Comparison of Prevalence of Micronuclei in Oral Exfoliated Cells Among Tobacco and Non-Tobacco Users

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ABSTRACT

Micronuclei serves as biomarkers of genotoxic damage and are indicative of chromosomal instability, which is linked to an increased risk of oral cancer. This study aims to compare the prevalence of micronuclei in oral exfoliated cells among tobacco users and non-tobacco users. Two groups are used, where 100 tobacco users are the test group and 50 non-tobacco users are for the control group. Oral exfoliated cells obtained via cytological smears from the buccal mucosa. The smear was placed on a cleaned slide. Papanicolaou (Pap) staining techniques were used to identify and quantify micronuclei under a light microscope. Data were analyzed using SPSS statistical software. There was a statistically significant association between tobacco use and the presence of micronuclei in oral exfoliated cells ($\chi^2(1) = 120.93$, $p < 0.001$). Micronuclei were observed in all examined high-power fields among tobacco users, whereas the majority of fields in non-tobacco users showed no detectable micronuclei. The findings indicate that tobacco users exhibit a significantly higher prevalence of micronuclei in oral exfoliated cells compared to non-tobacco users. This study confirmed a significant increase in the prevalence of Micronuclei in oral exfoliated cells among tobacco users compared to non-tobacco users, its highlight that tobacco use leads to increased genomic damage in oral epithelial cells, and also suggest that tobacco exposure, whether through smoking or smokeless forms, induces genotoxic damage, leading to chromosomal instability. Early screening, awareness programs, and smoking cessation efforts to reduce the risk of oral cancer.

Keywords: micronuclei, genotoxic, genomic, Papanicolaou, chromosome.

INTRODUCTION

Tobacco use is one of the main risk factors for oral cancer, which is a serious worldwide health issue (Almeida et al., 2021). Smoking, chewing, or snuffing tobacco is one of the main ways that tobacco use contributes to a number of oral health conditions (WHO 2020). One of the most common cancers in the world, oral cancer is particularly common in areas where tobacco use is common, like South Asia, parts of Europeans and Africa (Motgi et al., 2014). Buccal mucosa smears frequently contain oral exfoliated cells, which are readily available and can offer important information about the genotoxic effects of tobacco use (Gopal and Padma, 2018).

Oral mucosal cells are the first barrier for ingestion or inhalation route and are capable of metabolizing proximate carcinogens to reactive products (Chan et al., 2021). The carcinogenic compounds found in tobacco, such as nitrosamines, polycyclic aromatic hydrocarbons, and reactive oxygen species, cause genetic mutations and cellular damage in the oral epithelium (Ye et al., 2019). Carcinogenic chemicals found in tobacco, both

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smokeless and smoked, can damage DNA, resulting in genetic changes and a higher chance of malignant transformation in oral epithelial cells (Pradeep et al., 2019).

Extra nuclear chromatin structures called micronuclei (MN) are created when chromosomes break or missegregate during cell division (Di Bona et al., 2024). They have been extensively employed in cytogenetic investigations to evaluate DNA damage in a variety of tissues, including the oral mucosa, and are trustworthy indicators for genotoxicity (Fenech et al., 2021). Increased micronuclei in oral exfoliated cells are thought to be a precursor to genomic instability, which over time may develop into precancerous and malignant tumors (Upadhyay et al., 2019).

When chromosomes are broken apart or not fully integrated into the daughter nuclei during cell division, tiny extra nuclear entities known as micronuclei are created (Al-Bashaireh et al., 2018). Micronuclei in exfoliated oral cavity cells have been demonstrated to be a sign of genetic damage and can be employed as a biomarker to evaluate exposure to carcinogens (Yang et al., 2020).

The development of micronuclei (MN) in exfoliated oral cells is among the first signs of genetic damage (Krupina et al., 2021). A known biomarker of genotoxicity, cytotoxicity, and chromosomal instability—all important elements in the start of carcinogenesis is the presence of more micronuclei (He et al., 2022). Cytological analysis of exfoliated oral cells for the production of micronuclei can be a non-invasive and efficient way to identify early tobacco-induced DNA damage because the oral mucosa is immediately exposed to tobacco carcinogens (Kranthi et al., 2024).

An important way to understand the early consequences of tobacco-related genotoxicity is to compare the proportion of micronuclei in oral exfoliated cells between tobacco users and non-users (Heddle et al., 2019). In order to determine the role of tobacco in genetic damage and possible oral carcinogenesis, this study was intended to assess and compare the frequency of micronuclei in these two groups. The results of this study could raise awareness of public health issues and emphasize the value of early screening for high-risk populations.

This study was therefore, conducted to compare the prevalence of micronuclei in oral exfoliated cells between tobacco users and non-tobacco users to evaluate the genotoxic effects of tobacco.

MATERIALS AND METHOD

Study Area

This study was conducted in public areas like bus stops, bars, clubs, parks and kiosks where tobacco are sold in major towns such as Ibadan and Ogbomosho in Oyo state.

Study Population

A total of 100 participants of tobacco users were used as test group and 50 participants of non-tobacco users were used as control group. This is a cross-sectional study aimed to compare the prevalence of micronuclei in oral exfoliated cells among tobacco users and non-tobacco users. A self-structured questionnaire was administered to all consenting subjects to obtain their socio demographic data.

Ethical Clearance

The ethical approval for this study was obtained from the Ministry of Health, Oyo state, Nigeria. All participants were asked to sign a written informed consent.

Specimen Handling

A sterile cytobrush was gently rubbed against the buccal mucosa after participants have been instructed to rinse their mouths with clean water to remove debris. The collected cells were then immediately smeared onto

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clean glass slides, fixed with 95% ethanol. Following fixation, the smear was stained using Papanicolaou staining technique to identify micronuclei (Metgud and Neelesh, 2018).

SPECIMEN PROCESSING

Procedure for staining (Papanicolaou stain)

- The slide was fixed in 95% ethanol for at least 30mins.
- The slide was rinsed in water.
- The slide was stained with Harris Hematoxylin for 5mins and thereafter rinsed in water.
- It was differentiated in 1% acid alcohol immediately
- The slide was blued in running tap water for 10mins.
- It was then rinsed in 70% alcohol and 95% alcohol.
- The slide was stained in OG6 for 2mins.
- It was rinsed in 2 changes of 95% alcohol.
- The slide was stained in EA50 for 4mins.
- It was thereafter, flooded with 2 changes of 95% alcohol and dehydrated in absolute alcohol.
- The slide was then cleared and mounted using DPX.

Microscopic Examination

Each of the slides was viewed with a light microscope and carefully observed and examined for the presence of micronuclei.

Statistical Analysis

Chi-square test was used to explore associations. This was done to examine the relationship between the frequency and duration of tobacco use and the prevalence of micronuclei. The collected data were analyzed using SPSS 21. The significance level was considered at p-value of less than 0.05.

RESULTS

Micronucleus Frequency in Oral Exfoliated Cells

A total of 150 high-power fields (HPFs) were examined (using X10 and X40 objectives), comprising 100 HPFs from tobacco users (test group) and 50 HPFs from non-tobacco users (control group). Micronuclei (MN) were identified and recorded according to established cytological criteria.

The tobacco user group demonstrated a markedly higher frequency of micronuclei compared to the control group.

Descriptive Statistics

The tobacco users exhibited a mean micronucleus frequency of 4.27 MN per HPF, with values ranging from 1 to 10. In contrast, the control group showed a mean frequency of 0.14 MN per HPF, with most fields demonstrating no detectable micronuclei. In the tobacco group, MN counts were consistently elevated across examined fields, with most values clustering between 4 and 6 MN per HPF. In contrast, the control group exhibited a highly skewed distribution toward zero, with only occasional single MN occurrences (Table 1).

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Table 1: Descriptive Statistics of Micronucleus Frequency per HPF

Variable	Tobacco Users (n = 100 HPFs)	Controls (n = 50 HPFs)
Mean MN/HPF	4.27	0.14
Minimum	1	0
Maximum	10	1
Range	9	1

Cytological Findings

Microscopic evaluation revealed the presence of micronuclei in exfoliated oral epithelial cells predominantly in tobacco users. The micronuclei appeared as small, rounded chromatin bodies with staining intensity similar to the main nucleus and clearly separated from it (Fig 1).

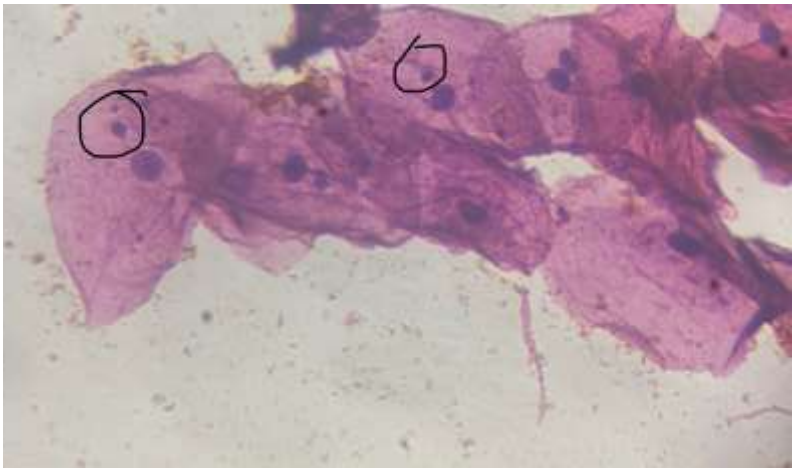

Figure 1: Photomicrograph of exfoliated oral epithelial cells from a tobacco user (×400 magnification) showing distinct micronuclei (circled).

Description: Micronuclei appear as small, rounded chromatin bodies separate from the main nucleus, with comparable staining intensity.

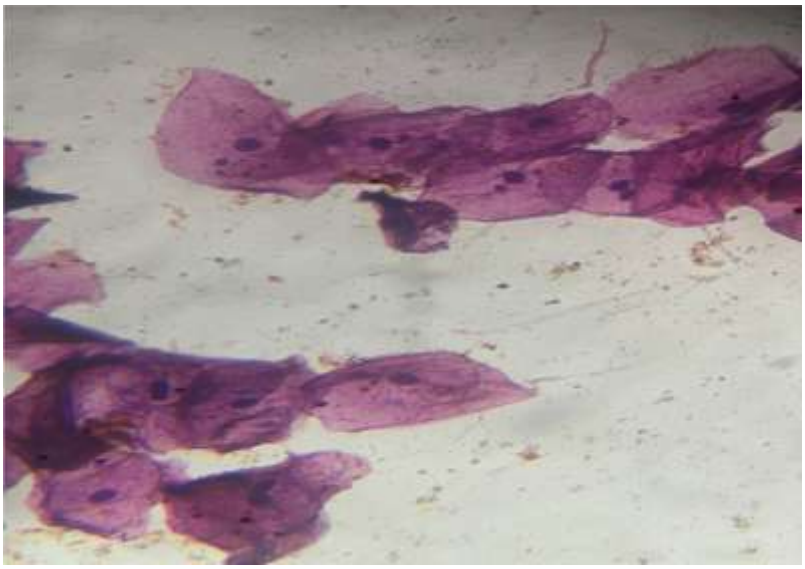

Figure 2: Photomicrograph of oral epithelial cells from a tobacco user (×400 magnification) demonstrating multiple micronuclei within the same high-power field.

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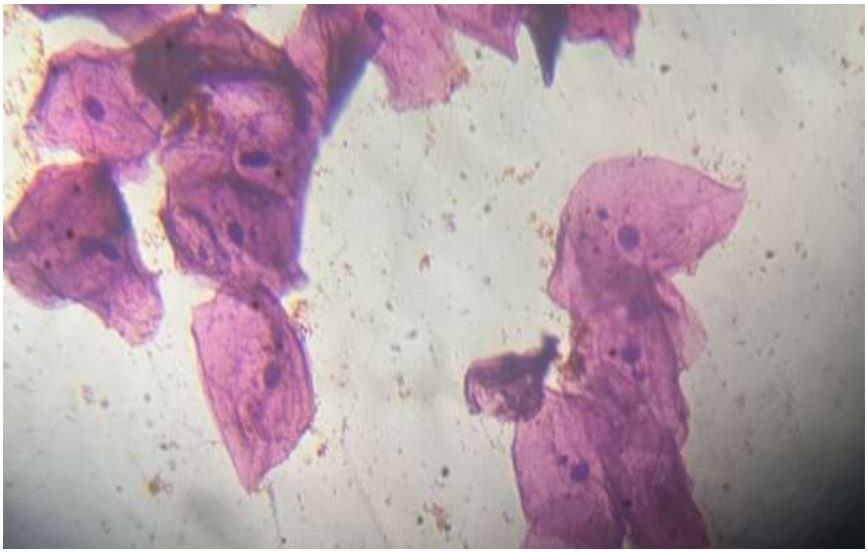

Figure 3: Photomicrograph of exfoliated oral epithelial cells from a non-tobacco user (×400 magnification) showing absence of micronuclei.

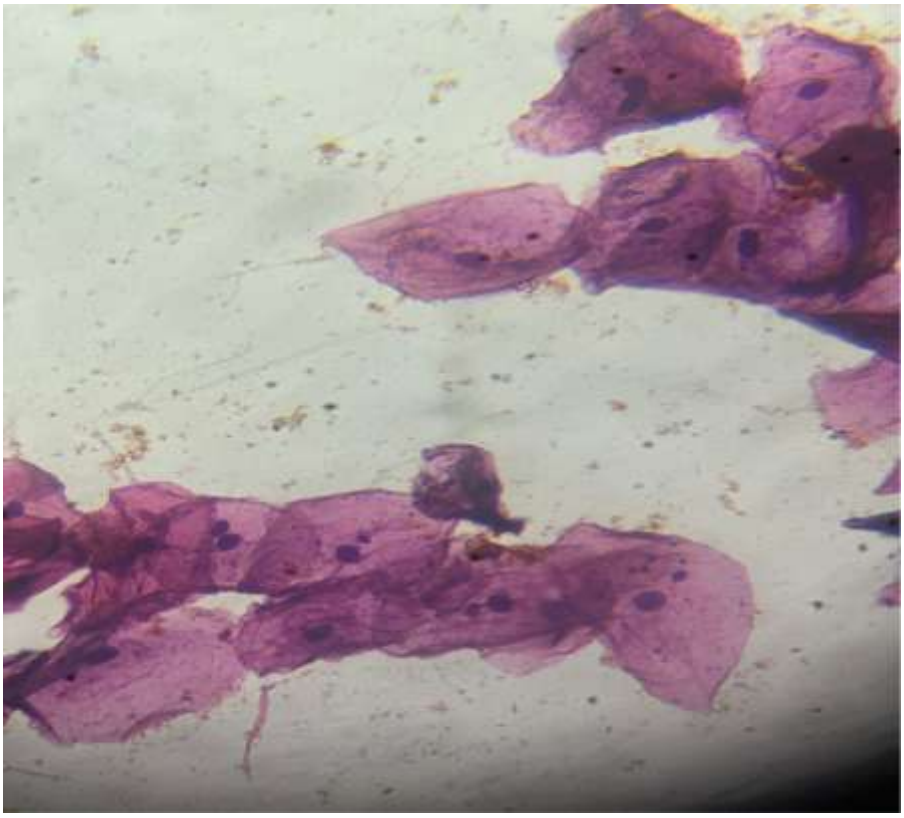

Figure 4: Comparative photomicrograph illustrating marked cytogenetic alterations in tobacco users relative to controls (×400 magnification).

Statistical Analysis

Table 4.2: Association Between Tobacco Use and Presence of Micronuclei

Group	MN Present	MN Absent	Total	χ^2	df	p-value
Tobacco Users	100	0	100	120.93	1	<0.001
Non-Tobacco Users	7	43	50			
Total	107	43	150			

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There was a statistically significant association between tobacco use and the presence of micronuclei in oral exfoliated cells ($\chi^2(1) = 120.93$, $p < 0.001$). Micronuclei were observed in all examined high-power fields among tobacco users, whereas the majority of fields in non-tobacco users showed no detectable micronuclei. The findings indicate that tobacco users exhibit a significantly higher prevalence of micronuclei in oral exfoliated cells compared to non-tobacco users.

Regression Analysis

Regression analysis was performed to determine the relationship between tobacco use and micronucleus frequency in oral exfoliated cells. The analysis showed that tobacco use was a significant predictor of increased micronucleus formation in oral epithelial cells. Participants who used tobacco demonstrated a significantly higher micronucleus count compared to non-tobacco users ($p < 0.05$). The findings indicate that exposure to tobacco contributes substantially to genotoxic damage and chromosomal instability in the oral mucosa. The regression model further suggests that increased duration and frequency of tobacco use may be associated with a corresponding rise in micronucleus frequency. Therefore, tobacco use can be considered an important risk factor for the development of oral cellular abnormalities and possible progression to oral cancer.

Table 4.3: Regression Analysis Showing Relationship Between Tobacco Use and Micronucleus Frequency.

Variable	Regression Coefficient (β)	Standard Error	t-value	p-value
Tobacco Use	0.82	0.07	11.71	<0.001

DISCUSSION

The current investigation evaluated the genotoxic effects of tobacco exposure by comparing and assessing the prevalence of micronuclei in oral exfoliated cells between tobacco users and non-users. According to the results, micronuclei were found far more frequently in tobacco users than in non-users, suggesting that tobacco use is linked to increased chromosomal damage and genomic instability. This is in agreement with the work of Gopal and Padma (2018) in the evaluation of cytogenic damage of micronuclei in oral exfoliated buccal cells in tobacco users.

Many carcinogenic and mutagenic substances included in tobacco products, including nicotine, polycyclic aromatic hydrocarbons, nitrosamines, and heavy metals, are responsible for the elevated micronuclei count among tobacco users. These compounds produce reactive oxygen species (ROS), which lead to chromosomal breakage, oxidative DNA damage, and mistakes in the creation of mitotic spindles, all of which eventually result in the formation of micronuclei.

This data is in line with results from a number of other researchers such as Motgi et al (2014), Heddle (2019), and Kranthi et al (2024) that consistently found higher micronuclei frequency in those who were exposed to tobacco in different ways, such as smoking cigarettes, chewing tobacco, or using snuff. These results' consistency supports the micronucleus assay's dependability as a sensitive biomarker for genotoxic damage early detection.

Micronuclei are much less common in non-smokers, which indicates that they have had less exposure to genotoxic substances and that their genomes are more stable. However, additional environmental and lifestyle factors like alcohol use, air pollution, occupational exposure, dietary deficiencies, infections, and aging may be connected to the small occurrence of micronuclei seen in certain non-tobacco users.

Additionally, individual vulnerability, nutritional state, dental hygiene, genetic predisposition, and the length, frequency, and kind of tobacco use can all affect the prevalence of micronuclei in tobacco users. The cumulative nature of tobacco-induced genetic damage is shown by the correlation between prolonged and heavy tobacco exposure and gradually increased micronuclei production.

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The results of this study highlight the clinical utility of cytogenetic monitoring in tobacco users, especially for early detection of persons at high risk for oral cancer and other potentially malignant illnesses. Timely intervention and prevention of disease progression are made possible by early identification using non-invasive methods like mouth exfoliative cytology.

Notwithstanding its advantages, the study might have certain drawbacks, such as a limited sample size, potential bias in selection, and dependence on self-reported tobacco use history. Furthermore, future research should thoroughly control for confounding variables such alcohol use, food habits, and environmental exposures as they may affect the incidence of micronuclei.

Overall, the study highlights the necessity of comprehensive tobacco control efforts and supports the use of micronucleus test as a straightforward, dependable, and affordable screening technique for evaluating the genotoxic consequences of tobacco.

CONCLUSION

In conclusion, the purpose of this study was to evaluate genotoxic damage by comparing the occurrence of micronuclei in oral exfoliated cells between tobacco users and non-users. The results showed that compared to non-tobacco users, tobacco users had a much higher prevalence of micronuclei. This suggests a clear correlation between increased chromosomal damage in oral epithelial cells and tobacco consumption.

Because nicotine products include several dangerous compounds that can damage DNA, they are genotoxic and carcinogenic, as seen by the higher frequency of micronuclei seen in tobacco smokers. According to the information now available, tobacco use is a significant risk factor for the emergence of oral cancer and other potentially harmful conditions.

The decreased frequency of micronuclei among non-smokers, on the other hand, indicates less exposure to genotoxic chemicals, highlighting the importance of lifestyle choices in preserving genetic stability. Thus, the micronucleus assay turns out to be a straightforward, affordable, non-invasive, and trustworthy biomarker for tracking populations at risk and for early diagnosis of genetic damage.

All things considered, this study underscores the significance of cytogenetic monitoring in tobacco users and the pressing need for preventative measures to lower the number of health issues associated with tobacco use.

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