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Clinical, statistical, histological and immunohistochemical aspects of periodontal changes in patients with diabetes mellitus

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Abstract

Background: Both diabetes mellitus (DM) and periodontal disease are the most widespread chronic inflammatory diseases that affect a very large number of the population worldwide. *Aim*: This study's aim was to compare the status of dental hygiene in a group of patients with DM, with patients in the control group, and to histologically analyzing the gum from the subjects with DM. *Patients, Materials and Methods*: The study sample was made up of 53 control subjects and 107 diabetics aged 19–80 years old. We evaluated the following parameters: the plaque index (PI) and the calculus index (CI), according to Simplified Oral Hygiene Index, and the gingival index (GI), according to the Löe & Silness criterion, correlated with glycosylated hemoglobin and the blood sugar levels. *Results*: For all hygiene indices, the mean values recorded for the control group were significantly lower than the mean values recorded for any sub-category in the diabetic groups. *Conclusions*: DM contributes unfavorably to the evolution of periodontal disease. The poor glycemic control and the improper oral hygiene have a negative impact on the health of the periodontium, highlighted by increased scores on PI, CI and GI scales.

Keywords: glycosylated hemoglobin, blood sugar, plaque index, calculus index, gingival index.

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by abnormally high levels of blood sugar caused by insufficient production of insulin, or poor performance, or both [1–3]. Type 1 DM (DM1), insulin-dependent, is the most common type of diabetes and it affects mostly young patients. Type 2 DM (DM2), non-insulin-dependent, is a chronic disease characterized by high levels blood sugar, resistance of cells to insulin action or poor secretion of insulin [4, 5].

Oral manifestations of diabetes can be devastating [6]. Such manifestations are: gingival and periodontal diseases, dental caries, xerostomia, candidiasis, precancerous lesions, burning mouth syndrome, oral wounds that heal poorly, periapical and periodontal pathology [7–10].

It is known that patients suffering from diabetes have a higher risk of developing periodontitis and that periodontitis is more severe in diabetic patients compared to those without diabetes [11]. Periodontal diseases are chronic microbial inflammations that affect the dental supporting structures [12].

Periodontitis etiology is associated with the presence of dental plaque and calculus deposits in the periodontal tissues [13]. Recent data show that the periodontal disease could increase the risk of systemic diseases, such as diabetes, because the presence of periodontal inflammation could be related to glycemic control in patients with DM. In the early 1990s, Löe referred to periodontitis as the "sixth complication" of diabetes [14].

Aim

Periodontal disease is evident in patients with systemic disorders, including DM, and in these patients, the ability to defend is affected. Based on these aspects, we performed both a clinical study, as well as a histological and immunohistochemical (IHC) study, which revealed the tissue changes occurring in the epithelium and the chorion, where we followed the cellular population of the inflammatory infiltrate and the appearance of the vascularization.

Patients, Materials and Methods

The study consists of 107 diabetic patients and 53 non-diabetic patients selected among the diabetic patients in the Clinic of Diabetes, Emergency County Hospital of Craiova, and among the patients of the Department of Endodontics, Faculty of Dental Medicine, University of Medicine and Pharmacy of Craiova, Romania, who needed dental examination and treatment.

The present study, conducted between June 2017 and July 2018, was approved by the Ethics Committee of the

University of Medicine and Pharmacy of Craiova. All the patients included in the study signed for informed consent. The patients were explained the objectives and steps of oral clinical examination. The dental diagnosis was communicated to all participants and they were referred for appropriate dental treatment.

The inclusion criteria for the study were: patients over 18 years of age; patients diagnosed with DM1 or DM2; patients with dental pathology; patients who agreed to participate in the study. The exclusion criteria were: patients under 18 years of age; pregnant patients; bimaxillary totally edentulous patients.

We recorded for every patient the demographic characteristics, the blood sugar and glycosylated hemoglobin (HbA1c) values at the time of the examination and the current status of the periodontium based on the indices: the plaque index (PI), the calculus index (CI) and the gingival index (GI). The patients were divided into three groups: the first group consisted of DM1 patients, the second group consisted of DM2 patients, and the third group was the control group.

Extraction of the dental units with the indication of extraction was performed and gingivectomy was performed for therapeutic purposes. The biological material obtained after the surgery was studied histologically.

To evaluate the periodontal health, the oral cavity was divided into sextants and the following teeth surfaces were utilized: the vestibular surfaces of the maxillary first molars, the lingual surfaces of the mandibular first molars, the maxillary right central incisor, and the left mandibular central incisor.

PI values were evaluated as: 0 – no bacterial plaque deposits; 1 – the plaque deposit did not cover more than 1/3 of the coronary area; 2 – the plaque deposit covered more than 1/3 but did not exceed 2/3 of the coronary area; 3 – the plaque covered more than 2/3 of the dental surface.

The values of CI were evaluated as: 0 – absence of calculus; 1 – supragingival calculus that covers less than 1/3 of the tooth surface; 2 – supragingival calculus that covers more than 1/3 and less than 2/3 of the tooth surface or presence of subgingival calculus islets; 3 – supragingival calculus that covers more than 2/3 of the tooth surface or continuous subgingival calculus deposits.

GI values were scored as: 0 – normal gingiva; 1 – mild inflammation (slight change in color, slight edema, no bleeding on probing); 2 – moderate inflammation (redness, edema and glazing, bleeding on probing); 3 – severe inflammation (marked redness and edema, ulceration, tendency to spontaneous bleeding).

According to the Guidelines of the *American Diabetes Association* (ADA) [15], the HbA1c values were classified as normal <5.7% (<39 mmol/mol), pre-diabetes 5.7–6.4% (39–47 mmol/mol), or DM ≥6.5% (≥48 mmol/mol) and the blood sugar values were classified as normal 100 mg/dL, pre-diabetes 100–125 mg/dL or DM ≥126 mg/dL.

To exclude possible false-positive measurements, a threshold of HbA1c of 7.5% and a threshold of blood sugar of 110 mg/dL have been proposed. The HbA1c values were divided into good controlled (≤7.5%) and poorly controlled (>7.5%) and, respectively, the blood sugar values were divided into good controlled ≤110 mg/dL and poorly controlled >110 mg/dL.

Data collection through clinical dental examination was conducted by two periodontologists. The information obtained was recorded on appropriate forms.

For the statistical analysis, we used Microsoft Excel (Microsoft Corp., Redmond, WA, USA), XLSTAT add-on for MS Excel (Addinsoft SARL, Paris, France) and IBM Statistical Package for the Social Sciences (SPSS) Statistics *ver*. 20.0 (IBM Corporation, Armonk, NY, USA). Continuous variables were summarized as the mean ± standard deviation (SD). Categorical variables were summarized as percentages. We calculated the frequency differences between various groups with the χ^2 (*chi*-square) test. Univariate comparisons of two means were carried out with an unpaired Student's *t*-test. The level of significance (p) was <0.05. We used the *chi*-square test to evaluate the association between qualitative variables (p-values lower than α=5% were considered statistically significant).

The histological material was represented by periodontal fragments collected from 50 patients diagnosed clinically and paraclinically with DM, who received dental treatments and from whom informed consent was obtained. Also, 10 paraffin blocks were obtained from the patients belonging to the control group, without DM. The collected material was fixed in 10% neutral buffered formalin for 24 hours and then processed through the paraffin inclusion histological technique, obtaining 3–5 μm thick sections. The sections obtained were stained with Hematoxylin–Eosin (HE), Goldner–Szekely (GS) trichrome, Masson's trichrome.

For the IHC study, 10 paraffin blocks of gingival mucosa were selected from the patients from which the histological study was performed. IHC processing used the Avidin–Biotin complex/Horseradish peroxidase (ABC/HRP – Avidin complexed with biotinylated peroxidase) technique as a working method. In this study, we used the following antibodies: anti-cluster of differentiation 3 (CD3) (monoclonal mouse anti-human CD3, clone F7.2.38, 1/25 dilution, Dako) for T-lymphocyte highlighting; anti-cluster of differentiation 20 (CD20) (monoclonal mouse anti-human CD20cy, clone L26, 1/50 dilution, Dako) for B-lymphocyte highlighting; anti-cluster of differentiation 34 (CD34) (monoclonal mouse anti-human CD34 Class II, clone QBEnd/10, 1/100 dilution, Dako) for endothelial cells highlighting.

Results

Our study included 160 subjects aged between 19 and 80 years, of which 107 diabetics and 53 subjects in the control group. In the diabetic group, 48 cases were diagnosed with DM1 and 59 patients were diagnosed with DM2. All of the patients, both diabetics, as well as controls, were grouped into five age groups whose distribution is shown in Table 1.

On distribution by age, we found highly statistically significant (HS) differences between the three groups regarding the patients' ages, the result of the *chi*-square test being p=0.0005 <0.05, the patients in the control group being younger than those in the groups with DM, between which there are no significant differences.

Performing the analysis of variance (ANOVA) test, we found that there are highly significant differences

between the average ages calculated for the three groups, the test result being p=0.0002 <0.001. As the result of the ANOVA test was significant, we used the Fisher's least significant difference (LSD) *post-hoc* test to identify the pairs of groups between which the differences are manifested. Thus, we found that there is a significant difference between the control group and both DM groups (Figure 1).

Table 1 – *Distribution of the total sample by age groups*

Age group	<30 years	30–39 years	40–49 years	50–59 years	≥60 years	Total
DM1	10 (20.83%)	3 (6.25%)	8 (16.67%)	12 (25%)	15 (31.25%)	48 (100%)
DM2	6 (10.17%)	10 (16.95%)	15 (25.42%)	9 (15.25%)	19 (32.2%)	59 (100%)
Control	15 (28.3%)	15 (28.3%)	14 (26.42%)	7 (13.21%)	2 (3.77%)	53 (100%)
Total	31 (19.38%)	28 (17.5%)	37 (23.13%)	28 (17.5%)	36 (22.5%)	160 (100%)
Chi-square test, p=0.000532 – HS						

DM: Diabetes mellitus; HS: Highly significant.

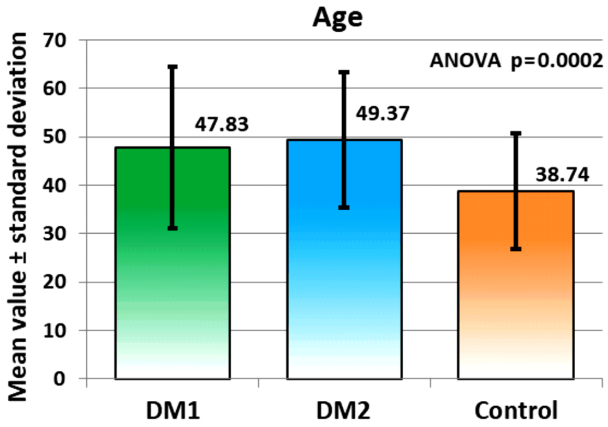

Figure 1 – *Comparison between the control group and both groups with diabetes mellitus (DM). ANOVA: Analysis of variance.*

The distribution of patients by gender is shown in Table 2. We found no statistically significant (NS) difference between male and female patients from DM1, DM2 and control groups (p=0.762298, *chi*-square test).

Table 2 – *Distribution of the total sample by gender*

Group	DM1	DM2	Control	Total
Females	24 (50%)	30 (50.85%)	30 (56.6%)	84 (52.5%)
Males	24 (50%)	29 (49.15%)	23 (43.4%)	76 (47.5%)
Total	48 (100%)	59 (100%)	53 (100%)	160 (100%)
Chi-square test, p=0.762298 – NS				

DM: Diabetes mellitus; NS: Not significant.

The distribution of patients according to the residence environment is significantly different between the three groups (p=0.011 <0.05, *chi*-square test), the patients in the control group coming from a significantly larger urban area than those in the DM groups (Figure 2).

By performing the Student's *t*-test, we demonstrated that there are highly significant differences between the blood glucose values of DM1 patients and those of DM2, those with DM1 having significantly higher values than the others (p<0.001) (Figure 3).

By performing the Student's *t*-test, we demonstrated that there are highly significant differences between the blood sugar values of DM1 patients and those of DM2, those with DM1 having significantly higher values than the others (p<0.001) (Figure 4).

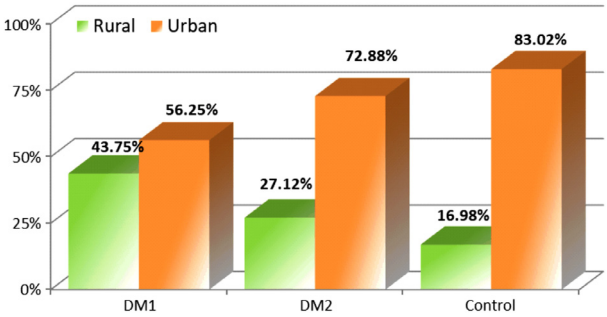

Figure 2 – *Distribution of patients according to the residence environment.*

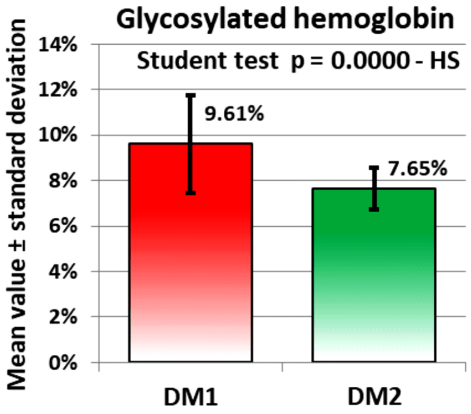

Figure 3 – *Comparison of diabetic groups according to glycosylated hemoglobin level. DM: Diabetes mellitus; HS: Highly significant.*

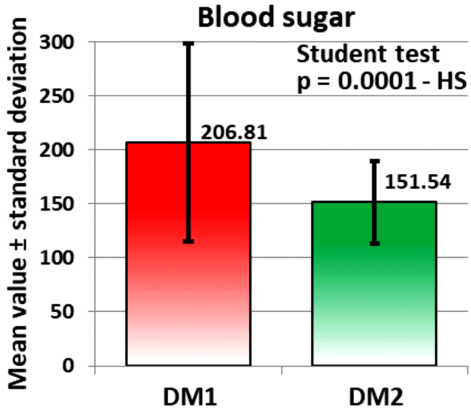

Figure 4 – *Distribution of diabetic patients according to blood sugar level at the time of examination. DM: Diabetes mellitus; HS: Highly significant.*

Within DM1 group, there is no statistically significant difference between the bacterial PI in patients with blood sugar above, respectively less than 110 mg/dL (p=0.574 >0.05), between CI (p=0.581) or GI (p=0.563) (Figure 5). There was only one patient with DM2 and blood sugar less than 110 mg/dL, so that value was not taken into account for the comparisons made. Patients in the control group have significantly lower bacterial PI values than those in the other groups, regardless of the blood sugar level (Figure 5).

For all hygiene indices, the mean values recorded for the control group were significantly lower than the mean

values recorded for any sub-category in the DM1 or DM2 groups (blood sugar less than or above 110 mg/dL and HbA1c less than or over 7.5%, respectively) (Figures 5 and 6). Within DM1 group, there was no statistically significant difference between the bacterial PI in patients with HbA1c above and less than 7.5%, respectively (p=0.934 >0.05), between CI (p=0.965) or GI (p=0.681) (Figure 6).

Within DM2 group, there was no statistically significant difference between the bacterial PI in patients with HbA1c above and less than 7.5%, respectively (p=0.914 >0.05), between the CI (p=0.781) or GI (p=0.704) (Figure 6).

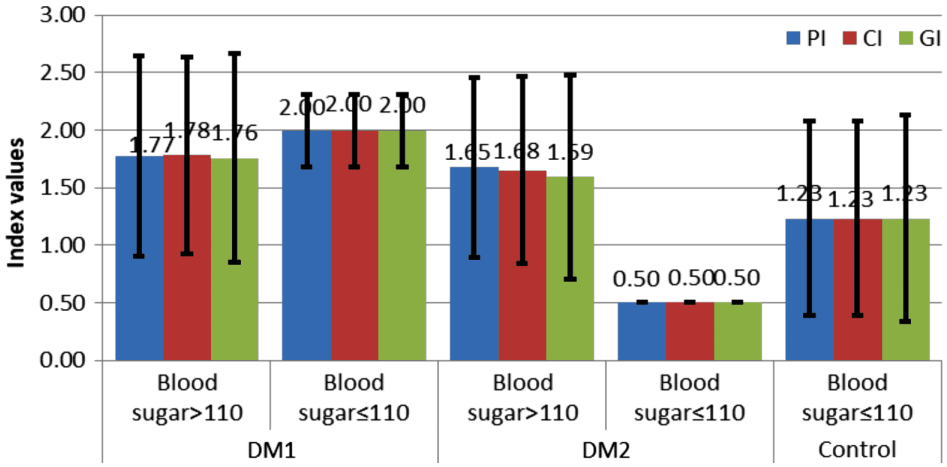

Figure 5 – ***The comparison of hygiene indices on groups of patients according to the blood sugar level. PI: Plaque index; CI: Calculus index; GI: Gingival index; DM: Diabetes mellitus.***

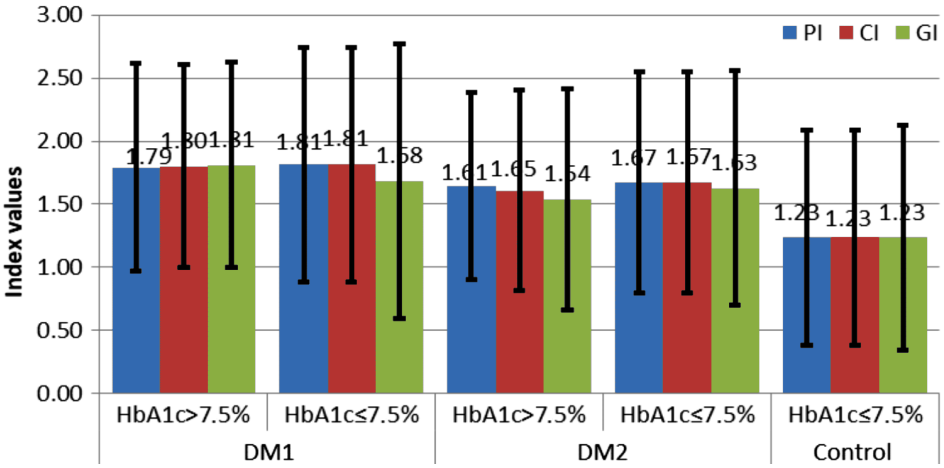

Figure 6 – ***The comparison of hygiene indices on groups of patients according to the glycosylated hemoglobin (HbA1c) level. PI: Plaque index; CI: Calculus index; GI: Gingival index; DM: Diabetes mellitus.***

Histological changes were observed in both the gingival epithelium and the lamina propria in sections from patients with DM. The histological changes were more intense in the patients whose DM was older and in those whose oral hygiene was deficient, these changes being influenced to a lesser extent by the blood sugar levels.

The microscopic examination of the sections obtained from patients with DM showed significant changes, both in the gingival epithelium and in the lamina propria. Thus, at the level of the gingival epithelium, on some sections, acanthosis was present, by multiplying cells in the intermediate layer, which led to an increase in the number of layers of the epithelium and, consequently, its thickness increase and the appearance of deep epithelial ridges (Figure 7A).

In some areas of the epithelium, the epithelial cells had a balloon-like aspect, through the vacuolization of the cellular cytoplasm and a process of acantholysis (Figure 7, B and C). Acantholysis was present especially in the sections where there was a lymphoplasmocytary infiltrate, at the epithelial level. On the contrary, in some sections, the epithelium presented an atrophic aspect by reducing the number of layers and the tendency to erase the epithelial ridges (Figure 7D).

At the level of the lamina propria, a chronic inflammatory process was identified, which varied in intensity from one section to another and even on the same section, areas with different intensities of the inflammatory process were identified. Chronic inflammatory infiltrate is associated with an angiogenesis process (Figure 7E). The most numerous cells present in the inflammatory infiltrate were lymphocytes. On the sections with inflammatory

process, the collagen fibers were abundant, arranged in the form of thick, perivascular bands. Collagen proliferation determines the reduction of the inflammatory process (Figure 7F). Collagen fibers, in areas with intense inflammatory infiltrate, appeared dissociated, fragmented and sometimes, their lysis caused disruption of connective tissue.

Most of the cells of the inflammatory infiltrate were of lymphocyte type. The specific highlighting of B-lymphocytes was made using the anti-CD20 antibody and the T-lymphocytes using the anti-CD3 antibody. On the microscopic sections, the distribution of the lymphocytes was variable, the B-lymphocytes being less numerous (Figure 8, A and B) compared to the T-lymphocytes (Figure 8, C and D). We identified, through the study of immunoreactivity at CD34, the presence of an angiogenesis process, angiogenesis being specific in the chronic inflammations and therefore, and in the casuistry studied. The angiogenesis process was exclusively capillary, with the starting point preexisting vessels, being identified both subepithelially and in the areas of the lamina propria where the inflammatory process was present (Figure 8, E and F).

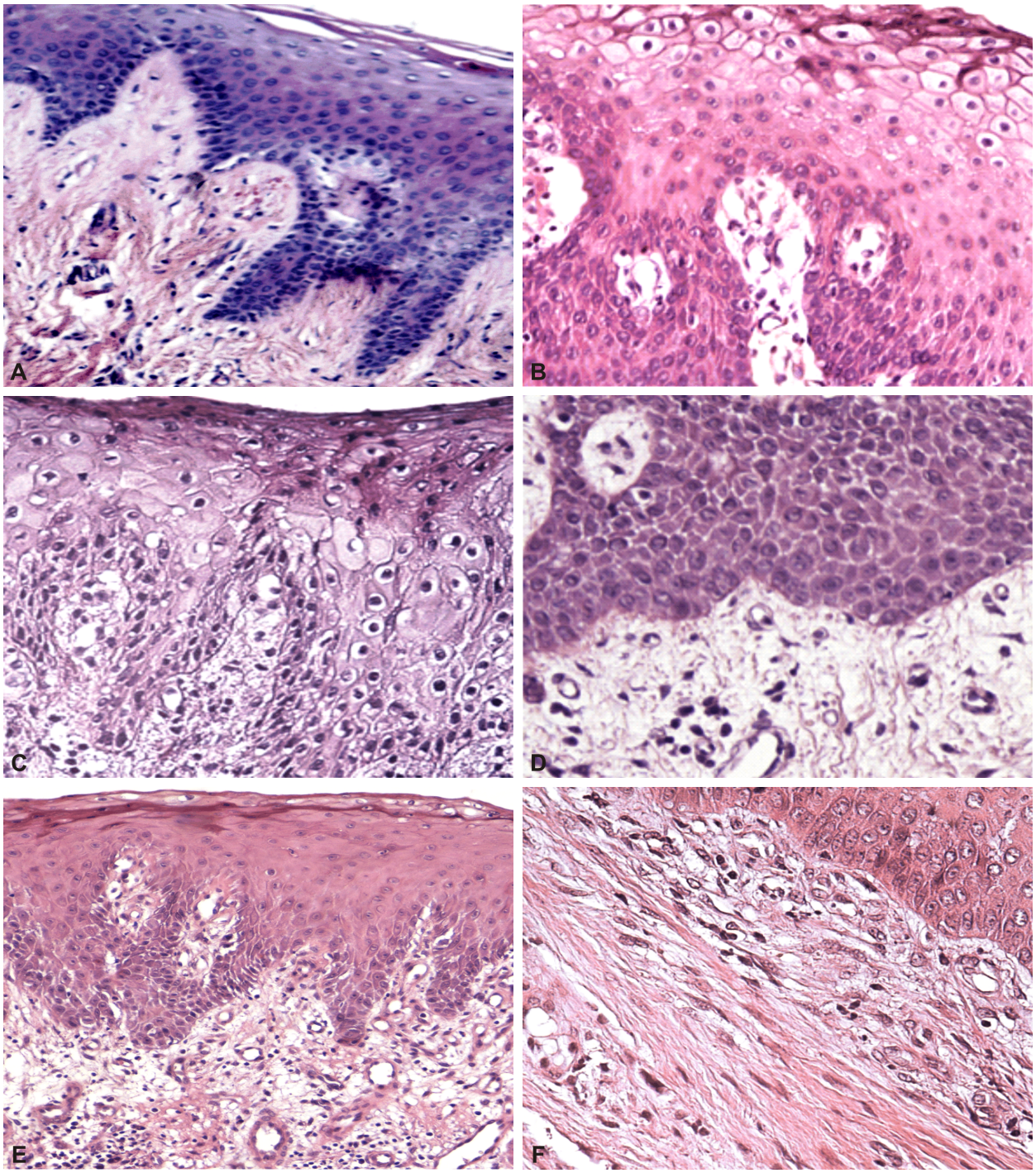

Figure 7 – ***(A) Acanthosis and discrete inflammatory infiltrate in the chorion; (B and C) Acanthosis, acantholysis and ballooning of epithelial cells; (D) Tendency to erase epithelial ridges; (E) Chronic inflammatory infiltrate associated with an angiogenesis process; (F) Collagen proliferation and decreased chronic inflammatory process. HE staining: (A, D–F) ×100; (B and C) ×200.***

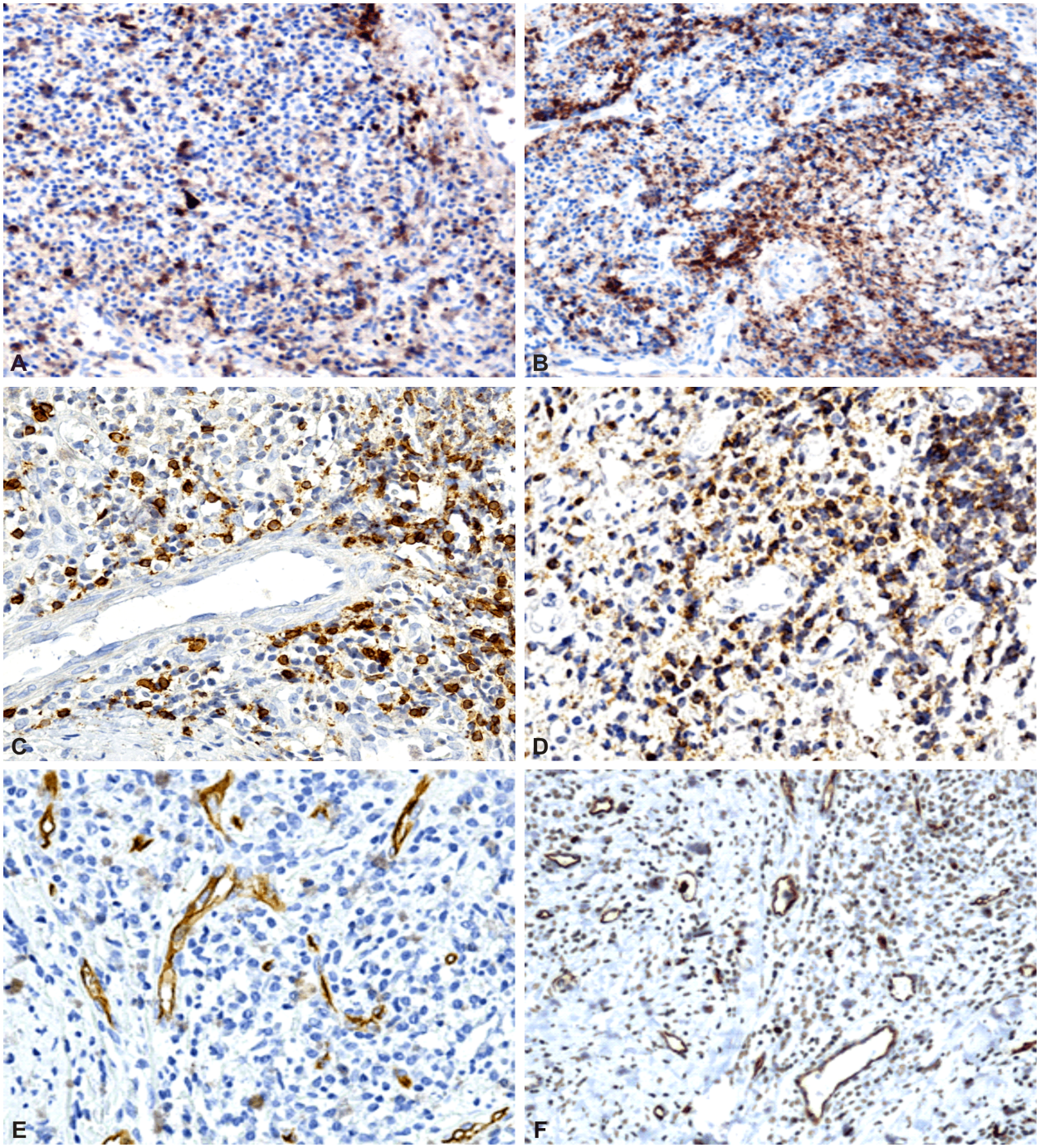

Figure 8 – ***(A) B-lymphocytes diffused in the lamina propria; (B) B-cells perivascularly arranged; (C and D) T-lymphocytes perivascularly arranged; (E and F) CD34-positive blood vessels in the areas of the lamina propria with chronic inflammatory infiltrate. Immunolabeling for: CD20, ×200 (A and B); CD3, ×200 (C and D); CD34, ×200 (E and F). CD: Cluster of differentiation.***

Discussions

Our study has shown that for all hygiene indices, the mean values recorded for the control group were significantly lower than the mean values recorded for any subcategory in the DM1 or DM2 groups (blood sugar less than or above 110 mg/dL, respectively HbA1c less than or over 7.5%).

Similar results were obtained by Moosa *et al.* (2018) [16], in their study in which the incidence of periodontal disease was higher in diabetic patients than in non-diabetic patients. Bharateesh *et al.* reported comparable results, in 2012 [17].

Other studies have also reported that DM is a possible cause of gingivitis and periodontitis and the degree of blood sugar control seems to be a significant factor in this correlation [18, 19]. However, another study concludes that there was no major difference between diabetics and non-diabetic groups regarding periodontal disease and oral hygiene [20], agreeing with the results reported by Velea *et al.*, in 2013 [21].

Periodontitis is the characteristic complication of DM and significantly varies with the glycemic control [22]. This conclusion supports the findings of many previous studies that state that diabetes is a risk factor for periodontal disease [18, 23]. The study of Tanwir & Tariq,

in 2012 [24], reports a higher frequency of periodontitis in the measures of indices used, and shows the marked difference in the controlled and uncontrolled group of diabetic patients. In that study, which included 141 individuals with controlled diabetes and 143 patients who had uncontrolled diabetes, were recorded and compared PI, GI and CI. GI and PI showed significant differences in both groups (p-value <0.001 and <0.002, respectively), while the difference of CI was not significant, *i.e.*, p=0.056. Uncontrolled diabetes had an important impact on periodontal health in the studied groups with poor hygiene; diabetic patients have more plaque [24].

The mean PI and mean CI scores recorded in this study for diabetic patients is similar to results of Rajhans *et al.* (2011) [25], who reported a mean PI and CI scores of 1.22±0.55 and 1.27±0.6 for diabetic patients and non-diabetics.

Ikimi *et al.* (2017) [26] found that the mean PI and CI scores were significantly higher in the diabetic group, suggesting a correlation between poor oral hygiene and DM.

There are studies that have reported that a higher incidence of periodontal disease exist among diabetics groups than in controls groups [27]. Regarding when diabetic subjects with DM1 and DM2 were studied together without distinction of diabetes types and compared with the control, the young and the adult subjects with DM presented a greater incidence of periodontal disease [28]. Furthermore, the high blood sugar levels in diabetics causes delay in wound healing, inability to respond adequately to infection, bone loss and, in most cases, tooth loss [29].

It has also been reported that sensitivity to periodontitis is increased approximately three times at the people with diabetes [30] and that there is an undesirable bidirectional relationship between periodontal disease and diabetes, so that a poor periodontal condition has a negative effect on blood sugar control and a hyperglycemic environment increases the susceptibility to periodontal disease [27, 30].

Periodontal disease is considered to be a complication of diabetes, which indicates the need to maintain oral health in these patients [31, 32]. The clinical, histological and IHC aspects present in the oral mucosa, within the periodontal disease, in patients with DM, underline the need for a better knowledge of the interrelationships between the two diseases, in order to apply an appropriate therapy, which will prevent other complications [33–35].

On the microscopic preparations examined, we identified changes of the gingival mucosa, both in the epithelium and in the lamina propria. At the epithelial level, we identified the acanthosis process with the presence of deep, penetrating epithelial ridges in the chorion, which determined, in many sections, a papillomatous aspect.

Periodontal disease is the result of bacterial aggression that triggers inflammation and mobilizes the immune defense system, as in diabetic patients in the study. Therefore, an inflammatory process was present in the sections, as a local response, as a reaction to bacterial invasion. The presence of defense cells, especially of the immune type, is due to the local presence of bacterial antigens that trigger a cascade of cellular and humoral events meant to identify the antigenic structures in order to annihilate them.

In the IHC study, we analyzed lymphocyte inflammatory infiltrate using CD20, immunomarker for B-lymphocytes and CD3 immunomarker for T-lymphocytes. The most numerous were CD3-positive T-lymphocytes. The lymphocytes were present both in the superficial area of the chorion, immediately subepithelially, and in the rest of the chorion, wearing the appearance of a diffuse infiltrate or, in some cases, they were identified in a grouped, nodular, especially perivascular aspect, indicating an increase in vascular permeability.

The inflammatory process causes an intense proliferation of fibroblasts, responsible for the formation and turnover of the extracellular matrix. Therefore, next to inflammatory cells, present in variable numbers, fibroblasts were present in the examined sections. They have been stimulated by both factors secreted by bacteria and cells of the immune system by fibroblast growth factor (FGF). Consequently, inflammation has been associated, to varying degrees, with collagen fibrillary proliferation.

The vascularization was increased, located both subepithelially and in the rest of the chorion, because of some angiogenic factors associated with mediators of the inflammatory process Through the study of CD34 immunoreactivity, we observed a process of capillary angiogenesis, with preexisting vessels starting point, angiogenesis being specific in the chronic inflammations and therefore also in the casuistry studied. The angiogenesis process was exclusively capillary, with preexisting vessels starting point [36–38]. The most intense changes were at the level of the lamina propria, where the inflammatory processes were associated with those of collagen fibrillar proliferation, in different proportions.

The histological and IHC changes observed in the group of patients with diabetes were not specific. They are also present in inflammations of the oral mucosa of local cause, as the specialized literature shows [39–42].

Conclusions

DM contributes unfavorably to the evolution of periodontal disease. The poor glycemic control and the improper oral hygiene negatively impact the periodontal health. This is highlighted by increased scores of GI, PI and CI. On the examined sections, changes of the gingival mucosa were present, both in the epithelium and in the lamina propria. Most commonly, the epithelium had a process of acanthosis and acantholysis, and at the level of the chorion, there was an inflammatory infiltrate associated with a process of capillary angiogenesis and a process of fibrillar collagen proliferation. In the sub-epithelial and diffuse localized lymphoplasmocytary inflammatory infiltrate, CD3-positive lymphocytes predominated.

Conflict of interests

The authors declare that they have no conflict of interests.

Authors' contribution

Cristina-Mihaela Fărcaş-Berechet & Irina-Anca Eremia equally contributed to the manuscript.

References

[1] Nazir MA, AlGhamdi L, AlKadi M, AlBeajan N, AlRashoudi L, AlHussan M. The burden of diabetes, its oral complications and their prevention and management. Open Access Maced J Med Sci, 2018, 6(8):1545–1553.

[2] Patel J, Dave B, Patel P, Patel S, Dave R. Relationship between diabetes mellitus and periodontal disease – a clinical study. BJKines–NJBAS, 2018, 10(1):22–28.

[3] Moin M, Malik A. Frequency of dental caries and level of risk among type II diabetics. Dentistry, 2015, 5(10):334.
[4] American Diabetes Association. 2. Classification and diagnosis of diabetes. Diabetes Care, 2017, 40(Suppl 1):S11–S24.
[5] Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care, 2003, 26(Suppl 1):S5–S20.
[6] Bajaj S, Prasad S, Gupta A, Singh VB. Oral manifestations in type-2 diabetes and related complications. Indian J Endocrinol Metab, 2012, 16(5):777–779.
[7] Mauri-Obradors E, Estrugo-Devesa A, Jané-Salas E, Viñas M, López-López J. Oral manifestations of diabetes mellitus. A systematic review. Med Oral Patol Oral Cir Bucal, 2017, 22(5): e586–e594.
[8] Veiga N, Marques T, Monteiro AS, Couto J, Conceição P, Matos S, Santos J, Pereira AR, Goolamhussen S, Grão R, André M, Coelho I. Oral manifestations and diabetes. Biomed J Sci Tech Res, 2018, 7(5):6168–6171.
[9] Albert DA, Ward A, Allweiss P, Graves DT, Knowler WC, Kunzel C, Leibel RL, Novak KF, Oates TW, Papapanou PN, Schmidt AM, Taylor GW, Lamster IB, Lalla E. Diabetes and oral disease: implications for health professionals. Ann N Y Acad Sci, 2012, 1255(1):1–15.
[10] Segura-Egea JJ, Castellanos-Cosano L, Machuca G, López-López J, Martín-González J, Velasco-Ortega E, Sánchez-Domínguez B, López-Frías FJ. Diabetes mellitus, periapical inflammation and endodontic treatment outcome. Med Oral Patol Oral Cir Bucal, 2012, 17(2):e356–e361.
[11] Polak D, Shapira L. An update on the evidence for pathogenic mechanisms that may link periodontitis and diabetes. J Clin Periodontol, 2018, 45(2):150–166.
[12] Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet, 2005, 366(9499):1809–1820.
[13] Demmer RT, Papapanou PN. Epidemiologic patterns of chronic and aggressive periodontitis. Periodontol 2000, 2010, 53(1): 28–44.
[14] Löe H. The gingival index, the plaque index and the retention index systems. J Periodontol, 1967, 38(6 Suppl):610–616.
[15] American Diabetes Association. 2. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes – 2019. Diabetes Care, 2019, 42(Suppl 1):S13–S28.
[16] Moosa Y, Shahzad M, Shaikh AA, Matloob SA, Anila, Khalid M. Influence of diabetes mellitus on oral health. Pak Oral Dent J, 2018, 38(1):67–70.
[17] Bharateesh J, Ahmed M, Kokila G. Diabetes and oral health: a case-control study. Int J Prev Med, 2012, 3(11):806–809.
[18] D'Aiuto F, Gable D, Syed Z, Allen Y, Wanyonyi KL, White S, Gallagher JE. Evidence summary: the relationship between oral diseases and diabetes. Br Dent J, 2017, 222(12):944–948.
[19] Indurkar MS, Maurya AS, Indurkar S. Oral manifestations of diabetes. Clin Diabetes, 2016, 34(1):54–57.
[20] Gul A, Banjar M, Redwan M, Elkwatehy WM. Influence of diabetes mellitus on dental health status. Int J Health Sci Res, 2017, 7(1):124–129.
[21] Velea OA, Kralev C, Onisei D, Onisei D, Nica LM, Velea IP. Diabetes mellitus and periodontal disease – a two-way road: current concepts and future considerations (literature review). Eur Sci J, 2013, 9(9):61–79.
[22] Orbak R, Simsek S, Orbak Z, Kavrut F, Colak M. The influence of type-1 diabetes mellitus on dentition and oral health in children and adolescents. Yonsei Med J, 2008, 49(3):357–365.
[23] Leong P, Tumanyan S, Blicher B, Yeung A, Joshipura K. Periodontal disease among adult, new-immigrant, Chinese Americans in Boston with and without diabetes – a brief communication. J Public Health Dent, 2007, 67(3):171–173.
[24] Tanwir F, Tariq A. Effect of glycemic control on periodontal status. J Coll Physicians Surg Pak, 2012, 22(6):371–374.
[25] Rajhans NS, Kohad RM, Chaudhari VG, Mhaske NH. A clinical study of the relationship between diabetes mellitus and periodontal disease. J Indian Soc Periodontol, 2011, 15(4):388–392.
[26] Ikimi NU, Onigbinde OO, Sorunke ME Adetoye JO, Enebong DJ. A study of periodontal disease and diabetes mellitus in Abuja Nigeria. IOSR J Dent Med Sci, 2017, 16(10):65–70.
[27] Teng YT, Taylor GW, Scannapieco F, Kinane DF, Curtis M, Beck JD, Kogon S. Periodontal health and systemic disorders. J Can Dent Assoc, 2002, 68(3):188–192.
[28] Demmer RT, Jacobs DR Jr, Desvarieux M. Periodontal disease and incident type 2 diabetes: results from the First National Health and Nutrition Examination Survey and its epidemiologic follow-up study. Diabetes Care, 2008, 31(7):1373–1379.
[29] Ogunbodede EO, Fatusi OA, Akintomide A, Kolawole K, Ajayi A. Oral health status in a population of Nigerian diabetics. J Contemp Dent Pract, 2005, 6(4):75–84.
[30] Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, Taylor R. Periodontitis and diabetes: a two-way relationship. Diabetologia, 2012, 55(1):21–31.
[31] Löe H. Periodontal disease. The sixth complication of diabetes mellitus. Diabetes Care, 1993, 16(1):329–334.
[32] Machtei EE, Hausmann E, Dunford R, Grossi S, Ho A, Davis G, Chandler J, Zambon J, Genco RJ. Longitudinal study of predictive factors for periodontal disease and tooth loss. J Clin Periodontol, 1999, 26(6):374–380.
[33] Aspriello SD, Zizzi A, Tirabassi G, Buldreghini E, Biscotti T, Faloia E, Stramazzotti D, Boscaro M, Piemontese M. Diabetes mellitus-associated periodontitis: differences between type 1 and type 2 diabetes mellitus. J Periodontal Res, 2011, 46(2): 164–169.
[34] Alves C, Andion J, Brandão M, Menezes R. [Pathogenic aspects of the periodontal disease associated to diabetes mellitus]. Arq Bras Endocrinol Metabol, 2007, 51(7):1050–1057.
[35] Ryan ME. Diagnostic & therapeutic strategies for the management of the diabetic patient. Compend Contin Educ Dent, 2008, 29(1):32–38, 40–44.
[36] Gawish A, Gamal-Eldeen AM, Sherif SH, Neamat A. Influence of the etiological factors for gingival enlargement on some angiogenic and inflammatory mediators: an immunohistochemical study. J Am Sci, 2010, 6(12):1754–1760.
[37] Cărămizaru M, Pleşea IE, Dragomir LP, Popescu MR, Uscatu CD, Şerbănescu MS, Alexandru DO, Comănescu TM. Quantitative assessment of morphological changes of dental pulp components of teeth affected by occlusal trauma. Rom J Morphol Embryol, 2018, 59(3):729–740.
[38] Butucescu M, Crăiţoiu Ş, Mogoantă L, Rusu MC, Iacov-Crăiţoiu MM, Stoenescu MD. The oral mucosa: epithelial professional phagocytes, lymphatics, telocytes, and false telocytes. Ann Anat, 2020, 229:151462.
[39] Drăghici EC, Crăiţoiu Ş, Mercuţ V, Scrieciu M, Popescu SM, Diaconu OA, Oprea B, Pascu RM, Crăiţoiu MM. Local cause of gingival overgrowth. Clinical and histological study. Rom J Morphol Embryol, 2016, 57(2):427–435.
[40] Crăiţoiu MM, Păuna M, Crăiţoiu Ş, Mercuţ V. Clinical and histoenzymatic interrelations of the edentulous ridge's mucosa. Rom J Morphol Embryol, 2011, 52(1):99–104.
[41] Bartold PM, Narayanan AS. Molecular and cell biology of healthy and diseased periodontal tissues. Periodontol 2000, 2006, 40(1):29–49.
[42] Berglundh T, Donati M. Aspects of adaptive host response in periodontitis. J Clin Periodontol, 2005, 32(Suppl 6):87–107.

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