ORIGINAL ARTICLE

Assessment of Genotoxic Effects in Buccal Cells of Tobacco Users via Micronuclei Assay: A Study from a Tertiary Care Centre in Kalaburagi

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ABSTRACT

Introduction: Oral cancer continues to pose a major public health challenge in India, driven by excessive tobacco consumption. Current study investigates the genotoxic effects of tobacco consumption—both smoked and smokeless—by analysing micronuclei (MN) frequency in exfoliated buccal mucosa cells. **Materials & methods**: The study involved 201 individuals who were divided into two distinct groups: smokers and users of smokeless tobacco. Buccal cell samples were collected and processed using the Papanicolaou staining technique, and MN were identified based on Tolbert's criteria. **Results**: This study revealed that smokers exhibited a significantly higher mean MN count (7.18) compared to smokeless tobacco users (3.8), indicating greater chromosomal damage. Melanosis was the most common clinical sign among smokers (84.8%), while 30% of smokeless tobacco users showed no clinical symptoms. The prevalence of MN exceeding 10 was notably higher among smokers with melanosis. Age-wise analysis showed younger individuals (18–30 years) predominantly preferred smoking, while older individuals leaned towards smokeless tobacco. **Conclusion:** The data indicate that smoked tobacco carries a greater genotoxic risk than smokeless types. The micronucleus assay is recognized as a reliable and non-invasive method for the early identification of genotoxic alterations.

KEYWORDS

Oral cancer, Micronuclei (MN), Genotoxicity, Buccal mucosa, tobacco, Melanosis

INTRODUCTION

Oral cancer is a major health concern in India, accounting for nearly 30% of all cancer cases.(1) Tobacco use—both smoked and smokeless—is a primary risk factor due to its carcinogenic compounds, including nitrosamines and polycyclic aromatic hydrocarbons.(1) The buccal mucosa, being the first point of contact, is highly susceptible to genotoxic damage.(2) One reliable marker of such damage is the presence of micronuclei (MN), these are extranuclear structures that originate from either chromosomal fragments or entire chromosomes that fail to be incorporated into the daughter nuclei during cell division.(3) Micronuclei formation is linked to exposure to tobacco, betel nut, alcohol, and environmental toxins.(3)

Exfoliative cytology offers a non-invasive method to detect early gesnotoxic changes in buccal cells. Among various assays, the micronucleus test stands out for its sensitivity and simplicity.(4) It does not require complex cell culture or DNA-specific staining and can be applied during the interphase stage of cell division.(4)

Aim & Objective:

- To assess the occurrence and structural characteristics of micronuclei in buccal mucosal cells among individuals consuming the two main, mutually exclusive tobacco usage patterns studied: smoked and smokeless tobacco.
- To establish a correlation between tobacco exposure and genotoxic damage, aiding early diagnosis and risk assessment of oral cancer.

MATERIAL & METHODS

Participants were recruited from the Outpatient Department of the Dental Department, primarily from rural areas. The study was a hospital-based cross-sectional design conducted over six months, from October 2024 to March 2025, in the Department of Dentistry at Gulbarga Institute of Medical Sciences, Kalaburagi. Approval from the Institutional Ethics Committee was obtained prior to initiating the study. Patient selection was based on their history of tobacco use. Written informed consent in the local language was secured before participation. Subjects were categorized into two groups:

Group A – users of smoking forms of tobacco

Group B – individuals using smokeless forms of tobacco

It is noted that while the study's overarching aim refers to "various tobacco usage patterns," the practical focus and design of this specific study were on comparing the genotoxic effects between two distinct, mutually exclusive groups: users of smoked tobacco and users of smokeless tobacco. A total of 201 participants were included in the study. The sample size was determined in collaboration with the Department of Community Medicine & Bio-statistics at Gulbarga Institute of Medical Sciences, Kalaburagi, utilizing the Open-Epi tool for accurate calculation. (5) A 5% margin of error and a 95% confidence level were used to set the parameters, based on an assumed 16% prevalence of the micronucleation rate in tobacco users. (6)

Inclusion Criteria

Individuals who regularly consumed five or more than five packets of smokeless tobacco each day over a minimum duration of five years.

Individuals who smoked 20 or more bidis/cigarettes daily for at least five years.

Exclusion Criteria

Individuals younger than ten years of age.

Subjects with systemic diseases, viral infections, alcoholism, prior radiation therapy/chemotherapy, ongoing medication, or occupational exposure to chemicals.

Individuals previously diagnosed or treated for premalignant or malignant oral lesions.

Cytology smears that were inadequate or unsatisfactory were excluded from evaluation.

Participants who both smoked and used smokeless tobacco products.

Sample Collection: Prior to collecting samples, each individual thoroughly rinsed their mouth with tap water. Buccal mucosal cells were then obtained by gently scraping the inner cheek using a dampened wooden spatula and subsequently transferred into 5 ml of saline solution (0.9% NaCl).

Sample Processing: The exfoliated cells suspended in saline were subjected to centrifugation at 1500 rpm for a duration of 10 minutes. After centrifugation, the supernatant was discarded, and the cell pellet was spread onto clean glass slides to prepare smears. These smears were fixed using 95% ethyl alcohol and stained using the Papanicolaou technique with a commercially available RAPID PAP staining kit. (7) All steps of sample processing, cytological preparation, and microscopic scoring for micronuclei were conducted by a single trained observer to ensure consistency and minimize inter-observer variability. Approximately 1000 cells per slide were examined under 400X magnification to locate cells with micronuclei, which were then further examined under 1000X magnification. Micronuclei were recognized based on the guidelines proposed by Tolbert et al.[6], which encompassed the following features:

- A smooth, rounded boundary suggestive of a distinct membrane.
- Dimensions smaller than one-third the diameter of the adjacent nucleus, yet sufficiently large to allow clear identification of shape and colour.
- Comparable staining intensity to that of the main nucleus.
- Similar internal texture to the nucleus.
- Located within the same focal plane as the nucleus.
- Absence of any connection, overlap, or bridging with the nucleus.

Statistical Analysis

Quantitative data were summarized as mean values accompanied by the standard error of the mean (SEM), whereas qualitative data were reported in terms of frequencies and percentages. Group comparisons were conducted using the unpaired Student's t-test. Additionally, Pearson's correlation and chi-square tests were employed to assess relationships and associations. A p-value of ≤ 0.05 was deemed statistically significant. All statistical analyses were carried out using IBM SPSS Statistics software, version 16.(8)

RESULTS

The 18–30 age group had the highest proportion of smokers, with 91.8% of individuals using the smoked form of tobacco. This trend slightly declined in older age groups: 84% in the 30–50 group and 80.4% in the 50–90 group. The use of smokeless tobacco was more prevalent in older individuals. Only 8.16% of the 18–30 group used smokeless tobacco, compared to 16.0% in the 30–50 group and 19.5% in the 50–90 group. The data suggests a clear age-related pattern in tobacco consumption habits. Younger individuals predominantly preferred smoking, while older individuals showed a gradual shift toward smokeless tobacco as depicted in **Table 1**. This trend may reflect changing health perceptions, accessibility, or cultural habits across age groups.

Table 1: Age-Wise Distribution of Tobacco Consumption

AGE	SMOKE	SMOKELESS	Total
18 - 30	45(91.8%)	4(8.16%)	49(100%)
30 - 50	89(84%)	17(16.0%)	106(100%)
50 - 90	37(80.4%)	9(19.5%)	46(100%)

df=2, χ^2 = 2.59, p=0.27

The average age of the participants was 39 years, with a median age of 36 years, and their ages ranged between 18 and 59 years. The average number of micronuclei observed was 7.18, with a standard deviation of 3.7. A higher concentration of micronuclei was noted in the 30 to 50-year age group, followed by the 18 to 30-year age group. Among those using tobacco in its smoked form, melanosis emerged as the most prevalent condition, with few other clinical symptoms reported as shown in **Table 2.**

Table 2: Demographic and Micronuclei Profile of Tobacco Users

Parameters	Smoke (n=171)	Smokeless (n = 30)
Mean age	39.44	42.47
Median age	36.00	43.50
SD Age	13.312	11.599
Minimum Age	18	21
Maximum Age	90	65
Mean micronuclei	7.18	3.8
Median micronuclei	8	3
SD Micronuclei	3.723	2.917
Minimum Micronuclei	0	0
Maximum Micronuclei	14	12

Melanosis was the most common manifestation or symptom among those who indulged in habit of smoked form of tobacco with 84.8% compared to 56.6% in those who took smokeless form of tobacco and vast majority of them, about 30% of them, those who took smokeless form of tobacco had no clinical sign compared to 12.9% of the subjects who took smoked form of tobacco. The prevalence of micronuclei exceeding 10 was primarily observed among patients with melanosis who consumed smoked tobacco, accounting for approximately 91.5%. This was notably higher compared to those with melanosis who used smokeless tobacco. The Pearson Chi-square value of 15.11, with a P-value of 0.001, indicating a highly significant association between the type of tobacco used and the presence of clinical signs. On other words the distribution of clinical signs was not due to chance—the type of tobacco used influenced the likelihood and nature of oral manifestations as shown in **Table 3.**

Table 3: Distribution of Clinical Signs Among Tobacco Users

	Clinical sign			
Type of tobacco	Melanosis	No clinical sign	Others	Total
Smoke	145 (84.8%)	22 (12.9%)	4 (2.3%)	171 (100%)
Smokeless	17 (56.6%)	9 (30%)	4 (13.4%)	30 (100%)

Pearson χ^2=15.11, P<0.05

Smoked Tobacco Users (n = 171): 35.7% had 0–5 micronuclei, indicating relatively lower cellular damage. The largest group (43.9%) had 6–10 micronuclei, suggesting moderate genotoxic effects. 20.5% had 11–15 micronuclei, reflecting higher levels of cellular abnormalities.

Smokeless Tobacco Users (n = 30): A significant majority (80%) had 0–5 micronuclei, indicating lower genotoxic impact compared to smokers. Only 16.7% fell in the 6–10 micronuclei range. Just 3.3% had 11–15 micronuclei, showing minimal high-level damage.

The data clearly demonstrates that individuals who consumed smoked tobacco exhibited a higher prevalence of micronuclei, especially in the 6–15 range, compared to those who used smokeless tobacco. This suggests that smoking tobacco may pose a greater genotoxic risk, potentially contributing more significantly to cellular mutations and oral cancer development. A Pearson Chi-square value of 20.7 with a p-value less than 0.05 demonstrates a notably significant correlation between the form of tobacco consumed and the occurrence of micronuclei, as illustrated in **Table 4 and Figure 1-6**.

Table 4: Distribution of micro-nuclei (MN) in buccal mucosal cells among users of smoked and smokeless tobacco

Type of tobacco	0–5 MN	≥ 6 MN	Total
Smoke	61	110	171
Smokeless	24	6	30

df = 1, χ^2= 20.5, p < 0.05, OR = 7.14, 95% CI (2.86 – 18.18)

Fig 1: Distribution of Micronuclei Counts Among Tobacco Users

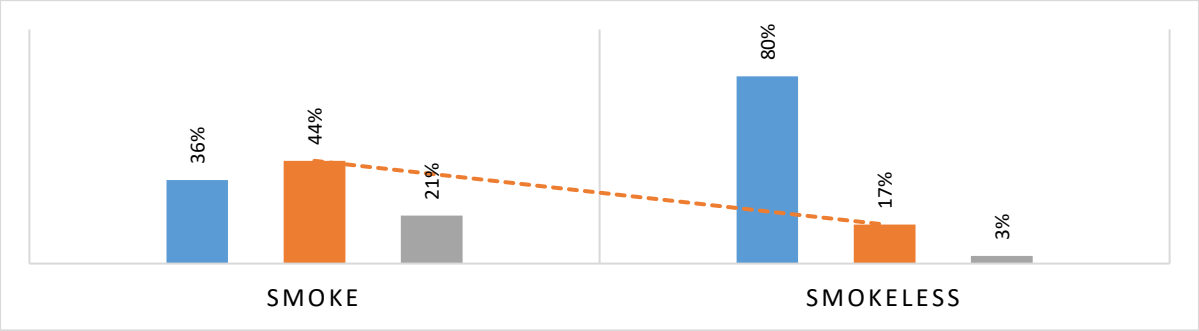


Fig 2: Exfoliated buccal epithelial cells in smokeless form of tobacco users (PAP STAIN 400X)

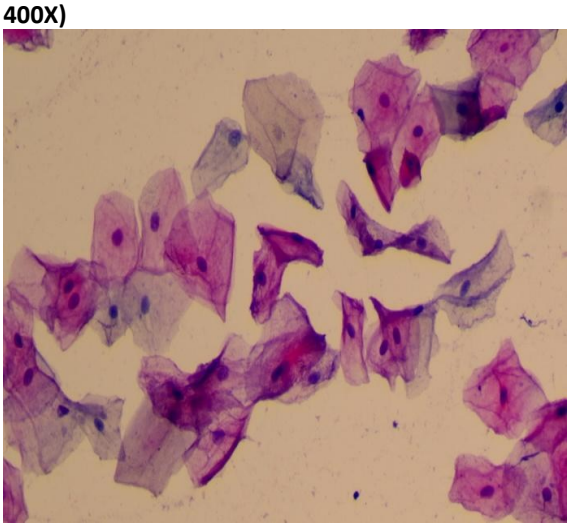


Fig 3: Exfoliated buccal epithelial cells with micronuclear count -8 in an individual with smoker (PAP STAIN 400X)

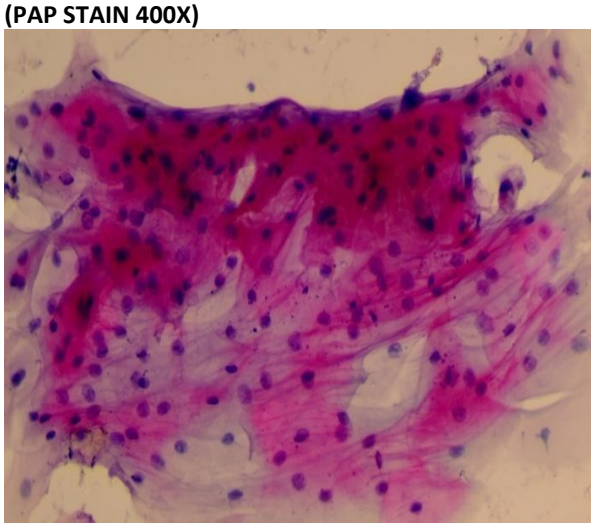


Fig 4: Exfoliated buccal epithelial cells with micronuclear count 8 in an individual with smoker (PAP STAIN 400X)

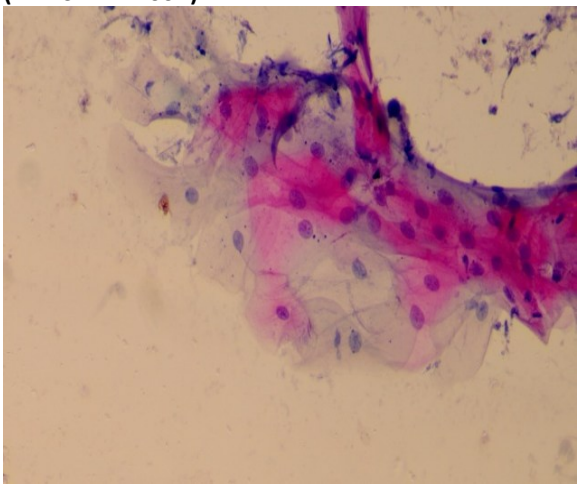


Fig 5: Exfoliated buccal epithelial cells with micronuclear count 13 in an individual with smoking (PAP STAIN 400X)

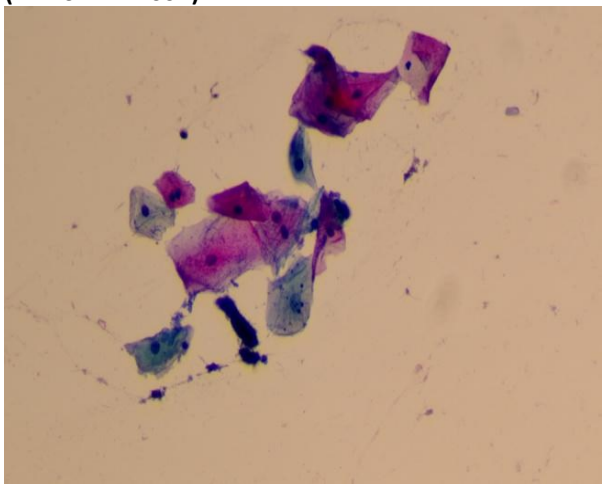
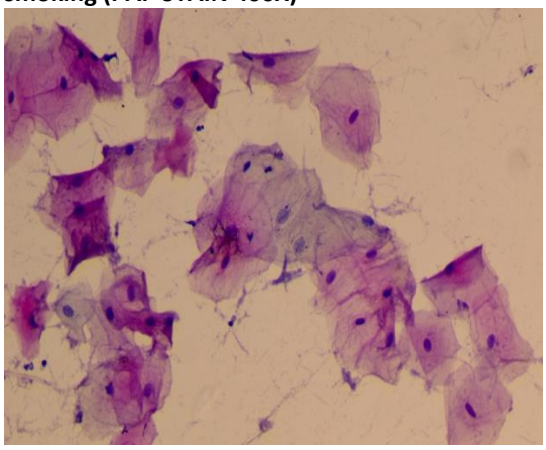


Fig 6: Exfoliated buccal epithelial cells with micronuclear count 15 in an individual with smoking (PAP STAIN 400X)



DISCUSSION

The present study demonstrated a clear and significant association between tobacco consumption and an increased frequency of micronuclei in exfoliated buccal cells. This finding robustly reinforces the established role of micronuclei as sensitive biomarkers indicative of genotoxicity.(9) Specifically, individuals who consumed smoked tobacco exhibited markedly higher micronuclei counts when compared to those who used smokeless tobacco, strongly suggesting a greater genotoxic burden among smokers.(10)

These results are in strong alignment with extensive previous research. A comprehensive systematic review and meta-analysis conducted by de Geus et al. conclusively confirmed that smokers consistently display elevated micronuclei frequencies within their oral mucosa when contrasted with non-smokers.(11) This empirical evidence strongly supports the prevailing hypothesis that tobacco smoke, which is densely packed with potent carcinogenic compounds such as polycyclic aromatic hydrocarbons and nitrosamines, directly induces chromosomal damage. This damage, in turn, manifests as the formation of micronuclei. Furthermore, Nersesyan et al. reported that individuals who smoked non-filtered cigarettes exhibited significantly higher levels of micronuclei and other distinct nuclear anomalies compared to those who used filtered cigarette variants. This observation critically suggests that the specific type and the overall intensity of tobacco exposure play a direct and influential role in determining the extent of cellular damage incurred.(12)

In stark contrast, the smokeless tobacco users within our study cohort displayed a more concentrated distribution of micronuclei predominantly in the lower range of counts. A considerably smaller fraction of this group exhibited counts exceeding. This observation is highly consistent with findings reported by Amin et al., who noted that while smokeless tobacco is indeed genotoxic, its detrimental impact is generally less severe in comparison to that of smoked tobacco.(10) However, Amin et al. also underscored the critical necessity for dynamic and adaptable cut-off limits to accurately identify high-risk individuals even among the users of smokeless tobacco. Pop et al. further expanded this understanding by conducting a comparative analysis involving smokers, e-cigarette users, and non-smokers. Their study concluded that both smokers and e-cigarette users presented remarkably higher micronuclei counts than non-smokers, although no statistically significant difference was detected between the two distinct nicotine delivery methods.(4) This finding crucially highlights the broader genotoxic potential inherent in nicotine-containing products, irrespective of their specific delivery form.(13)

The micronucleus assay, as meticulously employed in this study, continues to be validated as a highly valuable tool. It is widely acknowledged for being non-invasive, economical, and consistently dependable in identifying early genotoxic alterations in oral epithelial cells. Its valuable role in screening for potentially malignant conditions and oral cancer has been well-established across different population groups and patterns of tobacco use.(14,15)

CONCLUSION

The current findings, supported by existing literature, underscore the heightened genotoxic risk associated with smoked tobacco. While smokeless tobacco poses a lower risk, it is not devoid of genotoxic potential. The micronucleus assay remains a valuable biomarker for assessing cellular damage and identifying individuals at risk for oral cancer. These insights advocate targeted public health interventions and routine cytogenetic screening, especially in high-risk populations.

RECOMMENDATION

(Public health importance)

- Routine Cytogenetic Screening must be done for Implementation of regular screening using the micronucleus assay for individuals with a history of tobacco use, especially smokers, to detect early genotoxic changes and prevent progression to oral cancer.

- Targeted Public Health Campaigns must be undertaken to generate awareness programs emphasizing the greater genotoxic risk of smoked tobacco compared to smokeless forms especially Focusing on educating younger populations who predominantly use smoked tobacco.
- Clinical Monitoring of High-Risk Groups by prioritizing clinical follow-up for individuals showing micronuclei counts above 10, especially those with melanosis, as they may be at higher risk for malignant transformation.
- Further research in longitudinal studies need to be undertaken to track the progression of micronuclei frequency over time and its correlation with oral cancer development.
- Integration into Dental Practice by Training of dental professionals to recognize early signs of genotoxic damage and incorporate micronucleus testing into routine oral health check-ups for tobacco users.

Limitation of the Study

- **Cross-sectional design:** Limits causality and long-term effect observation.
- **Self-reported tobacco use:** Risk of recall bias or underreporting, especially in rural areas.
- **Limited biomarker:** Use of only micronuclei; other nuclear anomalies could offer a broader genotoxicity assessment.
- **Single-site recruitment:** Limits external validity to other regions or urban populations.

Relevance of the Study

This study is highly relevant as it addresses the genotoxic effects of tobacco use in a rural Indian population, where oral cancer is a major public health concern. By using the micronucleus assay, a non-invasive and cost-effective method, the research provides early indicators of cellular damage in buccal mucosa. The findings highlight the greater genotoxic risk posed by smoked tobacco compared to smokeless forms, reinforcing the need for targeted screening and preventive strategies. It also supports the use of cytogenetic biomarkers for early detection of oral cancer, especially in high-risk communities with limited access to healthcare

Authors Contribution

All authors have contributed equally.

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Conflict of Interest

NIL

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Declaration of Generative AI and AI Assisted Technologies in the Writing Process

NIL

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