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Cellular diameter in desquamated cells of smokeless tobacco users

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ABSTRACT

Background: Tobacco-induced mucosal changes have been identified in exfoliated cells. The morphology of the exfoliated cells depends on the nature of the changes taking place in the epithelial layer; conversely, alteration in cytological pattern may be attributed to the changes occurring in the epithelial layer. Applying this possibility, exfoliative cytological techniques have been applied to examine the effect of tobacco on the oral mucosa. **Aims and Objectives:** The study was conducted to study the alterations in the cellular diameter of buccal mucosal cells. **Materials and Methods**: In a case controlled study, 240 participants were divided into four groups according to their habit of consumption of tobacco leaves, tobacco with areca nut, participants with oral lesions, and participants without any such habit. The groups were, further, subdivided according to their age. Desquamated cells from the buccal mucosa of were collected and smeared on a slide. The cells were stained with Papanicolou (rapid kit). Fifty cells per slide were studied and photomicrographs were obtained at ×40 magnification. The cellular diameter was obtained using the Leica 1000 software. **Results:** The mean cell diameter was found to be highest in the group of normal subjects (49.51 ± 7.31 micron), followed by the group of tobacco users (43.52 ± 1.92 micron) and Gutkha users (39.05 ± 3.57 micron). The mean cell diameter was minimum (24.97 ± 3.13 micron) in the group of oral lesion patients. According to the ANOVA test, the difference in cell diameter among various groups is found to be highly significant ($P < 0.0001$). **Conclusion:** Tobacco used in any form can induce changes in the buccal mucosa. In our study, mean cellular diameter decreased from normal-cells to cells affected by oral lesions.

Key words: Exfoliative cytology; Papanicolou stain; Cellular diameter; Tobacco; Oral lesions

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INTRODUCTION

Oral mucosal cells are rapidly replenished and serve as a diagnostic tool for local and systemic diseases. The technique is minimally invasive and involves microscopic examination of desquamated cells, which can be obtained by simply scraping the mucosal lining.[1] The oral cavity exhibits a myriad of changes related to oral and systemic diseases. Normally, there is a physiological shedding of superficial cells, in case of malignancy the deeper cells also shed, along with the superficial cells.[2] Chewing tobacco and other smokeless tobacco products are known to be injurious to oral health. Tobacco usage shows variations in context to age, gender, and socioeconomic status in the Asian population.

The incidence of oral cancer is very high in this region, because people often use tobacco quid, which is a mixture of tobacco leaves with lime, areca nut, and catechu. The mixture is chewed and kept between teeth and the buccal mucosa.[3] The main categories of smokeless or chewing tobacco-induced oral mucosal soft-tissue lesions reported are as follows: oral squamous cell carcinoma (SCC) and verrucous carcinoma; oral potentially malignant disorders (leukoplakia, erythroplakia, and erythroleukoplakia); and tobacco pouch lesion (tobacco and lime users' lesion, oral submucous fibrosis when mixed with areca nut).

The morbidity and mortality rates of oral cancer have not decreased despite advances in therapeutic techniques, leading

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to increased treatment costs, and complications. Moreover, the early lesions look benign and people tend to ignore the lesion and report to doctors at a very late stage. An early diagnosis of oral cancers is critical for successful treatment. Oral exfoliative cytology is a useful tool for mass screening purposes since it has a sensitivity of 94% and specificity of 100%.[4] Parameters, such as nuclear area (NA), cytoplasmic area (CA), and ratio of NA/CA (N/C), may increase the sensitivity of exfoliative cytology for the early diagnosis since these are precise, objective, and reproducible.[5-8]

Aims and objectives

The study was conducted with the aim to analyse changes in the diameter of exfoliated cells in smokeless tobacco users.

MATERIALS AND METHODS

The Institutional Ethical Clearance

The study was conducted after the committee gave its clearance.

Place of study

Villages in and around Lucknow.

Screening tool

A structured questionnaire was used to collect information regarding the demographic details, occupation, and habits.

Inclusion criteria

Individuals falling in the age group of 15 years and above, irrespective of sex, caste or creed; individuals without any habit; and individuals habituated to smokeless tobacco and its various forms were included in the study.

Exclusion criteria

Individuals consuming alcohol, drugs, or smoking and individuals working in tanneries, chemical factories, or petrol pumps were also excluded from the study so that any environmental effect can be negated.

Our study was a case controlled study, 240 participants were included in the study after taking an informed consent. The participants were divided into four groups with 60 participants each depending on their habit of consumption of tobacco and gutkha (a mixture of tobacco and areca nut), a control group and a group with participants having oral lesions. The groups were, further, subdivided according to the age of the participants as follows 15–30; 30–40; 40–50; 50; and above. The data were collated according to the age group of the patient so that a trend toward the vulnerable age group could be estimated.

The buccal mucosa was scraped with a wooden spatula to collect desquamated cells. A smear was prepared and subsequently stained with papanicolou stain. Fifty cells per slide were observed under ×40 magnification. The cellular diameter was obtained using the Leica 1000 software (Figure 1).

The statistical analysis was done using the ANOVA test and Prosthoc Dunnet test.

RESULTS

The mean cell diameter was found to be highest in the group of normal subjects (49.51±7.31 micron), followed by the group of tobacco users (43.52±1.92 micron) and Gutkha users (39.05±3.57 micron) (Table 1 and Graph 1).

The mean cell diameter was minimum (24.97±3.13 micron) in the group of oral lesion patients.

According to the ANOVA test, the difference in cell diameter among various groups is found to be highly significant (P<0.0001) (Table 1).

The mean difference in cell diameter of oral mucosal cells from normal was found to maximum for oral lesion group with value 24.53 and according to Dunnett test, this difference was highly significant (P<0.001). The next mean difference from normal was seen for Gutkha group (10.46) and this difference was also highly significant (P<0.001) (Table 2 and Graph 2).

The least mean difference from normal was seen for tobacco group (5.99) and this difference was also highly significant (P<0.001).

Among the age group 15–30 years, the mean cell diameter was found to be highest in the group of normal subjects

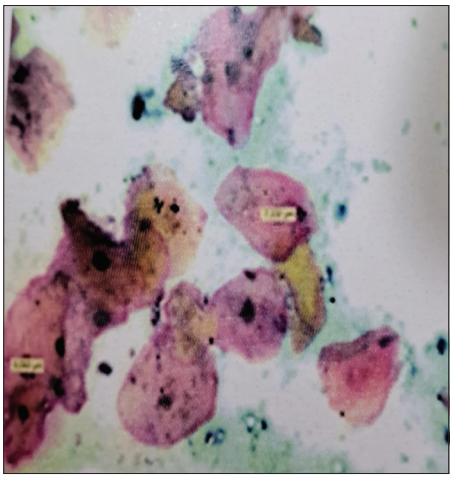


Figure 1: Exfoliated buccal cells, stained with PAP showing measurement of the cellular diameter using LEICA software

Table 1: Intergroup comparison of cell diameter of oral mucosal cells

Group	Cell diameter (micron)		F-value	P-value
	Mean	SD		
Normal	49.51	7.308	2191.30	<0.0001
Oral lesions	24.97	3.129		
Gutkha	39.05	3.574		
Tobacco	43.52	1.922		

Table 2: Bigroup comparison with normal group of cell diameter of oral mucosal cells using *post hoc* Dunnett test

Group versus normal	Mean difference	P-value
Oral lesions	24.53	<0.001
Gutkha	10.46	<0.001
Tobacco	5.99	<0.001

Table 3: Intergroup comparison of cell diameter of oral mucosal cells for the subjects belonging various age groups

Age group	Group	Cell diameter (micron)		F-value	P-value
		Mean	SD		
15–30 years	Normal	48.76	7.270	528.90	<0.001
	Oral lesions	24.39	2.648		
	Gutkha	38.57	4.305		
	Tobacco	42.87	1.781		
30–40 years	Normal	48.51	8.746	379.58	<0.0001
	Oral lesions	25.95	2.456		
	Gutkha	39.93	3.493		
	Tobacco	43.30	1.859		
40–50 years	Normal	51.71	6.953	671.83	<0.0001
	Oral lesions	24.26	4.194		
	Gutkha	38.69	3.188		
	Tobacco	43.75	1.817		
Above 50 years	Normal	49.05	5.561	813.21	<0.0001
	Oral lesions	25.30	2.618		
	Gutkha	38.99	3.069		
	Tobacco	44.17	2.002		

(48.76±7.27 micron), followed by the group of tobacco users (42.87±1.78 micron) and Gutkha users (38.57±4.31 micron) (Table 3 and Graph 3).

The mean cell diameter was minimum (24.39±2.65 micron) in the group of oral lesion patients.

According to the ANOVA test, the difference in cell diameter among various groups among the age group 15–30 years is found to be highly significant (P<0.0001).

Among the age group 30–40 years, the mean cell diameter was found to be highest in the group of normal subjects (48.51±8.75 micron), followed by the group of tobacco users (43.30±1.86 micron) and Gutkha users (39.93±3.49 micron) (Table 3 and Graph 3).

Table 4: Bigroup comparison with normal group of cell diameter of oral mucosal cells using *post hoc* Dunnett test for the subjects belonging various age groups

Age group	Group versus normal	Mean difference	P-value
15–30 years	Oral lesions	24.37	<0.001
	Gutkha	10.19	<0.001
	Tobacco	5.89	<0.001
30–40 years	Oral lesions	22.56	<0.001
	Gutkha	8.58	<0.001
	Tobacco	5.22	<0.001
40–50 years	Oral lesions	27.44	<0.001
	Gutkha	13.01	<0.001
	Tobacco	7.96	<0.001
Above 50 years	Oral lesions	23.74	<0.001
	Gutkha	10.05	<0.001
	Tobacco	4.88	<0.001

Table 5: Overall analysis of effects due to age and risk factors on diameter of oral mucosal cells

Source	F	P-value
Corrected model	452.8	<0.001
Intercept	127030.3	<0.001
Age	3.6	0.012
Group	2245.6	<0.001
Age and group interaction	4.8	<0.001

The mean cell diameter was minimum (25.95±2.65 micron) in the group of oral lesion patients.

According to the ANOVA test, the difference in cell diameter among various groups among the age group 30–40 years is found to be highly significant (P<0.0001).

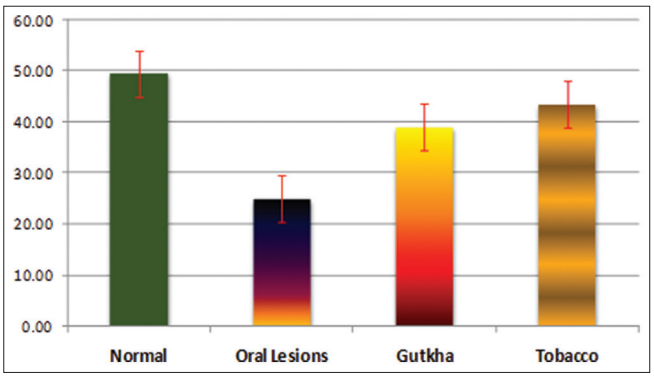
Among the age group 40–50 years, the mean cell diameter was found to be highest in the group of normal subjects 51.71±6.95 micron), followed by the group of tobacco users (43.75±1.82 micron) and Gutkha users (38.69±3.19 micron) (Table 3 and Graph 3).

The mean cell diameter was minimum (24.26±4.19 micron) in the group of oral lesion patients.

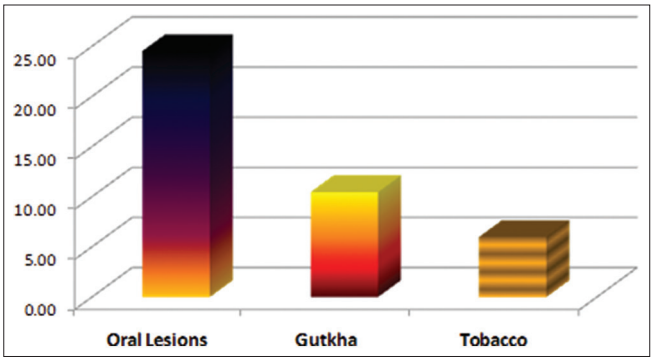
According to the ANOVA test, the difference in cell diameter among various groups among the age group 40–50 years is found to be highly significant (P<0.0001).

Among the age group of above 50 years, the mean cell diameter was found to be highest in the group of normal subjects (49.05±5.56 micron), followed by the group of tobacco users (44.17±2.00 micron) and Gutkha users (38.99±3.07 micron).

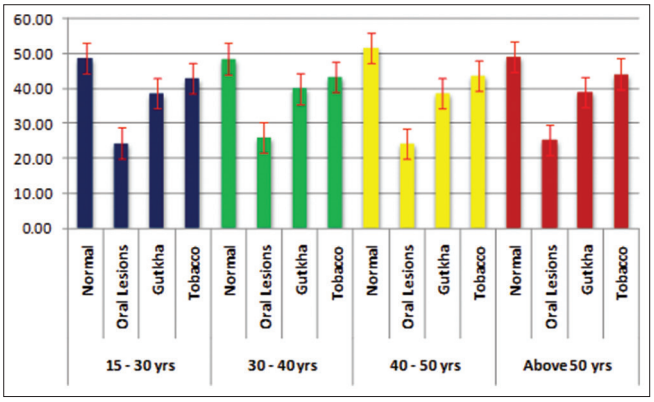
The mean cell diameter was minimum (25.30±2.62 micron) in the group of oral lesion patients.



Graph 1: Intergroup comparison of cell diameter of oral mucosal cells



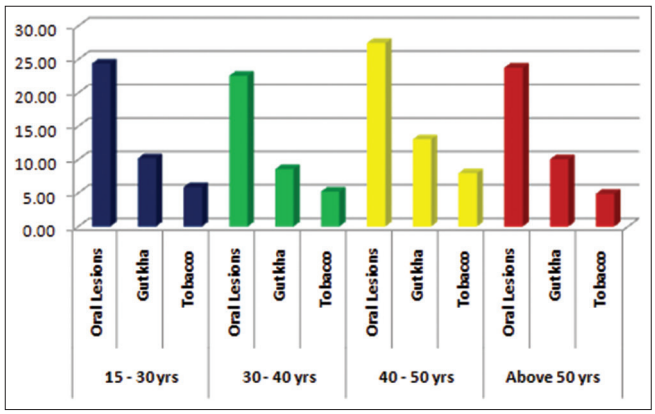
Graph 2: Bigroup comparison with normal group of cell diameter of oral mucosal cells



Graph 3: Intergroup comparison of cell diameter of oral mucosal cells for the subjects belonging various age groups

According to the ANOVA test, the difference in cell diameter among various groups among the age group above 50 years is found to be highly significant (P<0.0001).

Among the age group 15–30 years, the mean difference in cell diameter of oral mucosal cells from normal was found to maximum for oral lesion group with value 24.37 and according to Dunnett test that this difference was highly significant (P<0.001). The next mean difference from normal was seen for Gutkha group (10.19) and this difference was also highly significant (P<0.001) (Table 4 and Graph 4).



Graph 4: Bigroup comparison with normal group of cell diameter of oral mucosal cells using *post hoc* Dunnett test for the subjects belonging various age groups

The least mean difference from normal was seen for tobacco group (5.89) and this difference was also highly significant (P<0.001) (Table 4 and Graph 4).

Among the age group 30–40 years, the mean difference in cell diameter of oral mucosal cells from normal was found to maximum for oral lesion group with value 22.56 and according to Dunnett test this difference was highly significant (P<0.001). The next mean difference from normal was seen for Gutkha group (8.58) and this difference was also highly significant (P<0.001).

The least mean difference from normal was seen for tobacco group (5.22) and this difference was also highly significant (P<0.001) (Table 4 and Graph 4).

Among the age group 40–50 years, the mean difference in cell diameter of oral mucosal cells from normal was found to maximum for oral lesion group with value 27.44 and according to Dunnett test, this difference was highly significant (P<0.001). The next mean difference from normal was seen for Gutkha group (13.01) and this difference was also highly significant (P<0.001).

The least mean difference from normal was seen for tobacco group (7.96) and this difference was also highly significant (P<0.001).

Among the age group above 50 years, the mean difference in cell diameter of oral mucosal cells from normal was found to maximum for Oral lesion group with value 23.74 and according to Dunnett test this difference was highly significant (P<0.001). The next mean difference from normal was seen for Gutkha group (10.05) and this difference was also highly significant (P<0.001).

The least mean difference from normal was seen for tobacco group (4.88) and this difference was also highly significant (P<0.001).

After doing overall analysis of effects due to age and risk factors on diameter of oral mucosal cells, it was seen that the variation in diameter of oral mucosal cells was significantly influenced by the age of subject (P=0.012), highly influenced by the risk group (P<0.001), and also highly influenced by the interaction of age and risk group (P<0.001). The graph also depicts that from normal, maximum variation was seen in age group 40–50 years and among oral lesion patients, while the minimum variation was seen in age group 30–40 years and among tobacco patients (Table 5).

DISCUSSION

The use of smokeless tobacco is very common in India and its neighboring countries. Betel quid chewing and its variants predominate the prevalence data as far as use of tobacco is concerned. Packaged options these days are also quite popular since they are backed by a strong, promotion, and advertising. The consumption of smokeless tobacco is on the rise as people are of the opinion that smokeless form of tobacco is less harmful than its smoked form. Several studies have indicated that educating people about the ill effects of tobacco in times to come will not only decrease the use of tobacco but will also bring down the cancer mortality rate due to tobacco.

According to Critchley and Unal in 2003, some oral health implications include oral pigmentation, dental caries, gingival recession, oral mucosal lesions – tobacco pouch keratosis and potentially malignant lesions such as leukoplakia, erythroplakia, lichenoid reaction, and submucous fibrosis.[9] Jayalekshmi et al., in 2009, found that tobacco use is a major factor in the etiology of cancer.[10]

Pillai et al., in 2011, found that factors including areca nut chewing, ingestion of chilies, genetic, and immunologic processes cause OSMF.[11] Sujatha et al., in 2012, found that submucous fibrosis has a malignant potential of 7.6%.[12]

Exfoliative cytology is minimally invasive hence offers better patient compliance. A cytomorphometric analysis of buccal squames shows changes that are measurable.[13] Our study was therefore carried out to assess these measurable changes for the early detection of dysplasia or malignancy.[14]

We found that the cellular diameter of normal participants was the highest. The diameter progressively decreased from those who consumed smokeless tobacco, followed by Gutkha chewers. The lowest diameter was seen in participants with oral lesions.

Joshi and Kaijkar 2013[15] found the cellular diameter to be 52.73, Khandelwal and Solomon[16] 2010 found the diameter was 42.59±1.99, while Hande and Chaudhary found the cellular diameter to be 68.30±3.02. In our study, we found the cellular diameter to be 43.52. The cellular diameter of participants with submucous fibrosis in our study was 24.97, while there were other studies on premalignant lesions such as leukoplakia and according to Hegde[17]; Hande and Chaudhary and Ramaesh et al.,[18] the the diameter was 29.71±5.73, 57.75±4.66, and 41.32±0.13, respectively.

Multiple logistic regression analysis for all the habits as a whole for age groups revealed that subjects aged 30–39 years were at a higher risk (OR=0.69) and 50–59 years age group at a lower risk (OR=0.31) of developing lesions. However, only the latter was found to be significant.[19]

The risk of developing SCC increases with age, as this study concluded that the mean age of SCC patients was 53.64 years.[20]

Limitations of the study

We had to rely on the statements given by the participants, regarding their occupation and habits; our sample size was just enough to show a trend towards early recognition of cellular alterations.

CONCLUSION

Oral exfoliative cytology is an easy, minimally invasive and cost effective technique. A cytomorphometric analysis of exfoliated buccal squames can be done for mass screening purposes. Our study elucidates the importance of early recognition of cellular alterations in the absence of visible changes of mucosal surfaces. An application of quantitative techniques such as cytomorphometry to malignant or premalignant lesions can help to improve the diagnostic value of oral exfoliative cytology, thereby reducing the mortality and morbidity rates of high-risk premalignant lesions.

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Authors' Contributions:
SJ and PKS- Concept and design of the study, first draft of manuscript. Statistical analysis and interpretation of results; **TM-** Literature review and manuscript preparation; **SJ, TM, PKS-** Re revision of manuscript.

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