Correlation Between Collagen Fibers and Radiographic Patterns of Keratocystic Odontogenic Tumour

Dentistry Section

HARKANWAL PREET SINGH¹, SUJAN <u>SHETTY², PRASHANT PATIL³,NEERJA SETHI⁴, ABHAYJEET SINGH⁵, BN RAGHUNANDAN§</u>

ABSTRACT

Background: WHO has recently renamed odontogenic keratocyst as keratocystic odontogenic tumour (KCOT) depending on its tumour like behaviour.

Aim: To quantitate and qualitate different types of collagen fibers in KCOT using picrosirius red stain under polarising microscopy and to correlate with different radiographic patterns of KCOT to elucidate its biological behaviour in order to determine whether all KCOTs behave like a tumour.

Materials and Methods: Sixty histopathologically confirmed cases of KCOT were selected and stained histochemically using picrosirius red and examined under polarising microscope to evaluate colour of collagen fibers in the wall. Radiographic analysis of all the cases were also carried out and correlated with type of collagen of fibers.

Results: Greenish yellow collagen fibers were present statistically significantly more in multilocular KCOT and KCOT with multiple radiolucencies (both syndromic and non-syndromic) as compare to unilocular whereas orange red were significantly more in unilocular variety. Syndromic variety showed significantly higher number of greenish yellow collagen fibers than non-syndromic variety.

Conclusion: Quality, organization and packing of collagen fibers of unilocular type is different than other radiographic patterns which accounts for difference in biological behaviour of these lesion, so we conclude that aggressive treatment should be reserved for selected cases.

Keywords: Cyst, Neoplasm, Odontogenic, Picrosirus red, Radiograph

INTRODUCTION

Odontogenic keratocyst (OKC) is a clinicopathologically distinct form of odontogenic cyst known for its pathognomonic features i.e. aggressiveness and high rates of recurrence. Recently the WHO working group on odontogenic tumours recommended the term Keratocystic Odontogenic Tumours (KCOT) for these lesions to address their neoplastic nature [1,2] but reclassification has not yet been universally accepted and there is no worldwide agreement regarding treatment of this controversial entity. There is intricate relationship between epithelium and connective tissue of odontogenic cysts. Studies have shown that connective tissue play a significant role in the pathogenesis and expansion of odontogenic cysts [3]. Collagen forms the integral part of connective tissue stroma and plays a vital role in maintaining structural integrity and in determining tissue function [4,5]. Collagen has natural birefringence which is attributed to the arrangement of its fibers which is enhanced by special stains like Picrosirius red which serve as a procedure to differentiate procollagens, intermediate and pathological collagen fiber [5,6]. The typical radiographic features of KCOT are unilocular, multilocular or multiple well-circumscribed radiolucent lesions surrounded by a thin radiopague border with a smooth or loculated periphery [7]. Nonetheless, whether the connective tissue stroma and radiographic appearances of KCOT have any correlation is not been investigated in any previous study. So, we conducted a study to correlate collagen fibers of connective tissue stroma and radiographic patterns of KCOT in order to determine the behaviour of this controversial entity and define the line of treatment.

MATERIALS AND METHODS

Ethical permission from institution was taken before the commencement of study. Sixty formalin-fixed paraffin-embedded tissue blocks of histopathologically confirmed cases of KCOT, and five tissues of progressive stages of odontogenesis as control were taken from the Department of Oral Pathology and Microbiology, Dasmesh Institute of Research and Dental Sciences, Faridkot, Punjab, India. Sectioned of 4-µm thickness were the sections and after deparaffinization sections were incubated using 0.1% (w/v) Sirius red F3B in saturated Picric acid solution for 1h at room temperature. This was followed by rinsing with distilled water, staining with Mayer's haematoxylin, differentiation in 1% HCl, alkalinization with tap water, dehydration and mounting. The sections were then examined under polarising microscope birefringence colour of thin and thick collagen fibers were recorded under polarised light microscopy. With the application of image analyser software (Olympus) fibre thickness measuring 0.8µm or less was grouped under thin fibers and thickness between 1.4µm to 2.4µm were grouped under thick fibers. In each section, three separate high power fields with at least 50 fibers of each size were examined. The connective tissue in these slides showed polarization colours varying from greenish-yellow to yellow-orange to orange red. The colour of the collagen fibers was noted by two independent observers to eliminate the subjective bias. Radiographs of all the 60 cases were also taken and categorised into- Unilocular, multilocular, multiple radiolucency including Non syndromic and Nevoid Basal Cell Carcinoma Syndrome. The resultant data were analysed using Statistical Package for Social Sciences (SPSS, Chicago, IL, USA) and Epi-Info 6.04d software. Difference between the mean of two independent groups was observed by the t-test if data was normally (evenly) distributed. Differences between the different variables were analysed using the Tukey test. A p-value < 0.05 was considered significant.

RESULTS

In present study sample consist of 60 KCOT, which are radiographically categorised into Unilocular, multilocular, multiple radiolucency including Non-syndromic and Nevoid Basal Cell Carcinoma Syndrome [Table/Fig-1].

Radiographic Type	hic Type Number of Cases (n=60)			
Unilocular	28 (46.67%)			
Multilocular	18 (30%)			
Multiple (Non syndromic)	10 (16.67%)			
Multiple (NBCCS)	4 (6.66%%)			
[Table/Fig-1]: Distribution of cases of KCOT depending upon radiographic pattern				

Picrosirus red stained tissues under polarising microscopy show different birefringence were recorded. The progressive stages of embryonic odontogenic tissue were taken as control which showed prodominantly greenish-yellow birefringence interspersed with some amount of yellowish-orange birefringence [Table/Fig-2].



[Table/Fig-2]: A) Embryonic odontogenic tissue showed predominantly greenish yellow colored fibers. (PSR; X4); B) Unilocular KCOT showing predominantly orange red collagen fibers. (PSR; X10); C) Multilocular KCOT showing yellowish orange and greenish yellow colored fibers collagen fibers. (PSR; X40); D) Multiple (Non syndromic) KCOT showing predominantly yellow orange colored collagen fibers. (PSR; X10) and inset showing Multiple (Syndromic) KCOT with predominantly greenish yellow colored fibers collagen fibers. (PSR; X40); D) and inset showing Multiple (Syndromic) KCOT with predominantly greenish yellow colored fibers collagen fibers. (PSR; X40)

Color of Collagen Fibers	Unilocular	Multilocular	Multiple (Non- Syndromic)	Multiple (Syndromic)	f-value		
Greenish Yellow	12.06±1.65ª	22.40±1.50 ^b	22.86±1.07 ^b	28.08±2.65°	18.68		
Yellowish Orange	20.25±2.34ª	18.26±1.88ª	20.25±2.05ª	16.65±1.96ª	14.25		
Orange Red	28.84±2.24ª	14.26±1.48 ^b	16.84±1.81 ^b	10.25±0.45°	8.65		

[Table/Fig-3]: Distribution of polarization colors of thick collagen fiber in different radiographic patterns of KCOT. *Significant at 1%, Means followed by different superscript letters (a, b, c) within

a column are significantly different; Tukey's test: 0.05; KCOT: Keratocystic

odontogenic tumours

Unilocular variety showed significantly higher number of orangered birefringence of thick collagen fibers i.e. 28.84 ± 2.24 in KCOT than 14.26 ± 1.48 of multilocular, 16.84 ± 1.81 of multiple non-syndromic and 10.25 ± 0.45 of multiple syndromic varieties. Syndromic variety showed significantly higher number of greenishyellow fibers 28.08 ± 2.65 than 12.06 ± 1.65 of unilocular, 22.40 ± 1.50 of multilocular and 22.86 ± 1.07 of multiple non-syndromic and 10.25 ± 0.45 of multiple syndromic varieties. Multilocular and Multiple (non syndromic) varieties showed non-significant difference between greenish yellow and orange red birefringence of thick collagen fibers [Table/Fig-2,3].

Greenish yellow and yellow orange birefringence of thin collagen fibers in KCOT showed non-significant difference among all the radiographic patterns whereas orange red birefringence of

Color of Collagen Fibers	Unilocular	Multilocular	Multiple (Non- Syndromic)	Multiple (Syndromic)	f-value		
Greenish Yellow	22.66±2.40ª	26.25±2.45ª	24.65±1.86ª	26.28±1.40ª	22.25		
Yellowish Orange	24.8±2.32ª	26.65±2.32ª	28.32±2.6ª	24.32±2.40ª	24.32		
Orange Red	20.86±1.86ª	8.20±1.40 ^b	10.20±0.47 ^b	6.40±0.85°	6.40		
[Table/Fig-4]: Distribution of polarisation colors of thin collagen fiber in different radiographic patterns of KCOT.							

a column are significantly different; Tukey's test: 0.05; KCOT: Keratocystic odontogenic tumours.

 20.86 ± 1.86 thin fibers of unilocular variety was significantly higher than 8.20 ± 1.40 of multilocular, 10.20 ± 0.47 of multiple non-syndromic and 6.40 ± 0.85 of multiple syndromic varieties. Multilocular and Multiple (non syndromic) varieties showed non-significant difference between orange red birefringence of thin collagen fibers [Table/Fig-2,4].

DISCUSSION

When a thing ceases to be a subject of controversy it ceases to be subject of interest. Studies have been conducted to resolve such controversies. KCOT is always a subject of controversy since its first description Philipsen in 1956. Although, WHO has suggested term KCOT for OKT, there is no universal agreement regarding it. Present study also focussed on this controversy and analysed connective tissue and correlated with radiographic type to elucidate biological behaviour of each type and give opinion that whether treatment protocol for all KCOT should be same or aggressive treatment should be reserved for selected cases. The present study evaluated the clinicodemographic characteristics of 60 patients with KCOT, and has found 46.67% unilocular, 30.00% multilocular, 16.67% multiple (non-syndromic) and 6.6% syndromic KCOTs [Table/Fig-1]. Results revealed that unilocular radiographic type is most common type of KCOT.

Only few studies have focused on the importance of epithelialmesenchymal interactions for the expansion of odontogenic cysts. Stroma is essential for maintaining the epithelial tissues and both these make up an ecosystem with continuous molecular interactions. Stromal changes in cystic lesions can be depicted by picrosirus red stain and this stain imparts birefringence to collagen fibers only [8]. PSR impart different colours in shades of green-yellow to orange-red to collagen fibers in various cystic lesions. In our another published study we concluded that quality and organization of collagen fibers is different KCOT, Dentigerous cyst and Radicular Cyst which accounts for difference in biological behaviour of these lesions and it justify that neoplastic growth or expansion of cyst requires a functional stroma [9].

So, in order to elucidate the biological behaviour of different radiographic types of KCOT study was conducted to analyse the collagen fibers of connective tissue using picrosirus red under polarising microscope. Unilocular variety of KCOT showed significantly higher number of orange-red birefringence of thick collagen fibers than multilocular, multiple (non-syndromic) and multiple (syndromic) varieties. Syndromic variety showed significantly higher number of greenish-yellow fibers than other radiographic varieties [Table/Fig-3]. Hence, the greenish-yellow birefringence imparted in OKC can be attributed to the young and immature collagen fibers. According to Hirshberg [10] these greenish yellow collagen fibers in OKC probably represents procollagen, intermediate or pathological collagen. This could also probably partially validate the aggressive behaviour of OKC. High number of immature or pathological collagen in multilocular and multiple varieties than unilocular suggested that unilocular type is biologically less aggressive than other. Multilocular and Multiple (non-syndromic)

varieties showed non-significant difference between greenish yellow and orange red birefringence of thick collagen fibers. This suggests there is no considerable difference in biological behaviour of these lesions. Greenish yellow and yellow orange birefringence of thin collagen fibers in KCOT showed non-significant difference among all the radiographic patterns whereas orange red birefringence of thin fibers of unilocular variety was significantly higher than others [Table/Fig-4]. This suggests that mature fibers are seen significantly more in case of unilocular variety as compare to others. Hence, there is high propensity of cystic expansion in case of multilocular than unilocular. Thoma [11] advocated that multilocular cyst develops from an epithelial sprout that branches and forms, at each end of the branch, a small cyst. These become larger and, as they crowd together and fuse the intercystic tissue, are resorbed such that a single space results with a lobular outline and partial septa that appears to subdivide the cavity. Multilocular and Multiple (nonsyndromic) varieties showed non-significant difference between orange red birefringence of thin collagen fibers, so we suggest that there is no considerable difference in cystic expansion. High number of greenish yellow and less number of thick fibers in multilocular than unilocular type suggest that multilocular type have high aggressive potential. Shear also suggested that odontogenic keratocysts whose radiographic images showed a multilocular appearance had a higher recurrence rate than those with a unilocular appearance [12]. Recently Ba et al., [13] in his study concluded that solitary KCOT are more likely to be less biologically aggressive and should be classified as a cyst rather than a tumour. This means that more than half of KCOTs manifest themselves as ordinary cysts. We in our other published study have proved that majority of the unilocular cysts have a lesser proliferative potential than multilocular cysts and hence, are less biologically active and should not be treated as a tumour [14].

CONCLUSION

Quality and organization of collagen fibers of unilocular type is different than other radiographic patterns which accounts for difference in biological behaviour of these lesions. Majority of the unilocular cysts have a lesser aggressive potential than multilocular cysts and hence, are less biologically active and should not be treated as a tumour. We conclude that all odontogenic keratocysts do not behave like tumours and that aggressive treatment should be reserved for selective cases.

REFERENCES

- [1] Payne TF. An analysis of the clinical and histopathologic parameters of the odontogenic keratocyst. Oral Surg. 1972, 33:538-46.
- Philipsen HP. Keratocystic odontogenic tumour. In: Barnes L, Eveson JW, Reichart [2] P, Sidransky D (EDS). Head and neck tumours. Pathology and Genetics. WHO Classification of tumours. IARC Press, Lyon, 2005:306-07.
- [3] Vedtofte P, Holmstrup PD, Dabelsteen E. Human odontogenic keratocyst Transplant in nude mice. Scand J dent Res. 1982; 90: 306-14.
- [4] 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. [serial online] 2011 [cited 2011 Nov 3];48:211-15.
- [5] Whittaker P, Rich L. Collagen and picrosirius red staining: A polarized light assessment of fibrillar hue and spatial distribution. Braz J Morphol Sci. 2005:22:97-104.
- [6] Vij R, Vij H, Rao N. Evaluation of collagen in connective tissue walls of odontogenic cysts - A histochemical study. J Oral Pathol Med. 2011;40:257-62.
- [7] Blanchard SB. Odontogenic keratocysts: Review of the literature and report of a case. J Periodontol. 1997;68:306-11.
- [8] Constantine VS, Mowry RW. Selective staining of human dermal collagen. II The use of picrosirius red F3BA with polarized microscopy. The J of Invest Dermat. 1968;50;419-23.
- [9] Singh HP, Shetty DC, Wadhwan V, Aggarwal P. 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;3:15-20.
- [10] Hirshberg A, Sherman S, Buchner A, Dayan D. Collagen fibers in the wall of odontogenic keratocyst: a study with picrosirius red and polarization microscopy. J Oral Pathol Med. 1999; 28:410-12.
- [11] Thoma KH. Oral Surgery. Treatment of cysts of oral region. 5th ed. Saint Louis: The CV Mosby Company; 1969. 1002-15.
- [12] Shear M, Speight PM. Cysts of the oral and maxillofacial regions. 4th ed. Singapore: Blackwell Munksgaard; 2007. 14.
- [13] Ba K, Li X, Wang H, Liu Y, Zheng G, Yang Z, et al. Correlation between imaging features and epithelial cell proliferation in keratocystic odontogenic tumour. Dentomaxillofac Radiol. 2010;39:368-74.
- [14] Singh HP, Nayar A, Raj A, Kumar P. Are All Odontogenic Keratocysts Keratocystic Odontogenic Tumours? Correlation between Imaging Features and Epithelial Cell Proliferation. J Clin Imaging Sci. 2013;3:1-6.

PARTICULARS OF CONTRIBUTORS:

- Senior Lecturer, Department of Oral Pathology and Microbiology, Dasmesh Institute of Research and Dental Sciences, Faridkot, Punjab, India. Reader, Department of Periodontics, RKDF Dental College and Research Centre, Bhopal, India.
- 2.
- Reader, Department of Orthodontics, Malla Reddy Institute of Dental Sciences, Suraram Cross Road, Qutbullapur, Hyderabad, Andhra Pradesh, India. З.
- Senior Lecturer, Department of Oral Pathology, Desh Bhagat Dental College and Hospital, Sri Muktsar Sahib, Punjab, India. 4
- Senior Lecturer, Department of Oral Medicine and Radiology, Maharaja Ganga Singh, Dental College and Research Center Sriganganagar, Rajasthan, India. 5.
- Senior Lecturer, Department of Oral Pathology and Microbiology, Vydehi Institute of Dental Sciences and Research, Bangalore, India. 6.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Harkanwal Preet Singh,

Senior Lecturer, Department of Oral & Maxillofacial Pathology & Microbiology, Dasmesh Institute of Research and Dental Sciences, Faridkot, Punjab, India.

Phone: 09501544877, E-mail: hkps0320@gmail.com

FINANCIAL OB OTHER COMPETING INTERESTS: None

Date of Submission: Mar 26, 2014 Date of Peer Review: Jun 02, 2014 Date of Acceptance: Jun 11, 2014 Date of Publishing: Aug 20, 2014