UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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AVALIAÇÃO ESTRUTURAL E DIAGNÓSTICA DE TRÊS LESÕES FIBROSAS DA CAVIDADE BUCAL

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TESE APRESENTADA COMO PARTE DOS
REQUISITOS OBRIGATÓRIOS PARA A OBTENÇÃO
DO TÍTULO DE DOUTOR EM ODONTOLOGIA NA
ÁREA DE CONCENTRAÇÃO EM PATOLOGIA BUCAL

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RESUMO

O objetivo do presente trabalho é analisar os componentes celulares e de fibras do tecido conjuntivo nas hiperplasias inflamatórias (HI), nos fibromas (F) e na fibromatose gengival hereditária (FGH), além de investigar a imunocompetência e efetuar análises moleculares de pacientes com FGH. Para atingir os objetivos foram desenvolvidos 4 artigos, com diferentes metodologias e universos amostrais. No 1º artigo, pretendeu-se estabelecer critérios microscópicos válidos para diferenciar F e HI. Foram avaliadas em microscópio óptico 136 lesões coradas pela Hematoxilina-eosina (HE) e pelo Tricrômico de Masson quanto às características microscópicas. Os resultados mostraram que uma área central de fibras colágenas dispostas de forma enovelada e mais densa, circundada por uma camada de fibras dispostas de forma paralela são características dos F, enquanto a presença de hiperplasia epitelial, infiltrado inflamatório e fibras colágenas organizadas de forma paralela são características das HI. Tais resultados motivaram o 2º artigo, no qual estudamos 18 lesões de F e 13 de HI, que foram preparadas histologicamente e coradas pelo picrosírius red e pelo direct blue para avaliação quantitativa das fibras colágenas e de fibras do sistema elástico, respectivamente, em microscopia a laser confocal. Os resultados confirmaram a disposição estrutural das fibras colágenas observada no 1º artigo, além de apontarem diferenças nas áreas ocupadas pelas fibras colágenas em todas as regiões estudadas. A fim de proceder a uma avaliação dos componentes fibroso e celular das 3 lesões fibrosas, foi desenvolvido o 3º artigo. Espécimes das 3 lesões foram estudados em microscopia ótica, a fim de avaliar suas populações de fibroblastos e de células inflamatórias e os seguintes componentes fibrosos do tecido conjuntivo: fibras colágenas, sistema de fibras elásticas, fibras reticulares e fibras oxitalânicas. Os resultados mostraram disposição e concentração diferente das fibras colágenas nas 3 lesões e uma maior concentração de fibras reticulares na FGH. A análise dos componentes celulares mostrou um maior número de fibroblastos no F e uma maior contagem de células inflamatórias na HI. A partir do encaminhamento de uma família com FGH, optou-se por inclui-la no estudo, tendo em vista serem lesões do mesmo grupo. Com isso, foi desenvolvido um 4º estudo, que utilizou uma avaliação morfológica semelhante à dos 2 artigos anteriormente descritos. Dos pacientes com FGH foi obtido sangue periférico para

avaliação da proliferação celular de linfócitos através do teste do MTT e para o sequenciamento do gene *SOS-1*. Os resultados mostraram hiperplasia epitelial na porção externa da gengiva dos pacientes com FGH, maior concentração de fibras colágenas e poucas células inflamatórias. Os 3 pacientes com FGH não mostraram diferenças no seu índice de proliferação de linfócitos em relação aos controles e não apresentaram a mutação descrita no gene *SOS-1* de outras famílias com FGH. Pode se concluir que as 3 lesões apresentam estrutura conjuntiva diferente tanto no aspecto quantitativo quanto na disposição estrutural de seus componentes.

Palavras-chave: Hiperplasia inflamatória; Fibroma oral; Fibromatose gengival hereditária; Fibras colágenas; Fibras reticulares; Fibroblastos; Linfócitos; Proliferação celular.

ABSTRACT

The objective of this study was to analyze the cellular and fibrous components of connective tissue in inflammatory hyperplasia (IH), oral fibroma (OF) and hereditary gingival fibromatosis (HGF), and to investigate the immunocompetence and to perform molecular analysis in HGF patients. To achieve the goals were developed 4 articles, with different methodologies and sample universes. In the 1st article, we intended to establish microscopic criteria to differentiate F and IH. The microscopic characteristics of the lesions (n=136) stained by hematoxylin-eosin (HE) and Masson trichrome were evaluated in an optical microscope. The results showed that a central area of wound collagen fibers and arranged in a higher density, surrounded by a layer of parallel fibers are characteristic of F, while the presence of epithelial hyperplasia, inflammatory infiltrate and parallel collagen fibers are characteristics of HI. These results led the 2nd article, which studied 18 F and 13 and IH, histologically prepared and stained by picrosírius red and direct blue for the direct quantitative assessment of collagen fibers and elastic fibers of the system, respectively, in the confocal laser microscope. The results confirmed the structural arrangement of collagen fibers found in Article 1, and indicate differences in the areas of collagen fibers in all regions studied. In order to evaluate the cellular and fibrous components of the 3 fibrous lesions, was developed the 3rd article. Specimens of the 3 lesions were studied in optical microscopy, to assess their populations of fibroblasts and inflammatory cells and the following components of fibrous connective tissue: collagen fibers, elastic fiber system, reticular fibers and oxytalan fibers. The results showed different arrangement and concentration of collagen fibers in the 3 lesions and a higher concentration of reticular fibers in HGF. The analysis of cellular components showed a greater number of fibroblasts in F and a higher count of inflammatory cells in IH. With the identification of a family with HGF, we chose to include it in the study because the lesions belong to the group of benign fibrous lesions. With that, it developed a 4th study, which used a similar morphologic evaluation of the 2 articles described above. Periferic blood was extracted from the HGF patients in order to determine the proliferative capacity of the peripheral lymphocytes, by the MTT test, and in order to sequence the *SOS1* gene.

The 3 HGF affected patients did not present the described mutation for the *SOS1* gene, and the lymphocyte proliferative capacity in HGF patients was similar to those on controls. The results showed epithelial hyperplasia in the outer portion of the gingiva of patients with HGF, greater concentration of collagen fibers and few inflammatory cells. We can conclude that the 3 lesions present a different connective structure, considering both the quantitative aspect and the architectural disposition of their components.

Keywords: Inflammatory hyperplasia; Oral fibroma; Hereditary gingival fibromatosis; Collagen fibers; Reticular fibers; Fibroblasts, Lymphocytes; Cellular proliferation.

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LISTA DE ABREVIATURAS E SÍMBOLOS

ANOVA	Teste da análise da variância
β	Beta
DB	Direct Blue
DMSO	Dimethyl sulfoxide
DNA	Ácido desoxirribonucléico
ELISA	Enzyme Linked Immuno Sorbent Assay
F	Fibromas
FGH	Fibromatose gengival hereditária
GINGF	Autosomal dominant non-syndromic hereditary gingival fibromatosis
GR	Gomori reticulin
HE	Hematoxilina-Eosina
HGF	Hereditary gingival fibromatosis
HI	Hiperplasias inflamatórias
IH	Inflammatory hyperplasia
Ki	Kiel
LSD	Least Significance Difference
MT	Masson Trichrome
MTT	Tetrazolium salt
nm	namometer
OF	Oral fibroma
OR	Orcein
PBMC	Peripheral blood mononuclear cells
PCR	Polimersae chain reaction
PHA	Phytohaemagglutinin
PI	Proliferation index
PMA-PSR	Phosphomolybdic acid- Picrosirius red
PS	Picrosirius Red

RPMI	Roswell Park Memorial Institute
SOS1	Son of sevenless-1
Taq	Thermophilus aquaticus
TGFβ	Fator de crescimento transformante beta
TM	Tricrômico de Masson
UFRGS	Universidade Federal do Rio Grande do Sul

SUMÁRIO

INTRODUÇÃO

As hiperplasias inflamatórias são as patologias mais prevalentes da cavidade bucal, sendo mais comuns na gengiva e na mucosa jugal e, clinicamente, se apresentam, na maioria dos casos, como massas exofíticas, sésseis ou pediculadas, de coloração avermelhada, forma circular ou elíptica e superfície regular, que pode, ocasionalmente, estar ulcerada. Seu crescimento é geralmente lento e as lesões são indolores, exceto sob trauma mastigatório crônico (COELHO, ZUCOLOTO, LOPES 1994; WRIGHT, SCOTT 1992).

Tais lesões foram estudadas na dissertação de Badauy (2003). Durante o processo de confecção da dissertação e na submissão dos seus resultados na forma de artigo (BADAUY et al. 2005), constatou-se a ausência, na literatura científica, de um trabalho que definisse os critérios microscópicos para o diagnóstico histopatológico desta lesão. Por outro lado, observou-se que os critérios microscópicos descritos nas hiperplasias inflamatórias são muito semelhantes aos descritos para outras lesões hiperplásicas fibrosas do tecido conjuntivo, particularmente aos dos fibromas bucais (KFIR, BUCHNER, HANSEN 1980; MIGHELL, ROBINSON, HUME 1996; LUKES, KUHNERT, MANGELS, 2005; DALEI et al.1990; ZAIN, FEI 1990; PRIDDY 1992).

Uma pesquisa bibliográfica mais detelhada nos mostra que as hiperplasias fibrosas da cavidade bucal podem ser classificadas em 4 grandes grupos de lesões: as lesões de células gigantes, os granulomas piogênicos, os fibromas bucais e as hiperplasias fibrosas focais. As 2 primeiras lesões da classificação estão bem definidas quanto aos seus critérios microscópicos, enquanto em relação aos fibromas bucais e hiperplasias fibrosas focais existem subclassificações que tendem a sobrepor características microscópicas e provocam confusão no seu diagnóstico. Os fibromas bucais podem ser classificados em fibromas, fibromas de células gigantes (reconhecidos por alguns pesquisadores como entidade distinta pela presença de fibroblastos multinucleados, mas que podem estar presentes em outras lesões fibrosas também) e os fibromas irritativos, cuja classificação como entidade separada divide a opinião dos autores. As hiperplasias fibrosas focais podem ser sub-classificadas como idiopáticas, papilares, medicamentosas, FGH e HI, sendo que esta última possui características microscópicas semelhantes às dos fibromas irritativos. (CUTRIGHT 1974; TYLDESLEY

1974; KFIR, BUCHNER, HANSEN 1980; HOUSTON 1982; REIBEL 1982; SAVAGE, MONSOUR 1985; McGINNIS 1987; BAKOS 1992; PRIDDY 1992; GIUNTA 1999)

As diferenças de opinião dos autores quanto à subclassificação das 2 lesões produzem uma confusão entre fibromas irritativos e hiperplasias inflamatórias. Esta dificuldade diagnóstica acarreta tratamentos inadequados para as lesões e taxas discordantes de prevalência destas patologias, variando de 1,5% (WEIR, DAVENPORT, SKINNER, 1987) a 15% (COELHO, ZUCOLOTO, LOPES, 2000) no caso das hiperplasias inflamatórias, e 1% (HOUSTON 1982), 2,2 % (MAGNUSSON, RASMUSSON 1995), 13,2% (WEIR, DAVENPORT, SKINNER, 1987) a até 27% (KALYANYAMA, MATEE, VUHAHULA 2002) para os fibromas orais.

Com o objetivo de estabelecer critérios microscópicos válidos para o diagnóstico histopatológico das hiperplasias inflamatórias e dos fibromas bucais, foi desenvolvido o 1º artigo desta tese. Nesse trabalho, 2 examinadores calibrados realizaram uma análise em microscópio óptico das características microscópicas presentes nas 2 lesões. Os espécimes participantes do estudo foram corados pela hematoxilina-eosina, para exame das características microscópicas, e pelo tricrômico de Masson para confirmação se as fibras vistas na técnica anterior eram fibras colágenas ou musculares.

A partir dos resultados de alto conteúdo de colágeno e disposição das fibras do 1º trabalho, surgiu a oportunidade de se estudar a quantidade de fibras colágenas e de fibras do sistema elástico e sua disposição morfológica em hiperplasias inflamatórias e fibromas bucais. Para alcançar tal objetivo, os cortes de ambas as lesões foram corados pelo Picrosírius Red e pelo Direct Blue e foram analisados em microscopia a laser confocal, a fim de se avaliar as fibras colágenas e fibras do sistema elástico, respectivamente, originando o 2º artigo desta tese.

O comportamento clínico das lesões hiperplásicas fibrosas do tecido conjuntivo está bem documentado na literatura científica, mas as macromoléculas da matriz extracelular que estão envolvidas na patogenia dos diferentes aumentos de volume fibrosos da cavidade bucal ainda permanecem pobremente investigadas (HOUSTON 1982; REIBEL 1982; SWAN 1988; BAKOS 1992; BONNAURE-MALLET TRICOT-DOLEAUX, GODEAU 1995). Da mesma forma, estudos que se focam em características celulares e microscópicas das lesões hiperplásicas fibrosas do tecido conjuntivo são raros e, na maioria das vezes, inconsistentes.

Sabendo-se que a composição da matriz extracelular pode influenciar o comportamento clínico de um tecido, foi desenvolvido o 3º artigo desta tese. O objetivo do 3º artigo foi avaliar a composição de fibras colágenas, reticulares, do sistema elástico e oxitalânicas, além de quantificar fibroblastos e células inflamatórias nas três lesões até aqui estudadas. Para alcançar este objetivo, realizou-se uma avaliação de cortes obtidos das lesões em microscopia óptica, com lâminas coradas pela hematoxilina eosina (análise morfológica e quantificação celular), pelo Picrosírius Red (análise das fibras colágenas), pela reticulina de Gomorri (análise das fibras reticulares) e pela orceína (análise das fibras oxitalâmicas) e uma avaliação em microscopia a laser confocal com lâminas coradas pelo Direct Blue (análise do sistema de fibras elásticas).

Durante a confecção desses trabalhos, recebemos o encaminhamento de uma família que apresentava hiperplasia gengival, que recidivava mesmo após sucessivos procedimentos cirúrgicos e não estava ligada à presença de placa nem ao uso de mediação que predispusesse esta condição. Um exame mais detalhado permitiu-nos concluir que a enfermidade era associada à hereditariedade, permitindo classificar os pacientes como portadores de fibromatose gengival hereditária.

As hiperplasias associadas à Fibromatose gengival hereditária são classificadas como lesões hiperplásicas fibrosas do tecido conjuntivo, assim como hiperplasias inflamatórias e fibromas bucais. As lesões hiperplásicas fibrosas do tecido conjuntivo podem ser subdivididas em 4 grupos principais, de acordo com a sua prevalência: hiperplasias fibrosas localizadas, fibromas orais, granulomas piogênicos e lesões periféricas de células gigantes. (DALEY et al.1990; Mc GINNIS 1987). As hiperplasias associadas à Fibromatose gengival hereditária e as hiperplasias inflamatórias são lesões classificadas dentro da denominação geral de hiperplasias fibrosas localizadas. A fibromatose gengival hereditária é uma doença hereditária, com herança variável, caracterizada por um aumento lento e progressivo no volume gengival, de consistência fibrosa, (BAPTISTA 2002; COLETTA, GRANER 2006), enquanto a hiperplasia inflamatória é uma lesão reacional dos tecidos epitelial e conjuntivo desencadeada principalmente pelo trauma crônico (COELHO, ZUCOLOTO, LOPES 1994; WRIGHT, SCOTT 1992). Os fibromas são considerados por alguns autores tumores benignos, normalmente localizados na mucosa bucal, de crescimento lento e indolor (MAGNUSSON, RASMUSSON 1994; OLES 1968).

Embora seja considerada como uma doença hereditária rara, autossômica dominante, a Fibromatose gengival hereditária apresenta um padrão heterogêneo de herança, com vários graus de penetrância e expressividade e casos ocasionais de transmissão autossômica recessiva têm sido relatados (SINGER et al. 1993). Os locus genéticos para as formas autossômicas dominantes da Fibromatose gengival hereditária foram localizados no cromossomo 2p21-22 (GINGF) (HART et al. 1998), onde uma mutação no gene *SOS1* (*Son of sevenless*-1) foi descrita como responsável pelo desenvolvimento de Fibromatose gengival hereditária em uma grande família, com mais de 40 indivíduos afetados (HART et al. 2000), também no cromossomo 5q13-q22 (GINGF2) (XIAO et al. 2001), e, mais recentemente, no cromossomo 2p22.3-p23.3 (GINGF3) (YE et al. 2005). É importante salientar que o trabalho de Hart et al. (2000) foi desenvolvido através de mapeamento molecular e genético de uma família brasileira, enquanto os 2 outros estudos analisaram famílias chinesas, sugerindo a existência de variação na suscetibilidade genética à Fibromatose gengival hereditária associada à origem étnica.

Inflamação não é uma característica que tem sido associada à à Fibromatose gengival hereditária, tanto em investigações clínicas quanto microscópicas. No entanto há relato de associação entre Fibromatose gengival hereditária e periodontite agressiva generalizada, sugerindo um possível componente inflamatório nesta doença (CASAVECCHIA, UZEL, KANTARCI, 2004). Fator de crescimento transformante beta (TGFβ) é uma citocina importante na inibição da proliferação das células T, na inibição da ativação de macrófagos e também está envolvida na mudança de classe da imunoglobulina A. Estas características poderiam explicar os baixos níveis de inflamação observados em pacientes com Fibromatose gengival hereditária, no entanto, poucos estudos analisaram, de forma sistêmica, a imunocompetência de pacientes com Fibromatose gengival hereditária e os baixos níveis de inflamação descritos nestes pacientes permanecem como uma situação a ser explicada.

Diante da semelhança clínica e microscópica entre a Fibromatose gengival hereditária e as 2 lesões que já vinham sendo estudadas, optou-se por incluir os pacientes com Fibromatose gengival hereditária no grupo de estudos, o que originou o 3º e o 4º artigos desta tese. O 4º artigo teve como objetivo avaliar as características microscópicas, principalmente em relação à presença de inflamação, e quantificar as fibras colágenas nas hiperplasias gengivais da Fibromatose gengival hereditária. Também foram objetivos do 4º artigo testar a

imumocompetência e realizar análises moleculares nos pacientes afetados. Realizou-se uma avaliação em microscópio óptico de cortes histológicos de hiperplasias gengivais dos pacientes com FGH e de HI corados pelas técnicas da hematoxilina-eosina (análise das características microscópicas e da presença de células inflamatórias) do Tricrômico de Masson (análise da disposição das fibras colágenas) e uma avaliação em microscopia a laser confocal (quantificação das fibras colágenas). Obtiveram-se linfócitos do sangue periférico dos pacientes com Fibromatose gengival hereditária, sendo que uma parte destas células foi cultivada em meio de cultura apropriado para se avaliar a imunocompetência destes pacientes. Do restante dos linfócitos extraiu-se DNA a fim de se seqüenciar a região do gene *SOS-1* descrita como portadora da mutação causadora da Fibromatose gengival hereditária em uma grande família brasileira.

OBJETIVOS

OBJETIVO GERAL

Avaliação dos constituintes da matriz extracelular nas hiperplasias inflamatórias, nos fibromas e na fibromatose gengival hereditária, além de estudar a imunocompetência e alterações moleculares em pacientes com fibromatose gengival hereditária.

OBJETIVOS ESPECÍFICOS

1. Apontar critérios microscópicos válidos, que permitam diferenciar histopatologicamente hiperplasias inflamatórias e fibromas.
2. Estudar os componentes fibrosos e celulares característicos do tecido conjuntivo em lesões gengivais diagnosticadas como hiperplasias inflamatórias, fibromas e fibromatose gengival hereditária.
3. Estudar a imunocompetência e alterações moleculares em pacientes com fibromatose gengival hereditária.
4. Avaliar a população de fibroblastos e células inflamatórias nas hiperplasias inflamatórias, nos fibromas e na fibromatose gengival hereditária.

A MICROSCOPIC STUDY OF ORAL FIBROMAS AND INFLAMMATORY HYPERPLASIA.

RUNNING HEAD: Microscopic study of hyperplastic lesion

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KEYWORDS

Diagnosis; Pathology; Medicine; Examination.

ABSTRACT

Aim: To evaluate microscopic characteristics of Inflammatory Hyperplasia and Oral Fibroma and indicate criteria which facilitate their diagnosis.

Methods and Results: Histological 4μm sections (n= 136) were stained by hematoxilyn-eosin and by Masson Trichrome. The samples were evaluated in an optic microscope (100 x) by two independent observers. The distribution of the microscopic characteristics was classified according to the following scale: 0: absence of the characteristic; 1: characteristic present up to 1/3 of the microscopic fields; 2: characteristic present up to 1/2 of the microscopic fields; 3: characteristic present in more than 1/2 of the fields. The evaluation of the MT stained samples was made by describing the collagen orientation, its distribution and its density. The data were compared with the Mann-Whitney's "U" test (α=0.05). The wound fibers (fibers loosely oriented) and inflammatory infiltrate were statistically more frequent and higher in Oral Fibroma while parallel fibers and epithelial hyperplasia were more frequent in Inflammatory Hyperplasia. Epithelial hyperplasia, parallel fibers in the body of the lesion and inflammatory infiltrate are criteria for Inflammatory Hyperplasia diagnosis.

Conclusion: The presence of thick collagenous wound fibers in the centre of a lesion surrounded by a layer of parallel fibers is the main criteria for Oral Fibroma diagnosis.

CLINICAL RELEVANCE

Scientific rationale for study: The oral fibroma and inflammatory hyperplasia are lesions with very similar clinical and microscopic characteristics, and this fact can lead to a misdiagnosis and an incorrect treatment of those lesions.

Principal findings: There are microscopic characteristics that permit the correct diagnosis for oral fibroma and inflammatory hyperplasia.

Practical implications: The treatment of oral fibroma is surgical, while the diagnosis of inflammatory hyperplasia determines the removal of the irritation factor before the surgery. The procedure must be performed in this order at the risk of recurrence of the lesion.

INTRODUCTION

Fibrous hyperplastic connective tissue lesions are the most common group of lesions in the oral cavity and may be similar both clinically and histologically[1-3]. Oral fibromas (OF) and inflammatory hyperplasia (IH) account for the great majority of fibrous hyperplastic connective tissue lesions, more prevalent as localized swellings in the gingiva of the anterior maxilla [4-5].

These two lesions clinically look like an exophytic red round mass of the oral mucosa, sessile or pedunculated, with a regular surface which can occasionally be ulcerated. The growth is slow and the lesions are painless, except under traumatic conditions[1;6-8].

The prevalence of these lesions are variable i.e. 1.5%[9] to 15%[10] for IH; and 1%[11], 2.2 %[12], 13.2%[9] to 27%[13] for OF. The differences in lesion prevalence can be a result of the microscopic features variability described in the literature. The OF are microscopically described as a well differentiated fibrous tissue, with loosely arranged collagenous fibers involving stellate cells, and focally scattered lymphocytes just beneath the epithelium, covered by squamous epithelium showing the festooning of the rete ridges[11;14-17]. However, the OF microscopic appearance is also described as a connective tissue showing multiple large stellate-shaped cells occasionally containing several oval nuclei in an abundant eosinophilic cytoplasm. The cells can be distributed through loose collagenous fibers or be more copious just under the epithelium[11-12;16;18-21].

The microscopic features of inflammatory hyperplasia show an increase in irregular dense collagen fibers with focally scattered lymphocytes just beneath the epithelium presenting hyperplasia or acanthosis[1;4;10].

The clinical similarity between these lesions and the parameter variability for microscopic diagnosis causes confusion, which is reflected in the terminology, i.e. irritative fibroma; giant cell

fibromas; fibrous hyperplasia; epulis fissuratum; denture granuloma of the lesions[6;21-24]. The objective of the present study is to evaluate the morphologic characteristics of IH and OF through microscopic analysis and indicate criteria to facilitate their diagnosis.

MATERIALS AND METHODS

Preparation of the samples

Samples were retrieved from the files of the Oral Pathology Laboratory, Federal University of Rio Grande do Sul (UFRGS) between 2002 and 2005. The criteria for inclusion were as follows: histopathological diagnosis of IH and OF and total biopsies.

Two histological 4μm sections of each paraffin block (n= 136) were stained by hematoxilyn-eosin (HE) and by Masson Trichrome (MT) respectively (in the Pathology Laboratory of Institute Oswaldo Cruz, FIOCRUZ – Rio de Janeiro).

The patients' clinical history was investigated in order to determine the presence of irritation factor that could be associated with the etiology of the lesion

Morphological analysis

Each sample was independently evaluated in the total extension of the lesion using an optic microscope (100 x) and two independent observers (CMB and GGS) who were uninformed about the diagnosis of the lesions.

The morphological characteristics analyzed in the HE sections were wound fibers (fibers loosely oriented), parallel fibers (fibers in the same direction), inflammatory cells and hyperemia in the connective tissue; the following were analyzed in the epithelial lining: hyperplasia, acanthosis, hyperkeratosis and hydropic degeneration. The evaluation of the morphological characteristics was made in all the extension of the respective tissues and the number of microscopic fields was recorded according to the tissue in each lesion. The distribution of the microscopic characteristics was classified by lesion according to the following scale: 0:

absence of the characteristic; 1: characteristic present in up to 1/3 of the microscopic fields of the lesion; 2: characteristic present in up to 1/2 of the microscopic fields of the lesion; 3: characteristic present in more than 1/2 of the fields of the lesion.

In order to corroborate the morphologic approach in the collagen fibers, a complementary analysis was made. The evaluation of the samples stained by Masson Trichrome (a specific collagen stain) was qualitative, describing the collagen orientation, its distribution in the lesion (central or peripheric)[25] and the density of the collagenous fibers, classified according to Otasevic et al.[26] in a mild, moderate or severe density.

The data obtained from the morphological analysis of the two examiners were tabulated and confronted. The cases of discordance were reevaluated and an agreement was reached with the help of an expert pathologist (MSF). The agreement between the two examiners was evaluated in the beginning and at the end of the evaluation (κ=0.54 and κ=0.73, respectively).

The data obtained from that agreement were expressed by mean rank and compared with the Mann-Whitney's U test (α=0.05)

RESULTS

From the 3602 biopsies received by the Laboratory in the period of the study 106 had a histopathologic diagnostic of OF and 306 of IH (2.94 and 8.4% respectively). From these cases 61 OF and 75 IH fulfilled the criteria for inclusion and were investigated in the present study.

Concerning the lesions' clinical history, we observed the presence of irritation factor in 12 OF and in all cases of IH.

Analysis of the data obtained from the connective tissue showed that wound fibers (fibers loosely oriented) were statistically more frequent and higher in OF, while parallel fibers were more frequent in IH (Table 1). The collagen fibers density was also different, with the OF ones showing a higher level (Table 3). The inflammatory infiltrate was more frequent in IH and the

difference was statistically significant. (Table 1; figure 1). The epithelial hyperplasia was more frequent in IH (table2). The frequency of the scores of microscopic characteristic obtained by each lesion can be observed in table 4.

DISCUSSION

The OF and IH are lesions with very similar characteristics. The clinical and microscopic similarity can lead to a misdiagnosis and an incorrect treatment of these lesions. The microscopic characteristics observed in the literature vary according to authors and institutions and this produces variability in the prevalence of these lesions i.e. 1.5%[9] to 15%[10] for IH; and 1%[11], 2.2%[12] and 13.2%[9] for fibromas.

Several researchers describe the microscopic characteristics of fibroma as a well differentiated fibrous tissue, with collagenous fibers and focally scattered lymphocytes just beneath the epithelium, covered by squamous epithelium showing the festooning of the rete ridges[11-12;27]. However, these microscopic characteristics are not specific and are analogous to other connective tissue lesions[28-30], specific to IH[10;23-24;31-33].

The absence of a consensus in the literature regarding the microscopic diagnosis leads to confusion, which is reflected in the terminology (irritative fibroma) and mainly in the treatment of the lesions[6]. The IH are lesions associated with mechanical irritations and the treatment requires removal of the lesion and the irritation factor[6;10] , while the fibromas are of benign tumoral etiology and the treatment is surgical[12;34].

Therefore, the objective of the present study is to evaluate the microscopic characteristics of IH and fibromas and point out valid criteria for their diagnosis. In the same way, we evaluate the microscopic features of the 2 lesions.

Microscopic analysis of the HE staining showed that the collagenous wound fibers was the main microscopic characteristic for diagnosis of OF. This feature obtained score 3 in 92.1%

of OF, whereas most IH cases had score 0 (absent) or score 1 (table 4). These results corroborate several research findings in the literature[12;34]. Moreover, we have observed that spatial disposition of collagenous fibers tends to present a lot of wound fibers in the center of the lesion, the body of the OF extending for over half of the microscopic fields.

The periphery of the OF was a layer of parallel fibers simulating a fibrous capsule that was responsible for one third of most lesions with an average score of 1 to 2 in accordance with Oles[34]. This was the opposite of the IH where the collagenous parallel fibers determined its body, concentrating in the center of the lesion and thus, determining that most cases obtained scores 2-3 (table 4). This characteristic was important for the differential diagnosis between OF and IH and the findings were observed before[32].

The analysis of MT staining showed that the fibers observed in HE corresponded to collagenous fibers. The spatial distribution of the fibers was wound in OF and parallel in IH, but the fibers in the OF looked thicker and with a stronger bluish staining than the IH. Similarly, it was observed that the density of the collagenous fibers was higher in OF than in IH and this could suggest a difference of collagen type or in the velocity of collagen biosynthesis. These microscopic arrangements reinforce the theory previously described[12;35] of a neoplastic origin for OF.

The giant cells are described like large stellate, sometimes multinucleated cells with a well demarcated cytoplasm and a vesicular nuclei which contained preeminent nucleoli[11;16]. These cells are identified like fibroblasts, active in the collagen biosynthesis[3;12;16]. In the present study we have not observed statistical differences regarding the presence and distribution of these cells between the two lesions. This can be explained because the giant cells are not specific for giant cell fibroma but are found in other fibrous hyperplasias like inflammatory hyperplasia and

fibro epithelial polyp of the oral mucosa[20;35-36]. This fact leads many authors not to accept the GCF as a separate pathology[20-21;36] and we agree with this opinion.

Since IH is a reactive lesion of connective and epithelial components, the inflammatory cells have been considered a frequent feature in microscopic observations of this lesion[32-33], but not for OF. The explanation for the difference is that IH is related to traumatic etiology while fibromas are related to a benign neoplastic origin. The results from investigation of the irritation factor presence, and the differences in the scores of inflammatory infiltrate (Table 4) observed in the lesions of this study support this hypothesis. However, OF present exophytic growth which makes it susceptible to trauma. Therefore, a secondary inflammatory reaction can appear, and this is an explanation for 38.5% of OF cases where inflammatory infiltrate was observed.

The observation of epithelial hyperplasia is very common in the IH[10;23] but it is not exclusive of this lesion and can be a microscopic finding in OF[11-12;37]. However, epithelial hyperplasia can be considered as an unusual finding in oral fibromas and can be the result of traumatic factors. In the same way other features such as acanthosis, hyperkeratosis, hydropic degeneration and atrophy in the epithelial tissue, hyperemia and giant cells in connective tissues are occasional features and are not considered in differential diagnosis.

It was concluded that epithelial hyperplasia, parallel fibers in the body of the lesion and inflammatory infiltrate are important criteria for IH diagnosis and the presence of thick collagenous wound fibers in the centre of the lesion surrounded by a layer of parallel fibers is the main OF diagnosis criteria. Furthermore, we can suggest in accordind with this study, with our previous results and with other studies that the 2 lesions are of distinct nature: fibromas are neoplastic lesion and IH are inflammatory lesions.

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REFERENCES

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

2. Mighell AJ, Robinson PA, Hume WJ. Immunolocalization of tenascin – C in focal reactive overgrowths of oral mucosa. J Oral Pathol Med 1996; 25:163 –9.

3. Lukes SM, Kuhnert J, Mangels MA. Identification of a giant cell fibroma. J Dent Hyg 2005;79:1-14.

4. Dalei TD, Wisocky GP, Wisocky PD, Wisocky DM. The major epulides: clinicopathological correlations. J Can Dent Assoc 1990;56:627-30

5. Zain RB, Fei YJ. Peripheral fibroma/fibrous epulis with and without calcifications. A clinical evaluations of 204 cases in Singapore. Odontolstomatol Trop 1990;13:94-6.

6. Priddy RW. Inflammatory Hyperplasias of the oral mucosa. J Can Dent Assoc 1992;58:311-5, 319-21.

7. MCGuff HS, Alderson GL, Cale Jones A, Keller TA. Oral and Maxillofacial pathology case of the month. Giant cell fibroma. Tex Dent J 2005;122:688-9, 692.

8. Braga MM, Carvalho AL, Vasconcelos MC, Braz-Silva PH, Pinheiro SL. Giant cell fibroma; a case report. J Clin Pediatr Dent 2006;30:261-4.

9. Weir JC, Davenport WD, Skinner RL. A diagnostic and epidemiologic servey of 15,783 oral lesions. J Am Dent Assoc 1987;115:439-42.

10. Coelho ZL, Zucoloto MD, Lopes RA. Denture-induced fibrous inflammatory hyperplasia: a retrospective study in a school of dentistry. Int J Prosthodont 2000;13:148-51.

11. Houston GD. The giant cell fibroma. A review of 464 cases. Oral Surg Oral Med Oral Pathol 1982;53:582 –7.

12. Magnusson BC, Rasmusson LG. The giant cell fibroma. A review of 103 cases with immunhistochemical findings. Acta Odontol Scand 1995;53:293-6.

13. Kalyanyama BM, Matee MI, Vuhahula E. Oral tumours in Tanzanian children based on biopsy materials examined over a 15-year period from 1982 to 1997. Int Dent J 2002;52:10-4.

14. Nevile BW, Damm DD, Allen CM, Bouquot JE. Oral and maxillofacial pathology. Philadelphia (PA): WB Sanders, 2002:439-40.

15. Regezi JA, Sciubba JJ. Oral pathology: clinical pathologic correlations. 4thEd St Louis (MO): WB Sanders, 2000:200-60.

16. Weathers DR, Campbell WG. Ultrastructure of the giant cell- fibroma of the oral mucosa. Oral Surg Oral Med Oral Pathol 1974;38:550-61.

17. Bakos LH. Giant cell fibroma: a review of 116 cases. Ann Dent 1992;51:32-5.

18. Weathers DR, Callihan MD. Giant cell- fibroma. Oral Surg Oral Med Oral Pathol 1974;37:374-84.

19. Schneider LC, Weisinger E. The true gingival fibroma. An analysis of 129 fibrous gingival lesions. J Periodontol 1978;49:423-4.

20. Reibel J. Oral fibrous hyperplasias containing stellate and multi-nucleated cells. Scand J Dent Res 1982;90:217-26.

21. Savage NW, Monsour PA. Oral fibrous hyperplasias and the giant cell fibroma. Aust Dent J 1985;30:405–9.

22. Tyldesley WR. Oral medicine for the dental practitioner. 7. Inflammatory overgrowths and neoplasms. Br Dent J 1974;136:111-6.

23. Cutright DE. The histopathologic findings in 583 cases of epulis fissuratum.Oral Surg Oral Med Oral Pathol 1974;37:401-11.

24. Giunta JL. Gingival fibrous nodule. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;88:451-4.

25. Fowble VA, Vigorita VJ, Bryk E, Sands AK. Neovascularity in chronic posterior tibial tendon insufficiency. Clin Orthop Relat Res 2006;450:225-30.

26. Otasevic P, Popovic ZB, Vasiljevic JD et al. Relation of myocardial histomorphometric features and left ventricular contractile reserve assessed by high-dose dobutamine stress

echocardiography in patients with idiopathic dilated cardiomyopathy. Eur J Heart Fail 2005;7:49-56.

27. Waldron CA. Fibro-osseous lesions of the jaws. J Oral Maxillofac Surg 1993;51:828-35.

28. Wilkins SA Jr, Waldron CA, Mathews WH, Droulias CA. Aggressive fibromatosis of the head and neck. Am J Surg 1975;130:412-5.

29. Kurihara K, Ohba S, Hashimoto N. Benign fibroblastic tumor-like lesion simulating malignancy in the maxilla. J Oral Pathol 1984;13:679-85.

30. Odel EW, Lombardi T, Barret AW, Morgan PR, Speight PM. Hybrid central giant cell granuloma and central odontogenic fibroma-like lesions of the jaws. Histopathol 1997;30:165-71.

31. Cutright DE. Morphogenesis of inflammatory papillary hyperplasia. J Prosth Dent 1975;33:380-5.

32. Badauy CM, Barbachan JJD, Rados PV. Inflammatory hyperplasia: a microscopic study. Rev Fac Odontol 2002;43:48-51. (Article in Portuguese)

33. Badauy CM, Barbachan JJD, Rados PV, Sant'Ana Filho M, Chies JAB. Relationship between candida infection and immune cellular response in inflammatory hyperplasia. Oral. Microbiol Immunol 2005;20:89-92.

34. Oles RD. Incidence and distribution of various connective tissue fibers in oral fibromas. Oral Surg Oral Med Oral Pathol 1968;26:487-95.

35. Regezi JA, Courtney RM, Kerr DA. Fibrous lesions of the skin and mucous membranes which contain stellate and multinucleated cells. Oral Surg Oral Med Oral Pathol 1975;39:605-14.

36. Souza LB, Andrade ES, Miguel MC, Freitas RA, Pinto LP. Origin of stellate giant cells in oral fibrous lesions determined by immunohistochemical expression of vimentin, HHF-35, CD68 and factor XIIIa. Pathology 2004;36:316-20.

37, Anneroth G, Sigurdson A. Hyperplastic lesions of the gingiva and alveolar mucosa. A study of 175 cases. Acta Odontol Scand 1983;41:75-86.

Table 1. Results from microscopic analysis of connective tissue in hematolxilin-eosin staining.

Microscopic characteristics	Group	Mean rank	"U" and p value
Wound fibers	OF	48.53	189
	IH	23.06	<0.001*
Parallel fibers	OF	24.16	177
	IH	50.29	<0.001*
Giant cells	OF	37.45	610
	IH	35.44	0.66
Hyperemy	OF	33.09	516.5
	IH	40.31	0.123
Inflammatory infiltrate	OF	28.82	354
	IH	45.09	0.01*

Comparison with the Mann-Whitney's U test (α=0,05).

(* Statistically significant differences).

Table 2. Results from microscopic analysis of epithelial tissue in hematolxilin-eosin staining.

Comparison with the Mann-Whitney's U test (α=0,05).

Microscopic characteristics	Group	Mean rank	"U" and p value
Hyperplasia	OF	24.08	174
	IH	50.38	<0.001*
Acanthosis	OF	36.94	634
	IH	36.16	0.74
Hyperkeratosis	OF	36.26	637
	IH	36.76	0.876
Hydropic degeneration	OF	32.97	512
	IH	40.44	0.108
Atrophy	OF	36.87	632
	IH	36.09	0.72

(* Statistically significant differences).

Table 3. Results from microscopic analysis of colagen fibers in Masson Trichrome staining. Comparison with the Mann-Whitney's U test (α=0,05).

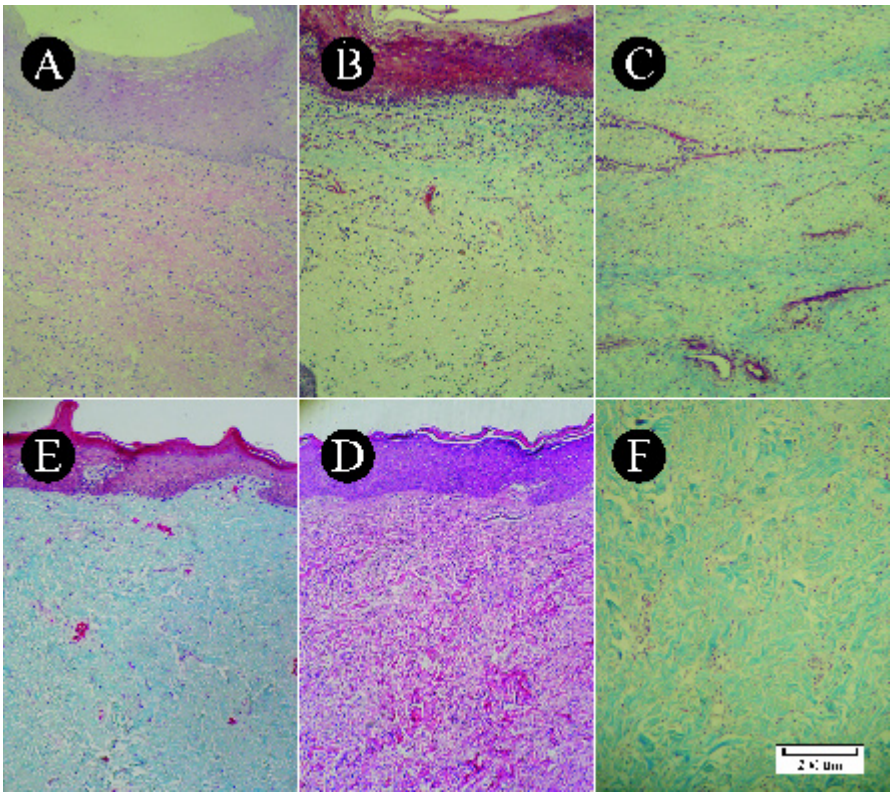
Trichrome	Group	Mean rank	"U" and p value
Density of collagen fibers	OF	40.84	481
	IH	31.65	0.045*

(* Statistically significant differences).

Table 4. Frequency of the scores observed in each microscopic characteristic by lesion that had statistical difference in the comparison whith Mann-Whitney's "U" test. (Oral fibromas, OF, n=61; Inflammatory Hyperplasia, IH, n=75). SCORES 0: absence of the characteristic; 1: characteristic present in until 1/3 of the microscopic fields of the lesion; 2: characteristic present in until1/2 of the microscopic fields of the lesion; 3: characteristic present in more than 1/2 of the fields of the lesion.

Microscopic characteristic	Score 0 (%)		Score 1 (%)		Score 2 (%)		Score 3 (%)	
	OF	IH	OF	IH	OF	IH	OF	IH
Wound Fibers	0	33 (44%)	0	16 (21.3%)	5 (8.2%)	7 (9.3%)	56 (91.8%)	19 (25.4%)
Parallel Fibers	11 (18%)	2 (2.66%)	36 (59.02%)	6 (8%)	13 (21.3%)	33 (44%)	7 (11.7%)	34 (45.34%)
Density of collagen fibers	0	0	12 (19.7%)	40 (53.3%)	35 (57.4%)	20 (26.7%)	14 (22.9%)	15 (20%)
Inflammatory Infiltrate	38 (62.29%)	6 (8%)	19 (31.1%)	26 (34.7%)	4 (6.6%)	20 (26.6%)	0	23 (30.7%)
Epithelial Hyperplasia	42 (68.85%)	13 (17.33%)	15 (24.6%)	4 (5.3%)	4 (6.55%)	13 (17.37%)	0	45 (60%)

Figure 1- Microscopic characteristics of IH in the periphery of the lesion showing parallel collagen fibers and an infiltrate of inflammatory cells in the connective tissue (A- HE staining and B- MT staining; original magnification 100x). C – Parallel disposition of collagen fibers in the centre of the IH (MT staining). D and E - Microscopic characteristics of OF in the periphery of the lesion showing a superficial layer of parallel collagen fibers covering the wound fibers of the lesion's centre. (D- HE staining and E- MT staining). F- Wound fibers in the centre of the OF. Note the higher level of the collagen fibers density confronted with figure C (MT staining).



A LASER CONFOCAL STUDY OF COLLAGEN AND ELASTIC FIBERS IN ORAL FIBROMAS AND INFLAMMATORY HYPERPLASIA.

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ABSTRACT

The objective of this study was to analyze collagen and system of elastic fibers in Inflammatory hyperplasia (IH) and Oral fibroma (OF). Histological 3µm sections (18 OF and 13 IH) were stained by Picrosirius for collagen fibers and Direct Blue for system of elastic fibers evaluation. The sections were evaluated using a confocal laser microscope. To improve contrast, images were captured in white, grey tones and black and a histogram was used to obtain the proportion of collagen in each area. A morphologic analysis was carried out considering the parallelism of the fibers, distinction of the layers and density of the fibers. Comparisons were performed using Analysis of variance and Fisher's exact test for quantitative and morphologic analysis, respectively. The results showed a higher ratio of collagen fibers in the center of OF, and a lower ratio in the center of IH. The center of OF was different from the others regions regarding the morphologic characteristics anlyzed. The elastic system do not showed staining. We can conclude that the OF have more proportion of collagen fibers and there is a different disposition of these fibers in OF and IH.

INTRODUCTION

Oral fibromas (OF) and inflammatory hyperplasia (IH) account for the great majority of fibrous hyperplastic connective tissue lesions, the most common group of lesions in the oral cavity[1,2]. This lesions are more prevalent as localized swellings in the gingiva of the anterior maxilla and may be similar both clinically and histologically[3-6]

The development of these lesions and tissue remodeling show a great increase of the connective tissue that compounds the stromae of the lesion[3,5-8]. The connective tissue had a structural function and plays a role as misregulation of the cell interactions and fiber production, like developmental morphogenesis, tissue organization, age changes and cell behavior[9-13].

The normal extracellular matrix of the oral tissues and the connective tissue of the OF and IH are mainly formed by connective tissue and its major component are collagen fibers and elastic system fibers[14-16]. The collagen and the elastic fibbers has been studied in normal tissue of

the mouth, in the bone surrounding the teeth, in the periodontal ligament in dentin and dental pulp by confocal scaning laser microscopy[11,17-20]. This method allows a three-dimensional or two-dimensional evaluation of the architecture of connective fibers, including the application of quantitative assessment techniques[9]. However, these evaluations not have focusing in the oral lesions, especially in OF and IH.

Considering the importance of connective fibers for the tissue architecture, tissue interaction and cell behavior, and that there is no previous report of morphometric and morphological study of connective fibers in OF and IH, the objective of this paper was to evaluate the amount and the arrangement of collagen and elastic fibers in this lesions.

MATERIALS AND METHODS

Samples were randomly retrieved from the files of the Oral Pathology Laboratory, Federal University of Rio Grande do Sul. The criteria for inclusion were as follows: histopathological diagnosis of IH and OF in the gingival and total biopsies.

Two 3μm section were obtained of each paraffin block (13 IH and 18 OF). One was stained by Phosphomolybdic acid- Picrosirius red (PMA-PSR) modified to confocal microscopy[21] (a specific collagen staining) and one by Direct Blue (DB) modified to confocal microscopy[22]. A normal skin slide was used as a posite control. The stainings were done at the Pathology Department of the Instituto Oswaldo Cruz (IOC – Fiocruz – RJ, Brazil).

The microscopic fields selected in the center and in the periphery of the lesions were obtained at 100x magnification. Images of specimens stained with PMA-PSR and DB were examined on a Zeiss LSM 510 meta confocal laser scanning microscope (Zeiss, Oberkochen, Baden-Würtemberg, Germany) with HeNe 543nm laser and an LP 560 filter to improve resolution.

Analysis

For the quantitative analysis, the images were used for collagen fibers quantification. Images were captured in white, grey and black tones to improve the contrast. Therefore, the connective tissue appeared in white and grey tones – corresponding to collagen fibers - and in

black, which corresponded to the interstitial space. The epithelial tissue appeared in black, meaning that the examiner was effectively blind.

Images were captured using a binocular microscope, CX41RF model (Olympus Latin America, Inc., Miami, Florida, USA) with a camera QColor 5, Coolet, RTV (Olympus Latin America, Inc., Miami, Florida, USA) coupled to a computer Dimension 5150 (Dell, Porto Alegre, RS, Brazil) and imported to the Image-Pro® Plus, version 5.1 (Media Cybernetics, Inc., Silver Spring, Maryland, USA), which was used to obtain the number of white, grey and black pixels. A histogram was used to obtain the proportion of collagen in each area.

For the statistical analysis the proportion of collagen fibers of the centre and the periphery of the lesions were considered as different groups and were compared by Analysis of Variance (ANOVA, $p \leq 0.05$).

A morphological analysis of the images of the lesions was carried out according to the following criteria:

✓ Fibers parallelism in relation to epithelial tissue, where:

1 = parallelism absent;

2 = parallelism present.

✓ Distinct fiber layers, where:

1 = no distinction between layers of collagen fibers;

2 = distinct layers of collagen fibers;

✓ Density of collagen fibers, where:

1 = densely arranged fibers

2 = loosely arranged fibers;

For the qualitative analysis, the data from the center and the periphery of the lesions were considered as different groups. The Fisher's exact test with a significance level of 5% was used for statistical analysis.

The examiner was calibrated by an experienced pathologist. Intra-examiner calibration was performed by means of a second analysis of one in every 10 fields observed, applying the Student's "t" test ($p>0.6$) and the Kappa coefficient test ($p>0.7$) in order to determine the degree of agreement for quantitative and qualitative analyses, respectively. The results revealed that there was no statistical difference between readings for either type of analysis.

RESULTS

The quantitative analysis showed differences statistically significant in collagen ratio between the region adjacent to the epithelium layer and center of both lesions and between the two studied regions of the same lesion (Table 1). The proportion of collagen fibers was higher in the center region of OF, following by adjacent to epithelium layer in OF, by region adjacent to epithelium in IH and by the center region of IH.

The qualitative analysis of collagen fibers' parallelism showed the presence of this microscopic characteristic, in the IH lesion and in the region adjacent to epithelium of OF. The center region of OF showed a wound disposition of collagen fibers, with absence of it's parallelism and the differences were statistically significant (Table 2).

The qualitative analysis of collagen fibers' distinction and its density showed that center region of OF was different of the region adjacent to the epithelium in this lesion and different of the IH. The center region of OF tended to have a higher prevalence of no distinction between layers of collagen fibers, which fibers densely arranged and these differences were statistically significant (Tables 3 and 4).

The analysis using the Direct Blue staining did not detect any label representing elastic fibers in the lesions, only around the blood vessels elastic system fibers were seed. (Figure 2)

DISCUSSION

The extracelular matrix is a complex integrated system responsible for the tissue architecture and for the properties of the tissue. The interstitial collagen and system of elastic fibers are the major components of the connective tissue in normal gingivae and in mouth's lesions of this tissue[14-16, 23-24]. The proportion and amount of the contents of connective tissue are determined by the finely tuned balance between syntheis and degradation of extracellular matrix that are consequence of cellular behavior and extraellular matrix interations[25-26]. Variations in the amount and in the ratio of collagen fibers and in the system of elastic fibers can occur in many diseases and are frequently the only morphological findings wich can be

related to the presence of structural defects, lesion's behavior, age changes and clinical symptoms[15,27].

The OF and IH are lesions with very similar clinical characteristics and high prevalence in the mouth (Weir 1987; Coelho 2000). The suggested pathogenesis of both lesions consists of increases in the connective fibers, especially collagen and elastic system that are responsible by the lesion's stromae (Lukes 2005; Priddy 1992; Braga 2006; Magnusson 1995; Bakos 1992). However, the anatomic disposition and distribution of these fibers are different in OF and in IH (Badauy 2002; Oles 1968). Since the architecture of extracellular matrix can play a role in the cell interaction (Toral 2007, Grunheid 2005; Friedl 2000) the differences in the fibers disposition and in the amount of collagen and elastic system fibers could be responsible by the differences in the clinical behavior and different recurrence rate after surgery of OF and IH.

The confocal laser scaning microscopy is a method that offers a high degree of image resolution and allows a quantitative and a morphologic analysis of connective tissue fibers'. (Dolber, Spach 1993; Fung 2003; Carvalho 1996). In this study we used a confocal laser microscopy to analyze the ratio of collagen and elastic system fibers in relationship to a total area of connective tissue in OF and in IH and we used same methodology for to evaluate the morphological disposition of these fibers in these lesions.

The quantitative analysis of the collagen fibers showed a higher concentration of the studied fibers in the central region of the OF compared to the layer adjacent to the epithelial tissue in the same lesion. In the IH an inverse situation was observed, once the region adjacent to the epithelium had more proportion of collagen fibers that the lesion's center. Besides, the lesions were different from each other in the ratio of collagen fibers in every studied region. Since there is a lack in the literature of quantitative studies of collagen fibers in OF and IH we can only speculate, based on studies about gingival enlargement, that the different amount in the fibers can be the result of increase in collagen synthesis, inhibition of collagen's degradative enzymes or both (Sakamoto 2002; Gagliano 2005).

The studies in gingival fibromatosis, that concern to the group of fibrous hyperplastic connective tissue lesions, argue if the differences in the collagen fibers' contents are result of cell behavior or an altered extracellular matrix that influence in the cellular signalizing (Meng 2007; Hakkinen 2007; Coletta 2006). In despite of this controversy, the fact is that different

lesions have a different level of collagen fibers. The investigations in idiopathic gingival enlargement, hereditary gingival fibromatosis and drug induced gingival overgrowth showed a varied level of collagen deposition and a different clinical behavior, in spite of it's similar clinical appearance and morphology (Gagliano 2005; Bonnaure-Mallet 1995; Barros 2001). In the same way, others studies suggested that the collagen deposition is higher in the lesion's growing site. (Sakamoto 2002) Therefore we can suggest that IH and OF are lesions of different nature. The IH is characterized by the presence of inflammatory infiltrate located near to epithelial tissue (Badauy 2002; Badauy 2005), the same region of collagen deposition in this lesion, and we can suppose that this lesion is of inflammatory origin. For the other side the inflammatory infiltrate in extremely rare in OF and the central region concentrated the major part of collagen fibers (Lukes 2005; Magnusson). This can reflects a different etiology, and despite of the controversy in the literature about the origin of these lesion (Priddy 1992; Kfir 1980; Dalei 1990) we can suppose that IH is an inflammatory lesion and the OF is a tumor characterized by a higher collagen deposition in the center of the lesion surrounded by a capsule which a minor collagen content.

In the qualitative analysis we observed a distinct pattern of collagen fibers' parallelism in each studied lesion. The IH showed a similar grade of collagen fibers' parallelism in the two evaluated regions and an optic microscopic study evaluating slides of this lesion stained by hematoxylin and eosin find a similar pattern of collagen disposition (Badauy 2002). In the OF the collagen fibers' parallelism were practically absent in the center of the lesion, but the periphery showed a more high degree of this characteristic. This is in accordance which morphologic investigations in OF present in the literature (Magnusson Rasmusson; Bakos 1992) and a histochemical study in OF showed a mass of fine, medium and coarse collagenous fibers in the center of the lesion surrounded by a well-defined lamina propria which clearly defined extent of bulk of the tumor (OLes 1968).

The analysis of the collagen's fibers layers and the density of those collagen fibers increased the differences in the architecture between the two lesions. In the two regions of IH predominated the distinct layers with loosely arranged fibers characteristic. Take into consideration that IH have an important inflammatory component, the exudative phenomenon of inflammation and it's chemical mediators can undergo changes in the connective tissue, like separation of connective's fibers, make it looser (Staszyc 2004). This statements are in

accordance with our data that showed a higher prevalence of the distinct layers with loosely arranged fibers characteristic in the region adjacent to the epithelial tissue in IH, the region that concentrate the inflammatory infiltrate (Badauy 2002; Badauy 2005). The OF presents a high prevalence of distinct layers with loosely arranged fibers characteristic in it's region adjacent to the epithelial tissue. In the central region of OF, for the other side, we do not observed distinction between layers and we observed densely arranged collagen fibers. These results can be confirmed by a higher proportion these fibers obtained from the quantitative analysis. In benign neoplasm, the major growth is in the lesion's center and leads to the compression of the adjacent tissue. These was observed in the periphery of OF with a higher prevalence of collagen fibers in a parallel disposition, surrounding the central region (Provenzano; Oles 1968; Hirshberg 1999). This reinforce the hypothesis that the OF is possibly a benign neoplasm.

The elastic system is the second more important component of connective tissue and is responsible by mechanical proprieties of the tissue (Ushiki 2002). In the mouth there are variations in the distribution of the types of elastic fibers within the tissues of the masticatory mucosa. The alveolar mucosa has heavy deposits of elastin which decrease in quantity and size as it merges with the attached gingival (Bourke 2000). A previous study of elastic fibers system in gingiva in relation to age showed a regular increase in the thickness of elastic fibers proportional to the age (Gogly 1997).

In the present study we do not observe staining for elastic fibers system in the connective tissue of OF or in IH, but the control of the staining technique showed a positive staining, certifying to us that the staining technique was correctly performed and that our results were reliable. The results the present study are in partial accordance with an immunohistochemical analysis that find elastic system fibers in IH denture induced but not in OF (Mighel, Robinson Hume 1997). The differences can be explained by the methodology used in the cited analysis: the authors considered positive the label around the blood vessels while in this study we only considered the staining of the lesion's connective tissue. Others studies of elastic fibers system showed a very low prevalence of this fibers in OF (Oles 1968) and the absence of these fibers in IH, that are in accordance which our results (Dourov 1990). An ultrastructural investigation in Hereditary Gingival Fibromatosis, a lesion of the same group of OF and IH, observed that elastic fibers system is rare (Barros et al. 2001). These

observations raise the problem of the elastogenesis by the fibroblast in healing and neoplasia, and confirm the role played by the proteolytic enzymes during inflammation (Dourov 1990).

We can conclude that OF have a higher ratio of collagen fibers than IH and this phenomenon is possibly consequence of the stronger stimuli for collagen fiber biosynthesis in benign neoplasms than in inflammatory process. The architecture of collagen fibers in OF and IH is too very distinct. The absence of collagen fibers' parallelism in the central region of OF and the absence of distinction between layers of collagen fibers can be the result of a quick biosynthesis process of these fibers that occurs in the growth center of a benign neoplasm. The presence of parallelism of collagen fibers in the whole extension of IH and high prevalence of distinct layers of collagen fibers with loosely arranged fibers are probably consequence of the exudation from the blood vessels in inflammatory process that distends the connective tissue fibers in a same direction and make it looser. The two observed lesions do not present elastic fibers system and this can be the result of immature connective tissue that is the component of OF and IH and the fact that elastic fibers are rarely synthesize in the presence of inflammation and in the situation of quick fiber biosynthesis by fibroblast.

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REFERENCES

1. Dalei, TD., Wisocky. GP., Wisocky, PD., and Wisocky, DM. (1990) The major epulides: clinicopathological correlations *J Can Dent Assoc* 56:627-630

2. Zain, RB., and Fei, YJ. (1990) Peripheral fibroma/fibrous epulis with and without calcifications. A clinical evaluations of 204 cases in Singapore *Odontolstomatol Trop* 13:94-96.

3. Kfir, Y., Buchner, A., and Hansen, LS. (1980) Reactive lesions of the gingiva. A clinicopathological study of 741 cases *J Periodontol* 51:655-661.

4. Mighell, AJ., Robinson, PA., and Hume, WJ. (1996) Immunolocalization of tenascin – C in focal reactive overgrowths of oral mucosa *J Oral Pathol Med* 25:163 –169.

5. Lukes, SM., Kuhnert, J., and Mangels, MA. (2005) Identification of a giant cell fibroma *J Dent Hyg* 79:1-14.

6. Magnusson, BC., and Rasmusson, LG. (1995) The giant cell fibroma. A review of 103 cases with immunhistochemical findings *Acta Odontol Scand* 53:293-296.

7. Badauy, CM., Barbachan, JJD., and Rados, PV. (2002) Inflammatory hyperplasia: a microscopic study *Rev Fac Odontol* 43:48-51. (Article in Portuguese)

8. Badauy, CM., Barbachan, JJD., Rados, PV., Sant'Ana Filho, M., and Chies, JAB. (2005) Relationship between candida infection and immune cellular response in inflammatory hyperplasia *Oral Microbiol Immunol* 20:89-92.

9. Petroll, WM. (2006) Differential interference contrast and confocal reflectance imaging of collagen organization in three-dimensional matrices *Scanning* 28:305-310.

10.Glass-Brudzinski, J., Perizzolo, D., and Brunette, DM. (2002) Effects of substratum surface topography on the organization of cells and collagen fibers in collagen gel cultures *J Biomed Mater Res* 15:608-618.

11. Kingsmill, VJ., and Boyde, A. (2001) Collagen fiber orientation affects osteoclastic resorption *J Dent Res* 80:2085-2088.

12. Gao, Y., Kostrominova, TY., Faulkner, JA., and Wineman, AS. (2008) Age-related changes in the mechanical properties of the epimysium in skeletal muscle of rats *J Biomech* 41:465-469.

13. Provenzano, PP., Elicieri, KW., Campbell, JM., Inman, DR., White, JG., and Keely, PJ. (2006) Collagen reorganization at the tumor-stromal interface facilitates local invasion *BMC Med* 4:38.

14. Ushiki, T. (2002) Collagen fibers, reticular fibers and elastic fibers. A comprehensive understanding from a morphological viewpoint *Arch Histol Cytol* 65:109-126.

15. Gogly, B., Godeau, G., Gilbert S., Lengrand JM., Kut, C., Pellat, M., and Goldberg, M. (1997) Morphometric analysis of collagen and elastic fibers in normal skin and gingival in relation to age *Clin Oral Invest* 1:147-152.

16. Oles, RD (1968) Incidence and distribution of various connective tissue fibers in oral fibromas. *Oral Surg Oral Med Oral Pathol* 26:487-495.

17. Reichenberger, E., Baur, S., Sukojo, C., Olsen, BR., Karimbux, NY., and Nishimura, I. (2000) Collagen XII mutation disrupts matrix structure of periodontal ligament and skin *J Dent Res* 79:1962-1968.

18. Kagayama, M., Sasano, Y., Tsuchiva, M., Watanabe, M., Mizoguchi, I., Kamakura, S., and Motegi, K. (2000) Confocal microscopy of Tomes' granular layer in dog premolar teeth *Anat Embryol (Berl)* 201:131-137.

19. Ohsaki, Y., and Nagata, K. (1994) Type III collagen is a major component of interodontoblastic fibers of the developing mouse molar root *Anat Rec* 240:308-313.

20. Hirschberg, A., Buchner, A., and Dayan, D. (1996) The central odontogenic fibroma and the hyperplastic dental follicle: study with Picrosirius red and polarizing microscopy *J Oral Pathol Med* 25:125-127.

21. Dolber, PC., and Spach, MS. (1993) Conventional and confocal fluorescence microscopy of collagen fibers in the heart. *J Histochem Cytochem* 41:465-469.

22. De Brito Gitirana, L. and Trindade, AV. (2000) Direct blue staining plus polarization microscopy: an alternative dye for polarization method for collagen detection in tissue sections. *J Histotechnology* 23:1-3.

23. Everts, V., Niehof, A., Jansen, D., and Beertsen, W. (1998) Type IV collagen is associated with microfibrils and oxytalan fibers in the extracellular matrix of periodontium, mesenterium and periosteum *J Periodont Res* 33:118–125.

24. Bourke, K., Haase, H., Li, H., Daley, T., and Bartold, P. (2000) Distribution and synthesis of elastin in porcine gingiva and alveolar mucosa *J Periodont Res* 35:361–368.

25. Birkedal-Hansen, H.(1993) Role of matrix metalloproteinases in human periodontal diseases *J Periodontol* 64:474-484.

26. Woessner, FJJr. (1991) Matrix metalloprtoteinases and their inhibitors in connective tissue remodeling *FASEB J* 5:2145-2154.

27. Holbrook, KA., and Beyers, PH. (1982) Structural abnormalities in dermal collagen and elastic matrix from the skin patients with connective tissue disorders *J Invest Dermatol* 79:7-16.

28. Weir, JC., Davenport, WD., and Skinner, RL. (1987) A diagnostic and epidemiologic survey of 15,783 oral lesions *J Am Dent Assoc* 115:439-442.

29. Coelho, ZL., Zucoloto, MD., and Lopes, RA. (2000) Denture-induced fibrous inflammatory hyperplasia: a retrospective study in a school of dentistry *Int J Prosthodont* 13:148-151.

30. Priddy, RW. (1992) Inflammatory Hyperplasias of the oral mucosa *J Can Dent Assoc* 58:311-315, 319-321.

31. Braga, MM., Carvalho, AL., Vasconcelos, MC., Braz-Silva, PH., and Pinheiro, SL. (2006) Giant cell fibroma; a case report *J Clin Pediatr Dent* 30:261-264.

32. Bakos, LH. (1992) Giant cell fibroma: a review of 116 cases *Ann Dent* 51:32-35.

33. Toral, C., Solano-Agama, C., Reyes-Márquez, B., Sabanero, M., Talamás, P., González del Pliego, M., and Mendoza-Garrido, ME. (2007) Role of extracellular matrix-cell interaction and epidermal growth factor (EGF) on EGF-receptors and actin cytoskeleton arrangement in infantile pituitary cells *Cell Tissue Res* 327:143-153.

34. Grunheid, T., and Zentner, A. (2005) Extracellular matrix synthesis, proliferation and death in mechanically stimulated human gingival fibroblasts in vitro *Clin Oral Invest* 9:124-130.

35. Friedl, P., and Brocker, EB. (2000) The biology of cell locomotion within three-dimensional extracellular matrix *Cell Mol Life Sci* 57:41-64.

36. Fung, D., Ng, D., Leung, M., and Tay, D. (2003) Investigation in the collagen fibril distribution in the medial collateral ligament in a rat knee model *Connect Tissue Res* 44:2-11.

37. Carvalho, HF., and Taboga, SR. (1996) The applicability of Hematoxylin-eosin staining plus fluorescence or confocal laser scanning microscopy to the study of elastic fibers in cartilages. *C R Acad Sci III* 319:991–996.

38. Kather, J., Salgado, MA., Salgado, UF., Cortelli JR., and Pallos, D. (2008) Clinical and histomorphometric characteristics of three different families with hereditary gingival fibromatosis *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 105:348-352.

39. Sakamoto, R., Nitta, T., Kamikawa, Y., Kono, S., Kamikawa, Y., Sugihara, K., Tsuyama, S., and Murata, F. (2002) Histochemical, immunohistochemical, and ultrastructural studies of gingival fibromatosis: a case report *Med Electron Microsc* 35:248-254.

40. Gagliano, N., Moscheni, C., Dellavia, C., Masiero, S., Torri, C., Grizzi, F., Stabellini, G., and Gioia, M. (2005) Morphological and molecular analyses of idiopathic gingival fibromatosis: a case report *J Clin Periodontol* 32:1116-1121.

41. Meng, L., Huang, M., Ye, X., Fan, M., and Bian, Z. (2007) Increased expression of collagen prolyl 4-hydroxylases in Chinese patients with hereditary gingival fibromatosis *Arch Oral Biol* 52:1209-1212.

42. Hakkinen, L., and Csiszar, A. (2007) Hereditary gingival fibromatosis: characteristics, and novel putative pathogenic mechanisms *J Dent Res* 86:25-34.

43. Coletta, RD., and Graner, E. (2006) Hereditary gingival fibromatosis: a systematic review *J Periodontol* 77:753-764.

44. Bonnaure-Mallet, M., Tricot-Doleus, S., and Godeau, GJ. (1995) Changes in extacelullar matrix macromolecules in human gingiva after treatment with drugs inducing gingival overgrowth *Arch Oral Biol* 40:393-400.

45. Barros, S., Merzel, J., Araújo, V., Almeida, O., and Bozzo, L. (2001) Ultrastructural aspects of connective tissue in hereditary gingival fibromatosis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodon* 92:78-82.

46. Staszyk, C., and Gasse, H. (2004) Oxytalan fibres in the periodontal ligament of equine molar cheek teeth *Anat Histol Embryol* 33:17–22.

47. Hirshberg, A., Sherman, A., Buchner, A., and Dayan, D. (1999) Collagen fibres in the wall of Odontogenic Keratocysts: a study with Picrosirius Red and Polarizing Microscopy. *J Oral Pathol Med* 28:410–412.

48. Bourke, KA., Haase, H., Li, H., Daley, T., and Barthold, PM. (2000) Distribution and synthesis of elastin in porcine gingival and alveolar mucosa *J Periodontal Res* 35:361-368.

49. Mighell, AJ., Robinson, PA., and Hume, WJ. (1997) Histochemical and Immunohistochemical localization of elastic system fibers in focal reactive overgrowths of oral mucosa *J Oral Pathol Med* 25:153–158.

50. Dourov, N. (1990) The demonstration of a system of elastic fibers in a study of fibromatous lesions of oral mucosa *Bull Group Int Rech Sci Stomatol Odontol* 33:125-130.

TABLES

Table1 Quantitative assessment of collagen ratio in confocal laser images extracted from OF and IH. Analysis of Variance (ANOVA) followed by Least Significance Difference (LSD). Means followed by different letters are different from each other.

Collagen ratio	Mean (SD)	p value
Oral Fibroma		≤0.05
- Adjacent to the epithelium layer	62.46 (5.56) A	
- Lesion center	75.90 (3.78) B	
Inflammatory hyperplasia		
- Adjacent to the epithelium layer	62.06 (3.67) C	
- Lesion center	57.56 (4.74) D	

Table 2. Analysis of parallelism of collagen fibers in IH and OF. Statistical analysis by Fischer's exact test ($\alpha \leq 0{,}05$)

Microsopic characteristic	Group			
Collagen fiber parallelism	Inflammatory Hyperplasia adjacent to the epithelium (A group)	Inflammatory Hyperplasia center of the lesion (B group)	Oral Fibroma adjacent to the epithelium (C group)	Oral Fibroma center of the lesion (D group)
Present	12 (92.3%)	11 (84.6%)	13 (72.22%)	2 (11.12%)
Absent	1 (7.7%)	2 (5.4%)	5 (27.78%)	16 (88.88%)
Difference statistically significant (P<0.05)				*

Table 3. Qualitative analysis of distinction of layers of collagen fibers. Statistical analysis by Fischer's exact test ($\alpha \leq 0,05$)

Microsopic characteristic	Group			
	Inflammatory Hyperplasia adjacent to the epithelium (A group)	Inflammatory Hyperplasia center of the lesion (B group)	Oral Fibroma adjacent to the epithelium (C group)	Oral Fibroma center of the lesion (D group)
Distinct layers of collagen fibers	13 (100 %)	13 (100 %)	18 (100 %)	3 (16.66 %)
No distinction between layers of collagen fibers	0 (%)	0 (0 %)	0 (0 %)	15 (83.34 %)
Difference statistically significant (P<0.05)				*

FIGURES

Figure 1: A. Photomicrography of region adjacent to the epithelial tissue from OF. Observe the lower density of collagen fibers compared with the figure C in a parallel arrange. B. Photomicrography of region adjacent to the epithelial tissue from IH. Observe the higher density of collagen fibers compared to the figure D in a parallel arrange. C. Photomicrography of central region from OF, showing the highest concentration of collagen fibers. Note the absence of the parallelism of the fibers. D. Photomicrography of central region from IH, showing the lowest concentration of collagen fibers. Note the parallelism of the fibers. (Photomicrography obtained with confocal laser microscopy; Picrosirius red staining; original magnification 100x).

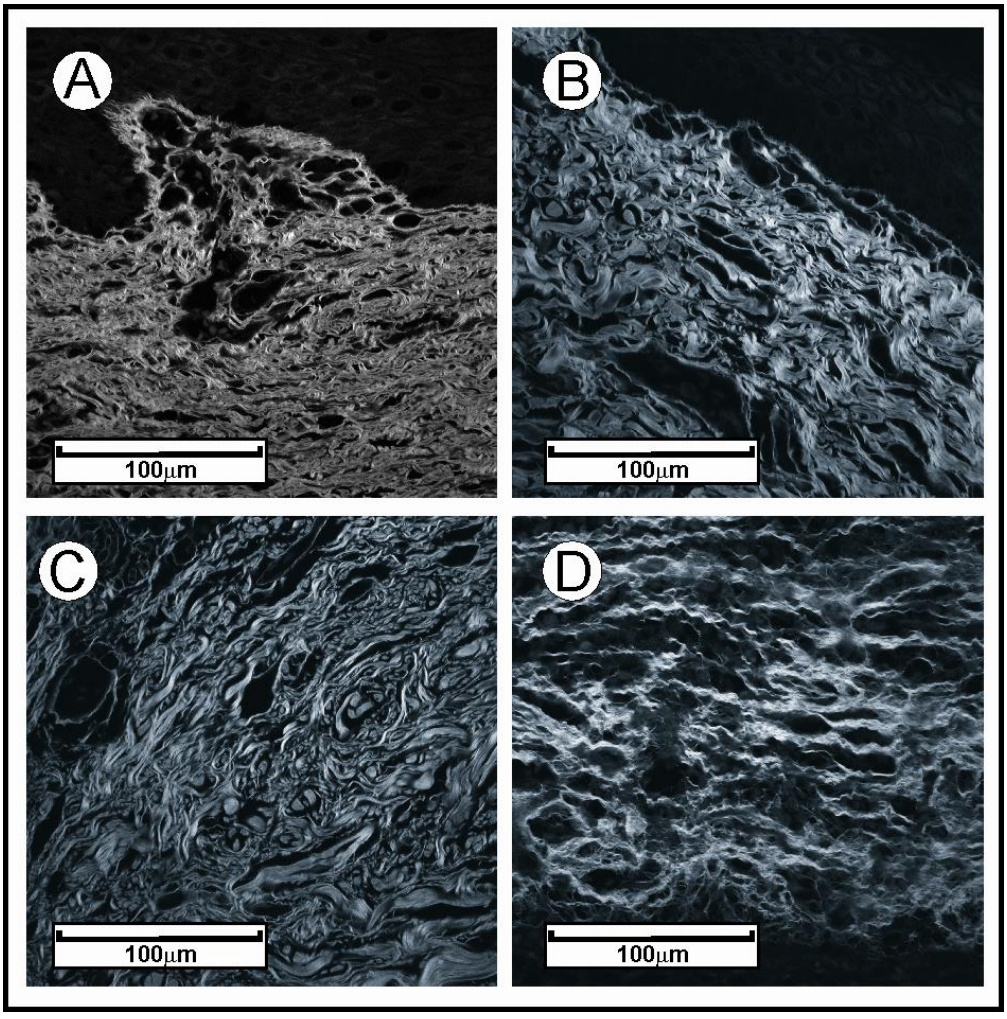
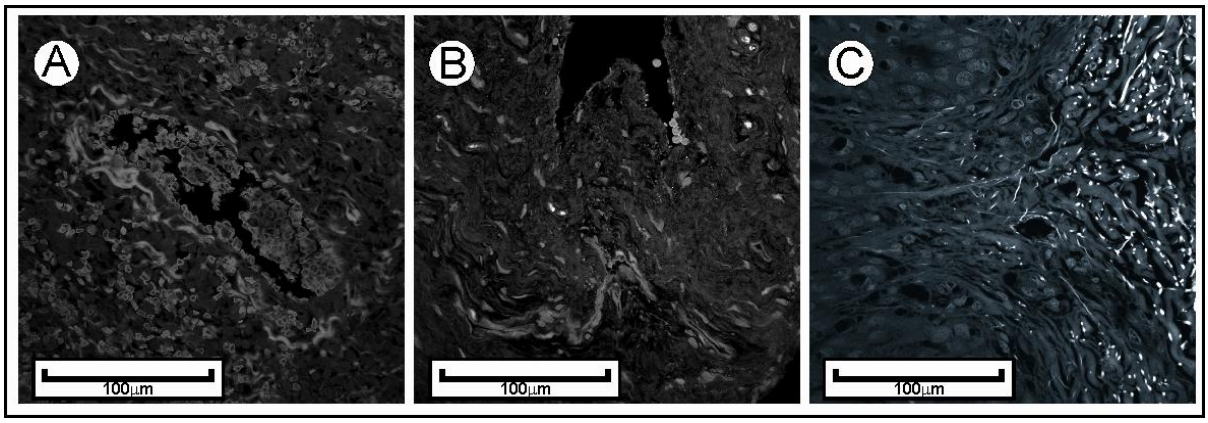


Figure 2: A. Photomicrography from the stroma of OF showing the absence of staining for elastic fibers system. B. Photomicrography from the stroma of IH showing the absence of staining for elastic fibers system. The white points seed in the figure are red blood cells. C. Photomicrography from technique's control showing the staining for elastic fibers system, certifying to us that the staining technique was correctly performed.
(Photomicrography obtained with confocal laser microscopy; Direct blue staining; original magnification 100x).



A STUDY OF CONNECTIVE COMPONENTS IN THREE FIBROUS GINGIVAL LESIONS

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ABSTRACT

Background: Fibrous hyperplastic connective tissue lesions are the most common group of gingival overgrowth, but the extracellular matrix and cellular characteristics of these lesions remain poorly investigated.

Methods: Histological sections from the gingiva of 3 patients with hereditary gingival fibromatosis (HGF), 13 inflammatory hyperplasias (IH) and 18 oral fibromas (OF) were stained for collagen, oxytalan, reticular and elastic fibers. The sections were analyzed in light and confocal microscopy for analysis of quantification, distribution and concentration of the fibers that were classified according to a scale. The fibroblasts and inflammatory cells were counted. The data were compared by Kruskall-Wallis test and by Analysis of Variance.

Results: The collagen fibers were more concentrated in HGF, followed by OF and by IH. These fibers were distributed in the center of HGF and OF and in the periphery of IH. The reticular fibers were more concentrated in HGF. These fibers were distributed in the center of HGF and OF and in the periphery of IH. The fibroblasts were more numerous in OF and the inflammatory cells were more numerous in IH, followed by OF and by HGF.

Conclusion: The studied lesions have a different connective structure.

Keywords: Fibromatosis, gingival; Inflammatory hyperplasia; Oral fibroma; Collagen fibers; Reticular fibers; Connective cells.

INTRODUCTION

Fibrous hyperplastic connective tissue lesions are the most common group of lesions in the oral cavity and may be similar both clinically and histologically. These lesions are most prevalent in the gingiva and clinically look like an exofitic red round mass of the oral mucosa, sessile or pedunculated, with a regular surface which can occasionally be ulcerated. Their growth is slow and the lesions are painless, except under traumatic conditions (Kfir 1980, Mighell 1996, Lukes 2005, Dalei 1990, Zain 1990, Priddy 1992).

The Fibrous hyperplastic connective tissue lesions were classified into major four groups according to their prevalence: focal fibrous hyperplasias, oral fibromas, pyogenic granulomas or peripheral giant cell granulomas. (Dalei 1990, Mc Ginnis 1987). The inflammatory hyperplasias (IH) and the Hereditary gingival fibromatosis (HGF) are lesions grouped in the general denomination of focal fibrous hyperplasias. The IH is a reactive lesion of connective and epithelial components, associated to a traumatic etiology (Coelho 1994, Wright 1992), while the HGF is an inherited disease characterized by a slow, progressive increase in gingival bulk and fibrosity (Baptista 2002, Coletta Graner 2006). The OF are localized swellings that growths slowly and have a benign tumoral etiology (Magnusson Rasmusson 1994, Oles 1968)

The clinical behavior of these lesions are well documented, however the extracellular matrix macromolecules involved in different types of gingival overgrowth remain poorly investigated (Houston 1982, Reibel 1982, Swan 1988, Bakos 1992, Bonnaure Mallet 1995). In the same way, reports of histological and cellular characteristics of gingival fibrous lesions are not always consistent, and our previous studies (unpublished data) showed a difficult in it's microscopic diagnosis.

The objective of the present study was to study fibrous and cellular characteristics of connective tissue in gingival lesions diagnosed like inflammatory hyperplasias (IH), oral fibromas (OF) and hereditary gingival fibromatosis (HGF).

MATERIAL AND METHODS

Preparation of the samples

The samples were obtained by total biopsies from gingival overgrowth with a clinical diagnosis of IH, OF and from a 3 generation family with a HGF diagnosis.
Five histological 4μm sections of each paraffin block (n= 34: 18 OF, 13 IH and 3 HGF) were obtained. One was stained with hematoxylin-eosin (HE) for cell count, one with Picrosirius Red (PR) for collagen fibers, one with Orcein (OR) for oxytalan fibers, one with Gomori reticulin (GR) for reticular fibers according to Bancroft and Stevens' description protocol and one by Direct Blue (DB) for elastic fibers system modified to confocal microscopy (De Brito Gitirana, Trindade 2000). The staining was done at the Pathology Department of the Instituto Oswaldo Cruz (IOC – Fiocruz – RJ, Brazil) and a normal skin slide was used as a posite control.

Microscopic evaluation

For the analysis, one calibrated examiner, that was blind for the sample's diagnosis, used an optical microscope CX41RF model (Olympus Latin America, Inc., Miami, Florida, USA) in a magnification of 100x.

The quantification of connective fibers was performed by sample according to the following scale: 0: absence of the characteristic; 1: characteristic present in up to 1/3 of the microscopic fields of the lesion; 2: characteristic present in up to 1/2 of the microscopic fields of the lesion; 3: characteristic present in more than 1/2 of the fields of the lesion.

The distribution of connective fibers was classified by sample according to the following scale: 0: absence of fibers; 1: fibers present predominantly in the periphery of the lesion; 2: fibers present predominantly in the center of the lesion; 3: fibers present in both center and periphery of the lesion.

The fiber's concentration was evaluated by a scale in absent (0), mild (1), moderate (2) and dense status (3).

For the fibroblast and inflammatory cells count, we used a previously described methodology (Badauy 2005).

This research was approved by the Research Commission and the Ethics Committee at the School of Dentistry of UFRGS.

Statistical analysis

The scores obtained from the fibers' analysis were compared by Kruskall-Wallis test, folowing by Mann-Whitney "U" test ($p \leq 0.05$). The data from the count cells methodology were compared by Analysis of Variance (ANOVA, $p \leq 0.05$).

The examiner had undergone calibration training in advance under supervision by an experienced pathologist. Intra-examiner calibration was performed by means of a second analysis of one in every 10 fields observed, applying the Student's "t" test ($p>0.6$) and the Kappa coefficient test ($p>0.7$) in order to determine the degree of agreement for quantitative and qualitative analyses, respectively. The results revealed that there was no statistical difference between readings for either type of analysis.

RESULTS

The analysis using the OR and DB staining did not detect any staining representing oxytalan and elastic system fibers in the lesions' connective tissue.

The results from PS staining (figure 1) showed that collagen fibers tended to concentrate in the periphery of IH, while in the OF and in the HGF the collagen fibers were preferentially in the lesion's center. The concentration of collagen fibers was higher in the HGF, following by OF and by IH and the differences were statistically significant (table 1).

The results from GR staining showed that reticular fibers tended to concentrate in the periphery of IH, while in the OF and in the HGF the reticular fibers were preferentially in the lesion's center (figure 1). The concentration of reticular fibers was higher in the HGF than the two other lesions (table 2).

The results from the count cells methodology showed that inflammatory cells were more numerous in IH, followed by OF and by HGF. The fibroblasts were more numerous in the OF than IH and HGF and the difference was statistically significant (table 3 and figure 2).

DISCUSSION

The extracelular matrix is a complex integrated system responsible for the tissue architecture and for the properties of the tissue. The extracellular matrix seems to play a role in the cell interaction (Toral 2007, Grunheid 2005; Friedl 2000) and the differences in the fibers amount and in it's disposition can be responsible by the differences in the clinical behavior

In this study we performed an analysis of the fibers and the connective cells. The results showed that the three studied lesions were different in their connective components. The results from the collagen fibers analysis showed a higher concentration of these fibers in the gingiva of HGF patients, followed by OF (a benign tumour) and by IH (an inflammatory lesion), suggesting a different mechanism of deposition of collagen fibers in this patients. This increase in collagen fibers is similar in HGF affected members of the same family although there are significant differences in the amount of synthesis/deposition of collagen fibers among families (Meng 2007, Kather 2008).

The HGF and the OF showed a collagen fibers distribution in the lesion's center while IH presented a peripheryc distribution of these fibers. The results obtained from HGF and OF microscopic analysis can reinforce the hypothesis of deposition of collagen fibers in the growing center of the lesion (Sakamoto 2002). Considering that IH has an important inflammatory component, the exudative phenomenon of inflammation, that occurs in this lesion in the layer adjacent to the epithelial tissue, and its chemical mediators can induce changes in the connective tissue, such as separation of connective fibers, making it looser and allowing an easier distinction between the bundles of collagen fibers (Staszyk 2004).

The elastic system is the second more important component of connective tissue and is responsible by mechanical proprieties of the tissue (Ushiki 2002). In the mouth there are variations in the distribution of the types of elastic fibers within the tissues of the masticatory

mucosa. In the present study we do not observe staining for elastic fibers system in the connective tissue of studied lesions, but the control of the staining technique showed a positive staining, certifying to us that the staining technique was correctly performed and that our results were reliable. The results the present study are in partial accordance with an immunohistochemical analysis that find elastic system fibers in IH denture induced but not in OF (Mighel, Robinson Hume 1997). The differences can be explained by the methodology used in the cited analysis: the authors considered positive the label around the blood vessels while in this study we only considered the staining of the lesion's connective tissue. Others studies of elastic fibers system showed a very low prevalence of this fibers in OF (Oles 1968) and the absence of these fibers in IH, that are in accordance which our results (Dourov 1990). An ultrastructural investigation in HGF, observed that elastic fibers system is rare (Barros et al. 2001). These observations raise the problem of the elastogenesis by the fibroblast in healing and neoplasia, and confirm the role played by the proteolytic enzymes during inflammation (Dourov 1990).

The oxytalan fibers are formed by a microfibrillar and an amorphous component (Chavrier 1990) and are an important component of the normal and of the regenerated periodontal ligament (Sculean 1999 e 1998). These fibers are an infrequent finding in human gingival, but there are reports of their ultrastructural distribution in the upper layers of gingival connective tissue (Chavrier 1988 e 1990). In this study we do not observed positive staining for oxytalan fibers in none of the studied lesions. In this way, there are no studies of these fibers in the studied lesions in the literature, and considering that the oxytalan fibers are a component of elastic system fibers (Chavrier 1988 e 1990) we can affirm that our results confirm the results from elastic system fiber methodology of this study.

Reticular fibers are fine fibers forming an extensive network in certain organs that are usually found mainly in the basement of epithelial tissues. The reticular fibers differ in structure, arrangement and function from collagen fibrils, but are continuous with collagen fibers with the objective of to form an extensive network so called the collagen fibrillar system (Ushiki 2002).

The results from the reticular fibers analysis showed a higher concentration of these fibers in the gingiva of HGF patients, than the OF and the IH. The reticular fiber's distribution analysis also showed a similar result to the collagen fibers analysis which a preferential

localization in the center of HGF and OF and in the periphery of IH. Since the reticular fibers are also a component of collagen fiber type I and type III, we can speculate if these fibers are oriented the new collagen fibers synthesized, justifying the coincidence of the results from the two methodologies (Montes 1980, Ushiki 2002).

The fibroblasts are cells responsible by the production and maintenance of extracellular matrix, including the collagen and reticular fiber synthesis (Agis 2008, Locke 2008). In this study, we observed a higher number of fibroblasts in OF when compared to HGF and IH, and this result can reinforce the hypothesis of tumoral ethiology for OF (Houston 1982, Reibel 1982, Magnusson Rasmusson 1995). There are no studies of fibroblasts' number in OF and in IH, but a study in three HGF affected families showed that the number of these cells is variable between the members of different families (Kather 2008). Thus, we can suggest that the number of fibroblasts have not any relation which the distribution and which the concentration of the collagen and reticular fibers in the three studied lesions.

The analysis of inflammatory cells showed a low number in HGF, a medium number in OF and a high number in IH of these cells. The results can be explained because inflammatory cells are rather rare in the gingiva of HGF patients (Gunhan 1995,Bitu 2006). Nevertheless, a histomorphometric study that quantified the inflammatory cells in the gingiva from 3 different HGF families show a high level of diversity among the analyzed families, suggesting that the pattern of inflammation could influence the pathogenesis of HGF (Kather 2008). In the other hand the inflammatory cells have been considered a frequent feature in microscopic observations of IH, related to traumatic condition or an opportunist infection (Badauy 2005, Badauy 2002).

Thus, we can conclude that the HGF, the OF and the IH have a different fibrous and cellular structure. The HGF have more concentration of collagen and reticular fibers that are localized in the center's lesion, and the lowest cellular number. The OF have more fibroblasts and a medium degree of collagen fibers that are localized in the center's lesion. The IH have more inflammatory cells, a medium number of fibroblasts and the lowest concentration of collagen fibers that are localized in the periphery of the lesion.

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REFERENCES

1. Kfir Y, Buchner A, Hansen LS. Reactive lesions of the gingiva. A clinicopathological study of 741 cases. *J Periodontol* 1980;**51**:655-661.

2. Mighell AJ, Robinson PA, Hume WJ. Immunolocalization of tenascin – C in focal reactive overgrowths of oral mucosa. *J Oral Pathol Med* 1996; **25**:163 –169.

3. Lukes SM, Kuhnert J, Mangels MA. Identification of a giant cell fibroma. *J Dent Hyg* 2005;**79**:1-14.

4. Dalei TD, Wisocky GP, Wisocky PD, Wisocky DM. The major epulides: clinicopathological correlations. *J Can Dent Assoc* 1990;**56**:627-630

5. Zain RB, Fei YJ. Peripheral fibroma/fibrous epulis with and without calcifications. A clinical evaluations of 204 cases in Singapore. *Odontolstomatol Trop* 1990;**13**:94-96.

6. Priddy RW. Inflammatory Hyperplasias of the oral mucosa. *J Can Dent Assoc* 1992;**58**:311-315, 319-321.

7. Mc Ginnis JPJr. Review of the clinical and histopathologic features of four exophytic gingival lesions – the pyogenic granuloma, irritation fibroma, peripheral giant cell granuloma, and peripheral ossifying fibroma. *J Okla Dent Assoc* 1987;**77**:25-30.

8. Coelho CM, Zucoloto S, Lopes RA. Denture-induced fibrous inflammatory hyperplasia: a retrospective study in a school of dentistry. *Int J Prosthodont* 1994;**9**:88-94.

9. Wright SM, Scott BJJ. Prosthetic assessment in the treatment of denture hyperplasia. *Br Dent J* 1992;**172**:313-315.

10. Baptista IP. Hereditary gingival fibromatosis: a case report. *J Clin Periodontol* 2002;**29**:871-874.

11. Coletta RD, Graner E. Hereditary gingival fibromatosis: a systematic review. *J Periodontol* 2006;**77**:753-764.

12. Magnusson BC, Rasmusson LG. The giant cell fibroma. A review of 103 cases with immunhistochemical findings. *Acta Odontol Scand* 1995;**53**:293-296.

13. Oles RD. Incidence and distribution of various connective tissue fibers in oral fibromas. *Oral Surg Oral Med Oral Pathol.* 1968;**26**: 487-495.

14. Houston GD. The giant cell fibroma. A review of 464 cases. *Oral Surg Oral Med Oral Pathol* 1982;**53**:582 – 587.

15. Reibel J. Oral fibrous hyperplasias containing stellate and multi-nucleated cells. *Scand J Dent Res* 1982;**90**:217-226.

16. Swan RH. Giant cell fibroma. A case presentation and review. *J Periodontol* 1988; **59**:338-340.

17. Bakos LH. Giant cell fibroma: a review of 116 cases. *Ann Dent* 1992;**51**:32-35.

18. Bonnaure-Mallet M, Tricot-Doleaux S, Godeau GJ. Changes in extracellular matrix macromolecules in human gingival after treatment with drug inducing gingival overgrowth. *Archs Oral Biol* 1995;**40**:393-400.

19. Brancoft JD, Stevens A. Theory and practice of histological techniques. London: Churchill Livingstone 1996.

20. De Brito Gitirana L, Trindade AV. Direct blue staining plus polarization microscopy: an alternative dye for polarization method for collagen detection in tissue sections. *J Histotechnology* 2000; **23**:1-3.

21. Badauy CM, Barbachan JJD, Rados PV, Sant'Ana Filho M, Chies JAB. Relationship between candida infection and immune cellular response in inflammatory hyperplasia *Oral Microbiol Immunol* 2005;**20**:89-92.

22. Toral C, Solano-Agama C, Reyes-Márquez B, Sabanero M, Talamás P, González del Pliego M, and Mendoza-Garrido ME. Role of extracellular matrix-cell interaction and epidermal growth factor (EGF) on EGF-receptors and actin cytoskeleton arrangement in infantile pituitary cells *Cell Tissue Res* 2007; **327**:143-153.

23. Grunheid T, Zentner A. Extracellular matrix synthesis, proliferation and death in mechanically stimulated human gingival fibroblasts in vitro *Clin Oral Invest* 2005; **9**:124-130.

24. Friedl P, Brocker EB. The biology of cell locomotion within three-dimensional extracellular matrix *Cell Mol Life Sci* 2000; **57**:41-64.

25. Meng L, Huang M, Ye X, Fan M, Bian Z. Increased expression of collagen prolyl 4-hydroxylases in Chinese patients with hereditary gingival fibromatosis. *Arch Oral Biol* 2007;**52**:1209-1214.

26. Kather J, Salgado MA, Salgado UF, Cortelli JR, Pallos D. Clinical and histomorphometric characteristics of three different families with hereditary gingival fibromatosi. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008;**105**:348-352.

27. Sakamoto R, Nitta T, Kamikawa Y, Kono S, Kamikawa Y, Sugihara K et al. Histochemical, immunohistochemical, and ultrastructural studies of gingival fibromatosis: a case report. *Med Electron Microsc* 2002;**35**:248-254.

28. Staszyk C, Gasse H. Oxytalan fibres in the periodontal ligament of equine molar cheek teeth. *Anat Histol Embryol* 2004;**33**:17–22.

29. Ushiki T. Collagen fibers, reticular fibers and elastic fibers. A comprehensive understanding from a morphological viewpoint *Arch Histol Cytol* 2002;**65**:109-126.

30. Mighell, AJ., Robinson, PA., and Hume, WJ. Histochemical and Immunohistochemical localization of elastic system fibers in focal reactive overgrowths of oral mucosa *J Oral Pathol Med* 1997;**25**:153–158.

31. Dourov N. The demonstration of a system of elastic fibers in a study of fibromatous lesions of oral mucosa *Bull Group Int Rech Sci Stomatol Odontol* 1990;**33**:125-130.

32. Barros S, Merzel J, Araújo V, Almeida O, Bozzo L. Ultrastructural aspects of connective tissue in hereditary gingival fibromatosis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodon* 2001;**92**:78-82.

33. Chavrier C. The elastic system fibres in healthy human gingiva. *Arch Oral Bio*l 1990;**35** Suppl:223S-225S

34. Sculean A, Donos N, Reich E, Karring T, Brecx M. Regeneration of oxytalan fibres in different types of periodontal defects: a histological study in monkeys. *J Periodontal Res* 1998;**33**:453-459.

35. Sculean A, Donos N, Windisch P, Reich E, Gera I, Brecx M, Karring T. Presence of oxytalan fibers in human regenerated periodontal ligament. *J Clin Periodontol* 1999;**26**:318-321.

36. Chavrier C, Hartmann DJ, Couble ML, Herbage D. Distribution and organization of the elastic system fibres in healthy human gingiva. Ultrastructural and immunohistochemical study. *Histochemistry* 1988;**89**:47-52.

37. Montes GS, Krisztan RM, Shigihara KM, Tokoro R, Mourao PAS, Junqueira LCU. Histochemical and morphological characterization of reticular fibers. *Histochemistry* 1980;**65**:131-141.

38. Agis H, Bauer M, Knebl G, Watzek G, Gruber R. Effects of platelet-derived growth factor isoforms on plasminogen activation by periodontal ligament and gingival fibroblasts. *J Periodontal Res* 2008;**43**:334-342.

39. Locke M, Hiland PL, Irwin CR, Mackenzie IC. Modulation of gingival epithelial phenotypes by interactions with regionally defined populations of fibroblasts. *J Periodontal Res* 2008;**43**:279-289.

40. Gunhan O, Gardner DG, Bostanci H, Gunhan M. Familial gingival fibromatosis with unusual histologic findings. *J Periodontol* 1995;**66**:1008-1011.

41. Bitu CC, Sobral LM, Kellermann MG, Martelli-Junior H, Zecchin KG, Graner E, Coletta RD. Heterogeneous presence of myofibroblasts in hereditary gingival fibromatosis. *J Clin Periodontol* 2006;**33**:393-400.

42. Badauy CM, Barbachan JJD, Rados PV. Inflammatory hyperplasia: a microscopic study *Rev Fac Odontol* 2002;**43**:48-51. (in Portuguese)

TABLES

Table1 Analysis of collagen fibers in microscopic images extracted from OF, IH and FGH. Comparison of the results with Kruskal-Wallis test, followed by Mann-Whitney "U" test. Mean rank followed by different letters are different from each other.

Microscopic characteristic/ Lesion	**Mean Rank**	**p value**
		≤0.05
Fiber's quantification		
- Oral Fibroma	18.67 A	
- Inflammatory hyperplasia	14.85 A	
-Hereditary gingival fibromatosis	22.00 A	
Fiber's distribution		
- Oral Fibroma	20.00 A	
- Inflammatory hyperplasia	12.77 B	
-Hereditary gingival fibromatosis	23.00 A	
Fiber's concentration		
- Oral Fibroma	20.06 A	
- Inflammatory hyperplasia	11.88 B	
-Hereditary gingival fibromatosis	26.50 C	

Table 2 Analysis of reticular fibers in microscopic images extracted from OF, IH and FGH. Comparison of the results with Kruskal-Wallis test, followed by Mann-Whitney "U" test. Mean rank followed by different letters are different from each other.

Microscopic characteristic/ Lesion	Mean Rank	p value
Fiber's quantification		≤0.05
- Oral Fibroma	17.00 A	
- Inflammatory hyperplasia	18.31 A	
-Hereditary gingival fibromatosis	17.00 A	
Fiber's distribution		
- Oral Fibroma	23.42 A	
- Inflammatory hyperplasia	8.38 B	
-Hereditary gingival fibromatosis	21.50 A	
Fiber's concentration		
- Oral Fibroma	17.08 A	
- Inflammatory hyperplasia	15.19 A	
-Hereditary gingival fibromatosis	30.00 B	

Table 3 Results from the fibroblasts and inflammatory cells counting. Comparison of the results with Analysis of Variance (ANOVA) followed by Least Significance Difference (LSD). Means followed by different letters are different from each other.

Cell counting/Lesion	Mean (SD)	p value
Number of inflammatory cells		≤0.05
Oral Fibroma	44.00 (5.44) A	
Inflammatory hyperplasia	62.69 (5.96) B	
Hereditary gingival fibromatosis	36.33 (2.51) C	
Number of fibroblasts		
Oral Fibroma	136.22 (9.45) A	
Inflammatory hyperplasia	96.00 (5.58) B	
Hereditary gingival fibromatosis	88.00 (2.00) B	

FIGURES

Figure 1. Photomicrography of collagen fibers in a fragment from an HGF (A), OF (B) and IH (C). (Picrosirius red staining; original magnification 100x)

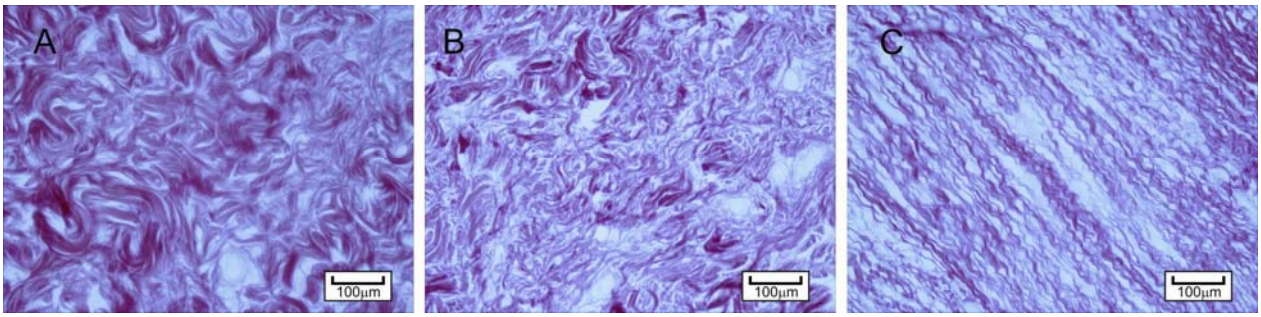
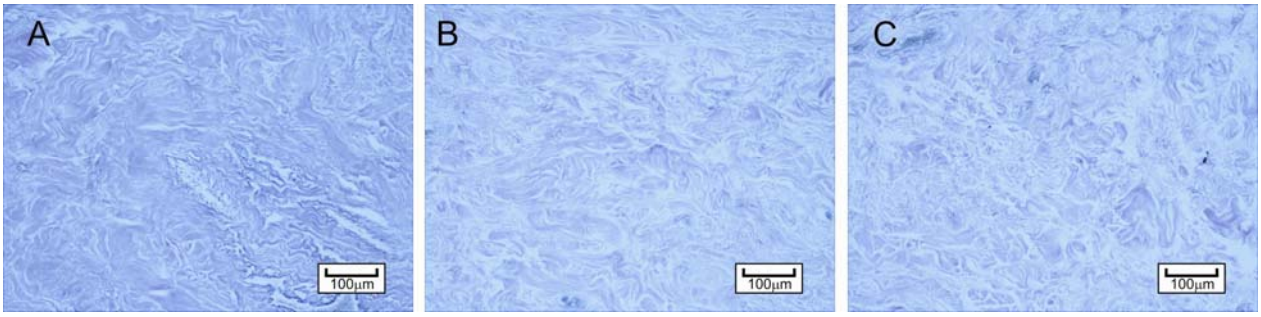


Figure 2. Photomicrography of reticulin fibers in a fragment from an HGF (A), OF (B) and IH (C). (Gomorri reticulin staining; original magnification 100x)



HISTOMORPHOMETRIC, MOLECULAR AND PROLIFERATIVE ANALYSIS IN HEREDITARY GINGIVAL FIBROMATOSIS (HGF) AND IN INFLAMMATORY HYPERPLASIA

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Number of tables: 5
Running title: Microscopic, molecular and proliferative analysis of HGF
Key findings: The gingiva of HGF patients presented a higher degree and a different structural disposition of collagen fibers when compared to the gingiva of patients with inflammatory hyperplasia. Peripheral T cell proliferative capacity, as a whole, was not impaired in HGF patients, and a mutation in the *SOS-1* gene, described to be associated to HGF, was absent in the patients.

ABSTRACT

Background: Hereditary gingival fibromatosis (HGF) is characterized by an increased production of collagen. The amount and disposition of collagen fibers as well as the degree of inflammation present in such disorder vary in different patients. The goal of this research paper is to analyze the histomorphometric characteristics of the gingival connective tissue, the presence of a mutation on the *SOS1* gene and to determine peripheral lymphocyte proliferative capacity in an affected family.

Methods: Histological sections from the gingiva of HGF patients and from inflammatory gingival hyperplasias (IH) were stained with hematoxylin/eosin, Masson Trichrome and Picrosirius Red. The sections were analyzed in light and confocal microscopy. Morphologic and quantitative analysis were carried out considering the fiber parallelism, distinction between collagen fiber layers, and inflammation presence and degree. The *SOS1* gene was partially sequenced and the proliferative capacity of the patients´ peripheral lymphocytes, determined by the MTT test, was compared to 18 normal control individuals.

Results: The HGF lesions have presented a higher proportion of collagen fibers. The distinction between the collagen fiber layers was more evident in the IH samples. The affected patients did not present the described mutation for the *SOS1* gene, although the possibility of other mutations in this same gene could not be ruled out. Lymphocyte proliferative capacity in HGF patients was similar to those on controls.

Conclusion: The collagen fibers were more prominent and arranged differentially in the gingiva of HGF patients. The peripheral T cell proliferative capacity, as a whole, was not impaired in HGF.

Keywords: Gingival hyperplasia; Immunology; Histology; Genetic(s); Pathogenesis of periodontal disease.

INTRODUCTION

Hereditary gingival fibromatosis (HGF) is characterized by a slow, progressive increase in gingival bulk and fibrosity. The clinical manifestations begin soon after the eruption of primary teeth and, in spite of gingival overgrowth, they generally do not involve inflammatory reactions, bleeding, periodontitis or changes in gingival color[1-3].

Different factors are believed to interfere on the expansion of the gingival bulk in HGF. Some of them are directly related to the connective tissue such as increased proliferation of gingival fibroblasts, increased collagen synthesis, and inhibition of enzymes that degrade collagen[4-11]. However, the increase in the amount of collagen fibers, deposition and the morphologic pattern of collagen in gingival tissues in HGF patients are not always consistent[12-16].

Other factors are less evident, but different soluble factors were already involved in HGF development. For example, the increase in collagen synthesis in HGF seems to be related to the production of the Transforming Growth Factor (TGF)-β1[6]. Also, it was suggested a correlation between TGFβ production and the fibroblast proliferation in HGF. A study comparing different TGF isoform production levels, collagen and fibronectin production among fibroblasts obtained from HGF patients and patients with a normal gingiva, identified elevated TGF levels in the cultures from HGF patients[17]. Although activated macrophages and lymphocytes are the main sources of TGFβ, other cell types are also TGFβ producers[7].

Inflammation is not a consistent feature associated with HGF, although gingival fibromatosis can be associated even with generalized aggressive periodontitis[18]. TGFβ is an important factor in the inhibition of T cell proliferation and macrophage activation, and is also involved in IgA class switching. This feature could explain the low levels of inflammation associated with HGF. Nevertheless, few studies have analyzed the immunocompetence of HGF individuals as a whole, and the low local levels of inflammatory response in HGF are still a puzzling feature.

Although considered as a rare autosomal dominant inherited disease, HGF presents a heterogeneous inheritance pattern, with variable degrees of expression and penetrance; occasional cases of recessive inherited HGF have been reported[19]. The genetic loci for autosomal dominant non-syndromic forms of HGF were localized in chromosome 2p21-22

(GINGF)[20] where a mutation in the *SOS1* (*Son of sevenless*-1) gene was reported as responsible for HGF development in a large family, with over forty members affected[21], as well as in chromosome 5q13-q22 (GINGF2)[22], and more recently in chromosome 2p22.3-p23.3 (GINGF3)[23]. It is important to point out that Hart et al´s research [21] was developed through the genetic analysis of a Brazilian family although the latest two studies analyzed Chinese families, suggesting the existence of genetic variation on HGF susceptibility associated to ethnic origin.

The objective of the present study is to analyze morphological and morphometrical features of collagen fibers comparing HGF and inflammatory gingival hyperplasias. The *SOS1* gene region was sequenced encompassing the previously described mutation in three affected individuals from the same family. Additionally, the proliferative capacity of peripheral T cells was determined in order to verify if the absence of inflammation observed in the gingiva from HGF patients could be associated to a systemic down regulation of the immune system.

MATERIAL AND METHODS

Subjects

The subjects with HGF were a 35-year-old African-Brazilian female, her two children (an 18-year-old female and a 12-year-old male) born from different fathers, and a 9-month-old grandson. Previous family history was unavailable as, having been adopted, the matriarch did not know her biological family.

The patients´ weight, height and blood count were within normal ranges, and they were not taking drugs known to induce gingival fibromatosis. The three older patients had more than one third of the crowns covered by gingival tissue and they did not show clinical evidence of gingival inflammation. The study had been previously approved by the Research Ethics Committee from the Federal University of Rio Grande do Sul (UFRGS) and participants signed an informed consent.

Microscopical evaluation

Preparation of the samples

After basic periodontal therapy and considering the absence of clinical signs of inflammation, cosmetic surgery of the gingiva was performed on all three older subjects and the tissue was sent for histopathological examination. Ten inflamatory gingival hyperplasias were used as a comparison group. The tissue samples were fixed in neutral formalin (10%), paraffin-embedded and cut in 3 μm histological sections. The sections were stained with hematoxylin/eosin (HE), Masson Trichrome (MT) and Picrosirius Red according to the protocol[24].

Morphological analysis

For the morphological analysis the slides were stained with HE and MT and evaluated in an optical microscope CX41RF model (Olympus Latin America, Inc., Miami, Florida, USA) in a magnification of 40 and 100x by one calibered examiner. In the slides stained with MT the presence of parallel fibers (fibers parallel in relation to epithelial tissue), presence of distinct layers of collagen fibers and absence of distinction between layers of collagen fibers were considered.

The microscopic characteristics were analyzed in captured images and were classified according to the following scale: 0: absence of the characteristic; 1: characteristic present in up to 1/3 of the microscopic field; 2: characteristic present in up to 1/2 of the microscopic field; 3: characteristic present in more than 1/2 of the field. The data obtained were expressed by mean and standard deviation and compared with the Mann-Whitney's "U" test ($p \leq 0.05$).

In the HE stained slides an anatomic observation of the collagen fibers was performed and the presence of inflammatory cells in the connective tissue subjacent to the junctional epithelium was studied. Both gingival sulcus (crevicular) epithelium and the gingival lining epithelium were analyzed. For scoring inflammation, histological sections were divided into three zones[25]. In the IH samples the evaluation was done in the region where the inflammatory cells were more concentrated.

Morphometrical analysis

A specific stain for collagen, Phosphomolybdic acid- Picrosirius red (PMA-PSR) modified to confocal microscopy, was used to stain collagen protein distinctly[26]. The slides were analyzed in a Confocal microscope.

Two images (one in the center and another in the periphery of the lesion) were captured (magnification 100x) and digitalized using an image analysis system with specific software (Image-Pro® Plus, version 5.1 (Media Cybernetics, Inc., Silver Spring, Maryland, USA). The images were analyzed in a histogram of gray, black and white colors. The collagen portion was assessed by the software on the basis of similarities in the white and gray colors of adjacent pixels. For the statistical analysis the proportion of collagen fibers of the center and the periphery of the lesions were compared by the Analysis of Variance test (ANOVA, $p \leq 0.05$).

Intra-examiner calibration was performed by means of a second analysis of one in every 10 fields observed, applying the Student's "t" test ($p<0.05$) and the Kappa coefficient test ($p>0.7$) in order to determine the degree of agreement for quantitative and qualitative analyses, respectively. There were no statistical differences between readings for either type of analysis.

DNA extraction, *SOS1* gene amplification and sequencing

Total DNA was extracted from peripheral blood cells as previously described[27] . Primers were designed in order to amplify the region encompassing the mutation described[28] in the *SOS1* gene exon 21 (dir ATCTCCTGGTGTTCGTCCATC and rev TACTTGAGTGAAAAGGGCTCG) yielding a 243bp fragment, and a PCR reaction was performed in a MJ Research PTC100, thermocycler (MJ Research Inc., Watertown, MA). PCR samples were prepared for a final volume of 25µl as follows: 1µl of DNA (0.2-0.5µg), 2.5µl of 10X PCR buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl], 1µl of 50mM $MgCl_2$, 1µl of 3mM dNTP mix, 1µl of 10pmol primer mix and 0.2µl *Taq* DNA polymerase 5U/µl (Invitrogen Corporation, California, USA). Samples were submitted to 40 cycles of amplification, each composed of 1 min at 94ºC, 1 min at 60ºC and 1 min at 72ºC. The sequence reactions were performed in a ABI310 (Applied Biosystems) automated sequencer using a Big Dye™

Terminator Cycle Sequencing kit (Applied Biosystem, Foster City, CA, USA), according to the manufacturer protocol.

Lymphocyte Proliferative MTT Assay.

The proliferative capacity of lymphocytes from the three HGF patients was compared with that of lymphocytes derived from eighteen normal controls, composed by 12 females and 6 males (mean age of 28.5 years).

The evaluation used an assay which measures the cleavage of the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl) and, thus measures mitochondrial activity in metabolically active cells. Briefly, peripheral blood mononuclear cells (PBMC, ie. monocytes and lymphocytes) from heparinized whole blood were isolated by density gradient centrifugation in Ficoll-Hypaque (Gibco, Gaithersburg, Maryland, USA). Cells were counted by means of microscopy (100 X) and viability always exceeded 95%, as judged from their ability to exclude trypan blue (Sigma, St Louis, Missouri, USA). PBMCs were cultured in a final concentration of 4 X 10^5 cells/ml in RPMI-1640 medium (Sigma), supplemented with 20% of autologous plasma, for 72 h at 37°C in 5% CO_2 atmosphere. Stimulation by the T cell mitogen Phytohaemagglutinin (PHA, Gibco) was performed in triplicate (100µl/well) to yield a final concentration of 1%.

The proliferative responses were determined by the MTT method previously described[29]. In the last 4 h of culture, 30 µl of freshly prepared MTT (Sigma) solution (5 mg/ml in RPMI-1640) was added to each well (in a 96-well plate), followed by the addition of 120 µl RPMI-1640. The cells were incubated for 4 h in the dark and centrifuged at 800 g for 7 min. After removal of the supernatant, 100 µl of dimethyl sulfoxide (DMSO, Sigma) was added to the wells, and the purple crystals were allowed to solubilize. The optical density (OD) was determined by using a Dynatech MR5000 ELISA plate reader at a 570 nm wavelength with a reference wavelength of 660 nm. The proliferation index (PI) was calculated as the ratio of proliferation in the absence of PHA and PHA-stimulated proliferation. The proliferation assay data from the HGF patients and controls were compared using Student's t-test (α =0.05).

RESULTS

Morphological analysis

The morphological evaluation of MT stained slides showed a similar level of collagen fiber parallelism on both groups studied. The distinction between the collagen fiber layers was more evident in IH, while samples with absence of distinction between collagen fiber layers were more prevalent in HGF lesions (Table 1). The MT technique showed a more evident staining in the central regions of HGF as compared to the IH lesions, coincident with the anatomic areas of hyaline aspect in the HE staining.

The microscopic evaluation of HE stained slides showed an increase in collagen fibers in the HGF patients´ gingiva, with condensation areas of hyaline aspect (Figure 1). Inflammatory infiltrate was generally absent in all specimens from the HGF patients (score 0), but scattered areas of chronic inflammatory cells limited to the crevicular epithelium and occasional round cells adjacent to the lining epithelium were observed (Figure 1). However, the inflammatory infiltrate was absent deep in the connective tissue in all cases, in contrast with the high degree of inflammation observed in IH ($p = 0.006$, Table 1).

In HGF, the gingival lining epithelium was hyperplastic with the presence of finger-like projections deeply penetrating into the connective tissue. The gingival sulcus epithelium presented a normal structure with uniform thickness of cell layers (Figure 1).

Morphometrical analysis

The confocal microscopic laser evaluation revealed the highest ratio of collagen fibers localized at the center of the HGF lesions. The region adjacent to the epithelial tissue in HGF lesions, followed by the same region in IH and the central region of IH showed lower ratios of collagen fibers in a decreasing order (Figure 2 and Table 2).

SOS1 gene amplification and sequencing

None of the three HGF affected individuals tested was identified as carrier of the previously described *SOS1* gene mutation (Table 3), being all homozygous to the normal, wild-type sequence.

Lymphocyte Proliferative MTT Assay

The Proliferation Indices (PI) calculated for each of the HGF patients were: 2.72; 2.93 and 3.32 respectively, as observed in Table 4. Normal controls had a PI ranging from 1.54 to 6.05 (mean 3.28; SD+/-1.27) and there were no statistical differences between the groups in the proliferation assay (table 5).

DISCUSSION

HGF is an uncommon condition characterized by a fibrotic enlargement of the gingiva that may be observed isolated or as part of a syndrome[30-33]. Males and females are equally affected by HGF, with a prevalence varying from 1:75.000[19] to 1:750.000[34]. Histological and cell culture studies have uncovered some of the molecular and cellular aspects of HGF; however, the pathogenesis of the disease is still largely unknown[8,11,13-15]. In this study a morphological and morphometric analyses of gingival samples obtained from HGF patients were performed in order to understand the architectural changes in the gingival tissue of these patients. An evaluation of inflammatory hyperplasia (IH), a similar fibrous lesion of traumatic origin, common in gingival region[35,36] was also performed.

The microscopic characteristics studied in the morphological analysis of the slides indicated a similar degree of collagen fibers parallelism between HGF and IH. The use of a specific collagen staining (MT – see figure 1) to evaluate the collagen fibers, in contrast with the use of HE, a general staining technique, as well as the occurrence of differences in the collagen deposition pattern among HGF families, can account for differences between our results and others in the literature[10,17,37-42]. Also, it is important to point out that rather than performing only a morphological evaluation of the collagen fibers, we used a scale to measure the proportion of microscopic fields showing the microscopic characteristic studied. Additionally, we performed a morphological analysis of the microscopic characteristic allowing for higher results reliability.

Concerning the distinction between layers of collagen fibers in the MT stained slides, different patterns were observed for samples derived from HGF and IH. We observed in the gingiva of HGF patients an absence of distinction among collagen fiber layers that could be the result of a higher accumulation of collagen fibers. This observation is in accordance with previous histological studies that report the presence of dense collagen fiber bundles in the gingiva of HGF patients[10,14,16,37-41]. Additionally, the difficulty in the individualization of the collagen fibers was higher in the center of the lesion and these areas are coincident with a hyaline area in the HE staining suggesting a high concentration of collagen fibers. This in an important finding, since a previous study suggested that collagen deposition is higher in the growing site of the HGF lesion[43]. In the same way, an immunohistochemical study[16] showed that myofibroblasts, a cell population that occurs in specific sites of the gingiva in a family affected by HGF, could be implicated in the pathogenesis of fibrosis in this lesion. Based on these evidences we can suggest that the synthesis of collagen fibbers occurs primarily in the center of the gingiva in HGF patients and later expands to the periphery where the collagen fiber layers present a higher degree of distinction.

The layers of collagen fibers in IH have a pattern that allows a higher distinction, in both the layer adjacent to the epithelial tissue and at the center of the lesion. This can suggest a lower deposition and concentration of collagen fibers in IH, as compared to HGF, although this lesion presents a higher concentration of collagen fibers than a normal gingiva[44-46]. Considering that IH has an important inflammatory component, the exudative phenomenon of inflammation and its chemical mediators can induce changes in the connective tissue, such as separation of connective fibers, making it looser and allowing for an easier distinction between the bundles of collagen fibers[47].

The microscopic evaluation of the HE stained slides showed, in HGF, a hyperplastic gingival lining epithelium with the presence of finger-like projections deeply penetrating into the connective tissue; these findings are in accordance with other studies[10,16,37,48].

Several studies have used different methodologies in order to evaluate the collagen production in HGF patients´ gingiva. The results have invariably shown an increase in the amount of collagen in HGF. This increase is similar in affected members of the same family although there are significant differences in the amount of synthesis/deposition of collagen fibers among families[5-7,9,10,14,15,17,42,49]. Nevertheless, the majority of these studies have not evaluated the

collagen amount in specific regions of the gingiva extracted from HGF patients, what seems to be an important data in that disorder[13,16,43].

In order to evaluate the proportion of collagen fibers at different regions of the HGF lesion, confocal laser scaning microscopy was used. This method permits a two or three-dimensional evaluation of the connective fibers architecture, including the application of quantitative assessment techniques[50-54]. The results of the morphometric analysis of this study have indicated a higher proportion of collagen fibers in the gingiva of our HGF patients (considering the amount of collagen fibers in the center of the lesion and in the region adjacent to the epithelial tissue) as compared to the results from another study[10]. This suggests a singular pattern of collagen deposition in the family under study, which is reinforced by the absence of the mutation in the *SOS-1* gene[28] (see further discussion). Another collagen quantitative study[42] showed an increase of only 10% in the total collagen in the gingiva of HGF patients as compared to the controls. Besides, we observed an increase of about 26% in the proportion of collagen fibers in the central region of the gingiva of HGF patients, compared to the region adjacent to the epithelial tissue in IH. Since there are no other quantitative studies regarding measurement of collagen fibers per gingival region, it can only be affirmed that the results of the morphometric analysis are consistent with the morphologic results of the present study as well as with a previous ultrastructural descriptive study[13]. In the same way, these findings reinforce the hypothesis of a growing site in the center of the gingiva in the family under study.

With the purpose of determining the genetic basis of HGF, Hart et al[20] studied an extended family that segregated HGF in an autosomal dominant form. Using a genomic search strategy, they identified genetic linkage of the HGF phenotype to polymorphic markers in the genetic region of chromosome 2p21 bound by the loci D2S1788 and D2S441. Another study of an HGF patient with a cytogenetic duplication involving the genetic region 2p13->p21 allowed to correlate the genetic interval of the HGF phenotype to a 8 Mb region in a more accurate way. This finding suggested that two gene loci in chromosome 2p could be involved in gingival fibromatosis[55]. This last suggestion was corroborated by further studies[21]. Hart et al[28] refined the chromosome 2p21-p22 candidate interval to ~2.3 Mb and the sequencing of 16 gene candidates in affected and unaffected members of a large Brazilian family with HGF1, identified a mutation in the *Son of sevenless–1* (*SOS1*) gene in affected individuals. They described the genomic structure of the *SOS1* gene and presented evidence that an insertion of a

cytosine between nucleotides 126,142 and 126,143 in codon 1083 of the *SOS1* gene was responsible for HGF1. This insertion mutation, which segregates in a dominant manner over four generations, introduces a frameshift and creates a premature stop codon, abolishing four functionally important proline-rich SH3 binding domains normally present in the carboxyl-terminal region of the SOS1 protein. The resultant chimera protein contains the wild-type SOS1 protein for the N-terminal amino acids 1–1083 fused to a novel 22–amino acid carboxyl terminus and is responsible for the HGF phenotype.

However, in the present study, none of the three HGF affected individuals was identified as carrier of the mutation previously described[28]. In spite of this, a possible existence of another mutation in the *SOS* 1 gene in this family cannot be ruled out. It should also be taken into account that these patients present an autosomal dominant pattern of inheritance (the children were descendent of different fathers with no history of HGF).

Inflammatory cells are rather rare[10,16,37,40] in the gingiva of HGF patients. Nevertheless, a histomorphometric study that quantified the inflammatory cells in the gingiva of 3 different HGF families show a high level of diversity among the analyzed families, suggesting that the pattern of inflammation could influence the HGF[10] pathogenesis. In the present study a scale was used in order to establish the degree of inflammation according to the density and distribution of the inflammatory cells[25]. The results showed a very low degree of inflammation in our HGF patients and only occasional inflammatory cells were seen in the slides. The difference in the degree of inflammation between the two groups under study (HGF and IH) was statistically significant. It can be suggested that the difference on inflammation observed is due to the fact that IH has an important inflammatory component[44,45]; however, inflammation in IH is frequently a result of a traumatic condition[35,36]. The gingiva of HGF patients is characterized by an enlargement that can favor trauma and, consequently could favor the presence of inflammatory cells. Considering the reduced levels of inflammation in this study HGF patients, a systemic analysis of the patients immunocompetence was proposed.

Concerning the cell proliferative capacity in HGF, there are conflicting results in the literature. It was shown that fibroblast strains derived from the gingiva of HGF patients presented higher proliferation rates as compared to fibroblast strains derived from normal gingiva and that they also presented an increased production of fibronectin and type I collagen[16,17]. It was also suggested that this increase in fibronectin and type I collagen could be

explained by an increased expression of TGF-beta in those cells[16,17], and it has been suggested more recently that the elevated proliferative rate of a HGF-derived fibroblast strain is related to elevated c-myc expression[56] . It should be pointed out that TGF-beta is associated with both increased collagen synthesis and T cell suppression[57]. This could explain why inflammatory reactions are not usually associated with HGF[5-7]. It is possible that the absence of inflammatory reactions directly associated with HGF results from a reduced T cell proliferative capacity. However, although statistical significant differences in the proliferation index of peripheral T cells obtained from HGF and from normal individuals were not observed, a tendency to a lower proliferation index on HGF patients could be suggested ($p=0.074$, see Table 5). It is also interesting to observe that the indices of proliferation among the HGF individuals ranged from 0.111 to 0.134, although among the controls this range presented a more clearly spread distribution (from 0.101 to 0.900). Epidermal growth factor and its receptor were also suggested to be involved in the epithelial cell proliferation observed in HGF patients[14] ; the possibility that TGF-beta (or other soluble factor) could cause local effects cannot be ruled out, suppressing inflammation and immune reactions specifically at the site of the gingival overgrowth.

Another study[48] examined the proliferation rate of fibroblasts in an HGF patient and 5 normal controls using Ki 67 immunohistochemical staining, concluding that there was no increase in the proliferation rate of the HGF fibroblasts. They suggested that the underlying mechanism of HGF probably involves an increase in the biosynthesis of collagen and glycosaminogycans rather than cell proliferation.

In accordance with the results of the proliferative assay, the microscopic analysis shows moderate inflammation infiltrates in only one specimen, and this inflammation was limited to the coronal portion in the junctional epithelium. This suggests that HGF development is independent of any inflammation.

Thus, this study suggests that peripheral T cell proliferative capacity, as a whole, is not impaired in HGF. The presence of hyperplastic finger-like projections in the lining of the epithelium, which penetrates deeply into the connective tissue, reinforces the idea that growth factors present in the gingiva of HGF patients could induce an increased proliferation in both connective and epithelial tissues, and act as important determinants in the development of HGF.

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REFERENCES

1. Baptista IP. Hereditary gingival fibromatosis: a case report. *J Clin Periodontol* 2002;29:871-874.

2. Doufexi A, Mina M, Ioannidou E. Gingival overgrowth in children: epidemiology, pathogenesis, and complications. A literature review. *J Periodontol* 2005;76:3-10.

3. Yalçin S, Yalçin F, Soydinc M, Palanduz S, Gunhan O. Gingival fibromatosis combined with cherubism and psychomotor retardation: a rare syndrome. *J Periodontol* 1999;70:201-204.

4. Coletta RD, Almeida OP, Graner E, Pagge RC, Bozzo L. Differential proliferation of fibroblasts cultured from hereditary gingival fibromatosis and normal gingiva. *J Periodont Res* 1998;33:469-475.

5. Coletta RD, Almeida OP, Ferreira LR, Reynolds MA, Sauk JJ. Increase in expression of Hsp47 and collagen in hereditary gingival fibromatosis is modulated by stress and terminal procollagen n-propeptides. *Connec Tissue Res* 1999;40:237-249.

6. Coletta RD, Almeida OP, Reynolds MA, Sauk JJ. Alteration in expression of MMP-1 and MMP-2 but not TIMP-1 and TIMP-2 in hereditary gingival fibromatosis is mediated by TGF-1 autocrine estimulation. *J Periodont Res* 1999;34:457-463.

7. Martelli-Junior H, Cotrim P, Graner E, SaukJJ, Coletta RD. Effect of transforming growth factor-beta1, interleukin-6, and interferon-gamma on the expression of type I collagen, heat shock protein 47, matrix metalloproteinase (MMP)-1 and MMP-2 by fibroblasts from normal gingiva and hereditary gingival fibromatosis. *J Periodontol* 2003;74:296-306.

8. Hakkinen L, Csiszar A. Hereditary gingival fibromatosis: characteristics and novel putative pathogenic mechanisms. *J Dent Res 2007*;86:25-34.

9. Meng L, Huang M, Ye X, Fan M, Bian Z. Increased expression of collagen prolyl 4-hydroxylases in Chinese patients with hereditary gingival fibromatosis. *Arch Oral Biol* 2007;52:1209-1214.

10. Kather J, Salgado MA, Salgado UF, Cortelli JR, Pallos D. Clinical and histomorphometric characteristics of three different families with hereditary gingival fibromatosi. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008;105:348-352.

11.Coletta RD, Graner E. Hereditary gingival fibromatosis: a systematic review. *J Periodontol* 2006;77:753-764.

12. Tipton DA, Howell KJ, Dabbous MK. Increased proliferation, collagen, and fibronectin production by hereditary gingival fibromatosis fibroblasts. *J Periodontol* 1997;68:524-530.

13.Barros S, Merzel J, Araújo V, Almeida O, Bozzo L. Ultrastructural aspects of connective tissue in hereditary gingival fibromatosis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodon* 2001;92:78-82.

14. Araujo CS, Graner E, Almeida OP, Sauk JJ, Coletta RD. Histomorphometric characteristics and expression of epidermal growth factor and its receptor by epithelial cells of normal gingiva and hereditary gingival fibroblasts. *J Periodontal Res* 2003;38:237-41.

15. Gagliano N, Moscheni C, Dellavia S, et al. Morphological and molecular analyses of idiopathic gingival fibromatosis: a case report. *J Clin Periodontol* 2005;32:1116-1121.

16. Bitu CC, Sobral LM, Kellermann MG, et al. Heterogeneous presence of myofibroblasts in hereditary gingival fibromatosis. *J Clin Periodontol* 2006;33:393-400.

17. Tipton DA, Dabbous MK. Autocrine transforming growth factor beta stimulation of extracelullar matrix production by fibroblasts from fibrotic human gingiva. *J Periodontol* 1998;69:609-619.

18. Casavechia P, Uzel MI, Kantarci A. Hereditary gingival fibromatosis associated with generalized aggressive periodontitis: a case report. *J Periodontol* 2004; 75:770-778.

19. Singer SL, Goldblatt J, Hallam LA, Winters JC. Hereditary gingival fibromatosis with a recessive mode of inheritance. Case reports. *Aust Dent J* 1993;38:427-432.

20. Hart TC, Pallos D, Bowden DW, Bolyard J, Pettenati MJ, Cortelli JR. Genetic linkage of hereditary gingival fibromatosis to chromosome 2p21. *Am J Hum Genet* 1998;62:876-883.

21. Hart TC, Pallos D, Bozzo L et al. Evidence of genetic heterogeneity for hereditary gingival fibromatosis. *J Dent Res* 2000;79:1758-1764.

22. Xiao S, Bu L, Zhu L et al. A new locus for hereditary gingival fibromatosis (GINGF2) maps to 5q13-q22. *Genomics* 2001;74:180-185.

23. Ye X, Shi L, Cheng Y et al. A novel locus for autossomal dominant hereditary gingival fibromatosis, GINGF3, maps to chromosome 2p22.3-p23.3. *Clin Genet* 2005;68:239-244.

24. Brancoft JD, Stevens A. *Theory and practice of histological techniques*. London: Churchill Livingstone 1996.

25. Tagge D, O'Leary T, El-Kafrawy A. The clinical and histological response of periodontal pockets to root planing and oral hygiene. *J Periodontol* 1975;46:527-533.

26. Dolber PC, Spach MS. Conventional and confocal fluorescence microscopy of collagen fibers in the heart. J Histochem Cytochem 1993;41:465-469.

27. Lahiri DK, Nurnberger JI Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991;19:5444.

28. Hart TC, Zhang Y, Gorry MC, et al. A mutation in the SOS1 gene causes hereditary gingival fibromatosis type-1. *Am J Hum Genet* 2002;70:943-954.

29. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;65:55-63.

30. Gorlin RJ, Cohen MM, Levin LS. *Syndromes of the Head and Neck.* New York:Oxford University Press; 1990:847-858.

31. Horning GM, Fisher JG, Barker BF, Killoy WJ, Lowe JW. Gingival fibromatosis with hypertrichosis. *J Periodontol* 1985;56:344-347.

32. Mangino M, Pizzuti A, Dallapiccola B, Bonfante A, Saccilotto D, Cucchiara E. Hereditary gingival fibromatosis (hgf) with hypertrichosis is unlinked to the HGF1 and HGF2 loci. *Am J Med Genet* 2003;116:312-314.

33. Holzhausen M, Gonçalves D, Correa Fde O, Spolidorio LC, Rodrigues VC, Orrico SR. A case of Zimmermman-Laband syndrome with supernumerary teeth. *J Periodontol* 2003;74:1225-1230.

34. Odessey EA, Cohn AB, Casper F, Schechter LS. Hereditary gingival fibromatosis: aggressive 2-stage surgical resection in lieu of traditional therapy. *Ann Plast Surg* 2006;57:557-560.

35. Coelho CM, Zucoloto S, Lopes RA. Denture-induced fibrous inflammatory hyperplasia: a retrospective study in a school of dentistry. *Int J Prosthodont* 1994;9:88-94.

36. Wright SM, Scott BJJ. Prosthetic assessment in the treatment of denture hyperplasia. *Br Dent J* 1992;172:313-315.

37. Fletcher JP. Gingival abnormalities of genetic origin: a preliminary communication with special reference to hereditary generalized gingival fibromatosis. *J Dent Res* 1966; Suppl 3:597-612.

38. Raeste AM, Collan Y, Kilpinen E. Hereditary fibrous hyperplasia of the gingiva with varying penetrance and expressivity. *Scand J Dent Res* 1978;86:357-365.

39. Nevin NC, Scally BG, Kernohan DC, Dodge JA. Hereditary gingival fibromatosis. *J Ment Defic Res* 1971;15:130-135.

40. Gunhan O, Gardner DG, Bostanci H, Gunhan M. Familial gingival fibromatosis with unusual histologic findings. *J Periodontol* 1995;66:1008-1011.

41. Marteli-Junior H, Bolzani G, Graner E, Bozzo L, Coletta RD. Microscopic and proliferative comparison of gingival fibroblasts from patients with normal gingiva and with hereditary gingival fibromatosis. *Pesqui Odontol Bras 2000*;14:123-129.

42. Lee EJ, Jang SI, Pallos D, Kather J, Hart TC. Characterization of fibroblasts with Son of Sevenless-1 mutation. *J Dent Res* 2006;85:1050-1055.

43. Sakamoto R, Nitta T, Kamikawa Y, Kono S, Kamikawa Y, Sugihara K et al. Histochemical, immunohistochemical, and ultrastructural studies of gingival fibromatosis: a case report. *Med Electron Microsc* 2002;35:248-254.

44. Badauy CM, Barbachan JJD, Rados PV. Inflammatory hyperplasia: a microscopic study *Rev Fac Odontol* 2002;43:48-51. (in Portuguese)

45. Badauy CM, Barbachan JJD, Rados PV, Sant'Ana Filho M, Chies JAB. Relationship between candida infection and immune cellular response in inflammatory hyperplasia *Oral Microbiol Immunol* 2005;20:89-92.

46. Gogly B, Godeau G, Gilbert S, et al. Morphometric analysis of collagen and elastic fibers in normal skin and gingival in relation to age. *Clin Oral Invest* 1997;1:147-152.

47. Staszyk C, Gasse H. Oxytalan fibres in the periodontal ligament of equine molar cheek teeth. *Anat Histol Embryol* 2004;33:17–22.

48. Saygun I, Ozdemir A, Gunhan O, Aydintug YS, Karslioglu Y. Hereditary gingival fibromatosis and expression of Ki-67 antigen: a case report. *J Periodontol* 2003;74: 873-878.

49. Chavrier C, Couble ML. Ultrastructure of the connective corium in hereditary gingival hyperplasia. *J Biol Buccale* 1979;7:191-203.

50. Reichenberger E, Baur S, Sukojo C, Olsen BR, Karimbux NY, Nishimura I. Collagen XII mutation disrupts matrix structure of periodontal ligament and skin. *J Dent Res* 2000;79:1962-1968.

51. Kagayama M, Sasano Y Tsuchiva M, et al. Confocal microscopy of Tomes' granular layer in dog premolar teeth. Anat Embryol *(Berl)* 2000;201:131-137.

52. Vardimon AD, Nemcovsky CE, Dre E. Orthodontic tooth movement enhances bone healing of surgical bony defects in rats. *J Periodontol* 2001;72:852-864.

53. Petroll WM. Differential interference contrast and confocal reflectance imaging of collagen organization in three-dimensional matrices. *Scanning* 2006;28:305-310.

54. Orsini G, Piatelli M, Scarano A et al. Randomized, controlled histologic and histomorphometric evaluation of implants with nanometer –scale calcium phosphate added to the dual acid-etched surface in the posterior human maxilla. *J Periodontol* 2007;78:209-218.

55. Shashi V, Pallos D, Pettenati MJ et al. Genetic heterogeneity of gingival fibromatosis on chromosome 2p. *J Med Genet* 1999;36:683-686.

56. Tipton DA, Woodard ES 3rd, Baber MA, Dabbous MKh. Role of the c-myc proto-oncogene in the proliferation of hereditary gingival fibromatosis fibroblasts. *J Periodontol* 2004;75: 360-369.

57. Alpagot T, Konopka K, Bhattacharyya M, Gebremedhin S, Duzgunes N. The association between gingival crevicular fluid TGF-beta1 levels and periodontal status in HIV-1(+) patients. *J Periodontol* 2008;79:123-130.

TABLES

Table 1: Morphological assessment of collagen fibers in samples from HGF patients and IH stained by MT and HE. Statistical analysis by Mann – Whitney "U" test ($\alpha \leq 0.05$).

Microscopic characteristic	Group		P
	HGF	IH	
	Mean Rank	Mean Rank	
Parallelism of collagen fibers	5.5	7.45	0.299
Distinction between layers of collagen fibers	2	8.5	0.007*
Absence of distinction between layers of collagen fibers	12	5.5	0.004*
Degree of inflammation	2	8.5	0.006*

* Statistically significant differences

Table 2 Morphometric assesment of collagen ratio in HGF patients and IH. Analysis of Variance (ANOVA) followed by Least Significance Difference (LSD). Different letters represent differences between groups with $p < 0.05$.

Collagen ratio	**Mean (SD)**
Hereditary Gingival Fibromatosis	
- Adjacent to the epithelium layer	64.64 (1.40) [a]
- Lesion center	88.54 (1.17) [c]
Inflammatory hyperplasia	
- Adjacent to the epithelium layer	62.70 (3.94) [a]
- Lesion center	57.69 (5.29) [b]

Table 3: Nucleotide sequence flanking the mutation described by Hart et al (2002) for the three HGF patients.

Sample	Sequence	Reference
SOS1 (normal)	GCACCAAATTCTCCAAGAACA	a)
SOS1 (mutation)	GCACCAAATTCTCC***C***AAGAACA	b)
HGF1	GCACCAAATTCTCCAAGAACA	present data
HGF2	GCACCAAATTCTCCAAGAACA	present data
HGF3	GCACCAAATTCTCCAAGAACA	present data

C Indicates an insertion of a C nucleotide in genomic sequence between nucleotides 126,142 and 126,143 and a consequent frameshift that results in premature termination of traduction at codon 1106.
a) GeneBank accession NM_005633
b) Hart et al, 2002.

Table 4: Peripheral Blood Lymphocyte Proliferation Index in Patients with HGF and in the Control Group.

Patient	Lymphocyte Proliferation Without PHA	Lymphocyte ProliferationWith PHA	Proliferation Index
HGF 1	0.134	0.364	2.72
HGF 2	0.119	0.349	2.93
HGF 3	0.111	0.368	3.32
Control1	0.883	1.940	2.20
2	0.202	0.760	3.77
3	0.150	0.980	6.05
4	0.887	1.370	1.54
5	0.240	0.680	2.84
6	0.220	1.030	4.68
7	0.206	0.560	2.71
8	0.215	1.050	4.88
9	0.900	1.890	2.10
10	0.149	0.510	2.85
11	0.138	0.580	4.20
12	0.151	0.380	2.52
13	0.476	0.940	1.98
14	0.177	0.330	1.86
15	0.237	0.620	2.60
16	0.101	0.466	4.61
17	0.229	0.730	3.18
18	0.210	0.950	4.53

Table 5. Comparison of means and standard deviation in the proliferation assay between the groups. Statistical analysis was done using the Student's t-test (α=0.05).

Group	Lymphocyte Proliferation Without PHA	Lymphocyte Proliferation With PHA	Proliferation Index (mean and standard deviation)
HGF	0.121^{+}- 0.011	0.360^{+}- 0.010	2.990^{+}- 0.30
Control	0.320^{+}-0.273	0.875^{+}- 0.463	3.283^{+}- 1.275
P-value	0.231	0.074	0.701

FIGURES

Figure 1: A. Photomicrography of a fragment from an HGF patient gingiva. The upper side is the region adjacent to the crevicular epithelium showing a normal structure with a uniform thickness of cell layers and scattered areas of chronic inflammatory cells. The center of the figure shows hyaline areas that can be interpreted as an increased deposition of collagen fibers. The lower side is the gingival lining epithelium (HE staining, original magnification 40x). B. High power magnification of gingival lining epithelium with the presence of finger-like projections deeply penetrating into the connective tissue (HE staining, original magnification 100x). C. The collagen fibers (in blue) in the center of an HGF patient gingiva. Note the parallelism and the higher diameter of the fibers. (MT staining, original magnification 100x). D. The collagen fibers (in blue) in the center of IH. Note the parallelism and the more evident distinction between the collagen fiber layers (MT staining, original magnification 100x).

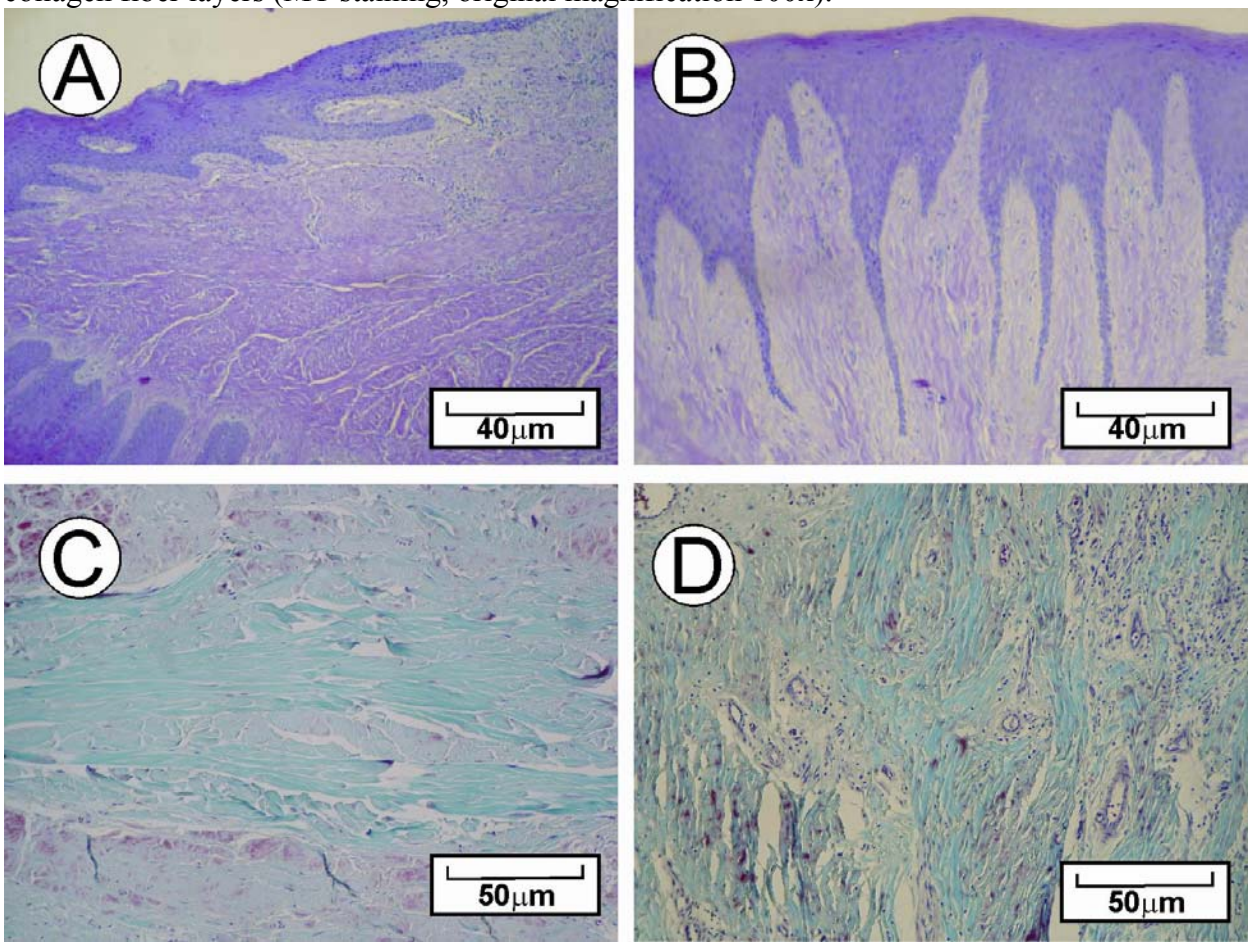
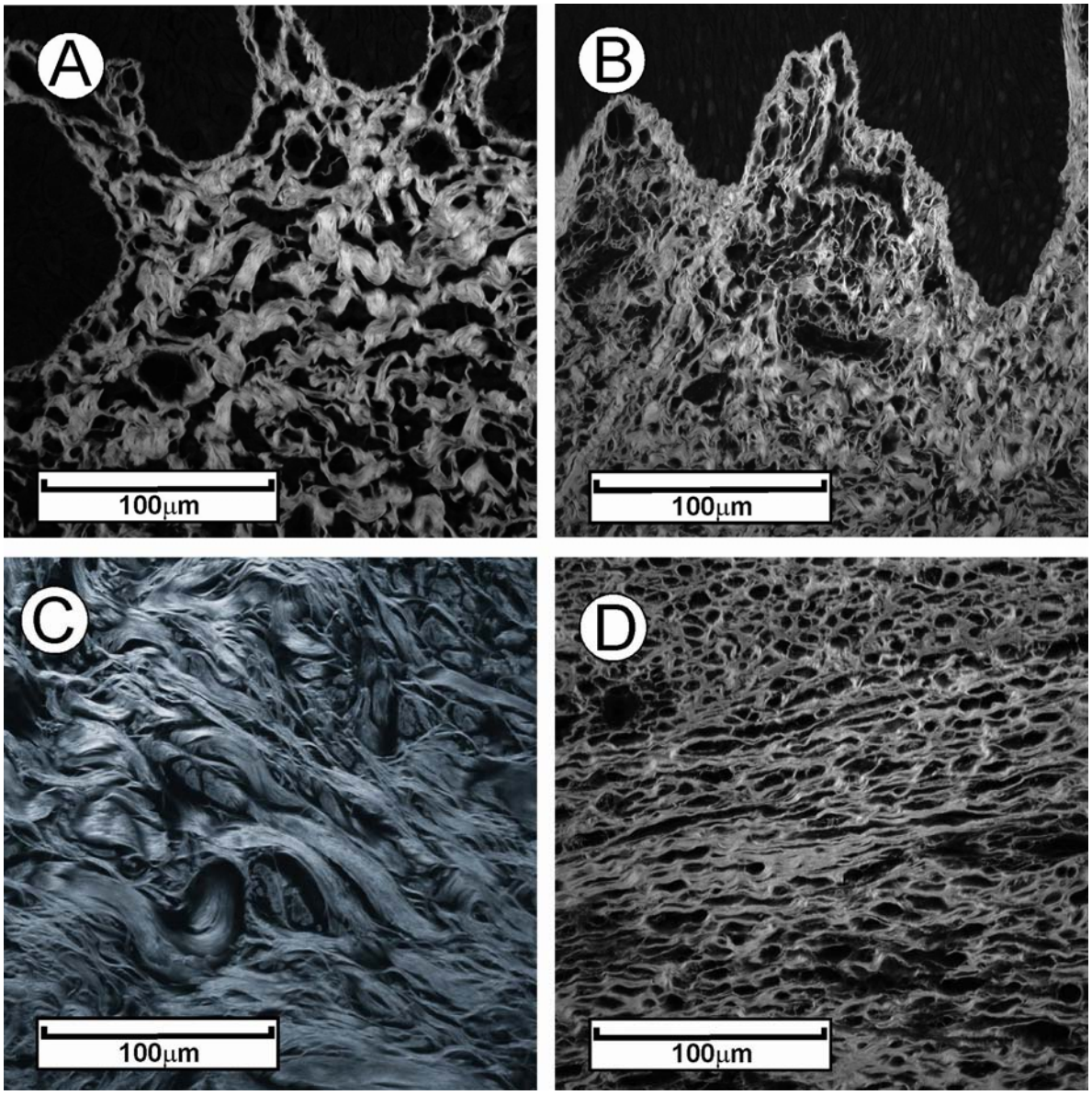


Figure 2: A. Photomicrography from the region adjacent to the epithelial tissue (upper side – in black) in the gingiva of an HGF patient showing the collagen fibers (in gray) in the connective tissue. B. Region adjacent to the epithelial tissue (upper side – in black) in IH. Note the higher proportion of collagen fibers as compared to the center of the lesion (D). C. Center of an HGF patient gingiva. Note the highest concentration of collagen fibers. D. The center of IH showing the lowest concentration of collagen fibers. (Picrosirius red staining; confocal laser scanning microscopy – original magnification 100x).



CONSIDERAÇÕES FINAIS

O presente estudo investigou, de forma ampla, os componentes microscópicos de 3 lesões fibrosas benignas da cavidade bucal, tanto no sentido de compreender o seu aspecto estrutural, quanto buscando critérios morfológicos que permitissem um diagnóstico correto das hiperplasias inflamatórias (HI) e dos fibromas (F). No 1º artigo aplicamos uma metodologia observacional de características microscópicas em HI e F corados pela técnica da Hematoxilina-Eosina (HE). Pode-se considerar que foi utilizada uma metodologia simples nesse artigo, se compararmos com estudos que fazem abordagens imunohistoquímicas ou mesmo com outros artigos do presente estudo, que trazem metodologias complexas de cultivo de células, análises moleculares e microscopia a laser. Contudo, a opção pela avaliação de lâminas coradas pela HE é que esta é a coloração de rotina dos laboratórios, permitindo a aplicação da metodologia proposta numa técnica que faz parte do dia-a-dia do patologista bucal e é perfeitamente compreensível pelo clínico geral e pelo cirurgião, público a quem estão direcionados os resultados do artigo. A utilização da coloração do Tricrômico de Masson (TM) objetivou comprovar se as fibras vistas na coloração de HE eram realmente fibras colágenas ou algum outro tipo de fibra, de modo a sustentar uma análise da disposição estrutural destas fibras.

Os resultados do 1º artigo mostraram que a presença de hiperplasia epitelial, infiltrado inflamatório e fibras colágenas dispostas paralelamente em toda a extensão da lesão são características microscópicas determinantes para o diagnóstico histopatológico de IH. Por outro lado, a presença de fibras enoveladas no centro da lesão, envolvidas por uma camada de fibras paralelas são características microscópicas determinantes para o diagnóstico histopatológico de F. Estes resultados, junto com o achado de maior densidade de colágeno observado nos F, contribuem, inicialmente, para a sugestão de que as lesões estudadas são de natureza diferente, dúvida presente em alguns trabalhos da literatura científica. A maior contribuição do 1º artigo certamente é o estabelecimento de critérios microscópicos para o diagnóstico histopatológico da HI e do F, algo ainda inexistente na literatura. Considerando os resultados citados, patologistas têm, a partir da publicação desse artigo, respaldo científico para embasar seus diagnósticos, contribuindo para uma padronização dos diagnósticos e

evitando multiplicidade de termos para denominar ou definir as duas lesões. A importância clínica do conhecimento produzido por esse artigo permitirá um adequado tratamento clínico das lesões diante do diagnóstico histopatológico, pois quando este for de HI, além da remoção cirúrgica (suficiente para o tratamento do F) dever-se-a remover também o agente traumático sob risco de recorrência da lesão.

O 2º artigo da presente tese utilizou uma metodologia mais complexa que a do 1º artigo, a fim de determinar a distribuição, a disposição e a quantidade de fibras colágenas e fibras do sistema elástico em HI e F. A utilização da microscopia a laser confocal para estudar os componentes fibrosos de ambas as lesões está embasada na dúvida presente na literatura quanto às duas lesões serem de natureza diferente ou representarem arranjos microscópicos diferentes de uma mesma lesão. Os resultados do 1º artigo quanto à diferença na densidade de fibras colágenas já sugeriram uma natureza diferente destas lesões, o que pôde ser reforçado pelos resultados do 2º artigo. Observou-se, no 2º artigo, que os F tiveram maior proporção de fibras colágenas por área, com maior concentração destas fibras na porção central da lesão, enquanto as HI tiveram menor proporção de fibras colágenas por área, com maior concentração destas fibras na porção periférica da lesão (camada subepitelial). Estes resultados, se interpretados sob a teoria de que o centro de crescimento das lesões hiperplásicas fibrosas do tecido conjuntivo localiza-se na porção de maior concentração de fibras colágenas nos mostram a natureza diferente das lesões estudadas.

Ainda como resultados da análise qualitativa realizada no 2º artigo, observamos que a avaliação do paralelismo das fibras colágenas apenas reafirmou o que foi observado na análise morfológica do 1º artigo: fibras paralelas em toda a extensão das HI e fibras enoveladas no centro dos F e paralelas em sua periferia. A análise da densidade das fibras por distinção das camadas de fibras mostrou um resultado semelhante ao da análise quantitativa: maior proporção de fibras densamente arranjadas com menor distinção das camadas e fibras no centro dos fibromas e fibras dispostas de forma frouxa e maior distinção das camadas de fibras em toda a lesão de HI, porém, com predomínio destas características na periferia das lesões. A concordância dos achados obtidos através de diferentes metodologias reforça a evidência científica e a consistência dos resultados dos 2 estudos. Por outro lado, não se observou coloração de fibras do sistema elástico nas lesões estudadas, provavelmente pela

opção metodológica de só considerar o parênquima da lesão, não se considerando a coloração das áreas ao redor dos vasos sanguíneos.

No 3º artigo realizou-se um estudo dos componentes fibroso e celular da HI, dos F e das hiperplasias gengivais de pacientes com FGH. A análise microscópica destas lesões mostrou um padrão diferente, na concentração, na distribuição e na quantificação de fibras das lesões, além de diferenças nas populações fibroblásticas e de células inflamatórias. As HI mostraram uma menor quantidade de fibras colágenas, com disposição predominantemente periférica e menor concentração de fibras, quando comparadas às outras lesões. As hiperplasias gengivais de pacientes com FGH obtiveram os maiores resultados na concentração de fibras colágenas e uma distribuição de fibras na sua região central, semelhante à observada nos F. As fibras reticulares mostraram resultados de distribuição e concentração semelhantes aos das fibras colágenas nas 3 lesões estudadas, achado ainda não descrito na literatura. O sistema de fibras elásticas e fibras oxitalânicas não mostraram coloração positiva. Com relação às populações celulares, as HI mostraram maior número de células inflamatórias que as outras lesões e os F mostraram maior número de fibroblastos.

O 4º artigo da presente tese estudou pacientes de uma mesma família com Fibromatose gengival hereditária (FGH), cuja matriarca desconhecia sua história familiar pregressa, por ter crescido em um orfanato e ter sido posteriormente adotada. Nesse estudo utilizou-se uma abordagem metodológica, centrada em 3 pontos principais: avaliação microscópica considerando a morfologia das lesões (lâminas coradas pela HE), análise qualitativa do colágeno (lâminas coradas pelo TM) e análise quantitativa das fibras colágenas (lâminas coradas pelo Picrosírius Red, avaliadas em microscopia a laser confocal) no 1º grupo de avaliações, utilizando-se HI como grupo de comparação. O 2º grupo de avaliações estudou as células inflamatórias, tanto na lesão gengival, quanto avaliou a atividades destas células obtidas do sangue periférico dos pacientes. O 3º grupo de avaliações realizou, considerando o desconhecimento da história familiar pregressa da matriarca, análise molecular de fragmentos dos genes dos pacientes, descritos como alterados em famílias brasileiras afetadas pela mesma patologia. Os resultados mostraram um padrão histológico de fibras condensadas na região central das hiperplasias gengivais, agrupadas em áreas de hialinização tecidual e hiperplasia epitelial simulando “tubos de ensaio”, enquanto as HI mostraram hiperplasia epitelial e fibras conjuntivas com baixa condensação, dispostas em uma direção paralela ao epitélio de

revestimento. A proporção de fibras colágenas depositadas nas FGH foi muito superior à obtida no grupo de comparação e diferente da descrita para outras famílias brasileiras afetadas. Nesse artigo, demontrou-se que as fibras colágenas concentram-se no centro da lesão gengival da FGH, algo ainda não descrito na literatura. A análise das células inflamatórias na hiperplasia gengival dos pacientes afetados mostrou quase que uma ausência destas células, em contraste com o infiltrado inflamatório abundante das HI, com um índice de inflamação mais baixo que o descrito para outras famílias brasileiras com FGH. Por outro lado não se pode sugerir que este resultado possa ser reflexo da situação sistêmica dos pacientes, pois a avaliação do índice de proliferação celular realizada mostrou a imunocompetência dos pacientes estudados, outro resultado não descrito na literatura. Finalmente, a análise molecular não mostrou evidência de mutações descritas em genes de famílias brasileiras. Tal achado reforça os resultados das outras metodologias utilizadas nesse artigo, se interpretados sob a teoria de que as características de quantidade de colágeno e índice de inflamação, em pacientes FGH, são semelhantes entre membros da mesma família, mas diferem entre famílias afetadas.

Todos estes achados em conjunto sugerem uma natureza diferente para as 3 lesões estudadas, embora apresentem semelhança clínica. A quase ausência de componente inflamatório nos fibromas pode contribuir para a rejeição do fibroma irritativo como lesão distinta dentro da classificação dos fibromas, enquanto o grande número de fibroblastos associado a um padrão distinto de fibras colágenas sugere uma etiologia tumoral desta lesão.

Apesar de acreditarmos ter contribuído para o entendimento dos componentes fibroso e celular do tecido conjuntivo das 3 lesões estudadas, estamos certos de que muita coisa ainda precisa ser elucidada. O estudo do componente protéico da matrize extracelular ainda pouco abordado, basicamente centrado na avaliação quantitativa e qualitativa das mucinas ácidas e básicas, poderia contribuir para um entendimento mais completo da estrutura e da patogenia das lesões fibrosas benignas e permanence como perspectiva de futura pesquisa. Além disso, a investigação de mecanismos de acumulação de colágeno, ainda desconhecido, na família com FGH e a localização da mutação gênica presente permitiriam um melhor entendimento da patogenia da doença, através da descrição de um tipo completamente novo desta patologia e um controle clínico mais eficaz do crescimento gengival nestes pacientes.

REFERÊNCIAS BIBLIOGRÁFICAS

BADAUY, C.M. **Estudo da Distribuição e Quantificação dos Linfócitos CD20, CD8 e CD4 nas Hiperplasias Inflamatórias e sua relação com a Infecção por *Candida sp*.** 2003. 68 f. Dissertação (Mestrado em Patologia Bucal) – Faculdade de Odontologia, Universidade Federal do Rio Grande do Sul, Porto Alegre.

BADAUY, C.M. et al. Relationship between Candida infection and immune cellular response in inflammatory hyperplasia. **Oral Microbiol. Immunol.**, Copenhagen, v. 20, no. 2, p. 89-92, Apr. 2005.

BAKOS, L.H. Giant cell fibroma: a review of 116 cases. **Ann. Dent.,** Wantagh, v. 51, no. 1, p. 32-35, Summer 1992.

BONNAURE-MALLET, M.; TRICOT-DOLEAUX, S.; GODEAU, G.J. Changes in extracellular matrix macromolecules in human gingival after treatment with drug inducing gingival overgrowth. **Archs. Oral Biol.,** Elmsford, v. 40 , no. 5, p. 393-400, May 1995.

BAPTISTA, I.P. Hereditary gingival fibromatosis: a case report. **J. Clin. Periodontol.**, Copenhagen, v. 29, no. 9, p.871-874, Sep. 2002

CASAVECCHIA, P.; UZEL, M.I.; KANTARCI, A. Hereditary gingival fibromatosis associated with generalized aggressive periodontitis: a case report. **J. Periodontol.**, Chicago, v. 75, no. 5, p.770-778, May 2004.

COELHO, C.M.; ZUCOLOTO, S. LOPES, R.A. Denture - Induced Fibrous Inflammatory Hyperplasia: a Retrospective Study in a School of Dentistry. **Int. J. Prosthodont**., Lombard, v. 13, no. 2, p. 148-151, Mar./Apr. 2000.

COLETTA, R.D.; GRANER, E. Hereditary gingival fibromatosis: a systematic review. **J. Periodontol.,** Chicago, v. 77, no. 5, p. 753-764, May 2006

CUTRIGHT, D.E. The Histopatologic Findings in 583 Cases of Epulis Fissuratum. **Oral Surg. Oral Med. Oral Pathol.**, St. Louis, v. 37, no. 3, p. 401-411, Mar. 1974.

DALEY, T.D. et al. The major epulides: clinicopathological correlations. **J. Can. Dent. Assoc.,** Ottawa, v. 56, no. 7, p. 627-630, Jul. 1990;

GIUNTA, J.L. Gingival Fibrous Nodule. **Oral Surg. Oral Med. Oral Pathol., Oral Radio. Endod.,** St Louis, v. 88, no. 4, p. 451-454, Oct. 1999.

HART, T.C. et al. Genetic linkage of hereditary gingival fibromatosis to chromosome 2p21. **Am. J. Hum. Genet.**, Chicago, v. 62, no. 4, p. 876-883, Apr 1998.

HART, T.C. et al. Evidence of genetic heterogeneity for hereditary gingival fibromatosis. **J. Dent. Res.**, Washington, v. 79, no. 10, p. 1758-1764, Oct. 2000.

HOUSTON, G.D. The giant cell fibroma. A review of 464 cases. **Oral Surg. Oral Med. Oral Pathol**., St Louis, v. 53, no. 6, p. 582 – 587, Jun. 1982

KALYANYAMA, B.M.; MATEE, M.I.; VUHAHULA, E. Oral tumours in Tanzanian children based on biopsy materials examined over a 15-year period from 1982 to 1997. **Int. Dent. J.,** Bristol, v. 52, no. 1, p. 10-14, Feb 2002

KFIR, Y.; BUCHNER, A.; HANSEN, L.S. Reactive lesions of the gingiva. A clinicopathological study of 741 cases. **J. Periodontol.**, Chicago, v. 51, no. 11, p. 655-661, Nov. 1980

LUKES, S.M. ; KUHNERT, J. ; MANGELS, M.A. Identification of a giant cell fibroma. **J. Dent. Hyg.,** Chicago, v. 79, no. 3, p. 9, Jul 2005.

MAGNUSSON, B.C.; RASMUSSON, L.G. The giant cell fibroma. A review of 103 cases with immunhistochemical findings. **Acta Odontol. Scand.**, Turku, v. 53, no. 5, p. 293-296, Oct 1995

MCGINNIS, J.P.Jr. Review of the clinical and histopathologic features of four exophytic gingival lesions – the pyogenic granuloma, irritation fibroma, peripheral giant cell granuloma, and peripheral ossifying fibroma. **J. Okla. Dent. Assoc.,** Oklahoma City, v. 77, no. 3, p. 25-30. Winter 1987

MIGHELL, A.J.; ROBINSON, P.A.; HUME, W.J. Immunolocalization of tenascin – C in focal reactive overgrowths of oral mucosa. **J. Oral Pathol. Med.,** Copenhagen, v. 25, no. 4, p. 163 –169, Apr. 1996.

OLES, R.D. Incidence and distribution of various connective tissue fibers in oral fibromas. **Oral Surg. Oral Med. Oral Pathol.**, St Louis, v. 26, no. 4, p. 487-495, Oct. 1968.

PRIDDY, R.W. Inflammatory Hyperplasias of the Oral Mucosa. **J. Can. Dent. Assoc**., Ottawa, v. 58, no. 4, p. 311-321, Apr. 1992.

REIBEL, J. Oral fibrous hyperplasias containing stellate and multi-nucleated cells. **Scand. J. Dent. Res.,** Copenhagen, v. 90, no. 3, p. 217-226, Jun. 1982

SAVAGE, N.W.; MONSOUR, P.A. Oral fibrous hyperplasias and the giant cell fibroma. **Aust. Dent. J.,** North Sidney, v. 30, no. 6. p. 405–409, Dec. 1985.

SWAN, R.H. Giant cell fibroma. A case presentation and review. **J. Periodontol.**, Chicago, v. 59, no. 5, p. 338-340, May 1988.

SINGER, S.L. et al. Hereditary gingival fibromatosis with a recessive mode of inheritance. Case reports. **Aust. Dent. J.,** North Sidney, v. 38, no. 6. p. 427-432, Dec. 1993

TYLDESLEY, W.R. Oral medicine for the dental practitioner. 7. Inflammatory overgrowths and neoplasms. **Br. Dent. J**., London, v. 136, no. 3, p. 111-116, Feb. 1974

WEIR, J.C.; DAVENPORT, W.D.; SKINNER, R.L. A diagnostic and epidemiologic servey of 15,783 oral lesions. **J. Am. Dent. Assoc**., Chicago, v. 115, no. 3, p. 439-442, Sep. 1987

WRIGHT, S.M.; SCOTT, B.J.J. Prosthetic assessment in the treatment of denture hyperplasia. **Br. Dent. J.**, London, v. 172, no. 8, p. 313-315, Apr. 1992.

XIAO, S. et al. A new locus for hereditary gingival fibromatosis (GINGF2) maps to 5q13-q22. **Genomics**, San Diego, v. 74, no. 2, p. 180-185, Jun. 2001.

YE, X. et al. A novel locus for autossomal dominant hereditary gingival fibromatosis, GINGF3, maps to chromosome 2p22.3-p23.3. **Clin. Genet**., Copenhagen, v. 68, no. 3, p. 239-244, Sep. 2005.

ZAIN, R.B.; FEI, Y.J. Peripheral fibroma/fibrous epulis with and without calcifications. A clinical evaluations of 204 cases in Singapore. **Odontolstomatol. Trop**., Dakar, v. 13, no. 3, p. 94-96, Sep. 1990.