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ABSTRACT

PHD THESIS

DENTINAL CHANGES IN CHRONIC MARGINAL PERIODONTOPATHIES

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INTRODUCTION

Chronic periodontopathies are bacterial infections affecting the periodontium, resulting in the apparition of an inflammatory process progressively damaging the mechanisms supporting and fixing teeth onto the alveoli, with the irreversible alteration of their physiological support. If not treated, the disease develops progressively, being the main cause of teeth loss (Novak MJ 2006). The progression of the disease is slow and its severity is measured based on the extent of destruction of the periodontal tissue. Periodontopathies are frequently associated to local inflammatory damage, bacteremia and a more or less manifest immunity response, depending on the individual reactivity of each patient.

The disease may appear in any person, irrespective of age, gender or race. A recent study of the National Health Institute of the US shows that 47% of the male population and 37% of the female population between 18 and 64 years old present a form of periodontopathy. In Romania, most adults over 40 years old present a form of periodontopathy.

According to Berezow AB et al (2011), periodontitis seems to have multiple ethiologies, of which the microbial and immunological ones are the most researched. Microbiology studies have shown that more than 700 species of bacteria exist in the oral cavity, of which more than half have not been cultivated (Aas JA et al 2005). Multiple research has shown that dietary changes, in combination with a flawed oral hygiene may cause changes in the composition of oral microflora (Al-Ahmad et al 2009). These dysbioses of the oral cavity may result in periodontopathy. A traditional hypothesis states that periodontitis develops with oral microbiosis passes from a mainly aerobic gram-positive germ format to a mainly anaerobic gram-negative germ format (Marsh PD 1994). Moreover, a significant reduction in the local immune response has been witnessed in patients with periodontopathy (Darveau RP 2009).

CHAPTER I
Histology and Physiology of the Dentine

The dentine is a mineralised, acellular, avascular conjunctive tissue, which forms the main structure of the tooth, surrounds the dental pulp at the level of the crown and root and has a similar composition to bones (Hubbard JM, Kon JC 2002). Of the three hard, calcified parts of a tooth, the dentine is the structure that is formed first, during the development and calcification of the tooth, due to the activity of odontoblasts (Craitoiu St 1999).

The cells responsible for the formation of dentine are odontoblasts. These cells are
organised in a single layer, in the palisade. They are highly polarised and differentiated, located at the interface between dental pulp and mineralised dentinal tubes (DT). The odontoblast processes (OP) begin at the distal end of cell bodies and then extend to dentinal tubes. In orthodentine, the most prominent morphological feature is represented by dentinal tubes (DT). The DT are extended from the dentino-enamel junction (DEJ) up to the mineralisation front in the pulp chamber, presenting numerous branches. The most narrow and branched part is found next to the DEJ, whereas the widest part is towards the dental pulp. The number, dimension (length, diameter), density and branches of the DT depend on their location within the tooth (internal, central or external part), on the crown or radicular dentine and the individual’s age (Nita M. 1992).

Depending on the relationship with dentinal tubes (DT), the dentine may be divided into peritubular dentine (PTD) and intertubular dentine (ITD), each of which have their own compositional and structural characteristics. PTD is the dentine surrounding the DT at the level of the dental crown. It is mainly made up of carbonated apatite crystals and only a small amount of collagen (Weiner S et al. 1999). ITD is the remaining dentine, found around and between dentinal tubes and occupying the space between two neighbouring dentinal units (Craitoiu Stefania et al. 1999); it contains larger amounts of collagen and less apatite crystals, which are closely connected to the collagen matrix and represent the largest volume of the dentine.

In topographic and structural terms, the dentine includes peripheral dentine (mantle dentine) and circumpulpal dentine (orthodentine). The mantle dentine (MD) is located immediately below the enamel, at the level of the DEJ; it has a stitched appearance, with cavities oriented towards the enamel and tends to become straight as it approaches the dental flange. It is 80-100 microns thick, is atubular, which shows that it is formed prior to the OBL differentiation and it presents an organic matrix of collagen fibres.

The circumpulal dentine (CPD) is the part of dentine found between dentine covering and predentine. Its main property is the presence of dentinal tubes (DT), which cross it from the DEJ to the dental pulp (Craitoiu St. 1999). The DT surround the cytoplasmic extensions of the OBL, which are elongated, as the cell body withdraws during dentinogenesis, pursuant to the successive apposition of dentine layers.

Due to its cellular component, odontoblasts, dentine is a living tissue, undergoing permanent regeneration. It changes with age, functional stress (chewing pressure) and pathological factors (dental decays). Compared to enamel, dentine regenerates permanently, as long as the living pulp is present. Dentine regeneration occurs as an apposition or a resorption process.
CHAPTER II
Dentinogenesis

The dental and maxillary system is formed from the first embrionary branchial arch, of which the maxillary, the mandible and adult teeth are developed. The development of these structures is based on signalling interactions between the stomodeal ectoderm and ectomesenchymal cells derived from the cranial neural crest (CNC) populating this region. Teeth are formed both from the ectoderm and the ectomesenchyme (Couborne MT, Sharpe PT 2003), by means of mutual interactions between the oral epithelium and the mesenchyme of the first branchial arch; the oral epithelium results in ameloblasts (enamel-forming cells) and mesenchymal cells differentiate into odontoblasts (secreting dentine) (Zhang Y et al 2003) and cementoblasts (secreting cement) (Sharpe PT 2001).

The odontogenesis initiated by these interactions starts with the migration of the cranial neural crest (CNC) cells (Couborne MT 1999; Linde AG et al 2002), shown by a thicker stomodeal epithelium which forms the oral epithelial band. Two processes arise from the free edge thereof, which get joined in the basic mesenchyme: an external one – the vestibular lamina, which will form the oral vestibule and an internal one – the dental lamina (DL), which will result in the teeth buds. The localised condensations of CNC-derived mesenchymal cells will form the dental papilla (DPa). The formation of the DPa begins with the transition of the dental germ from the bud stage to the cap stage and it is adjusted by epithelial signals from the primary enamel nucleus (EK) (Thesleff I, Keranen S 2001). Mesenchymal cells are condensed at the periphery, around the enamel organ and form the dental follicle. These dental tissues make up the dental germ (Couborne MT 1999). In the bell stage, the tooth is made up of the enamel epithelial organ (EEO) and the dental papilla (DPa). The enamel epithelial organ includes: proliferating preameloblasts and their successors, the cells in the inner enamel epithelium (IEE), the secondary enamel knot at the peak of current apices, the stellate reticulum (SR), the external dental epithelium (EDE) and the stratum internum (SI).

Odontoblasts (OBL) are cells of the pulp-dentine complex, derived from CNC cells, which synthesise and secrete all dentine compounds.
CHAPTER III
Study Objectives

In order to assess the histological changes arisen in the chronic marginal periodontopathies at the level of the odontium and the periodontium, the following objectives have been established:

- Assessing the microscopic changes of dentine;
- Evaluating the inflammatory changes in the superficial and marginal periodontium;
- Assessing the immunology, histology and chemistry of the population of lymphocytes and macrophages participating in the local immune response;
- Assessing the immunology, histology and chemistry of the vascular response;
- Assessing the matrix-metalloproteinase-8 (collagenase) reaction in the periodontal tissue;
- Assessing the changes in the dental pulp of patients with chronic periodontopathies.

CHAPTER IV
Histological Study of Dentinal Changes in Patients with Marginal Chronic Periodontopathies

The study dealt with fragments of odontium and periodontium collected from 67 patients ranging from 46 to 75 years old, who attended the dental medicine practice for pain, fetid breath, dental mobility, chewing disorders or physiognomic disorders. Of the 67 patients, 42 were of male gender and 25 of female gender. The clinical examination showed the presence of gum bleeding, gum oedema, periodontal pockets, dental mobility, gingival recession and even dental migration. Of the facilitating factors of periodontopathy, we have remarked: smoking in 39 patients (58%), diabetes mellitus in 12 patients (18%), bad tooth hygiene in 47 patients (71%), recurrent gingivitis in 33 patients (49%). All patients needed dental extraction, followed by alveolar curettage.

The collected biological material was fixed in a 10% solution of neutral formalin. The fixation time was 24 hours for fragments of periodontium and 7 days for teeth. Teeth were decalcified with trichloroacetic acid in an increasing concentration of 5% and 10% for 30 days. The biological material, i.e. the fragment of decalcified tooth, was processed according to the
classical histology technique, for paraffin inclusion. The histological cups were coloured with haematoxylin, eosin and trichromic with light green (the Goldner-Szeckeli technique).

The dentinal changes observed in patients with periodontopathies were present both in the crown dentine and the radicular dentine. Moreover, microscopic changes varied according to the gravity of the periodontopathy, the patient’s age and the associated risk factors (faulty hygiene, teeth plaque, consumption of alcoholic or acidulated beverages, smoking, etc).

The most frequent changes found in the histological examination under usual colouring were dentinal erosions and demineralisation. The microscopic appearance of dentinal demineralisation was found by the reduction in the tinctorial affinity for certain acid colouring substances, especially eosin. Most often, dentine had a slightly inhomogeneous acidophilic appearance. Using stronger microscopic objectives, we found a widening of dentinal canallicles, a clear proof of demineralisation, mainly of the peritubular dentine and partially of the intertubular dentine. If dentinal canallicles normally have a diameter of 1-1.3 microns, our study found an increase in their diameter, by more than 100% in some areas of the dentine.

Most often, the mechanism in charge with dentinal damage is the chemical mechanism, as it generates organic acids resulting in the demineralisation of the hard structures of the tooth. We consider that the microorganisms or cells of the immune system appearing at the level of the inflamed periodontium produce a multitude of enzymes (matrix-metalloproteinase) able to destroy organic matter, especially the collagen fibres in the dentinal structure. Thus, widened dentinal tubes and numerous missing images are found in dentine, not only because of the resorption of the mineral component, but also because of the destruction of the organic component.

The morphometric analysis of the average diameters of dentinal tubes in the three areas of the radicular dentine has proven that dentinal tubes have a significantly widened diameter in patients with periodontopathy, in comparison to known data from medical literature. Thus, their diameter was significantly widened and different among the three dentinal regions: $2.32 \pm 0.52 \mu$ for external dentine; $1.57 \pm 0.39 \mu$ for the medium dentine and $1.74 \pm 0.58 \mu$ for the inner dentine layer.

As for the relative areas occupied by canallicles and alveolar cavities, compared to the dentine mass, they were quite large and significantly different between the inner dentine layer ($20.89 \pm 7.41\%$), the medium dentine ($11.1 \pm 6.89\%$) and the external layer ($11.42 \pm 8.29\%$). Our morphometric data has shown that dentinal demineralisation and erosion has reached alarming levels (more than 25%) in some areas, which shows that dentinal resistance may be highly reduced through the apparition of microscopic erosion vacuoles in the dentinal structure.
CHAPTER V

The Histological, Immunological and Chemical Study of Periodontal Lesions

For the investigated batch of patients, histological changes were found both in the gum epithelium and the corion. Histological changes were extremely different from one patient to another and even for the same patient, which is why we consider that the etiology of periodontopathy is multifactorial and, besides the virulence of pathogen germs, the development of this disorder is also due to the entire host body, through the modelling of the immune response or through associated co-morbidities.

At the level of the covering epithelium, erosions and even necrotic areas were most frequently found. The necrosis of the junctional and perijunctional epithelium was also accompanied by the destruction of the basic membrane of the epithelium, so that the subjacent conjunctive tissue came into direct contact with the oral environment.

The presence of numerous lymphocytes, plasmocytes and macrophages was identified in the periodontal conjunctive tissue. Neutrophiles were very seldom found. This local defence reaction aims at locating and isolating the tissular aggression, in order to protect the healthy neighbouring tissue, as well as neutralising and disabling the toxic substances generated by microbial or viral factors or even own humoral factors, as well as the cellular enzymes released pursuant to the aggression of the bacterial plaque.

The presence of numerous fibroblasts has been seen in the inflamating periodontium. Their function is the synthesis of all the matrix components of the conjunctive tissue, which restores the structural and functional integrity of the periodontium. Most of the times, the response of fibroblasts did not result in the restoration of the periodontium, but the apparition of a scar tissue.

The same appearance of chronic inflammatory infiltration, dissociating or damaging alveolo-dental ligaments, was also frequently found in the deep periodontium. Moreover, extended necrotic areas associated to micro-haemorrhages were found in the marginal periodontium.

Immunology, histology and chemistry studies confirmed the cellular and tissular changes in the periodontium and provided new insight on the inflammatory response and the angiogenesis process. Of the immune system cells, the most numerous were positive T CD3 lymphocytes and positive CD68 macrophages.

Matrix-metalloproteinase-8 (MMP-8) was found in numerous cells in the inflammatory
infiltration, located close to the basic membrane of the gum epithelium, where large amounts of IV collaged are found, which may be interesting for pathological processes in the periodontal disease. In the areas where cellular necrosis and a severe inflammatory reaction were observed in the periodontium, the intensity of the response to MMP-8 was extremely high. In our study, the immunological, histological and chemical reaction of MMP-8, also known as collagenase, as derived from neutrophile leucocytes and the cells of the macrophage system, was most often correlated to the intensity of periodontal lesions and the inflammatory process.

CHAPTER VI
Histological Study of Pulpal Changes in Patients with Marginal Chronic Periodontopathies

The dental pulp presented more or less extended changes of fibrose, associated to the presence of immune system cells. An increased amount of collagen fibres may be the expression of periodontal stimuli or even the presence of immune system cells, stimulating the proliferation of fibroblasts. Another particular aspect of the dental pulp seen in patient with moderate periodontopathies was the presence of deposits of mineral salts, mainly calcium, under the form of well delimited knot structures. The form and dimensions of those “dystrophic mineralisations” varied from one patient to another. Some calcium deposits were located at the pulp periphery, other to the center. The histological structure of these formations consisted of calcium salts disposed on non-fibre structures, probably non-collagen proteins and proteoglycans.

Vascular pulpal changes in patients with moderate periodontopathy mainly consisted of degenerative changes, such as arteriosclerosis. In patients with severe periodontopathy, where periodontal damage reached the apex, pulpal changes were of an inflammatory type, with numerous mononuclear round lymphocytes, plasmocytes and macrophages and, more seldom, granulocytes. Moreover, pulpal necrosis was found, with wide areas being occupied by cellular and fibre detritus, due to the inflammatory process.

The chronic inflammatory process present at the level of the dental pulp also resulted in the alteration of odontoblasts and circumpulpal dentine. Thus, we identified areas where odontoblasts were absent, pursuant to necrosis, and dentine acquired a stitched appearance, with multiple areas of parcelled demineralisation. There are currently few histological studies on the influence of periodontitis of various degrees on the pulpal tissue.
CHAPTER VII

Conclusions

The most frequent changes in the odontium were:

Destruction of the crown enamel or cement;

Dentinal demineralisation and erosion;

Widened dentinal canallicles, especially by the demineralisation of the peritubular dentine and partially of the intertubular dentine;

If demineralisation, necrosis and dentinal resorption were limited, in young people, to the contact area between the periodontal inflammatory process and dentine, dentine damage was diffused in older people (over 50 years), on much larger areas, expressed by demineralisation and erosions in the form of 5-17 micron vacuoles, present in all the dentine: the circumpulpal, medium and peripheral dentine.

The histological, immunological and chemical study of the periodontium showed extremely different changes from one patient to another and even for the same patient, which is why we consider that the ethiology of periodontopathy is multifactorial and, besides the virulence of pathogen germs in the oral cavity, the development of periodontopathy is also due to the entire host body, through the modelling of the immune response or through associated co-morbidities.