

## Histological changes of gingival epithelium in diabetic patients with periodontal disease

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### Abstract

The pathogenesis of the periodontal disease is complex, because it derives from the initiation and maintenance of the chronic inflammatory process by the plenty microbial flora and its numerous bacterial products. The purpose of our study was to make evidence of the histopathological changes occurring in the gingival epithelium in patients with both diabetes mellitus and periodontal disease. *Materials and Methods:* Our study was carried out on gingival tissue from 68 diabetic patients with specific symptoms of periodontitis. The preparations were processed by Hematoxylin–Eosin staining technique and trichromic staining after the Goldner–Szekely method. *Results:* Although non-specific, since they have been generally found in chronic gingival inflammation, the noticed changes were significant and some of them had specific aspects for the diabetic patients: gingival epithelium changes like acanthosis type; the presence of inflammatory cells such as polymorphonuclear leukocytes and/or lymphocytes among the cells of the stratified squamous epithelium; surface ulceration areas covered by hematic and fibrino-leukocytary detritus; the epitheliocytes keratinization in the superficial layers. *Conclusions:* The diabetic patients with periodontitis frequently associate hypertrophy changes of the gingival epithelium with the epithelial permeability alteration and the presence of the intraepithelial inflammatory cells, subsequently leading to degenerative lesions and epithelial ulceration.

**Keywords:** diabetes mellitus, periodontitis, gingival epithelium.

### Introduction

The pathogenesis of the periodontal disease is complex, because it derives from the initiation and maintenance of the chronic inflammatory process by the plenty microbial flora and its numerous bacterial products. The host response in front of this infection mediates a complex cascade of tissue destructions [1].

The supplementary factors that contribute to disease initiation and progression include some systemic diseases, especially diabetes mellitus that can exacerbate the host response in front of local microbial factors leading to an intense and uncommon periodontal destruction. As a fact, the aggressive periodontitis is recognized as the sixth complication of the diabetic disease by Loe H [2], who concluded that multiple epidemiologic studies demonstrated that both type 1 and 2 diabetes are predictors for periodontal disease in the presence of a poor systemic control.

Although it is difficult to conclude about the specific effects of the diabetes upon the periodontium, there have been described a variety of alteration: the presence of gingival inflammation, sessile or pediculated gingival polyps, gingival proliferations, periodontal abscesses, periodontitis and dental losses [3–5].

Maybe the most impressive modifications appear at

individuals with uncontrolled diabetes mellitus, where the defense mechanisms are altered and thus the susceptibility for infections grow. These aspects lead in time to the onset of some serious periodontal conditions [6].

The severe gingival inflammation, the deep periodontal bags, the rapid bony loss and the frequent periodontal abscesses often appear in diabetic patients with poor oral hygiene [7–9].

The prevention and the control of the periodontal disease should be considered as a part of the control performed on the patients with diabetes. The major efforts must look towards the prevention of the periodontal disease in the patients with a risk of diabetes. The diabetic patients with low glycemic control should receive frequent periodontal treatment, especially if the periodontal disease is already present.

The histological study had as a purpose to make evidence of the histopathological changes occurring in the gingival epithelium, especially the gingival sulcus epithelium–sulcular epithelium and junctional epithelium.

### Materials and Methods

The studied material was represented by the gingival tissue fragments obtained by simple gingivectomy or after

tooth extractions performed on 68 patients known to have type 1 and 2 diabetes mellitus. The patients were selected from patients diagnosed with diabetes in the Nutrition and Metabolic Diseases Clinic of Emergency County Hospital from Craiova, Romania. The study was conducted over a period of two years, between 2006 and 2008.

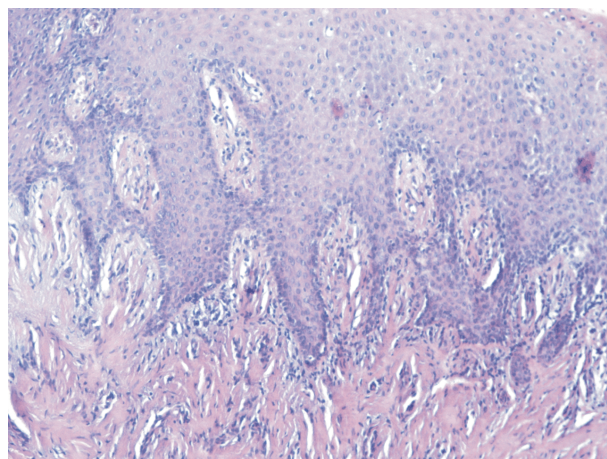
In the patients included in this study, we diagnosed specific symptoms for the periodontal disease such as gingival inflammation, gingival bleeding and recessions, mobility or even dental loss. There has also been taken into consideration the suggestive aspects for periodontal disease associated to diabetes: sessile and pediculate type of gingival outgrowing, bleeding gums, loss of periodontal attachment with great deep periodontal pockets. In these cases, there was performed the histopathological examination.

Mucosal fragments were fixed in 10% formalin and then processed by the usual technique of paraffin inclusion. The histological technique of paraffin inclusion used in this study performed the following sequences: dehydration, clarification, waxing, proper inclusion. The preparations were then processed by Hematoxylin–Eosin (HE) staining technique and trichromic staining after the Goldner–Szekely (GS) method.

## Results

Although non-specific, since they have been generally found in chronic gingival inflammation, the noticed changes were significant and some of them had specific aspects for the diabetic patients.

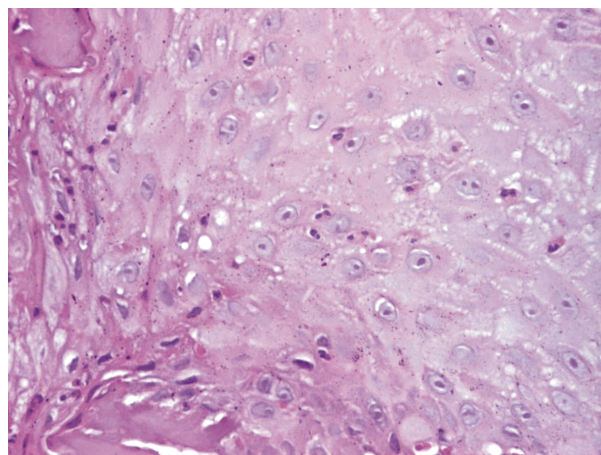
The gingival epithelium changes to the most patients (50 cases) were like acanthosis type with cell proliferation in squamous layer and the elongation of the interpapillary epithelial ridges that deeply penetrated into the chorion, taking a papilliform, digitiform aspect. As a result, the epithelium–chorion junction took an emphasized anfractuous look (Figure 1).



**Figure 1** – Patient with diabetes mellitus less than 10 years, marked acanthosis of gingival epithelium (HE staining,  $\times 100$ ).

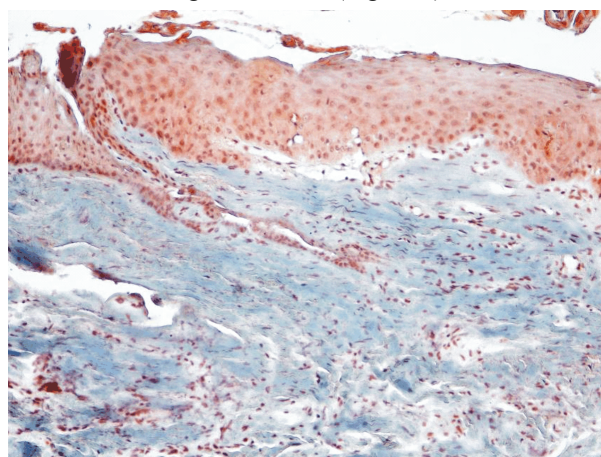
At some patients, there have been observed inflammatory cells such as polymorphonuclear leukocytes and/or lymphocytes among the cells of the stratified squamous epithelium (aspect called exocytosis). Also, in some cases the continuity of the gingival epithelium was interrupted

by the surface ulceration areas covered by hematic and fibrino-leukocytary detritus and sometimes with the presence of disintegrated polymorphonuclears. Especially in these cases, the cells of the remaining squamous epithelium presented degenerative lesions; they were swollen and sometimes with destroyed intercellular bridges (Figure 2).



**Figure 2** – Degenerate keratinocytes, intraepithelial inflammatory cells (polymorphonuclears), altered inter-cellular bridges (HE staining,  $\times 200$ ).

The other cases (represented by patients with diabetes for over 10 years) presented atrophic type epithelium with fewer layers of squamous cell, also with deletion of interpapillary ridges and tendency to rectilinear epithelium–chorion limit. Also, the epitheliocytes showed various degrees of keratinization in the superficial layers. The hyperkeratosis of parakeratosis-type was characterized by cells with elongated nucleus with compact chromatin. The parakeratosis and nuclear involution degree (characterized by karyopyknosis) was even bigger when the diabetic disease had a longer evolution (Figure 3).



**Figure 3** – Patient with diabetes for over 10 years, marked atrophy of the gingival epithelium (trichromic GS staining,  $\times 100$ ).

## Discussion

The histological study of the gingival fragments in patients with diabetes mellitus has proved in most of the cases significant changes in the gingival epithelium and lamina propria [10]. Therefore, in patients with diabetes with an evolution less than 10 years and in some of those

with diabetes for over 10 years, the acanthosis has prevailed into the gingival epithelium, which sometimes alternated with epithelial discontinuity due to its ulceration. The acanthosis with the elongation of the epithelial processes was observed in diabetic patients with a history of disease of one to five years [11, 12].

Another study based on patients with periodontal disease and type 1 diabetes mellitus show that the histological analysis on usual HE staining of the fragments collected from the gingival mucosa has revealed epithelial projections of the stratified pavementous epithelium to the underlying connective tissue and aspects of exocytosis. The penetration of the immune system cells into the layers of the covering gingival epithelium leads to degenerative lesions of the epitheliocytes with decrease of cell adhesion and appearance of ulcerations noticed to the studied patients [12]. The patients of the undertaken study presented at the intraepithelial level both polymorphonuclear leukocytes and rare lymphocytes that associated with epitheliocytes swallowing and intercellular bridges disintegration. The presence of the inflammatory cells at intraepithelial level suggested an emphasized vascular permeability, as well as an increased permeability of the gingival epithelium for this type of cells, with degenerative consequences over the epitheliocytes that finally evolved into ulcerations.

As the diabetic disease progresses at epithelial level, the atrophy phenomenon prevails, with the reduction number of the squamous cells layers, the deletion of the interpapillary ridges and the tendency for rectilinear epithelium-chorion limit, as noticed in our study. Also, as the diabetic disease progresses and especially in the poorly controlled diabetic cases with an evolution of more than 10 years, there has been prevailed the degeneration of the keratinocytes in the superficial layers, with the appearance of parakeratosis. The patients with a history of diabetes mellitus of five to 15 years and/or predominance of atrophic changes in gingival biopsy preparations show a mucosal thinning at the alveolar processes and atrophy of the stratified squamous epithelium, with keratinization centers appearance [11]. We consider that this excessive keratinization of the gingival mucosa represents a good way to strengthen the defense of the gingival epithelium to the harmful effect of the external factors, especially the bacterial plaque from these patients.

The electron microscopy of the cell populations from the biopsy fragments from the alveolar processes mucosa in patients with type 1 and 2 diabetes mellitus has shown degenerative changes of the endotheliocytes and epitheliocytes, and also the timing of these structural changes during the development of the diabetic disease. The epithelial degenerative changes of the epitheliocytes involve both the nuclear component and the cytoplasmic one: the number decreasing of the nucleoli of the basal cells, the karyopyknosis, the reduction of the organelles synthesized by proteins, the disruption of the mitochondrial crests, the decreasing of the tonofilaments number and the obliteration of the intercellular adhesion elements [13].

Numerous studies demonstrated the reciprocal interdependency between the periodontal disease and diabetes, as diabetic patients develop more severe periodontal diseases than non-diabetics [14–16]. The mechanisms

through which diabetes influences the evolution of periodontal disease seem to be immune/inflammatory. High level of blood glucose persisting in time seems to determine the chronic secretion of inflammatory mediators and in consequence an exaggerated periodontal response [17]. Diabetes patients present higher IL-6, IL-1 $\beta$  and TNF- $\alpha$  levels, as a response to the general irritation caused by bacterial plaque [18, 19].

We believe that the alterations of the gingival epithelium are caused not only by the local inflammatory reaction, but also by the composition of the bacterial plaque of the oral cavity. It was demonstrated that a higher concentration of *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Campylobacter*, etc. exist in diabetes patients [20, 21], which would explain the severity of the periodontal disease and the microscopic lesions of the gingival mucosa.

## Conclusions

The diabetic patients with periodontitis associate in most of the cases hypertrophy changes of the gingival epithelium with the epithelial permeability alteration and the presence of the intraepithelial inflammatory cells, subsequently leading to degenerative lesions and epithelial ulceration. Gingival epithelium atrophy is rarely met and is generally present in diabetics with the disease evolution of more than 10 years. To the patients with diabetes mellitus with long evolution, there is remarked an attempt to consolidate the defense of the gingival epithelium to the bacterial plaque action, by the means of parakeratosis appearance.

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