ORIGINAL ARTICLE

Oral Cytomorphometry of Smokers and Non Smokers

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ABSTRACT

Objective: The objective of the study was to observe and compare the changes in buccal exfoliated cells between smokers and Nonsmokers.

Study Design: Cross sectional comparative study.

Place and Duration of Study: Study was carried out at Islamic International Dental College, Islamabad and Post Graduate Medical Institute, Lahore. The duration of study was six months i.e 1st September 2013-1st March 2014.

Materials and Methods: Convinient, non-probability sampling technique was used. Quantitative data was obtained. The study groups consisted of 66 subjects divided into two equal groups of smokers S and non- smokers M, of ages between 15yrs-60yrs. Cellular diameter CD, nuclear diameter ND and nuclear to cytoplasmic ratio N/C ratio was assessed in buccal mucosal smears taken from clinically normal mucosa of smokers and normal subjects using exfoliative cytology. SPSS version 17.0 was used for data entry and statistical analysis. ANOVA and post-hoc tuckey were used for statistical analysis.

Results: The mean cellular diameter of smokers and non-smokers was 54.41± 3.30µm and 43.81±2.01µm respectively. The mean nuclear diameter of smokers and non-smokers was 12.68± 0.90µm and 9.97± 0.80µm respectively. And the mean N/C ratio of group smokers and non-smokers was 1: 4.43± 0.38 and 1: 4.42± 0.41 respectively. The ONE WAY ANOVA test showed significant results (p=0.000) for cellular diameter CD, nuclear diameter ND and N/C ratio both, while post hoc tukey test gave highly significant results for CD and N/C ratio i.e p=0.000.

Conclusion: Exfoliative cytology and cytomorphometry can help in the early detection of cellular changes as these techniques are easy, non-invasive and reproducible. Moreover, there is significant cause effect relationship between smoking and variables as nuclear diameter ND and N/C ratio.

Keywords: *Smokers, Oral Exfoliative Cytology, Cytomorphometry.*

Introduction

Oral squamous cell carcinoma comprises of 90-95% of all oral cancers.[1] In Pakistan, oral cancer is the second most common cause of cancer in women and third most common in men.[2] The five years survival rate for oral squamous cell carcinoma has remained at approximately 50% for the past several decades.[3] Prognosis of oral squamous cell carcinoma lacks improvement because most of the lesions are diagnosed or treated at advanced stages. The prognosis for patients with squamous cell carcinoma that is treated early is much better, with 5 years survival rate as high as 80%.[4]

Tobacco is an important causative factor for oral cancer. Different forms of tobacco like smokeless tobacco, naswar, cigarettes, cigars, pipes are proved etiological factors for oral cancers.3Many cellular alterations are caused by use of tobacco in the buccal cells. Studies have been carried out to find out role of techniques that can assess the cellular changes as a result of tobacco use.[5,6] Moreover, to evaluate their possibility to serve as a screening tool for early diagnosis of oral dysplastic lesions or that may lead to malignancy. Relating this prospect, exfoliative cytological techniques are being used to detect the influence of tobacco on the oral mucosa.[7]

Exfoliative cytology is a simple non-invasive diagnostic technique that is useful in early assessment of cellular changes in oral lesions.[8] But still this technique is not widely accepted as a screening tool because the results of various studies are quite variable and thus cannot be used as a standardized early diagnostic tool. Moreover, majority of studies carried out internationally assessed the cytomorphological and not the cytomorphometric cellular changes affecting the oral epithelium in tobacco chewers but not the smokers. Thus, objective of the present study was to evaluate the role of exfoliative cytology in differentiating the cytomorphometric parameters between smokers and non-smokers. Furthermore,

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quantitative techniques basedon the assessment of variables as nuclear diameter ND, cellular diameter CD and nuclear to cytoplasmic ratio N/C ratio may increase the sensitivity of exfoliative cytology for the early diagnosis of oral cancers as these techniques are accurate, objective and reproducible.[9]

Materials and Methods

The objective of the study was to:

1. Observe the cytomporphometric changes in buccal mucosal smears of smokers and non-smokers.
2. To compare these changes in buccal exfoliated cells between smokers and Non-smokers.

A cross- sectional comparative study was carried out at Histopathology Department, Post Graduate Medical Institute Lahore and Oral pathology department, Islamic International Dental College, Riphah International University, Islamabad from 1st September 2013 to1st March 2014.

The study group consisted of 66 adult males divided into two groups: smokers and non-smokers. The age group was 15 years and above. Smokers included were smoking cigarettes only for 3 years or more; 3-5 times daily and without any visible lesion in the oral cavity, respectively. While those included in control group were normal healthy individuals without any habit of using tobacco, pan or gutka. Also the subjects included in both groups were not having any chronic debilitating diseases.

Informed consent was obtained from all the subjects to obtain the cytological smears. Data was collected through convenient, non- probability sampling technique. Data that was collected was quantitative. Scrapings were obtained using a moistened wooden spatula. Using a gentle scraping motion cells were scraped from clinically normal looking buccal mucosa from the both groups. Three smears were taken from each individual to prepare three slides per case. The scrapings were smeared onto the centre of the previously marked glass slides and were immediately fixed in 95% Alcohol. All cytological smears were stained with hematoxylin and eosin, Giemsa and pap stains.[10] Each case has three slides and these three were individually stained with H&E, Giemsa and Pap stain. Two types of micrometers are used to measure an object under a microscope i.e stage micrometer and ocular micrometer. Ocular micrometer is precalibrated using a stage micrometer on required optical combination before making accurate measurements.[11] The ocular micrometer was precalibrated with the help of stage micrometer according to which one division of ocular micrometer was equal to 3µm using the following equation:

100 div on ocular micrometer = 30 divisions on stage micrometer (one div =10µm)

= 30×10

100 div on ocular micrometer = 300 um

1 div on ocular micrometer = x

x =3 µm

After calibration, variables like cellular diameter(CD) and nuclear diameter(ND) of the 50 cells in each smear were measured by using calibrated ocular micrometer fixed in eye piece of microscope on 40 x (Fig 1). The average of the values give the size of cell and nucleus in each subject, followed by calculating the N/C ratio (NCR). Data was entered in SPSS version 17.0 and all the mentioned variables were analysed. ONE WAY ANOVA and post hoc tuckey test were applied for two groups to compare the mean of CD, ND and their ratios.

Results

Subjects included in the study were all adult males with age range between 15yrs-60yrs; with peak age range in the 4th decade of life. After cytomorphometry following results were calculated in smokers and non-smokers: i.e cellular diameter, nuclear diameter, and N/C ratio table I. The smears in this study were analysed quantitatively and the mentined parameters were measured. Fifty clearly defined cells were measured in each slide with precalibrated ocular micrometer. The cellular diameter and nuclear diameter were recorded on both axis and mean was taken to calculate the values.

Discussion

On the whole, cytomorphometric results show that on all three stains i.e; H&E, pap and giemsa, the measurements were almost the same.

When variance analysis was conducted to analyse any difference in the cellular diameter between the two groups, a statistically significant difference was found ($p < 0.005$). The intergroup Post-hoc tukey analysis revealed that the difference in the cellular diameter between the smokers group (54.38 + 3.31µm) and the control group was significant.

Carcinomas in the oral cavity are caused by use of

Table I: Mean of CD, ND and NCR in smokers and non-smokers

Stain	Control			Smokers		
	CDμm	NDμm	N/C	CDμm	NDμm	N/C
H & E	43.81 ±2.01	9.97 ±0.80	1:4.4 ±0.41	54.38 ±3.31	12.66 ±0.92	1:4.3 ±0.39
GIEMSA	43.81 ±2.01	9.97 ±0.80	1:4.4 ±0.41	54.41 ±3.29	12.68 ±0.91	1:4.3 ±0.39
PAP	43.81 ±2.01	9.97±0 .80	1:4.4 ±0.41	54.36 ±3.32	12.63± 0.91	1:4.3 ±0.38

One way ANOVA and post hoc tuckey test showed significant results i.e p value 0.000 for ND and NCR.

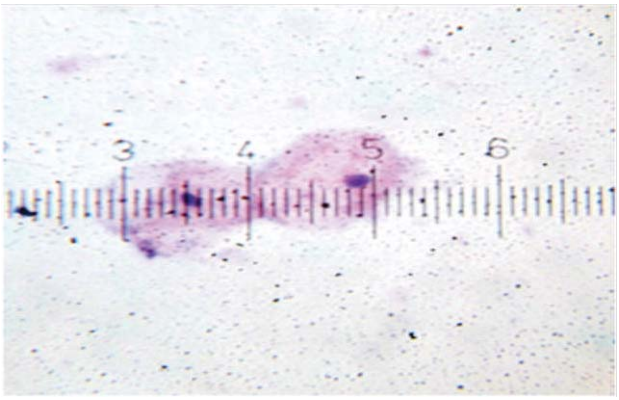

Fig 1: Image of Individual buccal mucosal cell of smoker superimposed with focused precalibrated ocular micrometer.

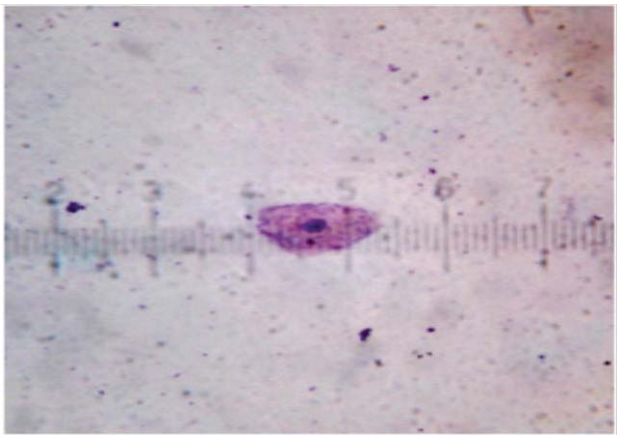

Fig 2: Image of Individual buccal mucosal cell of control group superimposed with focused precalibrated ocular micrometer

different forms of tobacco, thus making it possible to view the damage with naked eye.[12] Different forms of tobacco like smokeless tobacco, naswar, cigarettes, cigars, pipes are proven to be as prominent risk factors for oral cancers.[13]

The hostile effects of cigarrete smoking and smokeless tobacco use have been studied and documented by various studies.[14,15,16,17] Tobacco induced buccal changes at celluar level are also studied and documented in several articles.[18,19,20,21,22,23]

Oral exfoliative cytology has been proven to detect early changes in the cells even before the onset of the clinical lesion, and also this technique is inexpensive and easy with high sensitivity rates and diagnostic values.[24]

Hande and Chaudhary in 2010 conducted a study using cytomorphometry and showed that systemic and external factors affect the cytomorphometric variables such as ND, CD and N/C ratio.2 As the CD is increased in smokers in the present study, it may be due to any factor which is caused by smoking cigarettes. The results of the present study, i.e, increase in the CD of smokers as compared to the control group, contrasts with the other studies carried out like in case of Sumit babuta (2014) and Goregen (2011).[25,26] Whereby in a study conducted by Ramesh et al. (1999) CD was decreased in cigarette smokers.[27] Similarly, a study conducted by Ogden et al. (1997) also showed a decrease in CD of tobacco users.[7]

When variance analysis was conducted to analyse any difference in the nuclear diameter between the two groups, a statistically significant difference was found (p = 0.000). The intergroup Post-hoc tukey analysis revealed that the difference in the nuclear diameter between the smokers group (12.68 μm± 0.91) and the control group (9.97 μm± 0.80) was significant.

In the present study, smoker group showed an increase in ND in comparison with the control group. This may be due to various reasons including use of tobacco or increase in DNA content as stated by Hande and Chaudhary in 2010.[2] Einstein and Sivapathasundharam conducted a study in 2005 which showed that CD decreased while ND increased in the buccal mucosal cells of tobacco users in south of India.[28] Other studies which have been conducted in the past on the same subject showed the similar results which are consistent with the findings of the present study i.e smokers or tobacco users.[24,25] Ogden et al observed in 1989 5 % average increase in nuclear diameter of smokers when compared with those of the non-smokers.[8] While a study conducted

by Goregen in 2011 showed an increase of 16.5 % increase in ND of smokers as compared to non-smokers which was attributed to smoking.[26]

The analysis of variance test reported a significant difference in the N/C ratio between the two groups ($p<0.005$). The intergroup Post-hoc tukey analysis revealed that the difference in the N/C ratio between the control group (1:4.4 ± 0.37) and the smokers group (1:4.3 ± 0.38) was not significant ($p> 0.005$).

Franklin and Smith in 1980 carried out a study which showed that N/C ratio helps us to show the precise relationship in the altered cellular and nuclear diameter.[29] N/C ratio in the smokers was also higher when compared with the control group which could be because of increased CD and ND in the respective group. Increase in the N/C ratio can be indicative of an early dysplastic change because in squamous cell carcinoma the N/C ratio is increased to 1:1 from 1:4.[30]

The limitations of the study were that cytomorphometry can be computer assisted with the help of softwares that were not available for the present study. Computer assisted cytomorphometry can give more accurate and quick results as compared to manual cytomorphometric technique used in the present study. Cytomorphometry can measure the early changes in buccal smears of tobacco users which can help in the early detection of malignant changes to improve the prognosis of oral squamous cell carcinomas.

Conclusion

This study suggests that cytomorphometric analysis showed significant results in terms of changes in CD, ND and N/C ratio between the control and study group. However, it is important here to highlight the fact that these changes depict cause effect relationship only and association of these changes with dysplasia or pre-malignancy needs further verification with the help of specific immune-markers.

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