ORIGINAL ARTICLE

# Evaluation of Micronucleus Assay in Oral Exfoliated Buccal Cells in Potentially Malignant Disorders and Oral Squamous Cell Carcinoma

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## ABSTRACT

**Aims and objectives:** Exposure to carcinogens can lead to genomic instability which can be evaluated through various biomarkers that predict if a potentially malignant disorder is likely to develop into a tumor. Micronucleated cell (MNC) is one such biomarker that is easily detected in the exfoliated buccal cells. Early detection would improve the survival rate, thus reducing the morbidity and mortality.

**Materials and methods:** A study sample of 45 subjects was divided into three groups: Group I had 15 healthy subjects as controls, group II had 15 cases of potentially malignant disorder (speckled oral leukoplakia, erosive oral lichen planus, and oral submucous fibrosis), and group III had 15 cases of oral squamous cell carcinoma (OSQCC). These were clinically and histopathologically diagnosed and the smears from the buccal mucosa were stained and observed for the frequency of MNC.

**Results:** We observed stepwise increase in the frequency of MNC from groups I to III. The frequency of MNCs among the groups was statistically significant.

**Conclusion:** Therefore the assay of MNCs can be reliably used as an internal predictor of genomic damage as well as that of malignant transformation in potentially malignant disorders (PMD).

**Keywords:** Genomic damage, Micronucleated cell, Oral squamous cell carcinoma, Potentially malignant disorders.

## INTRODUCTION

Cancer is one of the major global threats to public health.[1] The influence of smoking, chewing tobacco, and alcohol has usually been considered as a relevant confounding factor for oral squamous cell carcinoma (OSQCC).[2] Oral squamous cell carcinoma has a tendency to be detected at a later stage, which is detrimental to the patients because of its high mortality and morbidity rates. It is, therefore, important to reduce the burden of this devastating disease.[3] This OSQCC is generally preceded by premalignant lesion or condition,[4] which is now termed as potentially malignant disorders (PMD), since 2007 by the World Health Organization (WHO).[5]

The clinical significance and the biological behavior of these PMDs are unpredictable, as some of them if left untreated can progress to malignancy.[3,6] It would be of practical importance to identify the risk group among them.[7] Buccal cells are the first barrier for the inhalation or ingestion route and are capable of metabolizing proximate carcinogens to reactive products.[8] The carcinogens from smoking and chewing of tobacco mixtures may either cause mutation of germ cells and genetic damage, resulting in accumulation of heritable abnormal genes or may lead to mutation of somatic cells giving rise to the appearance of micronuclei (MN) (Figs 1 and 2) like bodies in exfoliated cells.[9] Micronuclei are extranuclear cytoplasmic bodies associated with chromosomal aberrations.[10] The formation of MN takes place in the basal layer of the oral epithelium,[11] where a rapid turnover of epithelial tissues brings the cells to the surface where they exfoliate.[2,12] The MN can be used as a biomarker to define chromosomal aberrations in exfoliated buccal mucosal cells which can predict oral cancer as well as the malignant transformation in PMDs.[8] The buccal cell MN assay was first proposed in 1983.[6,8]

Though histopathology of biopsied material is a gold standard in diagnosing cancers, biopsy being an

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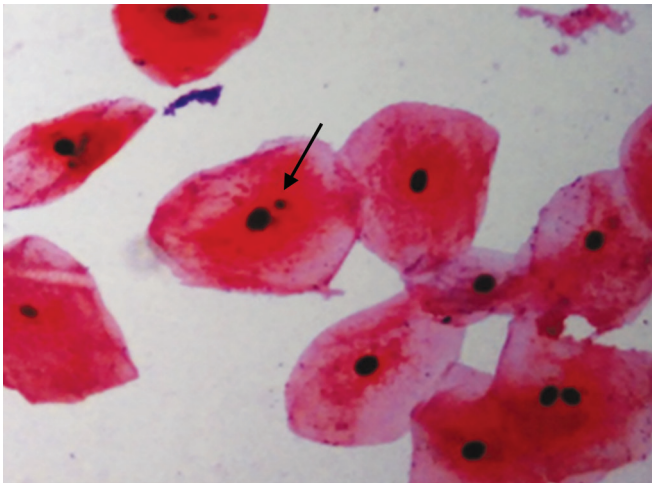
**Fig. 1:** Exfoliated epithelial cells with micronuclei
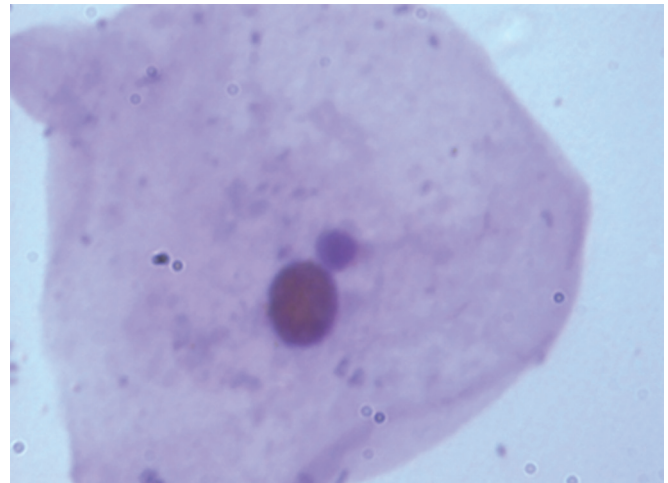($40\times$ magnification)



**Fig. 2:** Exfoliated epithelial cells with micronuclei
($100\times$ magnification)

invasive technique has limitations for some professionals and psychological implications for some patients. The buccal mucosa is an easily accessible tissue for sampling buccal cells in a noninvasive manner and does not cause undue stress to the subjects.[7,8] With this view in mind, the present study was carried out to assess the frequency of micronucleated cell (MNC) in oral exfoliated buccal cells in PMDs and oral cancer, which would help as a screening test for early diagnosis in individuals who are at a high risk of malignant transformation.

## AIMS AND OBJECTIVES

The present study is designed with the following aims:
- To evaluate the number of MNCs in oral exfoliated buccal cells from controls, PMDs, and OSQCC.
- Comparison of MN frequency between controls, PMDs, and OSQCC.
- To ascertain the reliability of MN as a diagnostic biomarker for early detection of OSQCC in high-risk group.

## MATERIALS AND METHODS

Forty-five patients with an age ranging from 25 to 72 years were selected for the study. After obtaining the informed consent from the patients, relevant case history was recorded, including their oral adverse habits, frequency, and duration. Detailed clinical examination was carried out.

The study samples were divided into three groups:
*Group I*: Includes 15 cases of normal healthy subjects as controls.
*Group II*: Includes 15 cases of histopathologically confirmed PMDs [Oral Speckled Leukoplakia (OSL) (n = 5), Erosive Oral Lichen Planus (EOLP) (n = 5), and oral submucous fibrosis (n = 5)].
*Group III*: Includes 15 cases of histopathologically confirmed OSQCC.

## Inclusion Criteria

*Group I*: Subjects having no obvious oral lesions, any habits of tobacco, areca nut, betel quid, any medications, and not exposed to diagnostic X-rays.
*Group II*: Patients with oral lesions histopathologically confirmed to be PMDs.
*Group III*: Patients with oral lesions histopathologically confirmed to be OSQCC.

## Exclusion Criteria

*Group I*: Subjects with the habits, oral lesions, and viral infections
*Groups II and III*: Subjects with treated cases of PMDs, OSQCC, and recurrent oral lesions.

## Collection of Exfoliated Cells

The selected subjects were asked to rinse their mouth gently with water to remove the residual food debris. Using a slightly moistened wooden spatula, oral mucosal cells were scraped from the lesional tissues and also from healthy buccal mucosa of control group. Fixed smears were stained with Papanicolaou stain commercially available with RAPID PAP™ kit. The smears were observed using research microscope under magnification of 40× for screening and counting and 100× magnification for confirmation of the MNCs. Zigzag method was followed for screening and counting the MNCs. From each slide, minimum of 1000 intact epithelial cells were screened and counted, which were expressed in numbers.

## Criteria used for Identification of Micronuclei by Tolbert et al[4]

The scoring criteria were based on the morphological status of the MN:
- Rounded smooth perimeter suggestive of a membrane

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**Table 1:** Mean age in the study sample

| Age (years) | Mean | Std. dev | SE of mean | Median | Min | Max |
|---|---|---|---|---|---|---|
| Group I | 44.93 | 11.46 | 2.96 | 46.0 | 27 | 65 |
| Group II | 41.07 | 10.65 | 2.75 | 40.0 | 26 | 58 |
| Group III | 56.00 | 10.43 | 2.69 | 60.0 | 36 | 72 |
| Overall | 47.33 | 12.39 | 1.85 | 49.0 | 26 | 72 |

**Table 2:** Gender and frequency of MNCs in the groups

| Gender | Groups | No. of subjects | Frequency of MNC | Total |
|---|---|---|---|---|
| Male | Control | 10 | 10 | 302 |
| | PMD | 11 | 94 | |
| | Oral cancer | 8 | 198 | |
| Female | Control | 5 | 5 | 191 |
| | PMD | 4 | 21 | |
| | Oral cancer | 7 | 165 | |

**Table 3:** Frequency of mean MNCs in the groups

| Groups | Mean | Std. dev | SE of mean | 95% CI for mean Lower bound | 95% CI for mean Upper bound | Min | Max | Kruskal–Wallis chi-square | p-value |
|---|---|---|---|---|---|---|---|---|---|
| I | 1.00 | 0.85 | 0.22 | 0.53 | 1.47 | 0 | 3 | 39.239 | <0.001* |
| II | 7.67 | 3.50 | 0.90 | 5.73 | 9.60 | 3 | 13 | | |
| III | 24.20 | 5.31 | 1.37 | 21.26 | 27.14 | 17 | 34 | | |

*Denotes significant difference

- Less than one-third the diameter of the associated nucleus, but large enough to discern shape and color
- Staining intensity similar to that of nucleus
- Texture similar to that of nucleus
- Same focal plane as nucleus
- Absence of overlap with, or bridge to the nucleus.

Nonparametric analysis was done using Kruskal–Wallis test and pairwise comparison of the groups was done using Mann–Whitney test.

## RESULTS

In our study, the mean age of group I was 44.93 years ± 11.46 SD, group II was 41.07 years ± 10.65 SD, and group III was 56.00 years ± 10.43 SD (Table 1). In this study, a total of 45 subjects were included, among which 29 cases were males (64.5%) and 16 were females (35.5%). In males the total frequency of MNC was 302 cells and in females 191 cells (Table 2). The frequency (%) of mean MNC in group I was 1 ± 0.85 SD, group II was 7.86 ± 3.50 SD, and that of group III was 24.20 ± 5.31 SD. The observed difference in mean MNCs among the groups was found to be statistically significant (p < 0.001) (Table 3).

On comparison of the mean differences among the groups, the MNCs were found to be highly statistically significant between groups I and II (p < 0.001), groups I and III (p < 0.001) as well as between groups II and III (p < 0.001) (Table 4).

## DISCUSSION

Oral squamous cell carcinoma is the most common malignancy, representing 90 to 95% of all oral malignancies.[13] Potentially malignant disorders, besides their clinical subtlety, are highly heterogeneous in their presentation; hence, it is desirable to identify high-risk individuals.[14]

**Table 4:** Comparison of the mean differences among the groups in the sample

| Groups (I) | Groups (J) | Mean difference (I – J) | Z | p-value | 95% CI for mean difference Lower bound | 95% CI for mean difference Upper bound |
|---|---|---|---|---|---|---|
| I | II | –6.667 | –4.680 | <0.001* | –10.04 | –3.30 |
| | III | –23.200 | –4.718 | <0.001* | –26.57 | –19.83 |
| II | III | –16.533 | –4.672 | <0.001* | –19.90 | –13.16 |

*Denotes significant difference

Smoking and chewing of tobacco mixtures are known to cause chromosomal breakage giving rise to the appearance of MN-like bodies in exfoliated cells,[8] which are much smaller than the principal nucleus. Micronuclei is situated around the main nucleus within the inner half of the cytoplasm with a diameter that is less than one-third of that of the main nucleus. Also, it is readily sourced from buccal mucosal cells and is clearly differentiated from binucleated cell as well as being easily identified by light microscopy.[15]

In the present study, group II consisted of young and middle-aged subjects that could be attributed to the ease of inculcation of the habits at an early age and thereafter. Group III showed increased incidence among elderly, as oral cancer develops after a long latency period. However, there was no correlation between age and number of MNCs in the sample. The findings are consistent with studies conducted by Saran et al.[13]

We also observed a male predominance, which may be due to the increased indulgence of different adverse habits in males, a semi-urban culture in addition to the socioeconomic background of subjects in the study group. This is in agreement with studies conducted by Sivasankari et al[16] and Dindgire et al.[17]

The frequency of the mean MNC observed was less in group I, moderate in group II, and high in group III. This can be explained by the gradual increase in the exposure to carcinogens and their genotoxic effects over a period of time. This finding correlated with that of studies done by Kamboj and Mahajan[6] and Anila et al,[18] where they found minimal MNC frequency in normal mucosal cells. The reason behind the moderately increased frequency of mean MNCs in group II could be due to the additive synergistic and antagonistic interactions between myriad compounds to which a cell is exposed. It also reflects the capacity of target tissues to activate procarcinogens into reactive species.[6] This result was similar to that of the studies on exfoliated cells in patients with PMD conducted by Kamboj and Mahajan[6] and Palve and Tupkari.[14] They concluded that there is highly significant increase in the mean MNC in PMD as compared to their control group.

High frequency of mean MNCs was found in OSQCC patients. This reflects the genomic instability associated with a malignant lesion. This could be attributed to the presence of a continuous habit with increased frequency and duration. The repeated exposure to cytotoxicants can result in chronic cell injury, compensatory cell proliferation, hyperplasia, and ultimately tumor development.[12] Our findings are in accordance with studies carried out by Devi et al,[4] Saran et al,[13] Palve and Tupkari,[14] and Sivasankari et al[16] where they found increased MNCs in oral cancer patients.

On comparison of the mean MNCs among the groups I and II, the observed mean difference was more in group II which was statistically significant (p < 0.001). On comparison between groups II and III the observed mean difference was less in group II than in group III, which was again statistically significant (p < 0.001). Also, the observed mean difference was found to be very high in group III than in group II. It portrays that the subjects in group II are high-risk individuals associated with precancerous nature, predisposing to malignant transformation.

A marked difference was observed on comparison between groups III and I. The age-related incidence suggests that time-dependent factors result in the initiation and promotion of genetic events that results in malignant change. It seems likely that the genomic damage is directly proportional to the exposure to carcinogens.

In this pairwise comparison, group III subjects appeared to be severely exposed to cytotoxic carcinogens for a longer period of time, giving rise to increased genomic instability that predisposes to malignant transformation either from preexisting PMDs or by itself. These findings are similar to studies conducted by Devi et al,[4] Palve and Tupkari,[14] and Sivasankari et al.[16]

Therefore, the present study showed a gradual increase in MNC counts from that of a normal mucosa to PMDs to carcinoma, suggesting a link between the presence of this biomarker, that is, MN with neoplastic progression.

## CONCLUSION

From this study the observations are:
- The frequency of MNCs was more in males when compared to females.
- Increased MNCs were observed in diseased than in healthy subjects.
- The frequency of MNCs in the PMD was high when compared to controls, suggestive of individuals at high risk for malignant transformation.
- The frequency of MNCs was observed to be very high in OSQCC when compared with PMD.
- There was a stepwise increase in MNCs frequency from normal mucosa to PMD to oral cancer, suggesting a link of this biomarker with malignant neoplastic progression.
- There was a direct correlation between the frequency of MN and the genomic damage.

We conclude that the presence of MNCs represents carcinogenic exposure on the target tissue which can be easily identified in exfoliated oral mucosal cells. Micronucleated cell scoring can be used as a biomarker to identify PMDs transforming to cancer at a much earlier stage and might specifically be exploited in the screening of high-risk population. This noninvasive approach may help in the screening of larger population as a sensitive and early diagnostic tool. Scoring MNC from exfoliated buccal cells is simple, easy to perform, and does not require special expertise.

We strongly endorse the view that evaluation of MNC in exfoliated oral mucosal cells is a reliable, economical, practical test that permits screening of large number of cells which can also be used in oral health centers. Micronuclei thus holds immense connotation in screening for PMDs and OSQCC.

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