Acta Histochemica 118 (2016) 451–455

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Histochemical analysis of collagen fibers in giant cell fibroma and inflammatory fibrous hyperplasia

Mônica Jarema Schmidt[a], André Tschoeke[a], Lúcia Noronha[a], Rafaela Scariot de Moraes[a], Ricardo Alves Mesquita[b], Ana Maria Trindade Grégio[a], Luciana Reis Azevedo Alanis[a], Sérgio Aparecido Ignácio[a], Jean Nunes dos Santos[c], Antonio Adilson Soares de Lima[d], Suelen Teixeira Luiz[a], Arieli Carini Michels[a], Maria Cássia Ferreira Aguiar[b], Aline Cristina Batista Rodrigues Johann[a,*]

[a] Pontifícia Universidade Católica do Paraná, School of Health and Biosciences, Rua Imaculada Conceição, 1155, Prado Velho 80.215-901, Curitiba, PR, Brazil
[b] Department of Oral Surgery and Pathology, School of Dentistry, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6667 sala 3202-D, Pampulha, 31.270-901, Belo Horizonte, MG, Brazil
[c] Department of Oral Pathology, School of Dentistry, Universidade Federal da Bahia, Av. Araújo Pinho, 62, Canela, 40110-150, Salvador, BA, Brazil
[d] Department of Stomatology, School of Dentistry, Universidade Federal do Paraná, Rua Prefeito Lothário Meissner 632 Campus II, Jardim Botânico, 80210170 - Curitiba, PR, Brazil

ARTICLE INFO

Article history:
Received 16 February 2016
Received in revised form 19 April 2016
Accepted 21 April 2016

Keywords:
Fibroma
Picrosirius red
Collagen

ABSTRACT

Objective: The aim was to investigate collagen fibers in giant cell fibroma, inflammatory fibrous hyperplasia, and oral normal mucosa.
Materials and methods: Sixty-six cases were stained with picrosirius red. The slides were observed under polarization, followed by the measurement of the area and the percentage of the type I and type III collagens. The age and gender were obtained from the clinical records.
Results: No differences could be observed in both the area and percentage of the type I and type III collagens within the categories of lesions and normal mucosa. In the giant cells fibroma, a greater area and percentage of type I collagen could be identified in individuals of less than 41.5 years ($p < 0.05$).
Conclusion: The distribution of type I and type III collagen fibers in the studied lesions followed a similar pattern to that observed in the normal mucosa, indicating a normal collagen maturation process of type III to I. The study supports that multinucleated and stellate cells of the giant cell fibroma appear to be functional within collagen types III and I turnover. The greater amount of type I collagen identified in giant cell fibroma in individuals of less than 41.5 years reinforce the neoplastic nature of lesion.

1. Introduction

Giant cell fibroma (GCF), a tumor lesion, appears to be unassociated with chronic irritation. Clinically, it is an asymptomatic, sessile or pediculate nodule of less than 1 cm in diameter, with a papillary or smooth surface (Weathers and Campbell, 1974), located on the tongue, gingiva, and alveolar mucosa (Houston, 1982; Kuo et al., 2009). A slight predilection for females (Bakos, 1992; Magnusson and Rasmusson, 1995) or no predilection have been reported (Kuo et al., 2009), and it is more frequently in patients of less than 30 years of age (Weathers and Campbell, 1974; Houston, 1982; Kuo et al., 2009). Histologically, GCF is characterized by the presence of mono or multinucleated fusiforms or stellate giant cells, which are mainly found in the subepithelial connective tissue (Weathers and Campbell, 1974; Magnusson and Rasmusson, 1995; Mighell et al., 1996).

In contrast to GCF, inflammatory fibrous hyperplasia (IFH) is a reactive lesion resulting from chronic irritation due to the use of removable partial or complete prostheses (Santos et al., 2011). GCF and the IFH constitute some of the most common fibrous lesions in the mouth (Miguel et al., 2003). The IFH affects patients who are older than GCF patients, most predominantly affected the sixth decade (Miguel et al., 2003; Corrêa et al., 2006) with a slight predilection for the females (Bakos,1992). Histologically, the IFH

* Corresponding author at: Clínica de Odontologia-Pontifícia Universidade Católica do Paraná, Rua Imaculada Conceição, 1155, Prado Velho 80.215-901 Curitiba, PR, Brazil.
E-mail address: alinecristinabatista@yahoo.com.br (A.C.B.R. Johann).

http://dx.doi.org/10.1016/j.acthis.2016.04.007
0065-1281/

is characterized by dense connective tissue, with variable chronic inflammatory infiltrate (Santos et al., 2011).

Picrosirius red technique has been used to evaluate collagen fibers in a wide range of oral and maxillary lesions, including: tumor or tumor-like lesions and cyst and odontogenic tumors (Junqueira et al., 1979; Toida et al., 1989; Dayan et al., 1994; Hirschberg et al., 1996; Allon et al., 2006; Hirshberg et al., 2007; Gajda et al., 2009; Vij et al., 2011; Allon et al., 2011; Moure et al., 2012).

A predominance of type III fibers suggests that the collagen molecules are not tightly packed, which often consist of pro-collagen, intermediate collagen, or collagen with pathological alterations, as compared to normal collagen fibers, which are packed fibers (Dayan et al., 1989). Considering that the GCF and the IFH are lesions that present different etiological, clinical, and histological characteristics, but in which both present an excess of collagen fibers deposition, the hypothesis of the present study would be that differences do in fact exist concerning collagen maturation as compared to the normal mucosa. In addition, it is unclear whether or not the morphology of the stellate and multinucleated fibroblasts of the GCF is in fact a degenerative or functional alteration (Odell and Lombardi, 1994). An evaluation of the maturation of the collagen in these lesions may indicate if these cells are functional or not. Thus, the present study aimed to investigate the differences in the collagen fibers in these lesions, as compared to the normal oral mucosa, in sections stained by the picrosirius red technique and examined under polarized light microscopy.

2. Materials and methods

The present work was approved by the Research Ethics Committee from Pontifícia Universidade Católica do Paraná—PUCPR (Number 29895).

2.1. Sample selection

Samples selected from the archives of three universities (Pontifícia Universidade Católica do Paraná—PUCPR, Universidade Federal da Bahia (UFBA) and Universidade Federal de Minas Gerais—UFMG), from 2007 to 2011, included biopsy forms, paraffin-embedded specimens, and histological hematoxylin and eosin (HE)-stained slides with the following diagnoses: a) giant cell fibroma, b) inflammatory fibrous hyperplasia, and c) normal mucosa of the alveolar mucosa and those which stem from the distal wedge performed to allow access for the extraction of the impacted third molar.

Two oral pathologists selected the cases using slides stained with HE in an Olympus BX40 optical microscope (Olympus, Tokyo, Japão), magnified at 100X, 200X, and 400X. The histological criteria used to confirm the diagnosis of GCF was the presence of abundant bundles of collagen fibers, generally arranged loosely; connective tissue presenting scarce or moderate vascularization; no inflammation; and the presence of giant mono or multinucleated, fusiforms or stellate cells (Fig. 1A). The connective tissue was covered with hyperplasic stratified pavement epithelium (Santos et al., 2011; Magnusson and Rasmusson, 1995). For IFH, the selected criterion was the presence of the fibrous connective tissue was dense and with variable chronic inflammatory infiltrate (Fig. 1C). The connective tissue was covered by the stratified pavement epithelium, generally hyperplasic and keratotic (Santos et al., 2011).

The exclusion criteria included fibrous hyperplasia caused by medication, the absence of paraffin-embedded specimens, cases with a small quantity of connective tissue for analysis, cases in which the intense inflammation modified the tissue architecture, and cases of normal mucosa fragments which appeared as moderate or intense inflammatory infiltrate.

Twenty-two cases were selected for each group: GCF, IFH, and normal mucosa (Fig. 1E), in a total of 66 cases. The following data were collected from patient clinical forms: age, gender, location, and time of evolution. The age was stratified as greater than 41.5 years of age and less than 41.5 years of age; this value corresponded to the median age value of all groups.

2.2. Picrosirius red staining

Sections of 4 μm in thickness were stained with picrosirius red to view the collagen fibers. After their deparaffinization in xylol, the sections were incubated in ethanol, hydrated in distilled water, followed by incubation in sirius red (Direct Red 80, diluted 0.1% in saturated picric acid Aldrich Chemical Company, Milwaukee, USA) for 1 h at room temperature. The sections were rinsed with distilled water, counter-stained with hematoxylin, washed in distilled water, dehydrated in alcohol solutions and in xylol.

2.3. Analysis of collagen fibers

The analysis was performed in such a way that the examiner did not know which slides contained lesions or normal mucosa. Eight images of connective tissue (four superficial and four deeper) were captured from each cut, magnified at 200X in an Olympus® BX50 optical microscope (Olympus, Tokyo, Japan with an Olympus® U-Pot polarization lens (Olympus, Tokyo, Japan)) attached to a Dinolite® AM 423X microcamera (AnMo Electronics Corporation, New Taipei City, Taiwan). The image acquisition parameters were set during the image capturing process (Zhang et al., 2011).

The type I and type III collagen fibers were differentiated by the polarization color. Against a black background, reddish-yellow thick fibers were considered to be type I collagen, while the fine fibers, similar to the yellowish-green network were considered to be type III collagen (Zhang et al., 2011; Junqueira et al., 1979). The images were measured in an image analyzer (Image ProPlus™ 4.5 Media Cybernetics Inc., Silver Spring, MD, USA) (Moure et al., 2012), which, after image segmentation, automatically measured the area and percentage (red or green) in each image in μm^2. The average of the type I and type III collagen was obtained by means of the average of the eight images. The results were expressed in area and percentage of type I and type III collagen fibers.

2.4. Statistical analysis

The SPSS 19.0 program (SPSS Inc, Chicago, Illinois, USA) was used for statistical analysis. To analyze the interaction of the variables of age and gender according to the type of lesion, the Chi-squared test was employed. When this test indicated differences among the variables, the test of difference between two proportions was applied. For the remaining variables, the Kolmogorov-Smirnov Normality Test was performed, which identified that the variables of type I collagen area, type III collagen area, % of type I collagen, and % of type III collagen when analyzed according to the group, according to the group versus age, and according to the group versus gender, these did not present a normal distribution. In this manner, the comparison among the groups was performed using the Kruskal-Wallis non-parametric test. To compare the collagen in each group stratified by gender and age, the Mann–Whitney test was applied. The significance level adopted in all the tests was of 5% ($p < 0.05$).

3. Results

The locations of the lesions and the normal mucosa are presented in Table 1. The average time of evolution for the GCF was of 50.8 months, varying from 4 to 240 months, there is no information in 7 cases (31.82%). For the IFH, the time of evolution was of 22.13