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Original Article

Dentistry Section

Evaluation of the Nature of Collagen Fibers in KCOT, Dentigerous Cyst and Ameloblastoma using Picrosirius Red Stain – A Comparative Study

YUKTI RAJ[1], MANNE SRINIVAS MUNI SEKHAR[2], SANJEEVAREDDYGARI SHYLAJA[3], SANGALA NAGENDRA BHAVANI[4], ORUGANTI VENKATA RAMANAND[5], SPANDANA PATHA[6], SHARATH KUMAR REDDY[7], AKULA SANDHYA RANI[8]

ABSTRACT

Background: Reciprocal interaction between dental epithelium and mesenchyme is thought to be crucial for normal odontogenesis. Thus, the mesenchymal influence of the fibrous capsules may play an important role in the maintenance of epithelial expression. Collagen is the major component of the extracellular matrix and possibly there is an alteration in the nature and structure of collagen in various pathological conditions. Studies by polarizing microscopy have also shown that there is a difference in collagen and probably these differences may play a role in their biologic behaviour.

Aim: The purpose of this study was to evaluate the nature of collagen fibers in keratocystic odontogenic tumour (KCOT), dentigerous cyst (DC), unicystic ameloblastoma (UA) and solid/multicystic ameloblastoma (SMA) and correlating this with their biological behaviour.

Materials and Methods: Five diagnosed cases each of UA, SMA, KCOT and DC were taken and stained using Picrosirius red stain kit and evaluated using a polarizing microscope.

Statistical Analysis: Chi-square test was used to analyse the results.

Results and Conclusion: Collagen fibers in dentigerous cysts showed predominant yellowish-red birefringence and fibers in KCOT and ameloblastomas showed a predominantly greenish-yellow birefringence. Hence, our study suggests that the nature and character of collagen fibers may influence the clinical behaviour of the lesion. Since ours is a pilot study, to corroborate our view, studies with larger sample size are required to substantiate the results.

Keywords: Collagen fibers, Odontogenic cysts and tumours, Picrosirius red, Polarizing microscopy

INTRODUCTION

The epithelial-mesenchymal interactions which take place during tooth development can also lead to various pathologies like odontogenic cysts and tumours [1]. Epithelium is chiefly held responsible for all these pathologies; however, connective tissue may also have a functional role to play in their development. The reciprocity between the epithelium and connective tissue can be assumed to play a significant role in the pathogenesis of odontogenic cysts and tumours [2].

Collagen encompasses one-third of the total protein content in the body, and also accounts for more than half of the dry weight of skin, making it the most predominant protein of extracellular matrix (ECM) [3]. Collagen fibers play an essential role in maintaining the structural integrity of the tissue and also determine its function. Despite the fact that the new molecular techniques like immunohistochemistry have a wide application in various diagnostic purposes, they have a limited role in demonstrating pathological collagen fibers. Collagen fibers are best demonstrated by conventional histopathology with the use of histochemical stains [4].

Various connective tissue stains such as van Gieson and Masson's trichrome have been used to detect collagen fibers in tissue sections. However, these stains may not be ideal for the detection of the nature of collagen, as they fail to reveal very thin, fine fibers; thus not giving the actual status of the collagen in the lesion [5]. Putchler and colleagues observed that thin, delicate collagen fibers stained constantly with Sirius red F3 BA in picric acid when viewed under polarized light microscopy. Moreover, the stain persisted with time [6]. Thus, picrosirius red stain in conjunction with polarized light microscope can serve as a valuable procedure to differentiate procollagens, intermediate and pathological collagen from normal collagen fibers [7].

Although some studies have shown the role of extracellular matrix in the pathogenesis of odontogenic cysts and tumours [4,5,7-14], there is still a lack of precise understanding of the role of connective tissue in the clinical behaviour of such lesions. The aim of this study was to analyse the nature of collagen fibers in Keratocystic odontogenic tumour (KCOT), Dentigerous cyst (DC), Unicystic Ameloblastoma (UA), and Solid/Multicystic Ameloblastoma (SMA) and to correlate the results with their known biological behaviour.

MATERIALS AND METHODS

The study material included a total of 20 histopathologically diagnosed cases of DC, KCOT, UA and SMA. Five formalin-fixed, paraffin-embedded tissue blocks of each of DC, KCOT, UA and SMA were retrieved from the archives of Department of Oral and Maxillofacial Pathology, SVS Institute of Dental Sciences, Mahabubnagar, Telangana.

Sections of 4-µm thickness were taken and floated on to the slides treated with Mayer's egg albumen (slide adhesive) and incubated at 47°C on a slide warmer for ensuring adhesion of the sections to the slides. Following deparaffinization, and later hydration in distilled water; the sections were stained with Harris's haematoxylin, differentiated in 1% HCl. The sections were then subjected to alkalization with tap water, followed by incubation in 0.1% (w/v) Sirius red F3B (Sigma-Aldrich) in saturated Picric acid solution for 1 hour at room temperature. The sections were rinsed in acidified water {5 ml acetic acid (glacial) to 1 liter of water (tap or distilled)}, followed by dehydration and then mounted with D.P.X. The sections were then examined under bright field and polarizing microscope. On examination under polarizing microscope, the collagen fibers exhibited birefringence. We stained another section with routine haematoxylin and eosin, to facilitate the comparision of similar sections with those stained with picrosirius red stain.

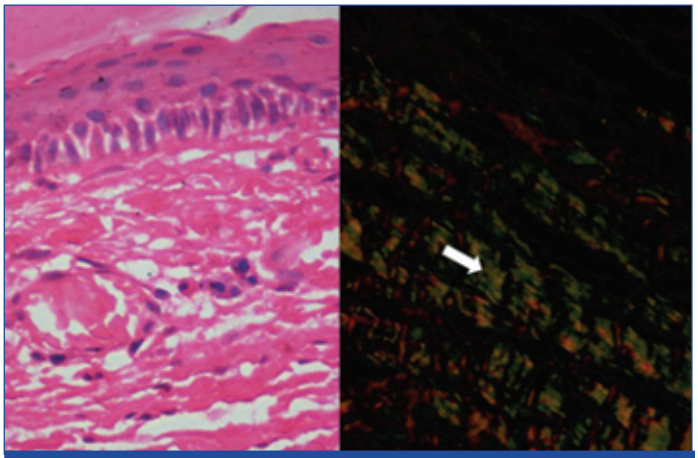
The analysis was performed under 10X magnification independently by two observers and the average value of their observations was taken. Areas of polarization colours were observed in five fields and

predominant colour was noted for each case. The findings of the study were subjected to chi-square test for statistical analysis.

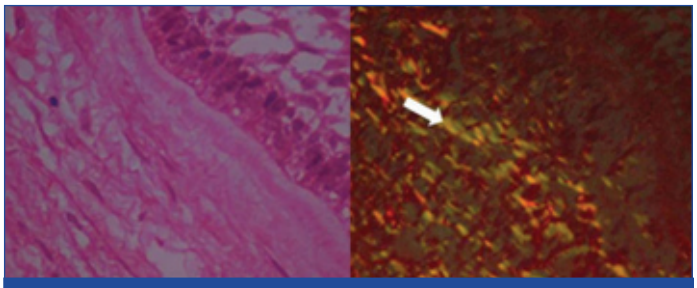
RESULTS

It was observed that 80% of KCOT, 80% of UA and 60% of SMA cases showed a predominant greenish-yellow birefringence whereas a yellow-red birefringence was observed in 80% of dentigerous cyst cases [Table/Fig-1-5]. [Table/Fig-6] shows staining *lesion cross-tabulation which depicts the percent of staining within the lesion and also overall staining. Of 20 cases studied, it was seen that most of the cases (60%; 12/20) showed a greenish-yellow birefringence (positive staining) and remaining 40% (8/20) of the cases showed a yellowish-red birefringence (negative staining) [Table/Fig-6].

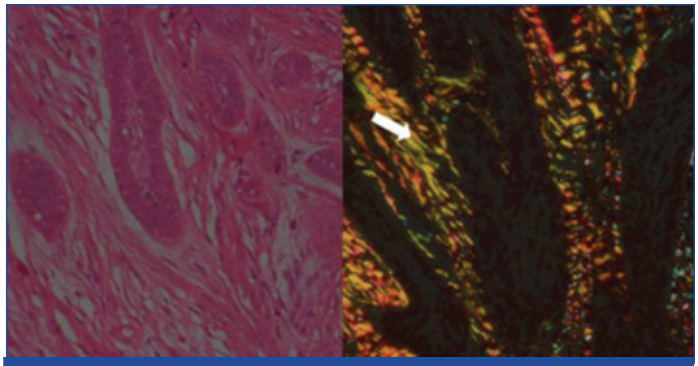
The greenish yellow polarization colour which denotes young and immature collagen increased with increasing aggressive nature of the lesions (KCOT, UA and SMA). However, the results were not statistically significant (p-value obtained = 0.172; p-value < 0.05 was considered significant) [Table/Fig-7].



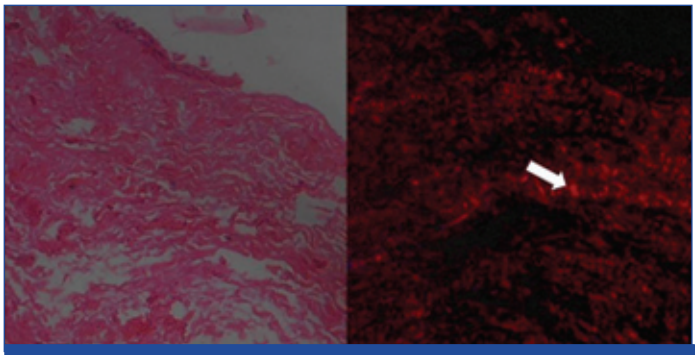
[Table/Fig-1]: KCOT (H&E) (40X); KCOT showing predominant greenish-yellow collagen fibers (arrow) (40X)



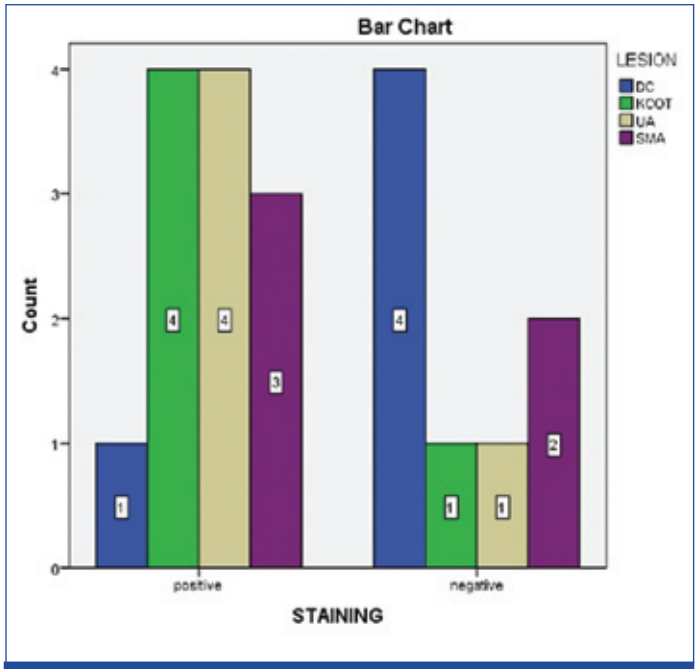
[Table/Fig-2]: Unicystic ameloblastoma (H&E) (40X); UA showing predominant greenish-yellow collagen fibers (arrow) (40X)



[Table/Fig-3]: SMA (H&E) (40X); SMA showing predominant greenish-yellow collagen fibers (arrow) (40X)



[Table/Fig-4]: Dentigerous cyst (H&E) (40X); DC showing predominant yellowish-red collagen fibers (arrow) (40X)



[Table/Fig-5]: Positive represents greenish-yellow birefringence and negative represents predominant yellowish-red birefringence

			LESION				Total
			DC	KCOT	UA	SMA	
STAINING	positive	Count	1	4	4	3	12
		% within STAINING	8.3%	33.3%	33.3%	25.0%	100.0%
		% within LESION	20.0%	80.0%	80.0%	60.0%	60.0%
		% of Total	5.0%	20.0%	20.0%	15.0%	60.0%
	negative	Count	4	1	1	2	8
		% within STAINING	50.0%	12.5%	12.5%	25.0%	100.0%
		% within LESION	80.0%	20.0%	20.0%	40.0%	40.0%
		% of Total	20.0%	5.0%	5.0%	10.0%	40.0%
Total		Count	5	5	5	5	20
		% within STAINING	25.0%	25.0%	25.0%	25.0%	100.0%
		% within LESION	100.0%	100.0%	100.0%	100.0%	100.0%
		% of Total	25.0%	25.0%	25.0%	25.0%	100.0%

[Table/Fig-6]: Table depicting Staining*Lesion cross-tabulation. This table shows that of twenty cases, 12 (60%) cases (inclusive of all the lesions) shows a positive staining (greenish-yellow birefringence) and remaining 8 (40%) showed negative staining (yellowish-red birefringence). This table also illustrates the % of staining within the lesion i.e. 1 of 5 cases of DC showed positive staining (20%)

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.000a	3	.172
Likelihood Ratio	5.178	3	.159
Linear-by-Linear Association	1.425	1	.233
N of Valid Cases	20		

[Table/Fig-7]: Chi-square test. the Pearson Chi-Square value as 0.172, which is not significant (p-value <0.05)
a. 8 cells (100.0%) have expected count less than 5. The minimum expected count is 2.00

DISCUSSION

During odontogenesis, morphogenesis and cell differentiation are controlled by epithelial–mesenchymal interactions [15,16]. Similarly, development of odontogenic cysts and tumours is also dependent on such interactions [17,18]. Majority of the studies have focused on the evaluation of the proliferative activity in the epithelium of the odontogenic lesions [19-21], with fewer studies focusing on the role of epithelial-mesenchymal interactions in the progression of these odontogenic entities [4,5,7-14].

Tumour stroma has a very important role to play in the progression of a neoplasm. The tumour stroma acts as a physical barrier to ward off the host immunological reactions. It also plays a vital role in supplying the essential nutrients to the tumour via blood and also serves to remove waste products [22]. Nevertheless, the amount of stroma differs from one tumour to another; some tumours have abundant stroma while others have minimal stroma.

Collagen is the major constituent of the extracellular matrix and there is an alteration in the nature and structure of collagen in various pathological conditions. Various studies done using polarizing microscopy confirm the above statement and suggest that these differences may influence their biologic behaviour. These studies observed that young and immature fibers imparted greenish-yellow birefringence and collagen fibers which were mature and densely packed showed a yellowish-red birefringence [4,7,9-13].

In the present study, collagen which forms the chief constituent of the connective tissue was evaluated, to note if there was any difference in the nature and character of collagen fibers in DC, KCOT, and SMA and to know if there was any correlation between the difference in the nature of collagen and the clinical behaviour of these lesions. It was found that the collagen in KCOT, UA, and SMA imparted a predominant greenish-yellow birefringence and collagen present in the stroma of DC imparted a predominant yellowish-red birefringence [Table/Fig-1-4].

In our study, 4 out 5 cases (80%) of DC showed a predominant yellowish-red birefringence which was in accordance with HP Singh et al., [4]. This yellowish-red birefringence of collagen fibers seen in DC could possibly attribute to the mature, densely packed collagen. As the collagen fibers mature, there is change in proteoglycan content of fibers causing dehydration of fibers resulting in increase in diameter of collagen fibers and intensity of birefringence. Hence, the change in polarizing colour of the fibers from greenish-yellow to yellowish-red is seen [8].

Further, a predominant greenish-yellow birefringence was observed in most of the cases of KCOT (80%), UA (80%) which was in agreement with HP Singh et al., VN Nayak et al., and JY Zhang et al., [4,9,10]. The greenish-yellow birefringence seen in KCOT, UA and SMA can be attributed to the young and immature collagen fibers. HP Singh et al., evaluated different types of collagen fibers in KCOT using picrosirius red stain under polarizing microscopy and correlated the results with different radiographic patterns of KCOT to elucidate its biological behaviour [11]. They found significantly more greenish yellow collagen fibers in multilocular KCOT as compared to its unilocular variant, indicating a more aggressive behaviour of the former lesion.

Kajikar MS et al., evaluated the role of inflammation in the connective tissue wall of infected and non-infected odontogenic keratocyst [12]. They observed that cases of OKC with no or mild inflammation had majority of poorly packed fibers with green and yellow birefringence and moderate to severely inflamed cases had well-packed and thick fibers showing orange to red birefringence. Thus, indicating that inflammation influences polarization colour and packing of collagen fibers in the connective tissue wall of infected and non-infected OKC. However, in our study, we did not take into account the role of inflammation in cysts and tumours. Hirshberg suggested that the greenish-yellow collagen fibers seen in these lesions represent procollagen, intermediate or pathological collagen [13]. This assumption can also justify the aggressive behaviour of these lesions.

Not many studies have evaluated the nature of collagen fibers in SMA. It was found in our study that 60% of the cases of SMA showed greenish-yellow birefringence whereas 40% showed a predominant yellowish-red birefringence, which were in contrast to study done by P Aggarwal et al., where a predominant orangish-red birefringence of the collagen fibers around the tumour islands was observed. It was suggested by them that due to the long-standing nature of the lesion more matured and densely packed collagen fibers were seen in SMA [14].

Eighty percent cases of KCOT showed predominant greenish-yellow birefringence as compared to 60% cases of SMA. This was not in correlation with the biological behaviour of these lesions as SMA is found to be more aggressive than KCOT [23]. However, this disparity could be due to the small sample size of our pilot study.

CONCLUSION

In the present study a predominant yellowish-red birefringence was observed in majority of cases of DC whereas a primary greenish-yellow birefringence was observed in most of the cases of KCOT, UA and SMA. However, the results were not statistically significant. Therefore, we opine that the quality of collagen fibers can have an impact on the biological behaviour of the lesion; thus, substantiating that connective tissue plays a vital role in the progression of odontogenic cysts and tumours. Owing to the small sample size, the results were not statistically significant. Hence, a study with a larger sample size will be favorable to corroborate our view.

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REFERENCES

[1] Prabhu SR, Wilson DF, Daftary DK, Johnson NW, editors. Oral Diseases in the Tropics. Oxford: Oxford University Press; 1993. pp. 367.
[2] Pindborg JJ, Hansen J. Studies on odontogenic cyst epithelium. Clinical and roentgenologic aspects of odontogenickeratocysts. *Acta Pathol Microbiol Scand.* 1963;58:283–94.
[3] Shoulders MD, Raines RT. Collagen structure and stability. *Annu Rev Biochem*. 2009;78:929–58.
[4] Singh HP, et al. A quantitative and qualitative comparative analysis of collagen fibers to determine the role of connective tissue stroma on biological behaviour of odontogenic cysts: A histochemical study. *Natl J Maxillofac Surg*. 2012;3(1):15-20.
[5] Rich L, Whittaker P. Collagen and picrosirius red staining: a polarized light assessment of fibrillar hue and spatial distribution. *Braz J Morphol Sci.* 2005;22:97–104.
[6] Sweat F, Puchtler H, Rosenthal SI. Sirius red F3BA as a stain for connective tissue. *Arch Pathol*. 1964;78:69–72.
[7] Vij R, Vij H, Rao NN. Evaluation of collagen in connective tissue walls of odontogenic cysts–A histochemical study. *J Oral Pathol Med*. 2011;40:257–62.
[8] Szendröi M, Vajta G, Kovács L, Schaff Z, Lapis K. Polarization colours of collagen fibres: A sign of collagen production activity in fibrotic processes. *Acta Morphol Hung*.1984;32:47–55.
[9] Nayak VN, et al. Comparative evaluation of collagen fibers in Odontogenic Keratocyst and Unicystic Ameloblastoma. *World Journal of Dentistry.* 2011;2(2):125-27.
[10] Zhang, et al. Differences in collagen fibres in the capsule walls of parakeratinized and orthokeratinized odontogenic cysts. *Int J Oral Maxillofac Surg*. 2011;40:1296–300.
[11] Singh HP, Shetty S, Patil P, Sethi N, Singh A, Raghunandan B. Correlation Between Collagen Fibers and Radiographic Patterns of Keratocystic Odontogenic Tumour. *J Clin Diagn Res*. 2014;8(8):ZC16-18.
[12] Kajikar MS, Joshi PS, Chougule M. A comparative study of influence of inflammation and collagen deposition in the cystic wall of infected and non-infected odontogenickeratocyst using picrosirius red and polarizing microscopy. *Indian J Oral Sci.* 2014;5:83-87.
[13] Hirshberg A, Sherman S, Buchner A, Dayan D. Collagen fibres in the wall of odontogenickeratocysts: A study with picrosirius red and polarizing microscopy. *J Oral Pathol Med*. 1999;28:410–12.
[14] Aggarwal P, Saxena S. Stromal differences in odontogenic cysts of a common histopathogenesis but with different biological behaviour: A study with picrosirius red and polarizing microscopy. *Indian Journal of Cancer*. 2011;48:211-15.
[15] Pispa J, Thesleff I. Mechanisms of ectodermal organogenesis. *Dev Biol*. 2003;262:195–205.
[16] Zhang YD, Chen Z, Song YQ, Liu C, Chen YP. Making a tooth growth factors, transcription factors, and stem cells. *Cell Res.* 2005;15:301–16.
[17] Carnelio S, Vij H. Expression of tenascin and nucleolar organizer region in ameloblastoma and ameloblastic fibroma. *J Oral Pathol Med*. 2010;39:223–29.

[18] Vedtofte P, Holmstrup P, Dabelsteen E. Human odontogenickeratocyst transplants in nude mice. *Scand J Dent Res.* 1982;90:306–14.
[19] Sudiono J, Zain RB. PCNA expression in epithelial linings of odontogenic cysts. *Annal Dent Univ Malaya*. 2003;10:1–5.
[20] de Oliveira MG, Lauxen Ida S, Chaves AC, Rados PV, Sant'AnaFilho M. Immunohistochemical analysis of patterns of p53 and PCNA expression in odontogenic cystic lesions. *Med Oral Pathol Oral Cir Buccal.* 2008;13:E275–80.
[21] Piattelli A, Fioroni M, Santinelli A, Rubini C. Expression of proliferating cell nuclear antigen in ameloblastomas and odontogenic cysts. *Oral Oncol.* 1998;34:408-12.
[22] Dvorak HF. Tumours: wounds that do not heal: Similarities between tumour stroma generation and wound healing. *The New England J Med*. 1986;315:1650-56.
[23] Gadbail AR, Patil R, Chaudhary M. Co-expression of Ki-67 and p53 protein in ameloblastoma and keratocysticodontogenic tumour. *Acta Odontol Scand.* 2012;70(6):529-35.

PARTICULARS OF CONTRIBUTORS:
1. Post Graduate Student, Department of Oral and Maxillofacial Pathology, SVS Institute of Dental Sciences, Mahabubnagar, Telangana, India.
2. Professor and Head, Department of Oral and Maxillofacial Pathology, SVS Institute of Dental Sciences, Mahabubnagar, Telangana, India.
3. Professor, Department of Oral and Maxillofacial Pathology, SVS Institute of Dental Sciences, Mahabubnagar, Telangana, India.
4. Reader, Department of Oral and Maxillofacial Pathology, SVS Institute of Dental Sciences, Mahabubnagar, Telangana, India.
5. Senior Lecturer, Department of Oral and Maxillofacial Pathology, SVS Institute Of Dental Sciences, Mahabubnagar, Telangana, India.
6. Post Graduate Student, Department of Oral and Maxillofacial Pathology, SVS Institute of Dental Sciences, Mahabubnagar, Telangana, India.
7. Post Graduate Student, Department of Oral and Maxillofacial Pathology, SVS Institute of Dental Sciences, Mahabubnagar, Telangana, India.
8. Post Graduate Student, Department of Oral and Maxillofacial Pathology, SVS Institute of Dental Sciences, Mahabubnagar, Telangana, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Yukti Raj,
Department of Oral and Maxillofacial Pathology, SVS Institute of Dental Sciences, Mahabubnagar, Telangana-509002, India.
E-mail: yukti.sahay90@gmail.com

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