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Cytogenetic Damage in Individuals Exposed to Vehicular Pollution

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KEY WORDS Peripheral blood lymphocytes; chromosomal aberrations; sister chromatid exchanges; satellite associations.

ABSTRACT Cytogenetic investigations on peripheral blood lymphocytes of 50 individuals exposed to different air pollutants on the road sides was made. This was compared to an equal number of exposed and matched controls w.r.t. age, sex, smoking and drinking habit and social status. The mitotic index (MI), chromosomal aberrations (CA), Sister Chromatid Exchanges (SCE) and satellite associations (SA) were analysed. All the parameters showed a significant increase ($P<0.01$) in the exposed sample compared with the controls, viz. MI, 3.75-6.80; CA 0.96-2.98; SCE 3.92-8.18 and SA 6.18-14.50. The occurence of the DG type of satellite association was highest and that of 3D type lowest. The frequencies of all the parameters also showed elevation with duration of exposure. MI however, showed decrease after continous increase upto 15 years of exposure. The vehicular fumes were thus found to be genotoxic.

INTRODUCTION

Air pollution is a complex mixture of toxic chemicals and particles, rarely can exposure to only one type of pollutant occur (Phillips 1997). Each of us is affected by this exposure regardless of occupation. Air pollutants are classified as primary or secondary. Primary atmosopheric pollutants are natural (dust, vegetation etc.) or anthropogenic in origin (e.g. smoke stack and vehicle emission). Secondary products may be generated from primary pollutants via atmospheric (Photochemical) reactions (Fishbein 1976). Non technological sources such as forest fires produce polycyclics, but technological sources (coal/oil gas burning facilities, motor vehicles, refuse burning) constitute the majority of the atmospheric load (National Research Council 1979).

The main pollutants emitted by automobile vehicles are carbon monoxide, oxides of nitrogen, oxides of sulphur, benzene and particulates, including smoke and lead compounds. Benzene is emitted from the motor vehicles together with petrol vapours during the operation and even when the vehicles are stationary. About 80 to 85% of benzene in atmosphere comes from automobiles. About 80-90% of the benzene from automobile sector is emitted from the exhaust (CPCB 2000). Epidemiological studies confirmed that individuals exposed to ambient air pollution, especially that generated from motor vehicles including diesel fumes, have increased risk of lung, abdominal and laryngeal cancers (Hayes et al. 1989; Steenland et al. 1990; Guberan et al. 1992; Balarajan and McDowall 1988 and Barbone et al. 1995). Genotoxic effect of various chemicals and other toxic substances on workers exposed at their occupational settings, is noteworthy. This exposure results in damage of DNA, thus affecting its replicating ability. Its ability to carry information is also altered. Cytogenetic testing of groups with known or suspected chemical exposure may reveal effect on the genetic material of exposed individual. The present investigation on the effect of pollution was carried out with this objective in mind.

MATERIALS AND METHODS

This study comprised 100 individuals in all, 50 individuals exposed to air pollutants and 50 occupationally unexposed controls matched with respect to age, sex, smoking and drinking habits.

An epidemiological survey was conducted using a proforma specially designed for this purpose.

The samples were taken from traffic policemen, vendors and shopkeepers of Motinagar area, New Delhi from Nov. 1999 to Jan. 2000. These individuals were exposed to low levels of SO_2 and NO_2 (0-30$\mu g/m^3$), and moderate level of SPM (70-140 $\mu g/m^3$) (CPCB 1997) and benzene (mean 17 $\mu g/m^3$) (CPCB 2000).

Blood samples were taken using heparinized syringes. Short-term lymphocyte cultures were set up within 4 h of sampling following the technique of Moorhead et al. (1960) with minor modifications. Lymphocytes were cultured by adding 0.5 ml of blood in 5 ml of RPMI 1640 medium (Hi Media) supplemented with 20% foetal calf serum and 0.1 ml of phytohaemagglutinin

(Sigma). Colchicine (10 µg/ml; Sigma) was added to the culture prior to harvesting. The lymphocytes were harvested after 48 h for assessing chromosomal abberrations (CA). The slides were prepared and stained with 4% Giemsa solution (E. Merck). For each person 100 well spread metaphases were analysed.

In order to calculate the mitotic index (MI) a minimum of 5,000 cells per individual were counted from Giemsa stained slides. The MI was calculated using the formula:

MI = (Number of dividing cells)/(Total number of cells scored) X 100

For sister chromatid exchanges (SCE) 5-bromo-deoxyuridine (BrdU, 10µg/ml culture) was added 24 h after establishing the cultures. Harvesting was effecting after 72 h. Slides were prepared by the air drying method, and stained with Hoechst 33258 (Sigma) and 4% Giemsa solution following the method of Perry and Wolff (1974). For calculating the frequency of SCE per cell, 30 metaphaes were analysed as per international norms.

For evaluating the frequency of satellite associations (SA), 100 good metaphases were scanned. The criteria described by Hansson (1970) were followed for evaluating the SA. These criteria were (1) the satellite ends of the associating chromosomes had to be directed towards each other with their longitudinal axes meeting between their short arms; and (2) distance between the centromeres of associated chromosomes should not exceed the total length of one G chromosome, its satellite excluded.

The coded samples were analysed by two observers, who were unaware of the identity of the sample, in order to remove possible laboratory scoring bias. The results were analysed statistically using the Student t-test.

Table 1: Mitotic Index (MI) in individuals exposed to vehicular pollution and controls

Duration of exposure (in years)	*Number of samples*	*Number of cells scored*	*Number of metaphases*	*MI ± SD*
Control Individuals	50	247225	9302	3.75 ± 0.21
Exposed Individuals	50	254795	17375	6.80 ± 0.26*
0-5	13	65194	4247	6.50 ± 0.31
6-10	15	76750	5261	6.84 ± 0.15
11-15	11	56551	3986	7.04 ± 0.07
16-20	11	56306	3881	6.92 ± 0.14

* Significant at P < 0.01

RESULTS

Epidemiological study showed that most of the exposed individuals complained of burning of eyes, nose, asthma and minor headache.

The data on various parameters obtained during the present investigation have been given in tables 1-6.

Higher values of MI were observed in exposed (6.80) as compared with controls (3.75) (Table 1). The increase was statistically significant (P<0.01). The highest values were observed in individuals exposed for a period of 11-15 years. With a further increase in the duration of exposure beyond 15 years a slight decline in the MI was recorded.

The mean percentage of the CA found in the exposed individuals was 2.98 and that in the controls was 0.96 (Table 2), resulting in highly significant values at P<0.01. Both chromosome type aberrations viz. dicentrics, rings, acentric fragments, chromosome gaps, chromosome breaks and chromatid type aberrations viz., chromatid gaps and chromatid breaks were encountered in the exposed individuals.

Table 2: Frequency of chromosomal aberrations in exposed individuals and controls

Group	*Exposed individuals*	*Control individuals*
No. of Individuals	50	50
No. of Metaphases	5000	5000
Chromosome Type Aberrations		
Dicentrics	5 (0.10)	1 (0.05)
Rings	2 (0.04)	-
Acentric Fragments	9 (0.18)	3 (0.06)
Translocations	2 (0.04)	-
Chromosome Gaps	12 (0.24)	4 (0.08)
Chromosome Breaks	8 (0.16)	-
Total (without gaps)	26 (0.52)	4 (0.08)
Chromatid Type Aberrations		
Gaps	161 (3.22)	77 (1.54)
Breaks	123 (2.46)	44 (0.88)
Isochromatid Exchanges	0	-
Total (without gaps)	123 (2.46)	44 (0.88)
Total Chromsomal Aberrations (without gaps)	149 (2.98)* ± 2.63	48 (0.96) ± 1.15

Values in parantheses indicate aberrations per 100 metaphases.
* Significant at P <0.01

Table 3 shows a significant difference in the frequency of CA among smokers as compared with non smokers. The exposed smokers showed higher values of CA (4.65) as compared with non smokers (1.86). A similar situation occurred among controls (2.05 and 0.34). The differences were statistically significants (P<0.01)

Table 3: Frequency of chromosomal aberrations (CA) in exposed and control individuals

Subject	*Control individuals*		*Exposed individuals*	
	n	*Mean ± S.D.*	*n*	*Mean ± S.D.*
Total Individuals	50	0.96 ± 1.15	50	2.98 ± 2.63*
Smokers	18	2.05 ± 1.16	20	4.65 ± 2.79*
Non-Smokers	32	0.34 ± 0.54	30	1.86 ± 1.85*
Alcholics	19	1.89 ± 1.24	17	4.64 ± 3.18*
Non-Alcholics	31	0.35 ± 0.55	33	2.12 ± 1.83*
Smoker Alcholics	12	2.41 ± 1.20	12	6.00 ± 2.73*

* Significant at $P < 0.01$
n - Number of Samples

Table 4: Frequency of CA and SCE with duration of exposure in individuals exposed to vehicular pollution and controls

Duration of exposure (in years)	*Number of individuals*	*CA*	*SCE*
		Mean ± SD	*Mean ± SD*
0-5	13	1.00 ± 0.81	7.76 ± 0.18
6-10	15	1.60 ± 1.24	7.94 ± 0.19
11-15	11	3.54 ± 1.50	8.31 ± 0.19
16-20	11	6.63 ± 2.41	8.85 ± 0.48

Table 5 shows the frequency of SCE in exposed individuals and their matched controls. A significant difference ($P<0.01$) was observed in the induction of SCE in exposed individuals (8.18) as compared with controls (3.92). The smokers, both exposed (8.52) and control group (4.04) showed higher values of SCE. Both CA and SCE were positively correlated with the duration of expsoure (Table 4).

Table 6 shows the frequency of SA in exposed and control individuals. The types of SA observed were DD, DG, GG, 2DG, 2GD, 2D2G and 3D. The SA showed highly significant values ($P<0.01$) in exposed samples (14.50) as compared with the controls (6.18). The DG type associations were the highest, while the 3D type were of lowest occurence.

DISCUSSION

Epidemiological study revealed that most of the exposed individuals complained of burning of eyes, nose, asthma and minor headache.

The mitotic index is known to respond to environmental pollutants. Mitotic index (MI) was found to be higher in exposed individuals as compared with controls. This shows that environmental pollution affects the cell cycle kinetics. The values of MI showed regular increase upto 15 years of exposure. Highest values of the MI were recorded in the individuals exposed for a period of 11-15 years. After 15 years the values of MI showed a decrease. It could be due to the fact that increased amount of air pollutants in exposed individuals could have started destroying the cells resulting in the decrease in the mitotic index. The MI also showed increase among SO_2, NO_x, and PAH exposed individuals up to an exposure period of 5 years (Yadav and Kaushik 1996; Yadav and Seth 1998a, b) and in those exposed to NH_3 up to 11 years (Yadav and Kaushik 1997) and declined thereafter. Rupa et al. (1989) recorded a significant increase in MI up to an exposure of 10 years. It decreased thereafter. The MI also showed a significant concentration dependent increase in individuals exposed to mosquito coil smoke (Das et al. 1994).

During the present investigation the individuals exposed to environmental pollution showed significantly increased CA as compared with their matched controls. For obvious reasons gaps were not considered. However, among controls as well as the exposed group the total chromatid type abberrations were far more than the total chromosome type aberrations. Comparable results were earlier obtained in workers of plastic industry exposed to polyvinyl chloride (Purchase et al. 1978; Anderson et al. 1981; Hrivnak et al. 1990; Kucerova et al. 1974, Yadav and Chhillar 2001a). In SO_2 and NH_3 exposed individuals of fertilizer factory Yadav and Kaushik (1996, 1997) also reported similar results.

Exposure to environmental carcinogens such as polycyclic hydrocarbon (Yadav and Seth 1998b), tobacco smoke (Yadav and Thakur 2000a,b), ethylene oxide (ETO) (Mayer et al. 1991), styrene (Norppa et al. 1980) , benzene (Yadav and Chhillar 2001c) and chemicals in the rubber industry (Yadav and Chhillar 2001b) have also been found to be associated with elevation in the frequencies of CA and SCE. Similarly significantly higher frequency of CA was also observed in exposure to mosquito coil smoke (Das et al. 1994) and in traffic policemen (Anwar and Kamal 1988; Bauchinger et al. 1972).

Significantly higher values of SCE as compared with the respective controls have been observed in the present study. The back ground level of SCE was 3.92 per cell. In earlier studies also it was reported to be less than 4 per cell (Yadav and Kaushik 1996, 1997; Yadav and Seth 1998a, b; Yadav and Thakur 2000a,b). Several studies showed an increase in frequencies of SCE in traffic policemen (Anwar and Kamal 1988), in petrochemcial workers (Zhou et al. 1986; Seth

Table 5: Frequency of sister chromatid exchanges (SCE) in individuals exposed to vehicular pollution and controls

Subject	*Control individuals*		*Exposed individuals*	
	n	*Mean ± S.D.*	*n*	*Mean ± S.D.*
Total Individuals	50	3.92 ± 0.21	50	8.18 ± 0.49*
Smokers	18	4.04 ± 0.09	20	8.52 ± 0.52*
Non-Smokers	32	3.84 ± 0.06	30	7.94 ± 0.30*
Alcholics	19	4.00 ± 0.12	17	8.48 ± 0.63*
Non-Alcholics	31	3.86 ± 0.07	33	7.98 ± 0.33*
Smoker Alcholics	12	4.08 ± 0.09	12	8.77 ± 0.52*

*Significant at $P < 0.01$

Table 6: Frequency of satellite associations observed in individuals exposed to vehicular pollution and control individuals

Parentheses	*Number of Individuals*			
	Control individuals (50)		*Exposed individuals (50)*	
Total Cells Scanned	5000		5000	
Type of Satellite Associations				
DD	68	(1.36)	146	(2.92)
DG	117	(2.34)	311	(6.22)
GG	47	(0.94)	91	(1.82)
2DG	33	(0.66)	63	(1.26)
2GD	22	(0.44)	50	(1.00)
2G2D	14	(0.28)	39	(0.78)
3D	8	(0.16)	25	(0.50)
Total	309	(6.18)	725	(14.50)
Associations per cell	6.18 ± 4.94		14.50 ± 9.14*	

Figures in parentheses indicate the satellite associations per 100 metaphases
*Significant at $P < 0.01$

1999). But Hedner et al. (1982) showed no correlation between frequencies of CA and SCE. Schmid et al. (1972) didnot find any significant differences in the frequency of CA in exposed and control groups.

Increased frequency of SCE and CA with the increasing duration of exposure have been observed in the present study. Age related increase in the cytogenetic damage has also been reported in Nordic samples (Anonymous 1990). Similar results were shown by Yadav and Kaushik (1996, 1997) and Yadav and Seth (1998a, b). Anwar and Kamal (1988) found that the exposed traffic policmen with exposure time of 10 years showed significantly higher frequency of chromosomal aberrations as well as mean sister chromatid exchanges.

It is well known that some human acrocentrics are preferentially involved in Robertsonian translocations and non-disjunction, and therefore, satellite association has received much attention because of their possible relation to these abnormalities. If sticky material from a large nucleolus organised by several acrocentrics persists through cell division, the risk of non-disjunction is most likely increased (Ohno et al. 1961). During the present investigation increased SA were observed in the individuals exposed to environmental pollution as compared to controls. Similar results were earlier reported by Rita et al. (1987) in grape garden workers occupationally exposed to different pesticides, by Yadav and Kaushik (1996, 1997) in fertilizer factory workers exposed to SO_2 and NH_3 gases, respectively and by Yadav and Seth (1998a,b) in workers exposed to PAH in coal tar workers. At low exposure levels (0.1 - 18.6 mg/m^3) workers exposed to benzene did not show biologically significant differences in the frequencies of chromosomes aberrations compared with the frequencies in control populations (de Jong et al. 1988). Sarto et al. (1984) also did not find any difference in the frequency of SCE in low level exposure (0.2-12.4 ppm) to benzene. However, a small increase in chromosome type aberrations was noticed, though there was no significant effect on the frequency of chromatid type aberrations. The cytogenetic effect of vehicular pollutions may be due the cumulative effect of SO_2, NO_2, SPM and benzene exposure in addition to several other pollutants present in the ambient air. Therefore, there is urgent need to check the level of air pollution due to vehicular emissions.

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