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Original

Evaluation of oral mucosa epithelium in type II diabetic patients by an exfoliative cytology method

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Abstract: Diabetes mellitus is a common metabolic disease that causes chronic hyperglycemia and disturbances in carbohydrate, lipid, and protein metabolism. Although diabetes can cause considerable cellular changes, this field has attracted little research. We therefore decided to evaluate the quantitative and qualitative changes in oral epithelial cells using an exfoliative cytology method. In 30 control individuals and 30 patients with type II diabetes, smears were obtained from two distinct oral sites: the buccal mucosa and tongue dorsum. The oral smears were stained using Papanicolaou solution. Quantitative and qualitative changes were evaluated in each slide. For this purpose, 50 clearly defined cells in each slide were microscopically evaluated, and photographs were subjected to computerized morphometric analysis. Cytoplasmic and nuclear areas in the diabetic group were significantly higher than in the control group. The cytoplasmic/nuclear ratio was lower in the control group. At both smear sites, the proportion of cells with nuclear changes was higher in the diabetic group. Diabetes mellitus can cause alterations in the oral epithelium that are detectable with this exfoliative cytology method. The method may be viable in evaluating this disease. (J. Oral Sci. 50, 335-340, 2008)

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Introduction

Diabetes mellitus is one of the most common endocrine metabolic disorders and its prevalence has been increasing worldwide. It affects about 14 million people in the United States with the number of new cases increasing by more than 700,000 per year (1). In Iran, 7.7% of adults aged 25 to 64, or 2 million adults, are estimated to have diabetes, of which half are undiagnosed (2). Type II diabetes mellitus accounts for approximately 95% of diabetes cases (1). This type of diabetes is the fifth most common chronic condition and the sixth most frequent cause of death among the elderly (3-5).

Hyperglycemia can be associated with several oral complications. Tissue repair is damaged and dysfunction of the oral mucosa occurs due to alterations in salivary flow (3,6,7) and constituents, changes in nutrition, and reduced immune defenses leading to changes in microbial oral flora and a greater tendency to infections (6,8). As a result, xerostomia, candidiasis, increased incidence of dental caries, gingivitis and periodontitis, periapical abscess, parotid enlargement, and burning mouth syndrome (BMS) are prevalent oral pathologies in patients with diabetes (6,8).

Several clinical and paraclinical techniques are available for evaluation of oral mucosal changes. Incisional or excisional biopsy is the most reliable technique for definitive diagnosis (9). For the evaluation of mucosal changes in patients with diabetes, some studies have used gingival biopsies showing an increase in thickness and hyalinization

of small capillaries (6). However, in diabetes, changes in blood glucose and the disease itself reduce the viability of such invasive techniques (11,12).

Accordingly, exfoliative cytology, which is a straightforward and noninvasive diagnostic method (13), can be considered as a more practical technique to evaluate the oral mucosa in diabetes. This method has been discussed over the last 30 years in the diagnosis of precancer in the cervical and vaginal mucosa and similar lesions of the oral mucosa. Most authors have come to the conclusion that exfoliative cytology is of some value in the diagnosis of precancerous lesions, and particularly in determining their prognosis. In recent years exfoliative biopsy has been introduced with good results in specification and diagnosis of the lesions (9,10,13). However, use of this method to evaluate quantitative and qualitative changes in oral epithelial cells in diabetes is debatable. A few studies have used exfoliative cytology to evaluate changes in the oral mucosa in diabetes mellitus and have shown that this disease can produce alterations in oral epithelial cells that are detectable by cytomorphometric analysis (10). The goal of our study was to evaluate the quantitative and qualitative changes in oral epithelial cells by exfoliative cytology in patients with type II diabetes.

Materials and Methods

Study population

We randomly selected 30 patients with a 5 to 10 year history of type II diabetes mellitus with last fasting serum glucose level ranging from 130-400 mg/dl from Tehran Endocrinology and Metabolism Research Center (as the case group) and 30 non-diabetic healthy persons with no risk factors for diabetes (as the control group). Diagnostic criteria for type II diabetes were as follows: symptoms of diabetes plus random serum glucose concentration ≥ 200 mg/dl (11.1 mM), or fasting serum glucose level ≥ 126 mg/dl (7.0 mM), or 2-hp ≥ 200 mg/dl (11.1 mM) (14). All participants ranged from 40 to 70 years of age. We designed a questionnaire that patients completed, including demographic characteristics such as age, race and sex and relevant medical history. Individuals who smoked, were dependent on alcohol, or had anemia or malignancy were excluded, to eliminate the effects of these conditions on cellular shape and morphology.

Smear collection

We chose two oral sites to take smears from, the buccal mucosa and tongue dorsum. Before samples were taken, patients washed their mouths with normal saline for about 5 minutes and then a surface smear was taken with the tip of a lancet. The smears were transferred to a clean dry glass slide and immediately spray-fixed with 95% ethyl alcohol. We then prepared the slides with a modified Papanicolaou staining process for cytomorphometric analysis (15).

Cytomorphometric assessment

In each slide, 50 clearly defined cells with predominant staining were selected manually in a random fashion from different fields, and in order to avoid measuring and counting the same cells again, we moved the microscope stage from left to right, and then down and across in a stepwise manner. Determining the mean lengths of the greater and lesser diameters of each cell (considering their oval shape and taking into account dimensions of the nucleus) we were able to calculate the cellular area (CA) and nuclear area (NA). Bilobed or multi lobed nuclei and the presence of inflammation, candidiasis, or cytoplasmic vacuolization were reported. We carried out all cytomorphometric analysis by transferring sample images at 100× magnification from a Leica Galen III microscope Buffalo, New York, USA) to a computer by means of digital camera (Sony EXWaveHAD Model No.SSC-DC58AP, Tokyo, Japan). We used Photoshop software for cytomorphometric assessments.

Statistical analysis

Results are indicated as mean ± SD for quantitative variables and as percentage for categorical variables. Variables were compared between two groups using the chi-square test or Fisher's exact test if required for categorical variables and the *t*-test for numeric variables. We used SPSS software version 13 for windows. *P* values < 0.05 were considered significant.

Results

Cytomorphometry showed that cytoplasmic area (CA) was significantly larger in patients with diabetes when compared to controls for both the tongue dorsum ($P < 0.0001$) and buccal mucosa ($P < 0.0001$). Nuclear area (NA) was also significantly larger in patients with diabetes ($P < 0.0001$ for both the tongue dorsum and buccal mucosa) (Table 1).

Cytoplasmic/nuclear (C/N) ratio was significantly larger in controls ($P < 0.0001$ for both smear sites) (Table 1).

Proportion of cells with no nuclear changes was significantly higher in the control group (buccal mucosa, $P < 0.0001$; tongue dorsum, $P < 0.009$). Karyorrhexis was significantly more common in the diabetic group, but only in the buccal mucosa ($P < 0.0006$). Frequency of other nuclear changes did not differ significantly between the two groups, although multilobed nuclei and cytoplasmic vacuolization showed a non-significant tendency to be

more frequent in diabetic patients (Table 2).

Frequency of inflammation, candidiasis, and cytoplasmic vacuolization was assessed and compared statistically between the two groups in both the buccal mucosa and tongue dorsum (results not shown); however, only inflammation in the tongue dorsum differed significantly between cases and controls ($P < 0.05$) and inflammation incidence was numerically higher in diabetic smears. We found a trend for candidiasis to be more frequent in the diabetic group than in the control group; however, this difference was not statistically significant.

Typical nuclear changes in the diabetic group (binucleation, karyorrhexis), cytoplasmic vacuolization, inflammation, and candidiasis) are shown in Fig. 1 (A-E, respectively), compared to a typical smear from the control group (F).

Discussion

In this study, we performed cytomorphometric analysis on smears obtained from patients with diabetes and compared the slides with those from healthy individuals. We aimed to clarify the findings of previous studies and determine the importance of this issue to public health. Our results showed a statistically significant increase in CA and NA in cells from patients with diabetes when compared to control cells, both in the tongue dorsum and the buccal mucosa. C/N ratio was considerably smaller in the diabetic group than in the control group. The present results are consistent with those of a similar study by Sandra et al. in terms of NA increase and C/N decrease in the diabetic group, but we also found CA enlargement in the diabetic group, a finding that their study did not show (10). This difference may be related to the disparity in the number of analyzed cases (30 in each group in our study compared to 10 in their study).

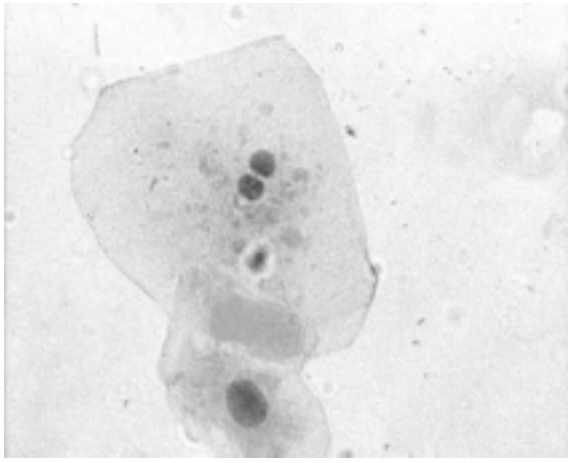
Inflammation is one of the factors that can increase NA and lead to a poorly preserved cytoplasm (16), but these characteristics are typically found only in young cells and are not representative of cellular atypias (10). In this study, we took the smears from different oral sites (the buccal mucosa and tongue dorsum) to reduce the effect of localized inflammation on our results. We also detected a range of cellular age in the smears, and cytomorphometric alterations were generalized rather than restricted to a certain generation of cells, suggesting that the changes we observed are not just related to inflammation.

Table 1 Cytomorphometric results of oral smears in both control and diabetic groups

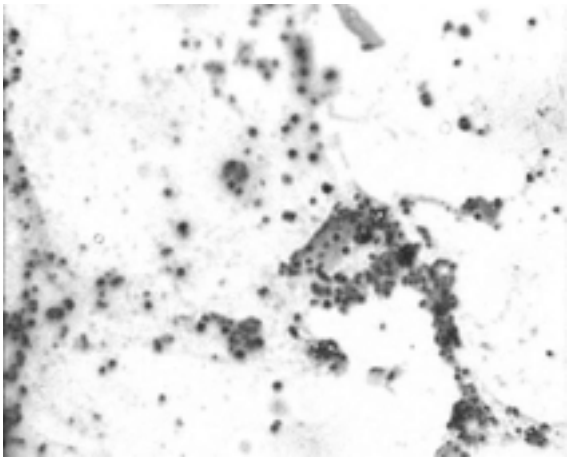
Determination	Control group (n = 30) Mean ± SD	Diabetic group (n = 30) Mean ± SD	*P* value
Nuclear area (μm²):			
Buccal mucosa	40.90 ± 0.68	60.48 ± 0.62	< 0.0001
Tongue dorsum	49.14 ± 1.14	52.33 ± 0.59	< 0.0001
Cytoplasmic area (μm²):			
Buccal mucosa	1659.44 ± 31.64	2129.85 ± 25.94	< 0.0001
Tongue dorsum	1669.23 ± 32.91	1716.21 ± 20.04	< 0.0001
Cytoplasmic/nuclear ratio			
Buccal mucosa	44.07 ± 0.492	39.04 ± 0.553	< 0.0001
Tongue dorsum	42.43 ± 0.716	37.41 ± 0.572	< 0.0001

Table 2 Nuclear morphology alterations in oral smears of both diabetic and control groups

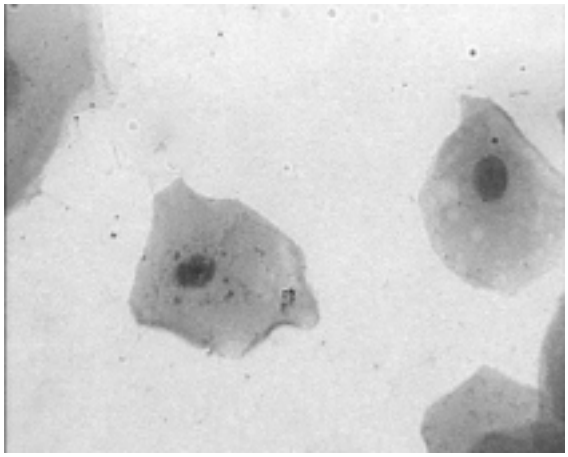
Nuclear position	Diabetic group (n = 30) %	Control group (n = 30) %	*P* value
No changes:			
Buccal mucosa	26.7	86.7	< 0.0001
Tongue dorsum	36.7	70	0.009
Multilobed nuclei:			
Buccal mucosa	23.3	6.7	0.072
Tongue dorsum	13.3	10	0.690
Karyorrhexis:			
Buccal mucosa	40	3.3	0.0006
Tongue dorsum	30	13.3	0.116



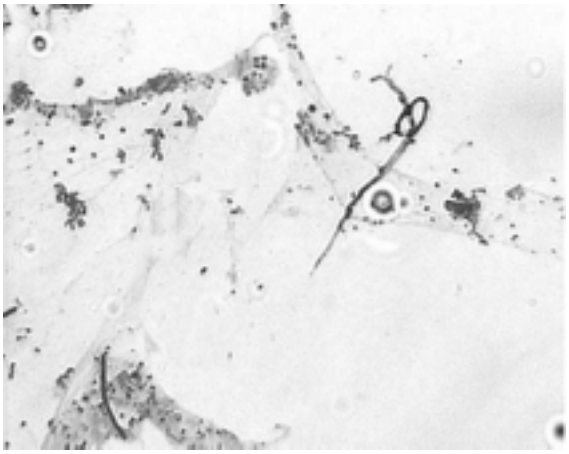
A



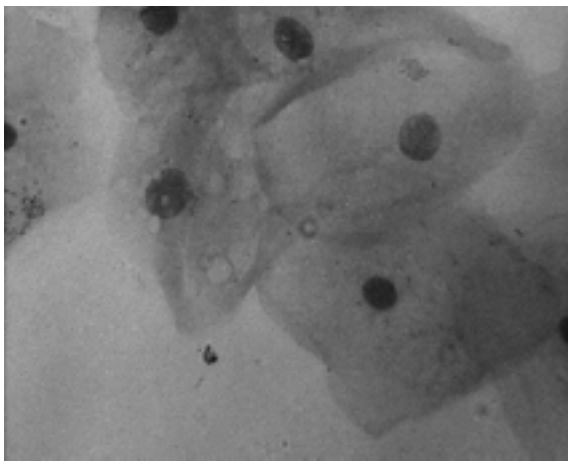
D



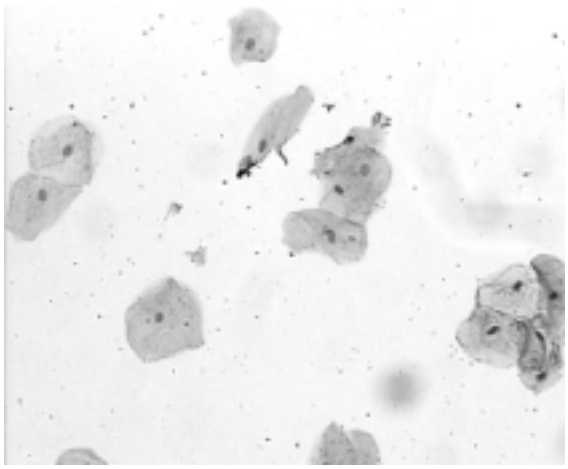
B



E



C



F

Fig. 1 Oral epithelial cells in smears stained by the Papanicolaou method: binucleation (A), karyorrhexis (B), cytoplasmic vacuolization (C), inflammation (D), and candidiasis (E) in typical smears from the diabetic group, compared to a typical smear from the control group (F). (A, B, C, and D: ×400, E and F: ×100)

We found that nuclear changes in both the buccal mucosa and tongue dorsum were significantly higher in the diabetic group than in the control group. This could be related to increased cellular age in patients with diabetes. Decreased cellular turnover might be a secondary reaction to ischemia caused by atherosclerosis in diabetic patients (12). Thus as a result of ischemia, cellular turnover would decrease and limited production of young cells would mean that the majority of cells are old or aged.

The overall process of aging in the individual is greatly affected by genetic factors, diet, and social conditions, as well as by age-related diseases such as diabetes. In addition, there is good evidence that cellular alterations induced by aging are an important component of aging of the organism. Cellular aging is the result of a progressive decline in the proliferative capacity and lifespan of cells; the effects of continuous exposure to exogenous factors cause the progressive accumulation of cellular and molecular damage. Morphologic alterations in aging cells include irregular and abnormally lobed nuclei, and pleomorphism (17). In the present study, multilobed nuclei and cytoplasmic vacuolization tended to be more frequent in diabetic patients, but this trend was not statistically significant. Advanced glycation end products are important in the pathogenesis of diabetes mellitus and they may also participate in cellular aging (17).

We found a trend for candidiasis to be more frequent in the diabetic group than in the control group; however, this difference was not statistically significant, which might be related to our small sample size. Guggenheimer et al. reported a much higher prevalence of candidiasis in diabetic patients (18).

We found a significant increase in inflammation in the tongue dorsum in the diabetic group in comparison with control group, and there was a similar trend in the buccal mucosa. This might result from decreased salivary flow in diabetes, due to hypofunction of the salivary glands secondary to adverse hormonal, microvascular, and neuronal changes (3,19).

In conclusion, we found noteworthy alterations in the cytomorphology of the oral mucosa in diabetes, and characterization of these changes give clinicians a more accurate image of what really happens during diabetes. Although we found significant cytomorphological changes in the oral mucosa in diabetes, these alterations cannot be considered predictive or diagnostic for diabetes because they are not unique to this disease. Even so, inflammation, candidiasis, and nuclear changes resulting from the diabetes disease process can collectively alert the clinician to the possibility of diabetes. However, further studies with greater sample size and comparison to other conditions causing similar cytomorphometric changes are needed to determine the predictive value of this method.

Moreover, we did not compare cytomorphometric alterations according to different stages of diabetes. It is possible that the degree of cellular change depends on the progression of diabetes, and this is another area for further research that could have considerable public health implications. This research area has received little attention to date and is worthy of further exploration.

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