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Analysis of Premalignant and Malignant Changes in the Exfoliated Oral Mucosal Cells

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Abstract
Tobacco and its end products are considered as the main etiological factors responsible for the pathogenesis of oral cancer. Various cytological alterations have been reported with tobacco usage like change in the nuclear size, cell size, nuclear to cytoplasmic ratio, nuclear shape etc. Of these parameters, the nuclear size, cytoplasmic size and their ratio have been shown to be significant. The objective of the present study is to assess the effect of tobacco chewing on buccal mucosa of 75 patients using cytomorphometry. Comparison was made between cytoplasmic area, nuclear area and the ratio between nuclear to cytoplasmic area of buccal squames of 25 normal subjects (N), 25 subjects using some form of tobacco for more than 5 years but not having any oral lesion (H) and 25 patients having habit of using tobacco for more than 5 years and having tobacco associated oral lesion (L). The statistical analysis of the result showed variation in the cytoplasmic area, nuclear area and ratio between nuclear and cytoplasmic area in the buccal smears taken from Normal, Habit and Lesional groups. Application of quantitative techniques to cytological smears could possibly improve the diagnostic value of oral exfoliative cytology.
Keywords: *Cytomorphometry, exfoliated mucosal cells, premalignant lesions.*

Introduction

The incidence of the head and neck cancer varies among different populations within the country and also among different population around the world. Oral cancer is the major health problem in the Asian subcontinent.[1] Enviormental factors play an important role in the pathogenesis of oral cancer predominantly, chewable and smoked tobacco. In the early stage, oral cancer appears as a premalignant lesion. Although, clinical lesions are seen before the malignancy develops but at times the tumor might develop in the normal appearing mucosa. The prognosis of the patients can be improved by early diagnosis and prompt treatment of the incipient lesions.[2]

Exfoliative cytology is a painless, simple, non invasive procedure generally popular as an oral cancer screening tool.[3] The quantitative exfoliative cytological procedures increases the chances for early detection of premalignant and malignant lesions. Cytomorphometry, a quantit-ative parameter, includes the study of nuclear size, nuclear shape, cell size, nuclear cytoplasmic ratio, nuclear texture, nuclear discontinutity and optical density. Amongst these, alterations in the nuclear and cellular size have shown significant changes in premalignant and malignant lesions. Exposure to stimuli such as tobacco enhances the proliferation of mucosal cells. [4]

The present study was carried out to study the morphological changes in the exfoliated buccal mucosal cells which will help us in differentiating the normal mucosa from the lesional mucosa.

Material and Methods

The material for the study was oral cytological smears obtained from the mucosa of healthy individuals and tobacco users.

The study group consisted of 75 (21-75 years) patients divided into three groups:

Group A: 25 Normal healthy subjects (N) without tobacco chewing habit and without any lesion. (Fig 1)

Group B: 25 Patients with a habit of consuming tobacco without any visible mucosal changes (H). (Fig2)

Group C: 25 Patients with the habit of consuming tobacco with tobacco associated lesion on buccal mucosa (L). (Fig 3)

The detailed information about the habit of tobacco chewing (duration, site and frequency) was recorded for each individual. Anemic patients (female patients with the hemoglobin concentration of less than 11 mg/dL and male patients with the hemoglobin concentration of less than 12 mg/dL), patients with the history of systemic disease and patients under medication were excluded from the study. To undertake the study ethical clearance was obtained from the institutional ethical committee. The procedure was described to the patient and a written consent was also obtained. Patients were made to rinse their mouth to remove the debris and the mucosa was cleaned with the swab dampened saline. The site of the smear for group A and group B was apparently normal appearing mucosa and site for group C was lesional mucosa. Oral smears were collected with the cytobrush moistened with normal saline. A gentle scrapping motion and a moderate pressure was applied to scrape off the cells from the Group A and Group B. In group C, if possible the entire lesion was scrapped off. Otherwise the most representative site was scrapped. The scrapings were smeared on two plain glass slides and fixed immediately in 95% methyl alcohol for one hour. All the smears were stained with the Papanicolaou stain. (Fig 4)

All the sections were examined under x200 magnification using Olympus BX51 light microscope and 100 cells were selected in each smear randomly. Well defined cells were identified and photographs of the same were taken using Olympus Live View Digital SLR Camera Olympus E-330. The photographs were analysed using Image Pro Express 6.0 for windows, (Media Cybernetics) and only well defined cells were included in the study. Mean values of Nuclear area and cellular area were calculated for every case. The nucleus and cell outlines were traced on screen with the help of trace wand tool and the software calculated the area automatically. (Fig 5) Nuclear to cellular ratio (N:C ratio) was calculated for the same cells.

Analysis of variance was performed for three groups to compare the mean cellular area, nuclear area and N:C ratio. Multiple comparision test Tukey-HSD procedure was applied to compare the mean values between the groups. The *P* value <0.001 was considered to be significant.

Results

All the subjects in the study group, except the group A (normal healthy subjects) were in the habit of consuming tobacco in any form for more than five years and minimum of 5-10 times daily.

The cellular area, nuclear area and the ratio of nuclear to cellular area values were plotted separately.

Nuclear Area: The nuclear area was found to gradually decrease from Group A to Group C and Group B. A statistically significant difference ($p<0.001$) was found between the mean values of different groups.

Cellular Area: The cellular area was found to gradually decrease from Group A to Group B to Group C. No statistically significant difference was found between the groups.

Nucleus/Cytoplasmic Area: The N:C ratio was found to gradually increase from Group A to Group B to Group C. A statistically significant difference was found between the groups.

Figure 1: Normal healthy subjects (Group A) without tobacco chewing habit and without any lesion.

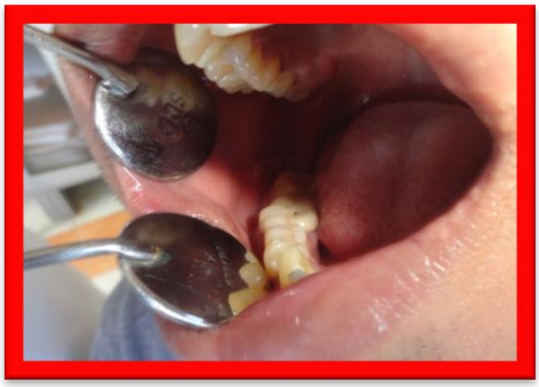

Figure 2: Patients with a habit of consuming tobacco without any visible mucosal changes (Group B).

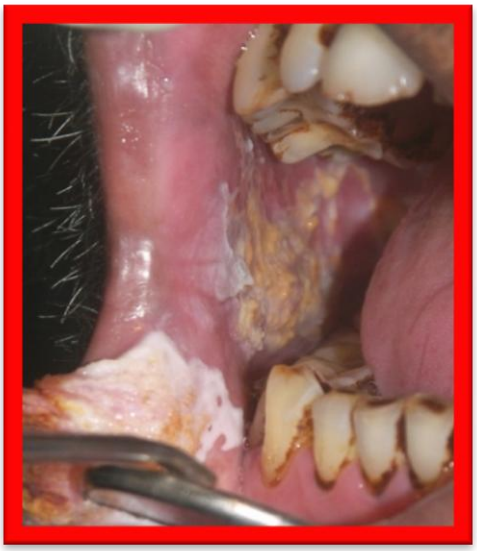

Figure 3: Patients with the habit of consuming tobacco with tobacco associated lesion on buccal mucosa (Group C).

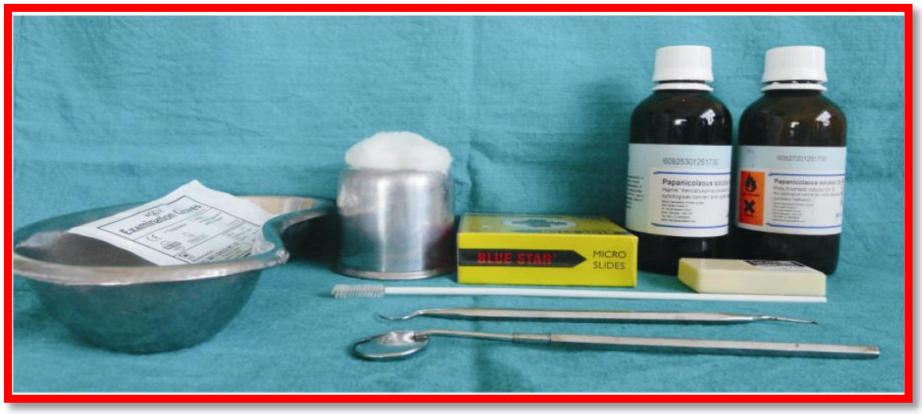

Figure 4: Figure showing material used for collecting and staining the smear. PAP stain, slides, cytology brush and 95% ethanol.

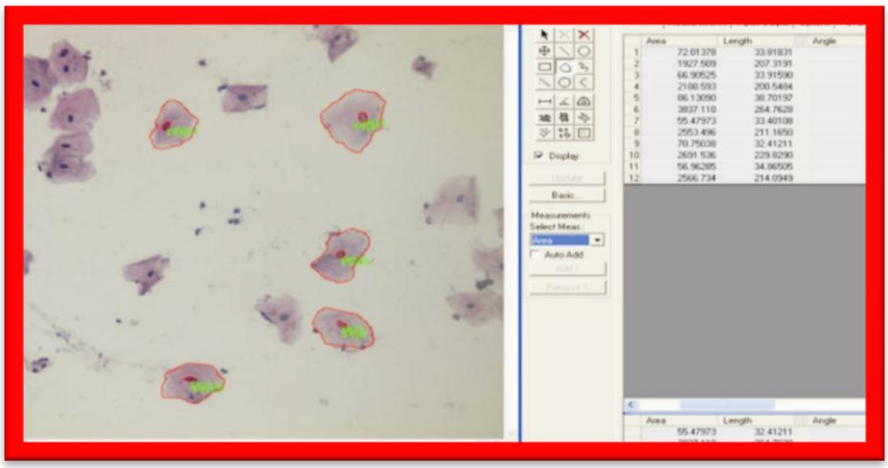

Figure 5: Cytomorphometric analysis done using Image Pro Express.

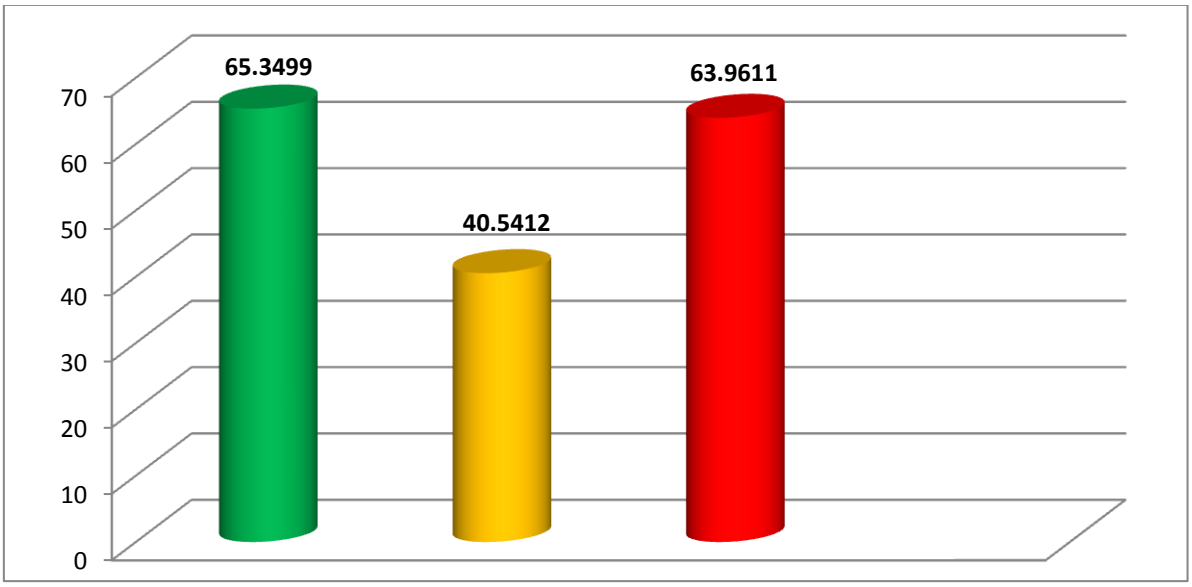

Figure 6: Mean value of nuclear area among the three groups.

F=29.967,p<0.001 ANOVA

Lesion vs Habit: p<0.001 Tukey HSD

Normal vs Habit: p<0.001 Tukey HSD

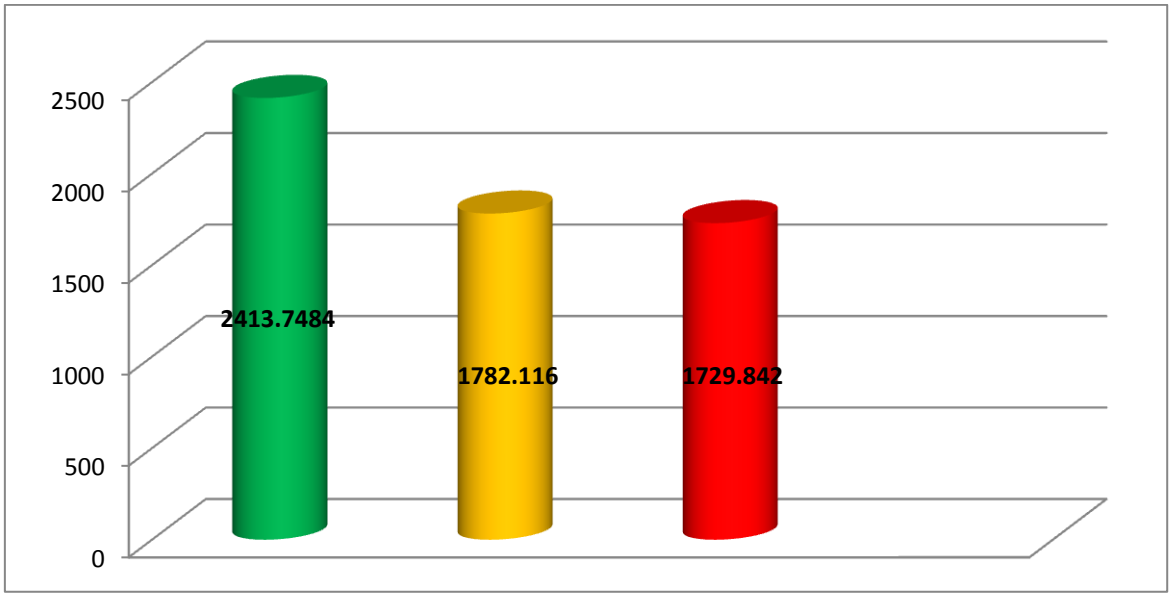

Figure 7: Mean value of the cellular area among the three groups.

F=2.065,p<0.134 ANOVA

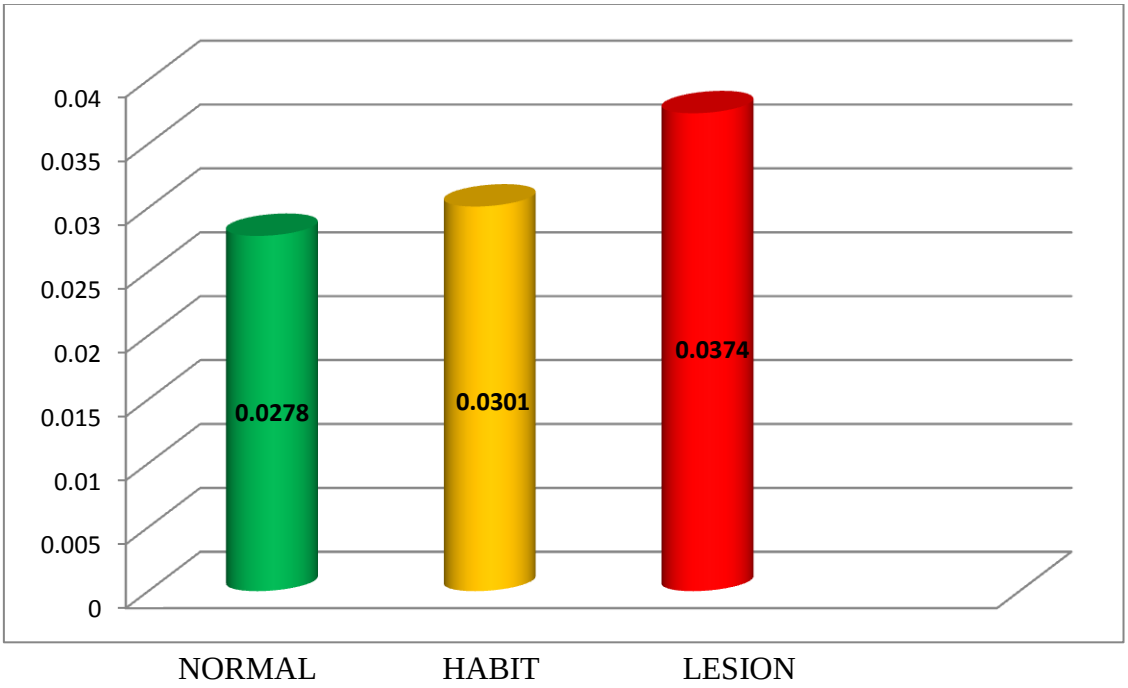

Figure 8: Mean value of the nuclear cellular area ratio among the three groups.

F=11.002,p<0.001 ANOVA

Lesion vs Normal: p<0.001 Tukey HSD

Lesion vs Habit: p=0.003 Tukey HSD

Discussion

Tobacco is consumed in a variety of ways. Tobacco consumption is basically categorized into two major forms- smokeless (chewing tobacco and snuff) and smoked (cigarettes, pipes, bidi and cigars). All these forms are known to cause oral cancer which is the sixth most common malignancy in the world. (Parkin *et al.*, 2000). [5] Lesions associated with the smokeless tobacco category are generally seen in the buccal or alveolar surface, mainly in the area where the quid is held.[6]

Despite the recent advances in the treatment modalities, the prognosis of the patients with Oral Squamous Cell carcinoma is only 50 %. (SEER, 1998). Lack of early detection and prevention is the most important factor associated with the poor survival rate of these patients. [3] Cellular changes are found to occur much before than the clinically visible lesion. Therefore, detection of the high risk premalignant lesion followed by the intervention at an incipient stage can probably reduce the mortality rate of such patients. High risk patients and patients with the habit of consuming any form of tobacco should be screened at regular intervals to detect the disease at an early stage.[7]

Exfoliative cytology is considered to be the most popular oral cancer screening tool. It is a painless, non invasive procedure and causes very little discomfort to the patients. Montogomery and Cowpe et al had pointed out that the principle of exfoliative cytology can improve the diagnosis of oral cancer.[8],[9]

Alteration in the cytological pattern of the exfoliated cells depends upon the changes taking place within the epithelium.[10],[11]

Quantitative oral exfoliative cytology techniques evaluates various parameters such as nuclear size, cell size, nuclear to cytoplasmic ratio, nuclear shape, nuclear discontinuity, optical density and nuclear texture to confirm the diagnosis. [4]

Amongst these parameters the nuclear-cellular size and ratio are found to significant in evaluating oral lesions.[12],[13]

Various studies have been done to evaluate the efficacy of nuclear- cellular area and N:C ratio in detecting malignant changes in exfoliated cells. It has been observed that the gradual deccrease in the cell diameter from normal-dysplastic to carcinomatous lesion and an increase in the nuclear area from dysplastic to carcinoma can be considered as early indicators of a malignant change.[14],[15]

In the present study comparision of cytological area showed a gradual decrease in the area from group A to group C. This decrease in cell size from group A to group C could be an early indication of malignant change as reported by Cowpe et al in 1985and Einstien et al in 2005.[14],[15]

The N/C ratio increased from group A to group C which indicates changes taking place in the nuclear content. Such changes are generally observed in active replicating cells.[16] Similar findings were also reported by Cancado et al 2004.[17]

The nuclear area is found to decrease in group B when compared to group C. This finding indicates cell shrinkage and pyknosis caused due to apotosis and subclinical keratinization. Prolonged usage of tobacco are found to induce such changes in the oral mucosa. These are the early indications of genotoxic effect of tobacco.

Conclusion

Application of quantitative techniques to smears obtained from oral premalignant and malignant lesions and suspicious area with clinical lesion, can possibly improve the diagnostic value of oral exfoliative cytology. However, evaluation of a greater number of cases is essential to establish the cut-off values of these parameters (*i.e.*, sensitivity and specificity) that can be used as definitive indicators.

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