Original Article

The role of stroma in the expansion of odontogenic cysts and adenomatoid odontogenic tumor: A polarized microscopy study

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Abstract

Objectives: To compare the polarization colors of collagen fibers of odontogenic keratocyst (OKC), radicular cyst, dentigerous cyst, and adenomatoid odontogenic tumor (AOT) with reference to their biological behavior. **Study Design:** Twenty cases each of OKC, radicular cyst, dentigerous cyst, and AOT were stained with picrosirius red stain and studied under polarized light. **Results:** A predominance of green to greenish yellow thick fibers was noted in OKC and AOT as compared to dentigerous cyst and radicular cyst. There was no significant difference between the polarization colors of the thin fibers in all the three groups. **Conclusion:** The stroma of OKC and AOT consists of poorly packed or pathologic collagen and plays a role in its neoplastic behavior.

Key words: Adenomatoid odontogenic tumor, collagen, odontogenic keratocyst, polarized microscopy

INTRODUCTION

No organ in the body is immune to disease and the oral cavity is no exception to this. The lesions affecting the jaws could be developmental, cystic, neoplastic, or inflammatory in origin. Cysts form a high percentage of pathologies affecting the jaws. The most commonly encountered cysts in the oral cavity are radicular cyst (52.3%), dentigerous cyst (18.1%), and the odontogenic keratocyst (OKC) (11.6%).[1] The OKC is notorious for its high recurrence rate which ranges from 10[2] to 62%.[3] Also, the growth of this cyst is more unremitting; Scharfetter in 1989 stated that the invasive growth of keratocysts is likely to be the result of active growth of the connective tissue wall. Human keratocyst collagenase degrades type I and II collagens at almost equal rates, but at the same time, no significant degradation of type III collagen occurs, implying that destruction of this connective tissue is associated with the growth of keratocysts.[4]

The adenomatoid odontogenic tumor (AOT), according to the histological typing of odontogenic tumors by the World Health Organization, is partly cystic and in some cases, the solid lesion may be present only as masses in the walls of a large cyst. Unusually large tumors are regarded as neoplasms rather than hamartomas.

To comprehend further the expansion of jaw cysts with special reference to the collagen present in its connective tissue capsule, the nature of collagen present should be studied. As collagen is an anisotropic substance, its packing and nature can be studied in the connective tissue walls of cysts and neoplasms with polarized microscopy. Many commonly used dyes exhibit dichroism and anomalous polarization colors. The enhancement of anisotropy by dyes and other agents has a histochemical meaning similar

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to that of metachromasia which also depends on orderly arrangement and spacing of certain reactive groups.

Thus, this present study was carried out with the aim of studying and comparing the polarization colors of collagen fibers in the walls of OKC, dentigerous cyst, radicular cyst, and AOT using polarized microscopy with reference to their biological behavior.

MATERIALS AND METHODS

Archival retrieved paraffin blocks of 20 cases each of OKC, dentigerous cyst, radicular cyst, and AOT were procured from the department of oral pathology of the institute, M.G.V's K.B, H Dental College and Hospital, Nasik, Maharashtra. Also, three cases of normal buccal mucosa and five cases of oral submucous fibrosis were used for comparison. All sections were cut to a thickness of 5 μm for the study. Picrosirius staining was done using Direct Red 80 (36554-8, Sigma-Aldrich). An Olympus BX 51 trinocular research microscope with an attached polarizer was used for the study. At the outset, the fiber thickness was determined with the aid of a calibrated ocular micrometer under an oil immersion ×100 objective. The polarization colors were determined separately for thin fibers (0.8 μm or less) and for thick fibers (1.6 to 2.4 microns). Five fields of each section were selected and the polarization colors of the fibers were noted as either green to greenish yellow or yellowish orange to orange red.

For statistical analysis, one-way analysis of variance was used to determine whether the polarization colors of the thick and thin fibers differed significantly between the four study groups. The *t*-test was used to find out whether there was a significant difference between the polarization colors of thick and thin fibers within a single clinical group.

RESULTS

A comparison of the polarization colors of the thick fibers in the four study groups showed that in AOT and in OKC, the mean number of green to greenish yellow thick fibers was significantly higher than the mean number of yellow red to orange red fibers [Figures 1 and 2]. However, in dentigerous cyst and radicular cyst, the mean number of green to greenish yellow thick fibers was significantly lower than the mean number of yellow red to orange red thick fibers [Figures 3 and 4] [Table 1].

Our results also showed that though the mean number of thick fibers showing green to greenish yellow colors in OKC was higher than that of AOT, there was not much difference in their mean value but when compared with radicular and

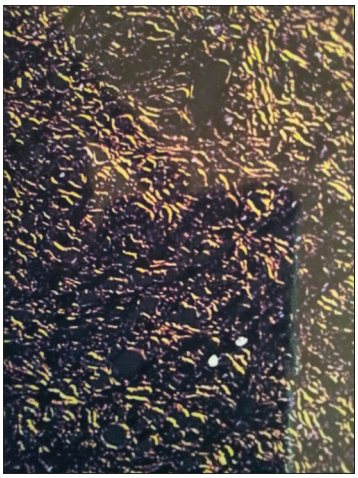

Figure 1: Picrosirius red-stained section of adenomatoid tumor showing a predominance of greenish yellow thick fibers

Figure 2: Picrosirius red-stained section of odontogenic keratocyst showing a predominance of greenish yellow thick fibers

Figure 3: Picrosirius red-stained section of dentigerous cyst showing a predominance of orange red thick fibers

dentigerous cyst, the mean value of green to greenish yellow thick fibers was significantly higher. [Table 2].

A comparison of the polarization colors of all the study groups showed that the mean number of green to greenish yellow thin fibers was significantly higher than the mean number of yellowish red to orange red thin fibers [Table 3]. There was no significant difference in the green to greenish yellow thin fibers in all the four study groups [Table 4].

DISCUSSION

Collagen is an anisotropic structure, which exhibits the phenomenon of birefringence, which can be selectively visualized using polarized light microscopy. Weak birefringence in biological specimens is enhanced by the addition of dyes or impregnating metals in an orderly linear arrangement. Thus polarized light has the ability to enhance histological assessment of tissue and can provide additional insight into the composition and structure of collagen. Following this line of reasoning, the present study was carried out. Polarized light microscopy of picrosirius red-stained sections enables us to visualize fibers of different thickness. This property is due to the fact that very thin fibril of collagen undetectable by normal microscopy becomes visible with this method as a source of light against a dark background. It was found in our study that when stained with picrosirius red, structures other than collagen did not exhibit any birefringence, proving that this histochemical stain is selective for collagen fibers. This is supported by several other studies.[5-7]

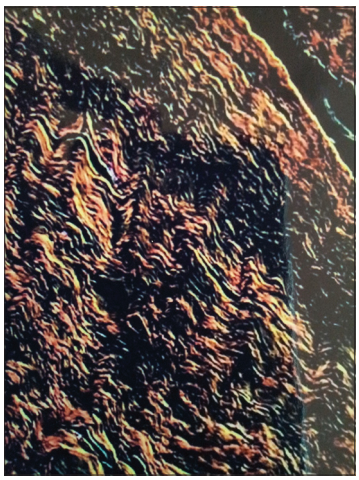

Figure 4: Picrosirius red-stained section of radicular cyst showing a predominance of yellow red to orange red thick fibers

Table 1: Comparison of the polarization colors of the thick fibers in AOT, OKC, dentigerous cyst, and radicular cyst

Groups	Mean number of green to greenish yellow fibers	Mean number of yellow red to orange red fibers	*P* value
AOT	57.8±15.63	42.2±15.63	*P*<0.01
OKC	58.8±11.17	41.2±11.17	*P*<0.01
Dentigerous cyst	34.2±6.64	65.8±6.64	*P*<0.01
Radicular cyst	27.8±8.55	72.2±8.55	*P*<0.01

AOT: Adenomatoid odontogenic tumor, OKC: Odontogenic keratocyst

Junqueira *et al*. in 1979 have stated that collagens of type I form thick fibers, are composed of closely packed thick fibrils, and present an intense birefringence with yellow to red color. On the other hand, collagens of type III form thin fibers, are loosely packed, and display a greenish birefringence.[7]

Our study showed that the number of thick fibers in OKC showing green to greenish yellow color was significantly higher than the thick fibers of dentigerous and radicular cysts. Similar findings have been reported by Hirshberg *et al*. in 1999.[8] Our study also showed that the findings in AOT were similar to those of OKC. It has been found that the stroma of AOT shows a strong immunoreactivity for collagen type III and VI,[9] also observed in ameloblastic fibroma.[10] This could explain the appearance of green to greenish yellow fibers in AOT. The predominance of green to greenish yellow thick and thin fibers could be due to the loosely structured nature of the collagen fibers and the predominance of type III collagen in the stromal tissue. In our study, it was observed that there was no significant difference in the polarization colors of thin fibers in all the four study groups. These results are comparable with those reported by Hirshberg *et al*. in 1999.[8] A predominance of green to greenish yellow color of thin and thick fibers has been found in several studies of different pathological conditions such as in connective tissue nevi collagen,[11] oxodipine-induced hyperplastic gingiva in dogs,[12] ameloblastic fibroma,[10] central odontogenic fibroma,[13] and walls of cysts.[8]

AOT was included in our study as this tumor of odontogenic

Table 2: Comparison of the thick green to greenish yellow fibers in AOT, OKC, dentigerous cyst, and radicular cyst

Group	AOT	OKC	Dentigerous cyst	Radicular cyst	'*F*' ratio
Thick fibers of green to greenish yellow color	57.8±15.63	58.8±11.17	34.2±6.64	27.8±8.55	11.526

AOT: Adenomatoid odontogenic tumor, OKC: Odontogenic keratocyst

Table 3: Comparison of the polarization colors of the thin fibers in AOT, OKC, dentigerous cyst, and radicular cyst

Groups	Mean number of green to greenish yellow fibres	Mean number of yellow red to orange red fibres	*P* value
AOT	85.2±9.45	14.8±9.45	*P*<0.01
OKC	81.6±9.21	18.4±9.21	*P*<0.01
Dentigerous cyst	77.4±11.98	22.6±11.98	*P*<0.01
Radicular cyst	78±9.31	22.0±9.31	*P*<0.01

AOT: Adenomatoid odontogenic tumor, OKC: Odontogenic keratocyst

Table 4: Comparison of the thin green to greenish yellow fibers in AOT, OKC, dentigerous cyst, and radicular cyst

Group	AOT	OKC	Dentigerous cyst	Radicular cyst	'*F*' ratio
Thin fibers of green to greenish yellow color	85.2± 9.45	81.6± 9.21	77.4± 11.98	78± 9.31	2.580

AOT: Adenomatoid odontogenic tumor, OKC: Odontogenic keratocyst

epithelium may be partly cystic and has varying degrees of inductive change in the connective tissue.

Extensive research has been done to ascertain the source of recurrence and enlargement of OKCs with greater emphasis being laid on the proliferative activity of its epithelial lining.[14-16] However, studies by Vedtofte *et al*. and Harris *et al*. have emphasized the role of stroma in cyst expansion.[17,18] Studies by Meghji *et al*. in 1989 have shown significant activity of interleukin1 in odontogenic cyst capsules implicating its role in cyst expansion.[19] Experiments by Scharfetter *et al*. have implicated that the active growth of the connective tissue wall was likely to be the cause of the invasive growth of the keratocyst.[4] High enzyme activity such as beta-naphthylamidase and leucine aminopeptidase in the walls of OKC are probably responsible for the collagenolysis leading to separation of keratocyst epithelium from the connective tissue contributing to its high recurrence rate.[20] It has been documented by various studies that collagen of the cyst wall in OKCs is pathologic and is subject to collagenolytic activity.[21,22]

The presence of green to greenish yellow thick fibers in OKCs as demonstrated by polarized microscopy indicated that the collagen fibers were poorly packed and were composed of procollagens, intermediate or pathologic collagen. Thus, the function of the stroma of keratocysts could possibly be regarded not just as a structural support but also as playing a part in their neoplastic behavior. On the other hand, the predominance of yellow red to orange red thick fibers in dentigerous cyst and radicular cyst indicates that the collagen fiber bundles are more closely packed and are probably not under the influence of intense collagenolytic activity as compared to OKCs. Also, the radicular cyst shows dense fibrosis in comparison with other cysts, as it is inflammatory in origin. This is because of the release of cytokines and growth factors which promote fibroblastic proliferation and possibly fibers as well.[23]

The impact of inflammation on the packing of collagen fibers in the wall of the connective tissue of OKCs was investigated by Hirshberg *et al*. in 2007. It was observed that in the presence of dense inflammation, the percentage of thick fibers with green birefringence decreases with an increase in thick fibers with red birefringence which appeared more packed.[24] Different patterns of radicular cysts suggest different biological behavior and a positive role of inflammation on polarization color of collagen fibers.[25]

CONCLUSION

The picrosirius red polarization method, therefore, is potentially useful for revealing the biology and pathology of collagen fibers in the connective tissue capsule of cysts and odontogenic tumors. It also emphasizes the importance of mesenchymal capsule in its neoplastic behavior. The nature and biologic behavior of these lesions, especially the aggressive ones can be assessed better by studying a larger group and with a more precise clinicopathologic correlation. Further comprehending the epithelial mesenchymal interactions at a molecular level using various epithelial and mesenchymal markers will give us a better insight into the exact nature and pathogenesis of these lesions.

REFERENCES

1. Jones AV, Craig GT, Franklin CD. Range and demographics of odontogenic cysts diagnosed in a UK population over a 30 year period. J Oral Pathol Med 2006;35:500-7.
2. Stoelinga PJ, Bronkhorst FB. The incidence, multiple presentation and recurrence of aggressive cysts of the jaws. J Craniomaxillofac Surg 1988;16:184-95.
3. Pindborg JJ, Hansen J. Studies on odontogenic cyst epithelium. Clinical and roentgenologic aspects of odontogenic keratocysts. Acta Pathol Microbiol Scand 1963;58:283-94.
4. Scharfetter K, Balz-Hermann C. Proliferation kinetics-study of the growth of keratocysts. J Craniomaxillofac Surg 1989;17:226-33.
5. Sweat F, Puchtler H. Sirius red F3BA as a stain for connective tissue. Arch Pathol 1964;78:69-72.
6. Constantine VS, Mowry RW. Selective staining of human dermal collagen II. The use of picrosirius red F3BA with polarization microscopy. J Invest Dermatol 1968;50:419-23.
7. Junqueira LC, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. Histochem J 1979;11:447-55.
8. Hirshberg A, Sherman S, Buchner A, Dayan D. Collagen fibres in

the wall of odontogenic keratocysts-a study with picrosirius red and polarizing microscopy. J Oral Pathol Med 1999;28:410-2.
9. Murata M, Cheng J. Enamel proteins and extracellular matrix molecules are colocalized in the pseudocystic stromal space of AOT. J Oral Pathol Med 2000;29:483-90.
10. Nyska A, Dayan D. Ameloblastic fibroma in a young cat. J Oral Pathol Med 1995;24:233-6.
11. Trau H, Dayan D, Hirschberg A, Hiss Y, Bubis JJ, Wolman M. Connective tissue nevi collagens. Study with picrosirius red and polarizing microscopy. Am J Dermatopathol 1991;13:374-7.
12. Dayan D, Waner T, Tal H, Nyska A. Polarization microscopy of picrosirius red stained collagen from oxodipine induced hyperplastic gingival of beagle dogs. Int J Exp Pathol 1993;74:225-8.
13. Hirshberg A, Buchner A, Dayan D. The central odontogenic fibroma and hyperplastic dental follicle. Study with picrosirius red and polarizing microscopy. J Oral Pathol Med 1996;25:125-7.
14. Main DM. The enlargement of epithelial jaw cysts. Odontol Revy 1970;21:29-49.
15. Toller PA. Autoradiography of explants from odontogenic cysts. Br Dent J 1971;131:57-61.
16. Li T, Browne RM, Mathews JB. Quantification of PCNA+cells within odontogenic jaw cyst epithelium. J Oral Pathol Med 1994;23:184-9.
17. Vedtofte P, Holmstrup P, Dabelsteen E. Human odontogenic transplants in nude mice. Scand J Dent Res 1982;90:306-14.
18. Harris M, Goldhaber P. The production of a bone resorbing factor by dental cysts *in vitro*. Br J Oral Surg 1973;10:334-8.
19. Meghji S, Harvey W, Harris M. Interleukin1 like activity in cystic lesions of the jaw. Br J Oral Maxillofac Surg 1989;27:1-11.
20. Magnusson BC. Odontogenic keratocysts: A clinical and histological study with special reference to enzyme histochemistry. J Oral Pathol 1978;7:8-18.
21. Donoff RB, Harper E, Guralnick WC. Collagenolytic activity in keratocysts. J Oral Surg 1972;30:879-84.
22. Sorsa T, Ylipaavalniemi P, Suomalainen K, Vauhkonen M, Lindy S. Type specific degradation of interstitial collagens by human keartocyst capsule collagenase. Med Sci Res 1988;16:1189-90.
23. Kaplan I, Hirshberg A. The correlation between epithelial cell proliferation and inflammation in odontogenic keratocyst. Oral Oncol 2004;40:985-91.
24. Hirshberg A, Lib M, Kozlovsky A, Kaplan I. The influence of inflammation on the polarization colors of collagen fibers in the wall of odontogenic keratocyst. Oral Oncol 2007;43:278-82.
25. Aggarwal P, Saxena S. Stromal differences in odontogenic cysts of a common histopathogenesis but with different biological behavior: A study with picrosirius red and polarizing microscopy. Indian J Cancer 2011;48:211-5.

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