"Evaluation of Micronucleus in Exfoliated Buccal Epithelial Cells of Petrol Station Attendants in Western Chhattisgarh Population (Durg–Rajnandgaon District)"

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ABSTRACT

Background: Petrol station attendants are chronically exposed to petroleum vapors containing benzene and other volatile organic compounds that may induce DNA damage and increase the risk of genetic alterations. The buccal micronucleus assay is a well-established, non-invasive biomonitoring method for detecting early cytogenetic damage.

Aim: To evaluate micronucleus frequency and nuclear abnormalities in exfoliated buccal epithelial cells of petrol station attendants in Western Chhattisgarh and compare the findings with healthy controls.

Materials and Methods: A comparative cross-sectional observational study was conducted among 90 male participants, including 45 petrol station attendants and 45 healthy controls. Exfoliated buccal epithelial cells were collected using a sterile cytology brush and stained by the Papanicolaou technique. A minimum of 1000 epithelial cells per participant were examined under a light microscope to assess micronuclei, binucleated cells, nuclear buds, karyorrhexis, karyolysis, and pyknosis. Statistical analysis was performed using SPSS version 25.0. Independent Student's *t*-test and Pearson's correlation analysis were applied, with $p<0.05$ considered statistically significant.

Results: Petrol station attendants demonstrated a significantly higher mean micronucleus frequency than controls (16.9 ± 4.2 vs. 3.3 ± 1.1 per 1000 cells; $p<0.001$). All other nuclear abnormalities were also significantly increased among exposed workers ($p<0.001$). A strong positive correlation was observed between duration of occupational exposure and micronucleus frequency ($r=0.68$, $p<0.001$).

Conclusion: Chronic occupational exposure to petrol vapors produces significant genotoxic and cytotoxic effects in buccal epithelial cells. The buccal micronucleus assay is an effective, simple, and non-invasive tool for occupational biomonitoring. Regular health surveillance and appropriate preventive measures are recommended to reduce exposure-related genetic damage.

Keywords: Micronucleus assay, Buccal epithelial cells, Petrol station attendants, Genotoxicity, Cytogenetic damage, Occupational exposure, Benzene, Nuclear abnormalities.

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INTRODUCTION

Occupational exposure to petroleum products represents a significant public health concern because petrol contains several volatile organic compounds, including benzene, toluene, ethylbenzene, and xylene (BTEX), many of which possess genotoxic and carcinogenic potential. Petrol station attendants are continuously exposed to these hazardous vapors during fuel dispensing, increasing their risk of cellular and genetic damage despite adherence to standard occupational safety measures.[1,2]

Genotoxic damage caused by chronic exposure to petroleum-derived chemicals can result in chromosomal instability, DNA strand breaks, and mutations, which may contribute to carcinogenesis. Early detection of such cellular alterations is therefore important for monitoring occupational health and preventing long-term adverse outcomes.[3,4]

The buccal micronucleus cytome assay has emerged as a simple, reliable, non-invasive, and cost-effective biomonitoring tool for evaluating chromosomal damage in human populations. Micronuclei are small extranuclear bodies formed from acentric chromosome fragments or whole chromosomes that fail to be incorporated into daughter nuclei during cell division, serving as established biomarkers of cytogenetic damage. In addition to micronuclei, other nuclear abnormalities such as binucleated cells, nuclear buds, karyorrhexis, karyolysis, and pyknosis provide valuable information regarding DNA damage, apoptosis, and cellular degeneration.[4-6]

Several studies have demonstrated a significantly increased frequency of micronuclei among workers occupationally exposed to petrol vapors compared with unexposed individuals, suggesting that prolonged exposure to petroleum hydrocarbons may induce measurable cytogenetic alterations.[7,8] However, data regarding petrol station attendants from Western Chhattisgarh remain limited.

Therefore, the present study was undertaken to evaluate micronucleus frequency and associated nuclear abnormalities in exfoliated buccal epithelial cells of petrol station attendants in the Durg–Rajnandgaon district of Western Chhattisgarh and to compare the findings with healthy individuals from other professions.

MATERIALS AND METHODOLOGY

Study Design

The present study was conducted as a comparative cross-sectional observational study to evaluate cytogenetic damage by assessing micronucleus frequency and other nuclear abnormalities in exfoliated buccal epithelial cells among petrol station attendants and healthy controls.

Study Setting

The study was carried out in the Department of Oral and Maxillofacial Pathology and Oral Microbiology in collaboration with selected petrol stations located in the Durg–Rajnandgaon district of Western Chhattisgarh, India.

Study Duration

The study was conducted from **April 2024 to January 2026**.

Study Population

A total of **90 male participants** were included in the study.

- **Group I (Exposed Group):** 45 petrol station attendants occupationally exposed to petrol vapors.
- **Group II (Control Group):** 45 healthy males from other professions with no occupational exposure to petrol vapors.

Sample Size

Sample size estimation was performed using **G*Power software (Version 3.1.9.7)** considering an effect size (r) of 0.29, α error of 0.05, and study power of 90%. The calculated sample size was 87, and after accounting for a 10% non-response rate, the final sample size was rounded to **90 participants**.

Inclusion Criteria

Group I

- Male petrol station attendants.
- Occupational exposure to petrol vapors for more than 1 year.

- Working more than 8 hours per day.

Group II

- Healthy males from other professions.
- No occupational exposure to petrol vapors.
- No history of tobacco or alcohol consumption.

Exclusion Criteria

- Acute or chronic systemic illness.
- Endocrine or immunological disorders.
- Previous radiation therapy within the last six months.
- Blood or coagulation disorders.
- Tobacco or alcohol users.
- Presence of oral mucosal lesions or significant oral pathology.

Ethical Considerations

The study protocol was approved by the Institutional Ethics Committee before commencement of the study. Written informed consent was obtained from all participants, and confidentiality of personal information was maintained throughout the study.

Collection of Buccal Cells

Participants were instructed to rinse their mouth thoroughly with clean water before sample collection. Exfoliated buccal epithelial cells were collected from the clinically normal buccal mucosa using a sterile cytology brush under aseptic conditions. The collected cells were immediately transferred into an alcohol-based fixative.

Preparation and Staining of Smears

Cell suspensions were smeared uniformly onto clean glass slides and fixed with 95% ethyl alcohol. Smears were stained using the conventional **Papanicolaou (PAP) staining technique**, including Harris hematoxylin, Orange G, and Eosin Azure, followed by dehydration, clearing in xylene, and mounting with DPX.

Microscopic Evaluation

Slides were initially screened under **10× magnification** and subsequently evaluated under **40× magnification** using a light microscope. A minimum of **1000 well-preserved epithelial cells** per participant were examined for the presence of micronuclei and other nuclear abnormalities.

Parameters Evaluated

The following cytological parameters were recorded:

- Micronuclei
- Binucleated cells
- Nuclear buds
- Karyorrhexis
- Karyolysis
- Pyknosis

Micronuclei were identified according to the standardized criteria proposed by the Human Micronucleus (HUMN) Project, including appropriate size, staining intensity, morphology, and separation from the main nucleus.

Figure 1. Collection of exfoliated buccal epithelial cells from petrol station attendants using a sterile cytology brush following oral rinsing prior to sample collection.

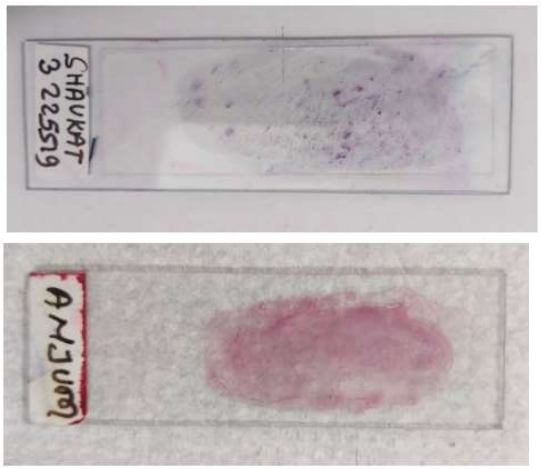

Figure 2. Preparation and cytological evaluation of buccal epithelial smears: (A) freshly prepared smear, (B) Papanicolaou-stained smear, and (C) microscopic examination under 40× magnification.

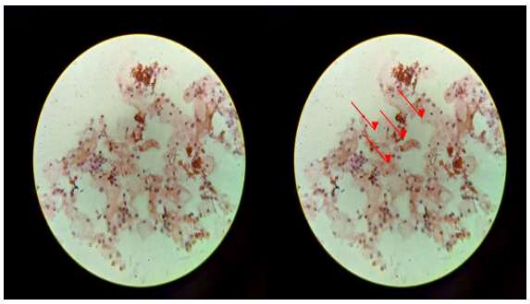

Figure 3. Representative photomicrographs of exfoliated buccal epithelial cells. (A) Normal epithelial cells showing intact nuclei. (B) Micronuclei (red arrows) adjacent to the main nucleus, indicating cytogenetic damage in petrol station attendants (H&E/PAP stain, ×40).

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using **SPSS version 25.0 (IBM Corp., Armonk, NY, USA)**. Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. Comparisons between groups were performed using the independent Student's *t*-test or Mann–Whitney *U* test, depending on data distribution. Pearson's correlation coefficient was used to evaluate the relationship between occupational exposure duration and micronucleus frequency. A *p*-value of <0.05 was considered statistically significant.

RESULTS

A total of **90 male participants** were included in the present study, comprising **45 petrol station attendants (Group I)** and **45 healthy controls (Group II)**. Exfoliated buccal epithelial cells were evaluated for micronucleus (MN) frequency and other nuclear abnormalities. Statistical analysis was performed using **SPSS version 25.0**, and a **p-value <0.05** was considered statistically significant.

Table 1. Comparison of Demographic Characteristics Between the Study Groups

Variable	Exposed Group (n=45)	Control Group (n=45)	p-value
Mean age (years)	32.8 ± 5.8	31.9 ± 5.2	0.462
Male (%)	45 (100%)	45 (100%)	—
Mean duration of occupation (years)	8.3 ± 4.7	—	—
Mean working hours/day	8.9 ± 0.8	—	—

The mean age of participants was comparable between the exposed and control groups (p=0.462), indicating that both groups were age matched. Petrol station attendants had a mean occupational exposure duration of **8.3 ± 4.7 years** and worked for an average of **8.9 ± 0.8 hours/day**, reflecting chronic occupational exposure to petrol vapors.

Table 2. Comparison of Mean Micronucleus Frequency Between Exposed and Control Groups

Group	Mean MN Count (per 1000 cells)	Standard Deviation	t-value	p-value
Exposed (n=45)	16.9	4.2	18.62	<0.001*

Group	Mean MN Count (per 1000 cells)	Standard Deviation	t-value	p-value
Control (n=45)	3.3	1.1		

Independent Student's t-test

The mean micronucleus frequency was **16.9 ± 4.2** in the exposed group compared with **3.3 ± 1.1** in the control group. The difference was highly statistically significant (**t = 18.62, p <0.001**). Petrol station attendants exhibited nearly a **five-fold increase** in micronucleus frequency, indicating significant chromosomal damage associated with occupational petrol vapor exposure.

Table 3. Comparison of Nuclear Abnormalities Between Exposed and Control Groups

Nuclear Abnormality	Exposed Group (Mean ± SD)	Control Group (Mean ± SD)	t-value	p-value
Binucleated cells	9.8 ± 2.6	1.5 ± 0.8	15.84	<0.001*
Nuclear buds	7.3 ± 2.1	0.9 ± 0.5	16.21	<0.001*
Karyorrhexis	11.9 ± 3.2	1.6 ± 0.7	17.05	<0.001*
Karyolysis	8.9 ± 2.8	1.4 ± 0.6	14.76	<0.001*
Pyknosis	10.8 ± 3.0	2.4 ± 0.9	13.92	<0.001*

Independent Student's t-test

All evaluated nuclear abnormalities were significantly more frequent among petrol station attendants than healthy controls (**p <0.001** for all comparisons). Among these abnormalities, **karyorrhexis** showed the highest mean value, followed by **pyknosis** and **binucleated cells**, indicating marked cytotoxic effects and nuclear degeneration associated with prolonged occupational exposure.

Table 4. Correlation Between Duration of Occupational Exposure and Micronucleus Frequency

Variable	Pearson Correlation (r)	p-value
Duration of occupational exposure vs Micronucleus frequency	0.68	<0.001*

Pearson correlation analysis

Pearson correlation analysis demonstrated a **strong positive correlation** between the duration of occupational exposure and micronucleus frequency (**r = 0.68, p <0.001**). This finding indicates that longer exposure to petrol vapors is associated with progressively increased cytogenetic damage in buccal epithelial cells.

DISCUSSION

The present study evaluated cytogenetic damage in exfoliated buccal epithelial cells of petrol station attendants using the buccal micronucleus assay, a well-established and non-invasive biomonitoring technique. The findings demonstrated a significantly higher frequency of micronuclei and other nuclear abnormalities among petrol station attendants compared with healthy controls. These observations indicate that chronic occupational exposure to petrol vapors is associated with both genotoxic and cytotoxic damage to buccal epithelial cells.

Petrol contains several hazardous volatile organic compounds, including benzene, toluene, ethylbenzene, and xylene (BTEX), which are capable of inducing oxidative stress, DNA strand breaks, chromosomal aberrations, and mitotic spindle disturbances. Continuous inhalation of these compounds during fuel dispensing exposes petrol station attendants to cumulative genetic damage over time. The significantly increased micronucleus frequency observed in the present study supports the hypothesis that prolonged occupational exposure to petrol vapors results in chromosomal instability and genomic damage. Similar findings were reported by **Celik et al.**, who demonstrated significantly elevated micronucleus frequencies among petrol station workers compared with unexposed controls, suggesting occupational exposure as an important determinant of cytogenetic injury.[9] Likewise, **Karahalil et al.** reported increased micronucleus formation in workers exposed to benzene-containing petroleum products, indicating that benzene metabolites play a major role in inducing DNA damage through oxidative mechanisms.[10] Comparable observations were also documented by **Sellappa et al.**, who found significantly higher frequencies of micronuclei in petrol station attendants in South India, emphasizing the adverse

biological effects of long-term exposure to petroleum hydrocarbons.[11]

Apart from micronuclei, the present study demonstrated significantly higher frequencies of binucleated cells, nuclear buds, karyorrhexis, karyolysis, and pyknosis among exposed workers. These nuclear abnormalities are recognized indicators of chromosomal instability, defective cytokinesis, apoptosis, and cellular degeneration. **Çelik et al.** also observed increased frequencies of these cytological abnormalities among gasoline station attendants and concluded that occupational exposure affects both DNA integrity and cellular viability.[12] The higher prevalence of karyorrhexis and pyknosis in the present study suggests enhanced nuclear fragmentation and apoptotic changes resulting from chronic exposure to toxic petroleum constituents.

The reliability of the buccal micronucleus assay depends largely on standardized staining techniques and scoring criteria. **Nersesyan et al.** demonstrated that appropriate staining methods improve the accuracy of micronucleus identification while minimizing false-positive results.[13] Similarly, **Bolognesi et al.** established standardized scoring criteria through the Human Micronucleus Project (HUMNXL), allowing consistent evaluation of micronuclei and other nuclear abnormalities across different laboratories.[14] The present study adopted these internationally accepted criteria, thereby improving the validity and reproducibility of the observations.

An important finding of the present study was the significant positive correlation between duration of occupational exposure and micronucleus frequency ($r = 0.68$, $p < 0.001$). This indicates that workers with longer exposure experienced greater cytogenetic damage, suggesting a cumulative dose-dependent effect of petrol vapor exposure. Similar exposure-response relationships have been reported in previous occupational biomonitoring studies, where prolonged employment at petrol stations was associated with progressively increasing DNA damage and chromosomal instability.[10–12] This finding highlights the importance of limiting occupational exposure duration and implementing preventive measures in the workplace.

The World Health Organization has emphasized that occupational exposure to hazardous chemicals should be minimized through engineering controls, adequate workplace ventilation, regular environmental monitoring, and the use of personal protective equipment.[15] In this context, periodic biomonitoring assumes considerable importance for the early detection of genetic damage before the development of clinically apparent disease. The buccal micronucleus cytome assay described by **Thomas et al.** provides a rapid, inexpensive, minimally invasive, and highly sensitive method for assessing occupational genotoxicity, making it particularly suitable for large-scale screening of exposed populations.[16] Furthermore, the methodological recommendations proposed by **Tolbert et al.** and **Nersesyan et al.** have established the assay as a standardized and reproducible technique for evaluating occupationally induced cytogenetic alterations.[17,18]

The present study has certain limitations. The relatively modest sample size and single-center design may limit the generalizability of the findings. Individual environmental factors, dietary antioxidants, genetic susceptibility, and environmental pollution were not evaluated and may have influenced micronucleus frequency. Future multicentric studies with larger sample sizes, detailed exposure assessment, biomarker analysis, and long-term follow-up are recommended to further elucidate the relationship between petrol vapor exposure and cytogenetic damage.

Overall, the findings of the present study are in agreement with previous occupational biomonitoring studies and provide further evidence that chronic exposure to petrol vapors results in significant genotoxic and cytotoxic alterations in exfoliated buccal epithelial cells. These observations underscore the importance of regular occupational health surveillance, implementation of appropriate protective measures, and periodic cytogenetic monitoring to reduce long-term health risks among petrol station attendants.

CONCLUSION

The present study demonstrated a significantly higher frequency of micronuclei and other nuclear abnormalities in exfoliated buccal epithelial cells of petrol station attendants compared with healthy controls. Chronic occupational exposure to petrol vapors was associated with both genotoxic and cytotoxic alterations, and the frequency of micronuclei increased significantly with longer duration of exposure. These findings confirm that the buccal micronucleus assay is a simple, reliable, and non-invasive biomarker for early detection of occupational DNA damage. Periodic cytogenetic screening, proper use of personal protective equipment, adequate workplace ventilation, and regular occupational health surveillance should be implemented to minimize exposure-related health hazards among petrol station workers.

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