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Occurrence of Chromosomal Aberrations in the Traffic Police Population in Tiruchirappalli

T. Ravimanickam[1,*], R. Sakthivel[2], I. Nandhini[1], J. Rawoofkhan[1] and M. Saiyad Musthafa[3]

[1]Department of Zoology, School of Science, Tamil Nadu Open University, Saidapet, Chennai, Tamil Nadu, India
[2]Department of Statistics, Presidency College, Chennai, Tamil Nadu, India
[3]Unit of Research in Radiation Biology & Environmental Radioactivity (URRBER), P.G. and Research Department of Zoology, The New College (Autonomous), Affiliated to University of Madras, Chennai, Tamil Nadu, India

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ABSTRACT Chromosomal rearrangements take place over a period of time because of the exposure of various biological and non-biological factors/stress from the environment. This study focused on translocation of DNA, deletion of DNA, loss or gain of chromosomes in mononuclear lymphocytes using selected biomarkers to assess exposure of affected chromosomes due to the pollution factors. The study focused on major changes in the chromosomes such as Dicentric, Acentric, Centric rings, Acentric rings, Chromatid breaks and Chromatid gaps. As a result, this study shows increased CA in the sample TP of the district Tiruchirappalli as compared to controls of the same district and majority of the TP showed drastic increase in acrocentric associated CA.

INTRODUCTION

Cytogenetic studies started during the early 1940's with the studies of Karl Sax on the induction of chromosomal aberration in *Tradescantia* microspores by X-rays and later it has been applied in the study of human chromosomes. Generally, the cytogenetic techniques are useful to estimate radiation doses received due to external radiation exposure, but it is difficult to estimate radiation doses except for certain radio-nuclides like tritium.

As cytogenetic indicators of genotoxic hazards in the workplace, chromosomal abnormalities, sister chromatid will exchange, and micronuclei in the peripheral blood lymphocytes have widely been used (Vellingiri et al. 2014; Bindhya et al. 2010; Sudha et al. 2009). Nowadays, during investigation of biomonitoring studies, it is common to measure the degree of DNA damages in terms of strand breaks as well as alkaline label site. It is simple to perform the assay on WBCs, and it has been used in numerous biomonitoring study of occupational exposure to substances like styrene, vinyl chloride, pesticides,1,3-butadiene, hair dyes, antineoplastic agents, organic solvents, sewage and waste materials, wood dust, and ionising radiation (Møller et al. 2000; Pero et al. 1983).

Cancer is known to be caused by chromosomal rearrangements that take place over time as a result of exposure to different mutagens from the environment and way of life (Sureshkumar et al. 2013; Balachandar et al. 2008; Manikantan et al. 2010b). Through the use of cytogenetic analysis, the association between particular chromosomal rearrangements and cancer has been demonstrated in a number of neoplastic tissues (Manikantan et al. 2010a). According to these findings, tumour cells have chromosome translocations, deletions, gains, or losses. In recent years, the karyotype of several tumours has been documented. The frequency of CA, such as chromatid breaks (ChB), gaps, dicentrics (DC), and sister chromatid exchanges (SCE), in peripheral blood lymphocytes was found to be higher in patients with Bloom's syndrome,

[*]Address for correspondence:
Dr. T. Ravimanickam
Molecular Human Cytogenetics and Aquatic Biology
Associate Professor
Department of Zoology
Tamil Nadu Open University
Saidapet, Chennai-15,
Tamil Nadu, India
Phone: +91 9786506522
E-mail: ravimanick2003@gmail.com

Fanconi's anaemia, and ataxia telangiectasia than in healthy individuals.

Dicentric (DC) chromosome assay is widely used to measure accidental radiation exposure. The number of DC formed due to radiation exposure is scored using the microscope. Since the frequency of DC chromosomes follow Poisson distribution, this technique can be used to differentiate whole and partial body exposures.

The primary influencing elements in commercial, residential, and industrial environments are vehicle exhaust and road dust. Since traffic officers work outside and are subject to exposure to road dust and vehicle exhaust, their safety is more at risk. Their health is more severely affected by air pollution, particularly heavy metals (Pb) pollution in metropolises. Urban air quality can be significantly improved by getting rid of extremely polluting businesses, upgrading the energy system, stepping up automotive pollution monitoring, and getting rid of junk cars (Liu et al. 2019).

Comparing smokers to non-smokers, cytogenetic damage was more obvious in smokers. Age and length of exposure also seem to be important factors in cytogenetic damage (Balachandar et al. 2007). As a result, the current study raises the possibility that the aggregate effects of smoking, age, and exposures to vehicle exhausts are to blame for the induction of cytogenetic damage (Sree Devi et al. 2009).

Additionally, a synergistic relationship of ambient air pollution and smoking has been seen in metropolitan dwellers. Furthermore, jobs that require a lot of exposure to the air pollution caused by traffic have linked to an increase risk of cancer. Chromosomal mutations are typically unplanned occurrences in the growth of neoplasia. Various biomarkers were used to gauge human exposures to genotoxic substances found in ambient air (Nielsen et al. 1996). In this respect, structural chromosomal abnormalities are of particular importance since high peripheral lymphocyte CA levels have been linked to an elevated risk of malignancy (Hagmar et al. 1994; Sellappa et al. 2010).

The objective of this study is create an awareness among the traffic police regarding the risk factors of over exposure to atmosphere pollution at the chromosome level. This study shows the various forms of CA that were identified in the traffic police population in the district of Tiruchirappalli.

MATERIAL AND METHODS

The single-cell agarose electrophoresis (SAGE) was used for detect and quantify DNA damage and repair. For the preparation of the sample, about 10-15 ml peripheral blood is collected aseptically in heparinized vials from the traffic police population. All fractions' lymphocytes are promptly isolated, cleaned, and suspended in PBS solution. A completely frosted micro-slide was gently dropped with 75 µl of 1 percent normal agarose in the PBS at 65 °C, covered right away with a cover slip, and placed for around 5 minutes over the frozen ice pack. After the gel had dried, the cover slip was taken off. At 37°C, a 3:1 mixture of the sample's lymphocyte suspension and 1 percent low melting agarose was added. The gel coating the micro-slide was swiftly covered with 75 µl of this combination, and it was then given the same amount of time to set as before. In the gel containing the lymphocyte suspension, a third coating of 75 µl of 1 percent low melting agarose was applied and let to set. Similar to this, multiple slides for each lymphocyte fraction were created. The slides were put in an ice-cold lysis solution once the agarose had solidified, and then they were placed in a refrigerator at 4°C for at least one hour. Low lighting was used for all of the aforementioned procedures in order to prevent further DNA deterioration. The slides were taken out of the lysis solution and put horizontally in an electrophoresis tank. Electrophoresis was performed at 0.6 V/cm for 15 minutes after the reservoirs were filled with electrophoresis buffer and the slides were allowed to stand in the buffer for 20 minutes to allow DNA unwinding. The slides were taken out after electrophoresis, cleaned, and a few drops of silver stain were added. The slides were then viewed under 20x and 40x magnification. Using a micrometre, the DNA migration's length was quantified.

Scoring Criteria

The slides were under microscope to record various types of unstable aberration like and used for the analysis of Di-centric (DC), Acen-

tric (AC), Centric ring (CR), Acentric ring (AR), Chromosomal gaps (CG), Double minutes (DM), and a Total chromosomal aberration (TCA).

RESULTS

In this study, in order to examine the reliability of these markers as predictors of exposure, a subset of biomarkers was used to measure exposure in a nation with allegedly low ambient air pollution levels. As demonstrated by cytogenetic investigations of lymphocytes from populations occupationally exposed to such agents, CA frequencies in human peripheral lymphocytes are well-established indicators of exposure to occupational or environmental genotoxic chemicals. In this study, to evaluate genetic biomarkers to assess exposure to the genotoxic compounds present in urban air, normal people and TP in the city of Tiruchirappalli were selected as the study population.

The first and important cytogenetic end point being the CA was studied for both the groups, namely the normal/control population and the TP (Table 1). The DC frequency ranged from 43 to 59 per 5000 cells in the control group, while in that of the traffic policemen group it had a range of 47 to 75. This is the preliminary indication that there is some kind of genetic effect of air pollution on the incidence of the DCs. However, the important observation in both the groups is that the aberration is restricted to only one DC per cell. The ratio between the aberrated and normal cells also follows an age dependent increase. The entire range had Poisson distribution (PD) only, which clearly indicates that the incidence of DC is a rare event only. Finally, the correlation between the two groups in the age range reflect that when the exposure to the pollutants is less, that is, in the lower age ranges such as <20 and 21-30, there seems to be no difference between the two groups, which is also reflected in the p-values also. In the middle age range of 31-40 years the correlation deviates and the p-value starts to show significant levels. Finally in the last two levels of 41-50 and >51 the correlation is very poor and concordantly the p-value shows statistically significant levels of difference.

Table 1: Statistical analysis of frequency of dicentrics (DC) in control population (CP) and traffic policemen (TP)

Distribution of DC among the normal people (NP) and traffic policemen (TP) among the various age groups												
Age group (in years)	*<20*		*21-30*			*31-40*			*41-50*		*>51*	
Nature of persons	*Control*	*Sample*	*Control*	*Sample*		*Control*	*Sample*		*Control*	*Sample*	*Control*	*Sample*
Dicentrics	43	47	49	52		51	59		55	67	59	75
Distribution of DC among the cells	1	1	1	1		1	1		1	1	1	1
Frequency (Aberrated cells) (DC/cells scored)	0.0086	0.0094	0.0098	0.0104		0.0102	0.0118		0.0110	0.0134	0.0118	0.0150
Frequency distribution with regard to absence of DC (μ)(Good cells)	0.0107	0.0103	0.01001	0.0098		0.0099	0.0092		0.0095	0.0086	0.0092	0.0081
Correlation between the age groups	0.993	0.990	0.853	0.799		0.608						
Nature of dispersion (^{2}/y)	PD	PD	PD	PD		PD	PD		PD	PD	PD	PD
p value (Levels of significance)	<0.05	<0.01	<0.005	<0.001		<0.001						
Interpretation of p value	NS	SS	S	ES		ES						

Sample Size = 10 per group
Number of Cells Scored Per Sample = 500
Total Number of Cell Scored Per Age Group = 5000

The Acentric ring, which is similar to CRs but very rare in occurrence, followed the same distribution per cell (only one CR per cell), correlation, and p-value projections (refer Table 2). Under dispersion (UD) of this aberration clearly indicates that it is very rare and not commonly seen.

The next level of aberration is not a chromosomal type of aberration even though it was included in the study. Strictly speaking it forms a lower level of double stranded break leading to the implication of only one strip of the chromosome, and thus it is a chromatid type of aberration. The difference between the chromatid gap and break is that the distance between the two separated pieces is less that the width of the chromatid it is former and if it is more it refers to the latter (refer to Tables 3 and 4). Occurrence followed the same distribution per cell (only one CG or CB per cell), correlation, and p-value projections for both the gaps as well as breaks. The main difference is that the gaps being more readily formed than the breaks show that in their occurrence, for example, <20 group – CG 14 nos., while only 8 CB were seen.

DISCUSSION

According to the findings, traffic police officers exhibit more chromosomal abnormalities than controls, both in terms of frequency and variety. Double minutes, acentric fragments, and rings were among the discovered numerical aberrations in these samples. Chromatid fractures and gaps, dicentrics, and ring chromosomes were among the structural aberrations identified. Traffic police officers had significantly higher acrocentric associations, metaphases with structural and numerical aberrations (including and excluding gaps), and mean percentage frequencies of all aberrant metaphases (multiple and single) than controls and those working less than 10 years.

Although 10 percent traffic police officers reported coughing and shortness of breath, both the TP and controllers appeared to be in good health. All married traffic officers and controls have healthy offspring. Traffic police officers work in an environment with more cars, bikes, etc., and exhausts. Increased chromosomal abnormalities compared to controls, and Acrocen-

Table 2: Statistical analysis of frequency of centric rings (CR) in control population (CP) and traffic policemen (TP)

Distribution of CR among the normal people (NP) and traffic policemen (TP) among the various age groups										
Age group (in years)	*<20*		*21-30*		*31-40*		*41-50*		*>51*	
Nature of persons	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*
Centric rings	5	7	8	11	12	19	17	29	22	34
Distribution of CR among the cells	1	1	1	1	1	1	1	1	1	1
Frequency (Aberrated cells)(CR/Cells scored)	0.0010	0.0014	0.0016	0.0022	0.0024	0.0038	0.0034	0.0058	0.0044	0.0068
Frequency distribution with regard to absence of CR (μ)(Good cells)	0.3015	0.2581	0.2425	0.2085	0.2000	0.1601	0.1690	0.1301	0.1490	0.1203
Correlation between the age groups	0.975	0.881	0.790	0.605	0.587					
Nature of dispersion	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
p value (Levels of significance)	< 0.05	< 0.01	< 0.005	< 0.001	< 0.001					
Interpretation of p value	NS	SS	S	ES	ES					

Sample Size = 10 per group
Number of Cells Scored Per Sample = 500
Total Number of Cell Scored Per Age Group = 5000

Table 3: Statistical analysis of frequency of chromatid gaps (cg) in control population (CP) and traffic policemen (TP)

Distribution of cg among the normal people (NP) and traffic policemen (TP) among the various age groups										
Age group (in years)	*<20*		*21-30*		*31-40*		*41-50*		*>51*	
Nature of persons	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*
Chromatid gaps	14	18	21	28	34	41	46	53	62	78
Distribution of CG among the cells	1	1	1	1	1	1	1	1	1	1
Frequency (Aberrated cells)(CG / Cells scored)	0.0028	0.0036	0.0042	0.0056	0.0068	0.0082	0.0092	0.0106	0.0124	0.0156
Frequency distribution with regard to absence of CG (μ)(Good cells)	0.1856	0.164301524		0.1324	0.1203	0.1097	0.1037	0.09667	0.0894	0.0798
Correlation between the age groups	0.9621	0.8325	0.0766	0.5902	0.4387					
Nature of dispersion	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
p Value (Levels of significance)	< 0.05	< 0.01	< 0.005	< 0.001	< 0.001					
Interpretation of p value	NS	SS	S	ES	ES					

Sample Size = 10 per group
Number of Cells Scored Per Sample = 500
Total Number of Cell Scored Per Age Group = 5000

Table 4: Statistical analysis of frequency of chromatid breaks (CB) in control population (CP) and traffic policemen (TP)

Distribution of CB among the normal people (NP) and traffic policemen (TP) among the various age groups										
Age group (in years)	*<20*		*21-30*		*31-40*		*41-50*		*>51*	
Nature of persons	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*
Chromatid breaks	8	11	15	21	26	34	39	46	47	56
Distribution of CB among the cells	1	1	1	1	1	1	1	1	1	1
Frequency (Aberrated Cells)(CB / Cells Scored)	0.0016	0.0022	0.0033	0.0042	0.0052	0.0068	0.0078	0.0092	0.0094	0.0112
Frequency distribution with regard to absence of CB (μ)(Good Cells)	0.2425	0.2085	0.1796	0.1524	0.1373	0.1203	0.1125	0.1037	0.1026	0.0940
Correlation between the age groups	0.8912	0.7548	0.6812	0.6104	0.5490					
Nature of dispersion	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
p Value (Levels of significance)	< 0.05	< 0.01	< 0.005	< 0.001	< 0.001					
Interpretation Of p Value	NS	SS	S	ES	ES					

Sample Size = 10 per group
Number of Cells Scored Per Sample = 500
Total Number of Cell Scored Per Age Group = 5000

tric connections in traffic officers may be caused by greater occupational exposure to car exhaust and suspended particulate matter (SPM) at city crossroads. There are a plenty of two- and three-wheelers and cars in Tiruchirappalli, and there were 77,391 registered vehicles from 2004 to 2009, equating to 25.23 vehicles per 1,000 inhabitants. Because there are no strong pollution standards for automobiles and because numerous Vehicle emissions are high because very old and even poorly maintained automobiles are still in use. Most three-wheelers utilise a diesel-kerosene fuel blend that is very polluting, and adulterated gasoline is in use (Ingle et al. 2005). Traffic officers are more at risk since they spend the majority of their time outside, where they are exposed to road dust and heavy metals like lead. The majority of the roadways are overcrowded and accommodate manually propelled carts, sluggish rickshaws, swift two and four-wheelers, and massively laden buses and lorries. Additionally, the sides of roadways are rarely cleaned of dust, which causes SPM to rise as traffic drives. As a result, the vehicles are not being driven at their best pace, which results in poor fuel efficiency and high emissions. The primary contributor to the emissions from automotive sources are increasingly regarded to pose a mutagenic/carcinogenic concern to residents of industrialised countries' urban regions. The soot that automobiles generate contains varying concentrations of adsorbed, highly genotoxic polycyclic aromatic hydrocarbons (PAHs) and their nitro derivatives, or nitroarenes. The latter are among the chemicals with the highest genotoxic activity, according to the Ames test (genetic point mutation on bacteria). Moreover, they are very common in the ultra-fine particulate matter (1.1 microns) that diesel engines release. Diesel exhaust has been designated by the International Agency for Research on Cancer as possible human carcinogenic. (IARC 1989). Therefore, those who are exposed to car exhaust emissions at work run a higher risk of developing health problems. Traffic police officers are particularly exposed to PAHs because cars are the primary source of these chemicals. According to studies, people exposed to PAHs have more chromosomal abnormalities and sister chromatid exchanges (SCEs). In Tiruchirapalli, gasoline with a high lead level was in use prior to 1997. In Tiruchirappalli, low lead gasoline with a lead content of 0.15 g/l and unleaded gasoline with a lead content of 0.013 g/l were introduced in January 1997 and February 2000, respectively (SPAAQMI 2002). Environmental lead comes through the air from gasoline in locations with a lot of traffic. Lead exposure can have long-lasting impacts because lead accumulates in the body over a lifetime, especially during pregnancy. Benzene is included in unleaded gasoline, and research has shown that both lead and benzene have genotoxic consequences. According to research, traffic police officers who are exposed to vehicle exhaust have greater blood lead levels than people who are not. Lead and benzene exposure has been linked to an increase in chromosomal abnormalities, according to studies (Hagmar et al. 1994). It is also possible that greater chromosomal abnormalities are caused by the increasing exposure of traffic police officers to PAHs, lead, and benzene. According to the findings, traffic police officers who have worked for more than 10 years exhibit greater levels of chromosomal abnormalities than those who have worked for less than 10. This demonstrates that chromosomal abnormalities increase with service time. The continuous and prolonged exposure of traffic police officers to air contaminated with SPM, PAHs, benzene, and diesel and gasoline pollutants could be one explanation. Because leaded petrol was still used in Tiruchirappalli before the year 2000, lead may also be the reason for an increase in chromosomal abnormalities among traffic police officers. These exhausts from leaded gasoline exposed traffic police officers who had been on the job for more than ten years. Chromosome abnormalities have been found to increase in association with lead exposure. The results are consistent with the earlier research on traffic police officers, in which the authors found that SCE values were higher in traffic police officers who continued to work after five years than in the first analysis. (Chandrasekaran et al. 1996). The outcome is also in line with earlier research on people exposed to fumes and diesel and gasoline exhausts, which found a link between longer exposure times and a higher incidence of chromosomal abnormalities (Sudha et al. 2010). Although traffic police officers and controls under the age of 35 had a higher percentage frequency of TAM than those above

that age, the differences were not statistically significant. As a result, the age effect was not readily apparent. The mean percentage of TAM was significantly higher for controls who were drinking, while the aberrations were not statistically different for traffic officers who were drinking. Because alcohol was used infrequently and in little amounts, the independent effect of alcohol on traffic cops could not be determined, but alcohol consumption may be having a synergistic effect on the chromosomal damage being produced by air pollution. According to reports, ethanol also increases the amount of physiologically active or mobile lead in the body and can make lead's genotoxic effects worse. While the amounts of iron and lead were lower in the police exposed to vehicle exhaust than in the control group, the levels of manganese were much lower (Shatie and Mathkor 2022). Consequently, the rise in chromosomal abnormalities brought on by increased pollution was also being influenced by ageing and alcohol consumption. The findings of this study are in line with those of earlier research on traffic police officers, which found that TP had a higher prevalence of chromosomal abnormalities and SCEs than controls (Anwar and Kamal 1988; Chandrasekaran et al. 1996). According to the study's findings, traffic police officers have more chromosomal abnormalities than controls do, as well as more acrocentric connections. This suggests that prolonged occupational exposure to air pollution with high levels of vehicle exhausts can have genotoxic consequences, and that the severity of these effects increases with exposure time. The genotoxic effects of occupational exposure to urban air pollution can thus be biomonitored using chromosomal abnormalities, including acrocentric relationships. In 100 percent male, 45.7 percent aged between 41-50 years, and 44.4 percent with a bachelor's degree and found that 72.2 percent of respondents had not received information, and 74.4 percent had not been trained. Most of the respondents had knowledge at a moderate level (48.4%). A minority of respondents had the perception of adverse health effects at poor level (2.7%) and had self-prevention behaviour from air pollution at a poor level (3.1%) (Wannalai et al. 2016).

The individual types of CA that were found are Dicentric Aberrations, Centric Rings, Acentric Rings, Double Minutes, and in the Chromatid type of Aberrations are the Gaps and Breaks and the Total CA.

CONCLUSION

The results have shown that rare types of aberrations like centric and acentric rings also occur, indicating that the pollutants have the capacity to inflict different types of complex aberrations that lead to the formation of these aberrations. The dispersion of these aberrations highlight their rarity in traffic policemen. Excessive acentric fragments are more indicative that the information of DC can be expected to increase if there is misrepair of these inti DC. Thus, there is a tendency to get even higher levels of DC in this type of environmental exposure. Another interesting feature is that the frequency of DM and EXACs are not significantly different from each other indicating the possibilities of events in DNA damage similar in both the instances. Finally, the overall views between the DSBs represented by the chromosomal aberrations like DC, EXAC, AR, CR, CG, GB and DM, and micronuclei follow a pattern of increasing complexity and general trends towards exposure related occurrence.

RECOMMENDATIONS

The recommendations of the present study leads to the conclusion that the traffic policemen have to be well equipped both in terms of safety equipment for air pollution as well as physiological stress related to air pollution causing free radical formation leading to formation of such chromosomal aberrations.

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