Original Research Article

Cytomorphometric analysis of oral squames Tobacco Smoker's using oral brush biopsy: An exfoliative cytological study

Rakshith Shetty[1], Somnath Mukherjee[2], Neerav Dutta[1], Divesh Kumar Bhagat[3], Sushma K N[4], Vijayendra Pandey[5*]

[1]Senior Lecturer, Department of Oral pathology, Vananchal Dental College and Hospital, Garhwa, Jharkhand, India
[2]Senior Lecturer, Department of Oral and maxillofacial Sugery, Vananchal Dental College and Hospital, Garhwa, Jharkhand, India
[3]PG Student, Department of Oral Pathology, Vananchal Dental College and Hospital, Garhwa, Jharkhand, India
[4]Senior Lecturer, Department of Oral and maxillofacial Sugery, Vananchal Dental College and Hospital, Garhwa, Jharkhand, India
[5]Reader, Department of Periodontology, Vananchal Dental College and Hospital, Garhwa, Jharkhand, India
[*]Corresponding author email: **drvijayendrapandey@yahoo.com**

Abstract

Background: Tobacco is mostly regarded as one of the primary etiologic factor in causing oral cancer. Literature quotes studies have depicted the alteration of nuclear (NA) and cytoplasmic area (CA) induced by tobacco smoking and chewing. Also some authors have stressed on quantifying the role of cytomorphometric analysis in analysing these cellular alterations. Therefore, we evaluated the cytological and cytomorphometric changes in the oral squames using brush biopsy from buccal mucosa of tobacco users.
Materials and methods: 200 patients of age group of 40-65 years attending the hospital OPD with history of smoking were included in the study. The patients were broadly divided into two groups;

smokers (n=150) and Non-smokers (n=50). The cytological samples were taken from clinically normal appearing oral mucosa, stained and analysed using Image analysing software. Independent-Samples T Test and One-Way ANOVA were used to assess the level of significance.

Results: significant increase in Mean NA was observed in smokers as compared to non-smokers. Also, Mean CA decreased in smoker's group as compared to non-smokers. Likewise, N/C ratio was found to be significantly elevated in smokers group. Cytomorphometric parameter changes showed significant alteration with increasing duration of smoking.

Conclusion: Cytomorhometric analysis can be used to analyse the alterations occurring in cellular and nuclear level.

Key words

Cytomorphometry, Exfoliative, Smoking.

Introduction

Oral cancer prevalence shows a geographic variation in its incidence among different countries of the world and also among different regions within a country. This variation seen can be contributed to the environmental variations and deleterious oral habits prevalent in different areas of the world. Tobacco chewing and smoking is often regarded as one of the primary etiologic factor for causing oral cancer. There has been reported a higher incidence of tobacco associated oral cancer in Asian countries [1]. Little quotes very limited references quantifying the cytologic assessment of the effects of smoking upon normal oral mucosa [2, 3]. The effects of primary potential etiologic factors, such as tobacco, should be appreciated first of all for the quantitative exfoliative cytology to be of value in the detection of dysplasia like mucosal disorders. Some authors have used quantitative cytomorphometric analysis to mucosa smears of oral cavity [4]. Some studies in the literature have depicted the alteration of nuclear (NA) and cytoplasmic area (CA) induced by tobacco smoking and chewing [5]. Hence, we undertook this study to assess the cytomorphometric changes in the squames of buccal mucosa obtained using brush biopsy in tobacco smokers as compared to non-smokers.

Materials and methods

The present study was conducted Vananchal dental college. The patients attending the OPD with different oral complaints were included in this institutional based cohort study. A total of 200 patients of age group of 40-65 years with history of smoking were included in the study. Cytological smears were taken from the buccal mucosa of the individuals using a cytology brush. Patients with history of any systemic illness, known drug allergy and anaemia were excluded from the study. In smoker's group, a total of 150 patients were placed while non-smokers group comprised of 50 patients. Since the most common age group of occurrence of oral cancer of buccal mucosa is 40 years, the age group of 40-65 years was selected for the present study [6]. Only those patients were included in the smoker's group who fulfilled the underlying criteria:

- Patients who smoked a minimum of 20 cigarettes or 3 cigars per day for minimum of past 4 years.
- Patients who gave a negative history of any systemic illness.
- Patients who had not undergone any kind of radiotherapy and/or chemotherapy in the past one year.
- Patients who gave negative history of drug consumption with known effect on oral mucous membrane in past 6 months
- Patients without any clinically observed oral lesions.

All the cytological samples were taken from clinically normal appearing oral mucosa. Patients who had negative history of smoking from past 5 years where included in the non-smokers group.

All the patients were pre-informed about all the procedures and written consent were obtained from them. All the groups were categorized into three groups based on the duration of smoking as shown in **Table - 1**.

Table - 1: Grouping of patients in various groups divided according to duration of smoking.

Groups	Duration of smoking
Group 1	4-9 years
Group 2	10-15 years
Group 3	> 16 years

Mouth mirror was used to examine the oral cavity of the patients. Self rinsing of the mouth was done by the patients and smear was obtained by gently scrapping the buccal mucosa with a cytological brush. The cells were smeared on a glass slide and fixed with varying concentration on ethanol. Papanicolaou stain was then used to stain the fixed smear slides. Research microscope was then used to analyse the stained slides. Fifty cells were randomly selected and counted while moving the slide upside down and left to right. Analysis of the cytomorphometric features was done using Image analysing software. Boundary was drawn around the cell contour using digital cursors. All the results were analysed by SPSS software. Independent-Samples T Test and One-Way ANOVA were used to assess the level of significance.

Results

Total of 150 smokers and 50 non-smokers were included for the study. **Table - 2** showed the cytomorphometric analysis of the buccal mucosa of smokers and controls. Mean nuclear area in smokers and non-smokers group was 73.25 and 65.39 respectively. Similarly, mean cytoplasmic area and mean cytoplasmic/ nuclear ration in smokers was 2412 and 0.032 respectively. Cytomorphometric analysis of the buccal mucosa of smokers based upon duration of smoking exposure is highlighted in **Table - 3**. The cytomorphometric alterations were found to

increase significantly in groups with increased duration of smoking.

Table - 2: Cytomorphometric analysis of the buccal mucosa of smokers and controls.

Variable	Smokers	Non-Smokers
Mean Nuclear area	73.25	65.39
Mean Cytoplasmic area	2412	2502.25
Mean Cytoplasmic/Nuclear ratio	0.032	0.028

Discussion

One of the most readily available and accessible method of diagnosing cancer is the Cytodiagnosis. In 1943, Papanicolaou was the first person to introduce the clinical application of exfoliative cytology for the purpose of diagnosis [7]. Being non-invasive; it is often regarded as a simple, cheaper, readily available method which can be easily performed on any kind of oral lesion [8]. Tobacco, in both smokeless and smoke form, is often regarded as one of the most common factor which initiates dysplastic changes in oral mucous membrane. In a survey, it was recorded that approximately 33 % of the world's population consumes tobacco in any form. Tobacco habit forms one of the most common etiologic factors of oral cancer [9]. Hence, we evaluated the cytomorphometric changes in the squames of buccal mucosa of the smokers using exfoliative cytology.

We selected the buccal mucosa as the primary site for collection of smear sample. The reason for selecting this site in oral cavity was, as stated by Baric, et al. [10], that cells removed from buccal mucosa are the principal cells for assessing changes occurring in oral mucosa due to smoking. While comparing the various cytomorphometric parameters, we observed an increase in mean nuclear area and a decrease in mean cytoplasmic area and Mean Cytoplasmic/Nuclear ratio as shown in **Table - 2** and **Graph - 1.** The results of our study were in

correlation with the results of Ogden, et al. who also reported approximately 5 % increase in mean nuclear area of smokers when compared with control group [11]. Smoking induced cellular adaptations can be considered as the primary reason causing increase and decrease in nuclear area and cytoplasmic area respectively, as found in this study. Smoking often leads to cellular irritation thereby causing increased proliferative activity of the cells resulting in cellular morphologic changes [12]. While comparing the cytomorphic parameters in groups associated with increasing duration of smoking habit, significant alteration in the values of nuclear area, cytoplasmic area and N/C ratio was observed as shown in **Table - 3** and **Graph – 2 to Graph - 4**. Similar results were obtained by Ogden et al. who observed increase in cellular alterations in relation to the progressive use of number of cigarettes smoked per day [11]. The possible reason for this change associated with duration of smoking, as hypothesized by Franklin and Smite, can be due to the fact that that changes in the size of the nucleus relative to the

size of the cytoplasm possibly reflects the significant changes occurring in a cell at the morphologic level [13]. Babuta, et al while evaluating the cytological changes of the exfoliated buccal mucosal cells in smokers as compared to non-smokers concluded that a progressive increase in nuclear area and a decrease in cytoplasmic area alond with a decrease in N/C ratio would appear to be due to smoking tobacco [14]. Hande, et al. cytomorphometricaly assessed the effect of tobacco chewing on buccal mucosa and observed a progressive decrease in cellular diameter, increase in nuclear diameter along with an increase in nuclear-cellular diameter ration in smears from all tobacco users and concluded that there could be cause–effect relationship between tobacco and quantitative alterations [15]. The results of this study highlights on a smoking dose-dependent cytomorhometric changes in smokers. Therefore, there is need to develop early diagnostic methods to evaluate the cellular changes occurring due to tobacco smoking.

Table - 3: Cytomorphometric analysis of the buccal mucosa of smokers based upon duration of smoking exposure.

Variable	Smoking duration		
	Group 1 4-9 years	Group 2 10-15 years	Group 3 > 15 years
Mean Nuclear area	68.93	72.02	75.11
Mean Cytoplasmic area	2495.52	2329.64	2249.56
Mean Cytoplasmic/Nuclear ratio	0.029	0.031	0.034

Graph - 1: Cytomorphometric analysis of the buccal mucosa of smokers and controls.

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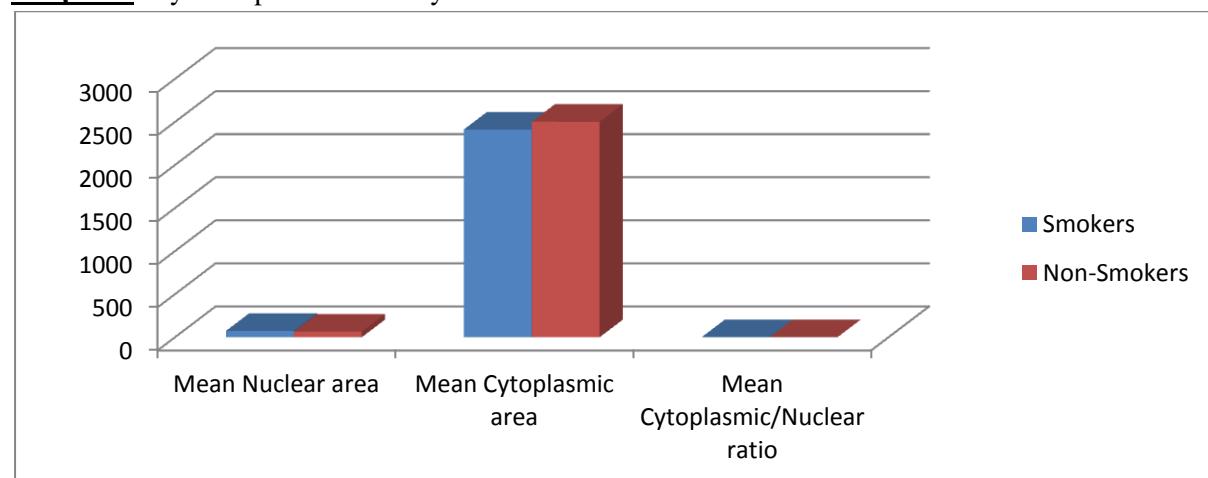
Graph - 2: Mean Cytoplasmic/ Nuclear ratio of the squames of buccal mucosa of smokers based upon duration of smoking exposure.
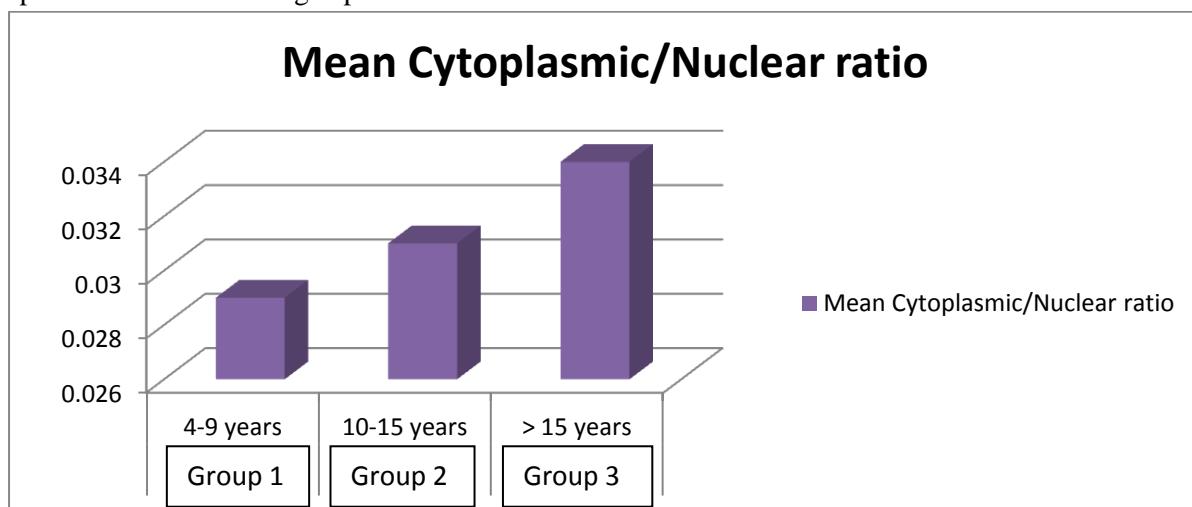
Graph - 3: Mean Cytoplasmic area of the squames of buccal mucosa of smokers based upon duration of smoking exposure.
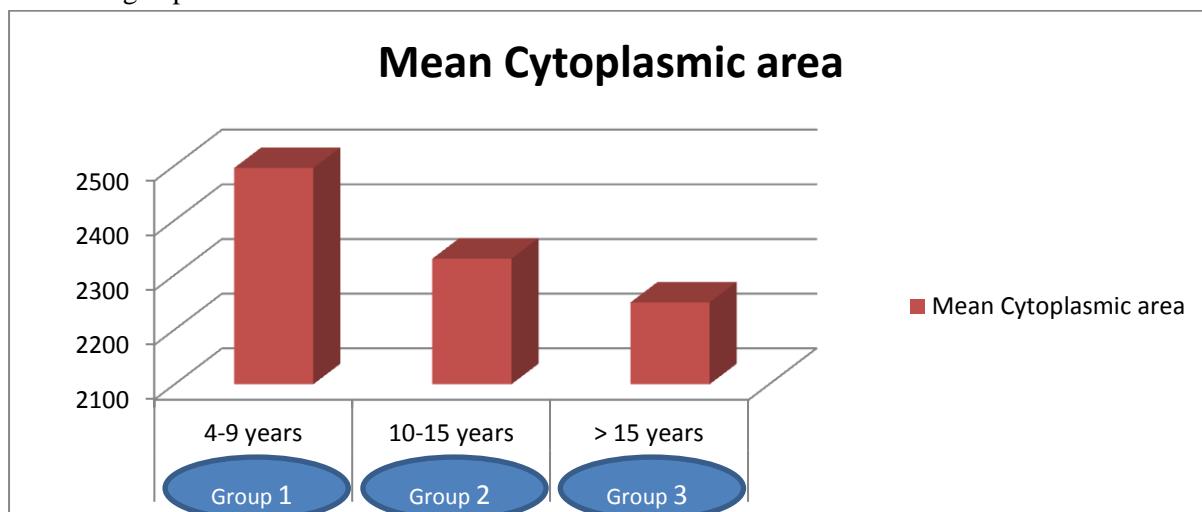
Graph - 4: Mean Nuclear area of the squames of buccal mucosa of smokers based upon duration of smoking exposure.
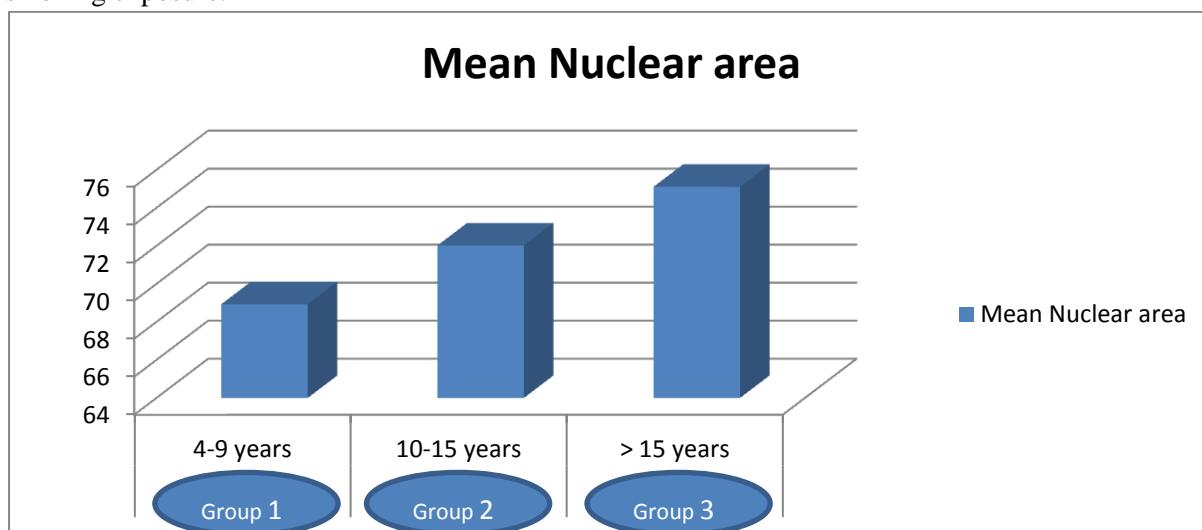
Conclusion

From the above results we can conclude that cytomorhometric analysis can be used to analyse the alterations occurring in cellular and nuclear level. Hence, we emphasize on using cytomorphologic aids in the assessment of tobacco induced changes occurring in oral cavity.

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