PhD THESIS

CONTRIBUTIONS AT THE HISTOLOGICAL STUDY OF GINGIVAL HIPERTROPHY

ABSTRACT

PhD COORDINATOR
Prof. Univ. Dr. ŞTEFANIA CRĂIŢOIU

PhD ELENA ANNABEL NIŢULESCU

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INTRODUCTION

Many specialisation studies insist on the high frequence of gingival hypertrophy which, no matter of the etiology, represents an invalidating dysfunction both from physical point of view affecting the general state of the body and causing oral functional troubles, and from a psychological point of view affecting facial estetics. Although there have been many researches, pathogeny and its mechanisms of apparition are not fully elucidated.

The origin of gingival hypertrophy is attributed to interaction of several genetical factors: hormonal ( during puberty and pregnancy ), different types of medication ( fenitoin, cyclosporine, dyltiasem, amphetamins ), growing factors (TNF-α) and neoplasical factors. The most common cause of gingival hypertrophy is the chronical gingival inflammation as a respons to the long exposure to the bacterian plaque. There is no known study between gingivite and gingival hypertrophy; if a preexisting gingivites stimulates gingival hypertrophy or if hypertrophy, by gathering gingival plaque caused by some hygienical difficulties it produces gingivites and by its development it determines a paradontal disease.

In this work we studied gingival hypertrophy of local cause induced by the microbian plaque. Therefore the goal of this study is to evaluate the presence of gingivite and/or of paradontite at patients with gingival hypertrophy and the percentage in which there is a relations between gingival hypertrophy and inflamatory process that can contribute to a high stimulation of the hypertrophy process.

KNOWLEDGE STUDY

CHAPTER 1
HISTOLOGY AND HISTOPHYSIOLOGY OF THE PERIODONTAL

The odont or dental organ represents the unit of structure and function of the dendo-alveolar archs. It is made of different kinds of tissue; they have the capacity to bear, soften and transmit the masticator pressures. It is made up of tooth or odont which consists of dental pulp, dentine and enamel and support tissues and link between the tooth and maxilar that make up the paradont. Dumitriu HT, 2009 shows that mantaining and support of the tooth in the maxilar bones is made by the marginal paradont. The notion of marginal paradont is used for the totality of tssues around the tooth except for those around the apex, for which one uses the notion on apical paradont. The marginal paradont has two main parts:
  A. Deep paradont, supporting or functional, made up of radicular cement, desmodont, alveolar bone;
  B. Superficial paradont or cover, made up of gum ( epithelium and chorion) and supraalveolar ligaments.

CHAPTER 2
PARODONTAL DISEASE

The paradontal disease is represented by a group of inflamatory conditions and infectious nature of the exterior paradontal caused by bacterian plaque which constantly appear on the teeth and develops toxins which affect the gum and the support tissues of the tooth. In its occurrence and evolution, a major importance is given to the general and localized factors. The paradontal disease consists of two anatomo-clinical entities : gingivites and paradontites. It's occurrence among pacients is in a continuous growth.

The paradontal disease is an affection of the paradontal structure, having an infectious cause and having chronical inflamatory lesions, being associated or not with distrophic or proliferative lesions, with a progressive evolution, which determins the distruction of the support tissues and, finally, causing the tooth to fall.
CHAPTER 3

CELLULAR MECHANISMS INVOLVED IN GINGIVAL HYPERTROPHY

It is a well known fact that the pathogeny of gingival hypertrophy is multifactorial and its development is overdeveloped by the growth of bacterial plaque.

Cytokines, (CTGF) metalloproteinases and miofibroplasts have a role in the development and evolution of this affections. There is still controversy regarding the role of the dental plaque:if this is important for the onset and worsening of gingival hypertrophy. Some studies have found a significant correlation between the occurrence and the severity of gingival hypertrophy and the accumulation dental plaque (O’Neil TC, Figures KH, 1982). Other studies show that a good oral hygiene can reduce gingival hypertrophy but can not prevent its apparition (Takada K, Sugiyama H, Umezawa K, Mega J, Hirasawa M, 2003).

PERSONAL CONTRIBUTION

CHAPTER 4

HISTOLOGICAL STUDY OF GINGIVAL HYPERTROPHY INDUCED BY BACTERIAL PLAQUE

MATERIAL AND METHODS

The study material was composed by fragments of gingival mucosis (53) which came from patients of the Surgical Clinic OMF of the Emergency Clinical Hospital Craiova and from some dental clinics from Craiova, ages ranging from 20 – 65 years, between october 2008 – march 2011. They have been divided in 3 categories :

- Group I – patients without lesions from which we have obtained 10 fragments of healthy mucosa during odontectomy of the included wisdom molars;
- Group II - patients suffering from gingival hypertrophy and gingivites but did not have affected sustaining tissues from which we have obtained 17 fragments of gingival mucosis;
- Group III - patients suffering from gingival hypertrophy and generalised chronical marginal paradontitis (15) and patients suffering from gingival hypertrophy and aggressive generalised marginal paradontitis (11) from which we have obtained 26 fragments of gingival mucosis.

Methods used

Harvested fragments of the gingival mucosis have been fixed in 10 % formalin for 24 hours and treated using the clasical procedure, in order to be included in blocks of paraffin. After that, we have used the microtome to cut 3-5 µ sections, which have been histological and histochemical stained.

RESULTS

The sections of the gingival mucosis obtained from group I after odontectomy of the included wisdom molars show a normal structure of the gingival mucosis, formed from the epithelium and the lamina propria.

The malpighian stratified epithelium sends deep prolongations into the chorion, the epithelial ridges, between which, the chorion also sends ridges, the connective papillae. Therefore, they form an interconjecture between the epithelium and the connective papillae which gives the mucosa a higher resistance to stress. On sections that came from groups II and III there have been identified various lesions, which affected both the surface epithelium and the adiacent chorion and whose intensity depended on the duration and evolution of the disease.

The epithelium has acantose and sometimes acantolise, hipercheratosis and some zones with paracheratinisation. On the chorion, the colagen fibrilar component is associated with an inflammatory chronical process formed mainly from lymfocites, macrophages, plasma cells and a higher number of fibrocites. The presence of the inflammatory limfoplasmocitar and macrophages infiltrate shows the existence of an immunological process but also a macrophagic one.
In the chorion, hialinised collagen fibers are predominant, organised in longitudinal strips, with a denser setting or with a more loose one. The dense network of thick collagen fibers is the consequence of an intense process of collagen synthesis.

We also identified exudative and vascular changes: an increased blood flow mainly in the subepithelial area, this localisation is justified by the epitheliums need for multiplication. The inflammatory infiltrate from the chorion is associated with neoformation capillaries which add to the development of the extracellular component through the addition of necessary substances that are needed for the increased synthesis that takes place.

In the chronic stage, gingivite can evolve into superficial marginal chronic paradontitis: the gum’s epithelium has hiperkeratosis and parakeratosis, has an unequal width and alternates with ulcerative areas. In the chorion there is a limfoplasmocitar infiltrate dens and pale or nodular, vasodilation, endarterita lesions with reduction of the vascular lumen. There is also a numerous number of neoformations capillaries with edema areas and with the disorganization of the fibrillar component. Gingival inflammation, in patients with gingivitis and periodontitis patients was associated with hypertrophic changes which may affect the conjunctival epithelial structures with reduced conjunctival reaction or may predominantly affect in the chorion’s connective fibrous structures with minimal response from the epithelial component. In our casuistry, I met mainly the involvement of the fibro-connective structures.

DISCUTION
The origin of gingival hypertrophy is attributed to interaction between several factors including: genetic predisposition, hormonal factors during puberty and pregnancy, treatment with various drugs (phenytoin, cyclosporine, diltiazem, amphetamines), various malignancies. The excessive growth of dental plaque and chronic gingivitis were also added to the predisposing factors of gingival hypertrophy. The inflammation inflicted by the dental plaque determines an increased proliferation of the conjunctival tissue, the catabolic capacity of collagenesis is saturated and therefore the degradation of the extracellular matrix is inhibited, resulting in local accumulation (Brown RS, Beaver WT, Bottomley WK, 1991; Brunet L, Miranda J, Farré M, Berini L, Mendieta C, 1996; Marsh PD, 2005).

Gingival overgrowth determined by the chronic or acute inflammation of the gingival mucosis – gingivites or paradontitis is defined as a pathology induced by the persistancy of a bad oral hygiene which determines the appearance of bacterial plaque and the infestation of the oral tissue by germs(Armitage GC 1999; Trackman PC ,Kantarci A, 2004; Bartold P, Narayanan AS, 2006; Lin K, Guihoto LMFF, Yacubian EMT, 2007). Most authors agree that histological changes are not specific, they consist, no matter the etiological factor, in various degrees of fibrosis or extracellular matrix overgrowth to which we add inflammatory tissue cumulation.

In gingival overgrowth there is a development of conjunctival tissue associated with an inflammatory process of different intensities and an epithelial hyperplasia (Clocheret K, Dekeyser C, Carels C, Willems G 2003).

The conjunctival tissue of the examined gingival mucosis sections is made of fascias of hialinized collagenous fibres, with a different distribution, either denser or looser. These are associated with an inflammatory infiltrate, especially limfoplasmocitar. We also met an increased vascularization localised mainly in the subepithelia, with dilated blood vessels, which indicates the presence of some angiogenic factors which are associated with mediators of the inflammatory process (Gawish A, Gamal-Eldeen AM, Sheriff SH, Neamat S, 2010).

A gingival overgrowth can show in the first part a proliferation of the collagen fibres and secondly it can be inflammatory, or it can be inflammatory and become fibrous (Lundergan, 2003).

There are still many uncertainties regarding the ratio between gingivitis and gingival overgrowth, if preexistent gingivitis stimulates gingival growth, or if overgrowth can, because of the difficulties in oral hygiene, cause the accumulation of bacterial plaque and gingivitis followed by destructive parodontal condition. The study developed by Pinheiro Feitosa MG, 2006 had as a goal the objective of evaluating if gingivitis is present to all patients with gingival hyperplazia, and if the inflammatory process responds to the antiinflammatory treatment.

Gingival overgrowth has increased, both to young people, adults and elderly, because of local cause or because of systemic condition and also because of medication prescribed for this conditions (Seymour RA, 2006). Although the emotion and psihological aspect of gingival
overgrowth is overlooked or slightly considered because the number of patients with this condition is increasing, this aspect becomes more and more important, the changes in the normal facial aspect can affect the quality of life.

CHAPTER 5

THE IMUNOHISTOCHEMICAL STUDY OF THE INFLAMATORY FIBROSYS IN GINGIVAL OVERGROWTH INDUCED BY BACTERIAL PLAQUE

MATERIAL AND METHODS

Research material
The research study was conducted on human tissue - gingival mucosis obtained after ortodontic treatment or after paradontal surgery. We have divided the study into these groups:

Group I - control group - 8 cases - patients without gum condition from which we have collected gingival mucosis as a result of dental extraction or ortodontic treatment;

Group II -8 cases patients which have been diagnosed with gingivites but without distructive lesion of the support tissue from which we have collected gingival mucosis as a result of dental extraction or ortodontic treatment;

Group III -15 cases patients who have generalised or localised parodontitis: III - a - chronic paradontitis 8 cases; III - b agressive paradontitis 7 cases;

We have excluded pations who had gingival overgrowth as a result of sistemic disease, diabetes, endocrinopathies or whose preliminar examination showed treatment with corticosteroids, immunosuppressive drugs, antiepilepsy; they have also excluded patients who were on antibiotics in the past 6 month.

Methods used
The study material was processed for immunohistochemical study, using the following antibodies: MMP-1, TGF(31, CTGF, TIMP1.

Results

THE STUDY OF EXTRACELLULAR MATRIX MODELLING IN INFLAMATORY GINGIVAL OVERGROWTH

The study of extracellular matrix modelling in inflammatory gingival overgrowth has been developed after rendering and adding the results which came through imunohistochemical staining.

The intensity of the imunohistochemical reaction ( brown cytoplasmatic precipitate) was considered absent ( negative or diffuse reaction ) or present ( strong or moderat reaction ) after evaluating the signal ratio on the whole slide.

Imunohistochemical response of MMP - 1

In a healthy or lesser inflamed gum the positive response was seldomly present and very discreet in keratinocites. In lamina propria the positive response for MMP - 1 was only found in the normal gum in a few cells from the superficial chorion.

In gingivitis the imunohistochemical response for MMP -1 was more proeminent at the lamina propria level.

In chronic paradontitis the epithelium has shown a high level of acanthosis, with numerous clear cells even in the spinous layer and variable response for MMP -1, with a higher level in the spinous and the granular layer, and absent in the bottom layer.

In agressive paradontitis the gingival epithelium has mentained the same morphological features. The chorion has shown an important increase in inflammatory cells ( plasma cells, macrophages, and rarely polymorphonuclears cells ) many of which had a positive reaction for MMP - 1.

Imunohistochemical response of TIMP -1
In gum inflammation the positive response was shown at the epithelium, but was mainly seen in the bottom layer, descending with epithelial crests in the depth of the conjunctival tissue.

In chronic gingivitis, the response for TIMP – 1 is a lot higher in the superjacent area of accumulation of fibrotic tissue. In areas in which the lesional aspect of the fibromucosa is dominated by the accumulation of inflammatory tissue, TIMP-2’s response is a lot more discrete in the epithelium. In aggressive parodontitis the immunohistochemical reaction for TIMP – 1 was mainly negative.

**Immunohistochemical response of HTRA-1**

In normal gum, the epithelium has shown a discreet positive reaction, the intensity is gradually increasing from the bottom layer towards the upper one. In lamina propria, the cells and the extracellular matrix were negative. The slides that came from gingivitis cases have also shown a discreet epithelial positive response.

In chronic parodontitis as in the aggressive one, the positive epithelial response for HtrA-1 was more intense than in gingivitis, and by comparison, in aggressive parodontitis the positive response was more prominent than in chronic parodontitis. The presence of HtrA-1 in the gingival epithelium and in proinflammatory cells is linked directly with the severity of the inflammation.

**THE STUDY OF GROWTH FACTORS INVOLVED IN ACCUMULATION OF THE EXTRACELLULAR MATRIX THAT CAME FROM INFLAMATORY GINGIVAL OVERGROWTH**

**The immunohistochemical response of TGF-β 1**

In gingivitis, we have seen a few keratinocytes with a relative intense immunoreactivity shown in the deep layers of the epithelium; in lamina propria the positive response was seen in the chorion’s papillae. In chronic parodontitis the immunoreactivity for TGF-β1 was more intense in lamina propria. In aggressive parodontitis, the reaction of the gingival tissue for TGF-β1 was greatly increased.

**The immunohistochemical response of CTGF**

In the gingival mucosa that came from patients from the control group, the reaction for CTGF was negative. In gingivitis we have seen an extremely low immunoreaction. Chronic parodontitis was best seen as a result of epithelial positive response, the groups of positive cells being distinguished from other groups by negative cellular clusters or those with diffused positive response. In aggressive parodontitis the positive response was revealed in the epithelium, and in the lamina propria we have seen the presence of diffused immunostains.

**DISCUSSION**

There is a permanent link between the inflammatory cells population and the extracellular matrix’s turnover in inflammatory gingival overgrowth. This connection is the result of synthesis and release of numerous biomarkers (cytokines, chemokines, growth factors) by epithelial cells or by mesenchymal cells, but the importance of these molecular cells on the reaction of the gingival fibromucosa is modulated, as recent studies show, on individual susceptibility (Garlet GP, Cardoso CR, Campanelli AP, Ferreira BR, Avila-Campos MJ, Cunha FQ, et al, 2007).

In our study we performed a series of immunohistochemical reactions for evaluating the activity of MMP-1, which is a part of the collagenases class, also named fibroblast-type collagenases FIB-CL (Birkedal-Hansen H, Yamada S, Windsor J, Pollard AH, Lyons G, Stetler-Stevenson W 2008).

Like many more MMP, although produced by a vast variety of “in vitro” cells, MMP-1 is not detected in normal tissue in a resting state (Pardo A, Selman M. 2006). Our results show a high presence of this metalloproteinases as the inflammation develops. The results indicate that a gingival overgrowth from the parodontal inflammation has an extremely polymorphic reaction for TIMP-1, ranging not only from one batch to another, but within the same batch in relation to lesion appearance noted: inflammation or fibrotic tissue deposition.

In this paper we tried to follow the dynamics of parodontal inflammation, of TGF –β1. The results have shown an increase of TGF -β1 from normal gum, to chronic parodontitis and to the aggressive one. The fibrous accumulation represents a normal action of the scarifying and repairing
process (Atamas SP, 2002), physiological healing processes, inflammation and fibrosis involving the action of the same molecules and cellular events (Bartold PM, Narayanan AS, 2006).

Among these molecules involved in connective tissue deposition by stimulating the synthesis we include connective tissue growth factor (connective tissue Growth Factor – CTGF).

CTGF also known as CCN2 is a protein, rich in cistein, made up of 349 aminoacid compounds, one of the six member of the profibrogenetic proteins, CCN, and can act as effector consecutive to the activation by TGF—β in various tissues that develop fibrotic lesions (Leask, A Abraham DJ, 2003). CTGF has a mitotic role, leading to the division of some cell categories, like fibroblasts (Brigstock DR, Steffen CL, Kim GY, Vegunta RK, Diehl JR, Harding PA, 1997; Asano M, Kubota S, Nakanishi T, Nishida T, Nakanishi T, Asano M, Shimo T, Takigawa M, 2005).

GENERAL CONCLUSION

1. Gingival overgrowth, with its aesthetics implication and the provider of growth places for microorganisms represents a serious concern, both for the patients and the physicians.

2. The epithelium, with different sizes in it’s different zones, develops acantosis and sometimes acantilosis, hipercheratosis and areas with paracheratosis. In chorion, the fibrillar collagen component is associated with a chronic inflammatory process consisting mainly of lymphocytes, macrophages and plasma cells and a larger number of fibrocites.

3. We identified vascular modifications: an increased vascularization, mainly in the subepithelia, justified by increased proliferation of the epithelium; dilated blood vessels are present, with thickened walls and with a reduced lumen and sometimes swelling which dissociates the upper chorion and neoformation capillaries that come to support the development of the extracellular component by adding necessary substances which are needed for the increased synthesis.

4. Both patients with gingivites and those with parodontitis have shown an increased gingival overgrowth pointed out by a process of acantosis, mixed with the increased number of fibroblasts, the development of collagenous fibrilar component layed out as a denser or looser fascia.

5. Our result suggest that gingival overgrowth, as a result of bacterial plaque is determinated by the alteration of the tissue homeostasis by altering the cellular group involved in the tissue turnover and those who participated in the inflamatory proces.

6. Different structural aspects found under the microscope, for the slides that came from different patients suggests that the reaction found in different slides represents a detereminant factor which added with the reaction of the microorganisms from the dental plaque determines more intense or mild changes at the gum level.

7. The gingival fibromucosis cells show a mixture of MMP – 1 and TIMP 1 depending on the stage of the condition. The response of MMP 1 in parodontal condition is variable in the same stage of the inflammation, the presence of varying immunohistochemical study detected from changes in the inflammation-fibrosis evolution.

8. The significant increase of the response in HtrA in the gingival epithelium but also in plasma cells suggests the active role of this molecule in the complex scenario of periodontal breakdown and infection control.

9. Tissue lesions coming from a parodontal condition are represented not only by tissue destruction but also the accumulation of fibrous tissue, the study of pathogenic mechanisms for the latter being the major contribution made by this paper.

10. This research offers the posibility of understanding some aspects regarding the pathogeny of the condition and indicates the posibility of developing a new strategy for reducing non-surgical overgrowth through inducing apoptosis in fibroblasts, associated with anti-inflammatory medication, which in turn would stimulate apoptosis
CURRICULUM VITAE

First name/Last name: Elena Annabel Nitulescu Date and Place of Birth: 03.06.1983; Craiova, Dolj Nationality: Romanian Telephone number: 0743104441 Electronic Mail: annabelnitulescu@yahoo.com; Education: - 1998-2002 - Mihail Sadoveanu `High School, Bucuresti; -2002-2008 - University of Medicine and Pharmacy of Craiova, Faculty of Craiova; -2008-2011- PhD student Foreign Languages: English, Italian Technical competence:- Microsoft Office

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- The novel role of HtrA1 in gingivitis, chronic and aggressive periodontitis. Lorenzi, Teresa; Nitulescu, Elena Annabel; Zizzi, Antonio; Lorenzi, Maria; Paolinelli, Francesca; Aspriello, 'simone Domenico; Banița, Monica; Craițoiu, Ștefania; Gaia, Gotei; Piemontese, Matteo; Lombardi, Tommaso; Corrado,' Rubini; Castellucci, Mario. Histopathology. In press Manuscript ID HISTOP-11-11-0586.

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