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Effects of Smokeless Tobacco on the Oral Epithelium of Tobacco Chewing Users

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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 the 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 multi-nucleation, bi-nucleation, pyknosis, karyorrhexis, karyolysis, and condensed chromatin in Papanicolaou stained buccal smears. The t-test was applied to know the statistical significance. Results: The distribution of frequency of multi-nucleation, bi-nucleation, 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 the early diagnosis of 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 the 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 several nuclear changes are related to an increase in the effects of carcinogens [5]. A lot of research work relating to the examination of oral epithelial cells has been done in the last decades [6].Histopathological studies of the 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 premalignant stage and often occur diffusely in the oral mucosa in establishing oral cancer.

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

MATERIAL AND METHODS

The study was carried out on 200 individuals selected from the patients attending outpatient department at ear nose and throat, tuberculosis and chest disease and radiotherapy departments, Sawai Man Singh Hospital, Jaipur, Rajasthan, India.

Exclusion Criteria:

- Patients who had an oral x-ray in the previous one month
- Patients who had received treatment for the buccal mucosal lesions like radiotherapy and/or chemotherapy for the oral lesions
- Chronic alcohol consumers 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, 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 to avoid confusion and the sample was spread on the slide. These slides were stained according to the Papanicolaou staining technique [7].

The smears were then observed under 40x and 100x magnifications. From each subject, a minimum of 1000 cells was screened for calculating the frequency of nuclear anomalies which includes multi-nucleation, bi-nucleation, pyknosis, karyorrhexis, and karyolysis.

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

RESULTS

In the present study outcomes scored were multi-nucleation, bi-nucleation, pyknosis, karyolysis, karyorrhexis, and condensed chromatin.

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

Table 1 Comparison of outcomes in tobacco users and non-users

Outcome	User status	Mean	SD	t-test	p-value
Multi-nucleation	User	1.87	2.94	2.7	<0.05
	Non-user	0.95	1.72		
Bi-nucleation	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 multi-nucleation was significantly higher in all age groups in males while in females in age groups 40-49 and 50-59 (Table 2).

Table 2 Distribution of frequency of nuclear changes

Age groups (in Years)		Multi-nucleation		Bi-nucleation		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	1.6	1.19	1.42	1.37	3.87	0	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	>0.05

30-39	t-test	2.54	1.58	2.49	0.97	1.8	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	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	1.3	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	6.77	2.23	1.45	1.77	4	1.7	0.57	0.55
	p-value	<0.05	>0.05	0.05	0	>0.05	0.05	>0.05	>0.05	>0.05	>0.05	<0.05	>0.05

The mean incidence of bi-nucleated cells was 0.72 ± 1.78 in tobacco chewers and was 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).

Among various ranges of age groups, the distribution of frequency of karyorrhexis was proved to be significant in 30-39 age groups in males and 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).

The mean frequency of pyknotic and karyolysis 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 ± 0.98 in users and 0.31 ± 0.82 in non-users and showed frequency at a significant level (Table 1).

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

Table 3 Correlation between the years of use of tobacco and incidence of nuclear aberrations

Years of use of tobacco	Multi-nucleation		Bi-nucleation		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	>0.05
>25 years												

No statistical significant could be identified between duration of use and incidence of multi-nucleation, bi-nucleation karyolysis, and condensed chromatin (Table 3).

DISCUSSION

Many studies have been done in India and abroad on the 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 the buccal mucosa of 100 tobacco users were studied and compared with 100 controls.

In the present study, the predominant cell types in the samples were squamous cells. The tobacco-chewing user group exhibited significantly higher cellularity than the 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]. By our findings, Indian studies showed the presence of bi-nucleated buccal mucosa cells in smokeless tobacco users [10,11].

In this study, pyknosis, bi-nucleation, 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 were 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 multi-nucleation, karyorrhexis, karyolysis, bi-nucleation, condensed nuclei were seen in tobacco users with increased frequency compared to controls, but only multi-nucleation 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, an increase in the number of micronuclei provides evidence that smokeless tobacco chewers may be at high risk for developing oral cancer. In comparison, the cellular changes associated with smokeless tobacco use were more than that in the control group, thus indicating the 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 the severity of the disease.

DECLARATIONS

Conflicts of Interest

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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