Research Article

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# Cytomorphometric Analysis of Exfoliated Cytology of Buccal Mucosal Cells in Non Smokers, Smokers and Tobacco Chewers in Different Age Groups

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## ABSTRACT

**Aim:** Exfoliative cytology is a minimal invasive procedure that has been used for detection of early changes in clinically normal oral mucosa of tobacco users.This study highlights the cause-effect relationship between tobacco usage in varied forms in different age groups structural cellular and nuclear alterations.

**Materials and Methods:** Study was correlated with different age groups it has been observed that nuclear diameter (ND) and cytoplasm diameter(CD) did not show significant difference in control group and in smokers and tobacco chewers it showed a significant difference which was statistically proved.

**Results:** The result showed that the mean ND and mean CD significantly higher in experimental groups when compared with the control group.

**Conclusion:** Increase in ND and decreased CD as well as altered N/C ratio would appear to be due to smoking tobacco. Cytomorphometric analysis can be used regularly to detect these cell alterations.

**Keywords:** Cytology, Oral Mucosa, Tobacco, Nuclear.

## INTRODUCTION

Oral cancer is the most common cancers in many developing countries constitutes the fourth most common cancer among males and the sixth most common cancer in females.[1]The incidence of oral cancer varies among different countries of the world and among different regions within a country. It specifies that environmental factorsmay play a vital role in the pathogenesis of thesemalignancies. Tobacco smoking and alcohol intakehave been attributed to as major risk factors. Strongassociation between cancers of the oral cavity andpharynx with the use of tobacco in any form is wellestablished.[2]The majority of oral cancers are diagnosed at advanced stages, resulting in poor prognosis and survival rate among patients, although there is implementation of cancer prevention programs in some countries.[3] In addition, despite advances in therapeutic techniques the morbidity and mortality rates of oral cancer have risen, leading to increased treatment costs and complications.[1] Hence, the early diagnosis oforal cavity cancers is critical in successful

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treatment of patients.[4] Oral exfoliative cytology is a simple, non-invasive, and painless method that involves microscopic analysis of cells collected from the surface of the oral mucosa .[5] However, this method had been abandoned because of certaincomplications such as inadequate tissue samples, technical errors, and the incorrect interpretation of findings.With advanced imaging techniques, computerized systems, and the use of quantitative techniques to verify the reliability of cytomorphometric analysis, this method is gaining in popularity once again.[6] Cytology can be used as a diagnostic technique in detecting earlychanges of diseases even in the absence of clinical manifestations.Easy and fast implementation,adequate diagnostic value, non-invasiveness, low cost and reproducibility are some advantages of this technique.[4]The literature shows controversial results with regard to the effects of smoking on oral cavity.[7] The present study was undertaken to evaluate the potential precancerous changes of oral cavityamong tobacco users.

## MATERIALS AND METHODS

Forty fivepatients of smokers and tobacco chewers were selected from the Out Patient Department of Dr. D. Y. Patil Dental College and Hospital, Nerul, Navi Mumbai for the study. 15 normal tissues were considered as case control. The patients were divided into 3 groups, group-I consists of patients less than 25 years of age, Group – II consists of patients of 25 -50 years of age and Group –III of patients more than 50 years of age. The patients who presented with the history of systemic illness, tobacco use or alcoholic consumption were excluded from the study.

**Inclusion Criteria:**

All subjects having tobacco habit in either smoking or smokeless form will be included in the study group.

**Exclusion criteria:**

**S**ubjects showing tobacco associated oral lesions with clinically evident coexistent haematological will be excluded from the study.

Scrapings were made from the buccal mucosa with moistened wooden spatula, smeared on to a clear glass slide and immediately fixed with 95% ethanol for a minimum of 15 minutes. The smear was visualized in 100X objective and focused on the stage micrometre scale. In all the cases the Nuclear diameter (ND) and the Cell diameter (CD) were measured in both the horizontal and vertical axes in micrometre. Only clearly defined cells were measured, excluding the clumped or folded cells. 100 cells were measured from each smear and the mean ND and CD values were obtained for each case. The Nuclear Cytoplasmic (NC) Ratio was also calculated for each case (Fig 1).

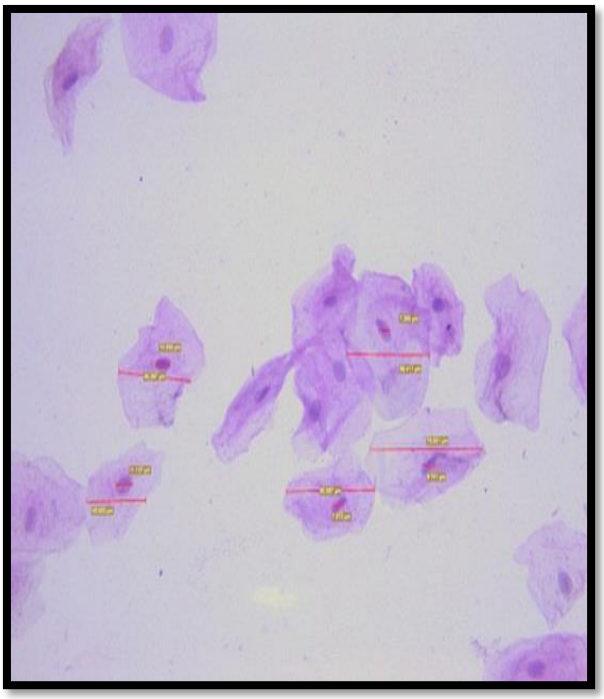


Fig 1: Nuclear diameter (ND) and the Cell diameter (CD) were measured in both the horizontal and vertical axes in micrometre.

## RESULTS

The study group were of age range between 20 to 60 years, which were divided into 3 groups as follows: Group 1- age group less than 25 years, Group 2- age between 25-50 years and Group 3- age more than 50 years. These following subjects had an habit of smoking and chewing tobacco. The mean values of ND,CD and N/C ratio is calculated among these groups shown in [Table 1, Graph1],[Table 2, Graph 2] and [Table 3, Graph3]. Statistically significant difference was observed in all the parameters between control group,smokers and tobacco chewers.

**Table 1: Width of Nucleus**

**Descriptive statistics:**

| | Control | | Smokers | | Tobacco chewers | |
|---|---|---|---|---|---|---|
| | Mean | Standard Deviation | Mean | Standard Deviation | Mean | Standard Deviation |
| Group 1 | 9.037 | .543 | 10.963 | .009 | 10.393 | .013 |
| Group 2 | 9.17 | 1.163 | 11.169 | .011 | 12.550 | .008 |
| Group 3 | 9.568 | 1.052 | 12.340 | .008 | 12.726 | .011 |

**Graph 1: Width of nucleus when compared between different groups**

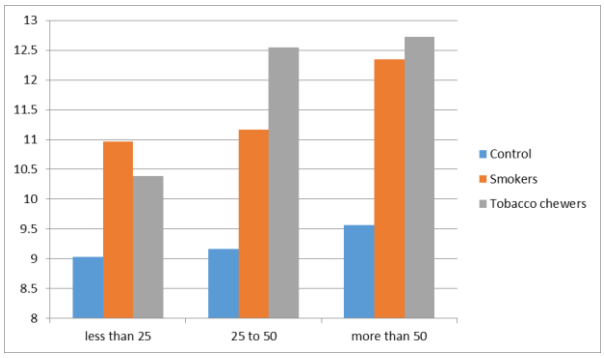



**Table 2: Width of cells.**

| | Control | | Smokers | | Tobacco chewers | |
|---|---|---|---|---|---|---|
| | Mean | Standard Deviation | Mean | Standard Deviation | Mean | Standard Deviation |
| Group 1 | 59.484 | 1.311 | 69.010 | .019 | 73.573 | 1.141 |
| Group 2 | 59.519 | 1.275 | 72.123 | .009 | 81.418 | .008 |
| Group 3 | 58.197 | 2.550 | 78.102 | .009 | 85.387 | .843 |

**Graph 2: Comparison between width of cells.**

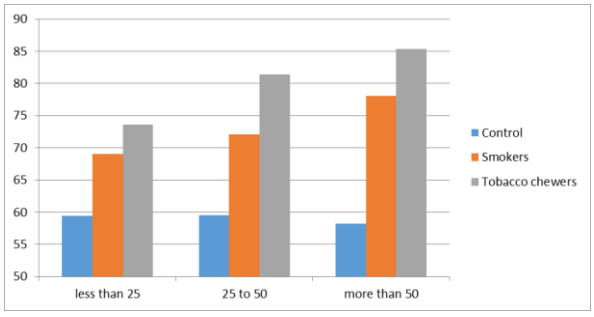
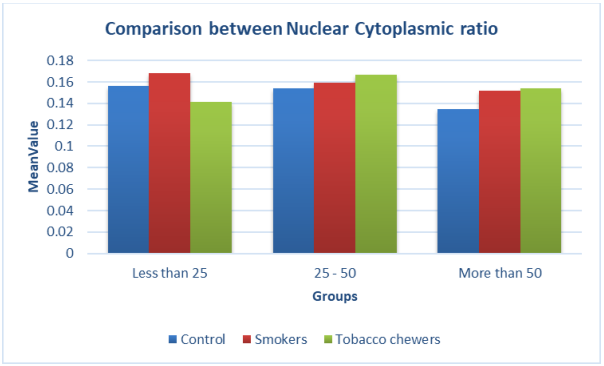



**Table 3: Nuclear Cytoplasmic Ratio.**

**Descriptive statistics:**

| | Control | | Smokers | | Tobacco chewers | |
|---|---|---|---|---|---|---|
| | Mean | Standard Deviation | Mean | Standard Deviation | Mean | Standard Deviation |
| Group 1 | 0.156 | 0.024 | 0.168 | 0.027 | 0.141 | 0.019 |
| Group 2 | 0.154 | 0.017 | 0.159 | 0.05 | 0.167 | 0.017 |
| Group 3 | 0.135 | 0.120 | 0.152 | 0.014 | 0.154 | 0.010 |




Graph 3: Comparison between nuclear cytoplasmic ratio.

## DISCUSSION

Oral exfoliative cytology is a simple and non-invasive diagnostic technique that can be used for early detection of potentially malignant lesions. Parameters such as cellular diameter (CD), nuclear diameter (ND), N/C ratio, nuclear shape, nuclear membrane continuity, optical density, and nuclear texture is been assessed by cytomorphology.[8] All of the major forms of tobacco such as cigarettes, cigars, pipes, and smokeless tobacco (chewing tobacco and snuff) are known to cause oral cancer. During malignant transformation, changes occur at the cellular level before clinical changes become evident. High risk potentially malignant disorders can be identified and intervened to reduce the mortality, morbidity, and cost of treatment associated with oral squamous cell carcinoma.[9]

In the South Indian population cytomorphological alterations in the form of a reduction in CD and increase in ND has been reported in buccal squames of tobacco users. In comparison to the non-smokers, there is an average increase of 5-16.5% in the ND has been reported in

the smokers.[10] In the present study, significantly alteredvalues were observed for all the three measured parameters (ND, CD, and N/C ratio) in the group using various forms of tobacco [Table 1]. These changes were directly related to the duration and frequency of habit. Increase in nuclear size is a cellular adaptation in response to an oral epithelial lesion. As there is decreased turnover rate of the oral epithelial cells which causes cells remain in the cell cycle for longer periods resulting in a delayed cell division, which in turn increases the nuclear size. The sizes of the nucleus and cytoplasm decline with aging.[11] The cellular changes seen in the present study can be partly attributed to the fact that tobacco on consumption releases various byproducts such as nitrosamine and nitrosonornicotine, which can influence cellular morphometry. In tobacco chewers and mishri users, there is a close contact of these byproducts thus facilitating their infiltration into the mucosa, which may be responsible for the pronounced cellular changes. Ramaesh et al. (9) reported that the nucleardiameter of the oral mucosa cells in individuals whosmoked cigarettes, chewed betel quid, or practicedboth these habits, was significantly greater thanthat of the control group individuals. They alsoreported that the cytoplasmic diameter of individualswho chewed betel quid and practiced both thesehabits was significantly smaller than that of the control group individuals.[12]

Our study emphasizes the importance of early recognition of cellular alterations caused by tobacco use. It showed a decrease in the CD and an increase in the ND and N/C ratio in cells isolated from the buccal mucosa and gingiva cells of tobacco users. These could be used as early indicators of dysplastic changes in the oral mucosa and should imposeproper early preventive measures to avoid the development of oral malignancies.

## CONCLUSION

Increase in ND and decreased CD as well as altered N/C ratio would appear to be due to smoking tobacco. Cytomorphometric analysis can be used regularly to detect these cell alterations. This method can also aid in motivating individuals to withdraw from adverse effects of tobacco smoking. Currently, use of exfoliative cytology has increased as an adjunct to screening of precancerous lesions and malignancies of the oral cavity.

## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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