

Genotoxic Effects of Exposure to Gasoline Fumes on Petrol Pump Workers

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Abstract

Background: Petrol pump workers are occupationally exposed to gasoline and its fumes consisting of several mutagenic chemicals.

Objective: To evaluate the genotoxic effects of exposure to gasoline fumes on petrol pump workers.

Methods: The study groups included 70 petrol pump workers (exposed group) and 70 healthy age-matched individuals with no known exposure (comparison group). Buccal micronucleus cytome assay (BMCyt) was performed to check the genotoxicity caused due to inhalation of gasoline fumes.

Results: The frequencies of micronucleated cells, nuclear bud, condensed chromatin cells, karyorrhectic cells, pyknotic cells, and karyolytic cells were significantly higher in the exposed workers compared to the comparison group.

Conclusion: Exposure to gasoline fumes is associated with increased frequency of cell abnormalities. This may lead to various health consequences including cancer in those occupationally exposed to gasoline fumes.

Keywords: Gasoline; Occupational exposure; Mouth; DNA damage; Micronucleus tests; Biomarkers

Introduction

Gasoline contains more than 150 chemicals including benzene, toluene, xylene, and phenolic compounds such as volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs). Many of the substances in gasoline and its fumes are released into the environment and create health risk to people. These chemicals react with the vital tissue macromolecules regulating the cellular functions leading to long lasting health disorders.¹ International Agency for Research on Cancer (IARC) has suggested

that exposure to gasoline vapors could be carcinogenic to humans, mainly on the basis of the well-established carcinogenicity of some components such as benzene.²

Many workers are prone to get exposed to bio-hazards or carcinogens, including dust particles, fibers, chemicals in the form of organic or inorganic raw materials, by-products or sometimes the end-products like petroleum fumes (hydrocarbons, benzene, etc).³ The association between exposure to such complex mixtures and certain types of leukemia has been shown in epidemiological studies.⁴

The buccal epithelium is composed of

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four strata including the basal cell layer, prickle cell layer, and the intermediate and superficial layers. The oral epithelium maintains itself by a system of continuous cell renewal in which new cells are produced by mitosis in the basal layer and migrate to the surface to replace those that are shed. Therefore, the mucosa is composed of progenitor and maturing cell populations.⁵ About 92% of the cancers originate from the external and internal epithelium.⁶ Buccal cell sample collection is an easy non-invasive method and it is thus convenient for large-scale monitoring studies in occupational and environmental toxic exposures.

Ahmedabad is the fifth most populated metropolitan city of India. It has a dense vehicular population and every second person owns a two wheeler or a vehicle, according to Economic Times in 2014.⁷ Petrol pump workers carry a higher risk of genotoxicity, as they are continuously getting exposed to gasoline fumes. We, therefore, conducted this study to investigate the possible genotoxic effects of gasoline fumes by performing buccal micronucleus cytome assay (BMCyt) in a group of petrol pump workers.

Materials and Methods

In this cross-sectional study, 70 male petrol pump workers (the exposed group) were selected from various heavy traffic areas of Ahmedabad—Ashram road, Lal darwaja, Sarkhej, Shahpur, and Relief road—which are in continuous air quality monitoring by Gujarat Pollution Control Board (GPCB). Another 70 age-matched healthy non-addicted men with no known exposure to gasoline fumes were also selected from a less populated area, Navrangpura (the comparison group). Each participant was asked about his age, work experience, duration of work-shift, allergy, and other health complaints.

Buccal Micronucleus Cytome Assay

Prior to buccal cell collection, participants were advised to rinse their mouth thoroughly with water to remove unwanted debris. A small headed toothbrush was used to collect cell samples from the inner wall of the cheek. The toothbrush was dipped in the marked centrifuge tube containing fixative—1:3 acetomethanol.⁸ After centrifugation, the cell suspension was used to make air dried slides, stained in Giemsa and studied under light microscope. A total of 1000 cells was scored per participant to determine the frequency of various cell types. The criteria proposed by Bolognesi, *et al*, were used for scoring nuclear anomalies.⁹

Ethics

The protocol of this study was approved by the Institutional Ethical Committee of Gujarat University. Samples were collected with prior consent of all the participants.

Statistical Analysis

Statistical analysis was done by *Student's t* test. Values are expressed as mean (SD) or median (IQR). A *p* value <0.05 was considered statistically significant.

Results

The mean age of participants in the exposed and comparison groups was 37 (SD 9) and 34 (SD 4) years, respectively. They had also a median work experience of 10.5 (IQR 14.5) and 18 (IQR 5) years, and median daily shift hours of 10 (IQR 2) and 9 (IQR 2) hours, respectively. The exposed workers had an 8–12-hour exposure to gasoline fumes every day for 14–30 years.

In BMCyt assay, eight cell types were distinctly identified based on their morphology. These included normal cells, binucleated cells, micronucleated cells, nuclear bud, condensed chromatin cells,

karyorrhectic cells, pyknotic cells, and karyolytic cells (see Figures).

Mean relative frequencies of the observed cell anomalies are shown in Table 1. The prevalence of all types of cell anomalies, except binucleated cells, was significantly ($p < 0.001$) higher in the exposed workers than in the comparison group. No significant difference was observed in the mean percentage of binucleated cells between exposed and comparison groups (Table 1).

Discussion

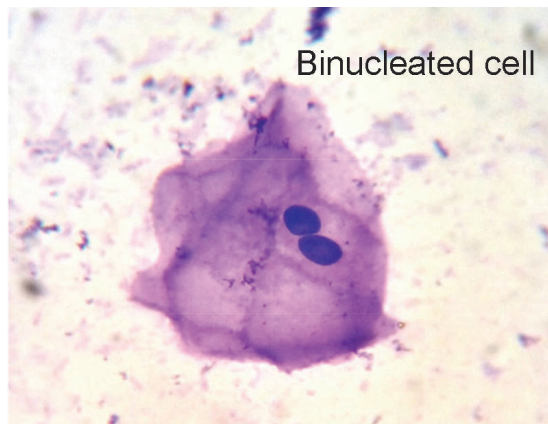
The major sources of gasoline fumes exposure for petrol pump workers are exhaust emissions, evaporation losses from motor vehicles, and evaporation during the handling, distribution and storage of petrol.¹⁰ In India, the workers recruited at the petrol pumps, refill the vehicles with petrol or diesel continuously on a daily basis, with long working hours and are thus, at a risk of having adverse health effects due to inhalation of volatile organic compounds released from these fuels—benzene, toluene, ethyl-benzene, xylene, *etc.* Half of the total benzene, toluene, ethylene, and xylene (BTEX) inhaled from air, is absorbed in the body that can further lead to many health hazards such as cancer, neurological diseases and teratogenic effects.¹¹

Petrol pump workers who refill gasoline are liable to absorb not only the products present in the fuel fumes, but also the products emitted by engines. The buccal cavity is the first target tissue to get exposed to the inhaled gases present in the fumes. The BMCyt has a set of biomarkers that can be used in epidemiological studies. The assay focuses on the occurrence of micronucleus as well as various cell types classified based on the nuclear morphology and cytological features that indicate the stages of DNA damage and cell death in exfoliated buccal cells. In our previous

Table 1: Percentage (95% CI) of cell anomalies in the exposed and comparison group

Cell anomalies	Exposed, (n=70)	Comparison, (n=70)
Binucleated cells	9.4 (7.5 to 11.3)	9.6 (7.7 to 11.5)
Micronuclei	2.5 (1.6 to 3.3)	1.7 (0.9 to 2.5)
Nuclear buds	1.3 (1.0 to 1.6)	0.3 (0.2 to 0.4)
Condensed chromatin	2.4 (0.9 to 4.0)	1.0 (0.2 to 1.8)
Karyorrhectic cells	9.1 (7.6 to 10.7)	3.4 (2.6 to 4.2)
Pyknotic cells	1.6 (1.4 to 1.9)	0.4 (0.3 to 0.5)
Karyolytic cells	8.4 (5.9 to 10.8)	3.6 (2.6 to 4.6)

study, the same methodology was used in a different group of exposed individuals (cellphone users). The images of various cell anomalies shown are a clear evidence of the cytological damage caused due to the prolonged exposure of harmful electromagnetic radiations.¹²



Binucleate cells are cells containing two main nuclei instead of one. The nuclei are usually very close and may touch each other and have the same morphology as that observed in normal cells. Although the significance of binucleate cells is still unknown, they are probably signs of failed cytokinesis following the last nuclear division in the basal cell layer.⁸ It has been shown that chromosomal non-disjunction occurs with a higher frequency in binucleate cells that fail to complete cytokinesis

TAKE-HOME MESSAGE

- Petrol pump workers are routinely exposed to toxic chemicals present in gasoline fumes during their work shifts.
- These workers were found to have increased frequencies of cell anomalies in buccal epithelium showing genotoxic effects of occupational exposure.
- It is important to educate petrol pump workers about the usage of precautionary equipment and the toxicity of fuels to ensure safe and healthy working environment.

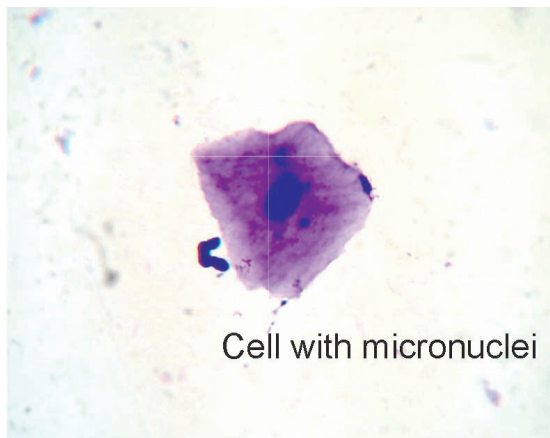
compared to those cells that have completed cytokinesis. This mechanism is thought to be a cytokinesis checkpoint for aneuploid binucleate cells.¹³ Another study on petrol pump workers showed significant increase in the frequency of binucleate cells in the exposed individuals.¹⁴ Thomas and Fenech¹⁵ have shown that binucleate cells might be associated with cell proliferation, while Celik, *et al*,¹⁶ considered it as an indicator of genotoxicity. In the current study, the frequency of binucleate cells was not significantly different between the exposed and comparison groups indicating no such effects.

Micronuclei in exfoliated epithelial cells are used to evaluate the genotoxic effects produced by low doses of carcinogenic substances or their mixtures, to which people are exposed.¹⁷ Micronuclei origi-

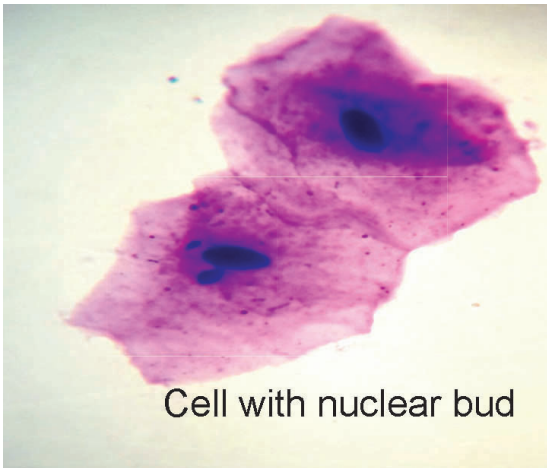
nate from acentric chromosome fragments or whole chromosomes that lag behind in anaphase and are left outside the daughter nuclei. The formation of micronuclei and the presence of chromosomal aberrations are strictly linked events; the presence of micronuclei in dividing cells is the result of chromosome breakage due to unrepaired or misrepaired DNA lesions or chromosome mal-segregation due to mitotic malfunctioning. These events may be induced by oxidative stress, exposure to clastogens, genetic defects in cell cycle checkpoint or DNA repair genes, and deficiencies in major cofactors in DNA metabolism and chromosome segregation machinery.¹⁸ Increased frequencies of micronuclei in buccal cells has been reported in smoking group of petrol pump workers, cell phone users, gutkha and pan masala chewers, bidi smokers, metal arch welders, tannery workers, and road paving workers.^{12,19-22}

In the present study, significant increase in the frequencies of micronuclei was observed in the petrol pump workers compared to the comparison group implying higher level of chromosomal damage in the exposed workers. Most of the micronuclei studies conducted previously were focused on analyses of lymphocytes. In the recent years, however, more investigators have become interested in using exfoliated cells from the oral cavity and other cell types that can be collected non-invasively, which is particularly important in pediatrics and occupationally exposed workers.²³

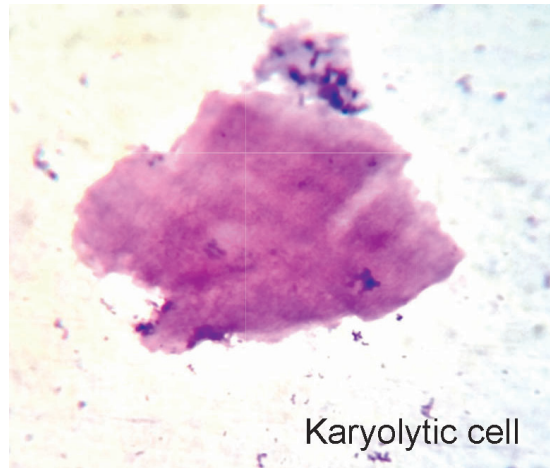
Some nuclear abnormalities such as breakage or loss of genetic material can also be measured in the form of nuclear buds, which are also known as “broken eggs.”²⁴ The main nucleus of nuclear buds has a sharp constriction forming a bud of nuclear material. Nuclear buds are attached to the main nucleus by a narrow or wide nucleoplasmic bridge. Nuclear buds and their associated nucleoplasmic bridges have similar staining intensity as the



Cell with micronuclei



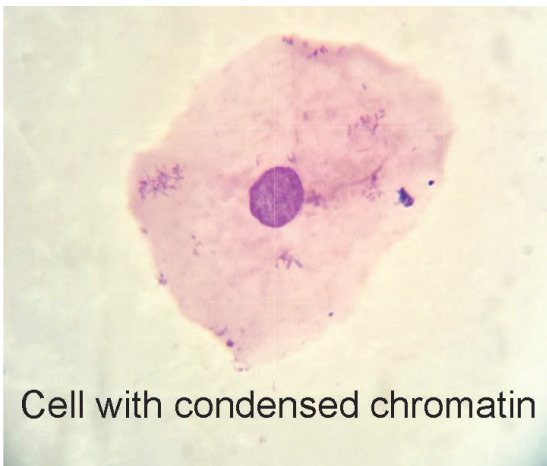
Cell with nuclear bud



Karyolytic cell

main nucleus. Nuclear buds usually have a diameter that is one-third to one-sixth that of the main nucleus; in some rarer cases, they could even be greater and almost up to the same size as the main nucleus.²⁵ In the current study, the frequency of nuclear buds in the exposed group was significantly higher than that in the comparison group, implying that the cells might be undergoing degeneration or exhibiting apoptosis due to the exposure.

Buccal cells with condensed chromatin show a roughly striated nuclear pattern in which the aggregated chromatin is intensely stained. Similar nuclear morphologies have also been shown in apoptotic cells.²⁶ In these cells, chromatin is aggregating in some regions of the nucleus while being lost in other areas. Although this has



Cell with condensed chromatin

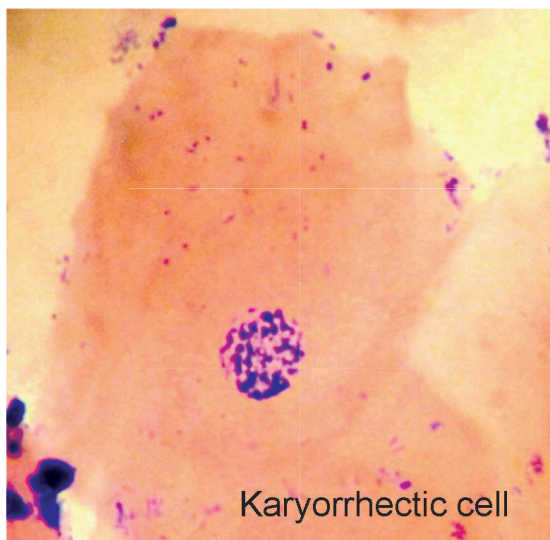
not been shown conclusively, these cells may be undergoing early stages of apoptosis. These cells, as well as karyorrhetic cells, invariably develop fragmented nuclei, leading to eventual disintegration. Sometimes, they appear to contain bodies similar to micronuclei, but these are not scored as micronuclei in the assay as their origin cannot be accurately determined, as suggested by Thomas, *et al.*⁸ In our study, the frequency of condensed chromatin cells in the exposed workers was significantly higher than that in the comparison group, which indicated that the cells might be undergoing apoptosis due to exposure to gasoline fumes.

Karyorrhetic cells have nuclei that are characterized by more extensive nuclear chromatin aggregation relative to condensed chromatin cells. They have a densely speckled nuclear pattern indicative of nuclear fragmentation ultimately leading to disintegration of the nucleus.²⁵ Although it has not been proven yet, these cells may be undergoing a late stage of apoptosis. In this study, the frequency of karyorrhetic cells in the exposed group was significantly higher than that in the comparison group, which showed that the nucleus was getting disintegrated due to gasoline fumes exposure.

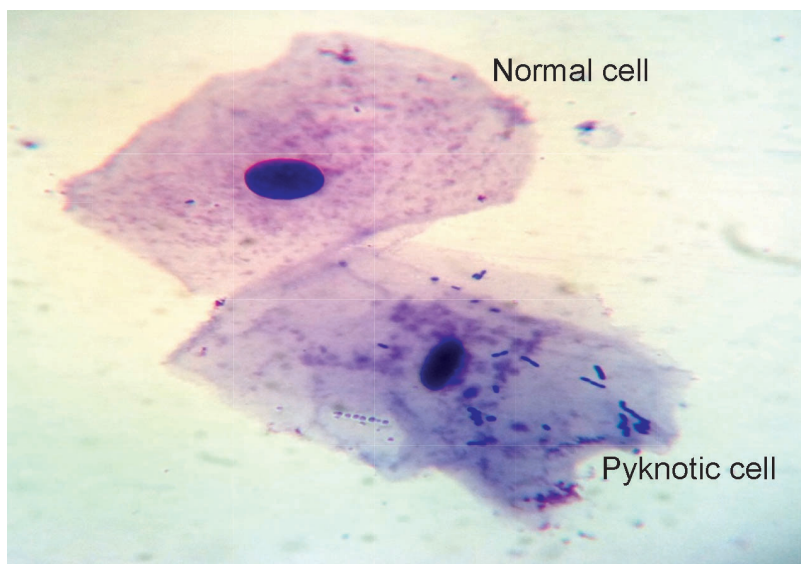
Pyknotic cells are characterized by a small shrunken nucleus, having a diameter

For more information on micronucleus, nucleoplasmic bridge, and nuclear budding in peripheral blood cells of workers exposed to low level benzene see <http://www.theijoem.com/ijoem/index.php/ijoem/article/view/785>





that is one-third to two-thirds of that in a fully differentiated nucleus of a viable cell and a high density of nuclear material that is uniformly but intensely stained. The biological significance of the pyknotic cells and the mechanism leading to their formation are unknown, but it is suggested that these cells may be undergoing a unique form of cell death.²⁵ They may represent an alternative mechanism of nuclear disintegration that is distinct from the process leading to the condensed chromatin and karyorrhectic cell death stages.^{27,28} In the present study, the frequency of pyk-



notic cells was significantly higher in the exposed workers compared to the comparison group, suggesting the cells might be undergoing apoptosis or cell death.

Among other nuclear anomalies in the present study, karyolytic cells were found with extremely elevated frequency in the exposed workers, similar to the results reported by Rajkokila, *et al.*²⁹ Karyolytic cells are cells in which the nucleus is completely devoid of DNA and appears as a ghost-like image that has no Giemsa staining; it represents very late stage in cell death process, which is indicative of necrosis.⁸

Degenerative nuclear phenomena are indicative of apoptosis (karyorrhexis, condensed chromatin and pyknosis) and necrosis (karyolysis), which reveal the genotoxic effects of exposure to gasoline fumes. Apoptosis is stimulated by exposure to mutagens and acts as a protective mechanism against cancer by eliminating genetically damaged cells. High levels of occurrence of apoptosis may constitute evidence of genotoxic damage that would be related to the initiation of the process of malignant transformation.³⁰ Karyolysis can occur in addition to these alterations, in cells undergoing necrosis, *ie*, cell death, consequent to the action of exogenous agents on the cell environment.³¹

Inhaled gasoline compounds such as benzene are absorbed by lung, metabolized in the liver and converted into a number of metabolites like benzene-oxide, phenol and catechol, which are considered toxic.³² Phenol concentration in the urine of the exposed workers represents 70%–85% of urinary benzene metabolites at air concentrations of 0.1–10 ppm.³³ Therefore, urinary phenol can be considered a valuable biomarker for external exposure to gasoline compounds. In our previous study, urinary phenol levels in petrol pump workers were found to be significantly high compared to the comparison group. The increased level of phenol was also correlated with the sig-

nificantly increased levels of antioxidant enzyme parameters, suggesting that the petrol pump individuals have exposure-related oxidative stress.³⁴ Moreover, reactive oxygen species (ROS) is a known contributor to the genomic instabilities leading to DNA strand breaks, gene deletions and rearrangements, chromosomal alterations, and oxidative DNA damage.³⁵ Based on the above findings, it is suggested that the increase in ROS parameters may have triggered formation of oxidative DNA damage, which ultimately resulted into genotoxicity in the exposed workers, as observed in significant increase in all cell anomalies but binucleate cells.

A study on those exposed to petrol has shown increased frequencies of micronuclei in buccal mucosal cells, which were linked to high risk for cancer as a long-term effect needing careful monitoring.³⁶ Biomarkers such as pyknotic cells, nuclear bud, karyolytic cells, karyorrhectic cells, *etc*, found in BMCyt have been associated with genetic defects in genome maintenance, exposure to genotoxic agents, accelerated ageing, risk of oral cancer, and neurodegenerative diseases.³⁷

Various occupational exposures, including gasoline components can increase the risk of many malignancies such as cancer of the urinary tract, skin, larynx, and pancreas, and leukemia.³⁸ The genotoxic effects in buccal mucosal cells of various exposures such as gutkha and pan masala chewing, bidi smoking, and prolonged use of cell phones have been reported earlier by our lab. The frequencies of all cell anomalies were directly proportional to the increased exposure time as well as some confounding factors (*eg*, addiction). Presence of these cell anomalies was also correlated with mild health problems in the exposed individuals.^{12,22}

It is clear that the micronucleus test in exfoliated epithelial cells is a useful biomarker for occupational exposure to

genotoxic chemicals increasing the risk of malignant and neurodegenerative disorders. In addition, other nuclear abnormalities, such as binucleates, karyolysis, karyorrhexis, pyknotic cells and cells with nuclear bud are also useful indices of chemical exposure and toxic response. A combination of micronuclei and nuclear abnormalities may therefore increase the sensitivity of the technique of using exfoliated epithelial cells to assess genotoxicity.

In conclusion, the results of this study showed that the petrol pump workers had a significant increase in the buccal cell anomalies implying higher degree of apoptosis, necrosis and cell death, which is a response towards adverse occupational exposure. It is evident that the long-term exposure to gasoline fumes can lead to a high susceptibility for the development of health risks including cancer. Therefore, they should be carefully monitored to prevent the long-term effects of the exposure. Further follow-up studies are suggested to check the effects of exposure including various other confounding factors such as age, diet, addiction, *etc*, in occupational workers.

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Conflicts of Interest: None declared.

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