PHD THESIS
ABSTRACT

CONTRIBUTIONS TO THE HISTOLOGICAL, IMMUNOHISTOCHEMICAL AND CLINICAL STUDY OF CHRONIC APICAL PERIODONTITIS

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ABSTRACT

INTRODUCTION 3

KNOWLEDGE STAGE 3

CHAPTER 1 3
Histology and histophysiology of the apical periodontium

CHAPTER 2 4
Etiopathogenic and clinico radiological aspects of chronic apical periodontites

PERSONAL CONTRIBUTION 4

CHAPTER 3 4
Clinico radiological study of chronic apical periodontites

CHAPTER 4 6
Histological study of chronic apical periodontites

CHAPTER 5 8
Immunohistochemical study of chronic apical periodontites

CHAPTER 6 General conclusions 10

SELECTIVE REFERENCES 11

Key-words: periodontitis, chronic apical periodontitis, apical granuloma, periapical lesions, immunohistochemical study, immunohistochemical markers
INTRODUCTION

Periapical lesions are among the most frequent odontal apical conditions in human teeth, generally called apical periodontites. Apical periodontitis is one of the most common endodontic conditions that doctors have to deal with in every day practice (Torabinejad M, Bakland LK, 1978).

We performed a clinical and immunohistochemical study of the periapical lesions, evaluating the various clinical and radiological aspects, in comparison to the results of the histological examination, in order to observe the relation between the clinico radiological and morphological aspects.

The PhD thesis is structured into two major parts:

I. Knowledge stage, where, in two chapters, we performed an update of the theoretical material in literature related to the histology and histophysiology of the apical periodontium and to the etiopathogenic and clinico radiological aspects of chronic apical periodontites (CAP).

II. The personal contribution part has as a main research objective the establishment of certain correlations between the clinical, imagistic, histological and immunohistochemical aspects and it is directed on performing a clinico statistical, histological and immunohistochemical study, each one having various objectives.

The theme of the PhD thesis refers to an interdisciplinary study. The study is important both for fundamental research, and especially for specialty clinical practice, bringing information with an applicability in the specialty medical practice.

KNOWLEDGE STAGE

CHAPTER 1
HISTOLOGY AND HISTOPHYSIOLOGY OF THE APICAL PERIODONTIUM

The periodontium is a group of tissues covering the tooth and supporting it in its dental alveola, being composed of structures originating in the dental follicle.

The periodontium, histologically speaking, is made up of two components, the support or apical periodontium, made of cement, alveolar bone and periodontal ligaments, and the covering or marginal periodontium, represented by the gingival fiber mucosa (Baniță M, Deva V, 2006, Ricucci D, 2009, Mjor IA, Heyeraas K, 2008, Nair PNR, 2005).

The periodontium has as main function the one of providing the connection between the tooth and the alveolar bone through a joint called gomphosis, thus allowing the tooth to move into its alveola (Ørstavik, 2008). It is a physiological mobility, easily to observe. Also, the sensorial function is another function of the periodontium that contributes to the interception of touch and pressure, due to the periodontal structures that are responsible for the tooth movement in the alveola.

The desmodontium, also called periodontium, represents the connection between the radicular and alveolar bones. Its functions are multiple ones, very important for the dental organ: tooth anchoring in the bone alveolae, control of dental movements and maintainance of periodontal integrity, control of transmitting exteroceptive and interoceptive stimuli, periodontium involvement in the process of teeth efflorescence (Crăiţoiu Ş, Floreşcu M, Crăiţoiu M, 1999, Nica I et al, 2005).
CHAPTER 2
ETIOPATHOGENIC AND CLINICO RADIOLOGICAL ASPECTS OF CHRONIC APICAL PERIODONTITES

Chronic apical periodontites are osteitic lesions, with a necrotic and destructive character that present a varied extension and that emerge as a result of the resorption processes of the radicular apex and of the apical periodontal tissue. The infection of the radicular channel usually emerges after the radicular pulp undergoes a process of necrosis that may take place as a consequence of cavity processes, trauma, periodontal diseases or iatrogenics: as a result of the incorrect techniques of endodontic treatment like an incorrect determination of the work length of the radicular channel, a mechanical aggressive treatment involving the pushing beyond the apex or exceeding radicular obturations (Sundqvist G, 1992 citat de Jose F Siqueira Jr, 2008, Bystrom A, 1986, Rocas IN, 2010).

In th CAP etiopathogeny, there are incriminated both local factors (microbial germs, traumatic and toxic factors), as well as general factors (systemic diseases, dysmetabolic diseases, avitaminooses, vascular lesions, factors decreasing the body resistance and reactivity). The action of the local factors may be increased by the action of the general ones, through vascular disorders causing a total or partial, progressive or sudden reduction of the apical blood flow (Stashenko P, 2002). The classification based on the radiological and anatomo-clinical criteria allows to choose an adequate treatment method (Crăiţoiu Ş, Florescu M, Crăiţoiu M, 1999, Răescu M, 2003):

A. Lesions of the apical periodontium with a contoured radiological image:
   1. Fibrous chronic periodontitis,
   2. Simple conjunctive granuloma,
   3. Epithelial granuloma,
   4. Cystic granuloma,
   5. Chronic apical periodontitis with hypercementosis,
   6. Chronic apical abscess,
   7. Specific chronic apical periodontites.

B. Lesions of the apical periodontium with an uneven radiological image:
   1. Progressive diffuse chronic apical periodontitis,
   2. Condensed chronic apical periodontitis.

PERSONAL CONTRIBUTIONS

CHAPTER 3
CLINICO-RADIOLOGICAL STUDY OF CAP

The clinical study was performed on a group of 132 patients diagnosed with chronic apical periodontitis, selected after the examination of 258 patients who presented for a specialty treatment, between nov 2012 – march 2016, in the Clinic of Dental Prosthetics within the University of Medicine and Pharmacy of, and also in a private clinic in Craiova.

The patients were clinically evaluated and divided in various groups, according to multiple parameters: age, sex, living environment, affected teeth, their localization, number of lesions found in a patient, objective and subjective clinical aspects, type of performed x-ray, radiological aspect of the apical lesion, as well as other associated pathologies (Table 3.1, Fig. 3.1- Fig. 3.6).
Table 3.1 Frequency of chronic apical periodontitis according to sex and age

<table>
<thead>
<tr>
<th>Age</th>
<th>&lt; 20</th>
<th>20 – 30</th>
<th>30 – 40</th>
<th>40 – 50</th>
<th>&gt; 50</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>5 (55.56%)</td>
<td>10 (62.50%)</td>
<td>28 (65.12%)</td>
<td>21 (58.33%)</td>
<td>11 (39.29%)</td>
<td>75 (56.82%)</td>
</tr>
<tr>
<td>Women</td>
<td>4 (44.44%)</td>
<td>6 (37.50%)</td>
<td>15 (34.88%)</td>
<td>15 (41.67%)</td>
<td>17 (60.71%)</td>
<td>57 (43.18%)</td>
</tr>
<tr>
<td>Total</td>
<td>9 (100.00%)</td>
<td>16 (100.00%)</td>
<td>43 (100.00%)</td>
<td>36 (100.00%)</td>
<td>28 (100.00%)</td>
<td>132 (100.00%)</td>
</tr>
</tbody>
</table>

Fig. 3.1 Frequency of chronic apical periodontitis according to age and living environment.

Fig. 3.2 Distribution of apical periodontitis according to localization, maxilla or jaw.

Fig. 3.3 CAP frequency according to the affected teeth.

Fig. 3.4 Distribution of CAP according to the local associated lesions.

Fig. 3.5 Distribution according to the type of affected root in molars 1 and 2.

Fig. 3.6 Distribution according to the used x-ray method.
CHAPTER 4
HISTOLOGICAL STUDY

The histological study was performed on the material taken during the surgical treatment of the endodontic periapical lesions (apical resections) or postextraction in a number of 65 patients belonging to a group of 132 patients diagnosed with chronic apical periodontitis, selected after the examination of 258 patients who presented for a specialty treatment, between nov 2012 – march 2016, in the Clinics of Dental Prosthetics within the University of Medicine and Pharmacy of Craiova, but also in a private clinic of Craiova. The material processing was performed by the paraffin inclusion technique.

In the microscopic examination of the histological samples of chronic apical periodontitis, there were diagnosed hyperplasia forms (granuloma), cystic forms (radicular cyst), dystrophic forms (fibrous chronic apical periodontitis). The conjunctive granuloma identified on the sections by the presence of a granulation tissue formed of a mixt cellularity, with various types of cells: fibroblasts, histiocytes, macrophages, plasmocyte lymphocytes, rare lymphocytes and numerous vessels (Fig. 4.1 A, B). Other times, the collagen fibrillary component was very well represented (Fig. 4.1 C, D).

Fig. 4.1 Conjunctive granuloma (A) Mixt cellularity (B) Numerous blood vessels. HE staining X 100

Fig. 4.1 (C) Conjunctive granuloma with intra granulomatous fibrilogenesis. HE staining X 100; (D) Collagen fibers, with a role of lining membrane of the granuloma. Masson trichrome staining X 100.
If vascularization of a mixt conjunctive-epithelial granuloma (Fig. 4.2 A, B) is not enough for ensuring the nutrition of epithelial cells, these may degenerate, thus determining the formation of the cystic granuloma (Fig. 4.2 C, D). On several samples, there were present lesions of fibrous chronic apical periodontitis (Fig. 4.3 A, B).

Fig. 4.2 (A, B) Mixt conjunctive epithelial granuloma mixt. Masson trichrome staining X 100 and GS Trichrome staining X 100; (C, D) Cystic granuloma. Masson trichrome staining X 200

Fig. 4.3 (A, B) Fibrous chronic apical periodontitis with areas of chronic inflammatory infiltrate. HE staining X 100.
CHAPTER 5
IMMUNOHISTOCHEMICAL STUDY

Of the 65 cases selected for the histological study, we performed an immunohistochemical analysis on a number of 40 apical lesions: granulomas and cysts.
In the immunohistochemical study we used a set of 8 antibodies (Table 5.1).

Table 5.1 Antibodies used for the immunohistochemical study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Epitope / marker</th>
<th>Manufacturer</th>
<th>Antigen demasking</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD 45</td>
<td>Leucocytes</td>
<td>DAKO</td>
<td>Buffering cytrate pH=6</td>
<td>1:100</td>
</tr>
<tr>
<td>CD 3</td>
<td>Lymphocytes</td>
<td>DAKO</td>
<td>Buffering cytrate pH=6</td>
<td>1:100</td>
</tr>
<tr>
<td>CD 4</td>
<td>Lymphocyte T</td>
<td>DAKO</td>
<td>Buffering cytrate pH=6</td>
<td>1:100</td>
</tr>
<tr>
<td>CD 8</td>
<td>Lymphocyte T</td>
<td>DAKO</td>
<td>Buffering cytrate pH=6</td>
<td>1:100</td>
</tr>
<tr>
<td>CD 20</td>
<td>Lymphocytes B</td>
<td>DAKO</td>
<td>Buffering cytrate pH=6</td>
<td>1:100</td>
</tr>
<tr>
<td>CD79-alfa</td>
<td>Plasmocytes</td>
<td>DAKO</td>
<td>Buffering cytrate pH=6</td>
<td>1:100</td>
</tr>
<tr>
<td>CD 68</td>
<td>Macrophages</td>
<td>DAKO</td>
<td>Buffering cytrate pH=6</td>
<td>1:200</td>
</tr>
<tr>
<td>Tryptase</td>
<td>Mastocytes</td>
<td>DAKO</td>
<td>Buffering cytrate pH=6</td>
<td>1:100</td>
</tr>
</tbody>
</table>

The purpose of our study was to compare the morphological characteristics of the apical granuloma structure and the periapical cysts to the immunohistochemical expression indicators for markers CD45, CD3, CD4, CD8, CD20, CD68, tryptase and MMP2.

On the microscopic sections of conjunctive granuloma, conjunctive epithelial mixt granuloma, cystic-prone granuloma, periapical cyst, there were identified positive T CD3 and CD45 lymphocytes in a higher number in comparison to positive B CD20 lymphocytes, thus showing the existence of a cellular immune defence process and that T lymphocytes, more than B lymphocytes play an important part in the pathogenesis of periapical lesions (Fig. 5.1-Fig. 5.6).

In our study, the macrophages were present in all the examined apical structures, presenting differences as a numeric representation from one structure to another, thus suggesting the fact that the intensity of the inflammatory reaction varies from one lesion to another, and even from one area to another, within the same section being observed certain differences (Fig. 5.7).

The microscopic examination indicated the presence of mastocytes in the active inflammatory areas, as well as in the peripheral areas of both periapical lesions (Fig. 5.8). In the group of periapical cysts, the mastocytes were located under the cystic epithelium, in the conjunctive tissue and in the intraepithelial parts. We observed the presence of a difference regarding mastocytes in the various types of periapical lesions. These were more numerous in the cysts in comparison to the apical granulomas. The mastocytes were most frequently arranged as compact and less isolated. Also, there were more localized in the areas with chronic inflammatory infiltrate and less frequent in the fibrotic areas.
Fig. 5.1 Conjunctive epithelial mixt granuloma. CD45+ T lymphocytes X 100 arranged perivascularly.

Fig. 5.2 Conjunctive granuloma. CD3+ T lymphocytes X 100 arranged perivascularly.

Fig. 5.3 Conjunctive granuloma. Frequent T helper lymphocytes CD4+, diffusely arranged x 200.

Fig. 5.4 Periapical cyst. Very frequent cytotoxic T lymphocytes CD8+ x 200.

Fig. 5.5 Conjunctive granuloma. B lymphocytes CD20+ x 200 arranged perivascularly.

Fig. 5.6 Conjunctive granuloma. Numerous plasmocytes CD79-alpha + X 200 arranged perivascularly.
CHAPTER 6
GENERAL CONCLUSIONS

Chronic inflammatory periapical lesions represent the most frequent pathology found in the alveolar bone, presenting different aspects determined by a varied etiology, by an individual reactivity and structural diversity of the apical periodontium, this inferring a histological study that, together with the clinical symptoms, radiological aspects and etiological factors, may provide important information regarding the pathology of the apical periodontium.

The examined apical structures, by their morphological particularities, were considered a particular form of chronic apical periodontitis. In all these forms, there was present an inflammatory infiltrate conjunctive tissue, associated or not with an epithelial tissue and, sometimes, with the presence of a cystic cavity.

The cellular component associated capillary blood vessels and a collagen fibrilary component. The report between the cellular component, the fibrillary component and the vascular one, as well as their arrangement, were different, thus indicating various aspects that may be correlated with the progressing particularities of these structures, therefore showing either a reduction of the inflammatory process or an active inflammatory process, with a progressing character.

The results of our study indicate the presence of a cellular immune process, and also of a humoral immune reaction in the examined tissues, immunohistochemically confirmed by the presence of T and B lymphocytes and of plasmocytes in the inflammatory infiltrate, with a predominant cellular immune process.

Understanding the immunobiology of these lesions leads to new perspectives upon novel ways of treatment, consisting in the intents of deactivating the host response. This thing may be performed by a prolonged local drug-administration with slow-release certain biodegradable devices, introduced through the radicular channel, thus providing the local medicine for a predetermined time period.
16. Stashenko P, Interrelationship of dental pulp and apical periodontitis in Seltzer and Bender Dental Pulp, Quintessence, Chicago, IL, 2002