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Clastogenic Effects in Human Samples Following Prolonged Exposure in Metal Industry

M. De,[1] S. Ghosh,[1] S. Palit,[1] A. Ghosh,[1] G. Talukder,[2] A. Sharma[1]

[1]Genetic Toxicology Unit, Centre of Advanced Study (Cell and Chromosome Research), Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Calcutta 700019, India
[2]Vivekananda Institute of Medical Sciences, 99 Sarat Bose Road, Calcutta 700026, India

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The need for determining the potential genotoxic effects of longterm exposure to complex workplace environment has been widely accepted (see IARC 1984, ICPEMC committee 1985, ICEM 1989) and a number of techniques have been adopted for the purpose (for review see Anderson 1988). Screening for chromosomal aberrations in cultured lymphocytes is now an accepted method for monitoring populations exposed to various chemicals, singly or in mixtures (Forni 1983, Galloway et al. 1986, Sorsa et al. 1982, 1983). Such studies are of particular importance in India, where industrialisation has progressed rapidly in certain belts during the past four decades. The present study was undertaken on groups of workers in metal industries, exposed directly and indirectly to complex environmental work conditions, using chromosomal aberrations as the endpoint for monitoring. Individuals were matched with respect to age, sex, nutritional status and addiction.

MATERIALS AND METHODS

Blood samples were collected from healthy male donors working in a factory for welding and exposed to metals like Fe, Zn, Cu, Ni, Pb and sulphuric acid fumes and also to oil, CO, oxy-acetylene gas and high temperature. The workers were grouped into two categories according to the degree of exposure -

Group I - directly exposed through working in electroplating, grinding, welding, painting and as fitters and millers.

Group II - indirectly exposed in the same factory through employment in stores, security and administration.

Detailed informations regarding the exposure of different individuals are given in Table 1. Routine blood tests were performed in all cases. Cultures of peripheral venous blood lymphocytes were grown in RPMI 1640 medium (Gibco), supplemented with 15% human AB^+ serum and phytohaemagglutinin

Correspondence to: M. De

Table 1. Detailed information regarding exposure and addictions of different individuals

Case No.		Age (in years)	Duration of exposure (in years)	Type of Job	Nutritional status	Addiction			
						Tobacco chewing	Smoking	Alcohol	Paan (Betel leaf)
Group IA (Directly exposed to metals)	1.	38	20	Fitter	Good	+	+	-	+
	2.	55	35	Fitter	Average	-	-	-	+
	3.	54	28	Fitter	Average	+	-	-	+
	4.	39	21	Mechinist	Average	+	-	-	+
	5.	50	28	Mechinist	Average	-	-	-	+
Group IB (Directly exposed to metal and heat)	1.	52	35	Welding	Average	-	-	-	+
	2.	55	38	Welding	Good	-	-	+	+
	3.	52	30	Bending	Average	-	+	-	-
	4.	50	30	Bending	Average	+	-	-	-
	5.	46	23	Furnace	Good	-	+	-	+
Group II (Indirectly exposed)	1.	50	30	Peon	Good	-	+	-	-
	2.	60	41	Manager	Very Good	-	-	+	-
	3.	54	33	Storekeeper	Very good	+	-	-	-
Group III (Controls)	1.	38	-		Good	-	-	-	-
	2.	50	-		Good	-	-	-	-
	3.	52	-		Good	-	-	-	-
	4.	55	-		Good	-	-	-	-
	5.	60	-		Good	-	-	-	-

Table 2. Cytogenetic changes in individuals with different levels of exposures

Case No.		Mitotic index	% of cells showing types of aberrations					Total chromosomal aberration (CA)	Total damaged Cells (DC)	Breaks/ cell	Numerical aberrations	Acro centric association
			G'	G"	B'	B"	RR					
Group I	1.	4.0	3	1	4	2	-	8.0	6.0	0.08	-	-
	2.	6.2	1	-	4	2	-	6.0	6.0	0.06	-	2
	3.	10.8	5	-	7	3	-	13.0	10.0	0.13	-	3
	4.	10.5	-	-	6	-	-	6.0	6.0	0.06	-	1
	5.	6.7	-	-	6	-	-	6.0	5.0	0.06	-	-
Mean±SD		7.6±2.9***						7.8±3.0**	6.6±1.9***			
Group IB	1.	7.7	6	-	5	-	-	5.0	5.0	0.05	-	-
	2.	9.0	3	-	4	2	-	6.0	6.0	0.06	1(2n=45)	2
	3.	8.5	-	-	5	-	1	7.0	5.0	0.07	-	-
	4.	3.9	7	-	6	-	1	8.0	6.0	0.06	-	3
	5.	3.8	4	-	4	2	2	12.0	8.0	0.12	-	-
Mean±SD		6.5±2.5*						7.6±2.7**	6.0±1.2**			
Group II	1.	4.5	6	-	7	-	-	7.0	5.0	0.07	-	2
	2.	4.5	2	-	1	-	-	1.0	1.0	0.01	3(2n=47)	1
	3.	2.2	1	-	-	-	-	0.0	0.0	0.00	-	-
Mean±SD		3.7±1.3						2.7±3.8	2±2.6			
Group III	1.	3.9	3	-	-	-	-	0.0	0.0	0.00	-	1
	2.	3.2	2	-	1	-	-	1.0	1.0	0.01	-	2
	3.	2.5	1	-	2	-	-	2.0	2.0	0.02	-	1
	4.	3.2	2	-	2	-	-	2.0	2.0	0.02	-	0
	5.	2.1	1	-	2	-	-	2.0	2.0	0.02	-	3
Mean±SD		3.0±0.7						1.4±0.9	1.4±0.9			

G' G" - Chromatid and chromosome gap; B' B" - Chromatid and chromosome breaks; RR - Rearrangements translocations and dicentrics); DC - Excluding numerical aberrations and acrocentric associations
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

(0.4 ml). For all the cases replicate sets of cultures were maintained. The cells were harvested after 72 h of incubation (Purchase et al. 1978) following the usual colchicine (0.02% ml), hypotonic (0.075 M KCl), acetic acid : methanol (1:3) fixation and air drying Giemsa schedule (Preston et al. 1987; Sharma and Sharma, 1980). Observations were taken from both culture sets and pooled. Slides were coded and scored blind. One hundred well scattered, evenly stained metaphase plates were scored per sample for chromosome aberrations (CA) and 1000 cells were observed for mitotic index.

Statistical analysis was carried out using 't' test and paired comparisons followed by ANOVA (analysis of variance) (Sokal and Rohlf, 1981). For calculation of breaks, a chromatid break was taken as one break and chromosome breaks, dicentrics and translocations as two breaks (Preston et al. 1987).

Table 3. ANOVA following paired comparisons for chromosomal aberrations between exposed workers and controls

Source of variation	F value
Between directly exposed workers to metals (Gr.IA) and controls	25.80***
Between individuals of Gr.IA	1.02 N.S.
Between directly exposed workers to metal and heat (Gr.IB) and controls	22.50***
Between individuals of Gr.IB	0.65 N.S.
Between indirectly exposed workers (Gr.II) and controls	0.21 N.S.
Between individuals of Gr.II	0.65 N.S.

Paired comparisons were made between exposed workers and age matched controls.

*** $P \leq 0.001$; N.S. - Not significant

RESULTS AND DISCUSSION

Under workplace conditions in factories, the workers are exposed to chronic subtoxic doses of mixed chemicals. In the present investigation majority of the workers had worked mostly for over 20 years under the industrial conditions. From detailed case histories it was observed that nutritional level ranged from average to very good and all of them were devoid of any chronic or infectious diseases. The blood pictures showed

78%-106% Hb (i.e. 11.4 gm%-15.3%) and all of them gave histories of addiction (Table 1). Aberrations observed in all cases involved chromatid breaks. The percentage of total abnormalities was high in all members of the directly and indirectly exposed group (Table 2). Paired comparisons between exposed workers and age matched controls showed significant differences with Gr.IA and Gr.IB (Table 3).

Prolonged exposure to various toxicants have been shown to lead to harmful effects, TCDD (2,3,7,8 tetrachlorobenzo P-dioxin) increased the number of CD_8 T-lymphocytes (Hong 1991) and exposure to mercury vapour reduced significantly the levels of IgA and IgG (Maszezunski et al. 1990). The incidence of spontaneous abortions was enhanced following exposure to organic substances from industrial noise (Lunge and Trubinkov 1990). Earlier studies from factories in West Bengal have shown a higher frequency of abnormal variants of lipoprotein and haemoglobins in directly exposed populations (Banerjee et al. 1987, De et al. 1992).

The relatively higher frequency of chromosomal aberrations in the 10 individuals directly exposed and 3 indirectly exposed for 20 years and more to the factory effluents indicate that the composite exposure is able to induce clastogenic changes. Such effluents, as mentioned earlier, include various metals and other chemicals. Composite mixtures of metals are known to be clastogenic following prolonged exposure to subtoxic doses in mammalian systems (Sharma and Talukder 1987 for review). The three individuals from the indirectly exposed group showed chromosomal aberrations comparable to the exposed ones. The data indicate that direct and indirect exposures are appreciably clastogenic to human systems following long exposure. The work has been limited to a selected group, keeping the factors like age, nutrition and period as similar as possible.

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