



Collagen and Its Role in predicting the Biological Behavior of Odontogenic Lesions

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ABSTRACT

Introduction: Odontogenic cysts and tumors have variable recurrence rates. Recurrence rate is mainly due to the activity of the epithelium. The epithelium of these lesions has been investigated extensively in regard to their role in proliferative and aggressive behavior of the lesions. However, the role of the connective tissue wall in their behavior has not been studied as extensively. Collagen is an essential part of the connective tissue as a whole and fibrous wall of cystic lesions especially. It is demonstrated by picosirius red dye staining combined with polarization microscopy. This method permits the evaluation of the nature of the collagen fibers in addition to their thickness.

Materials and methods: A total of 56 histopathologically diagnosed cases comprising odontogenic follicle, dentigerous cyst, unicystic ameloblastoma, keratocystic odontogenic tumor (KCOT), multicystic/solid ameloblastoma, and ameloblastic carcinoma were taken and stained using picosirius red stain and evaluated using a polarizing microscope.

Results: Collagen fibers in odontogenic follicles and dentigerous cysts showed predominant orange-red birefringence; fibers in unicystic ameloblastoma and KCOT showed both orange red and greenish-yellow birefringence; and fibers of multicystic/solid ameloblastoma showed predominant greenish-yellow birefringence and ameloblastic carcinoma that showed almost complete greenish birefringence. As the biological behavior of the lesions in the spectrum studied progress toward aggressive nature, increase in immature collagen fibers is noticed.

Conclusion: This study suggests that the nature of collagen fibers plays a pivotal role in predicting the biological behavior of odontogenic lesions.

Clinical significance: Aggressive nature of the odontogenic lesions is determined by both the epithelium and the connective tissue components (collagen). Studying the nature and type of collagen helps in predicting its biological behavior.

Keywords: Adult azo compounds, Birefringence, Odontogenic cysts, Odontogenic tumors.

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INTRODUCTION

Tooth development occurs as a result of array of epithelial mesenchymal interactions, which on the contrary can also lead to various pathologies, such as odontogenic cysts and tumors.¹ Jaws host wide variety of cysts and tumors encompassing the spectrum of lesions ranging from developmental cysts to malignant neoplasms.^{2,3} Few of these cysts and tumors exhibit a biologically aggressive course resulting in destruction of adjacent and deeper tissues.² Many researchers have emphasized on the role of epithelium in the biological behavior of these lesions, however, connective tissue especially collagen, which being understudied, may also have significant participation in its biological behavior.¹

Connective tissue comprises essentially of the cells and extracellular matrix (ECM). Extracellular matrix is composed of four intimately associated families of proteins: Collagens, elastin, adhesive glycoproteins, and proteoglycans.⁴ Collagen is the major component, which constitutes about 34% of the total ECM proteins. It forms the integral part of connective tissue stroma and plays an important role in maintaining structural integrity and in determining tissue function.⁵

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Collagen has got the natural birefringence attributed to the arrangement of its fibers.⁵ Connective tissue stains, such as Van Gieson and Masson's trichrome, are commonly used to detect the collagen fibers but these stains fail to demonstrate the very thin fibers, so the true nature of the collagen fibers in the pathologies cannot be evaluated.¹ Picrosirius red staining technique for collagen fibers was first developed by Junqueira et al in 1979. This method of staining is based on the property of birefringence exhibited by collagen under polarized light.⁴ When observed under polarized microscope this stain can differentiate procollagens, intermediate and pathological collagen fibers from normal collagen fibers.¹ Picrosirius red stain belongs to adult azo compounds group.

Inflammation also plays a role in the type of collagen synthesized and its arrangement. Studies have elucidated differing role of inflammation in type of collagen synthesized. In this study, the odontogenic lesions with secondary inflammation are excluded to understand the true role of the collagen in their biological behavior. Thus this study intends to evaluate the exact nature and role of collagen in the biological behavior of spectrum of odontogenic lesions comprising odontogenic follicle, dentigerous cyst, unicystic ameloblastoma, keratocystic odontogenic tumor (KCOT), multicystic/solid ameloblastoma, and ameloblastic carcinoma. To our knowledge, this study is first of its kind in English literature in assessing the role of collagen in the spectrum of odontogenic lesions encompassing from odontogenic follicle to ameloblastic carcinoma.

MATERIALS AND METHODS

The study material included a total of 56 histopathologically diagnosed cases of odontogenic follicle, dentigerous cyst, unicystic ameloblastoma, KCOT, multicystic/solid ameloblastoma, and ameloblastic carcinoma. These lesions are categorized into six groups according to their increasing order of aggressive biological behavior, with each group containing 10 cases except for ameloblastic carcinoma group, which consists of six cases (Table 1). Paraffin-embedded tissue blocks were retrieved from the archives.

Table 1: Groups of odontogenic lesions

Groups	Lesion	Number of cases
I	Odontogenic follicle	10
II	Dentigerous cyst	10
III	Unicystic ameloblastoma	10
IV	KCOT	10
V	Multicystic/solid ameloblastoma	10
VI	Ameloblastic carcinoma	6

Sections of 5 µm thickness were taken and mounted onto the slides treated with Mayer's egg albumin. These sections were deparaffinized and rehydrated by passing through series of graded alcohols and into distilled water. Harris hematoxylin was used to stain the sections, followed by the process of differentiation using 1% HCl and then washed with tap water for alkalization followed by incubation in 0.1% (w/v) Sirius red F3B (Sigma-Aldrich) in saturated picric acid solution for 1 hour at room temperature. Acidified water rinse was done for the sections followed by dehydration using alcohol and mounted with DPX. The sections were examined under bright field and polarizing microscope (Olympus CX41). Six fields from each section were analyzed under 10× magnification. The findings of the study were analyzed by Pearson's chi-square test. $p = 0.05$ or less is considered to be statistically significant.

RESULTS

Mature collagen fibers under the polarized microscope show orange-red birefringence, whereas immature collagen fibers show greenish-yellow birefringence. In this study, it was observed that 20% of odontogenic follicles, 28.3% of dentigerous cysts, 56.7% of unicystic ameloblastomas, 61.7% of KCOTs, 80% solid ameloblastomas, and 94.4% ameloblastic carcinomas cases showed greenish-yellow birefringence (Table 2).

Out of 10 cases of odontogenic follicle with total 60 fields examined, 48 fields (80%) have shown the predominance of collagen fibers with orange-red birefringence, indicating the presence of mature collagen and

Table 2: Lesion and birefringence cross tabulation

Groups	Orange-red birefringence				Greenish-yellow birefringence			
	Count (fields)	Percentage within this birefringence	Percentage within lesion	Total (%)	Count (fields)	Percentage within this birefringence	Percentage within lesion	Total (%)
I	48	31.2	80	14.3	12	6.6	20	3.6
II	43	27.9	71.7	12.8	17	9.3	28.3	5.1
III	26	16.9	43.3	7.7	34	18.7	56.7	10.1
IV	23	14.9	38.3	6.8	37	20.3	61.7	11
V	12	7.8	20	3.6	48	26.4	80	14.3
VI	2	1.3	5.6	0.6	34	18.7	94.4	10.1
Total	154	100	45.8	45.8	182	100	54.2	54.2

in 12 fields (20%) fibers have shown predominance of greenish-yellow birefringence, indicating the presence of immature collagen (Fig. 1).

Out of 10 cases of dentigerous cysts with total 60 fields examined, 43 fields (71.7%) have shown the predominance of collagen fibers with orange-red birefringence, i.e., mature collagen and in 17 fields (28.3%) fibers have shown the predominance greenish-yellow birefringence, i.e., immature collagen (Fig. 2).

Out of 10 cases of unicystic ameloblastoma with total 60 fields examined, 26 fields (43.3%) have shown the predominance of collagen fibers with orange-red birefringence, i.e., mature collagen and in 34 fields (56.7%) fibers have shown the predominance greenish-yellow birefringence, i.e., immature collagen (Fig. 3).

Out of 10 cases of KCOT with total 60 fields examined, 23 fields (38.3%) have shown the predominance of collagen fibers with orange-red birefringence, i.e., mature

collagen, and in 37 fields (61.7%), fibers have shown the predominance of greenish-yellow birefringence, i.e., immature collagen (Fig. 4).

Out of 10 cases of multicystic/solid ameloblastoma with total 60 fields examined, 12 fields (20%) have shown the predominance of collagen fibers with orange-red birefringence, i.e., mature collagen and in 48 fields (80%) fibers have shown the predominance of greenish-yellow birefringence, i.e., immature collagen (Fig. 5).

Out of six cases of ameloblastic carcinoma with total 36 fields examined, two fields (5.6%) have shown the predominance of collagen fibers with orange-red birefringence, i.e., mature collagen and in 34 fields (94.4%) fibers have shown the predominance of greenish birefringence, i.e., immature collagen (Fig. 6).

The results were statistically significant (p -value obtained = 0.001; $p \leq 0.05$ was considered significant) (Table 3).

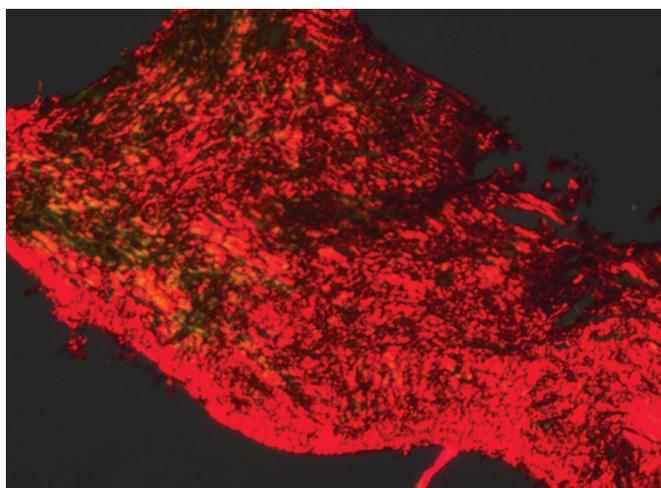


Fig. 1: Odontogenic follicle (group I) showing predominance of orange-red birefringence

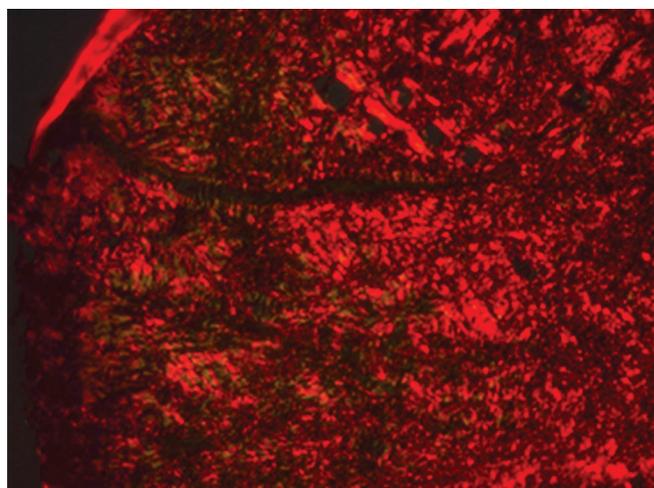


Fig. 2: Dentigerous cyst (group II) showing predominance of orange-red birefringence

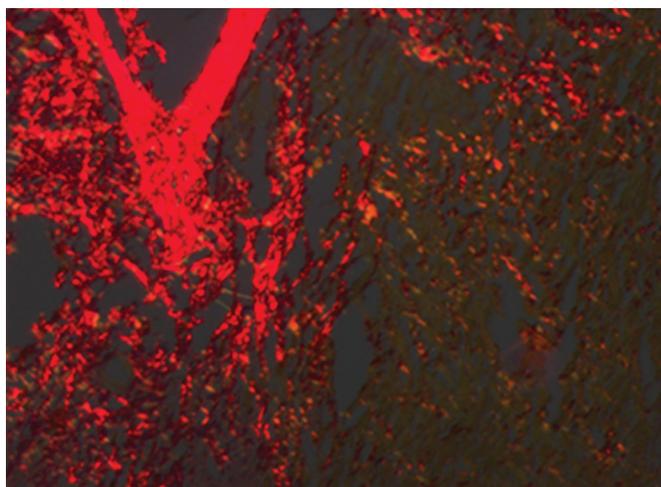


Fig. 3: Unicystic ameloblastoma (group III) showing almost equal proportion of orange-red and greenish birefringence

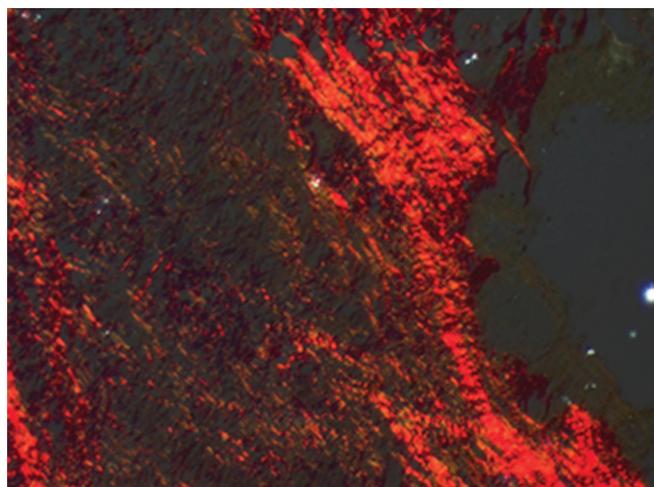


Fig. 4: Keratocystic odontogenic tumor (group IV) showing almost equal proportion of orange-red and greenish birefringence

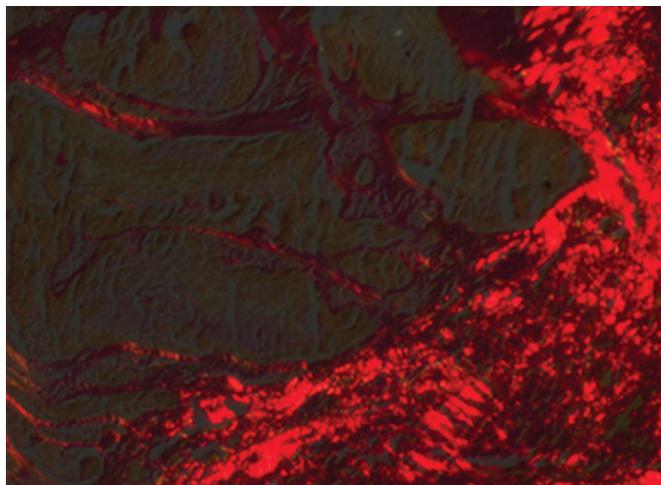


Fig. 5: Multicystic/solid ameloblastoma (group V) showing predominance of greenish-yellow birefringence

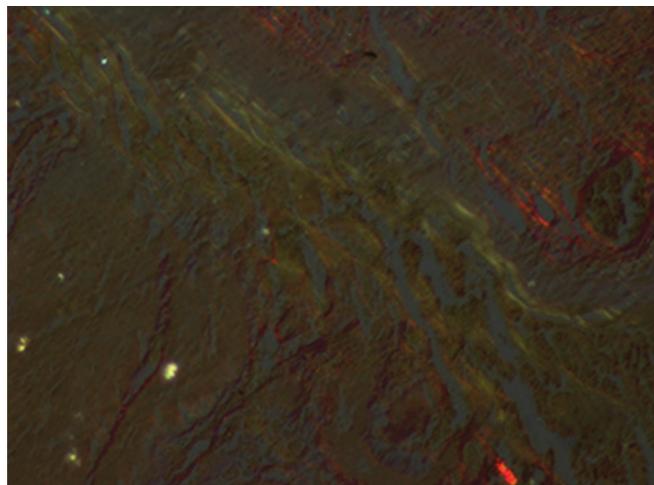


Fig. 6: Ameloblastic carcinoma (group VI) showing almost complete greenish birefringence

Table 3: Chi-square test

	<i>p-value</i>	<i>Degree of freedom</i>	<i>Asymp. sig. (2-sided)</i>
Pearson chi-square	85.505 ^a	5	0.001
Likelihood ratio	94.397	5	0.001
Linear-by-linear association	79.283	1	0.001
Number of valid cases	336		

^a0 cells (0.0%) have expected count <5. The minimum expected count is 16.50

DISCUSSION

Epithelial mesenchymal interactions play a very crucial role in homeostatic mechanisms of adult tissues. These interactions determine the development of the odontogenic apparatus and also the pathologies arising from it. Extracellular matrix plays a key role in growth, regulation, differentiation, and organization of tissues. By the modification in the ECM, the stromal cells can maintain control over cell size, function, response to wounds, and other pathologic conditions.⁶

Various researchers have proposed that epithelium of the odontogenic cysts play a role in the expansion of these cysts without the involvement of the nonepithelial component. Vedtofte et al⁷ postulated the importance of stromal component through their study on the transplanted keratocyst epithelium in the nude mice, which demonstrates that through epithelial mesenchymal interactions, stromal component plays a role in the biological behavior. The mesenchymal component rather than epithelium is the possible reason for primary defect in KCOT has been proved by Browne.⁸ The connective tissue stroma performs the function of barrier, thus preventing the evasion of host immune system. It plays an important role in supplying the nutrients and also removal of the waste products.⁹ However, the amount of the stroma present differs from lesion to lesion.

The connective tissue stroma is chiefly composed of collagen fiber bundles which can be categorized as thin and thick fibers. Type I and III collagens are predominant types, in which type I collagen consists of thick and mature fibers and type III collagen consists of thin and immature fibers.^{10,11} Collagen has the natural birefringence attributing to its anisotropic structure. Anisotropy of collagen is inhomogeneity of arrangement of collagen molecules in relation to collagen fibril axis. Birefringence of collagen can be better visualized using additives, such as picosirius red stain. Thus polarized light provides an additional insight into the composition and structure of collagen. This study was carried out based on this physical phenomenon. Picosirius red is the selective stain for collagen fibers. Thin fibers of the collagen are undetectable by normal bright field microscopy but they can be visualized with this method as a source of light against a dark background using picosirius red stain. Structures other than collagen does not show any birefringence when stained with picosirius red.^{3,12-14} The principle behind the picosirius red staining of the collagen fibers is that picosirius red is an acidic dye, which binds to the basic amino acids of the collagen (ionic bonding). Few researchers also state that interaction between the picosirius red and collagen can also be unionic. Thus the exact mechanism of action is still uncertain.

Depending on the type, collagen exhibits differential birefringence pattern, ranging from green-greenish yellow to orange red-red.^{14,15} This variation in the birefringence can be attributed to different patterns of physical aggregation of fibers seen in different types of collagen. Type I collagen fibers are composed of thick, mature, and closely packed fibrils presenting as an intense birefringence with orange-red to red color. Type III collagen is composed of thin, immature, and loosely packed fibrils presenting as a weak birefringence with greenish-yellow

to yellow color.^{14,16-19} This is in accordance with Hirshberg et al who states that greenish-yellow birefringence of collagen fibers in KCOT denotes its immature nature, which can be attributed to the precursor form of collagen (procollagen) or a pathologic collagen.²⁰

Within the spectrum of odontogenic lesions studied, we observed an increase in immature collagen (type III) component from odontogenic follicle (group I) to ameloblastic carcinoma (group VI). There is no significant difference between the percentage of immature collagen fibers in odontogenic follicles and dentigerous cysts. Similarly, there is no significant difference between the percentage of immature collagen fibers in unicystic ameloblastoma and KCOT. However, there is predominance of immature collagen fibers in multicystic/solid ameloblastoma (group V) and ameloblastic carcinoma (group VI), the latter almost completely showed immature collagen fibers. This suggests that the lesions with aggressive biological behavior have more amount of immature collagen fibers.

The results of this study are in concordance with Hirshberg et al²⁰; Mahajan et al³; Raj et al,¹ stating that aggressive lesions have more of immature collagen. However, our results are contrary to few studies, which states that there is increase in mature collagen with increasing aggressiveness of the lesion. This contrast in results can be because of the reason that when the stage of the polarized microscope is rotated, collagen bundles exhibiting particular birefringence reverses the birefringence.⁴ This physical phenomenon could be the reason for difference in birefringence observed between this study and studies contrasting it.

CONCLUSION

It has been proved that the epithelial component determines the biological behavior of various odontogenic lesions. However, the role of the connective tissue stroma has been understudied. This study has shown that the collagen of the connective tissue stroma also has an influence on the aggressiveness of the odontogenic lesions. The exact mechanism of how the epithelium and connective tissue interact with each other is not yet known. Hence, further studies pertaining to interactions between the epithelium and connective tissue of odontogenic lesions has to be carried out on larger samples.

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