Elevated Frequencies of Micronuclei and other Nuclear Abnormalities of Chrome Plating Workers Occupationally Exposed to Hexavalent Chromium

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Abstract

Background: Biomonitoring provides a useful tool to estimate the genetic risk from exposure to genotoxic agents. The aim of this study was to assess the potential cytogenetic damage associated with occupational exposure to hexavalent chromium by using micronuclei (MN) as a biomarker.

Methods: This was a cross-sectional study and all participants were males. Both the exposed and control individuals were selected from Coimbatore, Southern India. Exfoliated buccal cells from 44 chrome plating workers and 40 age and sex matched control subjects were examined for MN frequency and nuclear abnormalities (NA) other than micronuclei, such as binucleates, broken eggs, karyorrhexis, karyolysis and pyknosis.

Results: Results showed statistically significant difference between chrome plating workers and control groups. MN and NA frequencies in chrome plating workers were significantly higher than those in control groups ($p < 0.05$) and also significantly related to smoking habit ($P < 0.05$). A significant difference in NA was observed in workers exposed to chromium for longer duration. In addition to this, a higher degree of NA was observed among smokers.

Conclusion: MN and other NA reflect genetic changes, events associated with carcinogenesis. Therefore the results of this study indicate that chrome plating workers are under risk of significant cytogenetic damage. 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.

Key words: DNA damage; Buccal cells; Chrome platers; Micronucleus test

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Introduction

Electroplating was a very first categorically regulated industry. Electroplating involves the coating of an electrically conductive object with a layer of metal to achieve decorative or engineering requirement [1]. The metal processing involves mechanical (grinding and polishing), chemical and electrochemical methods (degreasing and scaling) employing solvents, alkaline and/or acid cleaners abrasive materials and/or water. Cadmium, chromium, copper, gold, nickel, and silver are the metals most commonly used to plate different objects.

Chromium plating continues to be the coating of choice for many metal finishing applications and is a process that involves the electroplating of a thin veneer of chromium onto an underlying metal for decorating purposes, protection from corrosion etc [2]. The chrome is found in various industrial uses, such as chrome plating, which uses the hexavalent form to protect from corrosion and improve the aesthetics. Hexavalent chromium (Cr VI) is an environmental carcinogen and a genotoxicant that is associated with respiratory cancers and induces several forms of DNA damage, including lesions that interfere with DNA replication. Cr VI is actively transported into cells through anionic non-specific channels, predominantly as a negative ion Chrome (CrO_4^-). Once reduced by glutathione, ascorbate and cysteine, in various stages of oxidation, is able to bind to proteins and DNA, causing gene mutations, chromosomal aberrations, altering the normal cell

cycle and inducing the genes responsible for apoptosis [3].

Several metals, including chromium, nickel, cobalt, cadmium and silica, have been classified as human carcinogens [4]. Although the mechanisms of their carcinogenesis are not clear yet, it is generally believed that generation of reactive oxygen species (ROS) and abnormal regulation of apoptosis play a critical role in neoplastic development in response to these metals [5]. In recent years, attention has been given to investigating the occurrence of genotoxic agents in the environment. The increasing concern of the general public for the welfare of humans requires the assessment of new sensitive and efficient methods for early detection of environmental genotoxic risk.

One parameter used in bioindication is the generation of genetic material fragments, known as micronuclei, due to the activity of clastogenic agents which provoke chromosome breaks. These fragments appear in the cytoplasm when either part of the chromosomes are not incorporated in the nuclei of the daughter cells in mitosis, frequently because these fragments do not have centromeres; these fragments left behind are incorporated in the secondary nuclei, called micronuclei [6,7].

Since DNA repair system is very sensitive to metallic ions, it is possible to visualize genomic structural damage such as the formation of micronuclei and cellular blebs, the latter typical of apoptosis [8]. There are only few reports on the health effects of chronic exposure to Cr VI in developing countries [9]. However no reports on human exposure to Cr VI are available. Hence the present study was under taken to monitor the chromium (VI) induced cytotoxicity in exfoliated buccal cells of electroplating workers. Degenerative nuclear changes, such as micronuclei (MN) binucleates (BN), broken egg (BE), karyorrhexis (KR), karyolysis (KL), pyknosis (PK) were analyzed in the exfoliated buccal cells.

Materials and Methods

A total of 84 male subjects (44 chrome plating workers and 40 controls) from Coimbatore city, an industrial city in southern India with a minimum number of 240 chrome plating industries were analyzed in this study. All of the plating companies identified was small autonomous businesses and not part of larger organizations. The workers had varying durations of exposure (5-15 years) and they were in the age group 28-51 years. The experimental group was further branched as smokers (24) and non-smokers (20). The control group included 21 smokers and 19 non-smokers. The control group was selected from the general population with no history of occupational exposure to chromium or any known physical or chemical agent in the workplace, but belonged to the same age group and socio-economic status as the chrome platers. The selection criteria for the subjects were based on a questionnaire. The questionnaire covered standard demographic questions (age, genetic disorders, number of X-ray diagnoses, vaccinations, medication, smoking, alcohol, etc.) and occupational questions (years of exposure). We ensured that the workers and the controls did not markedly differ from each other except for occupational exposure. We also ensured that all the subjects had not been taking any medicines nor had they been exposed to any kind of radiation for 12 months before sampling. The subjects who smoked >5 cigarettes/day at least for 1 year were considered as smokers and those who consumed >120gm of alcohol/day were considered as alcoholics in both groups. The workers and control subjects were informed of the objectives of the work, and gave expressed informed and written consent to participate in this study before the collection of buccal cell samples. The samples of buccal cells were coded and the anonymity of the workers and control population was guaranteed. The institutional ethical committee approved the research procedures used in this study. The present study was conducted, according to the protocol published by the International Commission for Protection against Environmental Mutagens and Carcinogens [10].

Buccal cells (BCs) were collected from consented volunteers at the end of the work shift according to the criteria established by Tolbert and his co-workers [11]. Prior to BC collection the mouth was rinsed thoroughly with water to remove any unwanted debris. Buccal cell samples were obtained by rubbing the inside of both cheeks using a wooden spatula. The cells were collected in tubes containing 3ml sterile saline.

The cells were smeared on slide, dried in air and fixed with cold methanol: acetic acid (3:1) solution in 0.1M phosphate buffer (pH 7.5) for 20 min. Then the slides were stained by Feulgen reaction essentially by the modified procedure of Belien and co-workers [12]. In briefly, hydrolysis in 5N HCl for 10 min at room temperature, washing in distilled water for 5 min, staining with fresh Schiff reagent (Sigma Chem, USA) for 90 min, and washing in tap water for 15min. The cells were counter stained in Coplin jars containing 1% Fast Green reagent for 2-5 min and rinsed with distilled water. Slides were analyzed under light microscope (Leitz, Germany) with 1000X

Table 1. General characteristics of the groups studied.

	Study group	(n=84)	Age (years) (mean ±SD)	Average no of cigarettes/day (mean ±SD)	Duration of employment (years) (mean ±SD)
Control (n=40)	Smokers	21	42.50±2.25	18.50 ± 2.83	
	Non-smokers	19	40.25±3.76		
	Alcoholics	18	44.93±3.05	280.56 ± 20.81	
	Non- alcoholics	22	38.75±4.20		
Workers (n=44)	Smokers	24	43.46±3.72	21.00 ± 3.05	8.24 ±1.44
	Non-smokers	20	37.65±2.99		9.55 ±0.84
	Alcoholics	19	41.50±3.51	312.47± 18.76	6.75 ±1. 26
	Non- alcoholics	25	41.00±2.66		7.86 ± 0 85

magnification. A total of minimum 1500 cells per individual were scored for analysis of micronuclei.

The slides were randomized and scored by a single observer. Micronuclei were scored in normal cells. In addition, the frequencies of nuclear anomalies, namely binucleates, broken eggs, karyolysis, karyorrhexis and pyknosis were recorded. MN and other nuclear abnormalities were classified according to Tolbert and co-workers. MN must satisfy the following conditions: a) consist of nuclear material; b) be completely separated from the parent nucleus; c) be less than 1/3 of the diameter of associated nuclei; d) be smooth, oval- or round-shaped; e) be on the same plane of focus and f) be of the same color, texture and refraction as the main nucleus. Cells with two nuclei were considered to be binucleate. Besides MN, other nuclear anomalies, such as Binucleates (BN), broken eggs' (nuclei that appeared cinched), karyorrhexis (nuclear disintegration), karyolysis (dissolution of nucleus) and pyknosis (nuclear condensation) were recorded separately.

The results of chrome-plating workers were compared with those obtained from non exposed matched controls. Similarly the results of workers having smoking habits were compared with matched non exposed smoker's control, correspondingly for the alcoholics and non-alcoholics as well. The role of nonparametric factors was analyzed by the Mann-Whitney test, while the Students t-test was used for age and time comparisons. The number of MN and NA were compared with the Mann-Whitney test. All calculations were performed using Windows statistical package, version 11.5 (IL, USA). 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 demographic characteristics of the study subjects are presented in Table 1. The age, alcohol consumption and smoking status distributions were parallel among exposed workers and controls. Among the smokers, the years of smoking and daily cigarette consumption were analogous in the two groups.

The frequency of micronuclei (MN) was studied in 44 chrome plating workers and in 40 controls. Workers revealed a significant induction of MN when compared with controls ($P < 0.05$). Individuals of the exposed as well as control groups with smoking habit and alcohol consumption showed an enhanced frequency of micronuclei in comparison to non smokers and non alcoholics. Workers who are smokers showed a highly significant increase ($p<0.05$) in MN frequency when compared to all other groups and subgroups.

Workers also showed an increased MN frequency with an increase in duration of exposure ($P<0.05$). A significant correlation was observed between MN induction and duration of exposure in chrome platers. Like MN, other nuclear anomalies were more prevalent in chrome platers compared with that of controls.

Among the three nuclear anomalies, karyolysis was predominant in smokers followed by alcoholics. In addition subjects with exposure period of more than five years had a higher degree of nuclear abnormalities than subjects with less than five years of exposure ($p<0.05$).

Discussion

Occupational exposure to carcinogenic forms of chromium occurs among workers in several professional groups particularly with high exposure among chromeplaters [13]. The carcinogenic potential of metals is a major issue in defining human health from exposure [14].

Table 2. The frequencies of micronuclei and other nuclear abnormalities in exfoliated buccal epithelial cells of control and exposed chrome plating workers.

Study group			N	Nuclear abnormalities					
				MN (mean± SD)	BN (mean± SD)	BE (mean ± SD)	KR (mean± SD)	KL (mean ± SD)	PK (mean ± SD)
Control	Smokers		21	1.41±0.42	4.75±0.88	2.52±1.48	9.10±2.97	39.23±2.49	2.62±0.23
	Non-Smokers		19	1.08±0.21	3.42±0.98	3.21±1.51	6.05±1.56	26.31±2.57	1.86±0.79
	Alcoholics		18	0.73 ± 0.13	3.82±1.02	1.83±0.49	8.12±0.92	32.05±1.78	1.95±0.81
	Non-alcoholics		22	0.65 ± 0.22	2.67±0.85	1.55±0.42	6.20±0.72	29.03±1.88	1.65±0.27
Workers	Smokers		24	4.52±1.11*,†	7.28±0.87*,†	7.16±1.59*,†	19.05±1.25*,†	128.90±8.43*,†	5.12±0.46*,†
	Non-smokers		20	3.19±1.19*	6.98±0.97*	5.09±0.73*	12.27±2.22*	87.10±4.27*	3.69±0.39*
	Alcoholics		19	3.52 ± 1.15*	6.27±0.52*	6.51±1.07*	16.42±1.87*	103.55±4.56*	3.44±0.06*
	Non- alcoholics		25	3.33 ± 0.88*	6.05±1.09*	6.16±0.49*	13.06±1.47*	19.92±6.43*	2.57±0.08*
	Duration of exposures (years)	≤5	23	3.06 ± 0.03*	5.82 + 0.33*	5.10± 1.03*	14.04 ± 0.55*	93.53± 4.07*	2.22±0.40*
		>5	21	3.92 ± 0.05*	6.87 ± 0.51*	6.91 ± 0.67*	17.26 ± 1.32*	107.4 ± 8.07*	3.73±0.41*

Results are mean ± SD; *, $p<0.05$ compared with their respective controls; †$p<0.05$ compared with non smokers

Most of the chrome plating workers expressed concern regarding excessive smoke levels in the workplace and inadequate ventilation. None of these workers wore protective gear and hence chances of exposure are more.

It is known, that the dermal uptake of metals is a relevant route of exposure. Hexavalent chromium, a potent skin penetrant can induce cancer in workers engaged in industries working with chromates, through inhalation and skin contact. Dermal, renal and hepatic toxicity have been reported in workers exposed to chromium (VI) [15-17]. Cr (VI) among the various clastogenics, awaken the interest about its genotoxic activity due to its ample industrial use [19, 20]. Hence the present study investigated chrome plating workers for genotoxic effects using micronucleus assay.

The MN test has been increasingly accepted as a reliable biomarker of genotoxicity in occupationally exposed groups [5]. Benova and co-workers found double the frequency of buccal MN in Cr platers when compared with control persons [21]. Similarly our results also showed a significant increase in MN frequency of South Indian chrome platers.

The present investigation suggests that chrome platers under their particular conditions of exposure (cigarette smoke and alcohol) revealed clear evidence of genotoxicity in exfoliated buccal cell when evaluated by MN test. Our study showed that smoking status affected genetic damage in both groups studied, but a significant association emerged only among exposed workers. This shows synergistic effect between smoking and occupational exposure. Previous investigations reporting genotoxic effects in chrome plating workers using the MN test are scanty. Dillon and his collaborators detected, in V79 cells, micronuclei and other alterations resulting from genotoxic action of chromium complexes [22]. Our study revealed a significant induction of MN in chrome platers when compared to controls with respect to their years of exposure.

Besides elevated MN frequency, the chrome plating workers exhibited raised prevalence of several other nuclear anomalies like binucleates, broken egg, karyorrhexis, karyolysis and pyknosis. Karyolysis is associated with cytotoxicity, pyknosis and karyorrhexis accompany apoptosis, a process under genetic control. They occur at elevated levels in response to cellular injury. Increased frequency of these nuclear abnormalities in buccal epithelial cells of chrome platers indicates adverse cellular reaction and/or a surveillance mechanism to eliminate cells with genetic damage.

The MN assay is regarded as an important biomarker to predict the relative risk of cancer [23]. Thus, greater prevalence of MN in chrome platers implies that they are highly exposed to mutagenic insults and therefore are at a greater risk of developing cancer. The present study revealed the presence of a high genotoxic risk in metal plating worker. The used biomarker proved to be a valuable biomonitoring test. The results confirm that MN and other nuclear anomalies is a sensitive indicator for use in monitoring heavy metal occupational exposure. Hence it is recognized that genetic damage may be a sign of possible health risks. Employees, who may be exposed directly or indirectly to chromium (VI), need to be made aware of the genotoxic effects and ensure safe and healthy working atmosphere to alleviate the health hazards that they may encounter.

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Conflict of Interest

There is no conflict of interest for this study.

Authors' Contribution

SS designed the study, literature review and wrote the first draft of the paper. KSK contributed to the study design, literature review, data analysis and English writing of the manuscript. PS and JS contributed to the data entry, data collection and writing-up process. All authors read and approved the final manuscript.

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