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THESIS

CLINICAL, HISTOLOGICAL AND IMMUNOHISTOCHEMICAL ASPECTS OF PERIODONTAL LESIONS AT PACIENTS WITH DIABETES AND CHRONIC MARGINAL PERIODONTITIS

SUMMARY

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INTRODUCTION

The periodontal disease is an inflammatory disease affecting the tooth supporting structures, resulting in the loss of periodontal supporting tissues with increased tooth mobility and eventually tooth loss. It is a highly common disease, but with large variations from country to country. In the US, more than half of the population aged 18 have an early form of periodontal disease; the population over 35 years old shows a rate of about 75% of different types of periodontal disease (Negrato CA, Tarzia O, Jovanovič L et al, 2013). The gravity of forms for periodontal disease is also variable. According to some studies, between 30 and 50% of these diseases are mild forms, and 5-15% are severe, generalized forms (Ridgeway EE, 2000; Friedewald VE, Kornman KS, Beck JD, et al, 2009).

Periodontitis is not only a localized disease, but it also influences the systemic health of the individual. Over the years, periodontitis has been associated with various systemic diseases such as mellitus diabetes (Gurav A, Jadhav V, 2011), atherosclerotic cardiovascular diseases (Dietrich T, Sharma P, Walter C, et al, 2013), rheumatoid arthritis (Kaur S, White S, Bartold PM, 2013), cancer, chronic kidney diseases (Ruospo M, Palmer SC, Craig JC, et al, 2014), inflammatory bowel disease, obesity (Suvan J, D'Aiuto F, Moles DR, et al, 2011), metabolic syndrome (Gurav AN, 2014), and so on.

The periodontal disease is an inflammatory disease induced by a chronic bacterial infection that affects the gums, alveolar-dental ligaments and bone (the tooth supporting structures), mainly caused by Gram-negative anaerobic microorganisms that are in the bacterial plaque that adheres to teeth (Negrato CA, Tarzia O, 2010). Recent studies have shown that approximately 500 different bacterial and viral entities are in the dental plaque (Amar S, Han X, 2003). The pathogens present in dental plaque (dental film), most commonly identified in the etiopathogeny of the periodontal disease, belong to three species of microaerophilic bacteria (Actinobacillus actinomycetemcomitans, Campylobacter rectus, și corrodens Eikenella) and seven anaerobic species (Porphyromonas gingivalis, Forsythus Bacteroides, Treponema denticola, Prevotella Intermedia, Fusobacterium nucleatum, Eubacterium and spirochete) (Socransky SS, Haffajee AD, Cugini MA et al, 1998). Also, various herpesviruses, such as Epstein-Barr virus and human cytomegalovirus

have recently appeared, as pathogens, in periodontal disease (Slots J, Kamma JJ, Sugar C, 2003).

For the occurrence of periodontal disease, there are needed, besides the microbial factor, also some host factors that facilitate the development of the disease. These factors include genetic susceptibility, smoking, alcohol drinking, consumption of toxic substances, serious systemic diseases etc.

Regarding diabetes, in 1995 the prevalence of the disease in adults worldwide was estimated to be approximately 4.0% and is expected to rise to 5.4% by 2025, this meaning an increase of 135 million in 1995 to 300 million in 2025. The bulk of this growth will occur in developing countries. An increase of 42% (51-72 million), is expected in the developed countries and an increase of 170% (84-228 million), is developing. It is therefore expected that by 2025, 75% of people with diabetes will live in developing countries, where most people with this disease will be aged between 45-64 years. The International Diabetes Federation has estimated that diabetes costs represent 5-10% of the total budget of several countries (International Diabetes Federation, 2006).

Chapter I. Microscopic structure of periodontal

The totality of structures in dental alveolar space form a morpho complex called desmodonal or periodontal. In the periodontal space there is provided a fiber joint, a dynamic structure, responding to a number of extrinsic factors to maintain the normal occlusal position (Hering SW, 2012; Ten Cate R, 1998). On radiographs, dental alveolar space appears as an area of increased radiolucencies between the tooth root and alveolar bone. The periodontal normal width ranges from 0.15 mm to 0.35 mm, which can decrease with age (White SC, Pharoah MJ, 2014; Nan Jiang, Weihua Guo, Mo Chen, et al, 2016).

Dental alveolar space has an hourglass form, being narrower in the rotation zone of the tooth - hypomochlion – and wider in the cervical area, respectively apical. Due to hypomochlion point closer to the apex, about the union of 2/3 of the coronary root to 1/3 of the apical root, the width of the dental alveolar space will be higher to coronal rather than to apical, due to higher amplitude of the lever arm of 2/3 to 1/3 of the root length.

The periodontal ligament has two main functions: to transmit and absorb mechanical stress of the tooth, and supply on vascular way with cementum nutrients, the alveolar bone and its structures themselves.

Changes in adjacent mineralized tissues may increase and / or decrease alveolar-dental space and, finally, modifies the periodontal ability to transmit optimal occlusal loads, leading to the appearance of some pathological conditions (Hurng JM et al, 2011).

The fibers constitute the majority of the extracellular matrix, forming the periodontal ligament. These are mainly collagen fibers, but there are oxitalanice, reticulin and elastic fibers. Bundles of periodontal collagen fibers are arranged in ways that reflect their functional properties (Sawhney RK, Howard J et al, 2004).

The oxitalanice fibers regarded as immature elastin fibers are more abundant in the apical and cervical regions of the ligament and at the level of tooth ligament subject to important occlusal tooth stresses. The collagen fibers are named according to their location and are classified into 3 groups of fibers: group of dental alveolar fibers, gingival ligament fibers and gum transseptal fibers.

Elastic and reticulin fibers are extremely rare, being located more in the walls of small blood vessels.

The desmodontal fundamental substance takes the form of a polysaccharide gel, strongly hydrated, occupying a large volume (65%) of the dental alveolar space. The basic substance contains,mainly, synthesized macromolecules by local fibroblasts, hyaluronic acid, proteoglycans, and glycoproteins (fibronectin). The proteoglycans containing large numbers of hydroxyl, carboxyl, and sulfate have an intensely hydrophilic nature. Together with hyaluronic acid, of which is anchored in a stereospecific bond, the proteoglycans control the degree of hydration of the extracellular matrix.

The cells are represented by the connective tissue cells, epithelial cells, defense cells and stem cells. The fibroblasts are the most numerous desmodontal cells, representing about 50% of all the periodontal cells (McCulloch CA, Bordin S, 1991). They are located between the collagen fibers, their spatial orientation is determined by the arrangement of fibrillar bundles. The periodontal fibroblasts are heterogeneous being composed of several sub-populations. They synthesize all fibrillar and molecular structures present in periodontal connective matrix. The periodontal fibroblasts have the ability to respond to mechanical stress, such as the

movement of the tooth (Phipps RP, Borrello MA, Blieden TM , 1996); when the mechanical forces are applied to the tooth, the periodontal fibroblasts respond by proliferation and differentiation (Yamaguchi N, Chiba M, Mitani H, 2002).

The immune system cells are present in different amounts in periodont. These are represented by the lymphocytes, plasma cells, macrophages, mast cells and polymorphonuclear rare neutrophils. They come from the bloodstream and intervene in maintaining the local homeostasis. In periodontal diseases the number of these cells increases more impressively.

The stem cells are immature and unspecialized cells that can self-renew and they undergo to the asymmetric differentiation, ie they produce exact copies of stem cells and at the same time, they differentiate themselves into types of specialized cells such as the fibroblasts and the osteoblasts.

The alveolar bone is a mineralized connective tissue consisting of inorganic salts, organic matrix and water. At the alveolar bone, 23% is mineralized tissue; 37% is the organic matrix, in which the bulk is represented by the collagen, while the remaining 40% is water (Moss-Salentijn L. Melvin L, 1997).

CHAPTER II. PERIODONTAL DISEASE - CURRENT DATA

Periodontitis is one of the most common illnesses and is characterized by destroying the periodontal connective tissue, as a result of a host inflammatory response, secondary to the infection with bacteria and viruses present in the dental plaque (Bascones-Martínez A, Muñoz-Corcuera M et al, 2009; Zhang L, Henson BS, Camargo PM et al, 2009). Clinic, periodontitis presents mild, moderate or severe forms.

In the etiology of periodontitis is incriminated the bacterial plaque that accumulates on the teeth surfaces in the absence of some optimal oral hygiene measures. In the initial phase of the pathological process, the disease affects the superficial periodontium and is called "gingivitis". If gingivitis is neglected, the inflammatory processes may extend to the marginal periodontium resulting "periodontitis", which is an irreversible process. Oral bacterial flora includes over 700 different types of bacteria, of which about 400-500 species have been found in the subgingival plaque (Berezow AB, Darveau RP, 2011). In periodontitis, the subgingival microflora can consist of hundreds of species of bacteria, but only a small number was associated with the progression of disease and are considered important in

terms of etiology. The increased plaque accumulations are due to poor oral hygiene and favored by local factors such as the presence of dental plaque from the dental restorations, dental crowding or misaligned teeth.

Risk factors for periodontal disease

1. Smoking. Tobacco smoke exert a significant deleterious effect on periodontal tissues and increases the rate of progression of periodontal disease (Zini A, Sgan-Cohen HD, Marcenes W, 2011). Smoking modifies the host response to the action of bacteria in the dental plaque (Shchipkova AY, Nagaraja HN, Kumar PS, 2010; Ozçaka O, Biçakci N, Pussinen P, et al, 2011).

2. Cardiovascular diseases. The association between periodontal diseases and cardiovascular diseases are well studied and possible mechanisms include some of the following: elevated cholesterol concentrations and the action of oral bacteria may accelerate atherosclerosis or participation of proteins to acute phase, which may increase the severity of chronic periodontitis (Izumi A, Yoshihara A et al, 2009; Kamil W, Al Habashneh R et al, 2011).

3. Drugs and drug use. Medications may be a contributing factor to periodontal diseases. Drugs, agents such as calcium channel blockers, anticonvulsants and cyclosporine can induce gingival overgrowth (Rees TD, Levine RA, 1995). Some medications significantly decrease salivary flow and thereby increase the number of bacteria in dental plaque and dental scaling grow.

4. The stress. Patients exposed to stress with inappropriate behavior at stress present a higher risk of severe periodontal diseases (Akhter R, Hannan MA, Okhubo R, 2005; Johannsen A, Rylander G, et al, 2006; Johannsen A, Rydmark I, Söder B et al, 2007). Stress is associated with poor oral hygiene, increased glucocorticoid secretion that can depress the immune function, insulin resistance increase and a potential increased risk of periodontitis

5. Obesity. Obesity has been reported as an important risk factor for periodontal disease. The submission of tartar is more influenced by the consistency of food than their content: the harsh, with pronounced mechanical action, delays the formation of the dental plaque. Also, it has been found that the deficiency of vitamins A, B6, PP favors the formation of plaque; also eating foods rich in calcium, bicarbonate, protein, carbohydrate;

CHAPTER III. PERIODONTAL DISEASE AND DIABETES

Systemic diseases that affect host defenses act as risk factors for the development of gingivitis and periodontitis. Some altered host responses are associated with increased incidence and severity of periodontitis in diabetics.

Patients with type 1 odiabetes and its duration for over 10 years experience a loss of attachment greater than those with diabetes and its duration less than 10 years, particularly at those older than 35 years. Patients aged between 40 and 50 years, diagnosed long ago with type 1 diabetes have several areas with aggressive periodontitis and bone loss compared to patients without diabetes and similar ages. It has also been demonstrated that in patients with type 1 diabetes and other complications of diabetes such as retinopathy or nephropathy, they presented a periodontal attachment loss showed much higher than in patients without such complications. Other studies have shown that those patients with diabetes type 1 and periodontitis have a high prevalence of ketoacidosis, retinopathy and neuropathy.

Some studies have focused on the role of periodontal infection and plaque microflora in patients with diabetes, but failed to be conclusive. Therefore, it is not clear whether a modified microflora contributes to the increased incidence and severity of infection and periodontal destruction in patients with diabetes. The increased accumulation of tartar reported in patients with diabetes may be due to elevated serum calcium concentrations in patients with type 1 diabetes. The groove crevicular fluid at diabetics presents double levels of glucose compared to the other patients, and the concentration of urea can also be increased. These changes, together with basement membrane thickening and hemoglobin glycosylation can promote a unique environment that changes the microbial flora. However, some studies reported no significant differences between patients with or without diabetes, suggesting that alterations in the host response to existing periodontal pathogens may be the main responsibles for more aggressive destruction observed at patients with diabetes.

CHAPTER IV. CLINICAL AND STATISTICAL STUDY OF PATIENTS WITH PERIODONTITIS AND DIABETES

Our study included a total of 75 diabetic patients, aged 26 and 90, who were presented at the dental office for various symptoms of maxillary. The age of diabetes was between 5 and 16 years. Clinical and laboratory investigations followed: patient

age, social environment, eating toxic (smoking and drinking), oral hygiene, blood glucose levels, the presence of periodontal lesions and other oro-dental lesions, local irritative factors and associated diseases.

The reasons of patients consult presentation were varied: gingival, helen, ulcers, tooth mobility, and functional chewing aesthetic disorders. Of all patients studied, 69 showed that they accused gingival and halitosis, and these had constant high blood sugar levels.

Following the distribution of the group of patients with periodontal disease and diabetes by age we have found that periodontal disease associated with diabetes can occur in younger people (under 30 years), but most of them were over 50 years (59 patients, accounting for 78.66%).

Following the distribution of cases of periodontal disease associated with diabetes it was observed that 45 patients (60%) were female and 30 patients (40%) were male.

The evaluation of patients in relation to the social environment has allowed us to see that 47 patients (62.66%) were from urban areas and 28 (37.34%) from rural areas.

The repeated evaluation of glucose showed that 34 patients (45.33%) had a good control over diabetes, the blood sugar being close to normal (less than 125mg / dl), while at 41 patients (54.67 %) the glucose values ranged from 125ml / dl to 230 mg / ml.

The clinical examination of the maxillary showed multiple and very varied lesions. Thus, the gum ulcers were present in 21 patients (28%), 19 of which being in the age groups older than 50 years. Gingival ulcerations were localized predominantly in the interdental papillae. Most patients who had gum ulcers had a poor metabolic control of diabetes and an unsatisfactory state of oral hygiene.

The local irritation factors, represented by root remnants with anfractuoase edges, obturations, overflowing crowns, fixed dentures incorrectly adjusted cervical, mobile prostheses incorrectly adjusted, dental tartar, were present in 63 (84%) of the cases studied. Also, at patients with increased tooth mobility and interspersed edentulous there were present constant tooth horizontal and / or vertical movements, with major occlusal disorders.

The highlighting and quantifying of the bacterial plaque were performed using index O'Leary card, a qualitative index and percentage of the bacterial plaque that

highlights the presence or absence on buccal, oral, mesial distal surfaces of each tooth except the third molar. Only 25 of the patients experienced optimal dental hygiene, 21 of the patients had values greater than 70%.

The gingival bleeding assessment was done using gingival bleeding index SBI. As it can be seen in Table 3, only 9 patients (12%) had a small SBI, while 37 patients (49.33%) had a very high index SBI 25%.

Between the systemic diseases associated periodontal diseases and diabetes in our group of patients, it was found that 50 patients (66.66%) have also suffered from cardiovascular diseases and 28 of them (37.33%) suffered from obesity. The complications of diabetes type diabetic retinopathy and neuropathy, and chronic renal insufficiency, were identified only at 12 people, all aged over 50 years.

CHAPTER V. HISTOLOGICAL PERIODONTAL STUDY IN PATIENTS WITH PERIODONTITIS AND DIABETES

At patients with periodontal disease, with excessive mobility, to whomwas imposed tooth extraction, with the patient's agreement, there were collected small pieces of periodontal for histopathology and immunohistochemistry studies. There were taken as 32 pieces of periodontal tissue from patients with diabetes and 9 fragments of the patients without diabetes.

The biological material collected was fixed in 10% neutral formalin solution, for 24-48 hours at room temperature, after which it was included in paraffin, using the classic histopathological protocol. Cutting of the biological material was carried to the rotary Microm HM325 microtome equipped with a transfer system of the sections in a water bath (STS, microM) and the Peltier cooling system. For the histopathological study there were used two stains: hematoxylin-eosin staining (HE) and trichromic staining with green light, Goldner-Szeckeli technique (GS).

Pacienții cu DZ au prezentat la examenul microscopic alterări marcate ale peretelui vascular cu microhemoragii la nivelul laminei propria, alături de vasele sanguine ectaziate. Patients with DM showed at the microscopic examination marked alterations of the vessel wall with micro bleeds in the lamina, with ectasia of blood vessels. The hematic interstitial infiltrates have stressed the gingival chorion disorganization.

Lymphocytic inflammatory infiltrate was predominantly moderate and high intensity. Compared to the distribution of lymphocyte infiltration, it was noted that

regardless of the period of development of diabetes and inflammatory intensity distribution was predominantly diffuse at the level of lamina. The collagen fibers appeared dissociated from the inflammatory cells that are inserted among them. The interstitial edema and hemorrhagic infiltrates were present in most cases and have contributed to disorganization of the chorion's architecture. Also, it has been noted the fragmentation of collagen and sometimes even their lysis with disorganization of periodontal connective tissue.

Marked alterations were reported also at the alveolar bone tissue. Here there were highlighted radiolucent phenomena with patchy destruction of the haversian and spongis bone tissue.

CHAPTER VI. IMMUNOHISTOCHEMICAL PERIODONTAL STUDIES AT PATIENTS WITH WITH PERIODONTITIS AND DIABETES

For the immunohistochemical study, the histological sections were collected onto histological slides coated with polylysine (poly-L-lysine) (Sigma) in order to increase the adhesion of sections to slides and then were transferred to an incubator at 45 ° C and kept overnight (18 hours). The next d ay the immunohistochemical classical protocol was applied consisting of dewaxing and hydration of sections, followed by antigen unmasking by boiling the sections in a solution of sodium citrate, pH6, in a microwave for 21 minutes (7 cycles of 3 minutes each). Next, it was performed the blocking of the endogenous peroxidase by incubating the biological material in 3% of hydrogen peroxide for 30 minutes at room temperature, followed by washing in distilled water for 10 minutes and a wash in a solution of phosphate buffered saline substance 1% (PBS) 5 minutes. The blocking of non-specific sites was achieved by passing of sections in a bath with 2% skim milk for 30 minutes. Then, the sections were incubated with primary antibodies for 18 hours (overnight) in a refrigerator at 4℃, and the next day the biotiny lated secondary antibody was applied for 30 minutes at room temperature. After washing the biological material with PBS 1% (three baths of 5 minutes each), the streptavidin-HRP was applied, 30 minutes at room temperature, followed by washing the slides in 1% PBS 3x5 minutes. The signal was detected using 3,3'-Diaminobenzidine (DAB) (Dako). There attended contrastation with Mayer's hematoxylin, dehydration in alcohol, xylene clarification and mounting of blades using the environment DPX (Fluka).

For immunohistochemical study I used antibodies:

- anti-CD3 for highlighting the T lymphocytes;
- Anti-CD20 for highlighting the B lymphocytes
- Anti-CD68 for highlighting the macrophages

The immunohistochemical study showed a heterogeneous distribution of inflammatory cells in the periodontal lesions both in patients with diabetes and in those without diabetes. What was noted in our study was that the inflammatory reaction in patients with periodontitis and diabetes was more severe than in patients periodontal disease without diabetes. From the cells of the immune system the best represented are the macrophages and B cells, while T-cells had a much reduced response.

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