

Periodontal disease in diabetic patients – clinical and histopathological aspects

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Abstract

Periodontal disease is one of the most frequent diseases affecting people all over the world. The relation between periodontal disease and diabetes mellitus raised the interest both of dentists and doctors treating metabolic diseases, as the two conditions influence one another. In our study, we analyzed a number of 75 patients with diabetes mellitus and periodontal disease that presented to the medical consultancy for conditions of the dental maxillary system. The clinical study showed that periodontal disease and diabetes may affect young adults as well, still this pathological association more frequently appears after the age of 50. The disease was identified especially in the women living in urban area. The clinical examination of the dental maxillary system identified the presence of gingival ulcerations, dental calculus, gingival bleeding, radicular leftovers with anfractuous margins, fixed prostheses with an inappropriate cervical adjustment. Of the systemic diseases associated to periodontal disease and diabetes mellitus, there was observed that 66.66% of the patients also suffered from cardiovascular diseases (high blood pressure, ischemic cardiopathy, heart failure), and 37.33% suffered from obesity. The histopathological and immunohistochemical tests highlighted the presence of an inflammatory chronic, intense reaction, mainly formed of lymphocytes, plasmocytes, macrophages and granulocytes, heterogeneously disseminated and alteration of the structure of marginal and superficial periodontium. The inflammatory reaction in the patients with periodontal disease and diabetes was more intense than in the patients with periodontal disease without diabetes.

Keywords: diabetes mellitus, chronic periodontitis, risk factors, metabolic disease, glycemic control.

Introduction

Chronic periodontal disease is an inflammatory condition of the gums and the structures supporting the tooth (periodontium and alveolar bone), most often caused by anaerobic Gram-negative microorganisms, adhering to the teeth forming the bacterial plaque [1–3]. Periodontal disease is an extremely frequent condition, with very high variations from one country to another. For example, in the United States, more than a half of the population aged more than 18 years old has an early form of periodontal disease; the population more than 35 years old presents a percent of about 75% of various forms of periodontal disease [2, 4]. The severity of periodontal disease is also variable. According to some studies, between 30 and 50% of these conditions are mild forms, and 5–15% are severe, generalized forms [2, 4, 5].

Regarding diabetes mellitus, numerous studies showed that this disease represents a major epidemic, as it affects more than 340 million people worldwide [6]. If at first there was thought that periodontal disease represented a

complication of diabetes mellitus, at present there is considered that the two conditions influence each other [7–9]. Periodontitis in diabetic persons presents much more severe progressive forms than in non-diabetic persons [10–11].

We proposed to make clinical correlations between glycemia values and severity of the periodontal disease, to identify associated diseases and to histologically evaluate the periodontal lesions, especially the inflammatory reaction, in persons with diabetes, in comparison to those without diabetes.

Patients, Materials and Methods

Our study included 75 diabetic patients, aged between 26 and 90 years old, who presented in the dentistry consultancy for various symptoms of the dental maxillary system. The duration of the diabetic disease was comprised between 5 and 16 years. The clinical and paraclinical investigations included: the patients age, social environment, toxic substances intake (smoking and alcohol intake), oral hygiene, glycemia values, presence of periodontal

lesions and other oro-dental lesions, local irritative factors and associated diseases.

In the patients with periodontal disease, with excessive mobility, where there was required a dental extraction, there were harvested small pieces of periodontium used for histopathological and immunohistochemical studies. Thus, there were harvested 32 fragments of periodontium from the patients with diabetes and nine fragments from patients without diabetes.

The harvested biological material was fixed in 10% neutral formalin solution, for 24–48 hours at room temperature, after which it was included in paraffin, by using the classical histopathological protocol.

The sectioning of the biological material was performed in the Microm HM325 rotary microtome, equipped with a water bath transfer system of the sections (STS, microM) and a Peltier cooling system.

For the histopathological study, there were used two stainings: Hematoxylin–Eosin (HE) and green light trichrome, the Goldner–Szekely (GS) technique. For the immunohistochemical (IHC) study, the histological cross-sections were harvested on histological blades covered with poly-L-lysine (Sigma), for the purpose of increasing the blade adherence of the sections, followed by their transfer to an incubator at 45°C and kept over night (18 hours). The next day, there was applied the classical immunohistochemical protocol, consisting in the deparaffinization and hydration of the sections, followed by the antigen demasking, by boiling the sections in a sodium citrate solution pH 6 in a microwave oven for 21 minutes (seven cycles of 3 minutes each). Subsequently, there was performed the blocking of endogenous peroxidase by incubating the biological material in 3% oxygenated water for 30 minutes at room temperature, followed by a washing in distilled water for 10 minutes and a washing in a 1% phosphate-buffered saline (PBS) solution for 5 minutes. The blocking of non-specific sites was performed by transferring the sections into a 2% skimmed milk bath for 30 minutes. Then, the sections were incubated with primary antibodies for 18 hours (over night), in a refrigerator, at 4°C, and the next day there was applied the biotinylated secondary antibody for 30 minutes, at room temperature. After washing the biological material in 1% PBS (three baths of 5 minutes each), there was applied Streptavidin–HRP (Horseradish peroxidase) for 30 minutes, at room temperature, followed by the blade washing in 1% PBS, 3×5 minute. The signal was detected by using 3,3'-Diaminobenzidine (DAB) (Dako). There followed the contrasting with Mayer's Hematoxylin, alcohol dehydration, xylene clarification and assemblage of blades by using a DPX environment (Fluka).

For the immunohistochemical study, the following antibodies were used:

- anti-CD3 (clone F7.2.38, 1/100 dilution, Dako) for highlighting T-lymphocytes;
- anti-CD20 (clone L26, 1/100 dilution, Dako), for highlighting B-lymphocytes;
- anti-CD68 (clone KP1, 1/200 dilution, Dako), for highlighting macrophages.

Results

The patients presented to the medical examination due to various reasons: gingivorrhagia, bad breath, dental mobility, physiognomic and functional masticating problems. Of the total of examined patients, 69 presented as main complaint gingivorrhagia and bad breath, these constantly presenting high values of glycemia.

By analyzing the distribution of the patients with periodontal disease and diabetes according to the age, we observed that periodontal disease associated to diabetes may also appear in young people (less than 30 years old), but most of them were aged more than 50 years old (59 patients, representing 78.66%) (Table 1).

Table 1 – Distribution of patients according to age

Age of patients [years]	No. of patients
<30	3
30–40	3
40–50	10
50–60	27
60–70	23
>70	9

By following the case distribution of periodontal disease associated with diabetes, there was observed that 45 (60%) patients were females and 30 (40%) patients were males.

The evaluation of the patients in relation to the social environment allowed us to observe that 47 (62.66%) patients came from the urban area and 28 (37.34%) from the rural area.

The repeated evaluation of glycemia showed that 34 (45.33%) of the patients had a good control over diabetes mellitus, glycemia values being close to normal (less than 125 mg/dL), while in 41 (54.67%) patients the glycemia values varied from 125 mg/dL to 230 mg/mL.

The clinical examination of the dental maxillary system showed multiple and quite varied lesions. Thus, gingival ulcerations were present in 21 (28%) of the patients, 19 of them being in the age groups older than 50 years old. Gingival ulcerations were localized mainly in the interdental papillae. Most of the patients who presented gingival ulcerations had a poor metabolic control of diabetes mellitus and a poor oral hygiene.

The local irritative factors, represented by radicular leftovers with anfractuous margins, fixed prostheses with an inappropriate cervical adjustment, mobile prostheses with an inappropriate cervical adjustment, dental calculus, were represented by 63 (84%) of the studied cases. Also, in the patients with high dental mobility and intercalated edentations, there were constantly present horizontal and vertical dental displacements, with important occlusal problems.

The highlighting and quantification of the bacterial plaque was performed with the O'Leary plaque index, a qualitative and percentage index of bacterial plaque that highlights its presence or absence on the vestibular, oral, mesial, distal surfaces of every tooth, except for the wisdom molars. Only 25 of the patients presented optimal values of dental hygiene, 21 of the patients presenting values higher than 70% (Table 2).

Table 2 – Values of O'Leary plaque index

O'Leary plaque index	No. of patients
<20%	9
20–30%	16
30–50%	9
50–70%	20
>70%	21

The evaluation of the gingival bleeding was performed with the gingival bleeding index (sulcus bleeding index – SBI). As observed from Table 3, only nine (12%) patients had a lower SBI, while 37 (49.33%) patients had a very high SBI index 25% (Table 3).

Table 3 – Gingival bleeding index SBI

Gingival bleeding index SBI	No. of patients
<25%	9
35–25%	19
70–35%	10
100–70%	37

SBI: Sulcus bleeding index.

Of the systemic diseases associated with periodontal diseases and diabetes mellitus, in our group of patients, there was observed that 50 (66.66%) of the patients also suffered from cardiovascular diseases, and 28 (37.33%) of them suffered from obesity. The complications of diabetes like retinopathy and diabetic polyneuropathy, as well as chronic kidney failure, were identified only in 12 persons, all aged more than 50 years old (Table 4).

Table 4 – Systemic diseases associated with diabetes and periodontal diseases

Systemic diseases associated with DM	No. of patients
Heart diseases (HBP, IC, HF)	50
Obesity	28
Diabetic retinopathy	4

Systemic diseases associated with DM No. of patients

Diabetic polyneuropathy	4
Chronic kidney failure	4

DM: Diabetes mellitus; HBP: High blood pressure; IC: Ischemic cardiopathy; HF: Heart failure.

The histopathological and immunohistochemical study evaluated the inflammatory reaction of the periodontium in patients with periodontal disease and diabetes, in comparison to the periodontium obtained from the patients with periodontal disease, without diabetes. In this way, we studied the reaction of macrophages and T- and B-lymphocytes. The classical stainings showed the presence of a chronic infiltrate in the periodontium, mainly formed of mononuclear round lymphocytes, plasmocytes and macrophages and a few granulocytes (Figures 1 and 2). The presence of inflammatory cells caused the disarrangement and remodeling of the periodontal conjunctive tissue. Thus, in some areas, the periodontium presented the image of a granulation tissue rich in angiogenesis vessels, which showed a very high intensity of the remodeling processes of the conjunctive tissues that fix the tooth in the alveola. Some angiogenesis vessels presented a discontinuous wall that allowed the extravasation of red blood cells, with the presence of microhemorrhages in the periodontium that secondarily amplified the local inflammatory and remodeling reaction of the periodontium (Figure 2).

The immunohistochemical study showed a heterogeneous distribution of the inflammatory cells in the periodontal lesions, both in the patients with diabetes mellitus and in those without diabetes. Our study showed that the inflammatory reaction in the patients with periodontium and diabetes was more intense than in the patients with periodontal disease, but with no diabetes mellitus. Of the immune system cells, the best represented were the macrophages and B-lymphocytes, while the T-lymphocytes had a more reduced reaction (Figures 3–8).

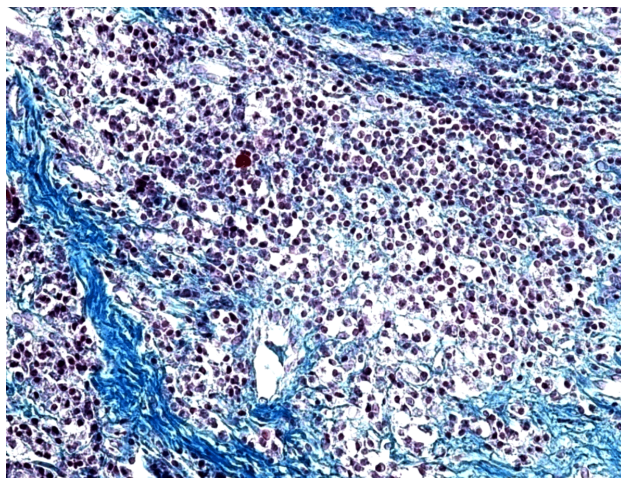


Figure 1 – Image of periodontium coming from a patient with periodontal disease and diabetes, partially destructured by the presence of a chronic inflammatory infiltrate mainly formed of round mononuclear cells. GS trichrome staining, ×200.

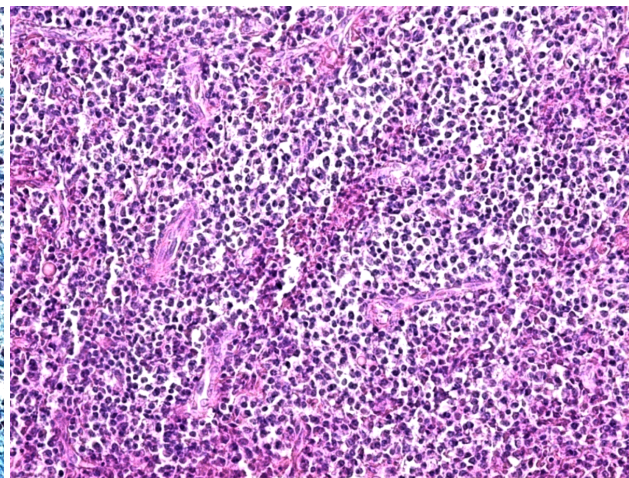


Figure 2 – Severe periodontitis, with intense inflammatory reaction, with numerous angiogenesis vessels and microhemorrhagic areas, in a patient with diabetes. HE staining, ×200.

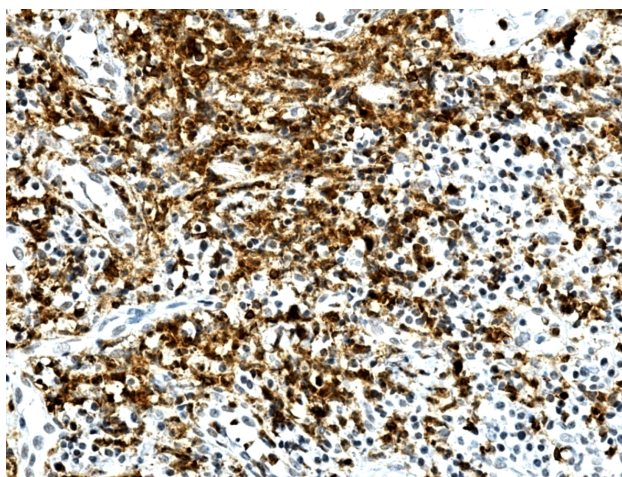


Figure 3 – Marginal periodontium with intense reaction of macrophages, in a patient with periodontal disease and diabetes mellitus. Anti-CD68 antibody immunostaining, $\times 200$.

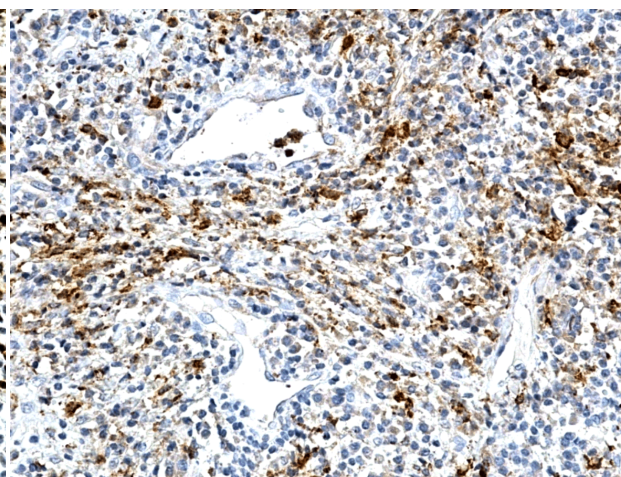


Figure 4 – Moderate macrophage reaction in a patient with periodontal disease without diabetes mellitus. Anti-CD68 antibody immunostaining, $\times 200$.

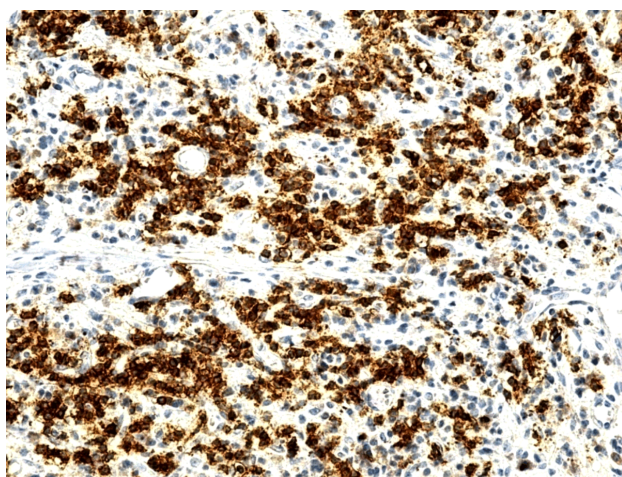


Figure 5 – Intense reaction of B-lymphocytes in a patient with periodontal disease and diabetes mellitus. Anti-CD20 antibody immunostaining, $\times 200$.

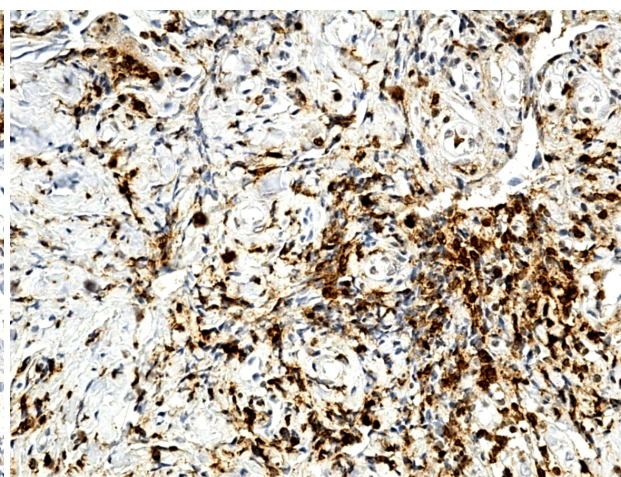


Figure 6 – Moderate reaction of B-lymphocytes in a case of periodontal disease without diabetes mellitus. Anti-CD20 antibody immunostaining, $\times 200$.

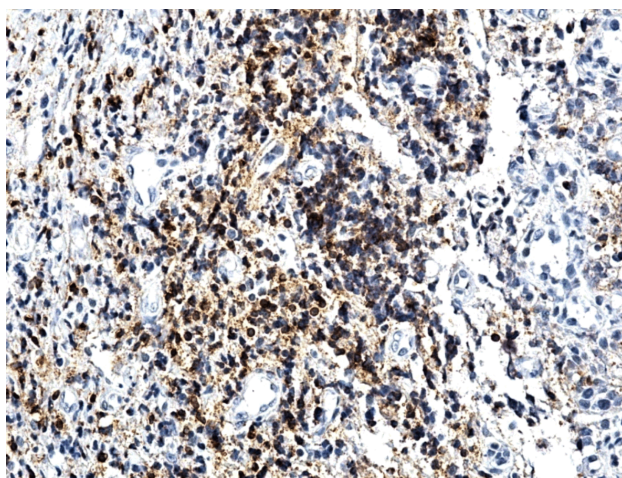


Figure 7 – Moderate reaction of T-lymphocytes in a patient with periodontal disease and diabetes mellitus. Anti-CD20 antibody immunostaining, $\times 200$.

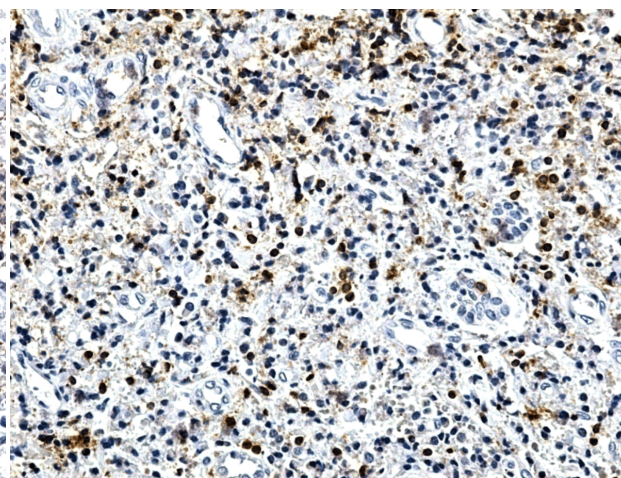


Figure 8 – Microscopic aspect of the periodontium in a patient with periodontal disease, without diabetes, with a moderate reaction of T-lymphocytes. Anti-CD20 antibody immunostaining, $\times 200$.

Discussion

Periodontal disease is one of the most frequent conditions affecting the populations all over the world, being the main cause of tooth loss in adult persons [4, 12]. It is considered an important health problem worldwide, as it affects the quality of life [13]. Periodontitis is not only a condition affecting the dental maxillary system, but also the health state of the whole body. Numerous studies associated periodontitis with various systemic conditions, such as diabetes mellitus [14], atherosclerosis cardiovascular diseases [15], inflammatory diseases of the intestine [16], rheumatoid arthritis [17], chronic nephropathies [18], Alzheimer's disease [19], dyslipidemia, obesity [20, 21], etc.

Diabetes mellitus is a very frequent metabolic disorder, characterized by the presence of chronic hyperglycemia, because both the insulin secretion and its action are low [22, 23]. Diabetes mellitus is considered a major risk factor for chronic periodontitis. Diabetes itself does not trigger periodontitis, but it predisposes and accelerates periodontal damages.

In our study, we observed that the association between periodontal disease and diabetes mellitus may also appear in young adults (less than 30 years old), but this association is more frequent in persons more than 50 years old. A study from Lithuania showed that in children with diabetes, aged between 10 and 15 years old, the prevalence of gingivitis was 27%, while in children of the same age, the prevalence of gingivitis was 13% [24]. Other studies showed that in children with diabetes mellitus, periodontal disorders usually occur in adolescence, and, sometimes, even earlier [25, 26]. Most studies showed that in persons with diabetes mellitus, periodontitis is more frequent (twice, three times) in comparison to the non-diabetic population [22, 27–30] and occurs at an earlier age than in the healthy population [31]. Also, the association between periodontitis and diabetes mellitus increases with age. For a long time, there was considered that periodontal disease is the sixth complication of diabetes mellitus [32], but recent studies showed that between the two conditions there is a bidirectional relation [33–34], negatively influencing one another.

The clinical examination performed by us showed that in the patients with periodontal disease and controlled diabetes (with low values of glycemia), the periodontal lesions had a lower intensity, in comparison to the patients with high values of glycemia. Some studies consider that hyperglycemia causes an exaggerated response to the aggression of periodontal pathogen bacteria, having as a result a more rapid and severe damaging of the periodontal tissue [35, 36].

In the last decades, numerous studies showed that periodontitis affects not only the dental maxillary system, but it also influences the systemic health of the individual. By evaluating the associated systemic pathology in our patients, there was observed that 66.66% had cardiovascular diseases, while 37.33% suffered from obesity. At present, there is considered that the inflammatory response in the periodontal tissues is not only a local pathology, but it also affects other structures of the human body, thus influencing the onset and progress of certain

systemic diseases. According to some studies, periodontal tissue inflammation is responsible for various cardiovascular diseases, including the atherosclerosis processes [37–40].

Regarding the relation between periodontal disease and obesity, various studies showed a strong association between the two conditions [41, 42], although the molecular mechanisms that make obesity influence periodontal lesions are still unknown [43].

The microscopic studies we performed highlighted a more intense inflammatory reaction in the patients with periodontitis and diabetes mellitus, in comparison to the patients with periodontitis without diabetes, which confirms the fact that diabetes causes the periodontal disease to have a more severe progress. Of the cells identified by us, we observed the presence of a higher number of macrophages and B-lymphocytes and a lower number of granulocytes. We consider that the inflammatory reaction is the essential and defining element in the progress of periodontal disease.

The inflammatory reaction is generated by the bacterial flora that forms dental plaque. If there is a gum superficial inflammation at first, a change in the bacterial flora, including the change of anaerobic Gram-positive bacteria with anaerobic gram-negative germs would constitute the essential elements through which there would be performed the transition to periodontitis [44, 45]. Still, we should admit that the host factors (genetic factors, local risk factors, systemic diseases) play an essential part in the onset and progress of the periodontal disease [46–48]. The damaging of the local defense systems of the gum and periodontium leads to the pervasion of bacteria in the periodontal conjunctive tissue and the triggering of a local inflammatory response, leading to the destruction of teeth fixing systems in the alveola.

Numerous studies showed that chronic hyperglycemia causes an exaggerated inflammatory response. Therefore, the polymorphonuclear neutrophils and monocytes synthesize a higher quantity of cytokines in the patients with periodontal disease and diabetes, in comparison to the patients with periodontopathy but non-diabetic, thus generating a more intense inflammatory response and more serious lesions in the periodontium [49–51].

Conclusions

Our study showed that periodontal disease associated with diabetes shows clear signs and a different evolution, according to the glycemia values, and to the presence or absence of irritative local factors. Periodontal disease and diabetes may also occur in young adults, but the incidence of the two conditions increases with age. Of the systemic diseases associated with periodontal disease and diabetes mellitus, there was observed that 66.66% of the patients also suffered from cardiovascular diseases, and 37.33% suffered from obesity. The histopathological and immunohistochemical examinations highlighted the presence of a chronic intense inflammatory reaction, mainly formed of lymphocytes, plasmocytes, macrophages and rare granulocytes, heterogeneously disseminated and damaging of the marginal and superficial periodontium. The inflammatory reaction in the patients with periodontal disease

and diabetes was more intense than in the patients with periodontal disease without diabetes.

Conflict of interests

The authors declare that they have no conflict of interests.

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