UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

DOCTORAL SCHOOL

PhD THESIS

ABSTRACT

CLINICAL AND MORPHOLOGICAL STUDY OF FIBROTIC GINGIVAL ENLARGEMENT

SCIENTIFIC COORDINATOR:
Prof.Univ.Dr. Mihai Surpăţeanu

PhD Student:
Eugen Ionuţ Pascu

Craiova
2013

CONTENTS

Keywords

Gingival enlargement, gingival fibromatosis, antibody,antigen,cyclosporine A (Csa),connective tissue growth factor (CTGF),matrix metalloproteinases (MMP),mesenchymal epithelial transition (TEM).

CHAPTER I

HISTOLOGY AND HISTOPHYSIOLOGY OF ORAL MUCOSA

1. Histology of oral mucosa

Oral mucosa has ectodermal origin, from the internal layer of the embryonic disc. Is coats the entire oral cavity, the initial part of the digestive tract. The oral cavity represents a virtual cavity located above lip, posterior to isthmus of the fauces, next to the cheeks, above the hard palate and under the tongue and sublingual space.

The oral cavity is divided by the alveolar-dental arches in two compartments:

- vestibule: a horseshoes shaped space located is between the cheeks, lips and alverolar and dental arches;
- the proper oral cavity located behind the alverolar and dental arches.

Regardless of the topographic area in the oral cavity that it coats, the oral mucosa consists of two distinct structures, outside the **epithelium oral,** squamously stratified, placed on a continuous base membrane and **chorion**, called the lamina propria, which is connective tissue well vascularised that supports the oral epithelium.

From a functional point of view, the oral mucosa is subdivided into three major histological varieties: coating mucosa -covering the cheeks - jugal mucosa, lips -labial mucosa, oral floor, the ventral side of the tongue and soft palate; chewing mucosa – coats the the gum the and hard palate and specialised mucosa - located on the back side of the tongue (Baniță and Deva, 2006).

Coating mucosa represents about 60% of the oral mucosa surface. This type of mucosa has great flexibility, being thus adapted to chewing, phonation and swallowing. It is formed by non-keratinised epithelium, located on a lax conjunctive chorion, highly vascularised, which links it to the subadjacent structures.

Specialised mucosa (sensory) represents approximately 15% of the oral mucosa surface covers the back side of the tongue. It is a mucosa with variable degree of keratinisation, with polymorphous bumps called lingual papillae, directly linked to the muscle surface of muscular and membranous structure of the tongue.

Masticatory mucosa, represents approximately 25% of the total oral cavity surface. As corresponding topographic area, this type of mucosa covers the gums and the hard palate, parts that are constantly stressed by the masticatory actions. It consists in keratinised epithelium, closely linked to subadjacent periosteum.

2. Vascularisation and innervation of the oral mucosa.

The oral mucosa is highly vascularised and innervated. Many blood arteries, emerged from the internal carotid artery, form in the mucosa structure two anastomosed arterial networks: one in the deep chorion and other in the superficial chorion. The two arterial networks give multiple arterioles and capillaries which form numerous papillary vascular plexus. Apparently, each of the chorion papillae has at least four capillaries. The veins have a similar tact to that of arteries. Innervation of oral mucosa is rich, somatic and vegetative. Sensory nerve fibers, mainly belong to the trigeminal nerve, which gives free nerve endings both at chorion level and at oral epithelium level. In addition, there are sensitive corpuscles in the chorion (Meissner, Golgi, Rufina). All these nerve structures provide tactile, thermal and painful general sensitivity of oral mucosa (Ross and Pawlina, 2003).

3. Histological characteristics of gingival mucosa.

Gingival mucosa or gum represents the part of the oral mucosa that covers the alveolar bone and teeth in the cervical region. Together with gum ligaments it forms the periodontal coating. The terms "clinically normal" or "clinically healthy" are used to designate the gum tissue characterised by the following aspects:

- shade of variable pale pink or coral according to the race and pigmentation. The factors that influence the color are the vascular contribution, the epithelial thickness, the degree of keratinisation. Melanin physiological pigmentation occurs frequently in African Americans, Asians,

Indians and Caucasians of Mediterranean countries.

- knife or blade aspect (or ”hemmed”), the gums that fit tightly around the toot.
- firmness and minimum depth of sulcus gingivae without bleeding when probed (Wilkins, 2013).

4. Functions of gingival mucosa

Protective function.

Gingival mucosa, through its keratinized epithelium, performs primarily the protective function of the subjacent structures and less the absorption and secretion function.

Sensory reception function

By receptors it contains for tactile, thermal, pain receptors, gingival mucosa contributes on one hand to organism defence against harmful substances penetration informing the central nervous system on the qualities physical and chemical properties of food introduced into the mouth and starts together with other structures in the oral cavity swallowing, salivation, mastication reflexes. (Nanci 2003, Baniță și Deva, 2006).

Absorption function .

Small quantity of some water-soluble substances are absorbed at gingival mucosa level: alcohol, cocaine, nicotine, nitroglycerin.

Excretion function (emonctory).

At gingival mucosa level are discharged - excreted, both by salivary gland channels and by phagocytic cells some harmful substances that have entered inside the body or resulted from metabolic tissue processes (urea, uric acid).

There were enzymes identified in the gum (alkaline phosphatase and acid phosphatase, glucose-6-phosphate-dehydrogenase, lactate dehydrogenase, β-glucuronidase) involved in the metabolic, inflammation, healing and scarring process (Ross și Pawlina, 2006, Telser, 2007).

CHAPTER II - CLINICAL-MORPHOLOGY ASPECTS OF GINGIVAL ENLARGEMENT

1. Gingival enlargement concept

Gingival enlargement is clinically defined as volume thickening or increase of soft tissue covering the alveolar ridges with more than 1 mm, the enlargement degree can be different, from limitation of interdental papilla to covering the entire tooth crowns (Desai and Silver, 1998,American Academy of Periodontology, 2004, Lin and colab., 2007, Kataoka and colab., 2005,Carey and colab., 2009).

2. Classification of gingival enlargement

Clinically detectable gingival enlargement is classified in relation to the etiological factor and pathological changes which it determines:

- **reactive gingival enlargement** produced by the existence of bacterial plaque, is the most common form, called focal reactive gingival enlargement, or inflammatory hyperplasia, also known as epulis. Generally, epulis are sessile or pedunculated lesions of gum, the term is clinical and topographical, without a histological description of the lesion, thus the term gum reactive is rather used (Kfir și colab., 1980).
- **gingival enlargement determined by local chronic irritations** as root cavities residues, cavities, defective prosthesis.
- **gingival enlargement caused by the treatment** with certain medicine as: anticonvulsants of phenytoin type, immunosuppressants – A cyclosporine, antihypertensive calcium channel blockers – nifedipine; which is also called fibrotic gingival hyperplasia.
- **gingival enlargement determined by systemic diseases** – diabetes mellitus, certain leukemias.
- **congenital gingival enlargement**- epulis or Neumann tumor
- **gingival enlargement determined by hormonal imbalances**, appeared during puberty or pregnancy, also called epulis (Clocheret and colab., 2003).

- **gingival fibromatosis** also called gingival elephantiasis, or idiopathic gingival fibromatosis, hereditary gingival hyperplasia, gingival lesion without bacterial plaque, gingival gigantism, or just hypertrophic gums (Bittencourt and colab., 2000, Coletta and Graner., 2006)

3. Epidemiological characteristics of gingival enlargement

The incidence of gingival enlargement varieties are very different, according to the social and economic level of the population concerned and the incriminated risk factors, the data reported being 1/9000 adults, most of them are of inflammatory type, followed by drug-induced hypertrophies , hereditary gingival fibromatosis is the least common (www.maxillofacialcenter.com, Academy Report, 2004). Regardless of the incriminated etiologic factor, the presence of bacterial plaque is incriminated as an etiology cofactor, especially in drug-induced enlargement. (Academy Report, 2004).

4. Clinical and morphologic characteristics of gingival enlargement

The following **clinical classification** is the most current classification of gingival enlargement:
Degree 0 - no gingival modification is identified
Degree 1 - enlargement located on interdental papilla
Degree 2 - enlargement involved in interdental papilla and marginal gum
Degree 3- enlargement that covers ¾ or more of the tooth crown (Bokenkamp, 1994, DeAngelo, 2007, Douzgou and Dallapicolla,2011).

5. Histological characteristics of gingival enlargement

It is modern and widely accepted that regardless the incriminated etiological factor, the gingival enlargement is characterized by increased volume of the conjunctive tissue with different degrees of inflammation and fibrosis and thickening of the epithelium. The degree of inflammation, fibrosis, cell hyperplasia depend on the duration, dose and drug type if the enlargement is determined by the medicines and the quality of oral hygiene or individual susceptibility and external factors (Trackman și Kantarci, 2004).

6. Treatment for gingival enlargement

All authors emphasize the importance of proper oral hygiene, supported by regular professional care for favorable evolution of gum disease and as important adjuvant in case of surgery, with the purpose to delay the occurrence of relapses(Oh and colab., 2002, Douzgou and Dallapicola, 2011). Most of the authors claim that oral hygiene represents the key to reduce the relapses and their severity.

Classical surgery must be performed on (scales), in several sessions, because tissue can bleed excessively. Using the CO_2 laser allows the problem to be solved in one session as the intraoperative bleeding is reduced, which improves visibility and speeds up the execution.

7. Pathogenic ways involved in gingival enlargement

Summary of specialized studies show that inflammation, in scarring or fibrotic lesions, involves the same molecules and biological events (Bartold and Narayanan, 2006). The same authors state that fibrosis may occur as an answer to an isolated factor or of a combination of factors such as:

- abnormal release of mediators during inflammation. The synthesis of some molecules causes the release of others and their dialogue may have synergistic, cumulative or antagonistic.
- persistence of abnormal modifications in the action of growing and cytokines factors.

Motivation and study premises

We intended a corroborated, clinical, histological and immunohistochemical study of a variety of gingival enlargement clinically defined as fibroid or fibrotic gingival enlargement. Many local etiological factors or general genetics or hereditary, as well as some medicine, especially phenytoin, cause this variety of gingival enlargement, hence the confusion in terminology frequently occurred in the relevant literature is defined as gingival fibromatosis.

Taking advantage of the new techniques of research, especially the genetic and molecular biology ones, fibromatosis enlargement was framed more precisely in terms of defining the etiologic factors, including the hereditary fibromatosis, genetically caused by mutations in SOS

gene, syndromic, which recognizes numerous genetic mutations determining other clinical acts besides gingival enlargement and idiopatic fibromatosis determined by the unknown etiological factors, often hereditary or syndromic fibromatosis are included in this name category.

Objectives of the study

The main objective of this study it was to compare and corroborate the clinical, histological and immunohistochemical aspects of patients diagnosed on specialised examination gingival fibromatosis enlargement. The studied clinical cases originate from patients with fibrotic gingival enlargement determined by several etiologic factors. We have excluded from our study the enlargement determined by phenytoin, also with fibrotic features, but which recognises different pathogenic mechanisms, studied along with other medicine that cause gingival enlargement

The specific objectives of this project were:

1. Clinical characterisation of fibrotic gingival enlargementaccording to the etiological factor.
2. Histological characterisation of gingival mucosa according to the determined ethiological factor and the description of particular aspects of the epithelium and lamina propria.
3. Corroboration of fibrotic loading degree with the presence of inflammatory gums lesions.
4. Immunohistochemical investigation of the main fibroblasts phenotypes using specific markers: vimentin, α-SMA and FSP1 and corroborating the results with the histological aspects representative for each case according to the risk factor.
5. Testing the hypothesis that active gum fibroblasts come from the difference in keratinocytes through epithelial-mesenchymal transition mechanism.
6. Testing the hypothesis that according to the ratio of incriminated etiological factor, the gingival epithelium has an important role in increasing the synthesis of extracellular matrix as a reservoir of synthesized cells by investigating the expression FSP1, Ki-67 and E-cad, or as a source of pro-fibrilogenetic growth factors.
7. Investigating the tissue sources, the synthesis level and the interdependence relations for the two major pro-fibrilogenetic growth factors: TGFβ1 and CTGF.

CHAPTER III

CLINICAL STUDY OF FIBROTIC GINGIVAL ENLARGEMENT

The purpose of study of the present chapter is the following:

1. to define and describe the basic components of fibrotic gingival enlargement examination.
2. to estimate the initiation and evolution of the gingival enlargement with the scope to create a useful database for the diagnosis and treatment of this disease.

1. Material and method

1.1 Studied material

The initial studied sample consisted of 15 patients, which came to OMF Surgery Clinic and Periodontics Clinic at the Faculty of Dentistry- U.M.F.- Craiova.

After an comprehensive scientific documentation that allowed, based on a systematic review of data from the relevant literature, the definition of current considerations and principles concerning the the clinical and laboratory aspects of gingival fibromatosis, we moved to select the cases that are the subject of this study.

The investigation began in 2010, and consisted in assessing the group of patients of both genders, aged between 7 and 59 years.

The inclusion criteria represented the *subjective* and *objective* clinical signs, that can guide the diagnosis to a form of gingival fibromatosis. The final number considered and included in the research group was of 10 cases

2. Results

The patients had local lesions of gingival mucosa determined by the presence of bacterial plaque and tartar deposits on both arches.

The initial lesion started at the marginal gingival level and interdental papilla round shaped. As the inflammation progressed, these size increasing of marginal and papillary gingival united and turned into a massive tissue fold that covered a significant part of the crown and interfered with the occlusion.

3. Discussions

Gingival fibromatosis can be assigned to several causes: inflammatory, hereditary disease, social disease associated with another syndrome and a disease related to side effects of some medications

Hereditary gingival fibromatosis comes with a series of complications, according to the increased volume:

- physiognomic disorder
- eruption delay
- tooth movements due to fibrous tissue
- malocclusion, pains, when mucosa partially the chewing surfaces and it is traumatized during mastication

As clinical image, the first signs of induced gingival volume increasings occur 3-4 months after the administration. In case of phenytoin, the volume increases 2-3 weeks after the administration and reaches its maximum after 18-24 months. (McDonald, 1994).

Gingival fibromatosis can be presented as an isolated non-syndromic variant and occasionally associated with epilepsy, mental retardation or hypertrichosis (Peeran and colab. 2013).

CHAPTER IV - HISTOLOGICAL STUDY OF FIBROTIC GINGIVAL ENLARGEMENT

1.Material and methods

The histological study was performed on selected cases after clinical examination, as presented in the previous chapter.

In relation to the initial diagnosis, the lesion aspect and clinical evolution after the established treatment, therapy gingivectomy was performed for a number of 12 cases clinically diagnosed with gingival fibrotic enlargement.

Some of the cases included in the histological study came from existing biological samples achieved during the research project ID 563 2008-20011.

Patients included in this study were aged between 7-59 years, 6 of them women and 5 men.

Setting up the study group:

- **group I -** reactive focal gingival enlargement - **4 cases**
- **group II** – hereditary gingival fibromatosis –**3 cases**
- **group III** – syndromic gingival fibromatosis - **2 cases**
- **group IV** - idiopathic gingival fibromatosis -**3 cases**

1.2. Used methods

1.2.1. Common histological methods

Histological processing of the biological material was performed in the Histology Laboratory of UMF Craiova. Histological techniques involved going through several steps which will be described below:

1. Inclusion in paraffin

- fastening;
- dehydration;
- clearing;
- paraffining;
- proper inclusion and block formation.

Parts that are well coated with molten paraffin are integrated in a paraffin block of

homogeneous consistency which becomes liquid at room temperature. As a shape for blocks there can be used casting molds or small plastic boxes supported by a metal base (stainless steel). Pour the molten wax into the preheated metal support. Then, with a spatula well flame heated passes through the paraffin in the casting shape to remove the possible air bubbles that have formed and to melt the thin wax formed at the surface. With a flame heated forceps remove the last piece from the paraffin bath located in the thermostat and it will be dipped into the paraffin mold.

2. Obtaining the histological section

2.1.Sectioning the paraffin block

Blocks sectioning was performed with a Leica microtome, cutting sections (cups) of 3-5µm. The sections were spread on glass slides which were previously cleaned and degreased.

2.2. Placing the section on glass slides

The sections were collected one by one from the surface treated with polylysine on the slides. Each glass slide was introduced into the crystallizer liquid placing it diagonally under the section, then it was slowly raised while the section was held with a needle in the middle of the glass slide.

3. Colouring the sections

3.1. Hematoxylin eosin colouring technique (HE)

This is the most common colouring technique for viewing tissue architectonics, coloring the structures in different shades according to their dyeing properties

3.2. Trichromic colouring technique method according to Masson (Masson modified)

3.3. Silver impregnation technique (Gömöri modified)

This method is used to detect reticular fibers sections of different tissues on fixed sections buffer shaped formalin and immersed in paraffin

3.4. AS-Alcian Blue technique (alcian blue)

This method is used to identify neutral mucopolysaccharides, mucoproteins, glycoproteins and glycolipids, as well as glycosaminoglycans in tissues.

2. Results

Common histological colourings showed significant differences during overall examination of cases belonging to the four groups. Thus, focal reactive fibrosis of gingival mucosa as a whole had thickening aspect, determined by increasing thickness of the epithelium and lamina propria. The overall images show accumulation collagen fibers in chorion in thick strips with the intersection that give insular narrow spaces with inflammatory infiltration well represented.

Epithelium appears on some thickened surfaces, papilloma-virus aspect, with deep and sometimes branched tops, with areas of parakeratosis alternating with others of hyperkeratosis, for the same case described the mucosa collected from the the sulcus gingivae has a very thick epithelium, with superficial round cells or having areas the intense parakeratosis and inflammatory cell infiltration. At this level there are frequent areas of acanthosis with acantholysis. Detailed images containing the chorion papilla on transversal section have an increased amount of extracellular matrix at their level, with fewer cells and sanguine vessels but with dense conjunctive fascicle, which transforms the papillary lax conjunctive into a dense conjunctive tissue. These are accompanied by fewer inflammatory cells, both in conjunctive and epithelial level. Capillary blood vessels are more present in chorion papillae without fibrotic accumulation, and dilated arterioles and venules appear in areas of inflammatory cells accumulation, along with small capillaries.

3.Conclusions and discussions

In this chapter we intended a systematic study of gingival fibromucosa in fibrotic gingival enlargement. The selected cases after clinical diagnosis were placed in four study groups. The groups were thus designed to include the accumulation of extracellular matrix determined by several risk factors and etiologic factors, so we can compare the histological aspects we have found.

Fibrotic gingival enlargement diagnosed during the clinical examination is histologically characterized by increased volume of gingival fibromucosa where the gingival epithelium and

connective subjacent tissue have equal roles.

Special colourings have great importance for the study of morphological changes because they indicate quantitative changes - organic matrix deposits that grow the consistency of gingival fibromucosa when clinically examined but not identified with fibrillar aspect on histological examination in the usual colorations.

CHAPTER V
IMMUNOHISTOCHEMISTRY STUDY OF PATHOGENIC WAYS INVOLVED IN COLLAGEN DEPOSITS IN GINGIVAL ENLARGEMENT

In this chapter we intend to emphasize the techniques of immunohistochemistry of certain molecules involved in multiplication, differentiate and adjust the synthesis functions of fibroblasts, main cells involved in the synthesis of extracellular matrix in gingival fibromucosa.

1. Material and methods

1.1. Studied material

During the immunohistochemical study, there were used the same gingival tissue samples that were processed for paraffin immersion technique and subsequently coloured with common histological colours, as presented in the previous chapter.

1.2. Methods used

1.2.1. Immunohistochemical techniques

Immunohistochemistry is a technique used for microscopic morphology study, used both for research purposes and for histopathological diagnosis.

Immunohistochemistry actually represents the *in vitro* adaptation of an antigen-antibody enhanced reactions, which will ensure the view of localization *in situ* for specific components of tissues and cells.

1.2.2. Classification of immunohistochemical techniques

The direct method. The direct method is performed in a single phase and involves a marked antibody (ie FITC conjugated antiserum) which reacts directly with the antigen in tissue sections. This technique uses a single antibody, it is quick and short. However, it is not very sensitive due to low signal amplification and it is rarely used in practice since the introduction of the indirect method.

The indirect method. The indirect method involves two antibodies: an unmarked primary antibody, which forms the first layer and which reacts with the tissue antigen, and a second antibody that will react with the primary antibody, forming second layer. It is imperative that the secondary antibody should be anti IgG of the animal species that produced the primary antibody.

1.2.3. Antibodies used in the study

The following table presents the antibodies used to detect the antigens assessed using immunohistochemical studies in this thesis, the source, dilutions and immunohistochemical method used for each antibody.

ANTIBODY Source	Code	Dilution	Method
Monoclonal mouse antihuman α-smooth muscle actin (1A4) Dako	M0851	1:100	EnVision
Monoclonal mouse anti Ki-67 Dako	M7020	1:50	ABC
Monoclonal mouse antihuman vimentin (V9) Dako	M0725	1:50	LSAB
Monoclonal mouse antihuman CTGF 6(B13) Santa Cruz Biotechnology Inc., SUA	Sc-101586	1:300	ABC

Monoclonal mouse anti human TIMP-2 (3A4) Santa Cruz Biotechnology Inc., SUA	Sc-21735	1:100	ABC
Monoclonal mouse anti human e-Cadherin (NCH38), Dako	M3612	1:100	ABC
Polyclonal rabbit anti-human S100 A4 , Dako	A5114	1.200	ABC

2. The results obtained

2.1. The study of expression immunohistochemical of fibroblast markers

To highlight the phenotype of fibroblasts involved in the synthesis of collagen, I have used anti-vimentin, anti α-sma and anti-A4 S100 (FSP1).

Immunoreaction for α-sma displays for group I, composed as I have shown in fibrotic growth of local reactive nature, a highly restricted positivity, consistently observed at the blood vessel wall. I used this aspect as an internal control of immunohistochemical reaction. It can thus be seen a relatively modest number of blood vessels both at chorionic papilla level and in the profound chorionic.

Immunohistochemical reaction for group I in case of patients with secondary fibrotic gingival enlargement wearing an orthodontic prosthesis and showed a much higher incidence of α-SMA positive cell compared to patients in the same group, but with enlargement produced by the local inflammatory causes.

2.2. The study immunohistochemical expression of Ki-67 and E-cadherin

Positive reaction for FSP1 of a large number of keratinocytes and fibroblasts in the lamina propria, determined us to continue the immunohistochemical study in order to determine the origin of these fibroblasts. Thus, I used two antibodies, E-cadherin (E-cad) and Ki-67.

Gingival fibroid enlargement cases from groups II and III - hereditary gingival fibromatosis respectively syndromic, were characterized by a higher number of cells in division at basic epithelial layer, specifically marked with Ki-67.

2.3. The study immunohistochemical expression of profibrilogenetic increase factors

The main growth factors known for their role in stimulating the synthesis of extracellular matrix are TGFβ1 and CTGF.

As in the case of markers, the results obtained after the immunohistochemical marking has been described, the two mentioned growth factors showed different reactions both according to the study group and within the same batch.

3. Discussions and conclusions

In this chapter we aimed to highlight certain molecules involved in the synthesis of collagen, certain molecules involved in multiplication, differentiation and metabolism of fibroblast occurrences in gingival chorion. Our results indicate a large number of mesenchymal positive elements for vimentin in the group I containing fibromucosa from patients with focal reactive fibrosis, while the same group of patients with fibrosis developed after wearing the orthodontic prosthesis had a reduced number of vimentin positive cells, like those in group IV.

Clinical diagnosis of gingival fibromatosis recognises multiple pathogenic pathways depending on etiological factor or risk.

Fibroblasts, the main cell occurrence involved in the synthesis of extracellular matrix presents a significant phenotypic variation, mostly marked by FSP1 and vimentin.

TGF-β1 has role not only in increasing the collagen deposits but also the epithelial-mesenchymal transitional mechanism, being constantly present in the tip of epithelial ridges as well as in isolated keratinocytes.

GENERAL CONCLUSIONS

Fibrotic gingival enlargement diagnosed during histological clinical examination reveals slightly similar histological aspects regardless the etiological determining factor.

There is a constant epithelial thickening and volume increasing of connective tissue.

The main conjunctive elements are the connective collagen fibers, but their structure varies according to the degree of inflammation, predominantly with collagen of type I when the inflammation is reduced, while the type III collagen increases in quantity in the case of a consistent inflammatory infiltration.

The degree of local inflammation varies according to the etiological factor: the reactive enlargement and the idiopathic reactive granulation tissue is much better represented than in the syndromic and hereditary one.

Lamina propria fibroblast occurrence is numerous and highly heterogeneous, cell phenotypes vary according to the established etiological factor. Fibroblasts are frequently positive for vimentin, mostly positive for FSP1 and positive myofibroblasts for α - SMA are scarcely found.

Further studies are needed to determine the ability of collagen synthesis for each of these occurrences.

TGF-β1 and CTGF profibrilogenetic growth factors are well represented in cases of accentuated fibrotic deposits, their immunohistochemical expression was present in all studied cases regardless of the etiology and collagen deposits

BIBLIOGRAPHY

American Academy of periodontology. Informational paper: drug associated gingival enlargement, . J.periodontol., 75:1424-31, 2004.

Baniţă M., Deva V.,Organul Dentar,Editura Alma,Craiova:68-78,96-102, 2006.

Bartold, P.M. , Narayanan A. S. Molecular and cell biology of healthy and diseased periodontal tissues, Periodontol 2000 vol.40, 29-49, 2006.

Bittencourt L.P., campos V., Moliterno L.F.M., Ribeiro D.P.B., Sampaio R.K., Hereditary Gingival Fibromatosis Review of the Literature and a case report, Oral Medecine, Quintessence International 2000; 31(415-418), 2000.

Bökenkamp A, Bohnhorst B, Beier C, Albers N, Offner G, Brodehl J. Nifedipine aggravates cyclosporine A-induced gingival hyperplasia., Pediatr Nephrol. Apr;8(2):181-5., 1994

Carey JC, Cohen MM Jr, Curry CJ, Devriendt K, Holmes LB, Verloes A., Elements of morphology: standard terminology for the lips, mouth, and oral region., Am J Med Genet A. Jan;149A(1):77-92. doi: 10.1002/ajmg.a.32602. 2009.

Clocheret K, Dekeyser C, Carels C, Willems G, Idiopathic gingival hyperplasia and orthodontic treatment case report. J Orthodontics, 30(1):13-19. 2003.

De Angelo S, Murphy J, Claman L, Kalmar J, Leblebicioglu B, Hereditary gingival fibromatosis: a review, Compend Contin Educ Dent, 28(3):138-143., 2007.

Desai P, Silver JG., Drug-induced gingival enlargements. J Can Dent Assoc. Apr;64(4):263-8., 1998.

Douzgou S., Dallapicolla B., The gingival Fibromatoses, www. intechopen.com, 2011.

Kataoka M, Kido J, Shinohara Y, Nagata T, Drug-Induced Gingival Overgrowth-a Review, Biol Pharm Bull, 28(10):1817-1821, 2005.

Kfir Y, Buchner A, Hansen LS. Reactive lesions of the gingiva. A clinicopathological study of 741 cases. J Periodontol. Nov;51(11):655-61, 1980.

Lin K., Guihoto L.M.F.F., Marcia Targas Yacubian E, Drug-Induced Gingival Enlargement –Part II . Antiepileptic Drugs Not Only Phenitoin is Involved, JEpilepsy and Clinical Neurophysiology, 13(2) 83-88, 2007.

McDonald A., Post/cores in dentistry: a review. J Ir Dent Assoc. ;40(3):69-71, 74, 77.,1994.

Nanci A.,Ten cate's oral Histology,Development,structure and function, Sixth,Mosby, 2003.

Oh TJ, Eber R, Wang HL. Periodontal diseases in the child and adolescent. J Clin Periodontol. May;29(5):400-10. 2002.

Peeran SW, Ramalingam K, Peeran SA, Mugrabi MH, Abdulla KA., Hereditary nonsyndromic gingival fibromatosis: report of family case series. Case Rep Dent. 2013;2013:835989. Epub Sep 26., 2013.

Ross M.H. Pawlina W. Histology, A text and Atlas, with correlated cell and molecular biology, Fith edition, Lipincott Williams & wilkins, 2003.

Telser A.G.,Integrated Histology Mosby,Elsevier, 2007.

Trackman PC, Kantarci A, Connective tissue metabolism and gingival overgrowth, Crit Rev Oral Biol Med, 15(3):165-175., 2004.

Wilkins Esther M.BS Clinical Practice of the Dental Hygienist: Text and Student Workbook, Package 11th Edition, 2013.

www.maxillofacialcenter.com