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Histological and immunohistochemical study on the dental pulp of patients with diabetes mellitus

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Abstract

Diabetes mellitus is a disease that brings numerous alterations in the human body, mainly on the blood vessels and nervous system, its complications being difficult to treat most of the time. Oral complications are largely known and studied. Changes that occur in the dental pulp are of importance for the dentists, considering regular procedures outcome. In early stages of the disease, new blood vessels appear especially under the odontoblasts layer as a reaction to stimuli. In later stages, the defense systems of the dental pulp are outnumbered, nervous branches will be destroyed and disorganized. When periodontal disease occurs as well the mortification of the dental pulp will be faster.

Keywords: diabetes mellitus, dental pulp, S100, α-SMA, factor VIII, endothelial cells.

Introduction

Diabetes mellitus is a complex and heterogeneous syndrome induced by genetic or gained alteration of insulin secretion and/or resistance to its peripheral action. It is characterized by glucose metabolism alteration leading to secondary alteration of the other metabolisms: lipid, protein, hydric, electrolytic, ionic and vitamin [1].

Diabetic patients usually have concomitant oral manifestations, of which most commonly known are: xerostomia, infections, poor healing, increased incidence and severity of caries, candidosis, gingivitis, periodontal diseases and burning mouth syndrome [2, 3].

There are just few clinical and paraclinical studies concerning modifications appeared at dental pulp level. Bender *et al.* and Russel, in the early sixties, have manifested interest for the first time for dental pulp study in diabetic patients [4–7]. This matter is still insufficiently researched because the pulp is difficult to isolate and to be prepared for microscopic study. Also, is very difficult to achieve correlations between the observed microscopic alterations and the patient status. The dental pulp is a connective tissue with special characteristics, one of the most important being that it has a microcirculatory system with no lateral blood vessels branches and is set between solid dentinal walls, making it unable to accept significant volume changes [7]. The dental pulp is therefore prone to irreversible inflammatory changes and this can be significantly increased for the patient with diabetes mellitus, as they are known to have tissue vulnerability caused by micro- and macro-circulatory disorders [8].

We considered being appropriate and challenging to study the alterations present at dental pulp level in diabetic patients, considering the existent state of knowledge and well known vascular and immune response alterations of diabetic patients.

Patients, Materials and Methods

This study was done on 39 dental pulps coming from 27 patients with type II diabetes mellitus. The patients were aged 39 to 67. From this group of patients, 11 had diabetes recently diagnosed and did not follow an insulin treatment and, the other 16 had diabetes diagnosed for at least seven years and were insulin dependent. As one major complication of the diabetes is the periodontal disease, 15 of them were on a follow-up program for periodontal disorders in the dental office. For these patients, we had to perform teeth extractions due to major bone loss and high mobility following periodontal complications. The rest of 12 patients arrived in the dental office accusing different other pathologies such as tooth wear or cavities, which also required teeth extraction.

The patients have been informed about the treatment and they signed a formal agreement considering the use of their data and the gathered biological materials. Knowing that dental pulp is highly aggressed during dental treatment and is built into an organ-like structure – dentine–pulp complex, we tried not to disrupt this structure while collecting the soft tissue and we collected the dental pulp by cutting the extracted teeth.

The samples obtained from teeth were treated with

10% formaldehyde for 48–72 hours and then embedded in paraffin, following the classical histological protocol.

For the histological study, we used the Hematoxylin–Eosin (HE) and Goldner–Szeckely (GS) trichrome stainings. And, for the immunohistochemical study, we used the ABC immunohistochemical method, considering its advantages of sensibility and specificity. The method relies on high Avidin affinity for Biotin. The antibodies used were: protein S100 (polyclonal rabbit S100, Dako), alpha-smooth muscle actin (α-SMA, monoclonal mouse anti-human alpha-smooth muscle actin, clone 1A4, Dako), factor VIII (monoclonal mouse anti-human von Willebrand factor, clone F8/86, Dako), collagen IV (monoclonal mouse anti-human collagen IV, clone CIV22, Dako).

The dental pulp samples have been separated in two groups as following: group A – samples obtained from teeth with periodontal disease, and group B – samples obtained from teeth without periodontal disease, all samples from patients with diabetes mellitus of different stages.

Results

The histopathological study of the dental pulp tissue samples obtained from patients with diabetes associated with periodontal disease has shown the presence in the apical area of a more or less intense chronic inflammatory infiltrate, mainly formed by lymphocytes, plasma cells and macrophages. Inflammatory infiltrate appeared most often diffused, dissociating the collagen fibers from the dental pulp (Figures 1 and 2). In some cases, the inflammatory infiltrate appeared much more abundant or was associated with pulp microcalcifications (Figures 3 and 4).

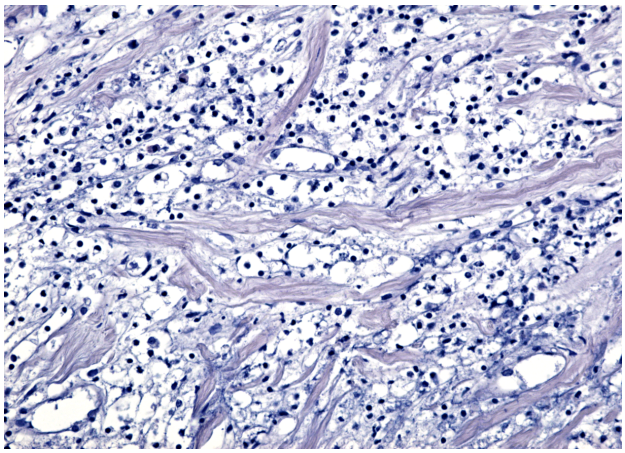

Figure 1 – ***Chronic pulpitis image, characterized by the presence of a diffuse inflammatory infiltrate, predominantly formed by lymphocytes and plasma cells. HE staining, ×200.***

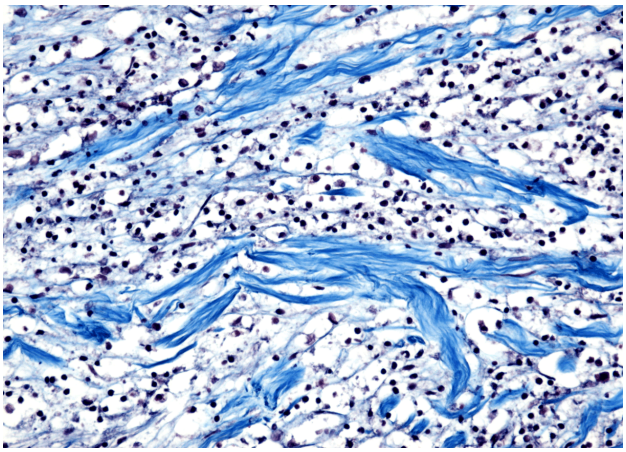

Figure 2 – ***Moderate pulp inflammatory infiltration that dissociates collagen fibers. GS staining, ×200.***

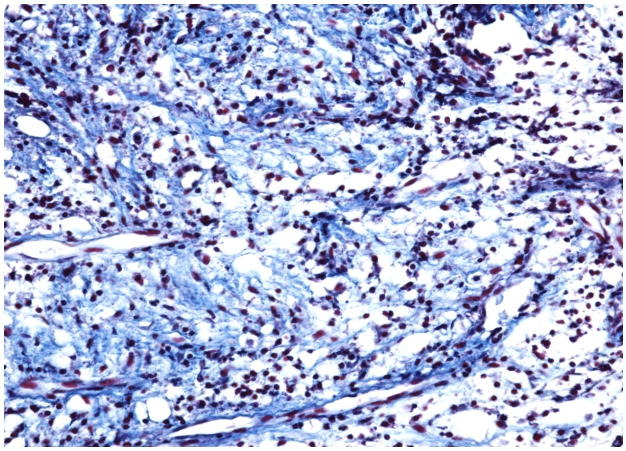

Figure 3 – ***Chronic pulpitis in the apical area with abundant inflammatory infiltration. GS staining, ×200.***

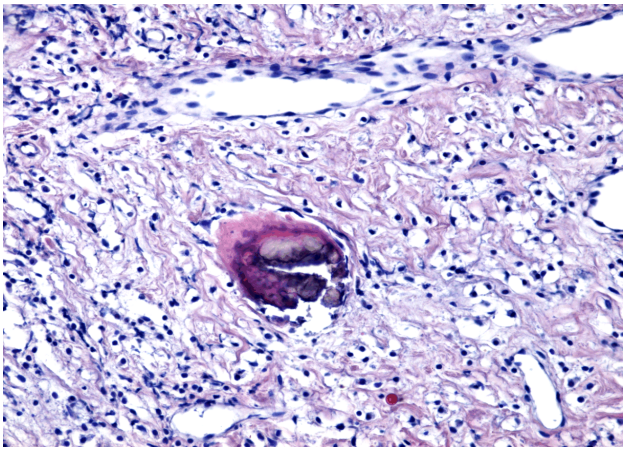

Figure 4 – ***Aspect of chronic pulpitis with microcalcifications. HE staining, ×200.***

In patients with diabetes mellitus without associated periodontal disease, we noticed the presence of an extensive collagen fibrosis process in both the apical pulp and the coronal pulp (Figure 5), the moderate proliferation of fibroblasts and the reduction of the blood vessels number (Figure 6). Frequently, the blood vessels have a thickened wall, with sclerosis. Also, in this group, microcalcifications were identified in the pulp stroma (Figure 7).

Through the immunohistochemical study, we aimed to evaluate some components of dental pulp in diabetic patients, namely, the nervous, vascular, myofibroblastic component and the collagen IV changes.

For the study of the nerve component, we used the S100 protein. Reaction for S100 protein is high positive for nervous fibers covered in Schwann cells. Immunostaining with the S100 protein allowed us to analyze the density of the nerve fibers in various areas of the dental pulp as well as their distribution in relation to other structural elements of the pulp connective tissue. In the apical pulp collected from patients with diabetes without

paradontopathy, we noticed that nerve fibers were numerous, grouped in coarse bundles, disposed almost parallel to the blood vessels (Figure 8), while in the coronary pulp they became thinner, with a plexiform layout, sending fine fibers to the dentinal canals. In patients with diabetes associated with periodontal lesions, the nerve fibers appeared more rarely, and they were more disordered (Figure 9), probably due to the development of the chronic inflammatory process at this level.

Because on the classic histology images, we noticed the presence of a high fibrous process in the dental pulp, we wanted to investigate the presence of myofibroblasts in patients with diabetes associated or not with a periodontal disease. For this, we used the immunostaining with the anti-human α-SMA antibody, which selectively marks smooth muscle fibers, myofibroblasts and pericytes. In our study, we did not identify myofibroblasts in the dental pulp, which would make us believe that the synthesis and deposition of collagen was done only by pulping fibroblasts. Instead, the reaction to α-SMA was highly positive in the blood vessels, marking the smooth muscle fibers from the arterioles and venules walls. We have identified numerous arteries and relatively large caliber disposed almost parallel to the apical pulp and the center of the coronal pulp (Figures 10 and 11).

For the evaluation of the blood capillary distribution, we used the anti-human von Willebrand factor (factor VIII) that selectively marks endothelial cells. We have identified numerous blood capillaries placed among the collagen fibers in the dental pulp, but especially at the periphery of the pulp in the immediate vicinity of the odontoblasts layer (Figure 12).

In patients with diabetes and periodontal disease, a higher number of blood capillaries with large endothelial cells with abundant cytoplasm have been identified, demonstrating the presence of an accentuated vascular angiogenesis process. In diabetic patients without periodontal disease, angiogenesis vessels have not been identified (Figure 13), which makes us believe that the inflammatory process in the dental pulp stimulates the occurrence of the vascular angiogenesis processes.

In our study, we sought to identify the presence of other types of collagen (other than collagen III) that occur in the remodeling processes of the connective stroma in the dental pulp from the patients with diabetes. In this regard, we sought to identify the presence of collagen IV by using a specific antibody. The immunohistochemical reaction was negative for collagen IV in both types of samples (Figure 14), proving that in the processes of pulp remodeling in diabetics, predominantly type III fibril collagen is formed.

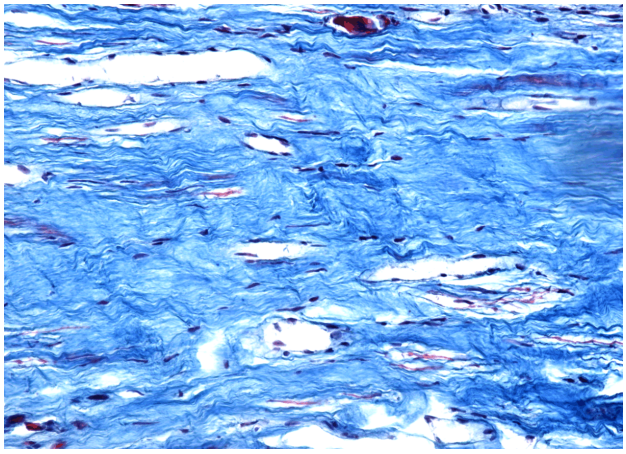

Figure 5 – ***Dental pulp with extensive collagen fibrosis. GS staining, ×200.***

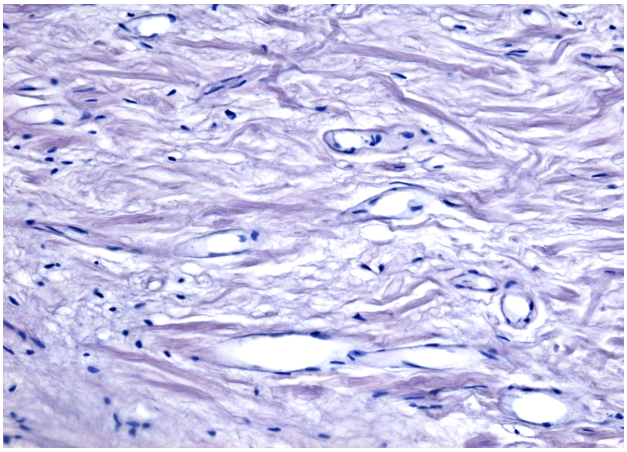

Figure 6 – ***Dental pulp with rough collagen fibers, slightly orderly arranged, with proliferation of pulp fibroblasts and reduction of vascular blood network. HE staining, ×200.***

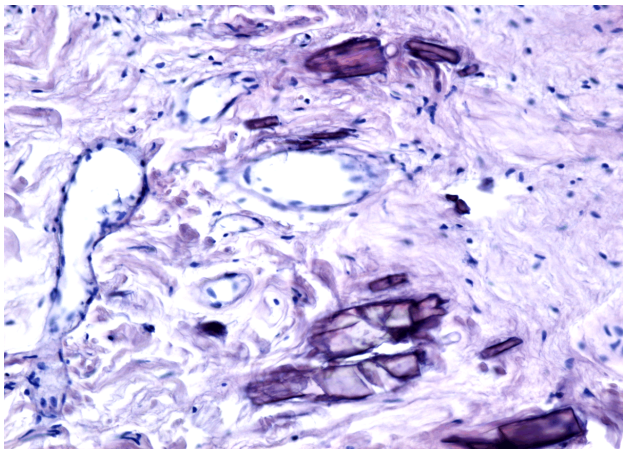

Figure 7 – ***Diffuse pulp calcifications. HE staining, ×200.***

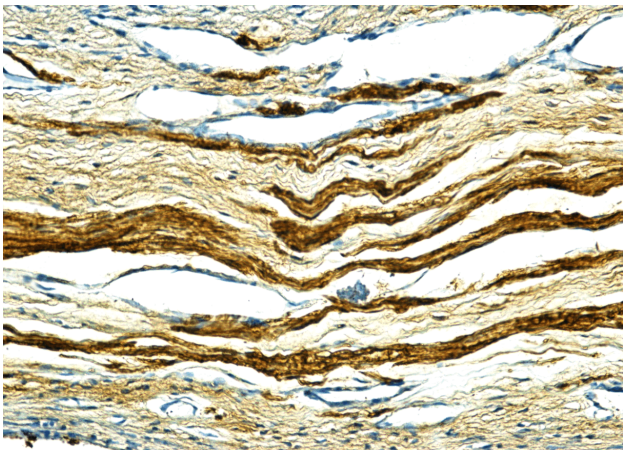

Figure 8 – ***Immunohistochemical image of the root pulp, in which it is noticed the presence of bundles of nerve fibers arranged nearly parallel to the blood vessels. S100 immunostaining, ×200.***

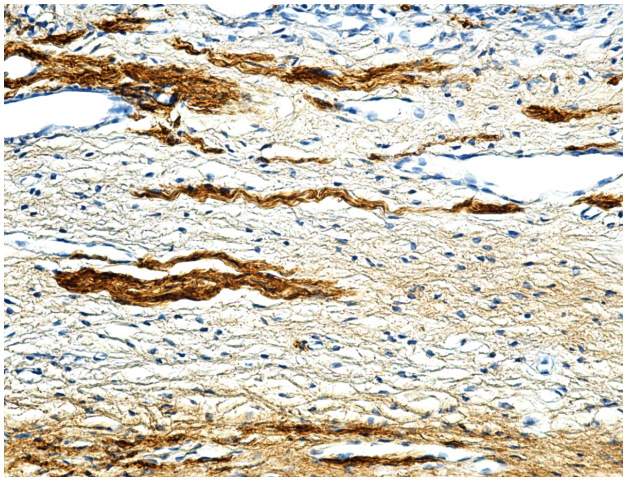

Figure 9 – ***Dissociated nerve fibers present in the dental pulp in a patient with diabetes and periodontitis. S100 immunostaining, ×200.***

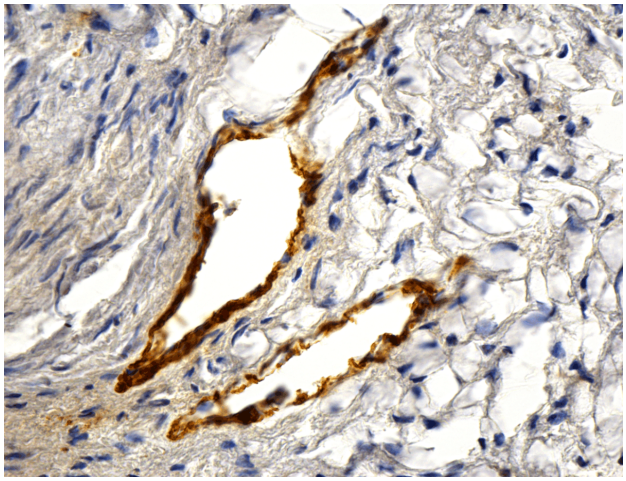

Figure 10 – ***Microscopic image of increased caliber arterioles present in the coronary pulp in a patient with diabetes without periodontal disease. α-SMA immunostaining, ×200.***

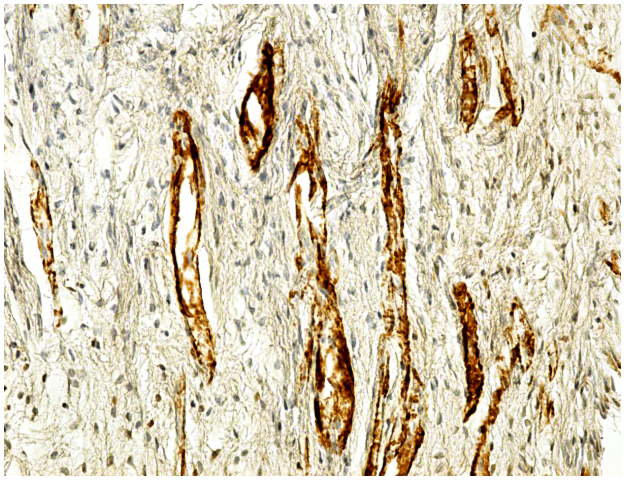

Figure 11 – ***Numerous arterioles and venules present in the apical pulp of a patient with diabetes and periodontal disease. α-SMA immunostaining, ×100.***

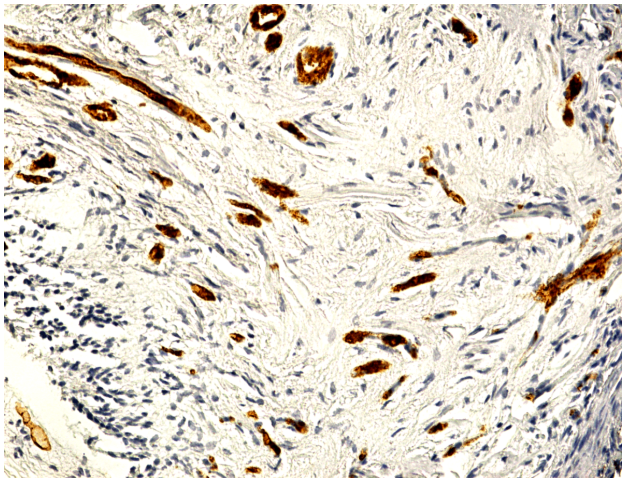

Figure 12 – ***Numerous blood capillaries arranged in the close proximity of the odontoblastic layer. Factor VIII immunostaining, ×200.***

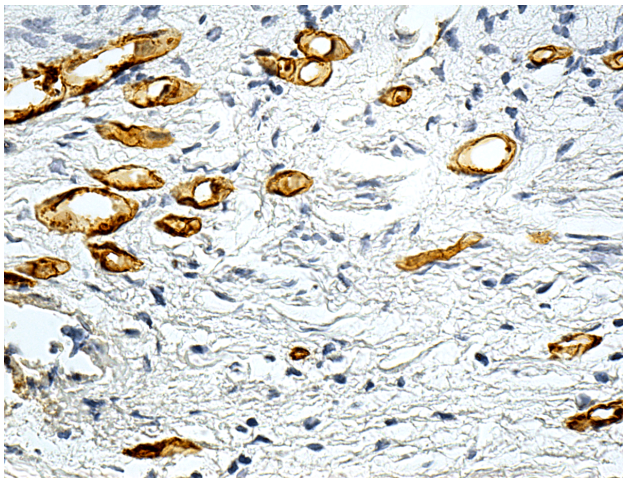

Figure 13 – ***Angiogenesis capillaries present in the center of the dental pulp from a patient with diabetes and periodontal disease. Factor VIII immunostaining, ×400.***

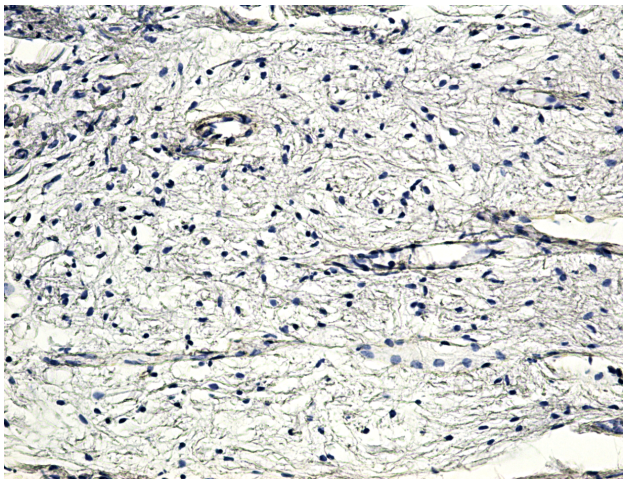

Figure 14 – ***Image of a dental pulp from a patient with diabetes and periodontal disease, in which it is noticed the absence of type IV collagen. Collagen IV immunostaining, ×200.***

Discussion

Diabetes mellitus is a chronic disease with a slower or faster development, which causes many debilitating complications in the long term [9]. As a multisystemic metabolic disease, diabetes alters the structure and functions of many organs and tissues, including dental pulp and periodontal tissues. Moreover, this disease can affect the outcome of endodontic treatment [10–12].

Although the relationship between oral health and diabetes has been extensively studied, especially in regard to the periodontal disease, data on pathogenesis, progression and healing of endodontic pathology in diabetic patients are extremely low [9], although diabetes has an accelerated increase of incidence and prevalence over the past 25 years [13].

In our study, we wanted to evaluate the histopathological changes of dental pulp collected from patients with diabetes, associated or not with periodontal lesions. The most constant histopathological change in dental pulp in patients with diabetes was the increase in fibrillar collagen by transforming the pulp from a loose connective tissue into a fibrous tissue. Another constant change was the thickening of the vascular wall by increasing its content in collagen, most blood vessels presenting microscopic aspects of arteriosclerosis. It is known that hyperglycemia causes vascular wall changes rapidly leading to diabetic microangiopathy and accelerating the process of arteriosclerosis [11]. Our data confirms that diabetes affects pulp arterioles, producing at this level arteriolosclerosis processes. We also believe that excessive fibrotic changes present in the dental pulp are due to ischemia that occurs following changes in blood vessels, which induces proliferation of fibroblasts. Several studies have shown that blood flow is reduced in the dental pulp in diabetic patients [10, 14].

In patients with diabetes and periodontal lesions, the presence of a chronic inflammatory infiltrate, predominantly of lymphocytes, plasmocytes and macrophages, has been revealed in the pulp tissue. Several studies have shown that diabetic patients have periapical and pulpar inflammatory lesions that can cause various histopathological changes, including necrosis of dental pulp [11, 15]. It appears that hyperglycemia result in a reduction in the bactericidal activity of neutrophil polymorphonuclear cells and which would explain the more frequent periodontal and odontal infections in diabetic patients [16].

As it was stated also in other studies [17], the S100 protein immunohistochemical marking allow the researchers to observe the trajectory of the pulp nervous structures in the dental pulp. We noticed that nerve fibers were numerous, grouped in coarse bundles, disposed almost parallel to the blood vessels in samples from patients with diabetes without paradontopathy, while in the samples collected from patients with diabetes associated with periodontal lesions, the nerve fibers appeared more rarely, and they were more disordered. We should mention that not only the nerve structures are positive for the S100 protein but also other human dental pulp cells such as macrophages or dendritic cells [17, 18].

During this study, we identify expressions of α-SMA only in the blood vessels walls. However, we noticed various intensities of the staining according to the diameter of the vessel and its localization. Arteriolar medium coat seems to be better developed in the apical vessels. Pale expressions of α-SMA go near the odontoblastic layer, which means the maintenance of the medium vascular coat almost to the capillary area. Although actin was discovered in muscles since 1940, in the last decade became obvious that it is an abundant protein in non-muscle cells and seems to participate in cellular mobility as well as in the cytoplasmic matrix structure [19].

Within *in vitro* studies, Alliot-Licht *et al.* [20] have proven the apparition of positive cells to α-SMA in mineralized pulp tissue, as response of the pulp tissue to different harmful factors. These cells show isolated or gathered around mineralized nodes. Furthermore, in electronic microscope studies, some of these cells present aspects characteristic to myofibroblasts or pericytes such as: fibronexus, stress fibers, dentate nuclei and gap like junctions [21]. Brock *et al.*, during their *in vitro* studies, find high levels of α-SMA in cell cultures obtained from pig dental pulp. They also prove that the pulp cells that contain α-SMA have a capacity to contract a matrix based on collagen and glycosaminoglycans, and this matrix is similar to extracellular matrix [22].

Based on these findings, we considered necessary making an immunological staining with the anti-human von Willebrand factor (factor VIII) with specificity for endothelial cells, allowing us to see small vessels. We have identified numerous blood capillaries placed among the collagen fibers in the dental pulp, but especially at the periphery of the pulp in the immediate vicinity of the odontoblasts layer.

The histiocytes and the macrophages are in different proportions in the dental pulp according to the local conditions; they present a large heterogeneity from point of view of the expression of the cytochemical markers [23]. The linkage to the molecules depends on the environment. The linkage can be determined by cytokines [24]. Macrophages have a high capacity of phagocytosis [25]. There are high concentrations of inflammatory mediators and enzymes in diabetic pulp [26].

The immunohistochemistry in this study shows a strong and homogenous staining of large and small vessels. These results are in agreement with Trubiani *et al.* [27], and Digka *et al.* [28] that show the existence of a well-defined microvascularization in the dental pulp in young adults, proven by the intense expression of the CD34 antigens on the endothelial surfaces, and giving hints of the ability of the dental pulp vessels to reshape and produce new vessels.

Dental pulp is an active metabolic tissue with a high regenerative capacity as an answer to different stimuli. Capillary endothelium of whole vascular network of the dental pulp is dynamic and capable to achieve a large number of synthetic and metabolic functions. The development, maintenance and frame of the vascular system requires a high level of control, in which CD34 positive cells its proven to have an important role, as they have also in the fetal development as well as early life stages.

Recent studies remark the involvement of the thick endothelium in autoimmune processes, but still no conclusive studies. The thick endothelium determines

hyperviscosity, a higher degree of stiffness in the blood cells walls, making them slower in the small vessels and has consequences towards tissues hypoxia [29].

With age, small vessels endothelium suffers morphological changes such as: increased transportation through the endothelium, changes in the cytoskeleton, hypertrophy of the Golgi system and cytoplasmic deposits. It has been proven the existence of microvascular interconnexions between the periodontal ligaments, gingival tissue and dental pulp [30].

Endothelial cells have an important role in chronic inflammation. They express molecular adhesion and presents chemokines that lead to a higher recruitment of leucocytes in the tissues. There are numerous endothelial markers but were not enough studies regarding their specificity.

Digka *et al.* [28] claims that using immunochemistry techniques with CD34 antibody, vascular endothelium of the pulp shows a strong coloration for CD34 by using ABC method. The fine capillaries are highly positive and the odontoblasts negative. The endothelium of all vessels, both arterioles and venules was positive, with a brown homogenous intense staining.

Our immunohistochemical reactions were negative for collagen IV in both types of samples, proving that in the processes of pulp remodeling in diabetics, predominantly type III fibril collagen is formed, sustaining also the findings of Martinez *et al*. [31] for these particular patients.

The diabetic dental pulp, with its various pathological changes, present additional challenge for treatment [8]. In order to provide competent care to patients with diabetes mellitus, dental clinicians must understand the disease, its treatment and its impact on the patient's ability to undergo and respond to dental care. The clinician must know the patient medical history and must be aware or the histological changes that may occur in relation to the general status of the patient [32]. All paraclinical researches conclude in improving the clinical approach of the diabetic patient.

There are studies focused only on the endodontic treatment outcome some done on human subject, some on laboratory rats. Research indicates increased prevalence of periapical lesions in diabetics with decreased success rate of endodontic treatment. A reciprocal relationship exists between glycemic control and chronic periapical lesions [33]. In our study, we conclude same result, as the age of the diabetes and its control is directly related with the dental health in general and dental pulp health in particular, considering its response to injury and even the "life" of the tooth after the endodontic treatment.

Considering the pulp response to injury, we consider it being slower and overwhelmed faster than in a non-diabetic tooth. Studies confirm lowered blood flow in diabetic pulp [34] as well as high prevalence of in pulp calcifications [35]. Also, studies done on diabetic rats confirm a lower reparatory response during chronic pulp inflammation and reduced dentine bridge formation [36].

For patients that receive insulin therapy appointments should be scheduled so they do not coincide with peaks of insulin activity [37]. A patient that has diabetes mellitus well controlled medically and free of general complications can be treated in the dental office as a non-diabetic person, and they do not require any extra-prophylactic measures such as antibioterapy during endodontic treatment [38]. However, studies prove that the rate of success of endodontic treatment is reduced in diabetic patients having pre-operative periapical lesions [39]. Wang *et al.* found higher frequency of tooth extraction after endodontic treatment in patients with diabetes mellitus, suggesting lower success rate of root canal treatment [40].

Conclusions

For teeth with periodontal disease, in diabetic patient, can occur morphological changes depending on the status of the periodontal disease and the glycemia control of the patient. The vascularization is increased due to the newly formed blood vessels under the odontoblasts layer, showing an increased activity at this level due to external factors, but only in early stages of the diabetes. Considering the immunosuppressed body in the diabetic patient, the defense system of the dental pulp is fast overwhelmed, conducting to inflammation or even necrosis. Structural alterations in the dental pulp occur in various degrees, related to the age of the diabetes, if the patient does not suffer from periodontal disease.

Conflict of interests

The authors declare that they have no conflict of interests.

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