DOCTORAL THESIS

GINGIVAL OVERGROWTH OF LOCAL CAUSES - CLINICAL, HISTOLOGICAL AND IMMUNOHistoCHEMICALLY STUDY

ABSTRACT

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CHAPTER I
ANATOMY, HISTOLOGY and the HISTOPHYSIOLOGY of the ORAL MUCOSA
1.1. THE ANATOMY OF ORAL MUCOSA

The oral cavity is the first segment of the digestive system and previously communicates with the outside and posterior with the pharynx.

It is lined on the inside by the oral mucosa, which is the mirror of various physiological and pathological conditions of the body as some diseases start with the oral cavity or occurs subsequently at that level (Squier C, Brogden KA, 2011).

1.1.1. Cavity and oral mucosa structure

Oral mucosa varies considerably in structure in various regions of the oral cavity, but three main types of mucosa can be individualized in relation to their main function: coating mucosa represents approximately 60% of the total surface, masticatory mucosa and specialized mucosa occupying the rest of 40%.

1.1.2. Clinical features

1.1.2.1. Coating mucosa

Under normal circumstances, oral mucosa is moist, here being found the minor salivary glands (Squier C, Brogden KA, 2011).

In the area of the upper lip and jugal mucosa are located the sebaceous glands. The colour of normal, healthy oral mucosa is pale pink. At negroid, metis, indian race etc. melanin deposits can be seen on the lips, vestibular or palatal gingiva and it appears as brown, black spots, well defined, asymptomatic (Romînu M, Bratu D, Uram-Țuculescu S et al, 1997). The lips colour is bright purple contrasting with the skin tone.

Oral mucosa vary considerably in firmness and texture. Lips or cheek mucosa, for example, is soft and flexible, while the gingiva and palate has a firm body, being deprived of mobility.

1.1.2.2. Gingiva

Gingiva is the part of the masticatory mucosa that covers the coronal end of the alveolar bone. Healthy gingiva have a pink colour and in areas of hyperkeratosis, of reaction to the traumatic impact of food, the colour of the gingiva is paler, slightly whitish (Squier C, Brogden KA, 2011).

The gingiva may be non-adherent, free (marginal gingiva) and fixed, attached (adherent gingiva). The boundary between free gingival margin and fixed gingiva is marked by the sulcus of free gingival margin (gingival sulcus). In the gingival sulcus is an serous exudate derived from the epithelial insertion, the crevicular fluid (the fluid of the gingival sulcus, sulcular fluid) (Dumitriu HT, 1999).
Interdental gingiva (interdental papilla) includes the marginal gingiva lying between two adjacent teeth, beneath the contact point, in the cervical embrasure. It consists of interdental papilla vestibular and oral, joined by a col, having an hour-glass shape.

I.2. THE HISTOLOGY OF THE ORAL MUCOSA

Histologically, the oral mucosa is composed of non-keratinised stratified squamous epithelium in permanent exfoliation, corium (lamina propria) richly vascularized and submucosa showing small salivary glands. Between the epithelium and corium is located the basal membrane. (Crăițoiu S, Crăițoiu M, 1995). This non-keratinised epithelium is met in the oral vestibule, jugal mucosa, oral floor, soft palate and tongue ventral face.

On the other hand, on the level of the hard palate, dorsal side of the tongue and gingiva there is a complete or incomplete keratinized epithelium (paracheratosis) due to the pressures they suffer during mastication (Romînu M, Bratu D, Uram-Țuțulescu S et al, 1997).

I.3. HISTOPHYSIOLOGY OF THE ORAL MUCOSA

Oral mucosa performs a variety of functions, the most important being the protection, forming a barrier against microbial aggression or mechanical trauma. Other features include sensory perception, absorption (nitrates are absorbed sublingually), secretion, excretion of various products of metabolism, thermoregulation (mostly in animals) and an aesthetic role represented by the mucocutaneous confluence (Squier C, Brogden KA, 2011).

CHAPTER II

HYPERPLASIA OF ORAL MUCOSA DETERMINED BY LOCAL CAUSES

II.1. THE AETIOLOGY OF GINGIVAL HYPERPLASIA

The aetiology of the gingiva outgrowth is not fully known, however it can be directly correlated with individual susceptibility, various systemic diseases, local factors represented by cavities or iatrogenics in the presence of bacterial plaque and the action of drugs or their metabolic products (Arvind KS, Hardik JS, Mallika AP et al, 2010; Rossmann JA, 2011).

II.2. THE CLASSIFICATION OF GINGIVAL HYPERPLASIA

Gingival overgrowth are classified according to several criteria: etiologic factors, pathological changes, location and distribution (Newman MG, Takei HH, Carranza FA et al, 2002).

II.2.1. Inflammatory gingival hyperplasia

Inflammatory gingival hyperplasia is the most common form of outgrowth being induced by local irritant factors and is particularly associated with interdental papilla (NW Savage, CG Daly, 2010).
II.2.1. Chronic hyperplastic gingivitis

Gingival inflammation can lead to chronic gingival hyperplasia under predisposing factors and it occurs especially at the interdental papillae or the free gingival line (Dumitriiu HT, 1999).

The gum is increased in volume, of soft or firm texture, red, with smooth surface. Progresses slowly without pain. The lesions may undergo a spontaneous reduction in size, followed by exacerbation and continuous expansion (Dumitriiu HT, 1999).

Chronic inflammatory gingival overgrowth show characteristics of exudative and proliferative chronic inflammation.

II.2.1.2. Reactive hyperplastic lesions of the gingiva

Reactive hyperplastic lesions that can be seen on gingiva include local fibrous hyperplasia, pyogenic granuloma, granuloma with giant cells and peripheral ossifying fibroma (POF). These injuries may occur as a result of local irritants, such as trauma, microorganisms, plaque, calculus, faulty restorations and dental appliances and appear as a gingival proliferation. It represents approximately 9% of total gum increases (Bhasin M, Bhasin V, Bhasin A et al, 2013; Nartey NO, Mosadomr HA, Al-Cailani M et al, 1994; Kfir Y, Buchner A, Hansen LS, 1980; Zhang W, Chen Y, An Z et al, 2007).

II.3. PATHOGENIC MECHANISMS

Gingival overgrowth occurred triggered by a hyperplastic and hypertrophic process consists in forming a fibrous connective tissue with different degrees of inflammation.

At the conjunctive level, cell proliferation and differentiation, the synthesis of extracellular matrix are dependent on the cytokines produced by the cells involved in the non-specific defence process (macrophages) and, as well, in the specific defence process (lymphocytes). These cells associated with the fibroblasts, initiate different signalling cascades. Growth factors, metalloproteinases and myofibroblasts are also involved. It is considered that there is an imbalance in the synthesis and degradation of extracellular matrix leading to a significant accumulation of collagen at this level. Associates inflammatory and immune responses to the extracellular matrix level through various very active mediators.
CHAPTER III
CLINICAL STATISTICAL STUDY OF GINGIVAL OVERGROWTH CAUSED BY LOCAL FACTORS

III.1. The material used

A group of 74 patients who addressed the dental surgery showing signs of gingival overgrowth to varied extension, associated with fillings, cavities, fixed prostheses, dentures, orthodontic appliances.

Fragments of hypertrophied gingival mucosa have been collected from these patients. The patients are not registered with systemic diseases and they are not under treatment.

III.2. Methodology

A personal data sheet has been filled out for each patient stating personal data and the local and systemic clinical status followed by:

1. Oral hygiene exam with the determination of simplified oral hygiene index (OHI-S) and O’Leary plaque index (1993),

2. Coating periodontal examination with the determination of the Loe / Silness gingival inflammation (Loe et al, 1967) and papillary bleeding index on survey (Papillary Bleeding Index or PBI Muhlemann and Son index 1971),

3. Periodontometry to determine the depth of periodontal pockets (probing pocket depth or PPD), gingival insertion level (clinical attachment level or CAL), gingival overgrowth index.

III.3 Results

In most patients, regardless of age or area of origin, although clues of oral hygiene emphasis a good or satisfactory hygiene, i found a gingival overgrowth of grade 2 and 3 in cases with important coronary destructions (proximal carries and scrap roots) and in cases with fixed dentures and fillings overflowing and moderate and severe gingivitis.

Periodontometry performed on areas affected by local irritant factors, highlights bleeding either line or drop or mass bleeding covering portions of the tooth, false gum pockets due to gingival outgrowth without significantly changing the level of gingival insertion.

III.4 Discussions

In this study, the reasons for addressing the dental surgery were spontaneous gingival bleeding on brushing or chewing, debris retention, halitosis. On intraoral examination we found the presence of dental injuries ranging from simple proximal caries to deep caries with major coronary destruction or radicular scrap and the presence of dental restorations represented by overflowing fillings with rough, unpolished surface, fixed prosthesis with
oversized edges or poor adaptation on gingival level.

In all cases studied, I found an overgrowth of the adjacent gingival tissue. Relative to those retentive areas, the gingiva is red-violet and the palpation of the gingival sulcus with the periodontal probe highlights important gingival bleeding, presence of plaque on the restorations surface and the presence of debris in the retentive areas.

The periodontal tests and gingival index is calculated on the affected areas and highlights the gingival tissue inflammation. Periodontal tissue can be injured from the stage of preparation of teeth through the used method or due to the devices used for the coronary reconstruction (gingival retraction wire, matrices, wedges, etc.), and subsequently if these restorations generate premature contacts or occlusal interferences if they have a poor marginal adaptation both vertically and horizontally or if their surface remains rough favouring retention of plaque (Tadumadze L, 2005).

Restorative therapy must take into account that well finished and polished surfaces, which show no edges or undercuts or rugosities, helps to maintain a healthy periodontal tissue.

CHAPTER IV
HISTOLOGICAL STUDY OF GINGIVAL OVERGROWTH OF LOCAL CAUSES

IV.1. Study material

Gingival fragments from 30 patients have been used as study material. The patients have been randomly picked from the study group with the clinical diagnostic of gingival overgrowth, particularly associated with fixed dentures, fillings, dental caries.

The biological material sampled was immediately fixed in neutral formalin solution 10% for 3-5 days at the laboratory temperature.

IV.2. Methods used for histological study

The histological paraffin embedding technique used in this study performed the following stages: dehydration, clarification, paraffination and the actual embedding.

The obtained paraffin blocks were sectioned at a thickness of 3.5 mm, using a microtome Microm HM325 with a cooling system of the paraffin block sections and transport of the sections on the water film. Serial sections thus obtained were placed on histological blades, pre-treated with albumin, for conventional histological staining.

IV.3. Results

On the material stained through histological technique it was observed a marked gingival hyperplasia of the gingival epithelium, with the occurrence of an inflammatory infiltrate in the both superficial and deep lamina propria.

The increase in thickness of the epithelium was in all the study cases triggered by
hypertrophy and hyperplasia as well.

It was also noted the frequent occurrence of the phenomenon of acanthosis and epithelial cell ballooning areas that led to the breaking of desmosomes in the middle layer. These phenomena were present in cases of inflammatory cells found in the thickness of the epithelial coating.

IV.4. Discussions

Gingival overgrowth may occur by an imbalance between synthesis and degradation of extracellular matrix, release mechanisms of this process are not fully understood, although literature presents numerous data in this regard. The histological support of the gingival overgrowth associates a hypertrophy process (increase in size) and a hyperplasia process (increased number of cells that may be associated with an increase in the extracellular matrix) (Janosi K, Popsor S, Ormenisan A et al, 2013).

The proliferation and differentiation of connective tissue cells and the extracellular matrix production is controlled by cytokines which initiate a signal cascade mediated by specific receptors. Factors determining gingival hypertrophy alters the normal balance of cytokines in gingival tissue. (Arora PD, Silvestri L, Ganss B, Sodek J, McCulloch CA, 2001).

CHAPTER V

IMMUNOHISTOCHEMICALLY STUDY OF GINGIVAL OVERGROWTH OF LOCAL CAUSES

V.1. Study method

For the performance of the immunohistochemically study, the tissue went through the same primary process steps as in the histologic study. Thus it was fixed in 10% buffered formalin and embedded in paraffin according to the above described technique. The sectioning the paraffin block was performed to a thickness of 3.5 m and sections thus obtained were placed on poly-l-lysine blades.

Primary antibodies used

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Producer</th>
<th>Antigen unmasking</th>
<th>Dilution</th>
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<tbody>
<tr>
<td>CD 20cy</td>
<td>DAKO</td>
<td>Citrate buffer pH=6</td>
<td>1:50</td>
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<td>1:50</td>
</tr>
<tr>
<td>MMP 8</td>
<td>abcam</td>
<td>Citrate buffer pH=6</td>
<td>1:500</td>
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<tr>
<td>MMP 9</td>
<td>abcam</td>
<td>Citrate buffer pH=6</td>
<td>1:200</td>
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The base used in this study for the detection of the immunohistochemically signal was DAB (3-3' diaminobenzidine tetrahydrochlorid) code K3466, DAKO.

The iconography was performed with a Nikon Eclipse 90i microscope and NIS Elements software.

V.2. Results

The immunohistochemically study allowed the identification inflammatory pattern of mucosal gingiva. I noticed that most cells of the inflammatory infiltrate are of lymphocyte and macrophages type. The inflammatory infiltrate was with diffuse disposal, with areas prone to nodular organization.

The patients with various forms of periodontitis have high levels of MMP-9 which is expressed in the gingival fibroblasts, keratinocytes, resident macrophages and neutrophils.

Through the immunomarking at MMP-8 was shown the presence of the enzyme at fibroblasts level from gingival lamina propria with chronic inflammatory infiltrate, and as well to the level of the inflammatory cells in particular the polymorphnuclear type, with cytoplasmic staining pattern.

V.3. Discussions

Extracellular matrix components form a structure which ensures the integrity of tissue and regulates cell migration and represents a reservoir for cytokines and growth factors. In response to various stimuli, extracellular matrix is in changing, in reconstruction. Out of the proteases involved in the proteolytic degradation of extracellular matrix, the most important is the metalloproteinases group (MMPs) (Stamenkovic I, 2003).

In diseases involving inflammation, such as periodontal affection, MMPs are present, having increased levels.

In our study the immunoreactivity to MMP-9 was intensely positive to patients in whom clinical cure was delayed and negative to subjects with evident clinically cure.

MMP-8 was present in all the cases studied, variable in intensity from one preparation to another, with disposition in chorion, and among the epithelial cells, as well.

CONCLUSIONS

Faulty restorative treatments with overflowing edges presents iatrogenics with different consequences on the gingival tissue over a long or short period, and the severity of impairment varies from one country to another and from one practitioner to another.

Histological study revealed an increase in thickness of the gingival epithelium, associated with cellular and vascular changes of the chorion.
Patients with positivity at MMP-9 and showed a positive immune response to MMP-8 and to patients with micro bleeds, MMP-8 has been highlighted to the hematic infiltrate and immune response to MMP-9 was absent.

REFERENCES
