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Chromosome Damage in Nickle-Chrome Electroplaters

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INTRODUCTION

Electroplating occupies a prominent position in the industrial world. Metal, plastic and rubber parts are plated to prevent rusting and corrosion, for appearance, to reduce electrical contact, for resistance, as a base for soldering operations, to provide electrical insulation and to improve wearability. The common plating metals include cadmium, chromium, copper, gold, nickel, silver and their alloys.

Increased mortality from cancer among platers occurred not only in bronchiopulmonary cases, but also to a similar extent in gastro-intestinal and other forms of the disease. This suggests that chromic acid is absorbed through the bronchial tree, after which some of it is deposited locally and some (perhaps after changing to trivalent form) is carried to different organs, where deposition may occur for lengthy periods (Royle, 1975a). Bronchial asthma had a high incidence in the platers as compared with the general population. The risk of skin and intranasal ulceration is shown to increase progressively in platers (Royle, 1975b).

Enough work has been carried out on the physiological effects and ambient air monitoring of electroplaters. However, there is not much reported work on the cytogenetic damage of electroplaters, especially among Indian population. Therefore, to fill the lacuna the present investigation was carried out. Mitotic index (MI), chromosomal aberrations (CA), sister chromatid exchanges (SCE) and satellite associations (SA) were recorded and analysed as genetic end points to assess the genotoxicity.

MATERIALS AND METHODS

Experimental subjects were 68 male workers exposed during electroplating in a cycle industry. Equal number of individuals not exposed to any chemicals were chosen as the control population. Those persons who smoked more than 10 cigarettes per day were designated as smokers and those persons who were used to taking drinks daily were designated as alcoholics. The electroplaters in the present study were found exposed to a mean concentration of 0.6 mg/m^3 of hexavalent chromium and 1.2 mg/m^3 of Ni (metal).

The health and occupational histories of the subjects were recorded on a standard proforma through personal examination of exposed workers and unexposed controls with respect to age, sex, duration of exposure to pollutants, smoking and drinking habits, drugs intake, if any. The data were taken over a period of two years from July 1994 to August 1996.

Heparinized venous blood samples were obtained from each subject and samples were transported to the laboratory in insulated ice box. Short-term lymphocyte cultures were set up within 3 h of sampling according to the method of Moorhead et al. (1960) with minor modifications. Briefly, 0.5 ml of blood was cultured in 5 ml of RPMI 1640 medium supplemented with 20% foetal calf serum and 0.1 ml phytohaemagglutinin (Sigma).

For mitotic index (MI) a minimum of 5000 cells per individual were counted from Giemsa-stained slides. Mitotic index was calculated by using the following formula:

$$MI = \frac{\text{Number of dividing cells}}{\text{Total number of cells scored}} \times 100$$

Lymphocytes were harvested after 48 h for chromosomal aberrations (CA). Routine chromosome preparations were made and slides were stained with 4% Giemsa. For each person 100 well spread metaphases were analysed:

For sister chromatid exchanges (SCE) 5-bromodeoxyuridine (10 µg/ml culture) was added 24 h after setting up of the cultures. Harvesting was done after 72 h. Metaphase chromosomes were prepared and stained with Hoechst 33258 and 4% Giemsa following the method of Perry and Wolff (1974). For calculating the frequency of SCE per cell, 25 metaphases were analysed as per standard practice.

The criteria described by Hansson (1970) were followed for evaluating the satellite associations (SA).

The data obtained were statistically analysed by applying the χ^2 test for mitotic index and students 't' test for CA, SCE and SA.

RESULTS AND DISCUSSION

Occupational exposure to electroplating did not cause any major clinical symptoms. A few workers did report of respiratory tract diseases, however, the controls were all healthy.

The Mitotic indices (MI) in platers exposed during electroplating for varying periods and their matched controls are given in table 1. The mean MI was 4.18 in controls, whereas it was 5.77, significantly higher ($P < 0.05$) in the exposed workers. MI showed a gradual increase with a maximum of 7.04 upto 15 years of exposure. Thereafter, the MI showed a gradual decline.

Table 1: Mitotic index (MI) in electroplaters and control individuals

Duration of Exposure (in Years)	*No.of Samples*	*No.of cells Scored*	*No.of meta-phases*	*MI±SD*
Control Individuals	68	345018	14439	4.18 ± 0.85
Exposed Individuals	68	344777	19911	5.77* ± 1.77
0-5	9	43767	2250	5.13 ± 1.36
6-10	23	117856	7262	6.15 ± 0.76
11-15	21	107088	7566	7.04 ± 1.67
16-20	12	60856	3754	6.16 ± 2.23
21-25	3	16210	476	3.51 ± 0.18

* $p < 0.05$

Different types of CA observed are summarised in table 2. Frequencies of all types of aberrations *viz.*, dicentrics, rings, chromosome gaps, chromosome breaks, chromatid gaps, chromatid breaks and isochromatid exchanges were significantly higher in the exposed sample as compared to the controls. The frequency of total chromosomal aberrations per 100 metaphases was 3.64 in exposed and 0.86 in controls, the difference being significant at $p < 0.01$.

The frequencies of SCE in electroplaters and controls have been presented in table 3. The mean number of SCE was 4.18 in controls and 6.40 in platers, the difference being statistically significant ($p < 0.05$). The frequency of SCE in smoker -alcoholics was the highest (7.40). The frequency of SCE in smokers (6.43) and alcoholics (5.59) among exposed workers was also higher than their frequency in controls (4.33), (4.33) the difference being statistically significant ($p < 0.05$). The frequency of CA was the highest in smoker-alcoholics (6.04), whereas it was only, 1.09 in controls, the difference being statistically significant (Table 4). Frequencies of both the CA and SCE showed a positive correlation with the duration of exposure (Table 5). Compared to the controls, chromosomes of the exposed workers showed a two fold increase for

Table 2: Types of chromosomal aberrations (CA) in lymphocytes of exposed platers and in control individuals

Group	*Control*	*Exposed*	*'t'*
No. of individuals	68	68	
No. of metaphases	6800	6800	
Chromosome type aberrations			
Dicentrics	-	6 (0.088)	
Rings	-	3 (0.044)	
Acentric fragments	-	16 (0.235)	
Chromosome gaps	-	1 (0.014)	
Chromosome breaks	-	1 (0.014)	
Total	-	27 (0.397)	
			P<0.01
Chromatid type aberrations			
Gaps	108 (1.58)	212 (3.11)	
Breaks	59 (0.68)	221 (3.25)	
Isochromatid abberrations	-	2 (0.000)	
Total (Gaps excluded)	59 (0.86)	221 (3.25)	
Total chromosomal aberrations (except gaps)	59 (0.86)	248 (3.64)	p<0.01

Figures in parantheses indicate aberrations per 100 metaphases.

Table 3: Frequency of sister chromatid exchanges in exposed platers and control individuals in relation to smoking and alcohol consumption

	Exposed		*Control*		*'t'*
	N	*Mean ± SEM*	*N*	*Mean ± SEM*	
Total SCEs	68	6.40 ± 0.92	68	4.18 ± 0.87	$p < 0.05$
Smoker-Alcoholics	24	7.40 ± 0.51	22	5.06 ± 0.57	"
Smokers	16	6.43 ± 0.28	13	4.33 ± 0.43	"
Alcoholics	13	5.59 ± 0.13	17	4.33 ± 0.14	"
Non Smoker Non Alcoholics	15	4.83 ± 0.15	16	3.32 ± 0.15	"

SEM : Standard Error of Mean, N = Number of individuals.

Table 4: Frequency of chromosomal aberrations (CA) in exposed platers and control individuals in relation to smoking and alcohol consumption

	Control		*Exposed*		*'t'*
	N	*Mean ± SEM*	*N*	*Mean ± SEM*	
Smoker-Alcoholics	22	1.09 ± 0.89	24	6.04 ± 0.73	$P < 0.05$
Smokers	13	1.00 ± 0.67	16	3.75 ± 0.43	"
Alcoholics	17	0.70 ± 0.74	13	2.07 ± 0.61	"
Non Smoker Non Alcoholics	16	0.62 ± 0.48	15	1.40 ± 0.48	"

SEM : Standard Error of Mean, N = Number of individuals.

Table 5: Frequency of CA and SCE with duration of exposure

Duration of Exposure (in Years)	*N*	*CA*	*SCE*
0 - 5	9	1.66 ± 0.81	5.39 ± 0.44
6 - 10	23	2.04 ± 0.85	5.68 ± 0.38
11 - 15	21	4.47 ± 0.91	6.76 ± 0.36
16 - 20	12	6.41 ± 0.49	7.41 ± 0.39
21 - 25	3	6.66 ± 0.47	8.37 ± 0.13

various types of SA per cell (Table 6). The increase (4.08-9.14) was significant ($p<0.05$). The D-G type of association was found to be the highest (4.22%), while 3D-type association was of lowest occurrence (0.14 %).

A perusal of the literature reveals that cytogenetic studies on electroplaters are limited (Sarto et al., 1982). These authors reported statistically significant increase of CA and SCE and also reported a negative correlation between the frequency of SCE and the level of urinary chromium which was strongly enhanced by smoking. The clastogenic and DNA damaging activity of chromates in human cells has been reported both in *vitro* (Nakamura et al., 1978; Whiting et al., 1979) and in *vivo* (Begaliev et al., 1977). The genotoxicity of chromium has also been well established in other experimental *in vitro* and *in vivo* systems (Levis and Bianchi, 1982; Bianchi and Levis, 1985 and Venitt, 1986). Sunderman (1993) has reported the formation of Ni_2+ complexes that could catalyze the formation of oxygen free radicals and thereby damage DNA and chromosomes. Nickel compounds have been reported to be carcinogenic to humans and

Table 6: Frequency of satellite associations (SA) in exposed platers and control individuals

No. of Individuals	*Total cells scored*	*Types of Satellite Association between D and G group acrocentric chromosomes*							*Total*	*Ass. per cell*	*± SD*
		D-D	D-G	G-G	2D-G	2GD	2D2G	3D			
Exposed 68	6800	117 (1.72)	287 (4.22)	106 (1.5)	58 (0.85)	33 (0.98)	11 (0.16)	10 (0.14)	622	9.14*	±4.07
Control 68	6800	67 (0.98)	139 (2.04)	47 (0.69)	4 (0.05)	6 (0.08)	7 (0.10)	8 (0.11)	278	4.08%	±1.56

*Significant at $p < 0.05$

Figures in parentheses represent SA per 100 metaphases

experimental animals (Herting et al., 1994).

The present investigation has shown a significant increase in the chromosome aberrations as compared to the controls. A statistically significant increase of chromosomal aberrations, mostly of the chromosome type was also observed in cultured lymphocytes obtained from workers occupationally exposed to chromic acid (CrO_3). The three ring chromosomes noted in our study were present in exposed electroplaters who were both chronic alcoholics and chain smokers'. Gill (1991) has reported the occurrence of rings in chain smokers. However, in our study the occurrence of rings may be due to the combined effect of alcohol and cigarette smoke. Obe and Herha (1975) showed that chronic alcohol users exhibit a significantly higher frequency of aberrations in peripheral lymphocytes compared with the controls. Alcoholics did show higher chromosome aberrations than the exposed non alcoholics and the exposed smokers, but the exposed smoker-alcoholics had the maximum values showing that both smoking and alcohol have synergistic effect on chromosome aberrations. Retired nickel workers have been reported to show an increased incidence of breaks and gaps as compared to the controls (Waksvik et al., 1984).

Increased chromosome aberrations have also been reported with hexavalent chromium compounds (Raffelto et al., 1977; Umeda and Nishimura, 1979, Koshi and Iwasaki, 1983).

There is a remarkable increase in the number of SCE per cell in electroplaters as compared with the controls. Sarto et al., (1982) have also reported similar results in a study carried out on electroplaters.

A significant increase in SCE by Ni salts has been reported by Sahu et al., (1989, 1995). However, no difference in the incidence of sister chromatid exchanges was noted between retired nickel workers and matched controls (Waksvik et al., 1984). Increased incidence of SCE has been reported in workers exposed to elemental chromium and nickel containing dusts (Gennart et al., 1993).

With the duration of exposure, mitotic index increased upto 15 years of exposure, thereafter it gradually decreased. A nickel dose-dependent reduction of mitotic index was earlier reported by Au et al., (1994). The effect of metal on cell division may be attributed to the affinity of metals for sulfur ligands and consequently for the spindle proteins (Cherian, 1985).

Of late, association between the human satellite chromosomes have received much attention because of their possible relation to nondisjunction and translocations (Hansson, 1975). An increase in satellite association is reported in meningioma cells in parents of children with Down's syndrome (Curtis, 1974; Zankl and Zang, 1978), in cells from individuals exposed to x-rays and after in vitro $CoCl_2$ treatments (Nazaret, 1976; Favah, 1982). In the present study, the exposed group showed 9.14 SA per cell, whereas the controls had only 4.08 associations per cell. In order to understand the basis of SA by chemical mutagens one must keep in mind that it could be due to either a direct mutagenic effect on DNA or an indirect mutagenic effect via the spindle (Kisch-Volders et al., 1978).

These results show that electroplaters exposed to nickel and chromium in the ambient air, even at low concentration, caused chromosomal damage. Therefore, there is an immediate need to improve the working conditions and carry out regular health surveillance of workers.

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KEY WORDS Mitotic Index. Chromosomal Aberrations. Sister Chromatid Exchanges. Satellite Associations. Electroplaters.

ABSTRACT The type of electroplaters in the present study are the ones dealing with Nickel-chrome electroplating. Heparinized venous blood samples were collected from 68 electroplaters and equal number of matched controls. Genotoxicity was investigated by assaying mitotic index (MI), chromosome aberrations (CA), sister chromatid exchanges (SCE) and satellite associations (SA) in short term lymphocyte cultures. The mean frequency of MI was 5.77 ± 1.77, CA 3.64, SCE, 6.40 ± 0.92, and SA 9.14, in the exposed group as compared to controls in which the mean

frequencies of these parameters were 4.18 ± 0.85, 0.86, 4.18 ± 0.87 and 4.08 respectively.

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