Industrial Health 2010, **48**, 852–856 **Original Article**

# Evaluation of Genotoxicity in Petrol Station Workers in South India Using Micronucleus Assay

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*Received December 15, 2008 and accepted February 25, 2010*

**Abstract: In this study, the micronucleus (MN) frequency was assessed as a measure of genotoxicity in exfoliated cells of buccal mucosa extracted from 110 petrol pump workers and 100 controls. For each individual, 3,000 exfoliated buccal cells were analyzed. The individuals used in the study were grouped based on their smoking, drinking alcoholic beverages, and tobacco chewing habits. There was a significantly higher frequency of micronucleated cells in the exposed workers to petrol than in the unexposed control population. Smoking and drinking (alcohol) habits, age and length of occupation represent significant factors in terms of increasing the MN frequency measured in the exposed population. This study demonstrates that, using MN assay, it is possible to assess the cytogenetic damage in exposed individuals and that the significant increase in the induction of the MN in the exposed population suggests that the studied individuals may be at a higher risk of developing cancer and therefore monitored for any long term adverse effects of the exposure.**

**Key words:** Micronucleus, Occupational exposure, Petrol pump workers, Exfoliated cells, Genotoxicity

## Introduction

Petrol chemical is a complex combination of hydrocarbons, about 95% of compounds in petrol vapors are aliphatic and alicylic compounds and less than 2% aromatics[1)]. It is well known that some of them are genotoxic, mutagenic and carcinogenic agents. In 1989, the International Agency for Research on Cancer (IARC) considered all refinery environments are potentially carcinogenic for humans[2)]. Epidemiological studies in workers exposed to oil vapors and derivatives in oil refinery and petrochemical industries as people living in the neighborhood exposed to this revealed a rise in the number of diseases[3–6)]. The volatile nature of petrol makes it readily available in the atmosphere at any time it is dispensed, especially at petrol filling stations. Although people are exposed to gasoline fumes during fuelling and refueling at gas stations, the gas station attendants are at higher risk of exposure by virtue of their occupation[7)]. Also, the atmospheric concentration of gasoline vapor is not safe when inhaled even for a brief period of time and during fuelling of vehicles; the concentration of gasoline vapor in the air is recorded to reach between 20 and 200 ppm[7, 8)]. This concentration can get higher when there is a long line of cars to be fuelled, which is a usual occurrence during fuel scarcity.

Micronucleus (MN) assay for exfoliated cells in epithelial cells have been used to evaluate the genotoxic effects produced by low doses of carcinogenic substances or carcinogenic mixtures, to which human populations are exposed[9, 10)]. The frequency of MN in human exfoliated cells can be used as an "endogenous dosimeter" in tissues that are specific targets of genotoxic and carcinogenic agents, when carcinomas will develop[11)].

Petrol evaporates more readily in tropical than in temperate countries[12)]. In South India petrol stations are located on streets and workers at the station, have a higher opportunity for exposure. Petrol bunk workers are engaged in petrol filling for eight hours a day and do not wear personal protective equipment and personal hygiene is variable in work place, and therefore the

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occupational exposure to such derivatives may posse's genotoxic risk. The present work aim to explore the cytogenetic damage in exfoliated buccal cells obtained from petrol station attendants and control subjects, using micronucleus (MN) assay.

## Materials and Methods

*Subjects*

The study population composed of 110 male petrol pump workers and 100 unexposed controls. The exposed group included 30 smokers and 30 non-smokers, 31 smokers with alcohol drinking and 19 smokers with tobacco chewing from 21 petrol stations located in the urban area of Coimbatore City, South India. The respective control groups were matched for age and sex (30 smokers, 30 non-smokers, 26 smokers with alcohol drinking, and 14 smokers with tobacco chewing) and had no occupational exposition to toxic agents. At the time of sample collection the subjects signed a term of informed consent. All subjects were selected based on questionnaire which included items about age, occupational exposure, smoking habit, use of drugs, such as alcohol, virus illnesses, recent vaccinations, and radiological exams. All the individuals who agreed to participate in the study were healthy, and they answered a detailed questionnaire according to the protocol published by the International Commission for Protection Against Environmental Mutagens and Carcinogens[13]. For the exposed group, a further questionnaire was completed to evaluate the use of protective measure. None of these study groups showed significant differences with regard to lifestyle and personal factors. The study procedures used in the present study were approved by the Institutional ethical committee.

*Cell sampling*

Before sampling, each subject rinsed the mouth thoroughly with tap water. Exfoliated buccal cells were obtained by gently rubbing the inside of both cheeks with a sterile extra soft toothbrush for 1 min each. The participant then rinsed the mouth with 20 ml of 0.9% saline and expectorated into a 50-ml conical-based tube. The toothbrush was then rinsed in the tube and 30 ml saline was added before the cells were pelleted and washed once with Phosphate buffered saline (pH 7.4). Cell suspension was dropped onto clean slides and cell density was checked using a microscope. The slides were allowed to dry and then fixed in 80% ice cold methanol[14].

*Micronucleus analysis*

Ten micro liters of buccal mucosal cell suspension was smeared on a microscopic slide, smears were air dried and fixed in methanol: acetic acid (3:1)[15] and the slides were stained by May- Grunwald Giemsa (Sigma, St louis, MO)[16]. The MN analysis was done with a light microscope, at 100 × magnification, using coded slides. 3,000 cells from each individual were examined. Only unfragmented cells that were not smeared, clumped or overlapped and that contained intact nuclei were included in the analysis. Cells undergoing degenerative processes, such as karyorrhexis, karyolysis and fragmentation of nucleus, broken egg, or pycnosis were recorded separately[17, 18]. Micronuclei had to: a) be less than 1/3 in diameter of the main nucleus, b) be on the same plane of focus, c) have the same color, texture and refraction as the main nucleus, d) have a smooth oval or round shape, and e) clearly separated from the main nucleus. Questionable micronuclei were disregarded.

*Statistical analysis*

The samples were coded at the time of preparation and scoring. They were decoded before statistical analysis for comparison. The significance of the differences between control and exposed group means were analyzed using Student's *t*-test, whereas Pearson's rank correlation analyses were performed to assess the association between end-points and the independent variables. The MNC, BNC and BEC distributions of individuals, grouped by each of two-class factors, were compared with the Mann-Whitney test. All the calculations were performed using SPSS 11.01 statistical software (SPSS Inc., Chicago, IL).

## Results

The characteristics of the subjects used in the study are shown in Table 1. The individuals were classified according to their age, length of occupation, smoking, and tobacco chewing, and alcohol drinking habits.

Results on micronuclei frequency and nuclear abnormalities are given in Table 2. Assessment of MN frequencies in exfoliated buccal cells revealed a significant difference ($p<0.001$) between exposed workers with smoking ($12.61 \pm 0.39$) and controls with smoking habit ($2.79 \pm 0.16$). Smoking also had a marked effect on MN frequency among unexposed control group (2.79 vs. 1.63 between smokers and non-smokers, respectively). The average MN frequency in the exposed non-smokers was $11.17 \pm 1.06$, and in the control non-smokers was $1.63 \pm 0.05$ ($p<0.001$). The average micronucleus frequencies were 13.33 and $3.73 \pm 0.13$ between exposed smokers with tobacco chewing and unexposed smokers with tobacco chewing, respectively.

**Table 1. General characteristics of groups studied**

| Study group | | n | Age (yr) M ± SD | Average no. of Cigarettes/day | Average alcohol intake in last 1 yr (g alcohol drinking/day) | Duration of work Exposure (yr) |
|---|---|---|---|---|---|---|
| Controls n=100 | Smokers | 30 | 31.90 ± 2.12 | 23 | 84.03 ± 32.05 | |
| | Non-smokers | 30 | 34.76 ± 1.99 | | | |
| | Smoking with drinking | 26 | 30.72 ± 2.73 | 20 | | |
| | Smoking with Tobacco chewing | 14 | 36.80 ± 3.02 | 18 | | |
| Workers n=110 | Smokers | 30 | 29.47 ± 1.73 | 22 | 82.8 ± 35.53 | 6.49 ± 1.75 |
| | Non-smokers | 30 | 30.49 ± 1.20 | | | 5.83 ± 0.92 |
| | Smoking with drinking | 31 | 31.39 ± 2.04 | 16 | | 5.92 ± 0.89 |
| | Smoking with Tobacco chewing | 19 | 30.57 ± 2.26 | 16 | | 5.64 ± 0.50 |

M ± SD=Mean ± Standard Deviation.

**Table 2. The frequencies of micronuclei and other nuclear abnormalities in exfoliated buccal cells of controls and exposed subjects**

| Study group | | n | MN (M ± SD) | BN (M ± SD) | BEC (M ± SD) |
|---|---|---|---|---|---|
| Controls n=100 | Smokers | 30 | 2.79 ± 0.16 | 6.58 ± 3.45 | 6.56 ± 2.49 |
| | Non-smokers | 30 | 1.63 ± 0.05 | 4.82 ± 3.55 | 4.11 ± 1.06 |
| | Smoking with drinking | 26 | 3.64 ± 0.02 | 6.17 ± 3.23 | 5.13 ± 1.15 |
| | Smoking with Tobacco chewing | 14 | 3.73 ± 0.13 | 6.54 ± 2.59 | 5.93 ± 1.85 |
| Workers n=110 | Smokers | 30 | 12.61 ± 0.39* | 13.57 ± 3.97** | 18.97 ± 5.99* |
| | Non-smokers | 30 | 11.17 ± 1.06* | 8.68 ± 2.08** | 17.51 ± 8.36* |
| | Smoking with drinking | 31 | 13.94 ± 0.09* | 12.13 ± 4.43** | 18.26 ± 7.58* |
| | Smoking with Tobacco chewing | 19 | 13.33 ± 1.27* | 12.48 ± 2.20** | 18.90 ± 8.36* |

MN=cells with micronuclei; BN=binucleated cells; BEC= broken egg cells.
*Significantly different, $p<0.001$; **significantly different, $p<0.01$(Mann-Whitney test).

The average MN frequencies in the exposed smokers with drinking were 13.94 ± 0.09, and the unexposed control smokers with drinking were 3.64 ± 0.02, respectively. These results show that individuals with smoking, drinking, tobacco chewing and exposure to petrol have significantly higher frequencies of MN induction, indicative of cytogenetic damage in these individuals.

## Discussion

Petrol station attendants are workers chronically exposed to petroleum derivatives primarily through inhalation of the volatile fraction of petrol during vehicle refueling[13]. Epidemiological studies of filling station attendees have shown genotoxic effects at very low benzene from gasoline vapor exposure[12, 19–21]. Brandt *et al.*[22] have demonstrated genotoxic effects in workers exposed to low levels of benzene from gasoline. Santos-Mello *et al.*[12] have shown chromosomal deletions in lymphocytes of workers exposed to gasoline as attendants and also reported hematopoietic malignancy in petrol exposed workers. Similar exposures to gasoline garage mechanics and filling stations have been reported[23].

Analysis of exfoliated cells of buccal mucosa also provides evidence of other nuclear abnormalities such as binucleates (presents of two nuclei in a cell), karyorrhexis (nuclear fragmentation) and karyolysis (nuclear dissolution)[23]. Revazova *et al.*[24] reported an increase in nuclear abnormalities in buccal cells of women living in a dioxin-contaminated area. Increased frequency of nuclear abnormalities in buccal cells of smoke less tobacco users were also shown by Livingston *et al*[25].

Whereas Hogstedt *et al.*[20] detected a significant increase in the frequency of micronuclei in pokeweed mitogen-stimulated lymphocytes from gas pumps mechanics, and Santos-Mello[12] found a significant increase in chromosome deletions. The study carried out in Brazilian pump attendants showed a significant increase in the frequency of MNC, BNC and BEC among these workers. Similarly higher frequency of structural chromosome aberrations in individuals exposed to petroleum vapors were reported by[20, 26, 27] also revealed a significant increase in chromosome aberrations in petrochemical workers. Huisingh[28] reported the emissions resulting from the internal combustion

of automobile engines consist of thousands of different compounds. And the cytotoxic and genotoxic properties, of these emission products were demonstrated by Hadnagy[29]. According to Dittberner *et al*[30], alcohol use can increase the number of micronuclei. Sarto *et al*.[31] and Piyathilake *et al*.[32] has reported smoking also increase the MN frequency in buccal cells.

In our study, smoking, tobacco chewing, and alcohol drinking is associated with a significant induction of MN among petrol station attendants. Our results on control population also demonstrate that cigarette smoking with alcohol drinking increases the number of micronucleus in the buccal cells of control population compared to unexposed non-smoking and alcohol drinking group.

In conclusion results make it clear that the petrol station workers had a significant increase in cytogenetic damage as tested by MN assay. The micronucleus assay in human exfoliated buccal cells is one of the effective methods used for detecting cytogenetic abnormalities in human populations. It may be concluded that these workers may be at higher risk of developing cancer at later time and therefore should be carefully monitored for the long term effect of the exposure.

## Acknowledgements

The authors gratefully acknowledge the interest and support of thank Dr. K. Sasikala, Professor and Head, Department of Zoology, Bharathiar University, Coimbatore for her valuable technical help, research supported by the Karpagam University, Coimbatore and the subjects who volunteered to take part in this study.

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