

## The cytomorphological analysis of buccal mucosa cells in smokers

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**Aim:** To examine the effect of smoking on normal buccal mucosa cytomorphologically.

**Materials and methods:** Forty individuals aged between 40 and 60, comprising 23 smokers (14 male, 9 female) and 17 and non-smokers (10 male, 7 female), were included in the study. The buccal epithelial cells of these individuals were collected with a brush and the cells were measured cytomorphometrically using software.

**Results:** The average nuclear area (NA), nuclear perimeter (NP), minimal nuclear diameter (D-min), and maximal nuclear diameter (D-max) were  $46.00 \pm 11.31$ ,  $28.18 \pm 2.43$ ,  $6.18 \pm 0.88$ , and  $10.00 \pm 0.94$ , respectively, in the control group, and  $53.61 \pm 7.29$ ,  $29.52 \pm 2.02$ ,  $6.87 \pm 0.63$  and  $10.52 \pm 0.67$  in the study group, respectively. While there was a statistically significant difference in NA, D-min, and D-max ( $t = 2.586$ ,  $P = 0.014$ ;  $t = 2.909$ ,  $P = 0.006$ ;  $t = 2.064$ ,  $P = 0.046$ , respectively), there was no statistically significant difference in NP between the 2 groups ( $P > 0.05$ ).

**Conclusion:** This increase determined in NA shows smoking-related cellular adaptation. It is possible to conclude that this adaptive change in the cell nucleus tends to be a dysplastic change.

**Key words:** Oral exfoliative cytology, cytomorphometry, smoking, buccal mucosa

### Sigara içen bireylerde normal yanak mukozasının kantitatif sitolojik olarak incelenmesi

**Amaç:** Araştırmanın amacı sigara kullanımının normal yanak mukozasına etkisinin sitomorfolojik olarak incelenmesidir.

**Yöntem ve gereç:** Çalışmaya yaşları 40-60 arasında değişen, sigara kullanan 23 (14 erkek, 9 kadın) ve kullanmayan 17 (10 erkek, 7 kadın) olmak üzere toplam 40 birey dahil edildi. Bireylerden fırça ile yanak epitel döküntü hücreleri alındı ve özel bir bilgisayar programı kullanılarak hücrelerin sitomorfometrik ölçümleri yapıldı.

**Bulgular:** Hücre nükleuslarına ait ortalama nükleer alan (NA), nükleer çevre (NÇ), minimal çap (Çapmin) ve maksimal çap (Çapmax) değerleri Kontrol grubunda sırasıyla,  $46,00 \pm 11,31$ ,  $28,18 \pm 2,43$ ,  $6,18 \pm 0,88$  and  $10,00 \pm 0,94$  olarak bulunurken; Çalışma grubunda sırasıyla  $53,61 \pm 7,29$ ,  $29,52 \pm 2,02$ ,  $6,87 \pm 0,63$  and  $10,52 \pm 0,67$  olarak bulundu. Her iki gruptaki bu parametrelerden NA, Çapmin ve Çapmax arasında istatistiksel olarak anlamlı farklılık bulunurken, ( $t = 2.586$ ,  $P = 0.014$ ;  $t = 2.909$ ,  $P = 0.006$ ;  $t = 2.064$ ,  $P = 0,046$ , sırasıyla), NÇ değerleri arasında istatistiksel olarak anlamlı farklılık bulunmadı ( $P > 0,05$ ).

**Sonuç:** Nükleer alanda belirlenen artış, sigaraya bağlı hücresel adaptasyonu göstermektedir. Hücre çekirdeğindeki adaptif değişimi, displastik değişikliğe yönelim olarak yorumlamak mümkündür.

**Anahtar sözcükler:** Oral eksfoliyatif sitoloji, sitomorfometri, sigara, yanak mukozası

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## Introduction

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 (1). However, this method had been abandoned because of problems such as inadequate tissue samples, technical errors, and the incorrect interpretation of findings. Today, with advanced imaging techniques, computerized systems, and the use of quantitative techniques to verify the reliability of cytomorphometric analysis, this method is gaining in popularity once again (2).

Many factors affect the cytomorphology of the cells collected from the oral mucosa. Some of these factors are systemic diseases, e.g., anemia (3) and diabetes mellitus (4); radiotherapy (5,6); alcohol consumption (7); and smoking (8-10). Cigarettes contain many carcinogenic substances, mostly DNA-toxic carcinogens. It is well known that these carcinogenic substances cause genetic mutations and chromosomal abnormalities and micronuclei (11).

The oral mucosa of smokers exhibits many changes. Exfoliative cytological methods have been employed to examine these changes, especially in cells collected from the buccal mucosa (8-10,12). In healthy individuals, epithelial cells of the buccal mucosa in the oral cavity are naturally exfoliated every day. Thus, buccal mucosa cells are similar to vaginal epithelial cells, and can be collected through excavation. Exfoliated buccal cells are at the final stage of cell differentiation, and rarely display mitotic features (13). Morphological examination of these cells reveals large volumes, a pancake-like shape, non-granular cytoplasm, small and oval nuclei that are centrally located, and large cytoplasm. With the help of these features, these cells can be easily distinguished from polymorph nuclear leukocytes and other cells present in the oral cavity (13).

Most oral cancer patients smoke cigarettes and consume alcohol. The effect of these factors on the cell and nucleus morphometry has been continually investigated. Cowpe and Longmore (14), in their cytological study of the buccal mucosa of young adults, used quantitative techniques to diagnose oral cancer because they found these techniques to be more sensitive. Ogden et al. (15) indicated that quantitative techniques based on parameters such as

nuclear area (NA), cytoplasmic area (CA), and NA/CA ratio could increase the sensitivity of exfoliative cytology in the early diagnosis of oral cancer because these techniques were more accurate, objective, and repeatable.

The purpose of this study was to analyze the cytomorphology of buccal mucosa cells of smokers using computerized image analysis based on quantitative parameters such as nuclear area (NA), nuclear perimeter (NP), minimal nuclear diameter (D-min), and maximal nuclear diameter (D-max), as well as to evaluate potential dysplastic transformation.

## Materials and methods

This study was carried out in the Department of Oral Diagnosis and Oral Radiology, School of Dentistry and Department of Pathology, Faculty of Medicine, Atatürk University. Forty individuals between 40 and 60 years of age, who visited our clinic because of dental problems, were recruited for this study. The study group was composed of 23 cigarette smokers (14 male, 9 female), and the control group consisted of 17 non-smokers (10 male, 7 female). Patients were selected by systematic random sampling, irrespective of gender, socio-economic background, or cultural orientation. However, we accepted into the patient profile that the eating habit be similar to that of Erzurum and its environs. Patients for the study group were selected because they fulfilled the following criteria:

1. Smoked at least 20 cigarettes a day for the last 10 years.
2. Did not suffer from systemic diseases such as anemia or diabetes.
3. Had not received radiotherapy and/or chemotherapy in the last month.
4. Women were not pregnant or menstruating.
5. Did not consume alcohol
6. Did not smoke cheroots or nargile.

Informed consent was obtained from all the patients in the study. All the patients filled out a form and specified their age, the frequency and duration of their smoking, and systemic diseases, if any. Oral examinations were performed using a mouth

mirror and artificial light. Patients were asked to rinse their mouths with water before samples were taken to eliminate debris and excess saliva from the oral mucosa. Exfoliated epithelial cells were obtained from the right buccal mucosa with the help of a brush. Samples were spread on a slide and immediately fixed with fixation spray (Merckofix, Merck, Darmstadt, Germany) to avoid exposure to dry air (otherwise the cells will degenerate). In the pathology laboratory, the samples were stained with Papanicolaou on the same day and were placed under Leica CV 5030 (Leica, Nussloch, Germany), and automatically closed with Entellan bonding material (Merck, Darmstadt, Germany) and lamellae. Images of the nuclei of epithelial cells were obtained under a 20× light microscope (Olympus BX51TF, Olympus Optical, Japan) (Figure). They were transferred to a computer via a camera (Sony DXC-390P, Sony, Japan) and analyzed using a special program (Samba IDB.01, France). Only clearly defined cells were measured, avoiding clumped or folded cells, and unusually distorted nuclei and cells. The sampling was done in a stepwise manner, moving the slide from the left upper corner to the right, and then down in order to avoid measuring the same cells twice. During the measurement, a perimeter was drawn around 100 randomly selected nuclei from each smear. Drawings were repeated for each observation. The average NA, NP, D-min, and D-max values of cell nuclei were obtained for each case.

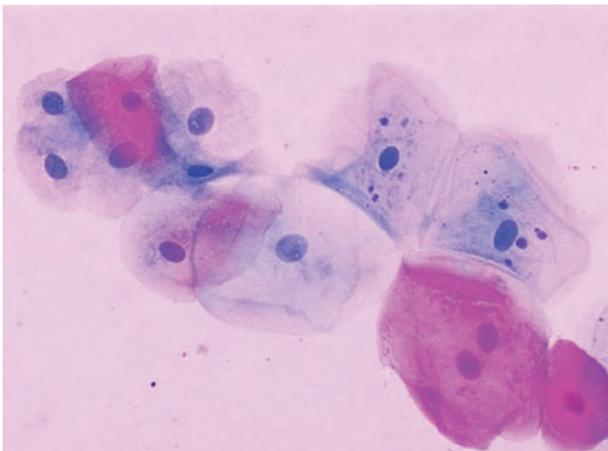


Figure. Microscopic views of buccal mucosa cells in smears stained by the Papanicolaou method.

**Data analysis:** Student's t-test was used to determine whether the differences between NA, ND, D-min, and D-max values of buccal epithelial cells collected from smokers and non-smokers were statistically significant.  $P < 0.05$  was considered statistically significant.

## Results

This study was conducted on 40 individuals, which included 23 smokers who smoked at least 1 pack of cigarettes a day for the last 10 years, and 17 non-smokers. The average NA, NP, D-min, and D-max values of the control and study groups, and the statistical differences are presented in the Table. As shown in the table, statistically significant differences were observed with NA, D-min, and D-max values ( $t = 2.586$ ,  $P = 0.014$ ;  $t = 2.909$ ,  $P = 0.006$ ;  $t = 2.064$ ,  $P = 0.046$ , respectively), while the differences in the values of NP ( $t = 1.910$ ,  $P = 0.064$ ) were not statistically significant between the control and study groups.

## Discussion

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 (16). Under normal conditions, epithelial cells are strongly held in place. However, the presence of benign diseases or the occurrence of malignant epithelial formations 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 (17).

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 (17-19). These parameters, especially NA and NA/CA ratio, have been shown to provide meaningful results in the diagnosis of oral lesions (15,17).

Table. The average of NA, NP, D-min, and D-max values of the control and study groups, and statistical comparison.

	Control Group		Study Group		t	P
	n	mean $\pm$ sd	n	mean $\pm$ sd		
NA ( $\mu\text{m}^2$ )	23	46.00 $\pm$ 11.31	17	53.61 $\pm$ 7.29	2.586	0.014*
NP ( $\mu\text{m}$ )	23	28.18 $\pm$ 2.43	17	29.52 $\pm$ 2.02	1.910	0.064
D-min ( $\mu\text{m}$ )	23	6.18 $\pm$ 0.88	17	6.87 $\pm$ 0.63	2.909	0.006*
D-max ( $\mu\text{m}$ )	23	10.00 $\pm$ 0.94	17	10.52 $\pm$ 0.67	2.064	0.046*

\*P < 0.05

Many cytomorphological studies have been conducted on premalignant and malignant lesions in the oral cavity (1,2,12,15,20-22). Quantitative cytomorphometric evaluation of exfoliated buccal mucosa cells obtained from premalignant and malignant lesions has revealed significant differences at the cellular level (20,21,23). However, few studies have compared exfoliated cells obtained from the normal mucosa of individuals.

The effect of smoking, as a risk factor for oral cancer, depends on the number of cigarettes smoked daily and the duration of smoking. Individuals who have been smoking for 10 years or more, and/or over 2 packs a day are defined as heavy smokers (24, 25). Shiffman et al. (26) considered individuals to be heavy smokers if they smoked over a pack a day. In this study, individuals comprising the study group smoked at least 1 pack a day and had been smoking for at least 10 years.

Smoking has led to different changes in the oral mucosa of many individuals. Smoking has been shown to be related to many pathologies, which range from harmless and reversible lesions, to oral cancer in oral mucous membranes (27-30). Since our study focuses on the effect of smoking on normal buccal mucosa, patients with lesions in the buccal mucosa such as epithelial dysplasia, leukoplakia, erythroplakia, and squamous cell carcinoma were not included.

The parameters used in our research were mean NA, NP, D-min, and D-max. These parameters of NP,

D-min, and D-max have not been previously used in studies in this field. A hundred exfoliated cells from the buccal mucosa of smokers and non-smokers were analyzed, and the average values for each individual were calculated according to the procedure described by Ogden et al. (10) and Ramaesh et al. (9).

Ogden et al. (10) studied the effect of smoking on the oral mucosa in individuals over 40 years of age using cytomorphological methods. They reported a 5% average increase in the NA values of smokers when compared to non-smokers. Our findings are consistent with those of Ogden et al. (10); however, we observed a 16.5% increase in the NA value of smokers over non-smokers. This increase in NA can be attributed to a cellular adaptation that depends on smoking. This adaptive change in the cell nucleus tends to be a dysplastic change.

Ramaesh et al. (9) reported that the nuclear diameter of the oral mucosa cells in individuals who smoked cigarettes, chewed betel quid, or practiced both these habits, was significantly greater than that of the control group individuals. They also reported that the cytoplasmic diameter of individuals who chewed betel quid and practiced both these habits was significantly smaller than that of the control group individuals. Similarly, Einstein and Sivapathasundaram (8) also analyzed the effect of smoking and betel quid chewing on the oral mucosa, using cytomorphological methods, and determined an increase in the average value of ND, and a decrease in cytoplasmic diameter values of smokers

and individuals with both these habits. Because we have discussed ND as D-min and D-max in our study, we can say that our findings are compatible with those of Ramaesh et al. (9), as well as Einstein and Sivapathasundharam (8). Our results showed a statistically significant increase in the D-min and D-max value of smokers. There was no statistically significant difference in the NP values between the 2 groups. Since these criteria have not been used

before, it is not possible to compare this finding with those of other studies. Further studies that use these parameters are needed.

Our results revealed that the NA, NP, D-min, and D-max values of the buccal mucosa cell nuclei of smokers were higher than those of non-smokers, and the difference was statistically significant in the case of NA, D-min, and D-max values.

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