10.5005/jp-journals-10037-1034

ORIGINAL RESEARCH

Evaluation and Comparison of Micronuclei from Intraoral Smears of Petrol Pump Attendants and Squamous Cell Carcinoma Patients

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ABSTRACT

Aim: Evaluation of nuclear anomalies in exfoliated buccal epithelial cells of petrol station attendants and oral squamous cell carcinoma patients.

Materials and methods: Study was carried out on 60 subjects. 20 petrol pump attendants, 20-squamous cell carcinoma patients and 20 healthy subjects.

They were asked questions regarding their life style 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 with Periodic Acid Schiff.

Results: There was a significant number of nuclear abnormalities seen in oral carcinoma group and then followed by the petrol pump workers.

Discussion: Micronuclei are identified with presence of a 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 and 1/16, the diameter of the main nucleus. 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.

Keywords: Micronuclei, Nucleus, Squamous cell carcinoma, Petrol, Benzene.

How to cite this article: Uppala D, Peela P, Majumdar S, Tadakamadla MB, Anand GS. Evaluation and Comparison of Micronuclei from Intraoral Smears of Petrol Pump Attendants and Squamous Cell Carcinoma Patients. Oral Maxillofac Pathol J 2015;6(1):550-555.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Oral malignancy is one of the tenth most common malignancies in the world and according to WHO oral cancer ranks fifth in males, sixth in females and seventh in both genders. The malignancy of the oral cavity constitutes the most important group of malignancies in the South and South East Asia. Its risk increases with the use of tobacco products (smoke form or smokeless form), and shows an augmented risk if associated with habit of alcohol consumption. There are various etiological factors that induce carcinomatous changes, carcinogens are the major cause for oral cancer.[1]

There are many people working in various occupations that have the potential to be exposed to bio hazards or carcinogens or carcinogen inducing substances. These substances may be dust particles, fibers, chemicals in the form of organic or inorganic, raw materials, byproducts or sometimes even the end products like petroleum fumes (hydrocarbons and benzene, etc.). Some of them can be present in the environment in the form of gases, vapors, mist or small dust particles and even fumes. Inhalation of these substances is the primary route of entry of substances or primary route of exposure. The workers in such environments are at a higher risk of cancer development over a long period of time.[2]

Gas station workers or petrol pump workers are more liable to exposure and also absorb the fuel fumes and the products emitted by engines (Santos—Mello and Cavalcante 1992). According to international agency for research on cancer (IARC) 1989, exposure to gasoline vapours is a possible carcinogenic nature of components such as benzene. This exposure increases the risk of development of cancers. Various studies have indicated a lower emission of mutagenic substances with alcohol blends and a positive correlation between the aromatic content of gasoline and its mutagenicity (Crebelli et al 1995). Major cause of death in gas station workers was reported due to lung cancers, who were exposed to petroleum products for a long period of time as stated by Grandian and Andersen 1991.[3]

There are various known hydrocarbons produced in petroleum refining and petroleum gas stations, which are also used as solvents. Benzene is the best known hydrocarbon whose exposure in high levels for a short duration may cause death in humans and animals. Lower levels of exposure show drowsiness, dizziness, headache and

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long-term exposures of benzene causes anemia, immune system dysfunction, effect on reproductive system and also shows genetic charges (Subrahmanyam et al 1991).[1,3]

Analysis of chromosomal damage in malignant cells and lesional cells provide a diagnostic and prognostic information. In carcinogenesis there are various steps which involve genetic damage, mutation in critical genes which relate to cell division. The normal functioning of DNA repair genes, cell proliferation and differentiation genes are lost as a consequence of mutations and the risk of cancer development is increased. Thus, evaluation of genetic damage or mutation or genetic instability can be used as a biomarker to predict the nature of the lesion. Micronuclei and cytoplasmic fragments have been reported as markers for high cancer risk as they increase in frequency in response to carcinogens.[4]

Micronuclei forms from the chromosome fragments or whole chromosome, which lag behind in anaphase or metaphase during nuclear division. Micronuclei in the oral epithelial cells represent a preferred target site for early genotoxic events involved by carcinogenic agents. There are various studies demonstrating the correlation of frequency of micronuclei and severity of this genotoxic damage. Few studies have tried to correlate the frequency of micronuclei in oral exfoliated cells and histopathological grading of oral squamous cell carcinoma.[6,13,18]

In the present study, we have made an attempt to assess the frequency of micronuclei from exfoliated buccal cells of petrol pump workers exposed to petroleum products and patient diagnosed with oral squamous cell carcinoma.

MATERIALS AND METHODS

The study was carried out on total 60 subjects divided into 20 petrol station attendants, 20 squamous cell carcinoma patients and 20 control subjects. Group I (Controls): Control group comprised of 20 healthy subjects with clinically normal oral mucosa. Group II: Comprised of 20 petrol pump attendants employed for atleast 5 years, Group III: Comprised of 20 patients diagnosed histopathological as squamous cell carcinoma (Fig. 1). The subjects were subjected to clinical examination and the demographics including age, sex, habits and medical history were obtained in the prepared performa and written consent was obtained.

Preparation of the Buccal Smears

The patients were asked to rinse his/her mouth with water and the buccal cells were scraped using moistened wooden spatula. The cells obtained were transferred on to the pre cleaned coded microscopic slides, air dried and fixed with spray fixative for 15 minutes and then subjected to papanicalou stain (PaP stain). The slides stained with PaP were subjected using olympus B×21i microscope under 40× magnification. The counting of the micronuclei was done using the slide in left to right direction. Care was taken to avoid the recounting of the same cells and overlapping cells.

Inclusion Criteria

Petrol pump workers working 5 years or more.

Exclusion Criteria

a. Petrol pump workers working less than 5 years.
b. Subjects with alcohol and tobacco habit (smokeless or smoke form).
c. Subjects with systemic diseases, endocrine and known immunological diseases since past 1 year.
d. Subjects exposed to radiation with in the since past 1 year.
e. Subjects with other occupation (fire fighters, shoe workers, cement factory workers, etc.).
f. Subjects with amalgam filling.

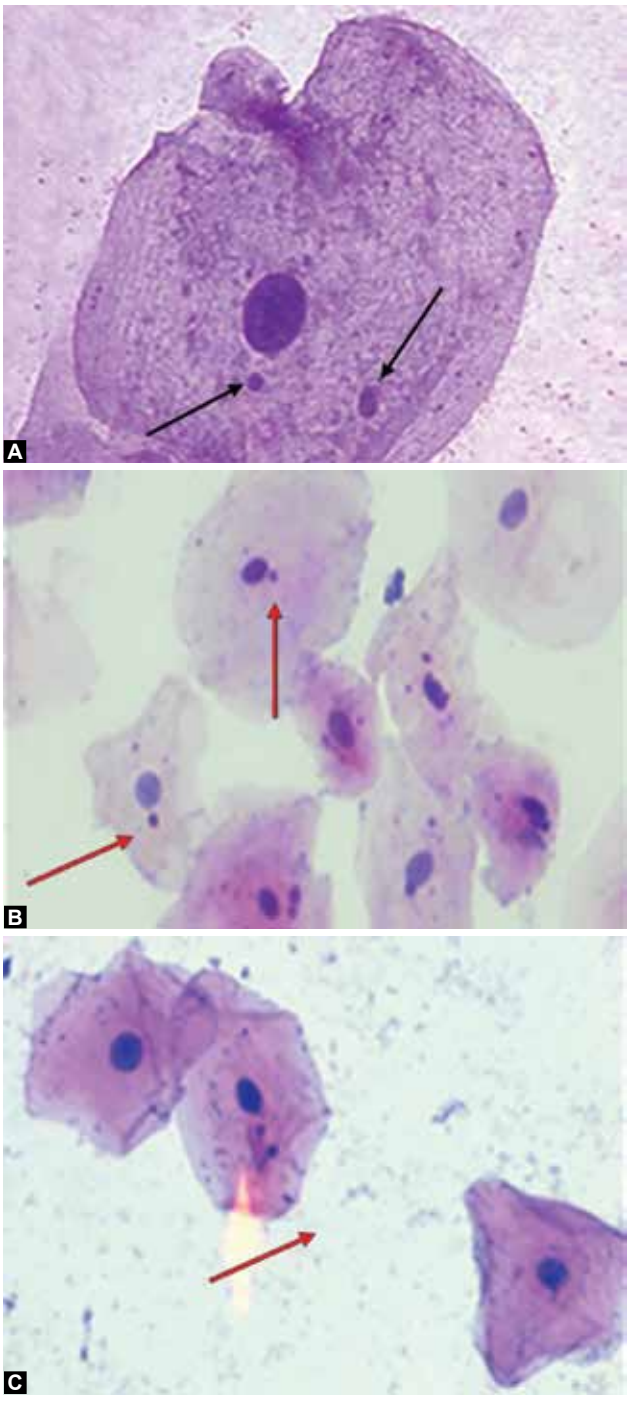
Parameters for Cell inclusion in the Cells to be Scored

a. Intact cytoplasm and relatively flat cell position on the slides.
b. Little or no overlap with adjacent cells.
c. Little or no debris.
d. Nucleus normal and intact, nuclear perimeter smooth and distinct.

Suggested criteria for identifying micronuclei are:
The micronuclei were scored according to the scoring criteria given by Tolbert et al 1992.

a. Rounded smooth perimeter suggestive of a membrane.
b. Less than a third the diameter of the associated nucleus, but large enough to discern shape and color.

Fig. 1: Oral ulcer on the right lateral border of the tongue (histopathologically diagnosed as well differentiated squamous cell carcinoma)



Figs 2A to C: Micronuclei adjacent to the nucleus in the exfoliated buccal cells

c. Staining intensity similar to that of the nucleus.
d. Texture similar to that of the nucleus.
e. Same focal plane as nucleus.
f. Absence of overlap with bridge to the nucleus.

All the sample slides were viewed by three observers so as to reduce the bias and error.[10-12]

Micronuclei

Micronuclei are small bodies that can be seen budding off from a newly divided daughter cell due to DNA damage. Micronuclei can contain a whole chromosome or part of a chromatid. The damage can be caused due to radiation, harmful chemicals and random mutations and also may be due to 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. Micronuclei are similar to the nucleus with a smaller size with 1/3rd to 1/16th of the normal nucleus, and seen or present adjacent to the nucleus (Figs 2A to C).

Five hundred cells were scored per subject to determine the frequency of micronuclei and the data were statistically analyzed as the mean ± standard deviation. 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 to 3 show the average age, male and female subjects in controls, cancer patients and petrol pump attendants, mean and standard deviation and mean difference respectively. The age range of the subjects was 25 to 50 years. The average age in controls, cancer patients and petrol pump attendants was 38.6, 48.4 and 36.2 years respectively. A total of 34 males and 26 females were in the study. The cytological observation in the subjects reveals numerous micronuclei, the average micronuclei in cancer patients was 5.85 with a standard deviation of 1.31, in petrol pump attendants the mean micronuclei was 3.45 with a standard deviation of 1.28 and the mean micronuclei in controls was 0.85 with a standard deviation of 0.75. When all the three groups were compared there was a statistical significance observed.

There was an increase in the micronuclei in cancer patients which is followed by petrol pump workers and controls. The increase in the micronuclei was more in female subjects when compared to male subjects in all the three groups. The mean difference of micronuclei in controls and petrol pump attendant is 2.40 and a statistical significance was observed, mean difference in cancer patients and control was observed to be 5.00 and statistical significance was observed and petrol pump attendants with controls showed a mean difference to be 2.60.

DISCUSSION

Cytomorphology is the most widely used method of oral exfoliative cytology and assesses parameters which include cell shape, cell size, cell area, cellular diameter, cytoplasmic area, nuclear diameter, nuclear area, nuclear

Table 1: Gender and age distribution of the subjects

	Control	*Cancer patients*	*Petrol pump attendants*
Average age	38.6	48.4	36.2
Males	10	09	15
Females	10	11	05

Table 2: Frequency of micronuclei

	Group						*p-value*
	Cancer		*Petrol pump*		*Control*		
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	
Micronuclei	5.85	1.31	3.45	1.28	0.85	0.75	<0.001 (S)

S: Significant

Table 3: Comparison between groups

Groups	*Mean difference*	*p-value*
Cancer *vs* petrol pump	2.40	<0.001 (S)
Cancer *vs* control	5.00	<0.001 (S)
Petrol pump *vs* control	2.60	<0.001 (S)

S: Significant

cytoplasmic area ratio, nuclear shape, nuclear membrane continuity, nuclear texture and its abnormalities. These parameters may change due to carcinogens and genetic damage. Chromosomal aberrations can be studied in exfoliated epithelial cells. In the oral epithelium micronuclei are considered to be important biomarkers for the risk of cancer development. Oral exfoliative cytology can reveal various cellular alterations including micronuclei, pyknosis, binucleation, karyorrhexis, karyolysis, broken egg nucleus and anucleation, etc. There are various factors responsible for chromosomal damage or genetic mutations like tobacco habits, carcinogens, drugs and pollutants, etc.[7,8]

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, binucleation, etc.[9]

Micronuclei are seen in both physiological and pathological conditions it could be formed from two basic phenomenon in mitotic cells, micronuclei are formed from eccentric chromosome, chromatid fragment whole chromosome or chromatids that are lag behind in anaphase of mitosis and are left outside the daughter nuclei in telophase. An early diagnostic test is highly beneficial to check the oral health of the individual and the progress of the lesion from precancer to cancer which can be followed by its early treatment.[14-16]

The international collaboration project on micronuclei frequency in human population (HUMN) organized and collected data on micronuclei frequency in different human populations and in various cell types to determine the extent to which micronuclei is a valid biomarker for detection of risk for diseases such as cancer and also for ageing. Hypothesis of a direct association between micronuclei frequency in target tissues or surrogate tissues and cancer development is supported by the findings like increase in the frequency of micronuclei in target tissues and also in peripheral lymphocytes in cancer patients.

In petrol station attendants there are various by products which induce micronuclei formation. Benzene and hydrocarbons are one of the main cause of genetic mutations or chromosomal damage and poses a risk of genotoxicity. Benzene is a genotoxic agent most commonly seen as a petrol component which is distributed in the environment as a contaminant. 98% of Benzene in the environment is derived from petrochemical and petroleum refining industries. A prolonged exposure to benzene in humans generally noticed in factories, refineries petrol stations, poses a risk of cancer development.

Individuals working in petrol stations are constantly exposed by nasal inhalation or oral inhalation of volatile organic compounds emitted during the vehicle refuelling. 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 increase frequency of micronuclei in oral squamous cell carcinoma patients compared to attendants working in petrol pumps. We observed a high frequency of micronuclei (117) in oral squamous cell patients with a mean of 5.85 ± 1.31. The increasing frequency of micronuclei in oral squamous cell carcinoma patients is due to chromosomal damage or genetic mutations. There was a marked increase of micronuclei in males when compared to females in oral squamous cells carcinoma patients this is attributed to the fact that male workers work for long shifts therefore increasing the period of exposure to genotoxic agents.

Micronuclei frequency in petrol pump attendants showed an increased micronuclei frequency compared to controls but decreased compared to oral squamous cell carcinoma patients. The frequency of micronuclei observed was higher with a mean of 3.45 ± 1.28 and also because number of females as petrol pumps attendants are less in this study compared to males according to male to female ratio to be 1;1.4, it is due to the fact that men are more engaged in this occupation. This is in accordance with Yager et al 1990, Tompa et al 1994, and Turel and Egeli 1994 showed a relationship between the increase

in micronuclei frequency in petrol pump attendants and exposure to benzene and benzene metabolites.

Micronuclei in exfoliated cells are useful biomarkers for early detection of occupational exposure to genotoxic chemicals, which influences the cytogenetic damage causing micronuclei in humans this was reported by Celik et al 2003. A significant increase of micronuclei in fire fighters was reported by Ray et al 2005 and Benites et al 2006 reported that gas station attendants showed an increase in micronuclei frequency if exposed to petroleum products for long period of time, which is in correlation to the present study.[19-23] Tikenko Holland et al 1996 and Surralles et al 1997 reported an increase in exposure to toxic chemicals such as hydrocarbons, Benzene and formaldehyde, which induces an increased micronuclei for mation in the buccal cells.[17] According to Sisenando HA et al 2012 the exposure to biomass burning seen in the Brazilian legal Amazon region showed an 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 an increased micronuclei formation in buccal cells which accounts for the genotoxic effects petroleum by products to which they are exposed.

CONCLUSION

A higher frequency of micronuclei were observed in cancer patients this may be due to the genotoxic effects of petroleum products to which the workers are exposed for a longer duration. 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 for the petrol station workers so as to alleviate the health hazards that they may encounter. Micronuclei increases with increasing age and also duration of exposure to genotoxic agents in the petrol pump by products. Thus, micronuclei frequency can be used as a biomarker for early detection of cancer.

ACKNOWLEDGMENTS

We would like to sincerely acknowledge Hindustan Petroleum Manager Vishakhapatnam, Andhra Pradesh, for the cooperation. We also would like to acknowledge the Chairman Dr MVVS Murthy, Principal Dr KV Gandhadar Prasad and vice principal Dr Ravi Shankar for their support in completing the study.

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