# CYTOMORPHOMETRY OF BUCCAL SMEARS OF NICOTIANA TABACUM (NASWAR) USERS: A PILOT STUDY

[1]RABIA MASOOD, BDS (MPhil Oral Pathology - Ongoing)
[2]NADIA ZAIB, BDS, MPhil Oral Pathology
[3]ROZINA JAFFAR, MPhil Histopathology and Morbid Anatomy
[4]KHOLA AHMED KHAN, BDS, (MPhil Oral Pathology - Ongoing)
[5]MUNNAZZA JAVED, BDS, (MPhil Oral Pathology - Ongoing)
[6]ALI RAZA, BDS, (MPhil Oral Pathology - Ongoing)
[7]NAILA UMER, BDS

**ABSTRACT**

*The objective of the current study was to asses the cytomporphometric changes in buccal mucosal smears of naswar users. The study groups consisted of 24 subjects divided into two groups i.e A control group and B naswar users of ages between 15yrs-60yrs. Cellular diameter CD,nuclear diameter ND and nuclear to cytoplasmic ratio N/C ratio was assessed in buccal mucosal smears taken from clinically normal mucosa of naswar users and normal subjects using exfoliative cytology. The mean cellular diameter of goup A and B was 43.93µm and 44.13µm respectively.The mean nuclear diameter of control group and naswar users was 9.99µm and 11.88µm respectively.And the mean N/C ratio of group A and B was 1: 4.42 and 1: 3.71 respectively.The independent T test showed significant results ($p \leq 0.000$) for nuclear diameter ND and N/C ratio both. Cytomorphometric changes in cellular diameter,nuclear diameter and N/C ratio assessed by our study depict only cause effect relationship between naswar and and association of these changes with dysplasia or premamlignancy needs further verification with the help of specific immunomarkers.*

**Key Words:** *Naswar users(dipping tobacco),oral exfoliative cytology,cytomorphometry.*

## INTRODUCTION

Naswar is a type of smokeless tobacco (Nicotiana tabacum) typically produced and used in Central and-Southeast Asia.[1] Presently, the use of tobacco products is the leading preventable cause of deathworldwide.[2] The number of deaths per year due to tobacco related diseases is about 5 million and ifcurrent smoking patterns continue, about 10 million deaths are expected to occur each year due to tobacco smoking by the year 2020.[3] Smokeless tobacco products (STP) are used without combustion and this eliminates the danger of direct exposure of toxic combustion compounds to the lung and other tissues of the user and of the people around. But the use of STP may result in other health hazards, local or systemic according to the content of various toxic products, including nicotine and tobacco-specific nitrosamines.[4]

Naswar contains tobacco, ash, cotton or sesame oil, water, and sometimes gum. It is held in the mouth for 10 to 15 minutes. Alkaloids are also found in larger quantity in naswar which may be responsible for nicotine like effects[5]. Nicotine from tobacco is absorbed through oral mucosa and from the mucous membrane of GIT as small amount of snuff is also ingested during the process.[6]

Naswar may not be as harmful as smoking cigarettes, but it should not be missed that all forms oftobacco carry serious health consequences, like oral and pharyngeal cancers.[7,8]

Cytomorphology is the most widely used method of oral exfoliative cytology, and asseses parameters such as cellular diameter (CD), nuclear diameter (ND) and nuclear to cytoplasmic ratio (N/C). These parameters, especially ND and N/C ratio, have been shown to provide meaningful results in the diagnosis of oral lesions.[9] Similarly, Einstein and Sivapathasundraham (2005) also analyzed the effect of smoking and betel quid chewing on the oral mucosa, using cytomorphological methods, and determined an increase in the average

[1] Lecturer,Oral Pathology Department Islamic International Dental College, Riphah International University, Islamabad. (NADER: National Academy of Dental Education and Research)
[2] Assistant Professor,Oral pathology Department. Islamic International Dental College, Riphah International University, Islamabad.
[3] Associate Professor,Histopathology. Post Graduate Medical Institute,Lahore.
[4] PGMI,Lahore.
[5] Dental surgeon, RHC,Mubarikpur.
[6] Senior Lecturer,Oral Pathology Faryal Dental College,Lahore. (NADER: National Academy of Dental Education and Research)
[7] Demonstrator, IIDC,Islamabad. Corresponding author: Dr Rabia Masood. 03216706404 .drrabiamasood@gmail.com. Lecturer Oral Pathology, Islamic International Dental College. Sector G 7/4. Islamabad.
**Received for Publication:** May 20, 2014
**Revision Received:** July 22, 2014
**Revision Accepted:** July 27, 2014

value of ND, and a decrease in cytoplasmic diameter values of smokers and individuals with both these habits.[10] Another study revealed that the ND values of the buccal mucosa cell nuclei of smokers were higher than those of non-smokers, and the difference was statistically significant.[11] Cytomorphometric changes could be the earliest indicators of cellular alterations. There is progressive decrease in cellular diameter, increase in nuclear diameter and increase in ratio of nuclear diameter to cellular diameter in smears from all tobacco users, as compared to normal subjects. This indicates that there could be cause – effect relationship between tobacco and quantitative alterations.[12]

## METHODOLOGY

The study was carried out at Histopathology Department, Post Graduate Medical Institute, Lahore and Oral Pathology Department, Islamic International Dental College (Riphah International University) Islamabad. The study group consisted of total 24 adult males with age 15 years and above, divided into two groups A and B that consisted of 12 subjects in control group (healthy individuals)and 12 subjects using only naswar, for 2 or more years; 3-5 times daily and without any visible lesion in the oral cavity, respectively. Informed consent was obtained from all the subjects to obtain the cytological smears. Scrapings were obtained using a moistened wooden spatula. Using a gentle scraping motion cells were scraped from clinically normal looking buccal mucosa from the control group A, and from the clinically normal looking buccal mucosa from the area where naswar is placed and held from the group B. The scrapings were smeared onto the centre of the previously marked glass slides and were immediately fixed in 95% Alcohol. All cytological smears were stained with hematoxylin and eosin staining.[13] Two types of micrometers are used to measure an object under a microscope i.e stage micrometer and ocular micrometer. Ocular micrometer is precalibrated using a stage micrometer on required optical combination before making accurate measurements.[12] The ocular micrometer was precalibrated with the help of stage micrometer according to which one division of ocular micrometer was equal to 3µm using the following equation:

100 div on ocular micrometer = 30 divisions on stage micrometer (one div =10µm)
= 30×10
100 div on ocular micrometer = 300 um
1 div on ocular micrometer = x
x =3 µm

After calibration, variables like cellular diameter(CD) and nuclear diameter (ND) of the 25 cells in each smear were measured by using calibrated ocular micrometer fixed in eye piece of microscope on 40 x (Fig 1). The average of the values give the size of cell and nucleus in each subject, followed by calculating the N/C ratio (NCR) Data was entered in SPSS version 17 and all the mentioned variables were analysed.Independent T test was also applied for two groups to compare the mean of CD,ND and their ratios.

## RESULTS

Subjects included in the study were all adult males between 15 years 60 years; with peak age range in the 4th decade of life (Fig 2).

After cytomorphometry following results were calculated in group A and B: i.e cellular diameter, nuclear diameter, and N/C ratio (Table 1). The smears in this study were analysed quantitatively and the mentined parameters were measured.Twenty five clearly defined cells were measured in each slide with precalibrated ocular micrometer. The cellular diameter and nuclear diameter were recorded on both axis and mean was taken to calculate the values.

The cytological variables CD, ND and NCR were also analysed according to duration of naswar use and frequency/day which revealed significant results (Table 2).

## DISCUSSION

Oral squamous cell carcinoma comprises of 90-95% of all oral cancers. Incidence of oral cancer varies with geographic distribution, varies from country to country in the world and region to region of the same country, indicating the involvement of environmental factors in the etiology of oral cancers. Tobacco is an important causative factor for oral cancer.[14] Chewing tobacco has been reported to cause oral cancer in Asia and in US Oral snuff is considered as a risk factor for oral cancers. Different forms of tobacco like smokeless tobacco, naswar, cigarettes, cigars, pipes are proved etiological factors for oral cancers. This is evidenced by the magnitude of the risks associated with greater amounts or longer duration of tobacco usage and the consistency of the findings for oral cancer across numerous cultures.[12]

The five year survival rate for oral squamous cell carcinoma has remained at approximately 50% for the past several decades.[14] Prognosis of oral squamous cell carcinoma lacks improvement because most of the lesions are diagnosed or treated at advanced stages. The reason for this delay is the late reporting of lesion by the patient and its superficial investigations by the health care workers, and it is presumed that such delays are longer for asymptomatic lesions. The prognosis for patients with squamous cell carcinoma that is treated

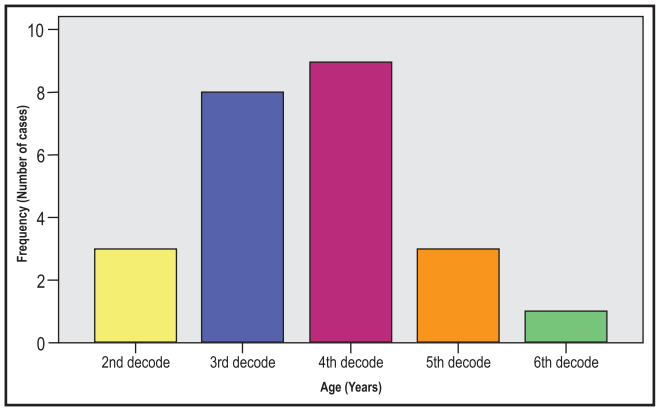


Fig 1: Age distribution.

TABLE 1: MEAN OF CD,ND AND NCR IN GROUP A AND B.

| Variable | Group | N | Mean (µm) | Std. Deviation |
|---|---|---|---|---|
| Cellular | naswar user | 12 | 44.13 | 2.66822 |
| diameter(µm) | control | 12 | 43.93 | 2.17880 |
| Nuclear diameter (µm) | naswar user | 12 | 11.88 | .55188 |
| | control | 12 | 9.99 | .86277 |
| NCR | naswar user | 12 | 1:3.71 | .26379 |
| | control | 12 | 1:4.42 | .42383 |

TABLE 2: MEAN OF CD ,ND AND N/C RATIO INRELATION TO DURATION OF NASWAR USERS AND FREQUENCY /DAY.

| | Duration of Naswar Use | | | Frequency /day | |
|---|---|---|---|---|---|
| | 4-5years | 6-8 years | 9-10 years | 5 Times/day | 5 Times /day |
| CDµm | 40.20 | 43.53 | 45.64 | 42.72 | 45.12 |
| NDµm | 11.52 | 11.9 | 11.94 | 11.6 | 12.0 |
| NCR | 1:3.49 | 1:3.66 | 1:3.83 | 1:3.6 | 1:3.75 |

The statistical significance of the variables was calculated by applying Independent T-test and ND and NCR showed significant results i.e p value 0.000

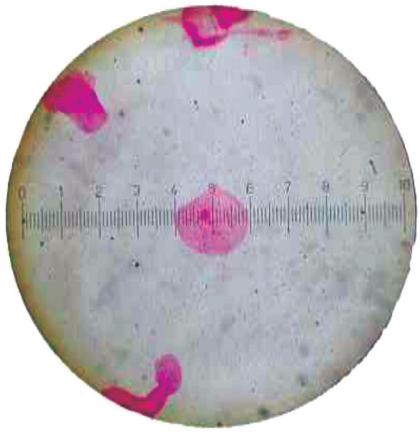

Fig 1: Image of Individual buccal mucosal cell of naswar user superimposed with focused precalibrated ocular micrometer.

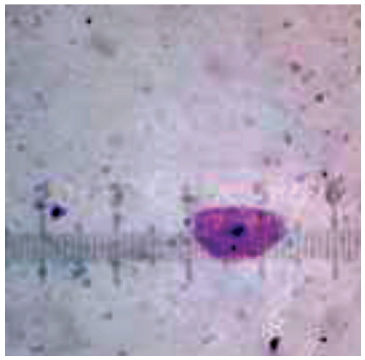

Fig 2: Image of Individual buccal mucosal cell of control group superimposed with focused precalibrated ocular micrometer.

early is much better, with 5 year survival rate as high as 80%; in addition, quality of life improves after early treatment.[15] Furthermore, before the onset of lesion of SCC, dysplastic changes in epithelium start before the appearance of that lesion. The diagnostic tool as exfoliative cytology can help in the early detection of these dysplastic changes in oral cavity as it has proven to be a gold standard in cervical cancer screening. 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.[16]

Exfoliative cytology a simple non-invasive diagnostic technique is useful in early assessment of dysplastic changes in oral lesions.[17,18] Quantitative techniques based on the assessment of variables as nuclear diameter ND, cellular diameter CD and nuclear to cytoplasmic ratio N/C ratio may increase the sensitivity of exfoliative cytology for the early diagnosis of oral cancers as these techniques are accurate, objective and reproducible.[19] In the present study cellular diameter was found to be slightly higher in naswar users as compared to the control group in contrast to the calculations carried out by Ramesh T et al, which shows the cellular diameter to be highest in normal mucosa and lower in dysplastic lesions.[20]

Nuclear diameter in the present study was increased significantly in the naswar users as compared to the control group showing comparable results to the study carried out in India in 2010 by Hande and Chaudry et al, which shows significantly increased nuclear diameter in the tobacco chewers.[12,21,22] In the present study,

nuclear to cytoplasmic ratio is increased in the naswar users as compared to the control group. This finding is consistent with the results obtained by different studies which also showed increase in the nuclear diameter.[17,23,24,25,26] This change relates to the significant increase in the nuclear diameter. Cowpe JG et al, 1998 suggested that nuclear to cytoplasmic ration increases due to increase in DNA content of the nucleus.[27]

Our study also showed CD and ND were increased in the subjects who used naswar more frequently and have been using it for long duration. N/C ratio was also significantly increased in the group using naswar for longer duration. These findings match with the findings of the study carried out by Saranaya et al, in 2014.[17]

The present study revealed that the changes similar to those occurring in histopathological sections of premalignant and malignant lesions are observed in the exfoliated buccal squames of naswar users, such alterations would have resulted from an increased cellular activity. Although there is increase in cellular diameter and increase in nuclear diameter and ratio of nuclear diameter to cellular diameter in clinically normal buccal mucosa squames of naswar users, it cannot be implied that these changes are only related to underlying malignant change. Our study shows that the quantitative changes observed in the cells are related to use of naswar ,and it may be predicted from these changes the cause-effect relationship between the naswar and these quantitative changes. Moreover, it is emphasized that traditional exfoliative cytology has its limitations, nonetheless introduction of special techniques like computer assisted oral brush biopsy and liquid based cytology have shown much better sensitivity and less false negative results and oral cytology seems promising to be used as a screening test like that used in cervical smears in near future. Furthermore, the results of this pilot study are statistically significant, we intend to carry out the major research project, in which comparison of cytomorphometric changes in buccal mucosal smears of naswar users and smokers will be analyzed.

## CONCLUSION

This study suggests that cytomorphometric analysis showed significant results in terms of changes in CD, ND and N/C ratio between the control and study group. However, it is important here to highlight the fact that these changes depict cause effect relationship only and association of these changes with dysplasia or pre-malignancy needs further verification with the help of specific immune-markers.

## REFERNCES


1 Gupta PC, Ray CS. Smokeless tobacco and health in India and South Asia. Respirology. 2003; 8(4): 419-31.

2 Brundtland GH. Achieving worldwide tobacco control. Jama. 2000; 284(6):750-751.

3 World Health Organisation. WHO Report on the Global Tobacco Epidemic.2008; The MPOWER package. Geneva, Switzerland: World Health Press.

4 Mogens Thomsen M, Ahlbom A, Bridges J and Rydzynski K. Scientific Committee on Emerging and Newly Identified Health Risks. 2008; Health Effects of Smokeless Tobacco Products. Brussels: European Commission

5 Ullah N, Asif AH, Khan MA, Ahmad W, Ali N, Khan T,Shah AA. Chemical analyses of naswar and cigarettes; a comparative study. International Journal of Basic and Clinical Research.2011; 1(1): 13-15.

6 Cohen DJ, Michel D, Donald E, Cutlip, Kalon KL, Jeffery J. Impact of smoking on clinical and angiographic restenosis after percutaneous coronary intervention. Circulation.2006; 104: 773.

7 Merchant A. Paan without tobacco: an independent risk factor for oral cancer. Int J Cancer.2000; 86(1): 128-131

8 Johnson N. Tobacco use and oral cancer: a global perspective J Dent Educ.2001; 65(4): 328-339.

9 Nayar AK, Sundharam BS.Cytomorphometric analysis of exfoliated normal buccal mucosa cells. Ind J Dent Res.2003; 14: 87-93.

10 Einstein TBA,Sivapathasundharam B.Cytomorphometric analysis of the buccal mucosa of tobacco users. Ind J Dent Res. (2005); 16: 42-46.

11 Göregen M, Akgül HM,Gündoğdu C. The cytomorphological analysis of buccal mucosa cells in Smokers. Turk J Med Sci.2011; 41 (2): 205-210.

12 Hande AH, Chaudhary MS. Cytomorphometric analysis of buccal mucosa of tobacco chewers. Romanian Journal of Morphology and Embryology.2010; 51(3): 527–532.

13 Gamble, M. The hematoxylins and eosin. In: Bancroft, J. D. and Gamble, M. (eds.) Theory and practice of histological techniques. 6th ed. China: Elsevier.2010; 121-134

14 Hashemipour MA, Aghababaie M, Mirshekari TR, Asadi-Shekaari M, Tahmasbi-Arashlow M, Tahmasbi-Arashlow F, Gandjalikhan Nassab SAH. Exfoliative Cytology of Oral Mucosa among Smokers, Opium Addicts and Non-smokers: A Cytomorphometric Study. Arch Iran Med. 2013; 16(12): 725 – 730.

15 Epstein JB, Zhang L, Rosin M. Advances in the diagnosis of oral premalignant and malignant lesions. J Can Dent. 2002; 68:617-21.

16 Babuta S, Garg R, Mogra K, Shekhawat S.Cytomorphometrical analysis of exfoliated buccal mucosal cells: Effect of smoking. Acta Medica International 2014; 1(1): 44-52

17 Saranya R S, Sudha S. Cytomorphological changes in buccal epithelial cells of khaini chewers in different age groups. Asian Journal of Biomedical and Pharmaceutical Sciences; 04 (30); 2014; 43-47.

18 Freitas MD, García–García A, Carneiro JLM, Crespo– Abeleira A, Gándara–Rey JM.Exfoliative cytology of the oral mucosa: a cytomorphometric comparison of healthy oral mucosa in oral cancer patients and healthy subjects. Revista Brasileira de Patologia Oral.2003; 2(4):2–6.

19 Ogden GR, Cowpe JG, Wight AJ.Oral exfoliative cytology: review of methods of assessment. J Oral Pathol Med.1997; 26:201-5 [pubmed].

20 Ramaesh T, Mendis BR, Ratnatunga N, Thattil RO.Cytomorphometric analysis of squames obtained from normal oral mucosa and lesions of leukoplakia and squamous cell carcinoma. J Oral Pathol Med. 1998; 27(2):83–86.

21 Prasad H, Ramesh V, Balamurali P.D. Morphologic and cytomorphometric analysis of exfoliate buccal mucosal cells in diabetes patients. J Cytol. 2010; 27(4): 113–117.

22 Gemitha G, Sudha S, Saranya R.S. Induction of cytomorphological changes in the buccal cells of khaini chewing South Indian population, Asian J Med Cli Sci, 2013; 2(1): 48-50.

23 Acharya S, Tayaar S.A, Khwaja T. Cytomorphometric analysis of the keratinocytes obtained from clinically normal buccal mucosa in chronic gutkha chewers. J Cranio Max Dis, 2013; 2:134-41.

24 Alka H.H, Minal S.C. Cytomorphometric analysis of buccal mucosa of tobacco chewers. Romanian Journal of Morphology and Embryology, 2010; 51(3):527–532.

25 Goregen M, Akgul H.M, Gundogdu C. The cytomorphological analysis of buccal mucosa cells in smokers. Turk J med sci, 2011; 41: 205–10.

26 Mustafa G, Hayati M.A, Cemal G. The cytomorphological analysis of buccal mucosa cells in smokers. Turk J Med Sci, 2011; 41 (2): 205-210.

27 Cowpe JG, Longmore RB, Green MW. Quantitative Exfoliative Cytology of abnormal oral mucosal smears. J R Soc Med. 1988; 81(9):509–513.