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Original Research Article

Micronuclear Assay in Petrol Pump Workers: A Prospective Observational Study

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Abstract: The presence of micronuclei in exfoliated buccal epithelial cells has proven a useful biomarker of occupational exposure to petroleum products. Genetic damage is the most important basic cause of developmental and degenerative disease. It is also well established that genomic damage is produced by environmental exposure to genotoxins, medical procedures (eg, radiation and chemicals), micronutrient deficiency (eg, folate), lifestyle factors (eg, alcohol, smoking, drugs, and stress), and genetic factors, such as inherited defects in DNA metabolism and/or repair. To investigate the cytogenetic damage in exfoliated buccal cells obtained from petrol pump attendants and control subjects, using the micronucleus test. The study sample consisted of 100 subjects (males) in the age group 21-60 years (Exposed group). The control group consisted of 100 individuals without clinically observed lesions and without any tobacco habits (Unexposed group). The petrol pump attendants (Exposed) group consisted of 50 subjects with tobacco habits such as tobacco chewing only, smoking only and both tobacco chewing and smoking(Exposed Group I); other 50 exposed subjects included were petrol pump workers without the above-said habits (Exposed Group II). The smear was obtained from the mucosa of the oral cavity by using the sterile cytopathology brush. Slides fixed in ethanol fixative and stained using Giemsa and PAP stain and 1000 cells were studied by using a light microscope. In this study, it was found that an average number of micronuclei (MN) were directly proportional to the years of exposure, 08.77 in subjects with 14 years of exposure and least in less exposed subjects (3-5 years). The average number of micronuclei (MN) in subjects who worked for more than 12 hours a day was high (08.23). A higher frequency of micronuclei was observed in petrol pump workers when exposed for a longer duration and much higher MN were seen in workers with tobacco use. It is necessary to educate the petrol pump workers about the hazardous and genotoxic effects so as to ensure the safety and healthy working atmosphere.

Keywords: Micronuclei, Petrol Pump Workers, Smoking, Tobacco, Oral Cancer, Squamous Cells.

INTRODUCTION

Petrol pump attendants are chronically exposed to petroleum derivatives primarily through inhalation of the volatile fraction of petrol during refueling [1]. According to the international agency for research on cancer exposure to gasoline vapors is a possible carcinogenic nature of components especially benzene [2]. Benzene is the best-known hydrocarbon whose exposure in higher concentrations even for the shortest duration can lead to a death of the living beings

including humans. Exposure to lower levels leads to drowsiness, dizziness, and headache. Continuous and long-term exposures of benzene cause anemia, immune system dysfunction, affects the reproductive system and can lead to genetic aberrations [3, 4].The physiology of DNA repair genes, cell proliferation, and differentiation genes are lost as a side effect of these mutations and the risk of cancer increases. Micronuclei (MN) are cytoplasmic chromatin masses with the appearance as small nuclei that arise from chromosome fragments or intact whole chromosomes lagging behind in the anaphase stage of cell division. Their presence in cells is a reflection of structural and/or numerical chromosomal aberrations arising during mitosis. These micronuclei are considered as markers for high-risk cancers.

AIM OF THE STUDY

To investigate the cytogenetic damage in exfoliated buccal cells obtained from petrol pump attendants and control subjects, using the micronucleus test.

MATERIALS AND METHODS

The study sample consisted of 100 subjects (males) in the age group 21-60 years (Exposed group). The control group consisted of 100 individuals without clinically observed lesions and without any tobacco habits (Unexposed group). The petrol pump attendants (Exposed) group consisted of 50 subjects with tobacco habits such as tobacco chewing only, smoking only and both tobacco chewing and smoking(Exposed Group I); other 50 exposed subjects included were petrol pump workers without the above-said habits (Exposed Group II). These 100 subjects were selected from different petrol pumps in Lingampally, Hyderabad working for more than 7 years with working shift not less than 12 hours. Smoking habit and tobacco usage by the groups was evaluated by using a questionnaire.

The oral smear was obtained from normal mucosa by using the sterile cytopathology brush. The oral sites included buccal mucosa, hard palate, gingiva and floor of the mouth. The smears were transferred and spread with a sterile glass slide onto the labeled, clean, dry glass slide. For Pap staining method, the slides were fixed at once by 95% ethanol for 20 minutes, whereas, they were air dried for Giemsa stain method. Two slides were made from each patient. One slide was stained with Papanicolaou stain and the other by Giemsa stain.

The buccal smear was stained with PAP and evaluated for MN in about 1000 cells. Scoring criteria for MN according to Tolbert *et al*. [24] were followed in this study [5,6].

Inclusion Criteria

Petrol pump workers working since 3 years or more.

Exclusion Criteria

- Petrol pumps workers working less than 3 years.
- Workers with alcohol and tobacco habits.
- Subjects with systemic disease, Endocrine disease and known immunological diseases for the past one year
- Subjects with previous employment in amalgam fillings, firefighters, shoe workers and cement factory workers were excluded.

Parameters for Scoring used in this study

- Monotonous cell position on the slides with flawless cytoplasm
- Little or no overlapping with adjacent cells.
- Little or no debris.
- Nucleus customary and undamaged, nuclear perimeter smooth and distinct.

Recommended standard for identifying micronuclei were according to the scoring criteria given by Tolbert *et al*. [24].

- < $1/3^{rd}$ of the diameter of the corresponding nucleus, but large enough to recognize shape and color.
- Staining strength homogenous to that of the nucleus.
- Texture homogenous to that of the nucleus.
- The Same focal plane as the nucleus.
- The absence of overlap with a bridge to the nucleus.

All the sample slides were viewed by three observers so as to reduce the bias and error [5,6].

RESULTS

In this study, it was found that most of the cases were in the age group of 21 to 30 years and with the exposure of 3 to 5 years (Table 1). It was found that more subjects were working overtime and increasing their exposure to the petroleum products.

In this study, it was found that an average number of micronuclei (MN) was directly proportional to the years of exposure, 08.77 in subjects with 14 years of exposure and least in less exposed subjects (3-5 years). However, in > 15 years of exposure, the average micronuclei (MN) should be higher but the numbers of subjects were less.

The average number of micronuclei (MN) in subjects working for more than 12 hours per day was high (08.23) (Table 2).

When a comparison was done between exposed subjects with habits of smoking, tobacco chewing & both the habits and with the exposed subjects without these habits, it was found that highest average micronuclei were seen in subjects with habits of both smoking and tobacco chewing than with tobacco chewing and smoking alone. The overall incidence of average micronuclei in the subjects without tobacco habits was lesser when compared to the tobacco using group (Table 3).

Table-1: Basis Charecteristics of Exposed and Unexposed Subjects

AGE IN YEARS	N=100 (Exposed)	N=100 (Unexposed)	Exposed (%)	Unexposed (%)
21-30	52	52	52	52
31-40	23	23	23	23
41-50	18	18	18	18
51-60	07	07	07	07
DURATION OF WORK IN YEARS	N=100 (Exposed)		Exposed (%)	
3-5 YEARS	48		48	
6-8 YEARS	17		17	
9-11 YEARS	13		13	
12-14 YEARS	09		09	
>15 YEARS	13		13	
WORKING HOURS	N=100 (Exposed)	N=100 (Unexposed)	Exposed (%)	Unexposed (%)
UPTO 6 HOURS	28	28	28	28
6-12 HOURS	59	59	59	59
> 12 HOURS	13	13	13	13
TOBACCO HABITS (N=50)	N=50 (Exposed)		Exposed (%)	
SMOKING	13		26	
TOBACCO CHEWING	29		58	
SMOKING AND CHEWING	08		16	

Table-2: AVERAGE NUMBER OF MICRONUCLEI (MN) PER 1000 CELLS IN VARIOUS SUBJECTS

DURATION OF WORK IN YEARS (n=100)	Exposed	Unexposed
3-5 YEARS (n=48)	05.12	NIL
6-8 YEARS (n=17)	06.41	NIL
9-11 YEARS (n=13)	07.92	NIL
12-14 YEARS (n=09)	08.77	NIL
>15 YEARS (n=13)	08.98	NIL
WORKING HOURS (n=100)	Exposed	Unexposed
UPTO 6 HOURS(n=28)	06.14	NIL
6-12 HOURS (n=59)	07.79	NIL
> 12 HOURS (n=13)	08.23	NIL

Table-3: AVERAGE NUMBER OF MICRONUCLEI (MN) PER 1000 CELLS IN TOBACCO AND NON-TOBACCO WORKERS OF THE PETROL PUMPS

TOBACCO HABITS (N=50)	Average number of MN
SMOKING (n=13)	08.11
TOBACCO CHEWING (n=29)	09.08
SMOKING AND CHEWING (n=08)	12.25
NO TOBACCO HABITS (N=50)	07.12

DISCUSSION

Micronuclei (MN) are cytoplasmic chromatin masses appearing as small nuclei that arise from the chromosomal fragments or intact whole chromosomes which lag behind in anaphase stage of the cell division. The presence of these micronuclei in the cytoplasm of the cells of the oral cavity depicts structural and genetic aberrations [7, 8].

Cytomorphology is the most widely used method of oral exfoliative cytology and assesses parameters which include shape of the cell, size of the cell, area of the cell, diameter of the cell, cytoplasmic area, diameter of the nucleus, area of the nucleus, nuclear-cytoplasmic area ratio, nuclear shape, nuclear membrane continuity, nuclear texture and its abnormalities. These parameters may become different due to various cancer causing agents and lead to chromosomal aberrations which can be studied in these shedding epithelial cells. In the oral epithelium, micronuclei are considered to be important biomarkers for the risk of cancer development. There are various factors responsible for chromosomal damage or genetic mutations like tobacco habits, carcinogens, drugs and pollutants [9]. Oral exfoliative cytology forms an early diagnostic test is highly beneficial to check the oral health of the individual and the progress of the lesion from precancerous stage to established cancer [9, 10].

Occurrences of chromosomal damage and their association with cancer have been evaluated using the micronuclei assay in both lymphocytes and exfoliated epithelial cells. Cancers are mainly caused due to tobacco habit, synthetic chemicals and natural chemicals of occupation or pollutants present in the environment. Most of the chemical carcinogens cause structural alterations in DNA which lead to genomic instability or genetic damage in the form of chromosomal abnormalities like micronuclei and bi-nucleation [9, 11].

Individuals working in petrol stations are continuously exposed to volatile organic compounds emitted during the vehicle refueling by nasal and oral inhalation. A significant increase in the micronuclei frequency in exfoliated buccal cells of petrol pump attendants was observed in the present study. This may be due to the presence of benzene in automobile exhaust and fumes of the petroleum products. There was an increased frequency of micronuclei in petrol pump workers with habits of tobacco use- smoking and chewing.

This study is in correlation with the recent study done by Shilpi *et al*. in 2016, where the study population comprised of 90 male petrol pump workers (exposed group) and 30 unexposed controls (healthy individuals). The oral smear was obtained from normal mucosa by using the sterile cytopathological brush. In that study, more than one-third (45.6%) of the cases were between 20-30 years of age. In 37.8% of the cases, the duration of work year was 5-10 years. The habit of smoking was among 24.4% of the cases while chewing was in 42.2% of the cases. The MN was significantly ($p=0.0001$) higher among the cases compared with controls [8].

In the study done by Divya uppala *et al*. [9], which involved 60 subjects (20 petrol pump attendants, 20-squamous cell carcinoma patients and 20 healthy subjects) and they were asked questions regarding their lifestyle and personal factors (age, duration of working in the petrol pump, alcohol consumption and smoking habits) were statistically analyzed. Buccal smears were taken from respective sites and stained. There were a significant number of nuclear abnormalities seen in oral carcinoma group and then followed by the petrol pump workers which is in correlation with the study.

Konopacka *et al*. [12] have reported that the micronuclei frequency was 1.50% ± 0.47% in smokers and 0.55% ± 0.32% for nonsmokers. Ozkul *et al*. [13] also have reported the mean micronuclei frequency in smokers was 1.99 ± 0.3.These findings are in agreement with the present study.

Our study showed significantly higher genotoxic or chromosomal damage in the form of the emergence of MN in gasoline workers, supported by Evans [14,15].

In the last 15-20 years, the MN assay has been applied to evaluate chromosomal damage for biologic monitoring of human populations exposed to a variety of mutagenic and carcinogenic chemical or physical agents, such as anti-cancer medications , arsenic in drinking water, dioxin used in fertilizers, ethylene oxide and formaldehyde used for hospital fumigation, lead oxide, solvents, benzene, ozone, polycyclic aromatic hydrocarbons, chlorates, toxic gases, pesticide mixtures, toluene, hexane, acetone, methyl-ethyl-ketone, 2-trans-hexol, and all forms of tobacco [16].

A wide range of baseline micronuclei frequencies has been reported (0.05-11.5 MN/1000 cells) with larger part of values between 0.5 and 2.5 MN/1000 cells. Large number of studies reported a statistically remarkable elevation of MN levels in exposed individuals compared with control groups, although the observed effects are relatively small [16].

Many studies report the age and sex of the study subjects, but only a portion of these studies were

able to ascertain a statistically significant effect by gender [17] or by age [18]. Smoking increases MN frequency by 2 to 3 folds compared with nonsmokers. However, some publications report no difference between smokers and nonsmokers and between men and women [19].

Lifestyle factors include smoking, alcohol consumption, and diet, especially vitamin deficiencies and supplementation. The majority of the studies with a significant increase in MN are related to a risk of oral cancer and were performed in subgroups of subjects with specific lifestyle habits, that is, chewers of betel quids (areca nut, betel leaves, slaked lime, and tobacco), reverse smokers, snuff dippers, Khaini tobacco (tobacco mixed with slaked lime), and other similar practices [20]. Results in these studies showed overvalue of the MN frequency because both smoking and chewing of tobacco mixtures are known to cause nuclear degeneration and appearance of MN-like bodies in exfoliated cells, were confused with MN. Hence, it is important to distinguish cell death events from genome damage in viable epithelial cells both in terms of biologic dosimetry and for evaluating cancer risk.Micronutrients and other vitamins have been shown to significantly decrease MN levels (1.4- to 4-fold) in healthy tobacco users, as well as in individuals with precancerous lesions [16, 21].

Some micronutrients, such as retinol, riboflavin, zinc, and selenium, however, failed to reduce the MN frequency in a study carried out in China in areas with a high incidence of esophageal cancer. One study showed a decline in MN frequencies in children and women who received controlled folate supplementation [22] and in patients with diabetes [23].

Ionizing radiation plays an important role in the treatment of many neoplasms, but it also produces genetic damage. Several studies evaluated micronuclei in buccal cells of patients undergoing radiotherapy in the head and neck region. The most striking increase in cytogenetic damage (150-300 MN/1000 cells) was observed in an early study of three patients exposed to a cumulative dose of 3400-4000 cGy. Some authors reported 68 MN/1000 cells after 2000 cGy and 16 MN/1000 cells after treatment with 1000 cGy for 3 weeks [24].

Moore *et al*. in their study showed more than 16-fold increase in MN frequency shortly after the initiation of radiotherapy, followed by the return to baseline 12 weeks later and 3 weeks after cessation of the treatment. In a more recent study, an increase in MN frequency in a group of head-and-neck cancer patients undergoing radiotherapy was evident both in buccal cells and peripheral lymphocytes [25]. Cao et al. found that the cytokines-blocked MN assay in peripheral blood lymphocytes was more sensitive than the buccal MN assay [26]. It was reported that radiation-sensitive oral tumors showed higher MN levels in exfoliated cells after radiation therapy than the more radiation-resistant ones, and the assay can be used as a predictor of tumor radiosensitivity [27]. According to Sisenando HA et al. 2012, the exposure to biomass burning seen in the Brazilian legal Amazon region showed a significant increase in micronuclei formation in the buccal cells, which is due to the exposure of the biochemicals released during the burning for long durations. This is in correlation with the present study where benzene and other petroleum products show increased micronuclei formation in buccal cells which accounts for the genotoxic effects petroleum by-products to which they are exposed [5].

CONCLUSION

A higher frequency of micronuclei was observed in petrol pump workers when exposed for a longer duration and much higher MN were seen in workers with tobacco use. It is necessary to educate the petrol pump workers about the hazardous and genotoxic effects so as to ensure the safety and healthy working atmosphere. Micronuclei increases with increasing age and also duration of exposure to genotoxic agents in the petrol pump by products. Thus, assessment of micronuclei frequency can be used as a biomarker for early detection of cancer. Regular master health check-ups should be conducted for petrol pump workers to reduce the risk.

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