The histopathological study of radicular dentinal changes in patients with chronic marginal periodontopathies

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Abstract
Periodontal disease is one of the most frequent conditions in individuals, having major health and social implications. Progressing as a chronic inflammation at the level of tooth support tissues, untreated chronic periodontitis may lead to a premature loss of the dental organ. Even though the main lesions caused by the periodontal disease affect the alveolo-dental ligaments, we tried to highlight the presence of certain lesions of radicular dentine in patients with chronic marginal periodontopathy. In our study, in the patients with chronic marginal periodontopathy, at periodontium level, there was highlighted the presence of a chronic inflammatory process with numerous lymphocytes and macrophages, microhemorrhages, and also areas with tissular necrosis. At radicular dentine level, in the cases of old, untreated periodontal disease, there were observed areas of more or less extended demineralization, erosion and necrosis. Our study showed that chronic periodontal disease may affect the tooth, as well, not only its support tissues.

Keywords: chronic periodontitis, inflammatory disease, periodontal tissues, dentinal lesions.

Introduction
Periodontal disease is an inflammatory disease mainly determined by the bacteria present in the biofilm of the dental plaque, affecting the tooth support tissues, thus leading to the destruction of alveolo-dental ligaments and alveolar bone [1–4]. Periodontal diseases (gingivitis and periodontitis) have quite a high prevalence, thus affecting up to 90% of the population all over the world [2]. A clinico-statistical study performed between 2009 and 2010 in the USA, including adults over the age of 30 years, showed that over 47% of adults had periodontitis, estimating that about 64.7 million individuals are affected by this disease at a national level [5]. Assuming that this incidence of periodontitis represents an average of the disease world widely, there was estimated that in Europe there exist about 400 million individuals suffering from various forms of periodontitis [6]. Due to a high prevalence and health and social implications, periodontal disease is considered a major problem of public healthcare.

Numerous studies showed that periodontal diseases are chronic conditions with an uncertain progress and a poor prognosis, determining a significant alteration of life quality, by tooth decay and preservation of halitosis at oral cavity level [7, 8].

The periodontal disease etiology is quite a complex one. The main factor is represented by the presence of pathogenic bacteria in the dental biofilm. There is estimated that, at oral cavity level, including at dental biofilm level, there exist over 700 bacterial species, but not all are involved in the etiopathogeny of periodontal disease [9]. Most of the pathogenic agents identified in periodontal disease are Gram-negative and anaerobic ones, having a synergic, simultaneous and complementary action. The most known bacterial species are represented by Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum, Prevotella intermedia, Prevotella nigrescens, Campylobacter rectus, Eikenella corrodens and Parvimonas micra [10–12]. Besides the bacterial infection, other factors are also involved in the onset and progress of the periodontal disease, such as: genetic factors, environment factors, toxic substances intake (alcohol, tobacco), immunological factors, coexistence of other chronic conditions (diabetes mellitus, obesity, chronic lung diseases, heart diseases), etc.

In the present study, we proposed to evaluate the radicular dentinal changes in patients with chronic marginal periodontopathies.

Materials and Methods
In our study, we included a number of 53 patients, aged between 44 and 76 years, who came to the Dentistry Consultory, complaining of local pain or pain in the trigeminal nerve trajectory, halitosis, dental mobility, mastication or physiognomy problems. The clinical and
radiological examination established the diagnosis of chronic marginal periodontopathy associated with irreversible periodontal lesions by conservative methods, thus requiring dental extractions, followed by alveolar curettage. After obtaining the informed consent from every patient, there were performed the dental extractions, which also allowed the harvesting of some small fragments of marginal periodontium. The extracted teeth and the periodontium fragments were immediately fixed in 10% neutral formalin solution for the performance of optical and immunohistochemical studies. The fixation process of the periodontium fragments lasted for 24 hours, at room temperature, while the teeth were left in the fixation solution for seven days. After fixation, the periodontium fragments were included in histological paraffin, while the teeth were subjected to a decalcification process in a 10% trichloroacetic acid solution for 14 days. For a rapid and homogenous decalcification, the decalcification solution was changed daily, and the bowel with the teeth and decalcification solution was placed on a magnetic stirring machine. After performing the decalcification, the teeth were longitudinally sectioned, washed in tap water for 24 hours and subsequently included in paraffin.

The sectioning of odontium and periodontium fragments was performed in the Microm HM350 rotary microtome, thus obtaining 4 μm-thick sections, stained with Hematoxylin–Eosin (HE) and Goldner–Szekely (GS) trichromic.

For the immunohistochemical study, the histological sections were collected on poly-L-Lysine covered blades for improving the biological material adherence to the histological blade. After blade drying in a thermostat at 37°C, there followed the deparaffinization, hydration and washing of the sections. The blocking of the endogenous peroxidase activity was performed by placing the histological sections in 3% oxygenated water 30 minutes at room temperature, followed by the washing in 1% phosphate-buffered solution (PBS – three baths of 5 minutes each). There was applied Streptavidin-HRP in 1% PBS for 3×5 minutes. The signal was detected using a 3,3’-Diaminobenzidine (DAB) (Dako), and the contrasting was performed with Mayer’s Hematoxylin. In our study, we used the following immunohistochemical markers: CD3 (L26 clone, Dako, 1:100 dilution) for T-lymphocyte highlighting; CD20 (L26 clone, Dako, 1:100 dilution) for B-lymphocyte highlighting; CD68 (KP1 clone, Dako, 1:200 dilution) for macrophage highlighting.

Results

The medical history taken in the dentistry consultatory highlighted that all the patients presenting with chronic marginal periodontopathy had signs and symptoms that have insidiously progressed, for several years, leading to a delay of a stomatological examination in some cases. Also, the discussions with the patients allowed the identification of several risk or favoring factors. Among the most frequent risk factors there were: gingivitis, present in 36 (68%) patients, diabetes mellitus, identified in nine (17%) patients, chronic cardiovascular diseases, present in 35 (66%) patients, chronic liver and kidney diseases, present in 16 (30%) patients. Among the most frequent favoring factors, we had: poor oral hygiene, found in 27 (51%) patients, chronic cigarettes smoking, practiced by 17 (32%) patients, and alcohol intake, assumed by eight (15%) patients.

The clinical examination highlighted the presence of gingival bleeding, gingival edema or gingival hypertrophies, the presence of 4–9 mm periodontal pockets, dental calculus, different grades of marginal recession, with excessive dental mobility and even dental migrations, as well. The radiological examination highlighted the enlargement of the periodontal area, often associated with more or less extended bone destructions (vertical and horizontal type), and even fractures of the dental roots. We need to mention that the odontal and periodontal changes we observed in the patients with chronic marginal periodontopathies were quite variable, according to the severity of the periodontal disease and the patient’s age. We believe that risk factors, such as poor oral hygiene, alcoholic or fizzy drinks intake, smoking, the presence of certain chronic diseases, marked this variability of the dento-periodontal lesions.

The histological and immunohistochemical study highlighted, at the periodontium level, the presence of a chronic inflammatory infiltrate, made up of lymphocytes, plasma cells, macrophages and rare granulocytes (Figure 1). Sometimes, in the marginal periodontium there were identified areas of edema and microhemorrhages (Figure 2) and even more or less extended areas of tissue necrosis (Figure 3). According to our observations, the periodontal tissue necrosis appeared more frequently in the deep side of the periodontal bags that exceeded 5 mm.

The immunohistochemistry techniques selectively highlighted the presence of macrophages (Figure 4), T-lymphocytes (Figure 5) and B-lymphocytes (Figure 6) within the periodontal conjunctive tissue. The immune system cells appeared heterogeneously arranged in the periodontal inflammatory infiltrate, sometimes disorganizing the alveolo-dental ligaments. In our study, the most numerous were the macrophage and T-lymphocyte cells. The number of B-lymphocytes was quite more reduced in comparison to the T-lymphocytes and macrophages.

The changes of the radicular dentine in patients with chronic periodontal disease varied quite a lot. This high variability may be due to the length of the disease, to the aggressiveness of the oral cavity microbial flora, the associated general diseases or the response reaction of every patient. Also, in the same tooth, the periodontal and dentinal changes were not identical on all radicular sides. In some patients, especially towards the apical area, the dentine did not present any microscopic changes (Figure 7), as the periodontal lesional processes had not reached that area. The invasion of the inflammatory process at the periodontium level and formation of periodontal bags resulted in the progressive degradation of the periodontium, with the emergence of a necrotic material in the periodontal area, the cement demineralization,
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alteration of its structure and extension of the lesional processes at radicular dentine level (Figure 8). The radicular dentine, in an incipient stage, presented some more or less extended demineralization areas, characterized by the color reduction of these areas.

In some patients, at radicular dentine level, there were observed erosion areas, from 100 μm up to 2–3 mm. In these areas, the periodontium, cement and part of the dentine were changed into necrotic material (Figures 9–11).

Figure 1 – Microscopic image of the marginal periodontium intensely infiltrated by lymphocytes, plasma cells and macrophages. HE staining, ×200.

Figure 2 – Marginal periodontium with necrosis areas and hemorrhage. HE staining, ×200.

Figure 3 – Extended area of periodontal necrosis. HE staining, ×100.

Figure 4 – Periodontium with an abundant inflammatory infiltrate, mainly made up of macrophages. Anti-CD68 antibodies immunostaining, ×200.

Figure 5 – T-lymphocytes present in a relatively high number in the periodontal inflammatory infiltrate. Anti-CD3 antibodies immunostaining, ×200.

Figure 6 – B-lymphocytes heterogeneously distributed within the periodontal inflammatory infiltrate. Anti-CD20 antibodies immunostaining, ×200.
The microscopic aspects observed in the patients with chronic periodontal disease lead us to the conclusion that the dentinal erosion process took place during more stages. In the first stage, there took place the dentine demineralization, followed by the degradation of the proteic component and its turning into a necrotic, unstructured material. In the dentin erosion areas, in the dentinal canaliculi there may gather bacteria, changing the dentine into a collector of microorganisms.
Periodontal disease is one of the most frequent diseases found in adults, characterized by inflammatory lesions of the gums, periodontal ligaments and alveolar bone [13, 14]. According to some authors, chronic marginal periodontopathies start with the disturbance of the gum homeostasis, caused by the bacterial plaque. Once the gingival inflammatory process starts, it may lead to periodontitis [15–17]. In the pathogenesis of periodontal disease, there intervene multiple types of bacteria present in the dental biofilm, but recent studies indicate that the onset and progress of periodontitis is conditioned by the presence of a microbial synergy and certain dysbiosis in the oral cavity flora [18–20]. The dental plaque bacteria may damage the gingival epithelium, the alveolo-dental ligaments, the alveolar bone in a direct manner or through some toxic, acid products, or proteolytic enzymes released in the periodontal space. The conflict between the bacterial flora and the periodontium results in the emergence of an inflammatory process.

In chronic periodontopathies, the host inflammatory response to the microbial aggression is an essential one and it is partially responsible for the periodontium damaging [13].

In our study, in the periodontium there highlighted an inflammatory infiltrate, richer or poorer in cells, made up of lymphocytes, plasma cells, macrophages and rare granulocytes. According to some authors [21], neutrophils, as well as the cells of the inborn immune response, are the first one to appear in the periodontal conjunctive tissue, because of the bacterial flora aggression. They may neutralize the bacterial flora action but, in certain circumstances, they contribute to the periodontal disease pathogenesis, through the excess release of proteolytic enzymes (matrix-metalloproteinases), which damage the conjunctive matrix, or through the release of oxygen reactive species [22, 24]. Numerous studies showed that the physical and biochemical properties of the extracellular matrix are essential for the differentiation and proliferation of local cells in the process of periodontium regeneration and reconstruction [24, 25].

In our study, the number of neutrophils was a reduced one, in comparison to the number of lymphocytes and macrophages, which explains the chronic characteristic of the disease. Of the lymphocytes, the most abundant ones were the T-lymphocytes. These cells play essential parts in the development of the inflammatory process, as they regulate the participation of other cells of the immune system, through various cytokines. Part of the mediators produced by the lymphocytes, or by the macrophages, maintain the local inflammatory process, with damaging effects [26]. Recent studies showed that the subset of T17 helper lymphocytes (Th17) is involved in the alveolar bone damaging in periodontal disease [27]. Other studies showed that the Th1-cells prevail in stable periodontal lesions (when there is a balance between the host and the microbial flora), while the Th2-cells predominate in the progressive lesions of periodontitis [28, 29]. The presence of B-lymphocytes and plasma cells, in the periodontal inflammatory foci, suggests the involvement of the humoral immune response in the etiopathogeny of the periodontal disease. At present, there is not exactly known whether B-lymphocytes and plasma cells are involved in the periodontium damaging process.

In our study, the presence of certain erosive lesions, at radicular dentine level, in the patients with chronic periodontal disease, indicates either a prolonged disease progress or an important flora aggression or a low immune response of the patient. The radicular dentine erosions appeared, in the presented cases, after the necrotic alteration of the cementum. Some studies showed that the cementum undergoes numerous physical and chemical changes during the periodontal disease pathogenesis [25].

In the first stage of the periodontal disease, the acellular cementum is irreversibly altered, but as the periodontal disease progresses, all the cementum and periodontium components are altered, which, from a physiological point of view, indicates the alteration of the periodontium mechanic and elastic properties [25].

Dentinal alterations may be complex and appear in a multitude of diseases. The dentinal alteration cannot take place as long as the dentine is protected by the odontoblasts and the predentine [30]. We believe that the erosions emerged at radicular dentine level are the result of the bacterial flora aggression, which has the ability to form an acid environment that dissolves the dentine mineral component, followed by the destruction of the organic component. The persistence of bacteria at this level makes that periodontal diseases become real infection foci that generate or complicate the systemic diseases. From the infectious periodontal focus, numerous bacteria may enter the blood, and from here they may colonize vital organs, including the brain, kidney, bone tissue or cardiac valves. Due to these reasons, we consider that the treatment of periodontal disease should be performed thoroughly and with a maximum care.

Conclusions

Periodontal disease may cause erosions at radicular dentine level, after the dento-alveolar ligaments and cementum damaging. The radicular dentine lesions are quite variable, according to the age of the disease, the intensity of periodontal lesions and the immune system reaction. The dentine necrosis and dentinal canaliculi alteration may change this histological structure through the bacteria persistence, at this level, into an infection focus from where there will be colonized vital tissues and organs.

Conflict of interests
The authors declare that they have no conflict of interests.

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