



Exfoliative Cytology of Type 1 Diabetic Patients

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ABSTRACT

Aim: The aim of this study was to analyze cytologically the buccal mucosa, tongue dorsum and floor of the mouth in diabetic patients and healthy volunteers to determine what cellular changes are affected by diabetes mellitus.

Method: In order to evaluate cellular changes induced by diabetes mellitus, exfoliative cytology was used for the analysis of buccal mucosa, tongue dorsum and floor of the mouth smears obtained from 30 type 1 diabetic patients and 40 healthy volunteers.

Result: Cytoplasmic geometric volume of these cells were 93974.37 in tongue, 82104.23 in floor of the mouth and 114373.33 in buccal mucosa in the type 1 diabetic patients. Cytoplasmic geometric volume were 133043.67 in tongue, 113914.45 in floor of the mouth and 165397.38 in buccal mucosa in the control group. Our nuclei geometric volume values were 454.907 in tongue, 626.771 in floor of the mouth and 652,868 in buccal mucosa in the type 1 diabetic patients. Nuclei geometric volume values were 347.149 in tongue, 445.427 in floor of the mouth and 342.592 in buccal mucosa in the control group. NA (nuclei) was markedly higher ($p < 0.005$) in the diabetic patient group, also, cytoplasmic volume exhibited a statistically significant difference ($p = 0.000$) between these two groups. Cytoplasmic volume was markedly higher ($p = 0.000$) in the control group.

Conclusion: The findings suggest that there were alterations in oral epithelial cells, detectable by microscopy and cytomorphometry in diabetic patients undergoing insulin treatment. Further research is needed to determine related factors. It may play an important role in the early detection of diabetes mellitus.

Key words: Oral epithelial cells, type 1 diabetes mellitus, Exfoliative cytology, nuclear volume, cell volume

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Tip 1 Diyabetli Hastalarda Eksfoliyatif Sitoloji

Amaç: Bu çalışmanın amacı diyabetli hastalar ve kontrol gruplarında diyabetes mellitustan etkilenen hücresel değişiklikleri tanımlamak için dil, yanak mukozası ve dil altı hücrelerini sitolojik olarak analiz etmektir.

Metod: Eksfoliyatif sitoloji diyabetes mellitustan etkilenen hücresel değişiklikleri belirlemek için 40 sağlıklı gönüllü ve 30 tip 1 diyabetli hastanın yanak mukozası, dil sırtı ve ağız tabanı içeren smearlarını analiz etmek için kullanıldı. Papanicolaou methodu ile boyanan her bir smear stereoloji methodu kullanılarak analiz edildi. Nükleus ve sitoplazmik volüm software (Steroinvestigator-MicroBrightField) programı ile belirlendi.

Bulgular: Bu hücrelerin sitoplazmik geometrik volümleri tip 1 diyabetli hastalarda dilde 93974,37, ağız tabanında 82104,23 ve yanak mukozasında 114373,33' dü. Sitoplazmik geometrik volümleri kontrol grubunda ise dilde 133043,67, ağız tabanında 113914,45 ve yanak mukozasında 165397,38' idi. Tip 1 diyabetli hastaların nükleus geometrik volüm değerleri dilde 454,907, ağız tabanında 626,771 ve yanak mukozasında 652,868' idi. Nükleus geometrik volümleri kontrol grubunda ise dilde 347,149, ağız tabanında 445,427 ve yanak mukozasında 342,592' idi. Nükleus diyabetik hasta grubunda belirgin bir şekilde yüksekti ($p < 0.05$), ayrıca sitoplazmik volümde de iki grup arasında istatistiksel olarak önemli bir fark olduğu tespit edildi ($p=0,000$). Sitoplazmik volüm kontrol grubunda belirgin bir şekilde yüksekti ($p=0,000$).

Sonuç: Bu bulgular insülin tedavisi altındaki diyabetli hastalarda sitomorfometrik ve mikroskopik olarak tespit edilebilecek oral epitelyal hücrelerde değişikliklerin olduğunu göstermiştir. Gelecek çalışmalar ilişkili faktörleri saptamak için gereklidir. Diyabetes mellitusun erken tanısında önemli bir rol oynayabilir.

Anahtar kelimeler: Oral epitelyal hücreler, tip 1 diyabetes mellitus, eksfoliyatif sitoloji, nükleus volümü, hücre volume

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, associated with irregularities in the metabolism of carbohydrates, lipids, and proteins (1). More than 200 million persons worldwide have diabetes mellitus (2). Diabetes mellitus affects approximately 14 million people in the United States, over a third of whom are undiagnosed (3). It is the third leading cause of mortality and morbidity in the United States, accounting for about 40,000 deaths per year (4). Type 2 diabetes mellitus prevalence is established 7.2% in Turkey (5-7).

Diabetes mellitus is a syndrome that results either from a profound or an absolute deficiency of insulin (type 1) or from target tissue resistance to its cellular metabolic effects (type 2) (4). Type 1 DM results in insulin deficiency secondary to autoimmune mediated destruction of B cells (8). The incidence of type 1 diabetes mellitus has increased in children and teenagers during the past 30 years (2). These patients usually have rapid onset of symptoms and are characterized by a virtually complete inability to produce insulin. A person may have type 1 diabetes develop at any age, although it predominates as the primary form of diabetes in children (2,3). The chronic metabolic complications are generally more severe in the person with type 1 diabetes. These include increased susceptibility to infection and delayed healing, neuropathy, retinopathy, and nephropathy (microvascular disease); accelerated atherosclerosis with associated myocardial infarction, stroke, atherosclerotic aneurysms

(macrovascular disease), and amputation (4). The oral complications of uncontrolled diabetes mellitus can include xerostomia, infection, poor healing, increased incidence and severity of caries, candidiasis, gingivitis, periodontal disease, periapical abscesses, and burning mouth syndrome (2).

Although many of the pathological processes affecting the oral mucosa are clinically distinguishable, most lesions require a definitive diagnosis before the appropriate therapy may be commenced. The most accepted clinical technique for the diagnosis of lesions in the oral mucosa is incisional or excisional biopsy (9). However, in specific clinical conditions, such as diabetes mellitus, a great many invasive techniques lose viability as a result of variations in blood glucose, infection, poor healing and the disease itself (10,11). In these cases, oral exfoliative cytology may be more appropriate (10). Exfoliative cytology is a simple non-aggressive technique that is well accepted by the patient, and allows a quick and fairly accurate assessment of suspicious lesions of the oral cavity (12). Exfoliative oral cytology can be defined as the obtention and characterization of cells from the surface of the oral mucosa (12). This technique, particularly, morphological and morphometric aspect of the cell, may yet provide the implementation of exfoliative cytology in public health programs (10).

The aim of this study was to measure and compare the nuclear and cell volume of cells present in smears collected from buccal mucosa, tongue dorsum and floor of the mouth in diabetic patients (13).

Table 1. Results of the cytomorphometric analysis of oral smears from the control and type 1 diabetic groups, and between groups correlation analysis

	Control group Mean±SD	Type 1 diabetic patients Mean±SD	f	p value
Cell Tongue	133043.67±22442.94	93974.37±23456.33	67,729	0.000
Cell floor of mouth	113914.45±19701.15	82104.23±18547.15	18,224	0.000
Cell Buccal mucosa	165397.38±35262.62	114373.33±25725.89	28,519	0.000
Nuclei Tongue	347.14±79.34	454.90±97.25	48,701	0.000
Nuclei floor of mouth	445.42±132.61	626.77±174.53	13,872	0.000
Nuclei Buccal mucosa	342.59±95.46	652.86±119.84	54,497	0.000

MATERIALS AND METHODS

A total of 30 patients with type 1 diabetes mellitus (16 men and 14 women) and 40 healthy volunteers (24 men and 16 women) were recruited from the Department of Internal Medicine, Ataturk University, Medicine of Faculty, Erzurum, Turkey. Before the enrollment, each subject consented to a protocol reviewed and approved by the Medical Ethics Committee of Ataturk University. A pro forma inventory was completed detailing name, age, sex and relevant medical history. In addition, biochemical and hematological measurements were carried out to exclude anemia and other systematic diseases.

Smears were obtained from clinically healthy buccal mucosa, tongue dorsum and floor of the mouth of patients with diabetes mellitus attending the private clinic and volunteer control individuals. After clinic examination, the tongue mucosa was dried with a gauze swab to remove surface debris and excess saliva. Smears were taken from the tongue dorsum of 30 type 1 diabetic patients and 40 healthy volunteers using a cytobrush and transferred to clean, dry glass slides. These were then immediately sprayed with a commercial fixative containing 95% ethyl alcohol. Smears from each individual stained by the Papanicolaou method were analyzed using stereological method, the nucleator. The smears were placed on a motor-driven stage attached to an microscope and cells were projected onto the monitor via camera at 200x magnification. Each clearly defined cell with predominant staining was examined by systematic sampling in a stepwise manner, moving the microscope stage from left to right and then down and across in order to avoid measuring the same cells again. The nuclear (NV) and cytoplasmic (CV) volume were evaluated for each cell using the software (StereoInvestigator-MicroBrightField).

The cytomorphometric data were compared between

diabetic and control groups by the Independent samples T test (SPSS). The statistical analysis was performed using the statistical software package SPSS (version 10.0; SPSS Inc., Chicago, IL, USA). Levels of significance were set at $p < 0.05$ and $p < 0.001$.

RESULTS

In our study, the mean age was 32.7 years in the type 1 diabetic patients (16 men and 14 women), 36.4 years in the control group (24 men and 16 women). The time of disease was greater than 1 year in % 90 of the diabetic patients, and medication was being used insulin. Cytomorphometric results showed that cytoplasmic geometric volume of these cells were 93974,37 in tongue, 82104,23 in floor of the mouth and 114373,33 in buccal mucosa in the type 1 diabetic patients. Cytoplasmic geometric volume were 133043,67 in tongue, 113914,45 in floor of the mouth and 165397,38 in buccal mucosa in the control group (Table 1). Our nuclei geometric volume values were 454,907 in tongue, 626,771 in floor of the mouth and 652,868 in buccal mucosa in the type 1 diabetic patients. Nuclei geometric volume values were 347,149 in tongue, 445,427 in floor of the mouth and 342,592 in buccal mucosa in the control group (Table 1). NA (nuclei) was markedly higher ($p=0.000$) in the type 1 diabetic patient group, also, cytoplasmic volume exhibited a statistically significant difference ($p=0.000$) between these two groups. Cytoplasmic volume was markedly higher ($p=0.000$) in the control group (Table 1).

DISCUSSION

In this study, we performed microscopic and cytomorphometric analyses of the oral epithelium in type 1 dia-

betic patients. Oral exfoliative cytology have important role because it could play in the diagnosis, prevention, control of the disease. This findings demonstrated that there was a real increase in the nuclear volume in the type 1 diabetic group present statistically significant differences. In addition, cell volume was increase in the control groups present statistically significant differences. There was the most increase in nuclear volume of buccal mucosa.

Alberti et al.(10) performed cytomorphometric analyses of the oral epithelium in type 2 diabetic patients and they found that there is a real increase in the nuclear area, as the cytoplasmic area did not present significant differences. They suggested that the cellular modifications may be related the chronic inflammatory process present in the oral cavity and partly by a delay in the keratinization process of the oral epithelium in type 2 diabetic patients (10). The inflammatory process can generate a microscopic picture identical to that found in the oral mucosa of diabetic patients. The findings may be understood by the presence of superficial erosions or ulcerations of the oral mucosa's squamous epithelium, which frequently occur in the course of inflammatory processes, such as diffuse stomatitis and gingivitis (2,10,14,15).

Other variables may lead to changes similar to those found in the oral smears of type 1 diabetic patients, such as in nutritional deficiencies like hypochromic anemia (iron deficiency) and megaloblastic anemia (deficiency of vitamin B12 and folic acid). Vitamin B12 and folic acid are essential substances in DNA synthesis. Thus, nutritional deficiencies involving both factors disturb DNA synthesis, with consequent increases in both cytoplasm and nucleus size (10).

The oral cavity is almost constantly flushed with saliva, which floats away food debris and keeps the mouth relatively clean. If the flow of saliva diminishes considerably, allow populations of bacteria to build up in the mouth (16,17). It is known that diabetic patients have lower salivary flow rates (18). Decrease in the salivary flow of diabetic patients probably related to systemic dehydration (polyuria), medication (diuretics) and/or membranopathy of the ductus (10). These may affect the cytomorphometric alterations of the oral mucosa cells. A number of factors that could influence the cytomorphology of cells removed from the oral mucosa have been investigated. These include radiotherapy, smok-

ing, alcohol, and malignant oral lesions. Therefore, the effects of such factors, if present, should be taken into account when assessing a lesion under investigation (19-21).

As a result of the fact that exfoliative cytology is a simple and rapid, non-aggressive and relatively painless: it is thus well accepted by patients and suitable for routine application in population screening programmes, for early analysis of suspect lesions, and for pre-and post-treatment monitoring of confirmed malignant lesions (12). The results observed in this study might contribute to the general understanding of the alterations in the cellular pattern of oral mucosa in diabetic patients (10).

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