Journal of Medical Genetics and Genomics Vol. 2(2), pp. 18-22, April 2010
Available online http://www.academicjournals.org/jmgg
ISSN 2141-2278 © 2010 Academic Journals

Full Length Research Paper

Nuclear anomalies in exfoliated buccal epithelial cells of petrol station attendants in Tamilnadu, South India

K. Rajkokila*, S. Shajithanoop and M. V. Usharani

Department of Environmental Sciences, Bharathiar University, Coimbatore -641 046, Tamilnadu, India.

Accepted 24 February, 2010

Petroleum derivative consist of a complex mixture of chemical compounds. Petrol station workers who pump fuel to vehicles absorb the products of fuel fumes and the products of combustion. The exposure posses a risk to human health and development of several types of cancers. A total of 120 petrol station workers and 105 control group of individuals in the age group of 17 to 35 years were recruited, a questionnaire based survey was conducted and buccal smears were collected from oral cavity and analyzed for nuclear anomalies. A higher frequency of karyolysis was observed among men (42.54 ± 0.76) than women (41.26 ± 0.59). A significant difference in nuclear anomalies was observed in workers exposed to petrol for longer duration. In addition to this, a higher degree of nuclear anomalies was observed among smokers.

Key words: Petrol station attendants, buccal cells, micronucleus frequency, genotoxicity, petroleum derivatives.

INTRODUCTION

Petrol station attendants are chronically exposed to petroleum derivatives primarily through inhalation of the volatile fraction of petrol during vehicle refueling (Celik et al., 2003). Occupational exposure to such derivatives poses genotoxic risk. Exposure to gasoline vapour is classified by the International Agency for research on Cancer (IARC, 1989) as possibly carcinogenic to humans, mainly on the basis of the well - established carcinogenic nature of some components, such as benzene. Benzene and other less known hydrocarbons are produced in petroleum refining, and are widely used as solvents. Oral or inhalation exposures at high levels may cause death in humans and animals, however, the main effects of these types of exposure are drowsiness, dizziness and head ache. Long term exposures to benzene may affect normal blood production, cause severe anemia, internal bleeding of immune system and human reproductive effects such as spontaneous abortion. Exposure to benzene has also been linked with genetic changes in humans and animals (Subrahmanyam et al., 1991). Furthermore, an increased cytogenetic damage in peripheral blood lymphocytes of level of workers occupationally exposed to petroleum and petroleum derivates has been demonstrated using different genetic end-points, such as sister chromatid exchange (SCE), DNA strand breaks and micronuclei (Hoegsted et al., 1991; Oesch et al., 1995; Bukvic et al., 1998). Nevertheless, about 92% of human cancers are derived from the external and internal epithelium. On the other hand, the micronucleus test in exfoliated epithelial cells has been shown to be an effective method to detect unstable chromosomal aberrations (Tolbert et al., 1991; Kayal et al., 1993; Pastor et al., 2001; Huvinen et al., 2002). Human population exposed to toxic chemicals such as benzene showed a significant increase in the buccal cell micronuclei (Surralles et al., 1997). Buccal epithelium cells provide an alternative source of tissue in human subjects monitoring for occupational and environment toxic exposures. Hence the objective of the present investigation was to study the extent of cytogenetic damage in exfoliated buccal cells obtained from petrol station workers. Degenerative nuclear changes, such as Micronuclei (MN) binucleates (BN), karyorrhexis (KR) and karyolysis (KL) were analyzed in the exfoliated buccal cells.

*Corresponding author. E-mail: gokilagenetics@gmail.com.

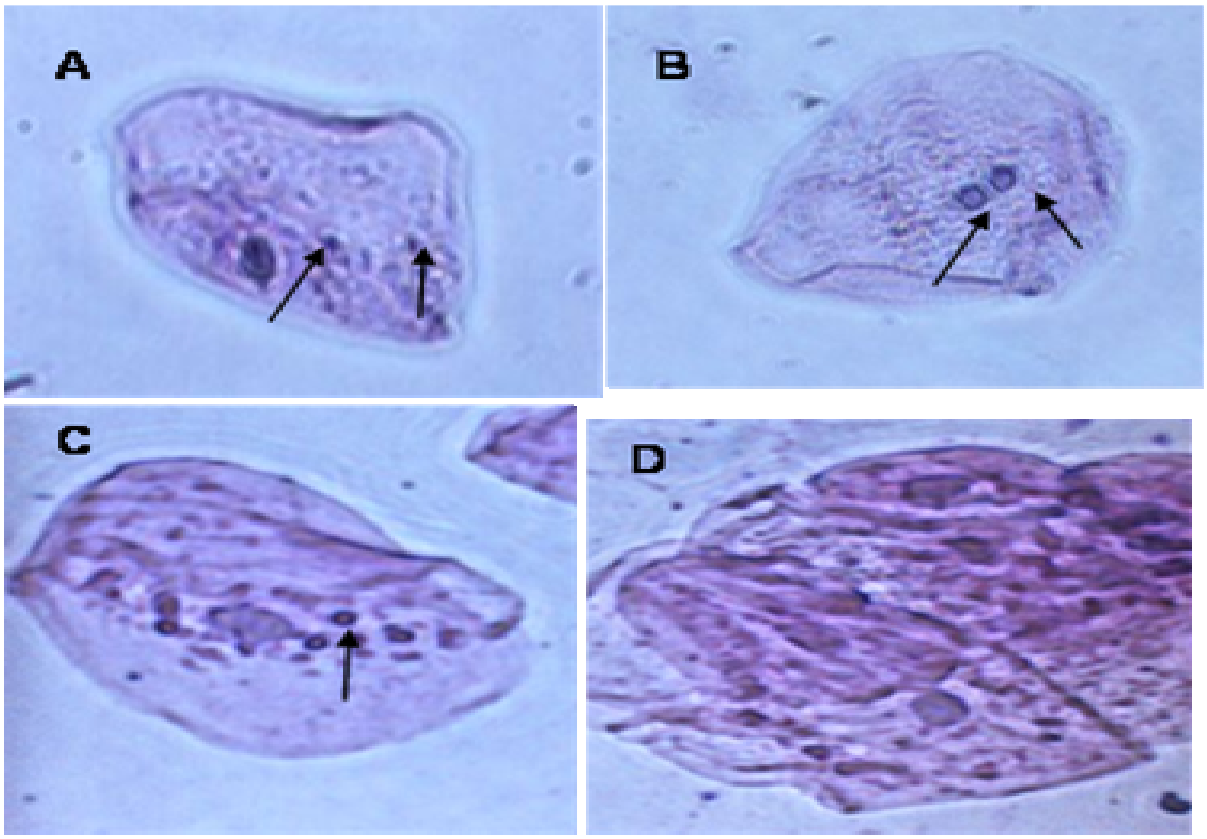

Figure 1. A: Micronuclei, B: binucleated cell, C: karyorrhetic cell, D: Karyolytic cell.

MATERIALS AND METHODS

Subjects

The study was carried out on 120 petrol station attendants. The control group consists of 105 healthy individuals with no exposure to any petroleum derivatives or other potential genotoxic substances. Participants were informed about the objectives of the study. They were asked to sign an informed consent form and to complete a questionnaire to obtain necessary information on their lifestyle and personal factors (age, working period, alcohol consumption and smoking habits, health, etc).

Buccal cell sampling, preparation and staining

Buccal cells originate from a multilayered epithelium that lines the oral cavity. Prior to buccal cell collection the petrol station attendants and control groups were advised to rinse their mouth thoroughly with water to remove unwanted debris. Sterile wooden spatula was used to obtain cell samples from buccal mucosa. The buccal mucosa was transferred into Eppendorf tubes with Phosphate Buffered Saline (PBS) at pH. 7.0 and centrifuged for 10 min at 1500 rpm. Supernatant was removed and replaced with 5 ml of fresh PBS solution and centrifuged for 10 min at 1500 rpm. This process was repeated thrice, because the PBS helps to inactivate endogenous, DNAases and aid in removing bacteria that may complicate scoring. After discarding the supernatant the pellet was smeared on to clean microscopic slides. Smears were air dried for 10 min, and then fixed in cold methanol: acetic acid (3:1) for 10 min. Slides were air dried for 10 to 15 min and stained in 2% Giemsa for 10 min and rinsed with double distilled water, air dried and viewed under a light microscope (Stich and Rosin, 1984).

Scoring criteria for buccal cytome assay

Three slides were prepared from each sample. Nuclear abnormalities were classified according to Tolbert et al. (1992). These criteria are intended to classify buccal cells into categories that distinguish between “normal” and “abnormal” based on their aberrant nuclear morphology. These abnormal nuclear morphologies are due to DNA damage and induced cell death. Photographic images showing distinct cell populations as scored in the buccal cytome assay are presented in Figure 1. Detailed descriptions of the various cell types observed in the present study are given below.

Micronuclei

Micronuclei are identified with presence of main nucleus and one or more smaller nuclei (micronuclei) in the cells. The micronuclei are usually round or oval in shape and their diameter may range between $^{1}/_{3}$ to $^{1}/_{16}$, the diameter of the main nucleus. Binucleated cells have two nuclei that are adherent to each other. This is indicative of failed cytokinesis. Karyorrhetic cells have dense network of nucleochromatin elements that lead to fragmentation and disintegration of the nucleus. In karyolytic cells, the nucleus is devoid of DNA and appears to have no nuclei. This indicates very late stage in cell death process. It has a cloudy appearance with no distinct features (Figure 1).

Table 1. Demographic characteristics of case-control study.

Control			Exposed		
Age (yrs)	**N (%)**	**Mean ± S.D**	**Age (yrs)**	**N (%)**	**Mean ± S.D**
Total	105	31.19 ± 1.26	Total	120	31.94 ± 1.60
Men	65 (62)	33.39 ± 1.62	Men	80 (67)	36.17 ± 1.23
Women	40 (38)	28.11 ± 1.04	Women	40 (33)	28.47 ± 1.11
Smoking habit			**Smoking habit**		
Men			**Men**		
Smoker	30 (29)	-	Smoker	45 (38)	-
Non - smokers	35 (33)	-	Non - smokers	35 (29)	-
Women			**Women**		
Non - smoker	40 (38)	-	Non - smoker	40 (33)	-
Alcoholism			**Alcoholism**		
Men			**Men**		
Alcoholics	26 (25)	-	alcoholics	40 (33)	-
Non - alcoholics	39 (37)	-	Non - alcoholics	40 (33)	-
Women			**Women**		
Non - alcoholics	40 (38)	-	Non - alcoholics	40 (33)	-

Table 2. Cytological observation in control groups.

Individuals	MNC	BNC	KRC	KLC
Smokers (N = 30)	2.96 ± 0.01*	3.42 ± 0.42*	9.60 ± 1.17*	29.49 ± 0.57*
Non-smokers (N = 75)	2.18 ± 0.02	2.82 ± 0.12	7.65 ± 0.76	24.42 ± 1.12
Alcoholics (N = 26)	2.59 ± 0.78*	3.12 ± 1.04*	14.23 ± 1.09*	24.49 ± 1.01*
Non-alcoholics (N = 79)	2.09 ± 0.05	2.67 ± 0.12	9.57 ± 0.22	22.27 ± 1.12
Age				
≤25 (N = 50)	2.58 ± 0.08*	3.11 ± 1.13*	9.17 ± 1.08*	27.14 ± 0.13*
>25 (N = 55)	2.20 ± 0.11	2.64 ± 0.69	6.02 ± 0.41	23.56 ± 0.12
Sex				
Female (N = 40)	0.98 ± 0.45	1.27 ± 0.52	7.52 ± 0.16	23.11 ± 0.61
Male (N = 65)	2.68 ± 0.17*	3.07 ± 0.28*	9.62 ± 0.61*	27.18 ± 0.06*

Data are reported as Mean ± SD. *P Value < 0.05 significant level.

Scoring method

1000 cells were scored per subject to determine the frequency of the various cell types outlined in the buccal cytome assay, consisted of micronuclei cell, binucleated cells, karyorrhectic, and karyolytic cells. A total of 1000 differentiated cells were scored in order to determine the frequency of micronuclei. Cells were scored by using both bright and low field.

Statistical analysis

All the data were expressed as the mean ± standard deviation. The synergistic effect between smoking and exposure were tested with a two-way analysis of variance. Multiple comparisons were made by using a least significant difference test. The error rate was accepted as 0.05 by student's test. All statistical analysis were performed using the program SPSS 11.

RESULTS

Tables 1, 2 and 3 show the main characteristics of the cases-controls studied. Age and sex ratio were nearly similar in both groups. The cytological observation in subjects

Table 3. Cytological observation in subjects.

Individuals	MNC	BNC	KRC	KLC
Smokers (N = 56)	9.81 ± 2.01*	7.82 ± 1.01*	19.81 ± 031*	41.26 ± 0.59*
Non-smokers (N = 64)	7.17 ± 3.11	4.80 ± 1.03	7.87 ± 0.51	32.84 ± 0.20
Alcoholics (N = 47)	9.41 ± 0.01*	6.27 ± 1.20*	18.68 ± 0.08*	39.57 ± 0.45*
Non-alcoholics (N = 73)	9.27 ± 0.88	4.18 ± 1.51	16.78 ± 0.4	30.72 ± 0.87
Age				
≤ 25 (N = 57)	8.68 ± 0.65	6.89 ± 0.54	12.18 ± 0.33	23.84 ± 0.28
> 25 (N = 63)	9.42 ± 0.81*	7.81 ± 0.08*	16.47 ± 0.87*	28.54 ± 0.48*
Duration of exposures (years)				
≤ 5 (N = 50)	6.15 ± 0.81	5.64 ± 1.55	16.82 ± 0.14	27.34 ± 1.70
> 5 (N = 70)	7.85 ± 0.45*	7.68 ± 0.10*	19.84 ± 0.45*	36.87 ± 1.05*
Sex				
Female (N = 40)	9.74 ± 1.41	3.97 ± 1.98	11.55 ± 1.87	26.53 ± 0.98
Male (N = 80)	12.46 ± 0.87*	6.87 ± 1.41*	21.56 ± 1.57*	42.54 ± 0.76*

Data are reported as mean ± SD. *P Value < 0.05 significant level.

reveals micronuclei and binucleated cells of buccal smears. The mean value of micronuclei in smokers was 9.81 ± 2.01 as against 7.17 ± 3.11 in non smoker exposed subjects. The mean value of binuclea-cells in men subjects without smoking was 4.80 ± 1.03 as against 7.82 ± 1.01 in subjects with a habit of smoking. In addition to this, males had a higher degree of micronuclei (12.46 ± 0.87) in exfoliated buccal cells than females (9.74 ± 1.41). Among the three nuclear anomalies, karyolysis was predominant in smokers followed by alcoholic subjects (39.57 ± 0.45). Comparatively males had a higher frequency of karyolysis (42.54 ± 0.76) than females (26.53 ± 0.98). In addition subjects with exposure period of more than five years had a higher degree of nuclear abnormalities than subjects with less than five years of exposure.

Male subjects had a higher degree of nuclear abnormalities than the females. The frequency of micronucleate cell, binucleate cell, karryorhexis cell and karryolysis cell were compared between smokers and non-smokers, alcoholics and non- alcoholics, age (less than 25 and more than 25) working experience (less than 5 and more than 5) and male and females in both control and subjects overall showed a higher level of nuclear abnormalities in males than the female subjects.

DISCUSSION

Benzene, an important component of petrol, is a widely distributed environmental contaminant. 98% of Benzene is derived from the petrochemical and petroleum refining industries. Therefore occupational exposure to benzene in human generally takes place in factories, refineries and other industrial settings. Population is exposed to benzene contained in petrol, vehicle exhaust, diesel fume and cigarette smoke (Zhang et al., 2002). Epidemiological studies showed a clear relationship between the increase in micronucleus frequency and exposure to benzene and benzene metabolites (Yager et al., 1990; Tompa et al., 1994; Turkel and Egeli, 1994).

In the present study, individuals working in petrol stations may have been exposed either by nasal or oral inhalation of the volatile organic compounds emanating during the vehicle refueling. Increase in the micronucleus frequency in exfoliated buccal cells of petrol station workers as observed in the present study may be due to the presence of benzene in automobile exhaust and tobacco smoke. In this study, 45 subjects (38%) were Smokers and 40 subjects (33%) were alcoholics. Predominantly all the male workers included in this study, were habitual smokers. A higher frequency of Karyolysis was observed in smokers than non- smokers.

We observed a higher degree of karyolysis among male subjects (42.54 ± 0.76) than females probably due to long working hours and continued exposure for men than women. Male workers also work on night shifts and therefore increased exposure. This may also be a significant factor for higher degree of karyolysis in buccal smears of men. Rural workers without formal education reported habitual chewing of tobacco. Smokers (smoking for more than 5 years) have higher degree of nuclear anomalies probably due to their excessive smoking habit (15 cigarettes/day). Alcoholics (including smokers) also revealed higher degree of nuclear anomalies. These factors are to be considered as an implicative parameter for high degree nuclear anomalies in buccal smears of men. In contrast to male workers, females do not work for

late hours or on night shifts. None of the females smoked or chewed tobacco and almost all female workers are non-smokers, because culturally Indian women do not smoke or consume alcohol. This could be a supportive factor for the low frequency of nuclear abnormalities in women than in men. The number of female subjects in this study is less than male subjects as it is customary for men to be engaged more in this occupation. Most of the fe-male workers in petrol stations do not serve for more than three years due to various socioeconomic reasons in India.

The micronuclei in exfoliated epithelial cells are useful biomarkers of occupational exposure to genotoxic chemicals. Cigarette smoking is one of the factor that may influence the rate of cytogenetic damage, such as micronuclei in humans (Celik et al., 2003) reported that cigarette smoking significantly increase the frequencies of micronucleus and other nuclear abnormalities in both controls and exposed subjects. Increase in micronuclei frequency was reported in fire fighters (Ray et al., 2005) gas station workers (Benites et al., 2006) and beedi smokers (Suhas et al., 2004).

Increase in exposure to toxic chemicals such as formaldehyde and benzene induces a significant increase in the buccal cell micronuclei (Titenko-Holland et al., 1996; Surralles et al., 1997). Occupational exposure in copper smelters have some risk of increased chromosomal damage measured as the frequency of micronuclei in peripheral blood lymphocytes and buccal epithelial cells of workers (Lewinska et al., 2007).Our results indicate clearly the petrol station attendants have an increased frequency of cells with micronuclei revealing genotoxic effects.

A higher frequency of buccal cells with micronuclei, binucleate, karyorrhexis and karyolysis was observed in the study subjects, probably due to the genotoxic effect of the petroleum derivatives to which they are exposed. It is necessary to educate the working population about the genotoxic effects of petrol exposure and to ensure safe and healthy working atmosphere for the petrol station workers to alleviate the health hazards that they may encounter. Private and public stake holders need to advocate stringent policies to safe guard the health of the neglected population of petrol station workers.

REFERENCES

Benites CI, Amado LL ,Vianna RAP, Martino-Roth MG (2006). Micronucleus test on gas station attendants. Genet. Mol. Res. 5: 545 - 554.

Bukvic N, Bavaro P , Elia G, Cassano F , Fanelli M Guanti G. (1998) Sister chromatid exchange (SCE) and micronucleus (MN) frequencies in lymphocytes of gasoline station attendants. Mutat. Res. 415: 25 - 3.

Celik A, Cavas T, Ergene-Gozukara S (2003). Cytogenetic biomonitoring in petrol stations attendants: micronucleus test in exfoliated buccal cells. Mutagenesis. 18: 417 - 421.

Hoegsted B, Holmen A , Karlson A , Raihle G , Nillus K, Vestlund K (1991). Gasoline pump mechanics had increased frequencies and sizes of mironuclei in lymphocytes stimulated by pokeweed mitogen.Mutat. Res., 263: 51- 55.

Huvinen M, Maekitie A, Jaerventaus H, Wolff H, Stjernvall T, ovi A, Hirvonen A, Ranta R, Nurminen M, Norppa H (2002). Nasal cell micronuclei, cytology and clinical symptoms in stainless steel production workers exposed to chromium. Mutagenesis, 17: 425 - 429.

IARC 1989. Monographs on the "Evaluation of the Carcinogenic Risks to Humans". 45: Petroleum Fuels. IARC, Lyon, France.

Kayal JJ, Trivedi AH, Dave BJ, Nair J, Nair US , Bhide SV, Goswami UC, Adhvaryu SG (1993). Incidence of micronuclei in oral mucosa of users of tobacco products singly or in various Combinations. Mutagenesis, 5: 31 - 33.

Lewinska D, Palns J, Stepnik M, Dziubaltowska E, Beck J, Rydzynski K, Natarajan AT, Nilsson R.. Micronucleus frequency in peripheral blood lymphocytes and buccal mucosa cells of copper smelter workers, with special regard to arsenic exposure (2007). Arch. Occup. Environ. Health, 80: 371 - 380.

Oesch F, Fuchs J, Vaupel J, Hengstler JG (1995). DNA single strand break analysis in mononuclear blood cells of petrol pump attendants. Int. Arch. Occup. Environ. Health, 67: 35 - 39.

Pastor S, Gutiearrez S, Creus A, Xamena N, Piperakis S, Marcos R (2001). Cytogenetic analysis of Greek farmers using the micronucleus assay in peripheral lymphocytes and buccal cells. Mutagenesis, 16: 539 -545.

Ray MR, Basu C, Mukherjee S, Roychowdhury S, Lahiri T (2005). Micronucleus frequencies and Nucleus anomalies in exfoliated buccal epithelial cells of firefighters. Int. J. Hum. Genet. 5: 45 - 48.

Subrahmanyam VV, Ross D, Eastmond DA, Smith MT (1991). Potential role of free radicals in benzene-induced myelotoxicity and leukaemia. J. Free Radic. Biol. Med. 11:495-515.

Surralles J , Autio K, Nylund L, Jarrventaus H, Norppa H, Veidebaum T, Sorsa M, Peltonen K (1997). Molecular cytogenetic analysis of buccal cells and lymphocytes from benzene - exposed workers. Carcinogenesis. 18: 817-823.

Suhas S, Ganapathy K S, Gayatridevi, Ramesh C (2004). Application of the micronucleus test to exfoliated epithelial cells from the oral cavity of beedi smokers, a high risk group for oral cancer. Mut. Res .561: 15 - 21.

Titenko-Holland N, Moore LE, Smith MT (1994). Measurement and Characterization of micronuclei in exfoliated human cells by fluorenscence in Situ hybridization with a centromeric probe. Mut .Res. 312: 39 - 50.

Tolbert PE, Shy CM, Allen JW (1992). Micronuclei and other nuclear abnormalities in buccal smears: methods and development. Mutat. Res. 271: 69 -77.

Tompa A, Major J, Jakab MG (1994). Monitoring of benzene exposed workers for genotoxic effects of benzene improved working-conditions related decrease in the frequencies of chromosomal aberrations in peripheral blood lymphocytes. Mutat. Res., 304: 164 - 165.

Turkel B, Egeli U (1994).Analysis of chromosomal aberrations in shoe workers exposed long term to benzene Occupant. Environ. Med .51: 50 - 53.

Yager JW, Eastmond DA, Robertson ML, Paradisin WM, Smith MJ (1990). Characterization of micronuclei involved in human lymphocytes by benzene metabolites. Cancer Res., 50: 393- 399.

Zhang L, Eastmond DA, Smith MT (2002). The nature of chromosomal aberrations detected in humans exposed to benzene. CRC Crit. Rev. Toxicol. 32: 1 - 42.