Original Article

Clinicopathologic Study of a Series of Giant Cell Fibroma Using Picrosirius Red Polarizing Microscopy Technique

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Abstract

Background: Giant cell fibroma (GCF) is a distinct type of fibroma with characteristic large, stellate mononuclear or multinucleated giant fibroblasts; the stroma of GCF is relatively unexplored. The Picrosirus red polarizing microscopy technique is used to characterize the collagen fibers. The aim of this study was to evaluate the staining properties of collagen fibers in GCF and to correlate it with fibroma using Picrosirius red under the polarizing microscope and van Gieson under bright field microscope.

Methods: In the present study van Gieson and picrosirius red stained slides of 7 cases each of GCF and fibroma were compared for the staining properties of collagen. Using picrosirus red polarizing microscopy; colors noted in fibroma included yellow, yellowish-orange and green, whereas the GCF showed predominantly yellow and orange colors. In Van Gieson stained sections it was observed that the collagen in GCF was densely packed and arranged perpendicular to the epithelium while the collagen in fibroma was loosely packed and arranged parallel to the epithelium.

Conclusion: Observable differences in the stroma of the collagen of GCF and fibroma were noted. Collagen in GCF was more mature and dense. The Picrosirius red polarizing technique can be used to assess the collagen in GCF.

Keywords: GCF, Picrosirius red, polarizing microscopy

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Introduction

The oral mucosa often presents with localized fibrous overgrowths and various names like irritation fibroma, focal fibrous hyperplasia, fibrous polyp, traumatic fibroma, fibrous hyperplasia and fibroepithelial polyp have been given to describe these lesions. Most of these lesions are reactive or reparative in nature and true fibromas are relatively rare.[1] Giant cell fibroma (GCF) is a fibrous tumor of the oral cavity with distinctive clinicopathologic features. These lesions were first described as a distinct entity by Weathers and Callihan in 1974.[2] They account for about 1% of all oral biopsies and constitute nearly 5% of all oral mucosal fibrous lesions.[3] Unlike fibromas, GCF is not associated with local trauma or irritation and clinically most of these cases are seen in the gingival.[2,3] This benign oral mucosal tumor often presents as a pedunculated, bosselated and exophytic mass that is usually asymptomatic. GCF is also known to be histopathologically different from fibroma as they are composed of large fibrous connective tissue that is usually loosely arranged with prominent vascular element and have the characteristic presence of giant, plump, spindle shaped and stellate fibroblasts some of which are multinucleated.[4] These cells are most prominent just beneath the epithelium and are less common or absent in the center of the lesion. Electron microscopic studies have demonstrated these large stellate and multinucleated cells to be atypical fibroblasts.[5,6]

Immunohistochemical studies have also proved that the phenotype of the cells of GCF is fibroblasts.[7] Even though, many studies have focused on the giant cells and their nature,[5–7] scanty research has been done till the date to study the collagen produced by these pathologic fibroblasts and their stromal characteristics.

The collagen molecules are orderly arranged in parallel fashion in tissue endowing them with property of normal birefringence with visible aggregated collagenous entities under optical and electron microscopes. Collagen molecules are rich in basic amino acids, hence they react with acidic dyes like Picrosirius red.[8] The molecules of this dye are long and oriented parallel to the collagen molecule, thus enhancing the birefringence of collagen.[8] The enhancement of birefringence of collagen using Picrosirius red poloarizing microscopy is a very sensitive and specific method to study the collagen qualitatively as reported by earlier studies. This method has also been utilized to study the collagenous stroma of some of the oral lesions like dental follicle, odontogenic cysts and tumours, oral submucous fibrosis, oral squamous cell carcinoma, peripheral ossifying fibroma and salivary gland tumor.[4,9–13] The polarization colors of collagen observed are in the spectrum of yellow, red, green, and also of mixed hues like greenish yellow and yellow orange.[8,9] The spectrum of polarization colors is the function of thickness of the sections, diameter of the fibers and the packing of the collagen fibers.[11,14–16] Yellow and red polarization color is indicative of mature thick fibers of collagen.[11] Predominant greenish hue of both the thick and thin collagen fibers suggests that the collagen fibers are loosely packed and could be

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composed of less mature collagen like intermediate molecules of collagen and pathological collagen.[16] A careful review of literature suggests that it has not been used till date to study the collagenous stroma of GCF. Therefore, the purpose of the current study was to provide an insight into the elusive stroma of GCF.

The purpose of the present study was to evaluate the nature of collagen fibers in GCF and fibroma by comparing the staining properties of collagen using van Gieson stained sections and determining the polarization colors of collagen in Picrosirus red stained sections.

Materials and Methods

Seven cases each of GCF and fibroma were included in the study. The clinical records and blocks of each were retrieved from the archives of the Department of Oral Pathology KLE's VK Institute of Dental Sciences. The clinical records were reviewed for the age and sex of the patients; location and duration of the lesion. Three 4 µm sections were made; one section was stained with H and E to confirm the diagnosis. The other section was stained with Picrosirius red stain for collagen using the procedure previously described.[13] In brief, after deparaffinization in xylene and ethanol the section were hydrated in distilled water, followed by counterstaining in 1% (w/v) Sirius red F3B (CI 35782) in Picric acid solution for 1 hour at room temperature.

The same protocol was followed for van Gieson technique except that the counterstain used was van Gieson (1% acid fuschin w/v in saturated picric acid solution) for 5 min. The slides were dehydrated by passing through 70% and 90% alcohol cleared and mounted.

The Picrosirus stained sections were viewed under a polarized microscope Leica DM 2500 and the van Gieson and H & E slides were viewed under bright field by two independent observers. In the van Gieson stained slides; sections were analyzed for the orientation of the fibers with respect to the epithelium and the arrangement of the fibers. The color of the fibers of the Picrosirius stained sections were analyzed under polarized microscope. Evaluation of the polarization colors was done as described by Montes GS and Junquiera LCU and colors were classified as yellow, red and green.[8] The hues in mixed or unequal intensities were denoted using terms like greenish yellow, yellowish red and so on.

Results

On reviewing the clinical records (Table 1 and 2) it was observed that, the age of patients with fibroma varied between 18 to 60 years and that of GCF ranged from 15 to 60 years. The duration of lesion ranged from 2 to 12 months for fibroma and for GCF it ranged from 3 months to 6 years suggests that the GCFs may represent a more slowly growing lesion. The most common site in which GCFs were noted to occur in the present study was in the gingiva. When the picrosirius red slides were viewed under polarizing microscopy the collagenous stroma of fibroma showed the spectrum of colors ranging from yellow, yellow-orange and green but the intensity of yellow color was more than the other colors. The GCF showed yellow and orange predominantly with equal intensity. Under van Gieson stained sections, it was observed that the collagen in GCF was densely packed and arranged perpendicular to the epithelium while the collagen in fibroma was loosely packed and arranged parallel to the epithelium.

Discussion

Wheathers and Callihan[2] introduced the term 'GCF', when they had identified around 108 out of 2000 oral fibrous nodules, with sufficient distinctive clinical and pathologic features to warrant separation and reclassification of the lesion. These differences were earlier restricted only to the epithelium and stellate fibroblasts in the GCFs. Based on clinical appearances, even though in the review by Houston GD[17] a female predilection was noted, in the present study the majority of cases were males. In coincidence with earlier data our study also showed that the GCFs had a predilection for gingiva. Over 60% of fibromas are noted to have a wart or nodular surface and these lesions are often mistaken clinically

Table 1. The clinical presentation of Fibroma patients

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Age/Sex	60/F	30/F	36/M	48/M	NA	22/F	26/M
Site of lesion	NA	Buccal mucosa	Buccal mucosa	Gingiva	Dorsal surface of tongue	Gingiva	Lateral border of tongue
Duration of lesion	8 months	12 months	6 months	8 months	6 months	2 months	2 months
Size	2 × 2 cm	1.5 × 1 cm	1 × 1 cm	3 × 3 cm	5 × 3 cm	NA	0.5 × 1cm

Table 2. Clinical presentation of patients with GCF

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Age/sex	48/M	54/M	50/M	48/M	50/F	60/F	15/M
Site of lesion	Buccal mucosa	Midpalatine suture	Buccal mucosa	Gingiva	Gingiva	Dorsal surface of tongue	Gingiva
Duration of lesion	72 months	24 months	3 months	3 months	NA	12 months	12 months
Size	3 × 2 cm	1 × 1 cm	1 × 1 cm	2 × 2 cm	0.5 × 0.5 cm	0.5 × 0.5 cm	0.5 × 0.5 cm

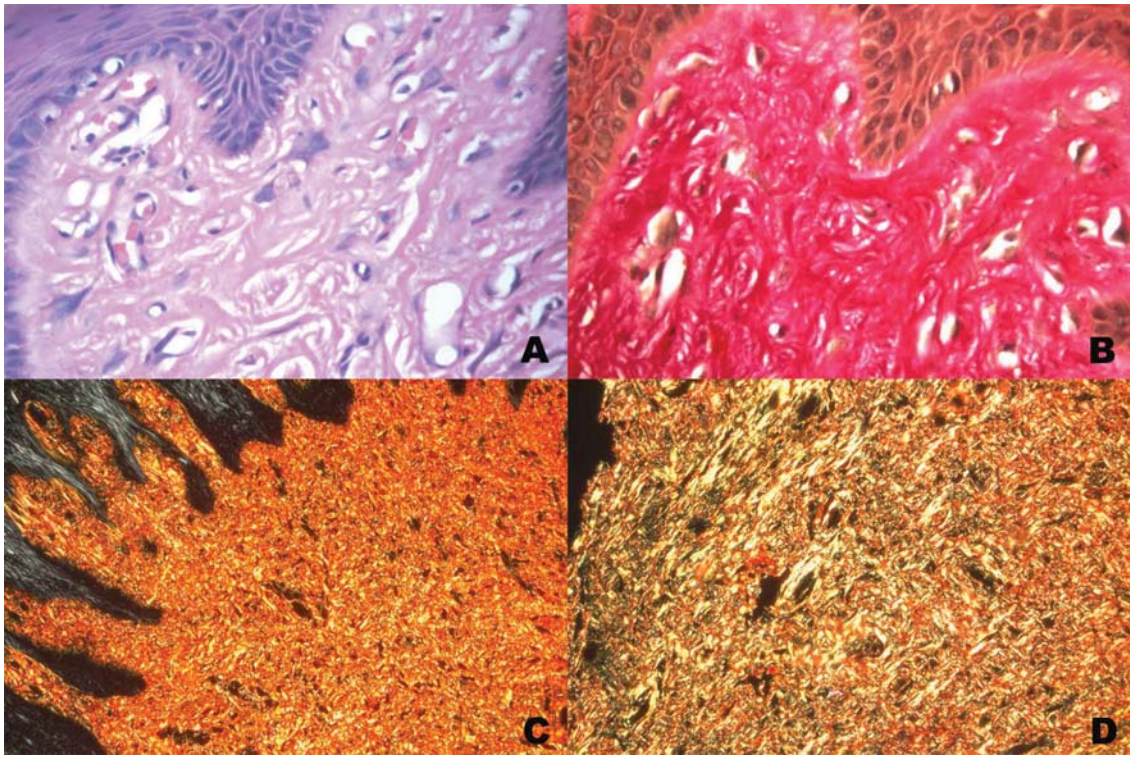

Figure 1. A) H & E stained section of GCF showing giant fibroblasts juxtaepithelially (40x); **B)** van Gieson stained section of GCF showing tightly packed collagen fibers interspersed with giant fibroblasts (40x); **C)** Picrosirius red stained section of GCF under polarizing light showing predominantly yellow polarizing colour. (10x); **D)** Picrosirius red stained section under polarized light shows predominantly green colour suggestive of immature collagen fiber.

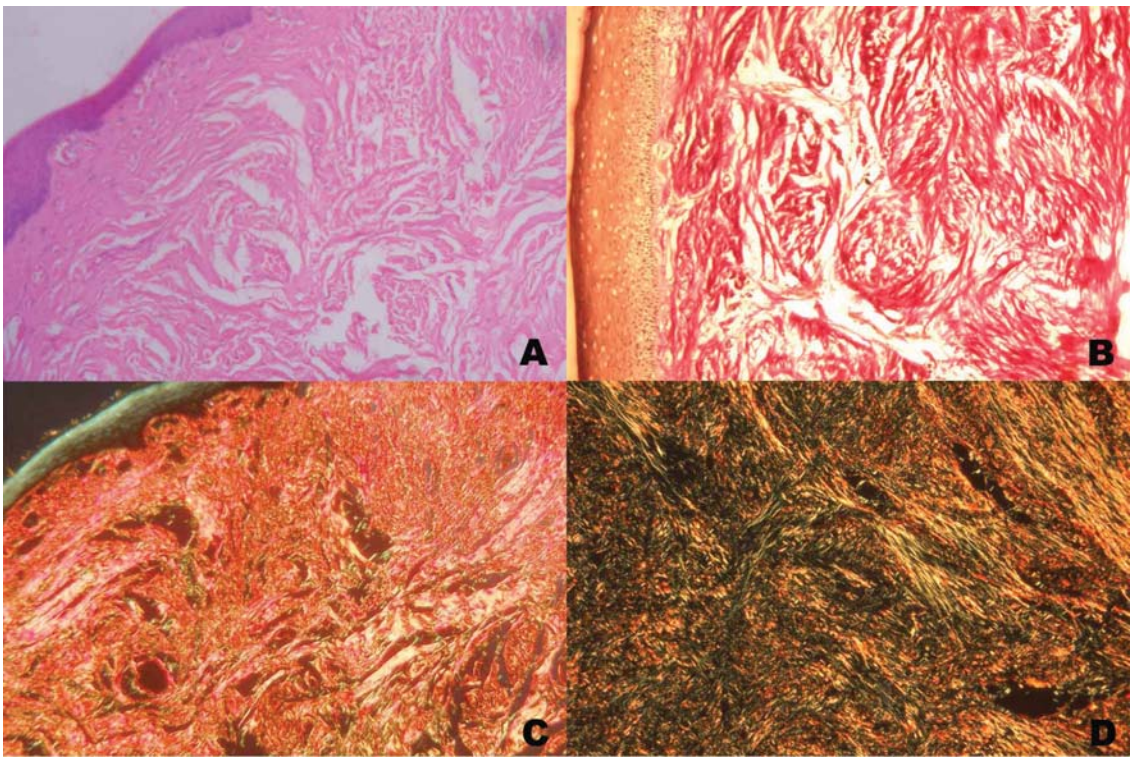

Figure 2. A H& E stained section of Fibroma at magnification (10x); **B)** van Gieson stained section of Fibroma showing loosely arranged collagen fibers (10x); **C)** Picrosirius red stained section of fibroma under polarized microscopy (10x). The section shows predominantly orange-red polarization colour; **D)** Picrosirius red stained section of fibroma under polarized microscopy (40x). The section shows predominant green colour indication presence of immature collagen fibers.

for papilloma. Few authors have also postulated a possible viral etiology for this elusive lesion as it had many histological features in common with the virus induced fibroblastoma of the deer. The viral origin was also based on the finding of atypical fibroblasts containing numerous intracellular microfibrils in electron microscopy. The multinucleated giant cells appear to occur by fusion of the mononuclear cells due to which the viral origin of the tumor was postulated.[5] Through the present pilot study, it can be postulated that the differences also extend to the stroma and the nature of collagen found in GCF.

Picrosirius red polarizing technique has been used previously in many lesions to study the possible significance of nature of collagen in the stroma on the biological behavior of the lesion.[9–14] Previous studies have shown polarization colors of thin fibers as green, greenish-yellow, whereas of thick fibers range from yellowish-orange to orange red.[8] A few studies have also shown that the polarization colors of Picrosirius red are determined by fiber thickness and packing of fibers.[14,15] Densely packed collagen give out longer wavelengths. It has also been proved that the polarization colors also depend on the age of the lesion. Longer duration of the lesions increases the amount of collagen fibers which exhibit yellowish-orange and orange polarization colors indicating tighter packing and better alignment of the micro fibrils, similar to normal mature collagen.[16]

In the present study, observation of Picrosirius red under polarizing microscopy revealed a different pattern of collagen fiber colors in GCF as compared to fibroma. The color spectrum, though observed was almost same, but the predominant color in GCF was yellow while that in fibroma was green. The collagen fibers of GCF were densely packed and their polarization colors were predominantly yellow. This suggests that stroma of GCF consists of more mature collagen, which is in concurrence to the review by Houston GD in which he reported that only two cases recurred.[17] Toida, et al.[18] reported two different histological patterns of fibers in irritational fibroma: one made up of perpendicular fibers (radiating type) and other with parallel fibers (circular type). In the present study GCF was found to have perpendicular fibers and fibroma with parallel fibers.

Traditionally the stroma of GCF has reported to be loosely arranged, but to the contrary van Gieson technique revealed that the collagen in GCF is densely packed.[3] In accordance with the earlier finding, in the present study we observed that GCF has a predilection for gingiva.

In the present study, an observable difference between the properties of collagen in GCF and Fibroma was revealed. The collagen of GCF was observed to be more mature and dense than that of fibromas. The Picrosirius red polarizing technique can be used to investigate the quality of collagen in GCF. In future the polarization colors of the collagen fibers should be evaluated separately for thick and thin collagen fibers. But more studies with larger sample size and advanced investigative methods are necessary to understand the significance, if any of differences noted in the stroma in these seemingly similar lesions.

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