

The influence of diabetes mellitus on periodontal tissues: a histological study

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Abstract

Objective: The aim of this study was to investigate histological changes that occur in the periodontium of subjects with type 2 diabetes mellitus without signs of periodontal disease and to establish the influence of this systemic condition upon periodontal structures. **Materials and Methods:** Gingival tissue samples were obtained from 12 adult patients with type 2 diabetes mellitus and 10 healthy adults, as control group. The specimens were examined using standard dyes as Hematoxylin and Eosin and PAS–Alcian stain, by a microscope with different magnifications. **Results:** Our results showed that periodontal disease in patients with type 2 diabetes mellitus is characterized by significant inflammation, affecting both epithelial and connective tissues, with degeneration of dermal papilla, increase in number of inflammatory cells, destruction of reticular fibers and accumulation of dense collagen fibers (fibrosis). **Conclusions:** Within the limits of this study, diabetic subjects presented distortion in periodontal attachment, with changes in both epithelial and connective tissues, when compared to the healthy controls, suggesting that diabetes mellitus has an independent effect on periodontal tissue. This effect is observed in both groups, so that we considered it to be independent of the periodontal condition.

Keywords: diabetes mellitus, periodontal disease, histopathology.

Introduction

Diabetes mellitus includes a group of metabolic diseases characterized by hyperglycemia that results from a deficiency in insulin secretion and/or its reduced action. Severe hyperglycemia can cause many symptoms, including polyuria, polydipsia, polyphagia, weight loss and impaired vision [1]. Peripheral vascular changes occur, leading to a reduced immunological capacity of the patient and increasing susceptibility for infection. Although diabetes mellitus has often been associated with periodontal breakdown, the exact role of this disease in the pathogenesis of periodontal disease is not completely understood [2]. Several epidemiological studies have shown that there is no difference in the periodontal status between diabetic and non-diabetic patients. Herring ME and Shah SK [3] suggested that patients with controlled diabetes have no more periodontal destruction than healthy controls. On the contrary, some investigators have found increased periodontal destruction in diabetic patients [4, 5].

The aim of this study was to investigate histological changes that occur in the periodontium of subjects with type 2 diabetes mellitus without signs of periodontal disease and to establish the influence of this systemic condition upon periodontal structures.

Materials and Methods

The experimental study group consisted of 12 diabetic

patients of both sexes, with age range 35 to 58 years, with no signs of established periodontal disease, who addressed to the Clinic of Odontology and Periodontology, Faculty of Dental Medicine, University of Medicine and Pharmacy of Târgu Mureș, for dental problems. In each case, bioptic specimens were obtained from a dental–periodontal unit in the posterior area of dental arches.

The control group consisted of 10 healthy subjects, of both sexes, with age range 32 to 50 years that addressed to the Emergency Department of our Clinic for different dental problems. All these subjects had no systemic disease and no medical treatment at the time of tissue examination. Extraction of teeth with no prognostic allowed us to collect tissue samples, from those sites that previously showed a healthy periodontium.

Tissue samples consisted of soft tissues (gingival fibromucosa). Tissue harvests were made with the patient's written approval and according to the technique and methodology in the international literature [6, 7]. We took care to harvest deep and narrow samples, not superficial and wide ones.

Tissue samples were fixed in Lillie neutral formalin for maximum five days, in adequate recipients, with a volume of formalin 50 times bigger than specimens. Gingival fibromucosa fragments were of 3–5 mm, gray-white colored, with some firm brown areas. The specimens were then treated as follows: three successively baths of one hour each in 80% ethylic

alcohol, three successively baths of one hour each in 95% ethylic alcohol, three successively baths of one hour each in absolute ethylic alcohol, three successively baths of one our each in xylene and two successively baths of one our each in paraffin. After inclusion, paraffin blocks were casted, labeled and sectioned with microtome at 4–5 μ m and displayed on glass slides. After dewaxing specimens were stained with Hematoxylin–Eosin and PAS–Alcian stains.

Results

When comparing microscopic aspects of fibromucosa of the control group with those of study group, we noticed that the latter group presented an epithelium of variable thickness, occasionally thin, with rare sites of superficial ulceration and acanthosis. The underlying connective tissue presents ectatic blood vessels surrounded by a rich inflammatory infiltrate, mainly lymphoplasmocytic. Biopsies from gingival mucosa of controls showed a squamous epithelium of normal histological aspect, with a thin layer of keratin, with rare and small sites of parakeratosis (Figures 1 and 2). The underlying connective tissue formed small papillae that enter through epithelium (dermal papillae).

Biopsies from the study group showed a pronounced

mitotic activity in the basal layer of the epithelium that accompanies the inflammatory process, cellular intra-epithelial vacuolization and acantholysis in stratum spinosum. Parakeratosis and remains of nucleus of epithelial cells were present in the superficial area of the epithelium. The capillaries and venules were dilated with perivascular lymphoplasmocytic infiltrate and thrombosis (Figures 3–5).

Degradation of connective tissue was present in diabetic subjects, due to a rich inflammatory infiltrate, destruction of reticular fibers and accumulation of dense collagen fibers (fibrosis) (Figures 6 and 7).

In our study, we also used PAS–Alcian stain in order to show neutral and acid mucopolysaccharides (glycogen colored in red and proteoglycans colored in blue-violet). In biopsies from the diabetic subjects, we noticed a thickening of the basal membrane of small blood vessels and accumulation of PAS-positive material in basal epithelial layer, glycogen respectively (Figure 8).

The presence of high quantities of glycogen at this level is responsible for volume modifications of the gingiva, as it is known that glycogen is a hydrophilic substance, attracting water and liquids into tissues. We also noticed mucopolysaccharides at the border between epithelium and connective tissue (Figures 9 and 10).

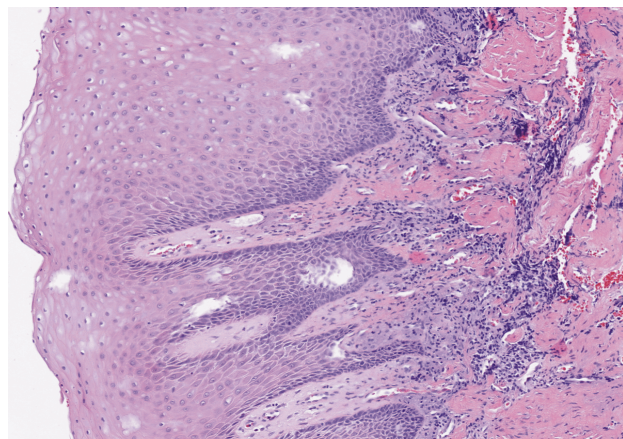


Figure 1 – Gingival biopsy taken from a 35-year-old healthy subject. Normal aspect of epithelium layers, dermal papillae and connective tissue (HE stain, ob. 10 \times).

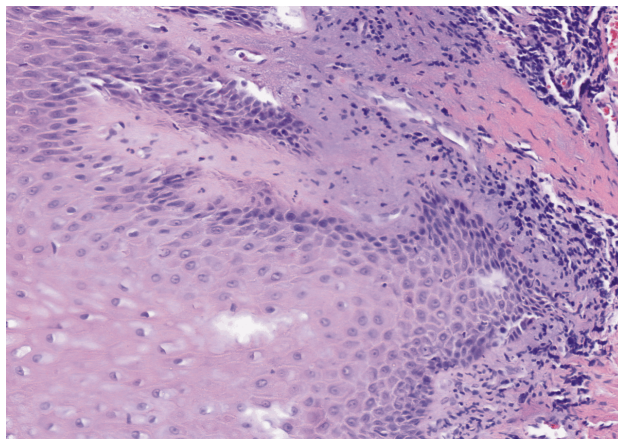


Figure 2 – Detail of previous figure. Vacuolization in spinocellular layer (HE stain, ob. 40 \times).

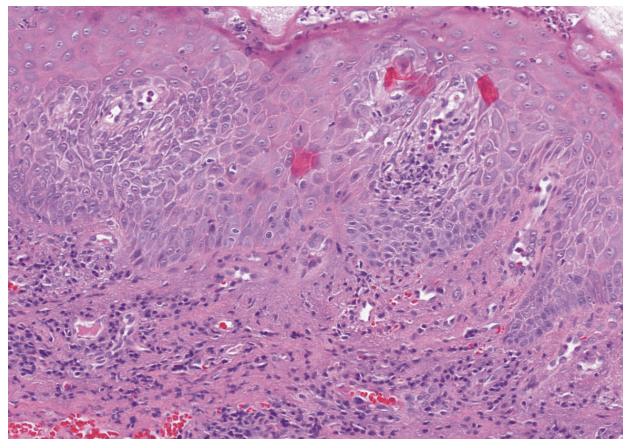


Figure 3 – Biopsy taken from a 46-year-old diabetic patient. Dermal papillae are flattened, dilated capillaries, vasodilatation and thrombosis in connective tissue vessels (HE stain, ob. 20 \times).

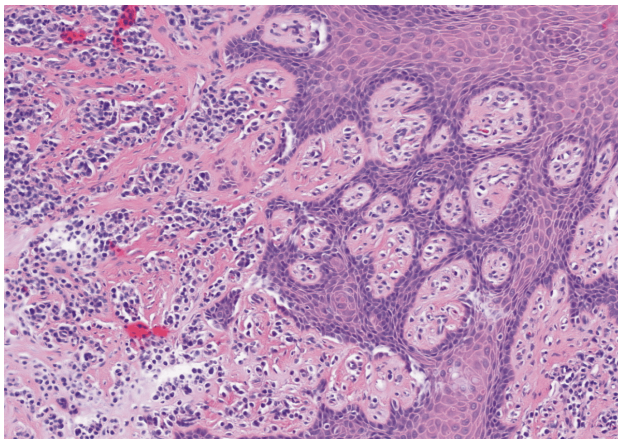


Figure 4 – Gingival biopsy taken from a 38-year-old diabetic patient. Acanthosis, proliferation of epithelium in chorion, islands of epithelium into the connective tissue (HE stain, ob. 20 \times).

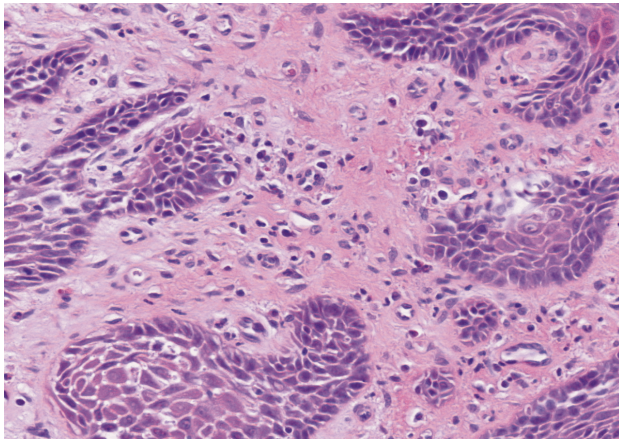


Figure 5 – Details of previous figure. Epithelial islands surrounded by connective tissue (HE stain, ob. 40×).

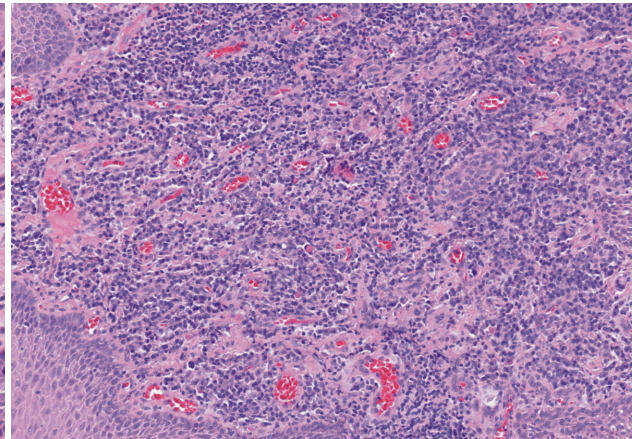


Figure 6 – Biopsy taken from a 40-year-old diabetic patient. Rich inflammatory infiltrate, rich vasculature of stroma (HE stain, ob. 20×).

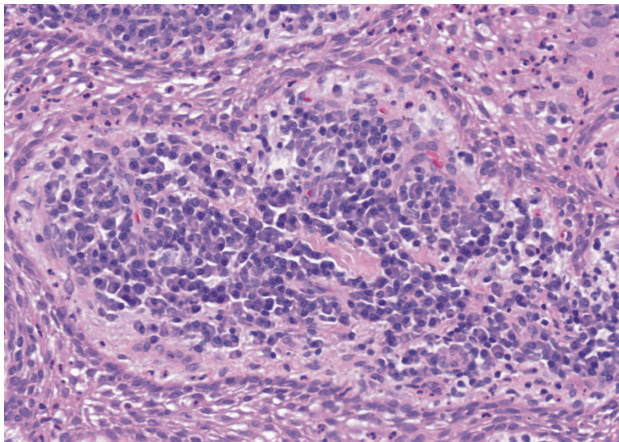


Figure 7 – Detail of previous figure. Rich mixed inflammatory infiltrate, with numerous plasmacytes and degradation of connective tissue due to the presence of inflammatory cells (HE stain, ob. 40×).

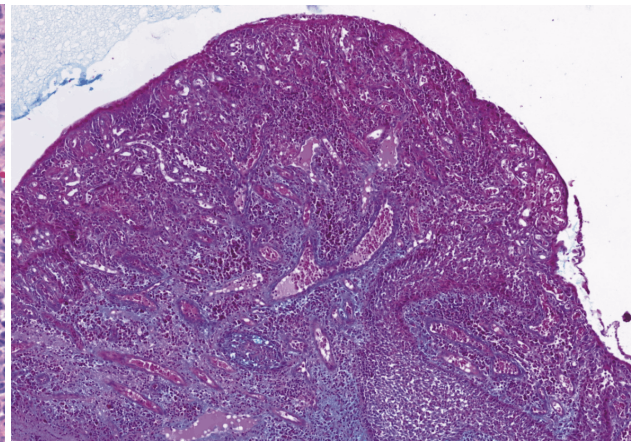


Figure 8 – Gingival biopsy taken from a 47-year-old diabetic patient. Rich inflammatory infiltrate, numerous dilated blood vessels, with thicker basal vascular membrane, both in epithelium and connective tissue (PAS-Alcian stain, ob. 10×).

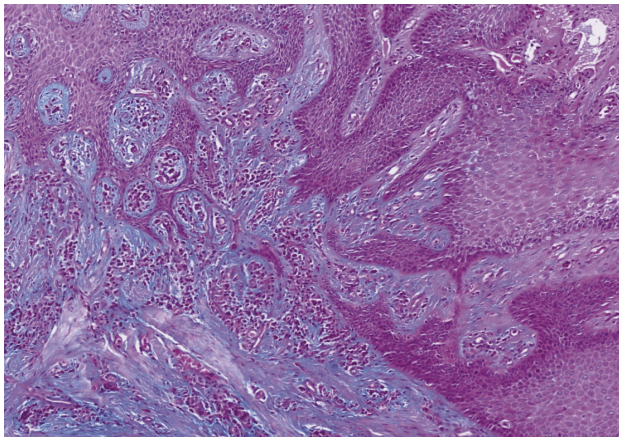


Figure 9 – Gingival biopsy taken from a 42-year-old diabetic patient. High quantities of acid mucopolysaccharides in the connective tissue. Collagen fibers lost their functional distribution (PAS-Alcian stain, ob. 20×).

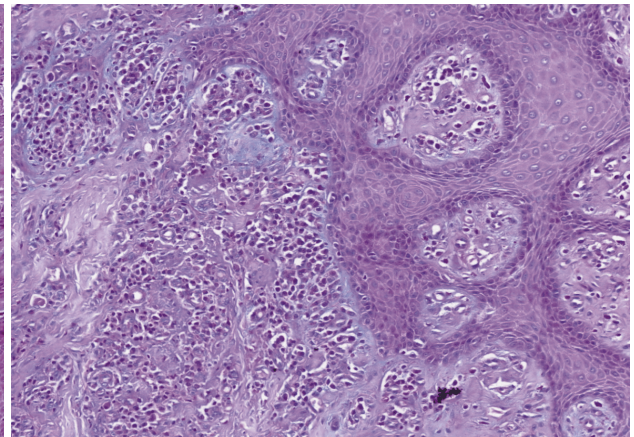


Figure 10 – Detail of previous figure (PAS-Alcian stain, ob. 40×).

Discussion

Our results are in agreement with several earlier reports. Many authors reported alterations of gingival connective tissue in subjects with diabetes mellitus. In 2008, Silva JA *et al.* [8] reported atrophy and

pleomorphism of the gingival epithelium, with decreased cellular organelles and increased intercellular spaces, and a thickened keratin layer with the reduction of height of dermal papilla. According to these authors, increased mitotic activity in epithelial basal layer

can contribute to the compromise of epithelial cells differentiation, leading to a thickening of the keratin layer and flattening of dermal papillae. Furthermore, they observed a reduction in collagen solubility and the distortion of reticular fibers.

Hillman G *et al.* [9], in 1998, noticed that under pathologic conditions such as gingival inflammation, stratum corneum is thicker (hyperkeratosis), with frequent fissures and flap lift off. Due to inflammation, cells with nuclear fragments appear (parakeratosis) because the time during which cells migrated to the surface was too short to permit the total transformation of cells content into keratin.

Other researchers observed that epithelial cells are involved in synthesis and secretion of thromboxane B₂ that appear during the inflammatory process in gingival tissue [10].

Takata T and Donath K [11] postulated that periodontal pocket formation seems to be initiated by degenerative changes that take place into the second interior layer of the epithelium, from the most apical part of junctional epithelium, which is in contact with subgingival dental plaque. Consecutively an intra-epithelial split occurs, followed by degeneration and desquamation of cells lining the split. The destruction of epithelial barrier and simultaneous penetration of bacterial or toxic products are supposed to be major factors involved in apparition of stable periodontal lesions.

Some studies were focused on various aspects of immune response in subjects with diabetes, including collagen metabolism, and have suggested new host modulatory approaches to address the altered host immune reactions [12,13]. Several collagen abnormalities have been identified, including a large reduction in collagen synthesis and solubility in gingiva, skin and bone, and an even more profound increase in the urinary excretion of hydroxyproline, an amino acid marker of collagen and its breakdown fragments. These findings suggest that the disease increases the degradation of newly synthesized collagen in various connective tissues throughout the body [14].

Altered collagen metabolism may predispose patients with diabetes not only to periodontal disease but also to other abnormalities of connective tissues, such as impaired wound healing. Elevations of collagenase activity in gingival crevicular fluid [12] and decreases in gingival collagen synthesis [15] in patients with diabetes have been observed.

Polymorphonuclear leukocyte (PMN) functions, such as chemotaxis and phagocytosis, are decreased in patients with diabetes and periodontal disease. Some studies have observed a decrease in PMN chemotaxis in patients with poorly controlled diabetes, and the severity of this PMN defect was correlated to the degree of glycemic control [16–18]. There was also reported an altered monocytic response in diabetic subjects.

Elevated levels of chemical mediators of inflammation known as prostanoids (prostaglandin E₂, or PGE₂) have been detected in the blood of patients with type 1 diabetes [19–21].

It is known that gingival vascular bed gives a clear

picture of the general state of the patients. Russel BG [22] found statistically significant abnormalities in a study of gingival tissue from diabetes mellitus. Particularly striking were the PAS-positive diastase resistant thickening of the vessel walls and the swelling and proliferation of endothelial cells, which in many cases produced luminal obliteration.

Histological studies have demonstrated that gingival blood vessels in subjects with long-term diabetes mellitus have more atherosclerosis compared to those of non-diabetic controls. Hove KA and Stallard RE [23] observed thickening of basement membrane of blood vessels in diabetic subjects in a light microscopic study. Clinical studies [24] have demonstrated that diabetes mellitus of long term duration and poor metabolic control shows clearly higher levels of gingival inflammation and periodontal diseases than non-diabetic control subjects. In diabetics, the periodontium is probably affected by increased collagenase action, functional abnormalities of neutrophil degranulation as a source of gingival crevicular fluid collagenase or other metabolic abnormalities in periodontal ligament fibroblast. Vascular changes in properly controlled long-term diabetes shows microangiopathy [25, 26].

Conclusions

According to the results of biopsies obtained from our study group, diabetes mellitus induces obvious histological and histochemical changes, in both epithelium and gingival connective tissue.

At epithelial level, we noticed accelerated mitosis in basal epithelial layer, acanthosis in spinocellular layer, parakeratosis and hyperkeratosis in superficial layer. In addition, at epithelial level, dilated capillaries with vascular stasis and thrombosis can be seen.

At connective tissue level, diabetes mellitus induced a rich lymphoplasmocytary infiltrate, which disorganizes the structure of conjunctive fibers. Gingival epithelium penetrates underlying chorion and epithelial cells islands occur into the connective tissue.

According to scientific evidence from different studies, all this changes depend on the duration of diabetic status and on the level of metabolic control of the disease, worsening as the diabetes is more poorly controlled and they are independent of the presence or absence of periodontal disease.

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