HISTOPATHOLOGICAL AND ULTRASTRUCTURAL INVESTIGATION OF GINGIVAL TISSUE FROM PATIENTS WITH CHRONIC PERIODONTITIS

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Summary

Periodontitis are infections that may cause systemic consequences. Due to that it is important to make an accurate histopathologic diagnosis as soon as possible. The aim of this study was to determine the main changes from the gingival mucosa biopsy taken from generalized chronic periodontitis (GCP) patients by light microscopy (LM) and transmission electron microscopy (TEM). The results were compared to those from healthy controls. GCP subjects important epithelium and subepitelial lesions, PMN infiltrates and bacteria were visualized. Conclusion: in GCP subjects bacterial infection is associated with important gingival mucosa lesions, that involve all the layer of the epithelium and connective tissue.

Keywords: periodontitis, light microscopy, TEM.

Introduction

Periodontal diseases are serious infections. In the past decade, the association of periodontal diseases with the development of systemic diseases has received increasing attention. It is not an exaggeration to state that periodontal infection represents a significant risk factor for various systemic diseases, because periodontal pathogens are translocated and released from the sulcus into the bloodstream, causing transient bacteremia. Therefore, the development of a method for periodontal disease diagnosis based on the concept that regards periodontal disease as a biofilm infectious disease is needed (Hiroaki and Atsuo, 2010; Wada and Kamisaki, 2010; Pao-Li, 2010).

The pathogenesis of periodontitis involves the interplay of microbiota present in the subgingival plaque and the host responses. Although periodontal disease is initiated and perpetuated by concerted interaction between host inflammatory and immunological responses to the specific infecting periodontopathogenic bacteria, it is characterized by destruction and exacerbation of gingival connective tissue and alveolar bone leading to eventual loss of teeth (Signat et al., 2002).

The aim of this study was to evaluate the structural and ultrastructural changes of gingival mucosa from generalized chronic periodontitis (GCP) patients.

Material and methods

Patients and study design

At the initial examination, the test and control group members were selected after a clinical and radiographic examination. The test group included 12 patients (6 male, 6 female) diagnosed with GCP in the Department of Periodontology, University of Medicine and Pharmacy Iuliu Hatieganu, Cluj Napoca, Romania from January to December 2010. A diagnosis of GCP was performed according to the
criteria established in 1999 (Armitage, 1999). The patients diagnosed with GCP had more than two areas with a 5 mm or more probing depth (PD) in the quadrant and bone loss > 30% of the root length on the radiographs. In a control group, 12 patients (6 male, 6 female) who did not have attachment loss or sites showing a PD of 3 mm or more with a sulcus bleeding index (SBI) < 10% were enrolled. Both the control and test groups had more than 20 functional teeth.

The periodontal status of potential study participants was examined by a single periodontists at recruitment (Table 1). A physical examination and structured interview was performed by a certified physician to assess eligibility (Table 2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Chronic periodontitis group</th>
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<tbody>
<tr>
<td>n (males/females)</td>
<td>12 (6/6)</td>
<td>12 (6/6)</td>
</tr>
<tr>
<td>Age</td>
<td>45.3±2.2</td>
<td>56.2±5</td>
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<tr>
<td>Probing depth - PD (mm)</td>
<td>2.2±4.2</td>
<td>3.5±1.02</td>
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<tr>
<td>Clinical attachment level – CAL (mm)</td>
<td>2.1±0.8</td>
<td>4.4±1.2</td>
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<tr>
<td>Plaque index – PI</td>
<td>10±3.7</td>
<td>40.2±6.8</td>
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<tr>
<td>Bleeding index - BI</td>
<td>12.2±5.5</td>
<td>56.3±14.7</td>
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Table 2: Inclusion and exclusion criteria

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<th>Inclusion criteria</th>
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<tr>
<td>• Male or female</td>
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<tr>
<td>• 25 years of age or older</td>
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<td>• Three or more periodontal pockets with a probing depth (PD) &gt; 5 mm</td>
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<td>• Have at least 16 natural teeth excluding third molars</td>
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<td>• Provide informed consent and willingness to cooperate with the study protocol</td>
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<th>Exclusion Criteria</th>
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<tr>
<td>• History of antibiotic use in the previous three months</td>
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<td>• Pregnant or lactating females</td>
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<td>• Treatment with antihypertensive, antilipemic, antiarrhythmic, and other cardiovascular drugs</td>
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<tr>
<td>• Systemic diseases such as diabetes, HIV/AIDS, liver disease, chronic renal failure, tuberculosis, and autoimmune diseases</td>
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<tr>
<td>• Previous history of cardiovascular disease: Acute myocardial infarct, stable angina, unstable angina, heart failure, atrial fibrillation, AV blockade, peripheral vascular disease, and cerebrovascular accident</td>
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<tr>
<td>• Patients who received periodontal treatment within the last 6 months</td>
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<tr>
<td>• Patients who require antibiotic prophylaxis before examination or treatment</td>
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<td>• Patients with some mental disability</td>
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Eligible subjects for the present study, control and GCP patients, were invited to the following visit for gingival mucosa samples collection. The protocol was approved by the Ethics Committee of University of Medicine and Pharmacy, Iuliu Hatieganu Cluj-Napoca. All patients gave informed consent to participate.

Collection of gingival tissues

Biopsy specimens of gingival mucosa were harvested from healthy subjects with normal mucosa (controls) and GCP patients. The biopsies were taken under local anesthesia (1% Lidocain).

Light and electron microscopy

Immediately after excision, all specimens were transferred to a 2.7% glutaraldehyde solution in phosphate buffered saline (PBS)0.1M, pH 7.2 for 90 min at 4°C for pre-fixation. Than they were washed in four successive baths with PBS 0.15M pH 7.2 for 4h at 4°C. Biopsies were post-fixed with 2 % osmic acid in PBS 0.15M pH 7.2 for 75 min at 4°C.
Dehydration was performed in a graded acetone series (50%, 70%, 80%, 90%, absolute) at room temperature, 30 min in each bath. The samples were infiltrated with Epon 812. Sections were cut on a Leica UC 6 ultramicrotome (DDK diamond wheel (Craciun and Horobin, 1989; Hayat, 2000). Sections of 500 nm thick were stained with Epoxy tissue stain for light microscopy (LM) and orientation purposes. For light microscopy were used an Olympus BX 51 microscope, a CCD Media Cibernetics camera, and Image Pro Plus software (Kuo, 2007; Kay, 1967).

Semi-thin sections of 20-40 nm were cut and stained with Epoxy Tissue Stain. For ultrastructural studies, sections was contrasted with uranyl acetate and lead citrate and examined on a JEOL JEM 1010 transmission electron microscope (Japan Electron Optical, Ltd., Tokyo, Japan). For TEM were used a Megaview III camera and Soft Imaging Analysis software (Pavelka and Roth, 2005; Ploaie and Petre, 1979).

**Results and discussions**

Several studies have described the normal gingival epithelium. It was subdivided into 3 sections, the oral epithelium, the sulcular epithelium, and the junctional epithelium. The oral epithelium extends from the mucogingival junction to the gingival margin. It is continuous with the sulcular epithelium that lines the lateral aspect of the gingival sulcus. The junctional epithelium forms the dento-epithelial junction apical to the sulcus. Its coronal end forms the bottom of the gingival sulcus and is overlapped by the sulcular epithelium. These epithelia differ from one another in their function and, therefore, in some of their histological characteristics.

**Histopathology of control subjects gingival mucosa**

Oral epithelium is a stratified, squamous keratinizing epithelium, that lines the vestibular and oral surfaces of the gingiva. It extends from the mucogingival junction to the gingival margin, except for the palatal surface where it blends with the palatal epithelium. It consists of a basal layer (stratum basale), a spinous layer (stratum spinosum), a granular layer (stratum granulosum) and a cornified layer (stratum corneum). It is designed primarily for protection against mechanical injury during mastication. Resistance to mechanical injury is mediated primarily by the numerous intercellular junctions, mostly desmosomes, that hold the cells tightly together and the cornified layer. The cornified layer and the relatively narrow intercellular spaces also contribute to the relative lack of permeability (Signat, 2002; Pavelka and Roth, 2005).

In the present study, LM examination of the control subject’s gingival mucosa outer surface showed that it is covered with a thick layer of keratinised stratified squamous epithelium, with finger like projections (RETE PEGS) into the dense collagen underneath (fig. 1). The strong attachment of collagen to epithelium between the RETE PEGS, causes the gingiva to have a stippled surface when it is normal and not inflamed.

Age and sex variations in connective tissue papillary density have been described. The connective tissue papillae have been regarded by several authors as adaptive structures which enlarge the epithelial-connective tissue interface in order to achieve a broader anchorage for the epithelium and to provide a larger exchange surface for nutritional purposes. It has been shown to possess a specific inductive influence on the differentiation of the contiguous epithelium (Signat, 2002; Bloom and Fawcett, 1975)

At TEM, the cells from the keratinised outer gingival surface of SC have lost their nuclei and cytoplasmic organelles and have accumulated keratin granules. Surface epithelial cell contains lipids and keratino-hyaline deposits, and are held to subsurface layer by desmosomes with no tight or intermediate junctions surviving (fig. 2, 3) (Andres J., 1977; Nita, 1992).

Cells are about to be desquamated. Subsurface epithelial cells have no nucleus,
no functioning cytoplasmic organelles, and periphery of cells still joined to adjacent cells by desmosomes, tight junctions and intermediate junctions (fig. 3).

Within the granular layer cells are flattened compared to those from the spinous layer, but they are still bound by desmosomes (fig. 4). This structure ensures the barrier function (Andres, 1977; Nita, 1992). The spinous layer is about 50-60% from the gingival epithelium. It contains polygonal or hexagonal cells bound by many desmosomes (fig. 5).

Another type of cell found in normal gingiva of the control subjects is Langerhans cell. These cells have dendritic extensions and are intercalated in the spinous layer between epithelial cells. They function as antigen processing cells and are modified macrophages (fig. 6).

The basal layer is attached to the basement membrane by hemidesmosomes, binding the epithelium to the connective tissue. Basal cells are columnar. As previously mentioned, they contain widely dispersed tonofilaments, extended to both hemidesmosomes and to the relatively few desmosomes found on the lateral and superior aspects of the cell (fig. 7) (Andres, 1977; Nita, 1992).

The basal cells have nucleus with one nucleoli, and are in different stages of cell division (fig. 8). They proliferate and pass through the various layers until they reach the surface and have become inactive and are desquamated off as demosomes fail to hold the cells together. Cell turnover is 10 to 12 days (Andres, 1977; Nita, 1992). Electron micrograph found Merkel cell in the basal layers of epithelium (Fig 9). They function as tactile proprioceptive cells and are connected to nerve fibers (Toader, 1996; Usineviciu, 1981).

The epithelial basal cell membrane facing the lamina propria is connected to it by a basal lamina, which consists of an electron-dense layer, the lamina densa and an electron-lucent layer, the lamina lucida. The lamina densa is composed of an afibrillar type of collagen, type IV collagen. The lamina lucida is composed of laminin and other glycoproteins (fig. 10) (Scalaletta and Maccallum, 1972; Hashimoto, 1972).

The connective tissue of gingiva is made up of mainly type I and III collagen bundles with some type IV fibers between the bundles and in the basement membrane. There are a few elastic fibers. The ground substance is mainly hyaluronic acid, chondroitin sulfate, as well as fibronectin a glycoprotein. There are also blood vessels which have terminal loops extending between the RETE PEGS. Healthy gingiva contains many fibroblasts and a few inflammatory cells such as macrophages, neutrophils, plasma cells and lymphocytes (fig. 10) (Scalaletta and Maccallum, 1972; Hashimoto, 1972).

![Figure 1: Gingival epithelia of healthy subject](image1)

![Figure 2: Cells from the keratinised outer gingival surface of healthy subject](image2)
Figure 3: Healthy subject subsurface epithelial cells have no nucleus, no cytoplasmic organelles, and periphery of cells still joined to adjacent cells by desmosomes.

Figure 4: Healthy subject granular layer with flattened cells, bound by desmosomes.

Figure 5: Healthy subject spinous layer with polygonal/hexagonal cells, bound by many desmosomes.

Figure 6: Healthy subject spinous layer with an intercalated Langerhans cell.

Figure 7: Healthy subject basal layer cells attached to the basement membrane by hemidesmosomes.

Figure 8: Healthy subject basal layer cells attached to the basement membrane.
Histopathology of GCP subjects gingival mucosa

LM examination of the GCP subjects gingival mucosa showed that epithelium is thin or destroyed. The structure of the connective tissue is completely disorganized by fragmented fibers, epithelial cells migration and leukocytes infiltration (fig. 11) (Bloom and Fawcett, 1975; Hillmann, 1998). TEM of gingival mucosa from GCP patients indicated that in contrast to the healthy periodontium, it exhibited conspicuous morphological differences. Stratum corneum is thin, with flat cells. Were it is detached, desmosomes are destroyed and lower layers degeneration was associated (fig. 12, 13).

Some bacteria were localized outside the epithelium and inside intercellular spaces (fig. 14).

In the spinous and granulous layer cells of GCP subjects are polymorph and many desmosomes were destroyed. Seriously damaged cells showed an abundance of large and often-disrupted intracytoplasmic vesicles. Bundles of filaments of intermediate size were present within the cytoplasm of the damaged cells (fig. 15, 16).

In the basal layer desmosomal connections between adjacent cells are enlarged. Degeneration affected individual cells while leaving the adjacent cells intact (fig. 16).

Where the basal lamina had lost its continuity and basal cells were destroyed, epithelial cells migrate into the connective tissue increasing connective tissue cell population (fig. 17,18). The effect of inflammation on the distribution of collagen types I, III, IV, V, and VI in the human gingiva was studied after staining them with antibodies to these proteins. The staining was sparse in areas of inflammation and leukocytic infiltration, because Collagen type I and III were almost entirely destroyed at sites of inflammation. At the GCP patients collagen filaments bundles were fragmentated or lysed and leukocytic infiltrate abundant (fig. 17, 18) (Bloom and Fawcett, 1975; Hillmann, 1998; Ghadily, 1978).

Conclusions

In GCP subjects bacterial infection is associated with important gingival mucosa lesions, that involve all the layer of the epithelium and connective tissue.

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Figure 11: GCP with thin or destroyed epithelium, fragmented fibers, epithelial cells migration and leukocytes infiltration in the connective tissue.

Figure 12: GCP thin stratum corneum, flat cells, lower layers degeneration.

Figure 13: GCP partially destroyed desmosomes.

Figure 14: GCP bacteria localized outside the epithelium and inside intercellular spaces.

Figure 15: Spinous and granulous layer cells of GCP subjects are polymorph, intercellular spaces are enlarged and many desmosomes were destroyed.

Figure 16: GCP basal layer desmosomal connections between adjacent cells are enlarged.
Figure 17: In the chorion of the GCP patients collagen filaments bundles were fragmentated or lysed.

Figure 18: At the GCP are abundant leukocytic infiltrate in the chorion.

References


