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# EFFECTS OF SMOKELESS TOBACCO ON THE ORAL EPITHELIUM OF TOBACCO CHEWING USERS

Anatomy

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## ABSTRACT

**Background and Objective:** The regular use of tobacco causes loss of cell cohesion, hyperkeratosis, and increased incidence of nuclear anomalies on oral mucosa. Thus, the study was conducted to observe the impact of chewing tobacco on the nuclear changes in oral epithelial cells. **Methodology:** This was a prospective observational study conducted on 100 tobacco chewing subjects ranging from 20 to 70 years in age. The nuclear aberrations such as multinucleation, binucleation, pyknosis, karyorrhexis, karyolysis and condensed chromatin in Papanicolaou stained buccal smears. The t-test was applied to know the statistical significance. **Results:** Distribution of frequency of multinucleation, binucleation and condensed chromatin proved to be significantly higher. The overall results of pyknosis, karyolysis, karyorrhexis did not reach the level of significance. **Conclusion:** Microscopically examined nuclear changes are a useful tool in early diagnosis of the oral carcinoma.

## KEYWORDS

Buccal mucosa, Papanicolaou, Chromosomal aberrations, Oral Cytology, Micronuclei, Oral epithelium, Chewing tobacco

## INTRODUCTION:

Cancer is one of the most common causes of morbidity and mortality today. The global burden of cancer continues to increase mostly because of increasing adoption of cancer-causing behaviors, particularly chewing tobacco forms in economically developing countries. Globally, about 5,00,000 new oral and pharyngeal cancers are diagnosed annually and three quarters of these are seen in the developing world, including about 65,000 cases reported in India. The World Health Organization (WHO) estimated that the proportion of deaths that result from tobacco-related diseases will rise in India from 1.4% of all deaths in 1990 to 13.3% of all deaths in 2020. The number of persons consuming tobacco is also likely to rise, according to the models presented in the 2002 report of the Economic and Social Council (ECOSOC) of the United Nations[1].

About 100 million people in India and Pakistan use smokeless tobacco[2]. The buccal cell nuclear changes were first proposed in 1983[3]. Micronucleus assay provides information on the cytogenetic damage in the tissues[4]. It is believed that number of nuclear changes is related to increase the effects of carcinogens[5]. A lot of research work relating to the examination of oral epithelial cells has been done in last decades[6].

Histopathological studies of oral mucosa of tobacco chewers have shown a connection between chewing tobacco and certain alterations in the epithelium specially metaplasia and cellular atypia. Such changes are considered to represent a pre malignant stage and often occur diffusely in the oral mucosa in establishing oral cancer.

Thus, the purpose of the study involved examination of oral epithelial smear to determine the prevalence of cells containing nuclear aberrations attributable to smokeless tobacco.

## MATERIALAND METHODS:

The study was carried out on 200 individuals selected from the patients attending Outpatient Department at Ear Nose and Throat, Tuberculosis & Chest Disease and Radiotherapy departments, Sawai Man Singh Hospital, Jaipur, Rajasthan, India.

## EXCLUSION CRITERIA:

1. Patients who had oral x-ray in previous one month.
2. Patients who had received treatment for the buccal mucosal lesions like radiotherapy and/or chemotherapy for the oral lesions.
3. Chronic alcohol consumer and smokers.

The sample size was calculated using decision analyst software at 95% confidence level alpha error 5%. After seeking ethical acceptance from the review board committee of the institution, a proper consent and the history of the patient were recorded. The patients were then subjected to sample collection. The subject was asked to rinse the mouth with drinking water. Taking all the aseptic precautions, a wooden spatula was then used to scrape the sample area (inner side of the cheek) three to four times with firm pressure. The slides were coded before scraping the mucosa so as to avoid confusion and the sample was spread on the slide. These slides were stained according to Papanicolaou staining technique[7].

The smears were then observed under 40X and 100X magnifications. From each subject, a minimum of 1000 cells were screened for calculating the frequency of nuclear anomalies which includes multinucleation, binucleation, pyknosis, karyorrhexis and karyolysis.

The data thus generated was analyzed using t-test, the significance level was considered at $p<0.05$.

## RESULTS:

In the present study outcomes scored were multinucleation, binucleation, pyknosis, karyolysis, karyorrhexis and condensed chromatin.

The mean frequency of multinucleation was 1.87±2.94 in user group and 0.95±1.72 in non-users (Table 1).

**Table no. 1: Comparison of outcomes in tobacco users and Nonusers**

| Outcome | user status | Mean | SD | T test | P value |
|---|---|---|---|---|---|
| Multinucleation | User | 1.87 | 2.94 | 2.70 | <0.05 |
| | Non user | 0.95 | 1.72 | | |
| Binucleation | User | 0.72 | 1.78 | 2.26 | <0.05 |
| | Non user | 0.29 | 0.86 | | |
| Pyknosis | User | 4.35 | 3.86 | 6.57 | >0.05 |
| | Non user | 1.26 | 2.72 | | |
| Karyolysis | User | 5.47 | 9.49 | 4.27 | >0.05 |
| | Non user | 1.41 | 1.91 | | |
| Karyorrhexis | User | 2.06 | 3.01 | 4.71 | >0.05 |
| | Non user | 0.55 | 1.11 | | |
| Condensed chromatin | User | 0.52 | 0.98 | 1.75 | 0.05 |
| | Non user | 0.31 | 0.82 | | |

The occurrence of multinucleation was significantly higher in all age groups in males while in females in age groups 40-49 and 50-59 (Table 2).

Mean incidence of binucleated cells was 0.72±1.78in tobacco chewers and found to be statically significant (Table 1). Incidence was significant in all age groups in males except the age group 40-49. No such correlation was found in females (Table 2).

**Table no. 2: Distribution of frequency of nuclear changes**

| Age groups (in Years) | | Multinucleation | | Binucleation | | Karyorrhexis | | Pyknosis | | Karyolysis | | Condensed chromatin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | male | female | male | female | male | female | male | female | male | female | male | female |
| 20-29 | t-test | 3.13 | 1.98 | 1.09 | 1.51 | 1.12 | 1.60 | 1.19 | 1.42 | 1.37 | 3.87 | 0.00 | 1.13 |
| | p value | 0.05 | >0.05 | 0.05 | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 | 0.05 | >0.05 | 0.00 | >0.05 |
| 30-39 | t-test | 2.54 | 1.58 | 2.49 | 0.97 | 1.80 | 1.34 | 2.14 | 1.43 | 3.69 | 1.88 | 0.79 | 0.01 |
| | p value | 0.05 | >0.05 | 0.05 | >0.05 | 0.05 | >0.05 | 0.05 | >0.05 | >0.05 | 0.05 | >0.05 | <0.05 |
| 40-49 | t-test | 2.00 | 0.21 | 1.85 | 1.54 | 1.07 | 1.55 | 2.13 | 1.04 | 2.43 | 2.74 | 2.11 | 0.17 |
| | p value | 0.05 | <0.05 | >0.05 | >0.05 | >0.05 | >0.05 | 0.05 | >0.05 | 0.05 | 0.05 | 0.05 | >0.05 |
| 50-59 | t-test | 1.23 | 3.13 | 0.65 | 1.91 | 1.30 | 2.34 | 3.34 | 3.77 | 1.66 | 7.25 | 1.28 | 0.95 |
| | p value | <0.05 | <0.05 | <0.05 | >0.05 | >0.05 | 0.05 | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 |
| 60-69 | t-test | 1.22 | 1.45 | 2.79 | 0.00 | 6.77 | 2.23 | 1.45 | 1.77 | 4.00 | 1.70 | 0.57 | 0.55 |
| | p value | <0.05 | >0.05 | 0.05 | 0.00 | >0.05 | 0.05 | >0.05 | >0.05 | >0.05 | >0.05 | <0.05 | >0.05 |

Among various ranges of age groups, distribution of frequency of karyorrhexis was proved to be significant in 30-39 age group in males and in females in age groups 50-59 and 60-69 (Table 2). Though mean frequency was not significant (2.06±3.01 in users and 0.55 ±1.11 in non-consumers) (Table 1).

Mean frequency of pyknotic and karyolitic changes did not reach the level of significance. (p>0.05) (Table 1), while a significant association of pyknosis was found in males, age group 30-49 (Table 2).

The occurrence of karyolysis in females was strongly associated in age groups ranges between 30-39 and 40-49 (p<0.05, Table 2).

The incidence of condensed chromatin was not found in males below 30 years of age (Table 2).

The mean of condensed chromatin was 0.52±0.98in users and 0.31±0.82in non users and showed frequency at significant level (Table 1).

Statistical analysis suggested that duration of use of tobacco (more than 25 years) and incidence of pyknosis and karyorrhexis was significantly higher (Table 3)

No statistical significant could be identified between duration of use and incidence of multinucleation, binucleation karyolysis and condensed chromatin (Table 3)

**Table no. 3 Correlation between the years of use of tobacco and incidence of nuclear aberrations**

| years of use of tobacco | Multinucleation | | Binucleation | | Karyorrhexis | | Pyknosis | | Karyolysis | | Consensed chromatin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | t test | p value | t test | p value | t test | p value | t test | p value | t test | p value | t test | p value |
| < 25 years | 0.33 | >0.05 | 1.48 | >0.05 | 2.33 | <0.05 | 2.19 | <0.05 | 0.32 | >0.05 | 1.00 | >0.05 |
| >25 years | | | | | | | | | | | | |

## DISCUSSION:

Many studies have been done in India and abroad on frequency of nuclear aberrations in 2 or more of the groups of non-tobacco users, users with premalignant lesion or carcinoma. In this study overall cytological findings in buccal mucosa of 100 tobacco users was studied and compared with 100 controls.

In present study, the predominant cell types in the samples were squamous cells. The tobacco chewing user group exhibited significantly higher cellularity than control group.

Oral carcinogenesis is a multistep process of accumulated genetic damage leading to cell dysregulation with disruption in cell signaling, DNA-repair, and cell cycle events, which are fundamental to hemostasis. These events can be conveniently studied in the buccal mucosa, which is an easily accessible tissue for sampling cells in a minimally invasive manner and does not cause undue stress to study subjects[8].

The micronucleus test has been receiving increasing attention as a simple and sensitive short-term assay for the detection of environmental genotoxicants[3].

By applying this test, an elevated incidence of micronuclei has been recorded in the buccal mucosa cells of smokeless tobacco users. *This form of tobacco use has many oral effects including leukoplakia, oral cancer, loss of periodontal support (recession), and staining of teeth and composite restorations*[9].

In accordance with our findings, Indian studies showed the presence of binucleated buccal mucosa cells in smokeless tobacco users[10,11].

In this study, pyknosis, binucleation, condensed chromatin and karyorrhexis were strongly associated with exposure. In accordance, smokeless tobacco users the incidence of micronuclei was twice and that karyorrhexis and karyolysis was 4.5 and 13 times more common respectively[12].

The presence of karyolitic cells in smears from oral cavities of tobacco users has been well documented in other studies also[13]. The duration and frequency of habits have a significant effect on the development of oral lesions[14,15].

In a study, nuclear anomalies like multinucleation, karyorrhexis, karyolysis, binucleation, condensed nuclei were seen in tobacco users with increased frequency compared to controls, but only multinucleation is seen significantly higher in tobacco users[16].

Casartelli *et al* observed micronuclei frequencies in exfoliated buccal cells in normal oral mucosa, precancerous lesions, and squamous cell carcinoma. They concluded that the gradual increase in micronucleus counts from normal mucosal to precancerous lesions to carcinoma suggested a link of this biomarker with neoplastic progression[1].

Similar to our findings, regarding the effect of the duration and frequency of smokeless tobacco use, many studies found that the duration and frequency of smokeless tobacco use were associated with cytological changes and malignant transformations[17,18].

## CONCLUSION:

From the present study, increase in the number of micronuclei provides the evidence that smokeless tobacco chewers may be at a high risk for developing oral cancer. In comparison, the cellular changes associated with smokeless tobacco use were more than that in control group, thus indicating more carcinogenic potential of smokeless tobacco. Micronucleus assay can be used as a biomarker of genotoxicity and epithelial carcinogenic progression. However, more research is required to establish it as a potential biomarker for oral carcinogenesis. Some precautions and recommendations put forward are the following. The method of obtaining the sample should be standardized and repeatable. Complete smear needs to be screened for counting the frequency of nuclear aberrations for more valid results. The clarification on the size of the nuclear aberrations, as to whether to consider a constant value or a range, demands further studies. Micronuclei assay is an effective tool that reflects severity of disease.

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## CONFLICT OF INTEREST:

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

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