

Differential Diagnosis Between Oral Fibroma and Inflammatory Hyperplasia: a Proposal for Histopathological Criteria

Diagnóstico Diferencial entre Fibroma Oral e Hiperplasia Fibrosa Inflamatória: uma Proposta para Critério Histopatológico

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Abstract

Aim: The present study proposed histopathological criteria for the differential diagnosis between those pathological entities. **Materials and methods:** Histological sections of lesions histopathologically diagnosed as Oral Fibroma (n=61) and Inflammatory Hyperplasia (n=75) and were submitted to different techniques (Hematoxylin-Eosin; Masson Trichrome and Phosphomolybdic acid - Picrosirius red) to allow quantitative and qualitative analysis. The qualitative analysis of collagen density was based on sections stained by Hematoxylin-Eosin and focused in the center and periphery of each lesion. **Results:** Wound and collagen fibers were more frequent and higher in Oral Fibroma, while parallel fibers were more frequent in Inflammatory Hyperplasia (Fisher's exact test, $p < 0.05$). The percentage of parallel collagen fibers beneath the epithelium was 72.22% and 92.3% in Oral Fibroma and Inflammatory Hyperplasia, respectively (Mann Whitney U test, $p < 0.05$). The parallel collagen fibers in the center of the lesion was found in 84.6% of Inflammatory Hyperplasia cases and was absent in 88.88% of Oral Fibroma. The central portion of Oral Fibroma had characteristically a dense and wound arrangement of collagen fibers. **Conclusion:** Oral Fibroma and Inflammatory Hyperplasia have distinctive features that may be useful in routine histopathological analysis, supporting the differential diagnosis.

Keywords: Neoplasms. Human. Connective tissue. Confocal microscopy. Pathology.

Resumo

Objetivos: O presente estudo propôs critérios histopatológicos para o diagnóstico diferencial entre as entidades patológicas. **Materiais e métodos:** Cortes histológicos de lesões diagnosticadas microscopicamente como Fibroma Oral (n=61) e Hiperplasia Fibrosa Inflamatória (n=75) foram submetidos a diferentes técnicas de coloração (Hematoxilina-eosina, Tricrômio de Masson e Ácido Fosfomolibdico- Vermelho de Picrosírius) para permitir análises quantitativa e qualitativa. A análise qualitativa da densidade do colágeno foi baseada nas lâminas coradas em Hematoxilina- eosina e observada no centro e periferia de cada lesão. **Resultados:** Fibras colágenas enoveladas eram mais frequentes e mais densas no Fibroma Oral, enquanto as fibras paralelas e ram observadas na Hiperplasia Fibrosa Inflamatória (teste exato de Fisher, $p < 0,05$). No centro da lesão, fibras colágenas paralelas foram encontradas em 84,6% dos casos de Hiperplasias Fibrosas Inflamatórias e ausentes em 88,88% dos Fibromas Oraís. A porção central do Fibroma Oral era caracterizado por um arranjo denso e frouxo das fibras colágenas. **Conclusão:** o Fibroma Oral e a Hiperplasia Fibrosa Inflamatória possuem características bem distintas que pode ser útil na rotina da análise histopatológica, auxiliando no diagnóstico diferencial.

Descritores: Neoplasias. Tecido Conjuntivo Humano. Microscopia Confocal. Patologia.

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Introduction

Lesions of connective tissue are among the most common group of lesions in the oral cavity and may be similar both clinically and histologically (Kfir et al., 1980; Mighell et al., 1996). Oral fibromas (OF) and inflammatory hyperplasia (IH) account for the great majority of the lesions (Dalei et al., 1990; Zain and Fei, 1990). Clinically, these lesions usually present as an exophytic red round nodule, sessile or pedunculated, covered by normal appearing mucosa, which may eventually be ulcerated. These lesions are typically slow-growing and painless, apart from acute traumatic conditions (Kfir et al., 1980; Priddy, 1992; McGuff et al., 2005; Braga et al., 2006).

The normal extracellular matrix of the oral tissues and the connective tissue of the OF and IH are mainly formed by collagen fibers and elastic system fibers (Oles et al., 1968; Gogly et al., 1997; Ushiki, 2002). Their clinical and histological similarities have caused some controversies in the literature, leading to the use of different terminology to refer to these entities. Particularly in relation to oral fibroma, it is debatable if it is a reactive lesion or a neoplasm in its nature. Based on the assumption that oral fibroma is a neoplasm and inflammatory hyperplasia is a reactive process, the aim of the present study was, based on a comparison of microscopic characteristics, to improve our knowledge of their histopathological patterns.

Materials and methods

Samples histopathologically diagnosed as IH and OF were retrieved from the files of the Oral Pathology Laboratory, Federal University of Rio Grande do Sul. The biopsy forms were assessed in order to obtain clinical information for determination of the inclusion criteria. For the IH group, only cases in which traumatic origin was evident, such as *Epulis fissuratum* were included. For the OF group, cases with possible involvement of irritation factors were excluded. The cases of discordance were reevaluated and an agreement was reached with help of an experienced pathologist (MSF).

Histological/Qualitative analysis (Hematoxylin-Eosin and Masson Trichrome)

Two histological 4µm sections of each paraffin block (n=136) were stained by Hematoxylin-Eosin (HE) and by Masson Trichrome (MT) techniques respectively. These stainings were performed in the Pathology Laboratory of Institute Oswaldo Cruz,

Fiocruz, Rio de Janeiro. Each sample was independently evaluated using an optical microscope (magnification x100) by two independent observers (CMB and GGS) who were unaware about the diagnosis of the lesions. The agreement between two examiners was evaluated in the beginning and at the end of the evaluation ($\kappa=0.54$ and $\kappa=0.73$, respectively). The following morphological characteristics were assessed:

Connective tissue: (a) wound fibers (loosely oriented fibers), (b) parallel fibers (fibers arranged in the same direction), (c) inflammatory infiltrate and (d) hyperemia;

Epithelial lining: (a) hyperplasia, (b) acanthosis, (c) hyperkeratosis and (d) hydropic degeneration.

Each criterion was classified as follows: 0 = absence; 1 = presence in up to one third of the microscopic fields; 2 = presence from one third to a half of the microscopic fields; 3 = presence in more than a half the microscopic fields of the section

In order to improve the histological evaluation of the collagen fibers, a complementary qualitative analysis was made. This evaluation was based on Masson Trichrome, which stains the collagen fibers, and which allowed the conduction of a descriptive analysis of density of collagen fibers - classified as mild, moderate or intense (OTASEVIC et al., 2005).

Quantitative analysis (Phosphomolybdic acid - Picrosirius red)

Two 3 μ m section were obtained from each paraffin block from the same site, gingival (13 IH and 18 OF). One was stained by Phosphomolybdic acid-Picrosirius red (PMA-PSR) and other by Direct Blue (DB). Both techniques were specific for collagen and elastic fibers evaluation and were modified for confocal microscopy (Dolber and Spach, 1993; Gitirana and Trindade, 2000). A section of normal skin was used as a positive control. The stains were performed at the Pathology Department of the Instituto Oswaldo Cruz (IOC - Fiocruz - RJ, Brazil).

The microscopic fields selected were, as follows: center and periphery of the lesions using 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-Württemberg, Germany) with HeNe 543nm laser and LP 560 filter to improve resolution. 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, assuring that the evaluation was supported only by the connective tissue features.

Images were recorded using a binocular microscope, CX41RF model (Olympus Latin America, Miami, FL, USA) with a camera QColor 5, Colet, RTV (Olympus Latin America) coupled to a computer Dimension 5150 (Dell, Porto Alegre, RS, Brazil). The Image-Pro® Plus software, version 5.1 (Media Cybernetics, Bethesda, MD, USA), was used to quantify the number of white, grey and black pixels. A histogram was obtained to calculate the proportion of collagen in each selected area.

Statistical analysis

The data obtained from morphological analysis of two examiners were tabulated and compared. Score values for presence of wound fibers, parallel fibers, giant cells, hyperemia and inflammatory infiltrate were compared by means Mann Whitney U Test. The qualitative analysis of the comparison between collagen density in the center and the periphery of each sample was carried out using the Fisher's exact test. For the quantitative (MT) and qualitative (PMA-PSR) analysis, Student t Test and Mann Whitney U Test were used. The level of significance was set at 5%.

Results

From 3,602 biopsies received by the Laboratory in the period of the study 106 had histopathological diagnosis 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.

Morphological/Qualitative analysis (Hematoxylin-Eosin and Masson Trichrome)

Analysis of the data obtained from the connective tissue showed that wound fibers (fibers loosely oriented) were more frequent and higher in OF, while parallel fibers were more frequent in IH (Table 1). The density of collagen fibers was also different, with the OF ones showing a higher level (Table 2). The inflammatory infiltrate was more frequent in IH (Table 1; Fig. 1). Regarding epithelial alterations, epithelial hyperplasia was more frequent in IH than OF.

Table 1. Results from microscopic analysis of connective tissue stained H&E.

Microscopic characteristics	OF	IH	p value
Wound fibers	48.53	23.06	<0.01*
Parallel fibers	24.16	50.29	<0.01*
Giant cells	37.45	35.44	0.66
Hyperemia	33.09	40.31	0.12
Inflammatory infiltrate	28.82	45.09	0.01*

*Mann-Whitney's U test ($\alpha=0.05$).

Table 2. Evaluation of density of collagen fibers by qualitative (Masson Trichrome staining -MT) and qualitative (Phosphomolybdic acid-Picrosirius - PMA-PSR) methods in the center of the lesion.

Methods	OF	IH	p value
MT	40.84	31.65	0.045*
Mean rank			
PMA-PSR	62.06	57.56	<0.05**
Mean (SD)	(3.67)	(4.74)	

* Mann-Whitney's U test ($p<0.05$)

**Student t Test

Quantitative analysis (Phosphomolybdic acid - Picrosirius red)

In relation to quantifications of collagen fibers beneath the epithelium, OF and IH showed similar values (data not shown). The analysis of collagen fibers presented a higher percentage ($p<0.05$) of densely arranged fibers in the center of OF when compared with that beneath the epithelium of OF and IH (Table 2).

The parallelism of the collagen fibers was found in different portions of IH and only beneath the epithelium of OF. The percentage of parallel collagen fibers under the epithelium was 72.22% and 92.3% in OF and IH, respectively. The parallel collagen fibers in the center of the lesion was found in 84.6% of IH and was absent in 88.88% of OF. Moreover, the central portion of OF had typically a dense and wound arrangement of collagen fibers (Table 3; Fig. 2). The analysis using the Direct Blue staining did not detect any label representing elastic fibers in the lesions, except for the blood vessels in the periphery.

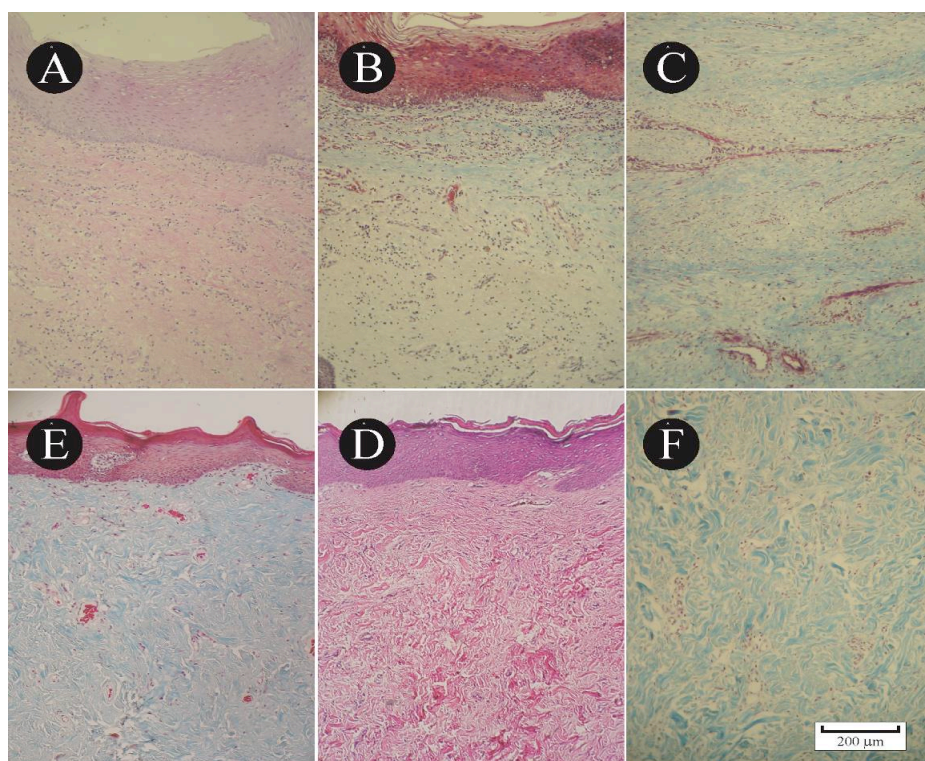


Figure 1. Microscopic characteristics of IH in the periphery of the lesion showing parallel collagen fibers and an inflammatory infiltrate in the connective tissue (A) HE staining and (B) MT staining; original magnification 100x). (C) Parallel disposition of collagen fibers in the centre of the IH (MT staining). (D, 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 centre of the lesion. (D, HE staining and E,- MT staining). (F) Wound fibers in the centre of the OF. Note the greater density of the collagen fibers compared with the microscopic appearance seen in figure C (MT staining).

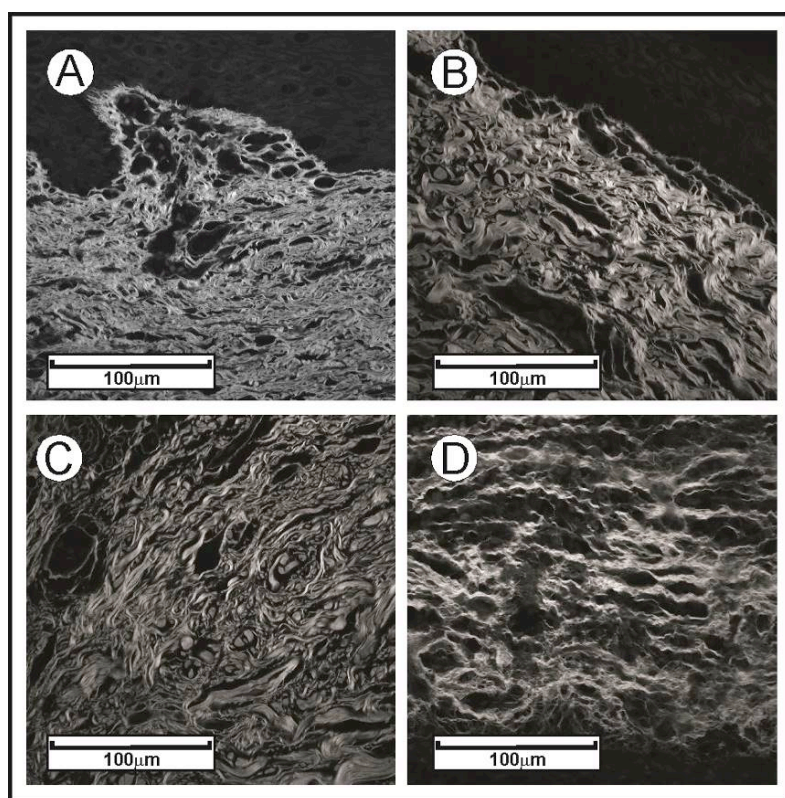


Figure 2. A, Photomicrography of region adjacent to the epithelial tissue from OF. Observe the lower density of collagen fibers compared with Fig. C in a parallel arrangement. B, Photomicrography of region adjacent to the epithelial tissue from IH. Observe the higher density of collagen fibers compared with figure D in a parallel arrangement. 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 parallel arrangement of the fibers. (Photomicrography obtained with confocal laser microscopy; Picrosirius red staining; original magnification 100x).

Table 3. Analysis of collagen fibers arrangement by means Confocal Laser Scanning.

Microscopic Characteristic	OF		IH	
	Beneath Epithelium	Center of Lesion	Beneath Epithelium	Center of Lesion
Densely arranged	0 (0%)	11 (61.11%)	1 (7.69%)	3 (23.08%)
Loosely arranged	18 (100%)	7 (38.89%)	12 (92.31%)	10 (76.92%)

Fischer's exact test ($p < 0.05$)

Discussion

The OF and IH are lesions that superimpose clinical and microscopical characteristics. Although the literature is controversial in relation to the etiology of oral fibroma, we assume, based on the literature (Oles et al., 1968; Magnusson; Rasmusson, 1995; Regezi; Courtney; Kerr, 1975; Provenzano et al., 2006; Hirschberg; Buchner; Dayan, 1996) that these lesions are neoplastic in nature. In order to depict their histopathological characteristics, quantitative and qualitative analysis were performed.

HE and MT staining analysis showed that wound and thick collagenous fibers in the centre of the lesion were the main microscopic characteristics and should be considered in diagnosis of OF. This was a remarkable finding, when values for this lesion were higher than IH (Tables 1 and 3). These results are in accordance with previous studies (Oles et al., 1968; Regezi et al., 1975). These data were reinforced by the confocal laser scanning microscopy analysis that also showed that the density of the collagenous fibers was higher in OF than in IH, probable due to a different collagen fiber type or different rate of biosynthesis. These microscopic arrangements support the theory that OF has a neoplastic nature (Oles, 1968; Regezi et al., 1975; Magnusson; Rasmusson, 1995; Hirschberg et al., 1996; Provenzano et al., 2006).

The periphery of the OF had a layer of collagen fibers with parallel arrangement, simulating a fibrous capsule, corroborating the findings of Oles (1968) in support of the theory of its neoplastic nature. In contrast, IH displayed parallel collagenous fibers in different portions of the lesion. This aspect appears to be important for the differential diagnosis between OF and IH, reinforcing a previous study from our lab (Badauy et al., 2002).

Since IH is a reactive lesion involving connective and epithelial tissues, the presence of inflammatory infiltrate and epithelial disturbances were expected and were microscopic features frequently found in that lesion (Badauy et al., 2002; Badauy et al., 2005). The explanation for the difference is that IH is clearly related to traumatic etiology, whereas fibromas have no clearly defined etiology.

Conclusion

The present data support that IH and OF have distinctive features that may be employed in routine histopathological analysis. We suggest that the thick and wound collagen fibers found in the central portion of OF surrounding by collagen fibers with parallel arrangement are associated with its neoplastic nature and are useful features for differential diagnosis. Furthermore, the presence of inflammatory infiltrate, epithelial hyperplasia and mainly the absence of different patterns of collagen fibers deposition favor the diagnosis of IH. The correct differential diagnosis is important, because IH requires identification and removal of irritative factors to avoid recurrences.

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