

## Research Article

# Evaluation of micronucleus frequency in oral exfoliated buccal mucosa cells of smokers and tobacco chewers: a comparative study

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### ABSTRACT

**Background:** Oral cancer is one of the tenth most common cancers in the world and constitutes the third most important group of malignancies in India. Majority of these cancers are diagnosed at an advanced stage resulting in poor prognosis and survival rates among patients. Hence early diagnosis of oral cancers seems to be the need of the hour. Analysis of exfoliated buccal cell micronuclei (MN) is a sensitive method of monitoring genetic damage. The present study aims to compare the frequency of micronucleus in tobacco chewers and smokers and thus evaluate the genotoxic potential of these habits.

**Methods:** The study was conducted on 60 subjects, divided into 3 groups each of 20 subjects. Group 1: with history of chewing tobacco, group 2: with history of chewing tobacco and smoking, group 3: healthy subjects without any habits as controls. Oral exfoliated cells were obtained from buccal mucosa of the subjects, 2 slides were prepared from each subject stained with Giemsa and H&E stain respectively. Atleast 1000 cells were examined for each subject and micronuclei frequency was scored according to criteria of Tolbert et al.

**Results:** The mean number of micronuclei was  $18.5 \pm 9.5$  in tobacco chewers,  $19.1 \pm 9.2$  in chewers with smoking habit and  $8.2 \pm 5.6$  in controls. Bonferroni multiple comparisons amongst these three groups showed the mean difference of micronuclei to be highly significant when chewers and chewers with smoking habit were compared to controls. Similarly based on the duration of addiction, a highly significant difference was noted in no. of micronucleated cells in subjects addicted to tobacco for more than 15 years.

**Conclusions:** Tobacco can cause and increase the rate of nuclear anomalies in both smoking and smokeless forms. Thus oral mucosal micronuclei frequency can be used as a marker of epithelial carcinogenic progression.

**Keywords:** Exfoliated cells, Micronuclei, Genotoxic, Tobacco users

### INTRODUCTION

Oral cancer is one of the tenth most common cancer in the world and constitutes the third most important group of malignancies in India.<sup>1</sup> Nearly 1,30,000 Indians die due to tobacco related oral cancer. The International Agency for Research on oral cancer (IARC) regards chewing of tobacco to be a known human carcinogen, which has a role in multistage progression of oral cancer.<sup>2</sup>

Indians chew tobacco rather than smoke it, especially in rural areas due to which 75,000 to 80,000 new oral cancer cases had been identified in 2012 and these proportions are expected to rise further by 2025.<sup>2,3</sup> Majority of these cancers are diagnosed at an advanced stage resulting in poor prognosis and survival rate among patients. Hence the early diagnosis of oral cancer seems to be the need of the hour.

Analysis of exfoliated buccal cell micronuclei (MN) is a sensitive method of monitoring genetic damage in human population. First proposed by Stich et al this test still continues to gain popularity as a biomarker of genetic damage due to its low cost, minimal invasiveness and ease of storage and slide preparation.<sup>4,5</sup>

The micronucleus is defined as a microscopically visible round or oval cytoplasmic chromatin mass next to the nucleus.<sup>6</sup> They originate from aberrant mitoses and consist of acentric chromosomes that have failed to incorporate into daughter nuclei during mitosis, events thought to be associated with increased risk for cancer.<sup>7</sup> The frequency of occurrence of micronuclei is a measure of chromosome breakage in early cell divisions and the number of micronuclei is known to increase with carcinogenic stimuli, long before the development of clinical symptoms.<sup>8</sup>

Thus the present study aims to compare the frequency of micronucleus in tobacco chewers and cigarette smokers and thus evaluate the genotoxic potential of these habits.

## METHODS

This cross sectional study was carried out in Department of Pathology, L. N. Medical College, Bhopal, India. The study population comprised of a total of 60 subjects, divided into three groups - Group 1: comprising of 20 subjects with a history of chewing tobacco, Group 2: comprising of 20 subjects with a history of chewing tobacco and cigarette smoking and Group 3: comprising of 20 age and sex matched healthy subjects without any habits as controls. A written informed consent was taken from all the subjects. The study was approved by the institutional ethical committee.

### Inclusion criteria

The inclusion criteria for smokers and tobacco chewers were the use of cigarettes and/or tobacco for at least last six months.

### Exclusion criteria

- Subjects with a history of recent viral infection.
- Subjects with use of antibiotics within 2 months before sample collection.
- Subjects with recent history of exposure to potential genotoxic agents, including X rays, chemotherapy and potential occupational exposures.
- Subjects who are chronic alcoholics.
- Subjects with any oral pathological lesions

### Sample collection, staining and cytological analysis

Prior to sampling each subject was asked to rinse his mouth thoroughly with tap water. Oral exfoliated cells were scraped from the buccal mucosa of control and study group with a moistened wooden spatula. The

scraped cells were placed onto pre cleaned slides. 2 slides were prepared for each subject. One Slide was air dried and stained with geimsa and other wet fixed in 95% alcohol and stained with H & E stain. At least 1,000 cells were examined for each subject and MN frequency scored according to the criteria of Tolbert et al.

The suspected nucleus is required to meet the following criteria in order to be considered as Micronucleus: (a) rounded, smooth perimeter suggestive of membrane; (b) less than third the diameter of the main nucleus, but large enough to discriminate shape and color; (c) staining intensity similar to that of nucleus; (d) same focal plane as nucleus.

### Statistical analysis

Statistical analysis was done using SPSS version 20. The findings are presented as mean and standard deviation. To analyse the mean difference of micronuclei among the three groups, one way ANOVA was performed and p value of less than 0.05 was considered statistically significant.

## RESULTS

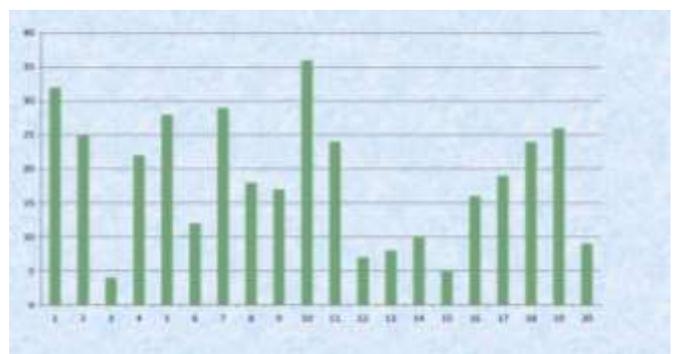
All the subjects included in the present study were males. No significant difference was observed regarding the age across the 3 study groups (Table 1).

**Table 1: Characterization of study subjects.**

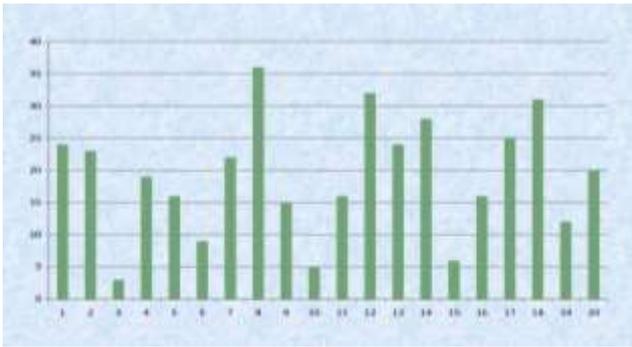
Groups	n=60	Age in years (Mean±2SD)
Chewers	20	44.3±12.1
Chewers with smoking habit	20	42.8±13.7
Controls	20	41.0±14.4

Chewers: Person chewing mixture of betel leaf, areca nut and tobacco. Controls : Non chewers non-smokers.

Figure 1-3 represents the frequency distribution of micronucleated cells in each of the 20 subjects across the 3 study groups.



**Figure 1: Frequency distribution of micronucleated cells in buccal mucosa smears of each of the 20 subjects (tobacco chewers).**

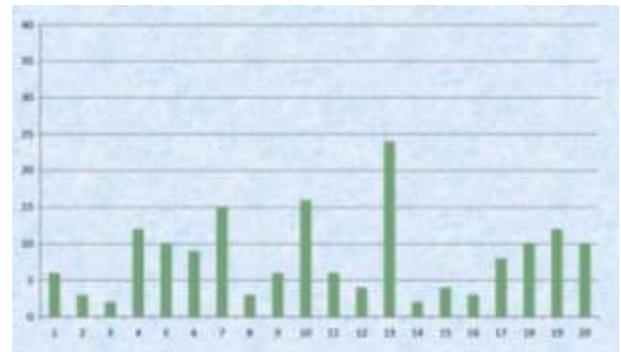


**Figure 2: Frequency distribution of micronucleated cells in buccal mucosa smears of each of the 20 subjects (tobacco chewers with smoking).**

The mean no. of micronucleated cells in the tobacco exposed group was found to be significantly higher as compared to the control group ( $p < 0.05$ ) (Table 2). Similarly the difference in the mean micronucleated cell count between the cases and controls was also highly significant ( $P < 0.001$ ).

Bonferroni multiple comparisons showed the difference in the mean micronucleated cell count to be highly significant when the tobacco exposed group was compared to the controls. However intragroup

comparisons amongst the two tobacco exposed groups (chewers and chewers with smoking habit) showed no significant difference in the mean micronucleated cell count (Table 3).



**Figure 3: Frequency distribution of micronucleated cells in buccal mucosa smears of each of the 20 subjects (controls).**

In an attempt to examine the impact of the duration of exposure of tobacco on micronucleus frequency a comparison was made between subjects having a period of addiction  $>15$  years and  $<15$  years. A highly significant difference was seen between the two groups (Table 4).

**Table 2: Comparison of mean no. of micronucleated cells across 3 study groups by one way ANOVA.**

Characteristic	Chewers	Chewers with smoking habit	Controls	P- value	S
No. of micronucleated cells/1000cells	18.5±9.5	19.1±9.2	8.2±5.6	0.000	S

All values in the three groups are reported as Mean±2SD.

**Table 3: Bonferroni multiple comparisons across 3 groups.**

		Mean difference	Standard error	Significance
Chewers	Chewers with smoking habit	0.550	2.9	0.997
Chewers	Controls	10.30	2.5	0.001*
Chewers with smoking habit	Controls	10.85	2.4	0.000*

\*The mean difference is significant at the 0.05 level.

**Table 4: Comparison of mean no. of micronucleated cells with duration of addiction.**

Characteristic	Period of addiction (<15 years)	Period of addiction (>15 years)	P- value	S
No. of micronucleated cells/1000cells	15.21±8.3	25.5±7.0	0.000	S

All values are reported as Mean±2SD.

## DISCUSSION

The use of the micronucleus test on exfoliated cells from oral epithelium with the aim of undertaking bio-monitoring on human populations exposed to genotoxic

agents was first proposed by Stich *et al.* The micronuclei (MN) assay is potentially an excellent biomarker to detect chromosome loss or malfunction of mitotic spindle which is caused by aneugenic mechanisms. The efficacy of this test for this purpose has been highlighted in many studies.<sup>7,9,10</sup>

Tobacco use (either by smoking or chewing) has harmful effects on Buccal mucosa.<sup>11</sup> The major toxic components of tobacco are nicotine, tar and polycyclic hydrocarbons. This study showed that tobacco in both smoking and smokeless forms has significant genotoxic effects on the buccal mucosa cells as evidenced by a higher MN frequency in tobacco chewers and tobacco chewers with smoking habit. These results are in full agreement with other studies.<sup>12-14</sup>

The synergistic effect from the habits of chewing and smoking tobacco on micronucleus induction in oral epithelial cells was investigated by Kassie *et al* the findings of which corroborate with our study.<sup>15</sup>

Furthermore the study also showed the micronucleus frequency to be significantly higher in persons with a longer duration of exposure to tobacco as observed by Caplash et al in his study.

The results obtained from this study in which occurrences of micronuclei in relation to the habits of smoking and chewing tobacco were investigated, the accumulated evidence indicates that the risk of this exposure is sufficient to discourage these habits and stimulate quitting them.

## CONCLUSION

Tobacco can cause and increase the rate of nuclear anomalies in both smoking and smokeless forms. Thus oral mucosal micronuclei frequency can be used as a marker of epithelial carcinogenic progression. Simplicity, accuracy, multipotentiality and large tissue applicability of the MN technology made it attractive in the past and will ensure a key role in the evaluation of mutagenicity and primary prevention in the future.

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*Ethical approval: The study was approved by the Institutional Ethics Committee*

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