

**UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA
DOCTORAL SCHOOL**

DOCTORAL THESIS

**CLINICAL, HISTOLOGICAL AND IMMUNOHISTOCHEMICAL
STUDY OF PERIODONTAL AND DENTINAL CHANGES IN
PATIENTS WITH SEVERE PERIODONTITIS**

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Craiova – 2015

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KEYWORDS: chronic periodontitis, clinical evaluation, immunohistochemistry markers, dentinal changes.

CHAPTER I

TOOTH DEVELOPMENT

Dentition represents the dental units present in the alveoli maxillary in the mouth. There are two dentures, primary and secondary, the primary develops prenatal and consists of 20 temporary teeth that will be replaced after peeling them by permanent teeth (dentition secondary). Enamel develops from ectoderm, mesoderm pulp of (mesenchyme) and dentin and cementum are formed by direct connective tissue mineralization.

Tooth development is a complex process that takes place in several stages, involving epithelial-mesenchymal cell interaction between oral epithelium and subjacent mesenchyme. Interference occurring in developing teeth may occur during any stage of odontogenesis, they will have a direct physiological processes by affecting and will affect the necessary treatments for each patient.

Presentation of the dynamic development process includes the following milestones: initiation, proliferation, and histomorphodifferentiation and apposition. Title stages is based on morphology that becomes the germ dental epithelial structure.

The tooth development process includes, after crown formation, root formation and in parallel structure supporting tissues that anchor the tooth at mandibular achieved.

The sequence of the tooth in the development process is as follows:

- initiation - bud stage;
- proliferation - hooded stage;
- histomorphodiferențierea - bell stage;
- apposition - the finalization of the bell, the crown formation

Epithelialo-ecto mesenchymal complex interaction determines the structuring of ectomesenchyme. Studies have shown that dental ectomesenchimul and body contain all information necessary to create the entire tooth and its attachment device.

The existence of the epithelial-mesenchymal interactions determine initiate the development processes of the tooth. Experimentally it is established that the mesenchyme / ectomesenchyme in the state of a hood, the bell and the advanced bell stages, determines the type of tooth and tooth development in the epithelium and can induce nondentary, resulting in secretion of enamel.

Dentin and enamel is deposited in appositional manner, with a rhythmic deposition of extracellular matrix organic nature, which then matures by mineralization. The process is characterized by periods of activity and rest, alternating at precise intervals; by depositing enamel and dentin, the dental crown is formed. Tooth shape is dependent on when stopped this division mitotic cell transformation corresponding moment in ameloblaste and practical exercise of submission of enamel by them.

Root formation begins after completion of forming crown at the next junction and continue amelocementare to and after the eruption.

Submission of dentin is dependent on the formation of the odontoblasts and those that occur after induction process conducted by internal organ enamel epithelial cells. In this context, internal organ enamel epithelial cells are necessary to induce root formation.

In parallel with the formation and deposition of cement, epithelial cells epithelial root sheath outstanding Hertwig, grouped in small islands, continues to distance itself from the external surface of the root and settle in the future periodontal ligament form Malassez epithelial debris.

Termination of developing root sheath during training lasting jacket and produce a small gap. In this situation, the toothgenesis does not intervene at the level of the defect and forms a small channel between the accessory bag and dental pulp.

Ansa cervical epithelial root sheath behave transformation Hertwig and induces cell transformation odontoblaste dental papilla, which participates in the formation of root dentin. Hertwig sheath cell involvement in the formation of cementum is controversial.

These outstanding epithelial cells, grouped in small islands, continues to distance itself from the external surface of the root and settle in the future periodontal ligament form Malassez epithelial debris. Malassez epithelial remnants are more common in young people, but persists throughout life and have been highlighted in 70 years. Following inflammation, these residues may cause epithelial cystic formations or proliferate in places where osteitis phenomena occur in areas of bi- and trifurcation root, as in along the root. This creates conditions for inflammatory pathology, degenerative resorptive and even at these levels.

The main characteristics of the intrauterine period are the differentiation and specialization of the tissues. Birth is marked by important structural and functional changes characterized by growth and development up to the stage of maturity and adult organism.

In the first two weeks after birth - the neonatal period - there was a decrease in weight, important changes of heart and vessels that adapt to postnatal blood circulation and initiate functions of the digestive system which takes over the work of digestion and assimilation of food by new born. In humans, the tooth formation begins already in the 6th week of embryonic life. It continues until early adulthood, when three permanent molars roots reach completion. Development of these stages can be observed simultaneously during odontogenezei which runs from the embryonic stage into adolescence.

CHAPTER II

TOOTH BODY

The odontium (dental body) is a complex structured tissue differently but morphologically and functionally harmonized to receive and transmit masticatory pressures. It consists of two components: odontium (masticatory component) and periodontium (supporting part). Present inside the tooth pulp cavity containing the tooth pulp. It communicates in its lower portion through the apical foramen with periodontal space. Through this space it enters and leaves the pulp vessels and nerves.

Dental enamel is the outer layer of the tooth crown. Dentin is the hard dental tissue surrounding the dental pulp; it is covered on the outside of enamel at the crown and cement at the root. Cementum is dentin coating on the tooth root, the anatomical package to apex. It is fixing the dental periodontal fibers. The tooth is secured to the wall of the alveolar bone by a conjunctive structure called periodontium..

Enamel is the only calcified epithelial tissue, obtained by organic matrix mineralization synthesized and secreted by ameloblasts; it is made in a proportion of 96% of an inorganic component in the form of hydroxyapatite and an organic component represented by collagen, lipids, carbohydrates, citric acid and lactic acid, 1.3%. and 2.7% water..

Dentin is the hard tissue that develops from the pulp and papilla and is progressively mineralized; it is the most voluminous hard tooth tissue; mineral content is lower, 67% hydroxyapatite crystals being prevailed 200 times smaller; organic substances (20%) represented by collagen, lipids, mucopolysaccharides, glycoproteins, peptides, proteins, and water (13%). Non-collagenous proteins play an important role in the regulation of mineralization of dentine or pulp stem cells in inducing differentiation odontoblasts. Dentinal tubules contain cytoplasmic expansions of the odontoblasts and have a direction parallel to the long axis of the tooth dentin corresponding upper crown occlusal face, the side faces are oblique, and from the apex toward the package are arranged perpendicular to the axis of the tooth. Coronal dentin contains more ducts per unit area than the root. In the wearing out processes a tertiary dentin with irregular structure is quickly deposited, with rare ducts and stressed curled, cell inclusions (odontoblasts, fibroblasts, blood cells)

Dental pulp is a loose connective tissue, derived from mesenchymal papilla ; pulp chamber extends into the root canals and is open to the outside through a narrow apex of the apical aperture. Root canals often have caliber irregularities and trajectory or issue lateral channels. Histologically, the pulp is made from a tissue which keeps the morphological characteristics of the embryonic mesoderm, the teeth of younger there was a high cell density and fewer teeth adult cells and pronounced fibril component.

The fibers are of collagen, reticulin and Weill Korff area. Collagen fibers are the most abundant and are of type I. Collagen distribution is determined by the health pulp. Papilla cells derived from pulp cells are divided into 4 groups: basic cells: fibroblasts and fibroblasts; highly

specialized cells: odontoblasts; cell relay: Weil's round cells; cells involved in the processes of defense: macrophages, mast cells, plasma, lymphocytes, white blood cells..

Periodontal dental component is made up of all body tissues of maintaining and supporting the tooth anatomic, tissue located in an anatomical and physiological complete interdependence. All components are interdependent periodontal biologically. An assault on one of the dysfunctional consequences has consequences for other components.

Periodontal has two main components: the marginal periodontium and apical tissue.

Marginal periodontium is divided into: superficial periodontium consists of: gingival and periodontal ligament supra-alveolar and deep, consisting of: cement, desmodontium, alveolar bone.

Junctional epithelium is formed around the cervical portion of the tooth that follows the cemento junction. Junctional epithelium extends in the apical direction from the lower portion of the trench of the gum and forms a sleeve around the teeth.

Toothgingival junction is an adaptation of the oral mucosa comprising epithelium and connective tissue components; it is considered to be a stratified squamous epithelium incompletely developed, autonomous with a high turn-over. It is unique in the human body where soft tissue vulnerable in terms of mechanical strength, fit organically by a dense, hipermineralized structure. In time, this structure evolves differently, producing epithelial components attached, in continuous progression pattern and structures keratinized epithelium. The special pattern of the junctional epithelium reflects the the fact that the supporting connective tissue is functionally different from the adjacent epithelium sulcular, a difference with important implications for the progression of periodontal disease and periodontal regeneration junction after toothgingival surgery.

Cement is a tough connective tissue with mesenchymal origin, coming from the internal part of the dental follicle. It covers the tooth roots and allows attachment and attachment of main periodontal ligament fibers, is avascular and lacks innervation..

The report between the enamel and cementum delineates the enamel-cementary junction.. Cementum is mineralized at a rate of 45-47%. There is a repair cement that adheres well to the surface root if there is a phase that precedes the new matrix deposition.

Periodontal ligament is represented by the dense fibrous connective tissue that occupies the space between the tooth root and periodontal space. It is a unique tissue, multipurposely specialized, highly vascularized, with diversified cell content surrounding the tooth root which plugs it into the inner wall of the alveolar bone; it continues with the connective tissue of the gingiva and communicates with marrow spaces in the vascular bundles in the bone.

Alveolar bone is the bone of the jaw and mandible containing dental alveoli. Alveolar process performs morphological support of the tooth, alveolar formation process is performed concurrently with tooth eruption and tooth loss, its causes rezorbire. Alveolar bone and root cementum along desmodontiu root form dental appliance anchor and absorb masticatory forces applied to the crown.

CHAPTER III

PERIODONTOPATHIES

Approximately 700 different bacterial species can be detected in subgingival biofilm and the infection is caused, in many cases, by bacterial association with a predominance of gram-negative anaerobic bacteria that colonize periodontal pockets.

Pathogenic bacteria involved in periodontal disease outbreak have two features: invasiveness and toxigenicity (producing exo and endotoxins). Bacterial cells involved in periodontal pathogenicity mechanism by exotoxin, enzymes, metabolites; exotoxin and leukotoxin interfere toxic level PMN leukocytes destroy the gingival sulcus, causing periodontal tissue colonization and invasion. Inflammatory mediators causes gingival connective tissue destruction, periodontal ligament and alveolar bone. Surface modification coating loss occurs through the root cementoblast outside and resulting injuries shallow resorption of cementum. Endotoxins produced by bacteria have an irritating effect on soft tissue, impairing activation of redress mechanisms

Specific and nonspecific immunity, complement each other. Inflammation of the gingival level remains limited to the result of a balanced symbiosis between the biofilm and the host tissues and periodontitis development imbalance is the result of symbiosis, the modified environmental and genetic factors.

Saliva and gingival sulcus crevicular fluid continuously removes the bacterial products and the increased turnover of the gingival epithelial removes bacterially loaded superficial cells. Neutrophils participate in determining the pattern of periodontal health maintenance and disease severity is increased in all forms of neutropenia, agranulocytosis and altering the function of leukocyte adhesion.

Pro-inflammatory cytokines are produced during an inflammatory response and promotes its progression; interleukin-1 (IL-1), IL-6, IL-8 and tumor necrosis factor (TNF- α), IL-12, IL-18, interferon (IFN) and granulocyte macrophage.

Anti-inflammatory cytokines suppress the inflammatory process, by their ability to suppress the expression of genes for pro-inflammatory cytokines. They block the effects of inflammatory mediators -inflammatory cytokines IL-4, IL-10, IL-13, IL-16 and TGF. Activation of biochemical mechanisms of secretion of cytokines targets signaling mechanisms as a result of interfering between the pattern of molecular microbial associate (lipopolysaccharide, fimbriae) and pattern recognition receptors (Toll like receptors, NODlike receptors) that exist in cells in periodontal tissues and infiltrating leukocytes. Existing studies states the importance of many cytokines in the initiation and regulation of immune responses in periodontal disease, and tissue destruction (mainly through activation of matrix metallo proteinase MMP).

Saliva contains numerous antibacterial factors such as: lysozyme, lactoferrin, peroxidase system, but have a significant role in protection against periodontal diseases. Initially gingival liquid works by non-adherent bacteria diffusion plate and reduces bacterial metabolites by tissues to their dilution. Gingival fluid complement is activated by opsonized bacteria, their metabolic products and bacterial endotoxins released. Activated complement may cause lysis

or inhibition of a number of bacteria and interfere with bacterial phagocytosis by neutrophils and macrophages. Cell distinctions can participate in C3a and C5a components by causing the release of histamine from mast cells and other chemotactic factors and several cytokines in the periodontal tissue.

The development and progression of periodontal disease are caused by endogenous and exogenous factors.

Assessment, understanding and appropriate management of these factors facilitate or control disease prevention. A selection of risk factors of chronic periodontitis and aggressive patient's age, bleeding on probing, diabetes mellitus, impaired furcației history of periodontal surgery, the depth probing, the bone assessed radiographically restorations made under the gumline, tartar scaling polymorphisms uni -nucleotide, history of smoking, vertical bone lesions. Smoking has periodontal risk for injury and was confirmed in numerous studies. Genetic factors modulate how individuals interact with different environmental agencies, including biofilms and influence susceptibility to periodontitis. The interaction between genetic factors and environmental ones (not single genes), which determines the outcome. Thanks to this feature intrinsic factors related to lifestyle disease development permit. Periodontitis is often referred to as the sixth complication of diabetes. Several studies suggest that maintaining control periodontal disease may play a key role in controlling diabetes. There is a direct relationship between the degree of obesity and the risk of developing type 2 diabetes for both children and of adults For its. Obesity is a multisystem disease and contributes significantly to the development of hypertension, diabetes, arteriosclerosis and cardiovascular and cerebrovascular diseases. Outside of these risk factors, obesity has been suggested as a risk factor for periodontitis. Diet plays a modifier in the initiation and progression of periodontal disease; However, because of the many variables and predisposing factors for periodontal disease it is difficult to determine the individual effect of each nutrient periodontal.

CHAPTER IV

MIXED ENDO PERIODONTAL LESIONS

The interdependence between the endodontic and periodontal damage still develops much confusion, questions and controversy. Differentiating between periodontal and endodontic problems can be difficult.

American Association of Endodontics (AAE) uses in the dictionary of medical terms used in 1998 the term "endo-periodontal lesions" (AAE Glossary of Terms Endodontics / electronic edition).

American Association of Periodontology defines the endo-periodontal lesions as "areas of localized infection and periodontal tissues circumscribed origin and / or pulp."

Pulp and periodontal problems are responsible for more than 50% of the loss of dental units. An endo-periodontal lesion pathogenesis may be varied, ranging from simple to complex.

Although the relationship between dental pulp and periodontal topography is well documented, ways of spreading bacteria from the periodontal tissues and pulp are debatable and controversial.

Pulp and periodontium are closely interrelated, having an embryonic, anatomical and functional relationship. Periodontal dental pulp is interconnected with both the apical foramen to the lateral canals and accessories, these routes allowing the exchange of harmful agents between the two tissue compartments. Pulp inflammation causes toxic agents eliminated by the main channel, the apical foramen, side channels and accessory canals. While any endodontic lesion can develop periodontal ligament along with the ability to form fistulas, which is retrograde periodontitis. Pulp inflammation or pulp necrosis extending periapical tissues causes a local inflammatory response, accompanied by alterations in bone metabolism and sometimes root resorption.

Periodontal damage is a progressive character. It begins in the sulcus and migrates to the apex, it is characterized by deposits of plaque and tartar that cause or maintain inflammation; this inflammation gradually destroys the device supports and produces an infiltration of lateral canals, ditches or canals accessories exposed dentin. Maintaining vascular flow through the apical foramen allows activating defense mechanisms (calcification or degenerative changes, minor can be observed histologically). If the injury compromises the vascular supply to the periodontal apical foramen, pulp necrosis and periodontal deterioration is widening. The prognosis for such a tooth depends essentially on the nature of the lesion of periodontal. Although periodontal disease has been shown to have a negative cumulative effect on tooth's nerve, total disintegration of the tooth's nerve is installed only if the main apical foramen plaque involves and compromises the vascular supply.

There are views that the periodontal or endodontic treatment may not lead to a good prognosis and cure, and requires a bidirectional therapy. It is generally accepted that impairment could initiate or perpetuate pulp periodontal damage; inverse theory is controversial. Simring and Goldberg postulated that endodontic therapy is indicated for the treatment of periodontal disease that is not responsive to terminal periodontal therapy.

CAPITOLUL V

CLINICAL STUDY

The purpose of this study is to approach the controversy risen around the relationship between periodontal disease and the changes of periodontal and dentine tissues and also to evaluate the changes in severe periodontopathy, through the measurement of clinical and paraclinical status of the selected patients, affected by severe chronic periodontal disease. "Chronic" periodontal disease refers to the progression in time of the disease, with no treatment and it does not suggest that the disease is "untreatable". The severity is based on evaluating the loss degree of clinical attachment level (CAL) and/or the bone mass loss. The selected patients for the study were among the ones who had requested periodontal treatment in the private cabinet, during October 2011 and March 2015, having the symptomatology of periodontal medical condition: changes of shape, color or texture of the gingival tissue, edema, spontaneous or easily caused bleeding, pain and gingival sensibility, gingival itching, dental mobility, pockets of different depths, hyper-growths of different degrees, physiognomic changes.

The initial clinical evaluation has been made for 92 patients, amongst them, only 37 corresponded to the selection criteria for the study and have continued by signing the informed consent form. The clinical study was doubled by the histological and immunohistochemical studies of marginal periodontium and dentine for the extracted teeth.

In order to obtain structured results, the patients were separated into groups, organized by gender criteria (feminine/masculine), background (rural/urban) and another group was the one for smoker patients. The patients were clinically and radiologically evaluated and they were diagnosed with severe chronic periodontal disease.

The data obtained after the evaluation of the patients' antecedents has been closely monitored for the achievement of statistic studies. The following results were established: values obtained after evaluating the main/determining reason which sends the patient to periodontal treatment (table 1), values/graphic obtained after evaluating the specific behavior of treatment and the option for treatment for patients with periodontal medical condition (table 2), values/graphic obtained after evaluating the time interval necessary for treatment appointment since the appearance of symptomatology (table 3), values/graphic obtained after evaluating the preoccupation for dental hygiene for the masculine/feminine groups of patients (table 4).

Clinical and paraclinical evaluation of the patient proposes the identification of all anatomical aspects which suffered changes, for the purpose of establishing the diagnose, the treatment plan and the periodontal prognosis: *evaluating the bleeding value, measuring the probing depth, evaluating the recession, evaluating the furcations, evaluating the mobility, modifying the gingival structure/color, modifying the implanting spindle of the tooth, bony resorption vertical/horizontal/mixed type, number of missing teeth.*

After the examination and evaluation, the configuration of periodontal affectation was made (table/graphic), which allows the statistic analysis of patients included into the study to be made.

	Fem. Gender patients(20)	Masc. Gender patients (17)	Urban (29)	Rural (8)	Smokers (11)
Plaque index	1.76	2.44	1.99	2.35	2.33
Probing bleeding	2.8	3.1	2.9	3.06	3.02
Recession level	4.2	4.11	4.16	4.12	4.14
Furcation exposure (degree 4)/patient	2	1.41	1.93	1.14	1.1
Clinical attachment level	3.75	3.76	3.60	3.78	3.80
Alveolar bone loss $\geq 50\%$	4.2	4.5	4.3	4.6	4.7
Mobility degree 3&4	3.08	3.97	3.5	4.16	4.19
Absence of frontal teeth (number)	3.2	4,3	3.4	4.5	4.72

Table 5.

The results of this clinical study have clearly shown that there are severe changes of the periodontal parameters and important destructions of periodontal tissues (table 5). In accordance with the questionnaire made, these changes seem to be the consequence of a big waiting interval from the moment the therapy began until the moment the specialty therapy was set up. The targeted evaluation on the time interval that had passed since the appearance of periodontal symptomatology and until the moment the therapy began has configured a time unit of 2 to 5 years for the patients in the study area, and an address deficiency for specific treatments for the patients in the rural area. The highest percentage for being in treatment was the dental mobility, a symptom which can be capable of drawing patient's attention regarding the fact that degenerative changes appear/ have appeared. The data obtained allow a decryption of a noncompliant behavior for periodontal and/or stomatological therapy, the motivation connected to fear and the lack of importance in present symptomatology being omnipresent in the patients' answers in the questionnaire. The necessity for dental extractions to be made and the appearance of physiognomic problems remain concrete reasons for which patients request stomatological or periodontal treatment, but they are not the most important, being doubled by the appearance of pain as a determining factor (81.8%), in accordance with this study. The behavior of therapeutics option is oriented towards therapy through extraction, and the utilization of one of the secondary hygiene means has a low score. The plaque value made to the patients included in the study had high values, also the bleeding value and the loss of clinical attachment. Bone loss for the patients of this study had high percentage values for the patients in the rural group and for the smokers, 57.8% of them recording severe loss of alveolar bone. The results of this study have shown high values for the extraction of these teeth (with an average of 4.5 – 4.72 missing frontal teeth).

CHAPTER VI

HISTOLOGICAL STUDY OF PERIODONTAL CHANGES

Material, method

The initial clinical evaluation was made for 92 patients, amongst them, only 37 corresponded to the selection criteria for the study and have continued by signing the informed consent form. After accomplishing the dental extractions, small fragments of marginal periodontium were collected, which were immediately used in the fixing process in 10% neutral formol solution for the accomplishment of optical microscopy and immunohistochemistry studies and the volume of the fixing solution was 15-20 times bigger than the volume of the fixing piece. 10% formalin is a mild fixer, this is why periodontium fragments could have been kept longer without the alteration of cellular or intracellular structures. The fixing process of periodontial fragments lasted for 24 hours at the laboratory's temperature, they were included in histological paraffin (the classic histological technique for inclusion to paraffin), a technique that allowed us make seriated sections with the thickness of 3-5 μm . The technique comported the following periods:

- a) dehydrating
- b) clarifying
- c) paraffining
- d) inclusion itself
- e) sectioning the block
- f) attaching of the sections on the blades and drying them
- g) coloring the sections
- h) drying and depositing the histological material.

Sectioning the odontium and periodontium fragments was made at the rotational microtome Microm HM350, which has a water transfer bath for the sections, obtaining sections with the thickness of 4 μm . The sections obtained are attached on glass histological blades, cleaned and degreased. Each blade with histological crown on it was numbered after the paraffin block, which corresponded to the periodontium fragment gathered. The histological mixtures were colored with hematoxylin-eosin, the most used method to emphasize the tissues and with the trichromic based on light green after the Goldner-Szeckeli method. A part of the histological mixtures obtained in this way were packed in white papers, emblazoned and deposited in a colder place, safe from temperature variations, in order to make other coloring techniques when needed.

Results

Coloring technique with H.E. offers the following results:

- Cytoplasm pink
- Nuclei blue
- Nucleoli violet-blue
- Collagen fibers pale pink
- Elastic and reticuline fibers do not color

In the trichomic dyeing with light green GS the following results are seen:

- Cytoplasm violaceous-pink
- Nuclei dark red or brown
- Nucleoli black
- Erythrocytes orange-red
- Collagen fibers green
- Muscular fibers brown-red

Microscopic study of periodontium has shown very varied lesions. Most often, the periodontium has been the chronic inflammatory infiltrate, formed of lymphocytes, macrophages and rare granulocytes. The intensity of the inflammatory processes was very variable from a patient to another and even for the same patient, the inflammatory reaction was either diffused or in a granuloma shape.

Frequently, in marginal periodontium have been identified micro-bleeding areas, more or less drawn out. We consider that these micro-bleedings are determined by injuries of micro-vessels from the blood circulations directly by the pathogenic agents or indirectly by local pH changes or by the secretion of toxic factors or enzymes (collagenases, hydrolases) by pathogenic agents or the cells of the immunitary system. The intensity of local micro-bleedings and the vascular congestion correlated with the intensity of the periodontal inflammatory process, which denotes the fact that inflammatory mechanisms and the local micro-circulatory changes are interdependent. For some patients, the alteration of vascular structure has determined the extravasation of plasma liquid, with edema constitution in the superficial periodontium, and the plasma and red cells extravasation from the vascular bed contribute to the accomplishment of the periodontium tumefaction of layer, to the change of its color and to the appearance of local pain.

There have been observed areas of periodontal necrosis of different diameters either close to odontium or far from it. A particular microscopic aspect was the one of a reduced reaction of fibroblastic cells, suggesting that the regenerative processes are very reduced.

In our study, we remarked the fact that the angiogenesis vessels were in a small number in the periodontal conjunctive and with the immature or discontinuous wall, because numerous bleeding suffusions were observed and even micro-bleedings in the periodontium. We believe that this periodontal inflammatory process has certain particularities which make the proliferation of fibroblasts and angioblasts show a deficit. A particular microscopic aspect observed on our preparations was that of a reduced reaction of fibroblastic cells.

It is surprising the fact that on the histological preparations made by us, the reduced number of fibroblasts was not correlated with the intensity of chronic inflammatory process.

CHAPTER VII

IMMUNOHISTOCHEMICAL STUDY OF THE INFLAMMATORY REACTION IN CHRONIC MARGINAL PERIODONTITIS

Immunohistochemistry always represents a completion of the histopathological diagnosis, very useful in the following cases: in establishing a positive and differential diagnosis of lesions, in establishing the etiology or the histogenesis, in evaluating some cellular and tissual markers, in the therapy of some medical conditions. For the immunihistochemical study we used the same biological material, namely fragments of periodontium gathered from the 37 patients who have had the histological study, the number of extracted teeth being 63.

For the immunohistochemistry techniques, the most used fixer us also 10% formalin. If in the majority of histological techniques 4-5 μm thick sections are preferred, for a good quality observation of the results even with a big magnification lens, in the classic immunohistochemistry thinner sections are preferred. In contrast to the classic histological technique, where we used Mayer albumin for the growth of the histological crown's adhesive capacity on the port-object blade, for the immunohistochemistry techniques we used blades covered in special adhesives in order to prevent the tissue detachment during the process (Super Frost and Poly-L-lysine). The adhesion of the tissue on the blade is complete after one night drain in a 45 degrees Celsius incubator. The immunohistochemistry techniques use the same procedures for deparaffinization and hydration, respectively 3-4 baths with aromatic hydrocarbons and ethylic alcohol in decreasing concentrations. A particular time of immunohistochemistry techniques is represented by the so-called "antigenic exposure", we used the technique of "antigen exposure with the help of microwaves" with the help of citrate plugs (pH 6) and EDTA (pH 8).

The stepdavidin-biotin method is based on the direct conjugation of stepdavidin with enzymatic molecules. The detection molecule used by us was peroxidase. The chromogen used in the case of peroxidase is diaminobenzidine. The results of coloring are valid if it is excluded any interference which determines an unspecific coloring (the negative control is not coloring) and if the technique's sensibility is guaranteed (the positive control tissue is positive, containing the studied antigen un a reduced concentration).

Through the immunohistochemical study made by us, we tried to evaluate which are the cells of the immunity system that take part to the formation of the imflammatory infiltration from the marginal chronic periodontitis. In our studym we used the following immunohistochemistry markers:

Antibody	Epitope / marker	Dilution	Antigenic recovery	Source
CD45	Leucocytes	1/100	Boiling in citrate	Dako
CD3	T Lymphocytes	1/100	Boiling in citrate	Dako
CD4	T Lymphocytes	1/100	Boiling in citrate	Dako
CD8	T Lymphocytes	1/100	Boiling in citrate	Dako
CD20cy	B Lymphocytes	1/100	Boiling in citrate	Dako
CD79-alfa	Plasmocytes	1/100	Boiling in citrate	Dako
CD68	Macrophages	1/200	Boiling in citrate	Dako

CD45 marker was used in order to emphasize the amplitude of T lymphocytes presence in the inflamed periodontium from the chronic marginal periodontitis; in our study the T lymphocytes were present in a very high number in the periodontium. Also, we identified a few neutrophilic granulocytes in the inflammatory process from the chronic periodontitis.

By using the CD3 marker, we emphasized the presence of cytotoxic T lymphocytes and helper, we remarked the presence of a high number of CD3 positive lymphocytes in the chronic marginal periodontitis, the most often, they appeared vaguely and unequally disseminated, mostly perivascularly in the periodontium.

CD4 positive lymphocytes. In our study, they have been emphasized only extremely rarely.

In comparison with CD4-lymphocytes, CD8 positive lymphocytes were much more abundant in periodontium from chronic periodontitis.

The evaluation of B lymphocytes in the chronic periodontitis was done with the use of anti-CD20 antibody. In our study, the B-lymphocytes were present at all persons with chronic periodontitis, but they were not distributed uniformly in the conjunctive periodontal tissue.

The investigation of macrophage system cells' reaction was made by the utilization of anti-CD68 antibody. In our study, macrophage cells were not spread uniformly in the conjunctive tissue of the inflamed periodontium.

CHAPTER VIII

MICROSCOPIC STUDY OF DENTIAL CHANGES OF PATIENTS THAT SUFFER FROM CHRONIC MARGINAL PERIODONTITIS

After obtaining the informed consent of each patient, dental extractions were made. The extracted teeth (63) were immediately stored for the fixing process in 10% neutral formalin solution in order to make the optic microscopy studies. The fixing process of teeth was of 7 days. After the fixing, the teeth stood for a decalcification surgery in 10% trichloroacetic acid solution for 30 days. For a quick and homogenous decalcification, they were put on a magnetic agitator. We considered that decalcification was optimum when the pieces became soft and flexible enough. After the decalcification, the teeth were sectioned longitudinally, washed in water for 24 hours and entered again in paraffin.

The sectioning was made on the rotational microtome Microm HM350, sections of 5 microns thickness being obtained, which were colored with hematoxylin-eosin and Goldner-Szeckeli trichromic. From the study, we observed that dentinal changes are complex and varied enough. We consider that in the appearance and development of dental erosions many factors intervene, such as structural properties of the tooth, changes of the physiologic properties of salivary liquid, consumption of low pH substances.

We believe that the erosions that appeared at the level of radicular dentine are the result of the aggressiveness of the bacterial flora, that have the capacity to form an acid environment which dissolves the mineral component of the dentine, followed by the destruction of the organic component.

GENERAL CONCLUSIONS

The patients go to the cabinet for the initiation of the therapy, late, in an advanced phase of tissual destruction. The patients' option for treatment, the most common for the therapy of teeth affected by periodontopathy is extraction. The appointments for the initiation of the treatment are made in the 2 to 5 years interval from the beginning of syptomatology; there have been observed significant correlations between the postponing of specialty treatments and the negative value of periodontal parameters.

The chronic inflammatory periodontal process was frequently accompanied by local micro-bleedings and cellular and tissular necrosis, testifying in this way the aggressiveness of some bacterial species which have entered subgingival, forming periodontal pockets. The immunohistochemic study confirmed the fact that in the chronic marginal periodontitis, the inflammatory infiltration is preponderantly formed of T CD45 positive lymphocytes. Among T-lymphocytes, the most numerous were CD3 lymphocytes, CD8 lymphocytes, while CD4 lymphocytes were poor represented. There are also emphasized big quantities of B-lymphocytes (CD20 positive), plasmocytes (CD79-alpha positive) and macrophages (CD68 positive), which demonstrates that the defending reation in the chronic marginal periodontitis is complex, both the cellular and humoral immunities taking part at it.

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