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Genetic Risk Assessment in Traffic Policemen of Chennai City by Sister Chromatid Exchange Analysis

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ABSTRACT Urban atmosphere contains a variety of chemicals which are potentially genotoxic and carcinogenic. An increased risk of lung cancer in certain occupational groups is due to exposure to traffic-related pollution. Few reports are available in India on the genetic damage caused by exposure to automobile exhaust pollution on traffic policemen. Therefore, the present work was undertaken to study the genetic risk assessment in traffic policemen of Chennai city by Sister Chromatid Exchange Analysis. The study subjects consisted of 56 traffic policemen who worked in high density traffic areas of Chennai city and 25 office workers unexposed to traffic as controls were used. Sister chromatid exchanges (SCEs) were analyzed in peripheral blood lymphocytes drawn from both subjects. The results expressed as mean SCEs/cell was found significantly (p<0.001) higher in traffic policemen (10.62±0.57) compared to the unexposed control subjects (6.49±0.31). These subjects were divided further into two sub-groups such as smokers and alcohol consumers which were not influence the incidence of SCEs/cell. However, a statistically significant (p<0.001) difference was observed between smokers and alcohol consumers than that of control subjects. This is the first studyof the risk assessment of Chennai city traffic police exposed to vehicular pollution.

INTRODUCTION

Air pollution has been generally recognized as a health hazard (Crebelli et al. 2001; Maffei et al. 2005). A number of epidemiological studies have indicated an increased risk of cancer development (Zijno et al. 2006; Singh et al. 2007a). The urban atmosphere contains a large variety of carcinogenic and mutagenic compounds in the form of particulate (Hu et al. 2007). Genotoxins in the air of urban areas are mostly produced by the incomplete combustion of fossil fuels by automobiles (Burgaz et al. 2002). The composition of automobile exhaust is complex and contains carbon monoxide, carbon dioxide, nitrogen dioxide, polycyclic aromatic hydrocarbons (PAHs) including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, benzo(a)pyrene, 3-nitrobenzan-throne as well as benzene, 1,3-butadiene, particulates, lead and sulfur dioxide (Singh et al. 2007b; Bono et al. 2007). The significant amounts of organic carcinogens, in particularl benzene and benzo(a)pyrene in urban air, raises concern over the possible long-term adverse health effects in urban populations (Tomei et al. 2001; Kamboj and Sambyal 2006).

Diesel engine exhaust was reported to induce cytogenetic damage in both mammalian and rodent cell lines and also increased chromosomal damage in humans (Knudsen et al. 2005). Traffic wardens were considered to be a high-risk group (Liu et al. 2007). Several studies reported an increased frequency of sister chromatid exchanges (SCEs), micronuclei and chromosomal aberration among traffic policemen (Zhao et al. 1998; Sreedevi et al. 2006). In contrast, there was no significant effect among traffic policemen from Italy, despite an increased level of PAHs being found in their surroundings. Similar observations were made by other investigators from Italy (Carere et al. 2002; Leopardi et al. 2003; Singh et al. 2007b). These variable results were attributed to the variation in exposure levels of the pollutants by the traffic policemen (Bolognesi et al. 1997; Hu et al. 2007).

Chennai is the capital of Tamil Nadu and one of the major metropolitan areas among the cities in India. It is located on the south-eastern coast with more than a population of 8.5 million spread

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over 1180 sq. km and density of population was 24,681/sq. km (Senthilnathan 2008). The economy of Chennai city has ranged from providing utility services to being based on automobiles. Particulate matter (PM) levels in almost all the metropolitan cities in India have exceeded the standard levels recommended by the World Health organization (WHO). About 2.5 million premature deaths are caused annually due to PM exposure (Pandey et al. 2006). Estimated emissions from motor vehicles in Chennai city in 2005 were 431, 46, 6 and 4575 tons/days for carbon monoxide, oxides of nitrogen, PM and carbon dioxide, respectively (Nesamani 2009). The high level of pollution load in Chennai city is due to the increase in the number of vehicles (Mahendra and Krishnamurthy 2005).

The present study was carried out to assess the genetic risk of the traffic policemen of Chennai city in view of their exposure to automobile exhaust from an ever increasing number of vehicles of all varieties and thereby, assess the city's atmospheric air quality. The genetic risk was assessed by analyzing the SCEs in cultured peripheral blood lymphocytes by using a conventional protocol.

MATERIALS AND METHODS

Study Subjects: The study consisted of 56 male traffic policemen discharging their duties in heavy traffic areas of Chennai city, India. During their duties, they worked upto 8 hours per day and 7 days a week. Twenty- five volunteers participated as control subjects who had similar age group, sex, and habits with no exposure to traffic pollution . Information on personal identity, life style, smoking status, alcohol consumption, employment history, prior occupational exposure, health status, and reproductive history was recorded on a proforma prepared for the purpose. Both study subjects were informed about the nature of study and their acceptance to participate in the study was obtained in the regional language. Two mL of venous blood sample was collected from each subject during the period, April to July 2004, with permission from the administrative authority, Joint Commissioner of Police Office, Chennai city, India.

Chemicals: 5-Bromodeoxyuridine, Giemsa, Hoechst (33258) and Colchicines were obtained from Sigma-Aldrich, USA. RPMI (1640) medium and Fetal Bovine serum was purchased from Hi-Media, Mumbai, India. Other solvents used in the present study were of analytical grade.

SCEs Analysis in Peripheral Blood Lymphocytes: Genotoxic risk was assessed by analyzing the chromosomes for SCEs on 72 h cultured lymphocytes of peripheral blood, that were stained with 5-bromodeoxyuridine at 24 h by using the method of Perry and Wolff (1974). Briefly, the method involved inoculation of 0.5 mL of blood into 5 mL of RPMI 1640 medium containing 1 mL of fetal bovine serum and 0.2 mL of phytohaemagglutinin under aseptic conditions. The cells were grown for 72 h at 37°C. 5-Bromodeoxyuridine at a final concentration of 0.25 µg/mL was added after 24 h and incubated in dark. At the end of the incubation, the cultured cells were treated with colchicine (4 mg/mL) for 30 min. The cells were then pelletted and treated with prewarmed 0.075M KCl for 20 min and fixed in 3:1 (v/v) ratio of methanol and acetic acid. The slides were processed for differential staining with Hoechst (33258) for 20 min in the dark. Slides were mounted in 2 x SSC (double strength Standard Sodium Citrate) and photoreactivated by exposure to daylight for 2 h. The slides were stained in 1% buffered giemsa for 30 min. For each individual, 25 well-differentiated second division metaphases were analyzed under oil immersion objective of OPTECH (Germany) microscopy. Representative metaphases were photographed using NOVA 125 ASA (Japan) black and white film and scored for SCEs.

Statistical Analysis: Differences in mean values between the exposed and control groups were assessed using analysis of variance (ANOVA). All the statistical tests were two-tailed with p value of 0.05. The values were compared by student's t-test, using in-transformed data. Simple linear regression analysis was used to estimate the correlation between variables. All the statistical tests were carried out using SPSS - 10.1 statistical software package.

RESULTS

The range of variation of SCEs/cell was greater in traffic policemen (3-28 SCEs/cell) than in the control subjects (1-21 SCEs/cell). The mean SCEs/cell in traffic policemen was 10.62 ± 0.57 and 6.49 ± 0.31 in the control subjects. The frequency of SCEs/cell in traffic policemen was significantly ($p < 0.001$) higher when compared to the control subjects (Table 1). Smoking did

Table 1: Comparison of mean SCEs/cell between traffic policemen and control subject: Main characteristics of the study subjects

Study subjects	*No.*	*Mean age in years*	*Mean years of employment*		*Mean SCEs/cell*	*Range SCEs/cell*
			General duty	*Traffic control*		
Traffic policemen	56	41.46±0.75	10.77±0.46[1]	6.62±0.44	10.62±0.57*	6.88 - 20.04
Office workers (control)	25	42.53±0.33	18.33±0.37	#[2]	6.49±0.31	4.76 - 9.28

Values are expressed as mean±S.E; * p<0.001 when compared with control; [1] Years of service before being deployed in traffic regulation; #[2] = not applicable

not influence the baseline frequency of SCEs/cell within study subjects. Among the traffic policemen, 20 and 36 people were habitual smokers and non-smokers respectively. The mean SCEs of smokers were 10.07 ± 0.89 and non-smokers, 10.96 ± 0.75. The corresponding values among control subjects were observed 6.70 ± 0.60 from smokers and 6.30 ± 0.26 from non-smokers. The difference between sub-groups of the study subjects were not statistically significant (p>0.05). However, statistically significant difference (p<0.01) was observed between smokers of traffic policemen and of the respective control subjects. A similar significant difference (p< 0.001) was also observed among non-smokers of the study subjects (Table 2).

Among the traffic policemen, 40 people who were habitual consumers of alcohol and 16 people were non-alcoholics. The mean SCEs were 10.76 ± 0.72 in alcoholics and10.22 ± 0.89 in non-alcoholics. The corresponding values in the control group respectively were 5.88 ± 0.35 and 6.79 ± 0.40. There was no statistically significant (p>0.05) difference observed between sub-groups of the study subjects. However, a comparison of alcoholic traffic policemen and control subjects showed significant difference (p<0.001). Similarly, statistically significant difference (p<0.01) was also observed between non-alcoholic traffic policemen and control subject (Table 3). Stepwise linear regression analysis of the data indicated that the duration of service and age were not important variables for influencing the mean SCEs/cell (Data not shown).

DISCUSSION

The present study was carried out to assess the genotoxic risk from traffic policemen of Chennai city due to occupational exposure of vehicular pollution and thereby to assess the quality of atmospheric air of the city. With an ever-increasing vehicle density of the city, there was a need to evaluate the genetic risk on traffic policemen exposed to automobile exhausts. A

Table 2: Effect of smoking on SCEs/cell: Comparison of smokers and non-smokers of traffic policemen and control subject

Study sub-groups	*Study subjects*					
	Traffic policemen			*Office workers (control)*		
	No.	*Mean*	*Range*	*No.*	*Mean*	*Range*
Smokers	20	10.07±0.89*	6.88 – 15.76	11	6.70±0.60*	4.76 - 9.28
Non-smokers	36	10.96±0.75**	7.48 - 20.04	14	6.30±0.26	5.16 - 7.36

Values are expressed as mean±S.E of SCEs/cell; * p<0.01, ** p<0.001, when compared with control.

Table 3: Effect of alcoholism on SCEs/cell: Comparison of alcoholic and non-alcoholic traffic policemen and control subject

Study sub-groups	*Study subjects*					
	Traffic policemen			*Office workers (control)*		
	No.	*Mean*	*Range*	*No.*	*Mean*	*Range*
Alcoholic	40	10.76±0.72**	6.88 – 20.04	9	5.88±0.35	4.76 - 6.88
Non-alcoholic	16	10.22±0.89*	7.48 – 13.24	16	6.79±0.40*	5.16 - 9.28

Values are mean ± S.E of SCEs/cell; *p<0.01, **p<0.001 when compared with control

decade ago, the traffic load of Chennai city roads was estimated to be 10,000 petrol and diesel-powered vehicles/hour (Chandrasekaran et al. 1996). In recent years, this density has increased by 3 to 4 fold. Hence, it was pertinent to evaluate the magnitude of genetic risk due to automobile exhaust in traffic policemen who are the most exposed population. The present study showed an increase in baseline SCE frequency among traffic policemen which was ~1.7 fold higher than that of control subjects. Similar results were also reported from studies particularly carried out in developing countries with heavy traffics (Zhao et al. 1998; Sreedevi et al. 2006). However, the contrast results to the present study were also observed from the developed nations (Carere et al. 2002; Leopardi et al. 2003). Higher frequency of SCE in traffic policemen are mainly due to the PAH including benzene. A recent study indicated that the two- fold higher level of benzene exposure was observed from the traffic policemen and other outdoor workers than that of indoor officers (Kamboj and Sambyal 2006). Similarly, traffic wardens experienced greater exposure of benzene than office personnel of the police department (Crebelli et al. 2003; Carere et al. 2002). Another study from Italy, demonstrated increased exposure to benzene and other pollutants by traffic policemen (Leopardi et al. 2003). Increased PAH levels in individual traffic wardens were also demonstrated with less genotoxic risk (Bolognesi et al. 1997). Currently, there is no reference data available either for benzene or PAHs concentration in the ambient air of the Chennai city.

The impact of SCE frequency in both smokers and non-smokers were found to be in the range of 6.88-20.04, compared to the office workers, 4.76-9.28. The high frequency of SCE in smokers was mainly due to the influence of polymorphic genetic biomarkers such as CYP1A1 (*m1, m2* and *m4* variants), CYP2E1 (*PstI* and *RsaI*), NQO1 (*Hinf1*), GSTM1 and GSTT1 (*null* variants). Earlier study from Italy indicated that there was no statistically significant association was observed between smoker and genetic biomarkers. However, they found smoker to be an effective SCE inducer, particularly in the presence of certain variant genotypes, CYP2E1. Significant association between CYP1A1 variant genotypes and SCEs were also reported (Carere et al. 2002). Consequently, urinary biomarkers such as trans - muconic acid (TMA) and S-phenylmercapturic acid (SPMA) were also significantly higher with respect to SCE frequency in active smokers than in non-smokers of traffic wardens (Crebelli et al. 2003; Hu et al. 2007). Statistically significant difference was not observed between smokers and non-smokers of traffic policemen which is concluded with findings of Leopardi et al. (2003) in Italy which is in contrast to some studies carried out by Zhao et al. (1998), Sato and Aoki (2002). On other hand, alcohol consumption is also implicated as a factor for enhancing the genetic risk. In the range of SCE frequency observed from smokers and non-smokers were similar to be in the range of alcoholic and non-alcoholic traffic policemen. The results indicated that the influence of age and duration of service had no impact on SCE frequency as per the earlier reports (Carere et al. 2002; Maffei et al. 2005). Furthermore, a follow up study on traffic policemen of Chennai city also reported that there was no significant increase for SCE frequency over a span of five years service (Chandrasekaran et al. 1996).

In the present study on the population, a wide range of health related problems involving systemic disorders such as hypertension, eosinophilia, diabetics, dermal allergy, kidney stone, respiratory tract infections and other infectious diseases such as jaundice have been reported by the traffic policemen as general health complaints. The observed increase in SCE frequencies in traffic policemen in the present study may be due to exposure to complex environmental mixtures of urban air pollution and lack of protective measures and perhaps the genetic background of the people. At present, this is the only experimental data available for the risk assessment of Chennai city traffic police exposed to vehicular pollution. Therefore, further work is required to confirm these results by comparative analysis of polymorphic genetic biomarkers and degree of air pollution in Chennai city.

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