

Comparative Study of Frequency of Micronuclei Using Papanicolaou Stain in Potentially Malignant Disorders and Oral Squamous Cell Carcinoma

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ABSTRACT

Background: Early recognition is crucial for successful treatment and good prognosis of oral cancer. A simple, non-invasive, and reasonably effective technique for initial evaluation of a suspicious oral lesion are exfoliative cytology. Micronuclei being structures presenting after genomic damage can be used as a screening tool in early detection of cancer. **Method:** A total of 60 subjects, 30 with potentially malignant disorders (PMD) (oral lichen planus and oral submucous fibrosis), 15 with oral squamous cell carcinoma (OSCC), and 15 with apparently normal mucosa, were included in the study. Cytological smears were collected from these groups and micronuclei were examined using Papanicolaou stain. One-way ANOVA was used to compare the mean micronuclei distribution among different study groups. **Results:** An increase in the frequency of micronuclei from normal to PMD to cancerous lesions was observed. Analysis has shown significant differences between various study groups ($P < 0.05$), with highest score observed in OSCC group, intermediary in PMD, and least in controls. **Conclusion:** The oral mucosal micronuclei can be used as a marker of epithelial carcinogenic progression.


Key words: Lichen planus, micronuclei, oral potentially malignant disorders, oral squamous cell carcinoma, Papanicolaou stain

INTRODUCTION

Cancer is considered as one of the most common life-threatening diseases affecting the mankind. It is estimated that oral cancer is the sixth most common cause of mortality and highest incidence is seen in the Indian population.^[1] The term oral cancer is nearly synonymous with oral squamous cell carcinoma (OSCC) because of its great numerical dominance.^[2] Most of the oral cancers are preceded by the potentially malignant disorders (PMD) that give a warning of risk and provide an opportunity

for detection and preventive measures.^[3] The possibility of malignant transformation of PMD is reported to be between 6.6% and 36.4%.^[4] Early diagnosis may improve the survival and decrease the morbidity of patients.^[3] Oral cancer is attributed to complex karyotypes that include many chromosomal deletions, translocations, and structural abnormalities.^[5]

Micronuclei can be used as an indicator of genotoxic exposure as shown in the study carried out by Hedde *et al.* (1983).^[6] Micronucleus can be defined as a microscopically visible, round-to-oval cytoplasmic chromatin mass next to the nucleus. These are extranuclear cytoplasmic bodies formed by the exclusion of chromosome fragments or whole chromosomes lagging at mitosis. Micronuclei being structures presenting after genomic damage in buccal mucosal cells are good indicators of chromosomal alterations in cytological samples.^[3]

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The study was undertaken to ascertain the frequency of Micronuclei in oral exfoliated cells of normal individuals, patients with PMDs and OSCC and to assess the role of Micronuclei in diagnosing malignant transformation of PMD.

MATERIALS AND METHODS

A retrospective study was conducted with a sample size of 60 subjects which included 30 subjects with PMD (oral submucous fibrosis and oral lichen planus), 15 subjects with OSCC, and 15 subjects with normal mucosa.

Inclusion criteria

Patients with apparently normal mucosa were included in the study.

Clinically and histopathologically diagnosed cases of as follows:

1. Oral submucous fibrosis,
2. Oral lichen planus, and
3. OSCC.

Exclusion Criteria

1. Patients detected with malignancies other than OSCC.
2. Patients with a history of malignancy and under treatment for malignancy (surgery, chemotherapy, and radiotherapy).
3. Patients with metastatic tumors in jaws from systemic malignancies.

Sample collection

Collection of exfoliated cells^[8]

Subjects were asked to rinse their mouth gently with tap water before the start of the procedure. A slightly moistened wooden spatula was used to obtain the smear of exfoliated cells from the oral cavity (buccal mucosa in control group). Oral mucosal cells for obtaining the smear were scraped from the margins of the lesion. Pre-cleaned microscopic slides were used for smear preparation on which the cells were coated and fixed in alcohol for 15 min.

Cytological preparation and evaluation

Papanicolaou technique for staining of the smears was used. Screening of each slide was made in a Zigzag manner from one end to the other end of the slide. From each slide, 200 cells were examined using low magnification (10) for screening and high magnification (40) for counting of micronuclei under the light microscope.

Scoring criteria

The criteria developed by Tolbert *et al.* which was used for counting the micronuclei, as follows:^[7]

1. Rounded smooth perimeter suggestive of a membrane.
2. Less than a third the diameter of associated nucleus, but large enough to discern shape and color.
3. Staining intensity similar to nucleus.
4. Same focal plane as nucleus.
5. Texture similar to nucleus.
6. The absence of overlap with or bridge to the nucleus.

Only those structures fulfilling the above-mentioned criteria were recorded as micronuclei.

RESULTS

An increase in the frequency of micronuclei from normal to PMD to cancerous lesions was observed [Figures 1 and 2].

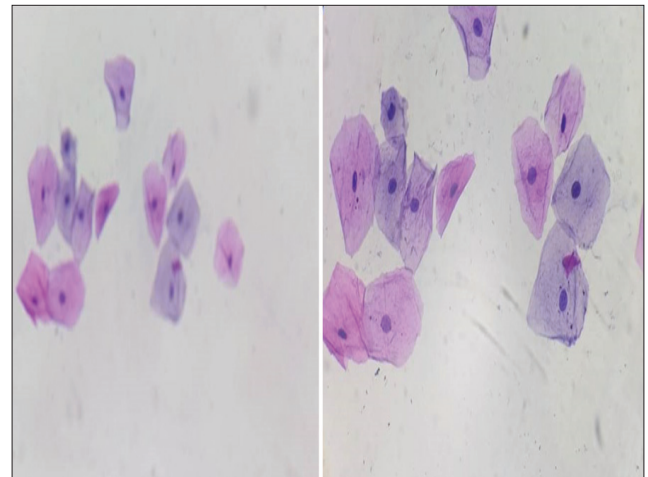


Figure 1: Photomicrograph showing micronuclei in oral potentially malignant disorders by Papanicolaou stain (10 and 40)

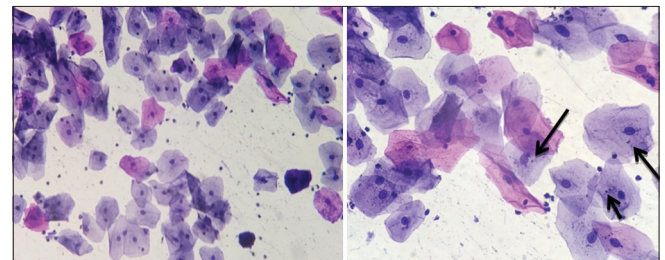


Figure 2: Photomicrograph showing micronuclei in oral squamous cell carcinoma by Papanicolaou stain (10 and 40)

Table 1: Age distribution among the groups

| Group | n | Minimum | Maximum | Mean±SD | P value |
|---------|----|---------|---------|------------------|---------|
| Control | 15 | 25.00 | 60.00 | 36.2000±11.79104 | P<0.001 |
| PMD | 30 | 21.00 | 60.00 | 40.1333±11.05701 | |
| OSCC | 15 | 39.00 | 64.00 | 50.4667±9.65747 | |

SD: Standard deviation, PMDs: Potentially malignant disorders, OSCC: Oral squamous cell carcinoma

Table 2: Gender distribution among the groups

| Group | Frequency (%) | P value |
|----------|---------------|---------|
| Controls | | |
| Male | 6 (40.0) | P<0.01 |
| Female | 9 (60.0) | |
| Total | 15 (100.0) | |
| PMD | | |
| Male | 21 (70.0) | |
| Female | 9 (30.0) | |
| Total | 30 (100.0) | |
| OSCC | | |
| Male | 13 (86.7) | |
| Female | 2 (13.3) | |
| Total | 15 (100.0) | |

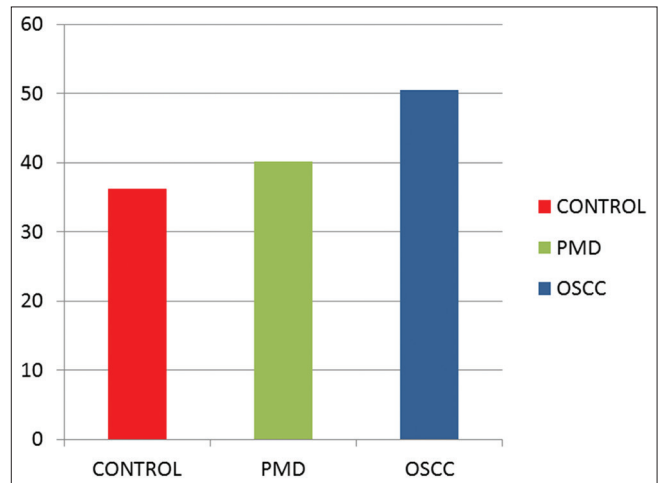
PMDs: Potentially malignant disorders, OSCC: Oral squamous cell carcinoma

One-way ANOVA test was performed among subjects of different age groups and found mean age distribution among controls, PMD, and OSCC was 36.2, 40.1, and 50.4 years respectively, the difference being statistically significant ($P < 0.05$) [Table 1 and Graph 1].

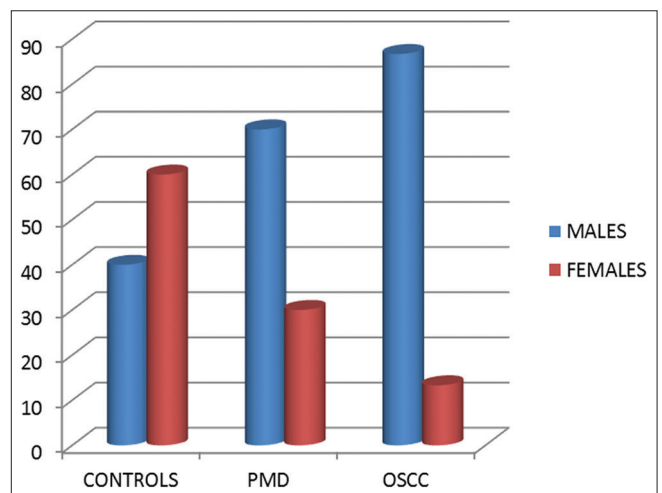
Gender distribution among different groups have shown 40% males and 60% females in controls, 70% males and 30% females in PMD, and among OSCC group 86.7% were male and 13.3% were female. Pearson’s Chi-square test was done between gender distribution among these groups, and a statistically significant ($P < 0.05$) correlation was observed [Table 2 and Graph 2].

Mean distribution of micronuclei among controls, PMD, and OSCC was 19.4, 32.0, and 56.7, respectively, and a one-way ANOVA was computed to estimate the mean micronuclei distribution among different study groups. This difference was significant statistically ($P < 0.05$) [Table 3 and Graph 3].

The results analyzed with *post hoc* test between controls and PMD ($P = 0.00$), controls and OSCC ($P = 0.00$), and PMD and OSCC ($P = 0.00$)



Graph 1: Age distribution among the groups



Graph 2: Gender distribution among the groups

were statistically significant and insignificant among potentially malignant groups (0.927) [Table 4].

DISCUSSION

OSCC is the most common cancer and accounts for 90% to 95% of all oral malignancies. Exfoliative cytology is a valuable tool for mass screening purposes, with a sensitivity of 94%,

Table 3: Distribution of micronuclei among the groups

| Group | n | Mean | Std. deviation | Std. error | 95% confidence interval for mean | | ANOVA |
|----------|----|---------|----------------|------------|----------------------------------|-------------|----------------------|
| | | | | | Lower Bound | Upper Bound | |
| Controls | 15 | 19.4000 | 3.66060 | 0.94516 | 17.3728 | 21.4272 | F=320.535 P<0.001 |
| PMD | 30 | 32.0000 | 3.53309 | 0.64505 | 30.6807 | 33.3193 | |
| OSCC | 15 | 56.7333 | 5.53517 | 1.42917 | 53.6681 | 59.7986 | |

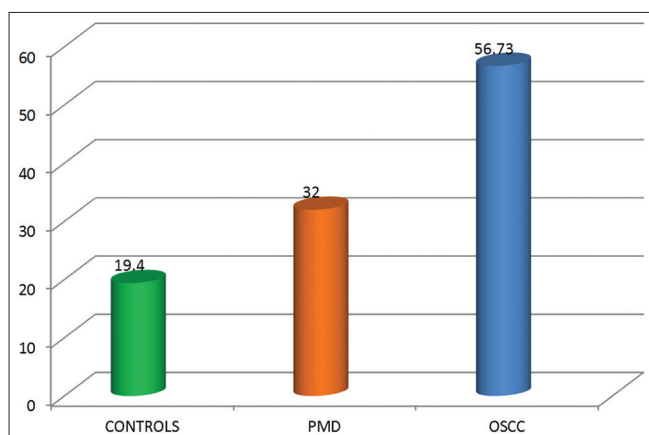
PMDs: Potentially malignant disorders, OSCC: Oral squamous cell carcinoma

Table 4: Mean difference of micronuclei among different groups

Dependent Variable: Micronuclei (Tukey HSD)

| (I) Type | (J) Type | Mean difference (I-J) | P value | 95% confidence interval | |
|---------------|---------------|-----------------------|---------|-------------------------|-------------|
| | | | | Lower bound | Upper bound |
| Controls | Lichen Planus | 13.06667* | 0.000 | 17.0948 | 9.0385 |
| | OSMF | 12.13333* | 0.000 | 16.1615 | 8.1052 |
| | OSCC | 37.33333* | 0.000 | 41.3615 | 33.3052 |
| Lichen planus | Controls | 13.06667* | 0.000 | 9.0385 | 17.0948 |
| | OSMF | 0.93333 | 0.927 | 3.0948 | 4.9615 |
| | OSCC | 24.26667* | 0.000 | 28.2948 | 20.2385 |
| OSMF | Controls | 12.13333* | 0.000 | 8.1052 | 16.1615 |
| | Lichen Planus | 0.93333 | 0.927 | 4.9615 | 3.0948 |
| | OSCC | 25.20000* | 0.000 | 29.2282 | 21.1718 |
| OSCC | Controls | 37.33333* | 0.000 | 33.3052 | 41.3615 |
| | Lichen Planus | 24.26667* | 0.000 | 20.2385 | 28.2948 |
| | OSMF | 25.20000* | 0.000 | 21.1718 | 29.2282 |

*The mean difference is significant at the 0.05 level. OSMF: Oral leukoplakia, submucous fibrosis, OSCC: Oral squamous cell carcinoma



Graph 3: Distribution of micronuclei among the groups

specificity of 100%, and an accuracy of 100% in detection of OSCC.^[8,9] A variety of substances, including genotoxic agents and carcinogenic

compounds in tobacco, betel nut, and alcohol, induce the formation of micronuclei in oral exfoliated cells.^[10] Micronuclei formation can occur by two basic phenomena in mitotic cells, which are chromosome breakage and dysfunction of the mitotic apparatus. They can be formed from eccentric chromosomes, chromatid fragments, whole chromosomes, or chromatids that lag behind in anaphase and are left outside the daughter nuclei in telophase.^[11]

The difference in the mean percentage of Micronuclei in PMD and controls was found to be statistically significant. Similar results were noticed in Sangle *et al.*^[3] The possible illustration for Micronuclei formation in preneoplastic conditions includes chromosome loss or breakage, chromosomal aberrations, mitotic apparatus dysfunctions, aneuploidy, and genetic instability.

A statistically significant ($P < 0.05$) increase in the occurrence of micronuclei from control to PMD to OSCC group was observed. These observations indicate an increase in cytogenetic damage of the epithelial cells. This is in accordance with the studies conducted by Sangle *et al.*, Devi *et al.*, and Halder *et al.*^[8,12,13] The mean difference between the numbers of Micronuclei in PMD group and OSCC group was statistically highly significant ($P < 0.05$). This is similar to the study conducted by Gupta *et al.* (2011).^[14]

The study conducted by Casartelli *et al.* concluded that the gradual increase in micronuclei frequency from normal mucosal to pre-cancerous lesion to carcinoma proposed a link of this biomarker with neoplastic progression.^[15] There was stepwise increase found in the percentage of micronucleated cells and micronuclei from control to pre-cancer patients and from pre-cancer to cancer patients in the studies by Saran *et al* and Grover *et al.*^[16-17]

In the study conducted by Kumar *et al.*, the cells from OSCC lesions were obtained by mincing the biopsy tissue and preparing single cell suspensions from it.^[10] This is in contrast to the present study where the smears were made by exfoliative cytology.

A statistical insignificance ($P = 0.92$) was seen among the PMD groups (lichen planus and OSMF). This is attributed to the fact that the rates of chromosomal aberrations in both these lesions were almost similar as stated by the Sarode *et al.* (2004).

Limitations

1. Smaller sample size.
2. Staining and counting of micronuclei is a technique sensitive procedure.
3. We used only single observer.
4. Inclusion of different grades of OSCC might give an accurate result.

Future prospects

1. Estimation of micronuclei with various nuclear stains.
2. Relative assessment of micronuclei with various morphometric parameters

CONCLUSION

With the findings of the current study, it is apparent that there is a gradual increase in the frequency of micronuclei from normal to pre-cancerous to cancerous lesions. Although the

sample size was small, it can be concluded that oral mucosal micronuclei may be used as a marker for epithelial carcinogenic progression.

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