

# Comparative Cytomorphometric Study of Exfoliated Oral Epithelial Cells from a Population of Smokers, Tobacco Users and Gutkha Chewers

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## ABSTRACT

### Introduction

Tobacco remains one of the most important preventable cause of addiction, sickness and mortality in the world as it affects the oral epithelium. Normal epithelium undergoes continuous exfoliation or shedding of its superficial cells, and it is replenished by new crop of cells from the basal layer. The rationale of exfoliative cytology lies in the epithelial physiology. The purpose of this study is to conduct quantitative cytomorphometric studies on squames obtained from a population of Tobacco and Gutkha users, to assess the cytomorphometric changes in Nuclear Area (NA) and Cytoplasmic Area (CA) of squames from tobacco smokers, tobacco chewers, gutkha chewers, to compare the results with control group normal healthy individuals and among themselves.

### Methods

Oral smears were taken from buccal mucosa of total 200 individuals. The smears were histochemically stained and cytomorphological assessment was done. Groups were compared by one way analysis of variance (ANOVA) and the significance of mean difference between the groups was done by Tukey's post hoc test after ascertaining normality by Shapiro-Wilk test and homogeneity of variances by Levene's test.

### Results

The mean Nuclear area of tobacco chewer (C) was the highest and the normal individual (N) was the least. The mean Cytoplasmic area of normal individual (N) was the highest and the tobacco chewer(C) was the least.

### Conclusions

This study supports and extends the view that cytomorphometric evaluation of keratinocytes can serve as a useful diagnostic adjunct for early detection of oral cancer.

**Keywords:** exfoliative cytology; cytomorphometric; tobacco users; gutkha users.

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## INTRODUCTION

Tobacco remains one of the most important preventable cause of addiction, sickness and mortality in the world. Tobacco can be chewed in form of smokeless tobacco or smoked as cigarettes, both manufactured and hand rolled. Pipes, cigars, bidis and other products are used to a lesser extent or predominantly in other regions. Smokeless tobacco is usually placed in the oral or nasal cavities against the mucosal sites that permit the absorption of nicotine into the human body. All forms of tobacco use are addictive and cause harm.<sup>1</sup>The above habits may cause some changes in the oral mucosa, which give rise to clinically detectable lesions in the mucosa. The oral Exfoliative cytology is quick, simple, less technically demanding, painless, non-invasive practical, reliable, cost-efficient, repeatable technique that can be used for multiple small/large lesions yet quite independent laboratory procedure for the microscopic investigation and diagnosis of different kinds of oral diseases, especially suspected malignant and premalignant lesions. The rationale of exfoliative cytology lies in the epithelial physiology. Normal epithelium undergoes continuous exfoliation or shedding of its superficial cells, and it is replenished by new crop of cells from the basal layer. Thus the thickness of the epithelium is maintained. When the epithelium becomes the seat of any benign or malignant disease the cells may lose their cohesiveness so that the deeper cells may be exfoliated along with the superficial cells.<sup>2</sup> With advanced technology, more reliable quantitative techniques like cytomorphometry, histometry etc, can be made using computer assisted image analyzer. This quantification aims at reproducibility, enables direct comparison from person to person. Morphometry can be used selectively on structures or samples, which are difficult

to assess accurately, like variation in cell and nuclear size and staining intensity<sup>3</sup>.The purpose of this study is to conduct quantitative cytomorphometric studies on squames obtained from a population of Tobacco and Gutkha users.

## METHODS

This study was undertaken to analyze the cytomorphometric changes in Nuclear area (NA), Cytoplasmic area(CA) of different study groups. The Study group consisted(buccal smears) of 50 cases of tobacco smokers, 50 cases of tobacco chewers and 50 cases of gutkha chewers and Control group consisted 50 smears of normal oral mucosa. Study groups were randomly selected from the Out Patient Department of Oral Medicine and Radiology, UCMS College of Dental Surgery, Nepal. The study was conducted from 4<sup>th</sup> January 2022 till February 2022.

### Selection Criteria

Male individuals aged between 20yrs. to 60 yrs with the habit of Tobacco Smoking, chewing and gutkha chewing since minimum 2yrs. with 5-10 packets per day

**Exclusion Criteria:** Patients with the combination of habits of Tobacco Smoking, chewing and gutkha chewing. Patients with systemic diseases.

Ethical clearance was obtained from the institutional ethical committee to undertake the study. Consent was taken from the patients to obtain cytological smears from their buccal mucosa.

**Procedure:** Smears of each case was taken from buccal mucosa were taken from above mentioned groups and then fixed with 95% ethanol + 3% glacial acetic acid (Biofix spray). The smears were studied by staining with Papanicolaou stain(Bio Lab Diagnostic)

according to modified rapid PAP method. The Papanicolaou stained slides were subjected to cytomorphometrical assessment. The stained smears were observed using Olympus Pentahead research microscope (BX 53F).

The cytoplasmic area (CA) and nuclear area (NA) of the cells were observed under 40X objective, photomicrographs were taken by the Olympus camera (DP22) and then these photomicrographs were transferred to a software (Cell A) that gave the required measurements. Only clearly defined cells were measured, avoiding clumped or folded cells and unusually distorted nuclei and cells. Twenty cells were randomly selected and measured for Cytoplasmic area and Nuclear area from each slide.

## RESULTS

### A. Distribution of subjects

The frequency distribution of subjects of four groups (Normal, Tobacco smokers, Tobacco chewers and Gutkha chewers) groups were summarized in Table 1. There were total 200 subjects in four groups i.e. 50 subjects in control group and 50 subjects in each comparing groups.

Group	Description	No. of subjects	Percentage
I	Tobacco smokers	50	25%
II	Tobacco chewers	50	25%
III	Gutkha chewers	50	25%
IV	Control Normal	50	25%

### Statistical analysis

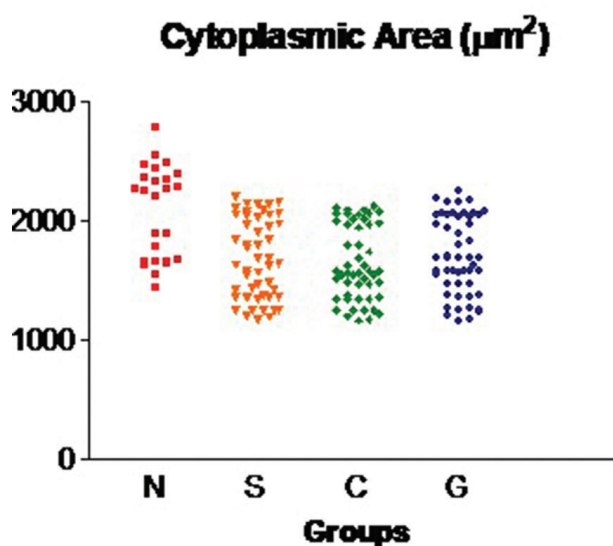
Data were summarized as Mean  $\pm$  SD. Groups were compared by one way analysis of variance (ANOVA) and the significance of

mean difference between the groups was done by Tukey's post hoc test after ascertaining normality by Shapiro-Wilk test and homogeneity of variances by Levene's test. The ANOVA was performed on log<sub>10</sub> transformed data. A two-sided ( $\alpha=2$ ) p value less than 0.05 ( $p<0.05$ ) was considered statistically significant. Analyses were performed on SPSS software (PSAW, Windows version 18.0).

### B. Primary outcome measures:

#### 1. Cytoplasmic Area

The observed Cytoplasmic Area (CA) of four groups at admission (enrollment) is depicted by scatter plot in Figure 1.



**Figure 1.** Scatter plot showing observed CA of four groups.

The observed CA of four groups is further summarized (Mean  $\pm$  SD) in Table 2. The CA of N, S, C and G groups ranged from 1442 to 2776  $\mu\text{m}^2$ , 1235 to 2333  $\mu\text{m}^2$ , 1244 to 2198  $\mu\text{m}^2$  and 1254 to 2277  $\mu\text{m}^2$ , respectively with 2076.12  $\pm$  385.41  $\mu\text{m}^2$ , 1792.30  $\pm$  320.92  $\mu\text{m}^2$ , 1722.57  $\pm$  312.35  $\mu\text{m}^2$  and 1752.79  $\pm$  333.38  $\mu\text{m}^2$ , respectively. The mean CA of N was the highest followed by S, G, and C, the least; i.e. found to be in the following ascending order:

CA: C < G < S < N

**Table 2.** Cytoplasmic Area (Mean ± SD) of four groups.

N (n=50)	S (n=50)	C (n=50)	G (n=50)
2076.12 ± 385.41	1792.30 ± 320.92	1722.57 ± 312.35	1752.79 ± 333.38

Comparing the CA of four groups together, ANOVA (Table 3) revealed significantly different CA among the groups (F=12.72, p<0.001).

**Table 3.** Comparison of CA among four groups by ANOVA.

Source of variation (SV)	Sum of squares (SS)	Degrees of freedom (DF)	Mean sum of squares (MS)	F value	P value
Groups	0.31	4	0.08	12.72	<0.001
Residual	1.19	195	0.01		
Error	1.51	199	0.09		

**Table 4.** Comparison of mean CA among four groups by Tukey test

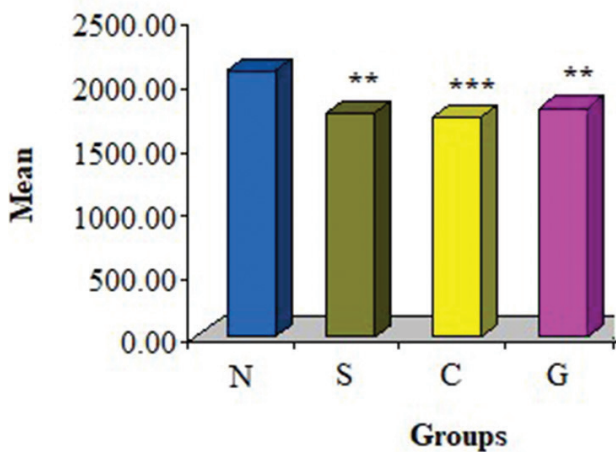
Comparisons	Mean Difference	Tukey q value	P value
N vs. S	283.80	4.71	P < 0.01
N vs. C	353.50	6.01	P < 0.001
N vs. G	323.30	5.43	P < 0.01
S vs. C	69.74	1.59	P > 0.05
S vs. G	39.51	0.89	P > 0.05
C vs. G	-30.22	0.71	P > 0.05

Further, comparing the mean CA of S, C and G groups with N group, Tukey test (Table 4) revealed significantly (p<0.01 or p<0.001) different and lower CA of S (13.7%), C (17.0%) and G (15.6%) groups as compared to N group (Figure 2).

\*\*p<0.01 or \*\*\*p<0.001- as compared to N

Similarly, comparing the mean CA of C and

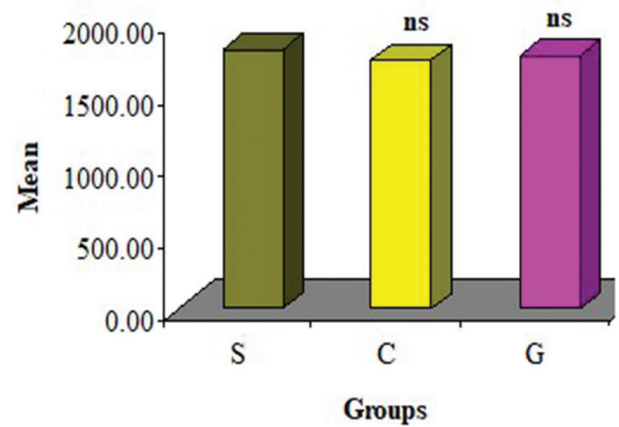
**Cytoplasmic Area (µm<sup>2</sup>)**



**Figure 2.** Scatter plot showing observed CA of four groups.

G groups with S group, Tukey test (Table 4) revealed similar (p>0.05) CA of both C and

**Cytoplasmic Area (µm<sup>2</sup>)**

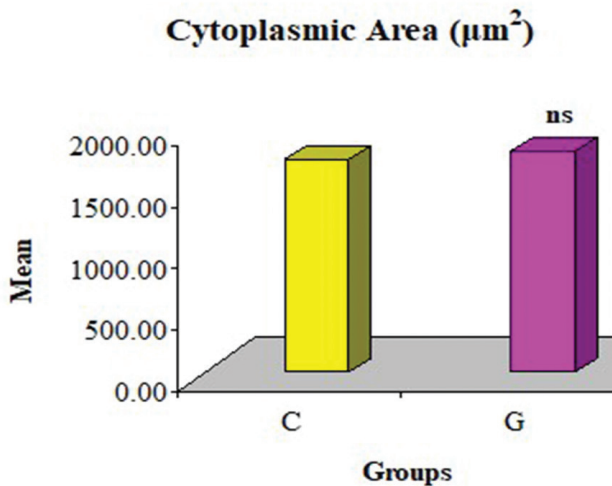


**Figure 3.** Mean CA of three groups.

G groups as compared to S group though it lowered 3.9% and 2.2% respectively in C and G groups as compared to S group (Figure 3).

<sup>ns</sup>p>0.05- as compared to S

Similarly, comparing the mean CA of G group with C group, Tukey test (Table 4) revealed similar (p>0.05) CA of G group as compared to C group though it was 1.7% higher in G group as compared to C group (Figure 4).

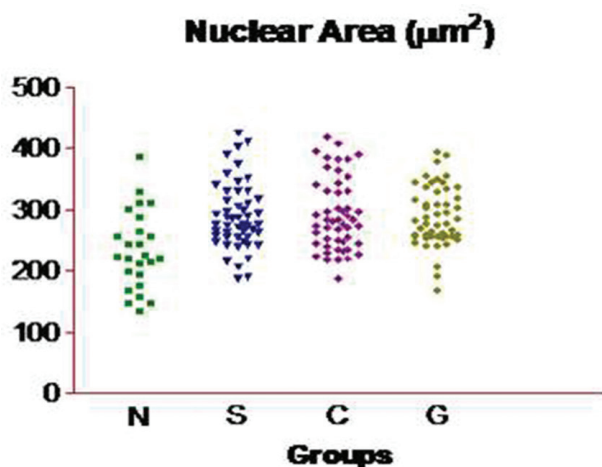


**Figure 4.** Mean CA of two groups.

<sup>ns</sup>p>0.05- as compared to C

## II. Nuclear Area

The observed Nuclear Area (NA) of four groups at admission (enrollment) are summarized graphically by scatter plot in Figure 5.



**Figure 5.** Scatter plot showing observed NA of four groups.

The observed NA of four groups is further summarized (Mean ± SD) in Table 5. The NA of N, S, C and G groups ranged from 133 to 385 µm<sup>2</sup>, 198 to 437 µm<sup>2</sup>, 198 to 429 µm<sup>2</sup> and 176 to 405 µm<sup>2</sup>, respectively with 232.34 ± 63.36 µm<sup>2</sup>, 299.93 ± 54.23 µm<sup>2</sup>, 302.42 ± 58.45 µm<sup>2</sup> and 298.67 ± 48.93 µm<sup>2</sup>, respectively. The mean NA of C was the highest followed by S, G and N, the least; i.e. found to be in the following ascending order:

$$NA: N < G < S < C$$

**Table 5.** Nuclear Area (Mean ± SD) of four groups.

N (n=50)	S (n=50)	C (n=50)	G (n=50)
232.34 ± 63.36	299.93 ± 54.23	302.42 ± 58.45	298.67 ± 48.93

Comparing the NA of four groups together, ANOVA (Table 6) revealed significantly different NA among the groups (F=36.79, p<0.001).

**Table 6.** Comparison of NA among four groups by ANOVA\*.

Source of variation (SV)	Sum of squares (SS)	Degrees of freedom (DF)	Mean sum of squares (MS)	F value	P value
Groups	0.97	4	0.24	36.79	<0.001
Residual	1.28	195	0.01		
Error	2.25	199	0.25		

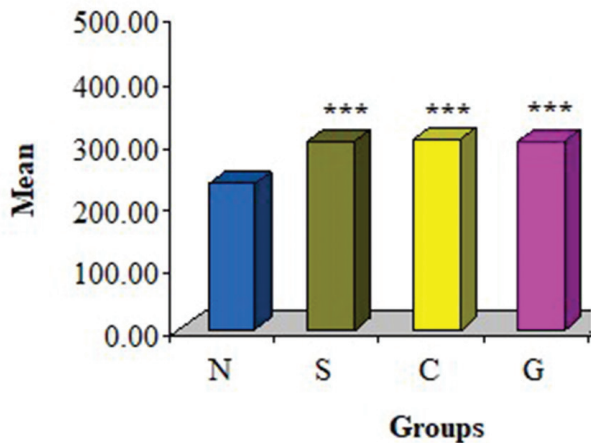
**Table 7.** Comparison of mean NA among four groups by tukey test.

Comparisons	Mean Difference	Tukey q value	P value
N vs. S	67.59	8.49	P < 0.001
N vs. C	70.09	8.73	P < 0.001
N vs. G	66.33	8.42	P < 0.001
S vs. C	2.50	0.30	P > 0.05
S vs. G	1.26	0.09	P > 0.05
C vs. G	3.76	0.38	P > 0.05

Further, comparing the mean NA of S, C and

G groups with N group, Tukey test (Table 7) revealed significantly ( $p < 0.001$ ) different and

### Nuclear area ( $\mu\text{m}^2$ )



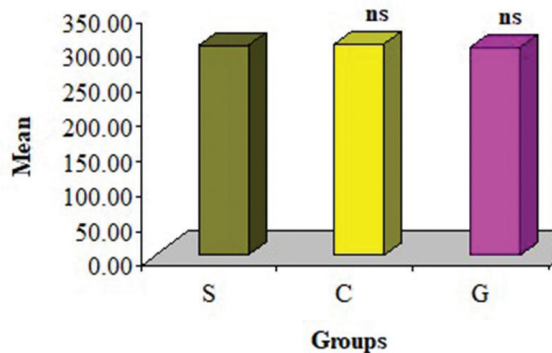
**Figure 6.** Mean NA of four groups.

higher NA of C (23.2%), S (22.5%), and G (22.2%) groups as compared to N group (Figure 6).

\*\*\* $p < 0.001$ - as compared to N

Similarly, comparing the mean NA of C and G groups with S group, Tukey test (Table 7) revealed similar ( $p > 0.05$ ) NA of both C and G groups as compared to S group though it was 0.8% higher in C group while 0.4% lower in C

### Nuclear area ( $\mu\text{m}^2$ )



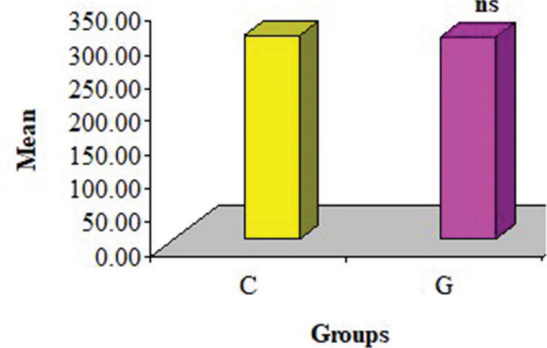
**Figure 7.** Mean NA of three groups.

group as compared to S group (Figure 7).

<sup>ns</sup> $p > 0.05$ - as compared to S

Similarly, comparing the mean NA of G group with C group, Tukey test (Table 7) revealed

### Nuclear area ( $\mu\text{m}^2$ )



**Figure 8.** Mean NA of two groups.

similar ( $p > 0.05$ ) NA of G group as compared to C group though it lowered 1.2% in G group as compared to C group (Figure 8).

<sup>ns</sup> $p > 0.05$ - as compared to C

## DISCUSSION

### NUCLEAR AREA (NA)

In the present study, of Nuclear area (NA) of (four groups) Normal (N), Tobacco smokers (S), Tobacco chewers (C) and Gutkha chewers (G) groups ranged (Mean  $\pm$  SD) 133 to 385  $\mu\text{m}^2$ , 198 to 437  $\mu\text{m}^2$ , 198 to 429  $\mu\text{m}^2$  and 176 to 405  $\mu\text{m}^2$ , respectively with 232.34  $\pm$  63.36  $\mu\text{m}^2$ , 299.93  $\pm$  54.23  $\mu\text{m}^2$ , 302.42  $\pm$  58.45  $\mu\text{m}^2$  and 298.67  $\pm$  48.93  $\mu\text{m}^2$ , respectively. Comparing the NA of four groups together, ANOVA revealed significantly different NA among the groups ( $F = 36.79$ ,  $p < 0.001$ ).

The mean Nuclear area of tobacco chewers was the highest followed by tobacco smokers, Gutkha chewers and Normal individuals the least; i.e. found to be in the following ascending order:

NA:  $N < G < S < C$

Goregen M, Akgul HM, Gundogdu C (2011)<sup>5</sup> conducted a study on smokers and non smokers and observed a 16.5% increase in the nuclear area value of smokers over non smokers. This increase in nuclear can be attributed to a cellular adaptation that depends on smoking. This adaptive change in the cell nucleus tends to be dysplastic change.

Our study was also in relevance with the studies done by,

Cowpe JG (1984)<sup>6</sup>

### Cytoplasmic area

In the present study, of Cytoplasmic area(CA) of (four groups) Normal(N), Tobacco smokers(S), Tobacco chewers(C) and Gutkha chewers(G) groups ranged (Mean  $\pm$  SD) 1442 to 2776  $\mu\text{m}^2$ , 1235 to 2333  $\mu\text{m}^2$ , 1244 to 2198  $\mu\text{m}^2$  and 1254 to 2277  $\mu\text{m}^2$ , respectively with 2076.12  $\pm$  385.41  $\mu\text{m}^2$ , 1792.30  $\pm$  320.92  $\mu\text{m}^2$ , 1722.57  $\pm$  312.35  $\mu\text{m}^2$  and 1752.79  $\pm$  333.38  $\mu\text{m}^2$ , respectively.

Comparing the CA of four groups together, ANOVA revealed significantly different Cytoplasmic area among the groups ( $F=12.72$ ,  $p<0.001$ ).

The mean Cytoplasmic area of Normal individuals was the highest followed by tobacco smokers, Gutkha chewers, and tobacco chewers the least; i.e. found to be in the following ascending order:

CA:  $C < G < S < N$

Tobacco remains one of the most important preventable cause of addiction, sickness and mortality in the world. Tobacco can be chewed in form of smokeless tobacco or smoked as cigarettes, both manufactured and hand rolled. Pipes, cigars, bidis and other products are used to a lesser extent or predominantly in other regions. Smokeless tobacco is usually placed

in the oral or nasal cavities against the mucosal sites that permit the absorption of nicotine into the human body. All forms of tobacco use are addictive and cause harm.<sup>7</sup>

The above habits may cause some changes in the oral mucosa, which give rise to clinically detectable lesions in the mucosa. As per the normal physiology, the oral epithelium renews itself rapidly (probably every 2 weeks). The rationale of oral exfoliative cytology is based on this physiological process, examining cells that are desquamated or abraded from the surface of the oral mucosa. Khandelwal S, Solomon MC (2010) quoted that Miller SC, Soberman A, Stahl SS were the first to study the cytology of the normal oral epithelium.<sup>8</sup> The superficial epithelial cells do contain nuclei and alterations in these cells can serve as reliable indicators of dysplastic or neoplastic changes.

Exfoliative cytology is based on epithelial physiology. A normal epithelium is exposed to regular exfoliation, namely the loss of cell surface, and the thickness of the epithelium is constant. Under normal conditions, epithelial cells are strongly held in place. The presence of benign disease or the occurrence of malignant epithelial formation causes the cells to lose their cohesive force and results in exfoliation. Loss of cohesion between the cells enables the collection of the exfoliated cells for microscopic examination. .

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. Advantage of exfoliative cytology included that it is a painless, bloodless non-invasive, quick and simple procedure, suitable in patients with systemic disease who are contraindicated for biopsy, guards against false negative biopsy, post biopsy complications can be eliminated.

The ideal instrument used for making a good cytological smear should be easy to use in any location, cause minimum trauma and provide an adequate and representative number of epithelial cells. It has been shown that a brush is an adequate instrument due to its ease in sampling and to the quality of the oral cytologic sample. Brush biopsy is a simple, relatively inexpensive, highly sensitive, risk free method of screening for cancer and serve as an aid to the clinical examination.

Cytobrush is a convenient instrument capable of sampling less accessible oral sites. Cytobrush pulls together an adequate number of cells and allows uniform dispersion of cells on a microslide which facilitates an accurate cytopathologic diagnosis.

Papanicolaou technique and its modifications have been used to study premalignant and malignant oral lesions. Advantage of PAP staining lies in the fact that the dehydration and clearing solutions help in causing cellular transparency. Cytomorphology is the most widely used method of oral exfoliative cytology, and assesses parameters such as Cellular Diameter (CD), Nuclear Diameter (ND), Nuclear Area (NA), Cytoplasmic Area (CA), NA/CA ratio, nuclear shape, nuclear membrane continuity, optical density and nuclear texture. These parameters, especially NA, CA have been

shown to provide meaningful results in the diagnosis of oral lesions.

The present study was conducted to assess the cytomorphometric changes in Nuclear Area (NA), Cytoplasmic Area (CA) in squames from Tobacco, gutkha consumers and compare the results with that of normal mucosa

## CONCLUSIONS

This study supports and extends the view that cytomorphometric evaluation of keratinocytes can serve as a useful diagnostic adjunct for early detection of oral cancer. An increase in the nuclear area and decrease in the cellular area are characteristics of malignant keratinocytes. This finding indicates that nuclear and cytoplasmic area may aid in establishing the prognosis of a dysplastic lesion. Usage of tobacco, gutkha can lead to pre malignant and malignant lesions. Evaluation of a greater number of cases is essential to establish the cut off values off values of these parameters that can be used as definitive indicators.

The early diagnosis of oral cancer is difficult, due to the asymptomatic nature and benign appearance of these lesions on initial presentation. The major advantage of exfoliative cytology is the non invasive character of the technique, which allow a simple and pain free collection of intact cells from different layers in the epithelium for microscopical examination and quantitative evaluation. The use of this

## REFERENCES

1. Warnakulasuriya S. Smokeless tobacco and oral cancer. *Oral Dis* 2004; 10:1-4
2. Tyler C, Charles P, Henry F, George E. Oral Exfoliative cytology. *Oral surg* 1972; 33(1): 61-74
3. Chen S. Effects of smokeless tobacco on the buccal mucosa of HMT rats. *J oral pathol med* 1989; 18:108-112
4. Ravi M, Mayank KS, Shruti P, Mamta S. The use of an oral brush biopsy without computer-assisted in the evaluation of oral lesions; a study of 94 patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008; 106: 246-53
5. Goregen M, Akgul HM, Gundogdu C. The cytomorphological analysis of



- buccal mucosa cells in smokers. *Turk J Med Sci* 2011; 41(2): 205-210.
6. Cowpe JG. Quantitative exfoliative cytology of normal and abnormal oral mucosal squames: preliminary communication. *J Roy Soc Med* 1984; 77: 928-31.
  7. Warnakulasuriya S. Smokeless tobacco and oral cancer. *Oral Dis* 2004; 10:1-4
  8. Khandelwal S, Solomon MC. Cytomorphological analysis of keratinocytes in oral smears from tobacco users and oral squamous cell carcinoma lesions-A histochemical approach. *Int J Oral Sci* 2010; 2(1): 45-52.
  9. Sivapathasundharam B, Kalasagar M. Exfoliative cytology. *J Oral Maxillofac Pathol* 2004; 8(2): 54-57.
  10. Offenbacher S, Weathers DR. Effects of smokeless tobacco on the periodontal , mucosal and caries status of adolescent males. *J Oral Pathol* 1985;14:169-81
  11. Schroeder KL, Soller HA, Chen MS, Neal J, Glover AD. Screening for smokeless tobacco associated lesions: recommendations for the dental practitioners. *J Am Dent Assoc* 1988; 116:37-42
  12. Rajesh SB, Reddy SB, Ramamurthy TK, Srinivas K, Patil S. Cytomorphometric analysis of obtained squames obtained from normal oral mucosa and lesions of oral submucous fibrosis. *J Indian Acad Oral Med Radiol* 2012;24:200-5.
  13. PC Gupta, CS Ray. Smokeless tobacco and health in India and South Asia. *Respirology* 2003; Vol.8; Issue 4: 419–431
  14. Ramaesh T, Mendis BRRN, Ratnatunga N, Thattil RO. The effect of tobacco smoking and of betel chewing with tobacco on the buccal mucosa: cytomorphometric analysis. *J Oral Pathol Med* 1999; 28: 385-388.
  15. Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of normal oral squames: an age, site and sex-related survey. *J R Soc Med* 1985; 78: 995-1004
  16. Ramesh TM, Ratnatunga N, Thattil RO. Cytomorphometric analysis of squames obtained from normal oral mucosa and lesions of oral leukoplakia and squamous cell carcinoma. *J Oral Pathol Med* 1998;27:83-6
  18. Robert WH, Benjamin K. Oral cytologic patterns in relation to smoking habits. *Oral Surg* 1976;42(3):366-74
  19. Ogden GR, Cowpe JG, Green MW. Quantitative exfoliative cytology of normal buccal mucosa and effect of smoking. *J Oral Pathol Med* 1990;19:53-5
  20. Einstein TB, Sivapathasundharam B. Cytomorphometric analysis of the buccal mucosa of tobacco users. *Indian J Dent Res*. 2005 Apr-Jun;16(2):42-6.
  21. Amith KN, Sivapathasundharam B. Cytomorphometric analysis of exfoliated normal buccal mucosa cells. *Ind J Dent Res* 2003;14(2): 87-94
  22. Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of abnormal oral mucosal smears. *JR Soc Med* 1988; 81:509-513

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