0.9% of saline. The saline was centrifuged at 1500rpm for 8min.the pellet once more with Carnoys fixative (methanol and glacial acetic acid 3:1). The cell suspension dropped in a glass slide and the cytomorphometric analysis are given in the **Table 2**.

Micronucleus Test

The MN test was carried out on buccal epithelial cells of 40 workers include 20 subjects and 20 controls. Oral buccal cells obtained were smeared on a precleaned slide, Cell suspension of 10 μ l was smeared on a microscopic glass slide, fixed and stained with Papanicolaou (PAP) stain. A total of 2000 cells per individual were scored for analysis of micronuclei.

Statistical Analysis

The samples were coded at the time of preparation and scoring. They were decoded before statistical analysis for comparison. Mean and standard deviation (SD) were calculated for biomarker. The significance of the differences between control and worker end-point means were analyzed using Student's t-test, whereas simple and multiple linear regression analyses were performed to assess the association between end-points and the independent variables. Mean values and standard deviations were computed for the scores and the statistical significance ($P < 0.05$) of effects (exposure, smoking and age) was determined using analysis of variance (ANOVA).

Results

The distribution of subjects with respect to age, smoking, alcohol consumption and years of exposure and their employment are indicated, as well as chromium levels in buccal epithelial cells using the micronucleus frequency that are given in the table 1 and the cytomorphometric analysis are given in the table 2.

Micronucleus Frequency in Buccal Cells

The frequency of micronuclei (MN) was studied in a total number of 40 workers including 20 subjects and 20 controls. Workers showing a major induction of MN when compare with controls (Table I). Individuals in the exposed as well as control groups with smoking habit and alcohol consumption showed a superior frequency of micronuclei (12.28 ± 0.61) and (6.94 ± 0.60) vs (12.61 ± 0.46) and (6.47 ± 0.44) when compare to nonsmokers and non-alcoholics (5.65 ± 0.24) and (3.12 ± 0.38) vs (7.07 ± 0.37) and (3.12 ± 0.41). Workers who are smokers and alcoholics showed a highly significant increase ($p < 0.05$) in MN frequency when compared to all other groups and subgroups (Table 1). A major increase in MN frequency of the exposure was very much increased and that was observed in smoking and alcohol consumptions in workers ($13.06 \pm$

1.66) and (13.61 ± 1.32) (Table 1). Also shows the age dependent increase in MN frequency was also noted both in controls and exposed (40.22 ± 4.14) vs (37.95 ± 4.26) (Table 1).

Cytomorphometric Analysis

Cytomorphometric analysis of the buccal mucosa of the subjects having smoking and alcohol consumption habit showed a significant variation in the size of ND and CD when compared to the controls (Table 2). The frequency of variation was high in subjects exposed to chromium with smoking and alcohol consumption habit and less comparing to controls which were consistent to Cytomorphometric is a simple, non-invasive [9] quantitative method used for the early detection of malignancy. This technique had been implemented in a number of studies to evaluate the effect of diverse systematic and external factors on [10], cell size and nuclear size using different experimental samples [11, 12]. An increase in the level of chromosomal aberration and micronuclei of smoking and alcohol. In the present study, we observed a significant reduction in the size of CD and increase in the size of ND. Increased ND to decreased CD ratio was one of the reliable changes during [13] progression from benign to a state of malignancy.

Discussion

Present study reports an elevated MN frequency among Cr and Cr compounds that are exposed in steel workers around Coimbatore region. The current analysis suggests that the steel workers under their particular conditions of exposure and using (smoke and alcohol) reveals the clear evidence of genotoxicity in buccal epithelial cells when evaluated by MN test. Our study revealed a significant induction of MN in workers

when compared to controls with respect to their age and years of exposure [14] also demonstrated a strong correlation between age and MN frequency and suggested that chromosome loss is a determining factor in this increase. And the same convincing association is apparent when assessing biomonitoring studies of genotoxicity. Fenech [15] showed that, after adjustment for age and sex, individuals with high cigarette usage [16] had statistically greater MN compared to non-smokers. An increase in MN has been observed in alcoholics consuming alcoholic beverages but not in abstainers of a year or more [17].

Therefore, our results demonstrate that MN assay performed in exfoliated buccal mucosa cells is an ideal methodology to measure potential risk related to Cr (III) exposure. However, the results of this study are not enough to establish any causal connection, although there is experimental evidence that supports the genotoxicity of Cr (III). Also, the possibility of unrecognized confounding factors is inevitable in studies such as this.

Workers in many occupational settings are exposed to certain genotoxic agents. These workers may not be aware that they have been exposed to genotoxic agents nor do they know the type and amount of agent to which they have been exposed. Therefore, there is a need to educate those who work with heavy metals about the potential hazard of occupational exposure and the importance of using protective measures.

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Table 1: General characteristics of the study population

Characteristics	Sample size (n=40)	Age (years)	Exposure duration (years)	No. of Cigarettes/day	Alcohol (gms/day)	MN (Mean \pm SD)
CONTROL	20	40.22 ± 4.14	–	–	–	3.71 ± 0.43
Total						
Smoking	Yes	8	38.7 ± 3.61	–	10.75 ± 2.91	5.625 ± 0.24
	No	12	36.16 ± 3.39	–	–	3.12 ± 0.38
Alcohol consumption	Yes	12	38.25 ± 3.93	–	171.66 ± 47.83	7.07 ± 0.37
	No	8	39.37 ± 4.27	–	–	3.12 ± 0.41
EXPOSED	20	37.95 ± 4.26	12.35 ± 1.89	–	–	7.94 ± 0.46
Total						
Smoking	Yes	15	36.6 ± 2.64	13.06 ± 1.66	12.53 ± 2.41	12.28 ± 0.61
	No	5	36.4 ± 3.64	11.20 ± 1.30	–	6.94 ± 0.60
Alcohol consumption	Yes	13	36.84 ± 2.33	13.61 ± 1.32	191.53 ± 47.23	12.61 ± 0.46
	No	7	38.57 ± 1.71	10.85 ± 1.34	–	6.47 ± 0.44

Table 2: Comparison of mean values of cellular diameter, nuclear diameter and nuclear cytoplasmic ratio in buccal epithelial cells of Samples and Controls

Parameter	Group	Group I	Group II	Group III
Nuclear diameter (µm)	Controls	6.89±0.28	7.83±0.15	6.65±0.25
	Users	7.31±0.38*	8.75±0.030*	8.24±0.51*
Cytoplasmic diameter (µm)	Controls	77.33±0.24	72.98±0.24	76.23±0.32
	Users	69.73±0.38*	46.59±0.31	59.2±0.28
Nuclear: Cytoplasmic ratio	Controls	0.11±0.01	0.13±0.008*	0.14±0.11
	Users	0.15±0.013*	0.20±0.012*	0.25±0.09*

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