

Increased Chromosomal Aberrations in Peripheral Blood Lymphocytes of Traffic Policemen of Amritsar City

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ABSTRACT Chromosomal aberrations were investigated in cultured Lymphocytes of 21 traffic policemen occupationally exposed to automobile exhaust and 13 residents of Amritsar city not occupationally exposed to automobile exhaust to assess the genotoxic effects of increasing air pollution of Amritsar city, Punjab (India). The mean percentage frequency of total aberrant metaphases (31.12 ± 10.12), metaphases with numerical aberrations (4.39 ± 2.62), structural aberrations including gaps (2.38 ± 1.91), structural aberrations excluding gaps (2.24 ± 1.68) and acrocentric associations (24.40 ± 7.36), was significantly higher in traffic policemen as compared to controls (13.02 ± 1.97 , 2.39 ± 0.80 , 1.30 ± 0.96 , 1.12 ± 0.68 , 9.41 ± 2.23 , respectively). The mean percentage frequency of total aberrant metaphases (40.03 ± 7.84), metaphases with numerical aberrations (5.65 ± 2.58), structural aberrations including gaps (3.62 ± 1.71), structural aberrations excluding gaps (3.34 ± 1.34), and acrocentric associations (30.76 ± 6.09) was also significantly higher in traffic policemen working more than 10 years as compared to traffic policemen working less than 10 years (24.52 ± 5.72 , 3.44 ± 2.49 , 1.45 ± 1.47 , 1.42 ± 1.27 and 19.63 ± 3.72 , respectively). The result indicates increased chromosomal aberrations and acrocentric associations due to occupational exposure to polluted air having increased concentration of automobile exhausts, and these effects increased with increasing duration of exposure.

INTRODUCTION

Air pollution in India has been aggravated by a number of developments, such as, growth in the size of cities, rapid economic development, industrialization, and increasing traffic and levels of energy consumption. Many urban air pollutants are recognized to be mutagenic and carcinogenic agents. Amritsar city in the state of Punjab (North India) has an area of 120.55 sq. km and the population is 1,229,561 with a high population density of 10,200 persons per sq.km (State Information 2003; STP 2004). The suspended particulate matter (SPM) levels of ambient air of the city as reported since 1997 is ~ 400 $\mu\text{g}/\text{m}^3$ which is almost three times the National Ambient Air Quality Standards for India (NAAQSI) (140 $\mu\text{g}/\text{m}^3$). Though there are no guideline values for long-term average SPM concentrations recommended by World Health Organization, studies have shown that the prevalence of bronchitis symptoms in children, and of reduced lung function in children and adults are associated with particulate matter exposure at annual average concentration levels

even below 20 $\mu\text{g}/\text{m}^3$ (as PM 2.5) or 30 $\mu\text{g}/\text{m}^3$ (as PM 10) (WHO 2003b). The current level of lead in Amritsar air is not known, though SO_2 and NO_2 are lower than the WHO limit (Table 1). In Amritsar, the major sources of air pollution are automobiles, and numerous small-scale industries, such as, textile processing, pharmaceutical, iron foundries, steel plants, glass and plastic mills, pulp and paper mills, dairy, woollen dyeing and electroplating.

The purpose of the present study was to investigate with cytogenetic method the effects of consistent exposure to polluted air containing automobile exhausts on traffic policemen of Amritsar city.

MATERIALS AND METHODS

The study includes 21 traffic policemen and 13 male controls of similar age. All the study participants were residents of Amritsar city exposed to similar level of ambient air pollution. The traffic policemen were consistently exposed to increased automobile exhausts at the busy road intersections of Amritsar city during their 8 h duty per day, 6 days a week. The control group consisted of residents (8 security personnel and 5 students) of Guru Nanak Dev University Campus situated at a distance of 7 km from the city centre, spread over an area having less traffic

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and adequate green cover with large number of open spaces. All participants were informed of the study aims and asked to complete a self-administrated standardized questionnaire form under informed consent. Personal history, such as past and present illness and medication, information about the total conceptions, number of live children, miscarriages, spontaneous abortions, postnatal death, birth defects and health of children, family income, family size, habitat and surroundings, diet pattern, such as frequency of consumption of non-vegetarian food, pulses, vegetables and fruits, was obtained from each participant.

Traffic policemen included in the study had worked for at least 8 months to 21 years with the mean duration of road duties being 8.36 ± 6.73 years. Traffic policemen and controls were in the age range of 28 to 45 years (mean 34.66 ± 4.06) and 23 to 51 years (mean 33.20 ± 7.91), respectively, at the time of study. All study participants were non-smokers. 61.90% of traffic policemen and 38.46% of controls were consuming alcohol, with the quantity and frequency of consumption being 100 to 150 ml, once or twice a week. The staple diet of all participants was vegetarian, but 71.43% of traffic policemen and 38.46% of controls were taking non-vegetarian food, eggs and meat, once or twice a week. All traffic policemen and controls were having similar diet pattern and socio-economic status.

Chromosomal Aberration Analysis: Peripheral blood culture was set up by standard protocol (Moorhead et al. 1960). 5 ml of peripheral blood sample per subject was taken in heparinized syringes. Lymphocyte cultures were set up by adding approximately 2 ml of autologous supernatant plasma along with lymphocytes from buffy coat to 8 ml of RPMI-1640 (HiMedia), supplemented with 0.125% gentamycine sulphate (Glenmark) as antibiotic

and 0.2 ml of PHA-M (Gibco) as mitogen. The cultures were incubated at 37°C for 71 h and exposed to 0.05 ml of colcemid (Sigma) at 10 mg/ml final concentration for the last 1 h before harvesting. The cells were harvested by the standard procedure using 0.075 M KCl as a hypotonic solution and methanol:acetic acid (3:1) as fixative. The slides were stained with Giemsa, coded and scored blind.

Scoring and Statistical Analysis: 100 well spread second division metaphases were scored for chromosomal analysis. The number of Total aberrant metaphases (TAM), metaphases with numerical aberrations, metaphases with structural aberrations (including and excluding gaps) and metaphases with acrocentric associations were scored. The latter were further scored as metaphases with single and metaphases with multiple acrocentric associations. The number of chromosomes involved in the association was also scored. Statistical analysis of the data was performed using Student's t-test.

RESULTS

The results have shown an increased frequency and variety of chromosomal aberrations in traffic policemen as compared to controls. Numerical aberrations found were polyploidy, double minutes, acentric fragments, hyper and hypoploid metaphases. Structural aberrations found were chromatid breaks and gaps, chromatid deletions, additions, radial chromosomes, diplochromosomes, pulverizations, endoreduplications, centromeric separations, dicentrics and ring chromosomes. Apart from numerical and structural aberrations, there was an unusually high incidence of acrocentric associations in the scanned metaphases. The number of chromosomes involved in an acrocentric association was higher and the number of

Table 1: Status of ambient air quality of Amritsar (NAAQSI 2003, WHO 2003a,b; PPCB 2004)

Year	SPM ($\mu\text{g}/\text{m}^3$)	SO ₂ ($\mu\text{g}/\text{m}^3$)	NO _x ($\mu\text{g}/\text{m}^3$)
	WHO limit – no guideline NAAQS – 140 $\mu\text{g}/\text{m}^3$	WHO limit – 50 $\mu\text{g}/\text{m}^3$ NAAQS – 60 $\mu\text{g}/\text{m}^3$	WHO limit – 40 $\mu\text{g}/\text{m}^3$ NAAQS – 60 $\mu\text{g}/\text{m}^3$
1997	140	17	44
1998	447	16	42
1999	439	20	43
2000	408	11	17
2001	396	17	20
2002	416	15	26
2003	433	13	30

acrocentric associations in a metaphase was significantly higher in traffic policemen as compared to controls. In controls, majority (84.33%) of acrocentric associations were involving two acrocentric chromosomes (D and G, D and D, and G and G group), and only 15.67% metaphases involved three acrocentrics (3D, 2D and G, and 2G and D). In traffic policemen, two chromosome associations were 75.94%, three chromosome associations were 21.88%, four chromosome (2D and 2G, 3G and D, 3D and G, and 4D and 4G) associations were 6.17%, and 0.58% was five chromosome (3D and 2G) associations.

The mean percentage frequency of total aberrant metaphases, metaphases with numerical and structural aberrations (including and excluding gaps) and metaphases with acrocentric associations (multiple and single) were significantly higher in traffic policemen as compared to controls (Table 2) as well as in traffic policemen working more than 10 years as compared to those working less than 10 years (Table 3).

The percentage frequency of TAM was higher but statistically non-significant in traffic policemen ≥ 35 years in age as compared to subjects < 35 years in age. The controls ≥ 35 years in age had slightly lower number of aberrations as compared to subjects < 35 years in age. The percentage frequency of TAM was significantly higher in controls taking alcohol but was non-significantly higher in traffic policemen as compared to those not taking alcohol (Table 4).

All traffic policemen and controls were apparently healthy, but seven (33.33%) traffic policemen reported coughing and breathlessness. All traffic policemen and married controls were having healthy children except one traffic

policeman who was infertile. Among traffic policemen, one case of spontaneous abortion, one case of miscarriage and one case of postnatal death were reported, and one control reported miscarriage twice. Any relation of these observations to the pollution could not be ascertained.

DISCUSSION

The results of the study have shown significantly higher frequencies of chromosomal aberrations and unusually high acrocentric associations in traffic policemen than the controls (Table 2). Acrocentric associations were considered as harbinger of aberrations, as they could result into Robertsonian translocation and aneuploidy in future cell divisions. Previous studies on traffic policemen have not reported frequency of acrocentric associations. Traffic policemen are occupationally exposed to increased automobile exhausts. Higher occupational exposure to automobile exhausts and SPM at the city intersections can be the cause of elevated chromosomal aberrations and Acrocentric associations in traffic policemen compared to the controls. In Amritsar there are a large number of two and three-wheelers and cars and the total number of registered vehicles from the year 1950 to April 2004 is 469,953 with the number of vehicles per thousand persons being 153 (DTO 2004). There are no strict pollution norms for vehicles and very old and even poorly maintained vehicles are still operating leading to high vehicular emissions. Adulterated petrol is in use, and majority of the three-wheelers use mixture of diesel and kerosene as fuel which causes a lot of pollution (Vehicular Pollution 2003). Most of the roads cater to manually driven

Table 2: Chromosomal aberrations found in controls and traffic policemen

Type of aberrations	Mean \pm SD in controls	Mean \pm SD in traffic policemen	Student's t-test distribution
% frequency of TAM	13.02 \pm 1.97	31.12 \pm 10.12	(p < 0.005), sig ^f
% frequency of metaphases with NA	2.39 \pm 0.80	4.39 \pm 2.62	(p < 0.005), sig ^f
% frequency of metaphases with SA including gaps	1.30 \pm 0.96	2.38 \pm 1.91	(p < 0.05), sig ^f
% frequency of metaphases with SA excluding gaps	1.12 \pm 0.68	2.24 \pm 1.68	(p < 0.05), sig ^f
% frequency of metaphases with AA	9.41 \pm 2.23	24.40 \pm 7.36	(p < 0.005), sig ^f
% frequency of metaphases with multiple AA	1.79 \pm 0.70	7.92 \pm 3.84	(p < 0.005), sig ^f
% frequency of metaphases with single AA	7.62 \pm 1.55	16.48 \pm 4.61	(p < 0.005), sig ^f

TAM = Total aberrant metaphases; NA = Numerical aberrations;
SA = Structural aberrations; AA = Acrocentric associations;
sig^f = Statistically significant difference.

carts, slow-moving rickshaws, speeding two and four-wheelers and heavily loaded buses and trucks and are over-congested. There is also infrequent cleaning of dust from the sides of roads which results in increased SPM as the traffic moves. Thus, the vehicles are not driven at optimum speed giving low fuel efficiency and high level of emissions. Automobiles are major sources of SPM and the dust present on the sides of the roads also leads to increased SPM as the traffic moves. Thus the traffic policemen are also more exposed to SPM. Seven (33.33%) traffic policemen reported coughing and breathlessness. The possible explanation can be their higher exposure to SPM. Impaired immune response has been reported (WHO 2003a) in rats exposed to resuspended road dust at 360 mg/m³ relative to exposure to either clean air or ammonium nitrate exposure, leading to lowered resistance to respiratory infection, at an exposure duration of 4 h/day for 4 days/week for 8 weeks. There are studies reporting increased chromosomal aberrations in individuals occupationally exposed to petrol and diesel exhausts and fumes (Hogstedt et al. 1981; Sobti and Bhardwaj 1993; Carere et al. 1995; Khalil 1995; Knudsen et al. 1999; Chitra et al. 2001; Burgaz et al. 2002). The urban airborne particles have also been shown to have genotoxic activity (Clonfero 1997; Zhao et al. 2002).

Emissions from automotive sources are estimated nowadays to be the main cause of mutagenic/ carcinogenic risk for people living in urban areas of industrialized countries. Soot emitted by motor vehicles contains variable quantities of adsorbed highly genotoxic compounds, polycyclic aromatic hydrocarbons (PAHs) and their nitro derivatives, i.e., nitroarenes. The latter are among the compounds with the highest genotoxic activity according to Ames test (genetic point mutation on bacteria)

and are particularly abundant in ultra fine particulate matter (<1.1 microns) emitted by diesel engines. Diesel emissions have been considered as probable carcinogenic agents for man by the International Agency for Research on Cancer (IARC) (Clonfero and Saia 1991; Clonfero 1997). Thus, the individuals occupationally exposed to pollutants in automobile exhausts are at increased health risk. As the automobiles are the main sources of PAHs, traffic policemen are more exposed to PAHs (Wang et al. 1978; Schenker 1980; Khesina et al. 1989; Clonfero 1997). There are studies reporting increased chromosomal aberrations and sister chromatid exchanges (SCEs) in individuals exposed to PAHs (Perera et al. 1992; Karahalil et al. 1998; Burgaz et al. 2002).

Petrol with high lead content was in use before the year 1997 in Amritsar. In January 1997, low lead petrol with a lead content of 0.15 g/l and in February 2000 unleaded petrol with a lead content of 0.013 g/l was introduced in Amritsar (SPAAQMI 2002). Air from petrol in dense traffic areas is a source of environmental lead. As lead has a long half-life and accumulates in human body over a lifetime, including prenatal life, its effects can persist in individuals exposed to it. Unleaded petrol has benzene (CAS 71-43-2) present in it, and both lead and benzene have been shown to have genotoxic effects. There are studies reporting higher blood lead level in traffic policemen exposed to vehicular exhausts than the non-exposed persons (Farsam et al. 1982; Ahmed et al. 1987; Kamal et al. 1991; Bavazzano 1994; Telisman et al. 2000). Studies have shown increased chromosomal aberrations in individuals exposed to lead and benzene (Jablonica et al. 1987; Huang et al. 1988; Smejkalova 1990; Tompa et al. 1994). As traffic policemen are more exposed to PAHs, lead and benzene, this can also be the cause of increased chromosomal aberrations.

Table 3: Chromosomal aberrations found in traffic policemen according to the duration of exposure

Type of aberrations	Mean±SD in traffic policemen working < 10 years	Mean±SD in traffic policemen working > 10 years	Student's t-test distribution
% frequency of TAM	24.52 ± 5.72	40.03 ± 7.84	(p < 0.005), sig ^a
% frequency of metaphases with NA	3.44 ± 2.49	5.65 ± 2.58	(p < 0.05), sig ^a
% frequency of metaphases with SA including gaps	1.45 ± 1.47	3.62 ± 1.71	(p < 0.005), sig ^a
% frequency of metaphases with SA excluding gaps	1.42 ± 1.27	3.34 ± 1.34	(p < 0.005), sig ^a
% frequency of metaphases with AA	19.63 ± 3.72	30.76 ± 6.09	(p < 0.005), sig ^a

TAM = Total aberrant metaphases; NA = Numerical aberrations; SA = Structural aberrations; AA = Acrocentric associations; sig^a = Statistically significant difference.

The results have also shown higher chromosomal aberrations in traffic policemen working more than 10 years as compared to those working less than 10 years (Table 3). This shows that chromosomal aberrations are increasing with increasing duration of service. The possible explanation can be that traffic policemen have a consistent and prolonged exposure to the polluted air having petrol and diesel emissions, SPM, PAHs and benzene with increasing duration of service. Lead can also be the cause of increased chromosomal aberrations in traffic policemen, because before the year 2000 leaded petrol was used in Amritsar. So, the traffic policemen working more than 10 years were exposed to the leaded petrol exhausts. Increased

chromosomal aberrations have been reported in individuals exposed to lead (Huang et al. 1988; Smejkalova 1990). The result is consistent with the previous study on traffic policemen, in which the authors have found higher values of SCEs after 5 years in traffic policemen who remained in service than in the initial analysis (Chandrasekaran et al. 1996). The result is also consistent with the previous studies on persons exposed to petrol and diesel exhausts, and fumes reporting a positive correlation between increasing exposure duration and higher incidence of chromosomal aberrations (Sobti and Bhardwaj 1993; Chitra et al. 2001).

The percentage frequency of TAM was higher in traffic policemen and controls ≥ 35 years

Table 4: Percentage frequency of total aberrant metaphases detected in traffic policemen and controls with reference to age and alcohol intake

Habits	No.	TAM (Mean \pm SD)	Student's <i>t</i> -test distribution
Traffic policemen < 35 years in age	10	27.91 \pm 10.49	(<i>p</i> > 0.05), non-sig ^b
Traffic policemen ≥ 35 years in age	11	35.04 \pm 8.28	
Controls < 35 years in age	4	13.92 \pm 2.45	(<i>p</i> > 0.05), non-sig ^b
Controls ≥ 35 years in age	9	12.61 \pm 1.58	
Traffic policemen taking alcohol	13	34.09 \pm 10.88	(<i>p</i> > 0.05), non-sig ^b
Traffic policemen not taking alcohol	18	27.67 \pm 6.86	
Controls taking alcohol	5	13.88 \pm 2.47	(<i>p</i> < 0.05), sig ^a
Controls not taking alcohol	8	12.48 \pm 1.33	

sig^a = Statistically significant difference.

non-sig^b = Statistically non-significant difference.

Table 5: Summary of previous studies on traffic police workers occupationally exposed to air pollution

No. of subjects	No. of controls	Location	Measured end point	Results (+) significantly higher /(-) non-significant difference	Reference
15	23	Ankara, Turkey	Chromosomal aberrations	+	Burgaz et al. (2002)
28	15	Cairo, Egypt	Chromosomal aberrations	+	Anwar and Kamal (1988)
23	23	Madras, India	SCEs	+	Chandrasekaran et al. (1996)
78	57	Lanzhou, China	Micronuclei	+	Zhao et al. (1998)
82	34	Genoa, Italy	Micronuclei	-	Bolognesi et al. (1997a)
54	35	Genoa, Italy	SCEs	-	Bolognesi et al. (1997b)
94	52	Genoa, Italy	³² P-postlabelling DNA adducts	+	Merlo et al. (1997)
15	20	Ankara, Turkey	Micronuclei	+	Karahalil et al. (1999)
133	57	Rome, Italy	SCEs	+	Carere et al. (2002)
44	45	Bangkok, Thailand	Alkaline comet assay	-	
			³² P-postlabelling DNA adducts	+	Ruchirawat et al. (2002)
			Serum PAH-albumin adduct level	+	

in age as compared to < 35 years in age, but the differences were non-significant. Thus, the clear effect of age was not seen.

In controls taking alcohol, the mean percentage of TAM was significantly higher, but in traffic policemen taking alcohol the aberrations were non-significantly higher than the subjects not taking alcohol (Table 4). Thus, alcohol intake could be having a synergistic effect on chromosomal damage being caused by air pollution. but as intake of alcohol was infrequent and in less quantity, the independent effect of alcohol in traffic policemen could not be ascertained. Ethanol reportedly also induces increase in the biologically active or mobile fraction of lead in body (Telisman et al. 2000) and can exacerbate the genotoxic effect of lead. Thus, the increasing age and alcohol intake were also contributing to the increase in chromosomal aberrations caused by increased pollution.

The results of this study are consistent with the previous studies on traffic police workers (Table 5), in which higher frequency of chromosomal aberrations and SCEs was detected in traffic policemen as compared to controls (Anwar and Kamal 1988; Chandrasekaran et al. 1996; Zhao et al. 1998; Burgaz et al. 2002).

The results of this study have shown increased chromosomal aberrations in traffic policemen as compared to controls and also an increase in acrocentric associations. This indicates occupational exposure to polluted air having increased automobile exhausts can have genotoxic effects and these effects increase with increasing duration of exposure. Thus, chromosomal aberrations, including acrocentric associations, can be used for biomonitoring the genotoxic effects of occupational exposure to urban air pollutants.

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