doi: 10.7213/archivesoforalresearch.09.002.AO06 ISSN 2236-8035
Archives of Oral Research, v. 9, n. 2, p. 185-191, May/Aug. 2013

Cytological analysis of epithelial cells in adolescents with type 1 diabetes mellitus

Análise citológica das células epiteliais de adolescentes com diabetes mellitus tipo 1

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Abstract

Introduction: Type 1 diabetes mellitus is a chronic metabolic disorder condition, which may causes oral mucosa changes in adolescents. The effect of glycemic control on the oral tissues has been scarcely reported as well as the low number of oral mucosal lesions found in this population. The goal of this study is to evaluate cytopathological changes in oral epithelial cells of adolescents with type 1 diabetes mellitus (DM1) and assess the glycemic control. **Materials and methods**: Oral smears were collected from normal mucosa by exfoliative cytology in 40 healthy adolescents and 40 with DM1. Cell morphology and cellularity were analyzed. Nuclear area (NA) and cytoplasmic area (CA) were measured; nuclear/cytoplasmic area ratio (NA/CA) was calculated. Time elapsed from the diagnosis of diabetes and glycated hemoglobin levels (HbA1c) were recorded. **Results**: There was no significant difference to NA, CA, NA/CA for both groups and also in relation to HbA1c ($P > 0.05$). No morphological differences were found among the groups ($P > 0.05$). There was a predominance of nucleated cells of the superficial layer in smears of both groups. Class I and Class II smears were predominant in both groups. **Conclusion**: This study revealed that type 1 diabetes mellitus and the glycemic control were unable to induce significant changes on oral epithelial cells in adolescents.

Keywords: Diabetes Mellitus. Type 1. Cytology. Mouth mucosa.

Resumo

***Introdução**: A diabetes mellitus do tipo 1 é uma doença metabólica crônica que pode causar alterações nas células do epitélio da mucosa bucal. O estudo do efeito do controle glicêmico nos tecidos bucais tem sido pouco relatado e o baixo número de lesões da mucosa bucal encontrada nesta população também. O objetivo deste estudo foi avaliar as alterações citológicas nas células epiteliais bucal de adolescentes com diabetes mellitus tipo 1 (DM1) e avaliar o controle glicêmico. **Materiais e métodos**: Esfregaços da mucosa normal foram coletados por citologia esfoliativa em 40 adolescentes com DM1 e 40 saudáveis. A morfologia celular e a celularidade foram analisadas. A área nuclear (NA) e área do citoplasma (CA) foram medidas, e a relação de área de núcleo/citoplasma (NA/CA) foi calculada. O tempo decorrido desde o diagnóstico da diabetes e os níveis de hemoglobina glicada (HbA1c) foram registrados. **Resultados**: Não houve diferença significativa para NA, CA, NA/CA para ambos os grupos e também em função da HbA1c (P > 0,05). Não foram encontradas diferenças morfológicas entre os grupos (P > 0,05). Houve um predomínio de células nucleadas da camada superficial em esfregaços de ambos os grupos. Esfregaços classe I e classe II foram predominantes em ambos os grupos. **Conclusão**: Este estudo revelou que o diabetes mellitus tipo 1 e o controle glicêmico não foram capazes de induzir alterações significativas nas células epiteliais da mucosa bucal de adolescentes.*

***Palavras-chave**: Diabetes Mellitus tipo 1. Citologia. Mucosa bucal.*

Introduction

Type 1 diabetes mellitus (DM1) is a chronic disease caused by total or relative insulin deficiency, characterized by chronic hyperglycemia and disturbances of carbohydrate, lipids and protein metabolism (1, 2). According to reports of the American Diabetes Association 75% of DM1 cases are diagnosed in persons under the age 18 years (3). Epidemiological studies demonstrated a strong association between lack of glycemic control (continuous or prolonged hyperglycemia) and the occurrence of oral and systemic complications in DM1 individual (4-7).

Quantitative changes of epithelial cells in the oral mucosa of individuals with type 2 diabetes were demonstrated (8-10), but no study was conducted with DM1 adolescents or has assessed the glycemic control.

Thus, the purpose of this study was to evaluate morphometrical and morphological changes in oral epithelial cells collected by exfoliative cytology in adolescents with DM1, and assess the glycemic control.

Materials and methods

The experimental protocol was approved by the Committee of Ethics at Hospital de Clínicas of Universidade Federal do Paraná Curitiba, Brazil, (CEP nº 1546). The adolescents and/or their parents or guardians were informed about the objective and other aspects of the research and have signed the terms of agreement.

Subjects. Forty adolescents with type 1 diabetes mellitus (DM1 group) and 40 healthy adolescents (Control group - C) aged from 14 to 19 years, paired in relation to sex and age, were considered for the study. The diagnosis of DM1 using the American Diabetes Association (ADA) classification was established as a criterion for inclusion in the sample (11).

The DM1 group was diagnosed with DM1 at Hospital de Clínicas at the Diabetes Ambulatory Care Service of Universidade Federal do Paraná, and healthy adolescents (C) were recruited from a public high school in Curitiba, Paraná. The adolescents with DM1 were followed up quarterly at the hospital. Name, age, relevant medical history were recorded in both groups. Adolescents who used alcohol, illegal drugs, mouthwashes or orthodontic braces as well as those presenting lesions in their oral mucosa were excluded from this sample, in order to minimize the possible effects of such conditions upon the epithelial cell morphology. In the control group, adolescents with any systemic disease were also excluded from the sample.

Evaluation of Glycemic Control. The results of glycated hemoglobin (HbA1c) tests were recorded

prior to cells collection. Patients with good glycemic control were considered to be those with HbA1c values of ≤ 8.0%, whereas poorly-controlled patients were considered to be those with HbA1c values of > 8.0% (11, 3). The time elapsed from the diagnosis of DM1, values of glycated hemoglobin (HbA1c) and capillary glycemia were collected from adolescents' medical registers at the hospital. The time elapsed from the diagnosis of DM1 was registered considering the period ≤ 9 years and > than 9 years.

Cells Collection. Exfoliated cells of the clinically normal oral mucosa were obtained by oral liquid-based exfoliative cytology. The squamous epithelial cells were collected using Universal Collection Medium (UCM) kit of DNA-Citoliq System™ (Digene, São Paulo, Brazil). The UCM brush provides an adequate and representative number of superficial and intermediate epithelial cells of oral mucosa.

Cytological preparations. The DNA-Citoliq System™ allows thin-layer preparations to be used through a filtration process. An aliquot of 200 µL of UCM was filtered through Filtrogene polycarbonate membrane filters™ (Digene, São Paulo, Brazil), pore size 5 µm, diameter 25 mm placed in Prepgene press™ (Digene, São Paulo, Brazil) attached to glass slides. Glass slides were immediately fixed in absolute alcohol for 20 minutes. Smears were then stained with routine Papanicolaou stain.

Cytomorphometric analysis. Each slide was assessed using the light microscopy by binocular Olympus BX50 microscopy™ (Olympus, Japan). Fifty randomly selected cells were measured in a stepwise fashion. Cell images were captured for digitalizing by Sony CCD Iris Color Video Camera™ (Sony Model DXC-107A, Japan) at x400 magnification. Nuclear (NA) and cytoplasmic (CA) areas were obtained by drawing around the nuclear and cell boundaries using the digitizer cursor and measuring mode of Image-Pro Plus image analysis system™ (Media Cybernetics, Silver Spring MD, USA), version 4.5.029 for Windows™ 2000. The NA/CA ratio of the epithelial cells of each sample was calculated. All smears were analyzed by the same person, who was previously gauged.

Cytomorphological analysis. Each slide was assessed using the light microscopy by binocular Olympus BX50 microscopy™ (Olympus, Japan). All cellular features were coded according to Papanicolaou classification (12). Papanicolaou staining is used as a routine method for the analysis of cytological aspects and allows the identification of basic inflammatory, dysplastic or malignant alterations (12). Additionally, the type of predominant cell (cellularity) in each smear was also analyzed.

Statistical Analysis. All data were tabulated, and statistical tests were performed with SPSS version 15.0 for Windows (SPSS Inc, Chicago, IL). Significant differences between groups and cytomorphological and cytomorphometric analysis were examined using t-test ($P < 0.05$). Significant differences in DM1 group, considering cytomorphometric variables (NA, CA and NA/CA) and the time elapsed from the diagnosis of DM1 and glycated hemoglobin were examined using t-test ($P < 0.05$). Chi-square test was performed for cytomorphologic variables ($P < 0.05$).

Results

From all 80 participants, 40 were from DM1 and 40 from C groups. The screened patients were 20 males and 20 females for each group. The mean age for DM1 was 17.35 years and for C it was 17.28 years.

The mean time elapsed from the diagnosis of DM1 was 8.52 years. The mean capillary glycemia and glycated hemoglobin (HbA1c) values were 203.87 mg/dL and 10.32% respectively, for DM1.

There was no significant difference ($P > 0.05$) between DM1 and C for morphometrical (Table 1) and morphological analysis (Tables 2 and 3). In cytomorphological analysis, in both groups, all analyzed slides were classified according to Papanicolaou's Class I or Class II, with normal smears with or without the presence of inflammatory cells (Table 2). Table 3 presents the type of predominant cell in each smear. It was observed a predominance of nucleated cells of the superficial layer.

According to the values of HbA1c, 27.8% (n = 14) of the subjects with DM1 were considered with a good glycemic control, and 72.2% (n = 26) with poor glycemic control. There was no significant difference ($p > 0.05$) between good and poor glycemic control for cytomorphometric variables (NA, CA and NA/CA) (Table 4).

Table 1 - Mean and standard deviation of NA (nuclear area), CA (cytoplasmic area), NA/CA (nucleous-to-cytoplasmic area ratio) in Type 1 Diabetes Mellitus (DM1) and Control (C) groups.

Groups	DM1	C	
Variable	Mean ± SD	Mean ± SD	P *value*
NA (μm2)	57.59 ± 5.58	57.28 ± 5.11	0.7985
CA (μm2)	2643 ± 42.88	2603 ± 44.73	0.6839
NA/CA (μm2)	0.03 ± 0.00	0.03 ± 0.00	0.0559

t -test, *Statistics difference ($P < 0.05$).
Source: Research data

Table 2 - Morphologic characterization of oral smears according to Papanicolaou's system classification in Type 1 Diabetes Mellitus (DM1) and Control (C) groups.

Classification	DM1 (%)	C (%)	P value
Class I	19 (47.50)	18 (45)	
Class II	21 (52.50)	22 (55)	0.8225
Total	40 (100)	40 (100)	

Chi-Square test, *Statistics difference ($P < 0.05$).
Source: Research data

Table 3 - Predominant cells in smears of Type 1 Diabetes Mellitus (DM1) and Control (C) groups.

Groups	Enucleated superficial layers cells n (%)	Nucleated superficial layers cells n (%)	Intermediated layer cells n (%)	Basal layer cells n (%)	Total	P value
DM1	-	35(87.50)	5(12.50)	-	40	0.5
C	-	33(82.50)	7(17.50)	-	40	31

Chi-Square test; *Statistics difference ($P < 0.05$).
Source: Research data

Discussion

It is well established that poorly-controlled diabetes can increase the glucose concentration in blood, and induce microvascular abnormalities and alterations in the response to infections (4). The systemic condition may affect the physical development of the adolescents (1). Furthermore, adolescence is a period of life marked by increased independence from family members, and adolescents with DM1 can not seriously follow the treatment and monitoring of disease (13, 5).

Clinical findings related to poorly-controlled diabetic patients affecting mainly the oral mucosa are candidosis, benign migratory glossitis, and xerostomia (8). Xerostomia frequently results from

Table 4 - Mean and standard deviation of NA (nuclear area), CA (cytoplasmic area), NA/CA (nucleous-to-cytoplasmic area ratio) in Type 1 Diabetes Mellitus group (DM1) related to glycated hemoglobin (HbA1c).

HbA1c - DM1 group			
Good Control (n = 14)		Poor Control (n = 26)	
Morphometric variables	Mean ± SD	Mean ± SD	P value
NA (μm2)	56.57±7.28	57.37±4.94	0.7062
CA (μm2)	2787±43.41	2642±41.44	0.3096
NA/CA (μm2)	0.03±0.00	0.03±0.00	0.2053

*t -test, * statistical difference significant (P < 0.05).*
**Reference values: good control ≤ 8% of HbA1c, poor control > 8% of HbA1c.*
Source: Research data

a reduction in saliva secretion, which is associated with an increase in the occurrence of dental caries due to systemic dehydration (polyuria), pharmacological interference (diuretics) and alteration in the membranes of excretory salivary ducts (5, 6). These alterations may cause damage to the oral mucosa epithelial cells in DM1 patients (10). Other reported manifestations include burning mouth syndrome, altered sense of taste, *lichen planus* and widening of the parotid (14). Although, the prevalence of these manifestations is frequent in type 2 DM and low during adolescence.

Few studies have demonstrated the relevance of the oral manifestations in patients with type 1 and 2 DM. However, the effect of glycemic control in the epithelial oral tissue still has not been widely investigated until now (8, 9, 15, 16).

In the present study, a morphological and morphometric analysis of the oral epithelium cells in adolescents with DM1 and the glycemic control were performed. Morphologically, all smears of both DM1 and C groups were classified as class I and II of Papanicolaou system for cytology. In the original Papanicolaou classification system, class I is defined as the absence of atypical or abnormal cells and class II as cells with normal morphology and inflammatory changes (12). This result was expected because all smears were obtained from a region of clinically normal mucous membrane. Furthermore, there is not a high occurrence of neoplasms in the oral mucosa of adolescents with diabetes. The inflammatory changes were observed in 50% of the smears analyzed. This result suggests that the oral mucosa might also be injured during mastication causing a chronic tissue irritation.

A predominance of nucleated cells of the superficial layer of epithelium in both groups indicates a regular maturation process without morphological variations. The oral mucosa epithelium is composed mostly by keratinocytes that are arranged in the basal, spinous, granular and superficial layers. Oral keratinocytes proliferate and differentiate along these layers until they are exfoliated (17). Diabetics could be more susceptible to develop oral epithelium changes, infections and poor healing, due to the pathophysiology of the disease (3).

However, morphometric changes (NA, CA and NA/CA ratio) were not observed in the DM1 group, neither related to glycemic control (HbA1c). This was the first cytological study with adolescents with DM1 (mean age 17.35). In comparison with other cytomorphometric studies, our sample was reasonable large (40 for each group), whereas in studies with type 2 DM, only 10 individuals were analyzed per group (9, 16).

Studies have demonstrated that the nuclear and cytoplasmic areas of epithelial cells in oral mucosa may be modified due to systemic conditions, such as anemia (18) or in response to the use of local agents like alcohol (19), tobacco (20,21) and illicit drugs such as crack (22).

Cytomorphometric studies of the oral normal mucosa epithelium in adults with type 2 DM demonstrated microscopic changes, such as increase or decrease in nuclear or cytoplasmic area and in nuclear/cytoplasmic ratio in the epithelium cells

when compared with healthy adults (9, 16). These alterations were explained by the authors as partially due to a delay in the keratinization process of the oral epithelium, something that increases nuclear size and decreases the cytoplasm (9). These findings could suggest an epithelial atrophy, once the thickness of epithelial layers is reduced. Moreover, keratinocytes do not exhibit a normal maturation process and are exfoliated before the decrease of the nucleus. This would justify a hypothesis that the nucleus increase in keratinocytes of oral mucosal epithelium of individuals with type 2 DM, but the delay in the keratinization process was not observed in adolescents with DM1.

In the present study, 65% (n = 26/40) of the DM1 group demonstrated poor glycemic control. The maintenance of low levels of HbA1c can be related to cellular aging (8). End products of the advanced glycation may be involved in the pathogenesis of DM and may also participate in the cellular aging process (8).

The decrease of cellular renewal process may be a secondary reaction to the ischemia caused by atherosclerosis in type 2 DM. Ischemia is related to a decrease in cell renewal – the proliferation of new cells is limited, as a result of a majority of old cells (8). Unfortunately, it was not possible to confirm this hypothesis, because morphological and morphometric changes were not found in the present study, despite of the fact that the mean time elapsed from the diagnosis of DM1 was 8.52 years.

The micro- (renal and ocular) and macrovascular (atherosclerosis) complications depends on the glycemic control and the time of DM evolvement. Approximately 3/4 of all DM cases are diagnosed in individuals younger than 18 years of age (1).

HbA1c can be used as a diagnostic test for diabetes and reflects average plasma glucose over the previous 8 to 12 weeks (2). It is the preferred test for assessing glycemic control in people with diabetes (2). The maintenance of the HbA1c levels as close as possible to normal has a great importance to reduce the susceptibility to infections and poor healing (4).

During the adolescence period there is a physiological increase in insulin needs, making difficult to control someone's capillary glucose and weight, demonstrating that the daily glycemic control is complex (7, 6). In this study, we found that 26 of the 40 adolescents with DM had a poor glycemic control in the previous 3 months.

Epidemiological evidence suggests that the burden of diabetes is likely to continue to increase globally. Due to an increase in the prevalence of adolescents with DM1 and the complexity and difficulty to control this disease, further studies should be performed to investigate the long-term impact of DM and its effects on oral mucosa.

Conclusion

This study revealed that type 1 diabetes mellitus and the glycemic control were unable to induce significant changes on oral epithelium cells.

Acknowledgments

The authors thank Dr. Sérgio Aparecido Ignácio for his help in statistical analysis. The authors also thank the Hospital de Clínicas at the Diabetes Ambulatory Care Service of Universidade Federal do Paraná for the opportunity of developing this study.

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Recebido: 02/11/2013
Received: 11/02/2013

Aceito: 13/01/2014
Accepted: 01/13/2014