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The Collagen Fibers Analysis of the Odontogenic Cysts: A Study with Picrosirius Red Staining Under Polarizing Microscopy

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Abstract

Objectives: This study aims to compare the polarization colors of the collagen fibers in the connective tissue wall (CNT) of radicular cyst (RC), dentigerous cyst (DC), odontogenic keratocyst (OKC), and calcifying odontogenic cyst (COC).

Methods: Collagen in the CNT of ten patients diagnosed with RC, DC, OKC, or COC was stained with picrosirius red staining and examined under a polarizing microscope. The birefringence of collagen fibers of odontogenic cysts (OCs) regarding the frequency and the labeling index (LI) scores based on the proportion of mature collagen (orange-red fibers) and immature collagen (greenish-yellow fibers) were compared.

Results: The orange-red polarization color was observed predominantly in the CNT of DCs (90.0%), RCs (70.0%), and OKCs (60.0%). Meanwhile, the greenish-yellow polarization color predominated in the CNT of COCs samples (50.0%). The mean LI values of RCs, DCs, OKCs, and COCs were 1.93±0.73, 1.90±0.44, 2.03±0.93, and 1.40±0.59, respectively. There is no statistically significant difference between the groups (p>0.05).

Conclusions: Although no statistically significant difference between OCs was observed, the collagen fibers of COCs were different from other OCs. The greenish-yellow polarization color predominantly observed in COCs suggested that the CNTs of some COCs might play a role in the cystic neoplasm behavior.

Keywords: collagen, odontogenic cyst, picrosirius red staining, polarizing microscopy

Introduction

Odontogenic cysts (OCs) are a heterogeneous group of diseases derived from the epithelial and remnant cells of tooth-forming apparatus.(1) They are classified into two groups including inflammatory cyst and developmental cyst.(1) OCs comprise three parts: lumen, epithelial-lined lumen, and surrounded fibrous connective tissue wall (CNT). About 7.0-12.0% of all biopsies from oral and maxillofacial region have been diagnosed with OCs including radicular cysts (RCs), dentigerous cysts (DCs), odontogenic keratocysts (OKCs), and calcifying odontogenic cysts (COCs).(1,2) RCs are inflammatory in origin and are associated with non-vital teeth. DCs are attached to cementoenamel junction of unerupted teeth.(2,3) OKCs are developmental cysts. They are characterized by locally aggressive behavior and tendency to recur in case of incomplete removal or in the presence of satellite cysts.(2,4) COCs show variable clinical behavior. Some of them show recurrence.(2,5) The most common cyst of the jaws is RCs (55.0%), followed by DCs (about 20.0%), OKCs (about 10.0%), and COCs (< 1.0%), respectively.(2,3)

The epithelial lining of such OCs has been exten-

sively investigated regarding their roles in cystic proliferation.[6] Although previous studies have reported various transcriptional and translational changes associated with lesion progression, the pathogenesis and biological behaviors of OCs remain relatively unknown.[2,6] For example, in 2005, World Health Organization (WHO) suggested that OKC and COC should be classified as cystic neoplasm (keratocystic odontogenic tumor and calcifying cystic odontogenic tumor, respectively).[2] However, in 2017, the WHO has reclassified the OKC and COC as a cystic lesion.[2,4,5] There is considerable debate regarding whether OKC and COC are a cystic neoplasm or a cystic lesion.[4,5] Previous studies have indicated that not only the epithelium but also the CNT of OCs is partly responsible for pathogenesis and biological behaviors of OCs.[7] The reciprocal interaction between epithelial and mesenchymal cells (epithelial-mesenchymal interaction) is considered to be important for normal odontogenesis and pathogenesis of OCs.[6,7] Thus, the mesenchymal influence of CNT may play a vital role in the growth and epithelial expression of OCs.[6,7]

Collagen is the major fibrous protein in CNT.[8] It provides structural support to the extracellular matrix of CNT and maintains integrity of cellular structure.[8] Collagen-related diseases usually arise from genetic mutation or nutritional deficiencies.[9] Collagen deposition and change in the collagen composition are the main pathological characteristic of some neoplasms including oral cancers.[8-10] Hence, the study of orientation and organization of collagen fibers may be useful in understanding the behaviors of OCs. Picrosirius red (PSR) is a strong and elongated anionic dye molecule that can react with cationic collagen fibers and enhance their birefringence effects under polarized light.[9,11] Combination of PSR staining and polarization microscopy has been used to study the fiber thickness and density of collagen packing.[9,12] This method serves as a tool for the structural analysis of collagen (procollagen, intermediate, and pathological collagen fibers).[9-12]

To the best of our knowledge, only a few studies have evaluated the nature of the collagen fibers in the CNT of OCs by determining polarized colors using PSR staining.[6] Moreover, the results still remain controversial.[6] There is no information about the polarization color pattern of the collagen fibers in the CNT of COC. In addition, precise knowledge of the roles of CNT in clinical behaviors of OCs still lacks. Thus, this research qualitatively and quantitatively investigates as well as compares the polarization colors of the collagen fibers in the CNT of RC, DC, OKC, and COC using PSR staining together with polarizing microscopy. The potential correlation between the nature of collagen fibers and the clinicopathological variables is also explored. The study results are expected to provide a better understanding about the biological behaviors of these lesions and be able to predict proper treatment plan as well as prognosis.

Sample collection and histological evaluation

The samples included 40 formalin-fixed paraffin-embedded (FFPE) tissue blocks which were achieved from the Department of Oral Surgery and Oral Medicine, Faculty of Dentistry, Srinakharinwirot University, from January 2015 to December 2020. RC, DC, OKC, and COC each had 10 blocks and irritation fibroma tissues from tongue as positive control had 2 blocks. The negative control (fibroma tissue omitted picrosirius red staining) was used. Two oral pathologists (intercalibration were assessed with kappa = 94) had confirmed the histological diagnoses based on the WHO classification of odontogenic cysts and tumors year 2017. For qualifying the OCs which were non-inflammatory cyst (DC, OKC, and COC). We selected only the cases with mild inflammation observed histopathologically for this study. This study was approved by the research ethics committee of Strategic Wisdom and Research Institute, Srinakharinwirot University, Bangkok, Thailand (reference number: SWUEC/X-499/2563).

Picrosirius red staining and evaluation

In Brief, All FFPE OCs were cut into sections (4-μM each). After deparaffinization in xylene and ethanol, each of sections was rehydrated in distilled water. Sections were subsequently incubated in picrosirius red (sirius red picrate solution obtained from Bio Optica Milano s.p.a., Italy) at room temperature for 1 h. After that, the samples were rinsed with distilled water, then counterstained with Mayer's hematoxylin, dehydrated, and finally mounted with coverslip. The staining procedures were performed according to the manufacturer's protocol to assure sensitivity and specificity of the staining. CU performed the picrosirius red staining in all tissues. All sections were examined by polarizing microscopy (Motic Asia, Hong

Kong). One thousand one cells from each of 3 random fields were examined per section by 2 oral pathologists. The polarization colors of the collagen in CNT were scored using a semi-quantitative method as the ratio of mature collagen (orange-red fibers) to the total number of collagens and the ratio of immature or pathological collagen (greenish-yellow fibers) to the total number of collagens. The scores were assigned as complete immature and pathological collagen (score 0), mixed mature and pathological collagen – predominantly pathological collagen presented – (score 1), mixed mature and pathological collagen – predominantly mature collagen presented – (score 2), and complete mature collagen (score 3). The scores were accepted if they agreed with both two investigations (intercalibration were assessed with kappa = 91). The mean score obtained from each case was calculated and used as labeling indices (LI) for each sample. To identify the nature of collagen fibers in the CNT of OCs, the samples were categorized into two groups based on the scores. Score 0 or 1 of 2 out of 3 random fields was categorized as greenish-yellow polarization color. Meanwhile, score 2 or 3 of 2 out 3 random fields was categorized as orange-red polarization color.

Statistical analysis

Fisher's exact test was used to examine the association between the polarization colors of collagen fibers in CNT of OCs and clinicopathologic information. Mean LI among RC, DC, OKC, and COC were compared using one-way ANOVA test. A p-value of < 0.05 was considered to be statistical significance.

Results

Clinicopathologic information of the OCs samples are summarized in Table 1. No correlation between nature of collagen fibers and clinicopathologic data (sex, age, largest cyst diameter, location, and histology) was observed ($p>0.05$) (Table 2-5).

In general, we found that the orientation pattern of collagen fibers in COC was different from other OCs (RC, DC, OKC). The comparison of the predominant polarization colors exhibited by collagen fibers in OCs is summarized in Table 6. The orange-red polarization color predominated in the CNT was observed in 9 out of 10 (90.0%), 7 out of 10 (70.0%), and 6 out of 10 (60.0%) in DC, RC, and OKC samples, respectively. Meanwhile, the greenish-yellow polarization color predominated in the CNT was observed in 5 out of 10 cases (50.0%) in COC samples (Figure 1 and 2). Figure 3 showed the positive control and negative control for the picrosirius red staining The mean LI values of RC, DC, OKC, and COC were 1.93±0.73, 1.90±0.44, 2.03±0.93, 1.40±0.59, respectively. Although there was a decrease in the mean LI of COC compared to other OCs, no statistically significant difference between the groups was observed ($p>0.05$) as shown in Table 7.

Discussion

Epithelial-mesenchymal interaction plays a crucial role in morphogenesis and cellular differentiation of the tooth bud and in pathogenesis of odontogenic lesions.[13,14] CNT may be regarded as not only a structural support of the cystic wall but also as a functional part indicating the behavior of the OCs.[6,7] Collagen is a major organic constituent of the CNT.[8,15] PSR staining combined with polarizing microscopy is a simple and specific method stained for collagen fibers.[9,11] The thickness, maturity, and packing of collagen cause the differences in polarization colors reflecting the nature of collagen fibers in OCs.[7,12,16] PSR comprises six sulfonate groups that can react with the basic amino acids in the collagen molecules and increase the birefringence property of collagen.[9,11,17] The increase in birefringence produced by PSR staining under polarizing light can differentiate the different types of collagen fibers such as type I and type III collagen fibers. Type I collagen fibers are coarsely packed, thick, and mature, showing a strong birefringence with orange-red polarization color. Meanwhile, type III collagen fibers form loosely packed, thin fibrillar, immature or pathological collagen, showing a weak birefringence of greenish-yellow color profile.[9,11] To date, although there are a few studies have reported the packing nature of collagen fibers in OCs and have suggested that the differences might influence their biological behavior, the study results remain uncertain.[6] Thus, this study assessed the nature of collagen fibers in the CNT of various OCs and correlated the results with their clinicopathological features to predict the aggressive behaviors of OCs using PSR and polarizing microscope. Our study showed that a predominance of greenish-yellow polarization was noted in COC samples as compared to RC, DC, and OKC samples. The mean LI of COC was decreased compared to other OCs,

Table 1: Clinicopathological characteristics of the odontogenic cysts included in this study.

Characteristics	RC n (%)	DC n (%)	OKC n (%)	COC n (%)
Samples	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)
Sex				
Male	5 (50.0)	5 (50.0)	3 (30.0)	6 (60.0)
Female	5 (50.0)	5 (50.0)	7 (70.0)	4 (40.0)
Age (years)				
≤ 35	5 (50.0)	6 (60.0)	4 (40.0)	7 (70.0)
> 35	5 (50.0)	4 (40.0)	6 (60.0)	3 (30.0)
Mean ± S.D.	32.1±16.1	35.0±20.6	40.3±15.5	26.1±16.5
Largest cyst diametera (cm)				
≤ 2	5 (50.0)	6 (60.0)	3 (30.0)	4 (40.0)
> 2	5 (50.0)	4 (40.0)	7 (70.0)	6 (60.0)
Location				
Maxilla	6 (60.0)	7 (70.0)	3 (30.0)	6 (60.0)
Mandible	4 (40.0)	3 (30.0)	7 (70.0)	4 (40.0)
Histology				
Daughter cyst presented	N/A	N/A	2 (20.0)	N/A
No daughter cyst	N/A	N/A	8 (80.0)	N/A

[a]Evaluated from the radiographic images,

RC = Radicular cyst, DC = Dentigerous cyst, OKC = Odontogenic keratocyst, COC = Calcifying odontogenic cyst N/A = Not available.

Table 2: Analysis of the correlations of nature of collagen fibers and clinicopathologic data in radicular cysts.

Characteristics (N)	Collagen birefringence		*p*-value[b]
	Greenish-yellow	Orangish-red	
Sex			
Male (5)	3	2	0.16
Female (5)	0	5	
Age (years)			
≤ 35 (5)	3	2	0.16
> 35 (5)	0	5	
Largest cyst diameter[a] (cm)			
≤ 2 (5)	2	3	1.0
> 2 (5)	1	4	
Location			
Maxilla (6)	2	4	1.0
Mandible (4)	1	3	

[a]Evaluated from the radiographic images

[b]Fisher's exact test, $p<0.05$ indicated statistically significant

Table 3: Analysis of the correlations of nature of collagen fibers and clinicopathologic data in dentigerous cysts.

Characteristics (N)	Collagen birefringence		*p*-value[b]
	Greenish-yellow	Orangish-red	
Sex			
Male (5) Female (5)	1 0	4 5	1.0
Age (years)			
≤ 35 (6) > 35 (4)	1 0	5 4	1.0
Largest cyst diameter[a] (cm)			
≤ 2 (6) > 2 (4)	1 0	5 4	1.0
Location			
Maxilla (7) Mandible (3)	1 0	6 3	1.0

[a]Evaluated from the radiographic images

[b]Fisher's exact test, $p<0.05$ indicated statistically significant

Table 4: Analysis of the correlations of nature of collagen fibers and clinicopathologic data in odontogenic keratocysts.

Characteristics (N)	Collagen birefringence		*p*-value[b]
	Greenish-yellow	Orangish-red	
Sex			
Male (3) Female (7)	0 4	3 3	0.2
Age (years)			
≤ 35 (4) > 35 (6)	2 2	2 4	1.0
Largest cyst diameter[a] (cm)			
≤ 2 (3) > 2 (7)	2 2	1 5	0.5
Location			
Maxilla (3) Mandible (7)	1 3	2 4	1.0
Histology			
Daughter cyst presented (2) No daughter cyst (8)	0 4	2 4	0.46

[a]Evaluated from the radiographic images

[b]Fisher's exact test, $p<0.05$ indicated statistically significant

Table 5: Analysis of the correlations of nature of collagen fibers and clinicopathologic data in calcifying odontogenic cysts.

Characteristics (N)	Collagen birefringence		*p*-value[b]
	Greenish-yellow	Orangish-red	
Sex			
Male (6) Female (4)	3 2	3 2	1.0
Age (years)			
≤ 35 (7) > 35 (3)	4 1	3 2	1.0
Largest cyst diameter[a] (cm)			
≤ 2 (4) > 2 (6)	2 3	2 3	1.0
Location			
Maxilla (6) Mandible (4)	3 2	3 2	1.0

[a]Evaluated from the radiographic images

[b]Fisher's exact test, $p<0.05$ indicated statistically significant

Table 6: The predominant polarization colors exhibited by collagen fibers in OCs.

Lesions	Total number of cases (%)	Number of cases showing predominant orange-red birefringence (%)	Number of cases showing predominant greenish-yellow birefringence (%)
RC	10 (100.0)	3 (30.0)	7 (70.0)
DC	10 (100.0)	1 (10.0)	9 (90.0)
OKC	10 (100.0)	4 (40.0)	6 (60.0)
COC	10 (100.0)	5 (50.0)	5 (50.0)

RC = Radicular cyst, DC = Dentigerous cyst, OKC = Odontogenic keratocyst, and COC = Calcifying odontogenic cyst.

Table 7: The mean labeling indices of collagen fibers between odontogenic cysts.

Lesions	Total number of Cases (%)	Mean LI±S.D.	*p*-value[a]
RC	10 (100.0)	1.93±0.73	0.266
DC	10 (100.0)	1.90±0.44	
OKC	10 (100.0)	2.03±0.93	
COC	10 (100.0)	1.40±0.59	

[a]One-way ANOVA test, $p<0.05$ indicated statistically significant.

RC = Radicular cyst, DC = Dentigerous cyst, OKC = Odontogenic keratocyst, and COC = Calcifying odontogenic cyst.

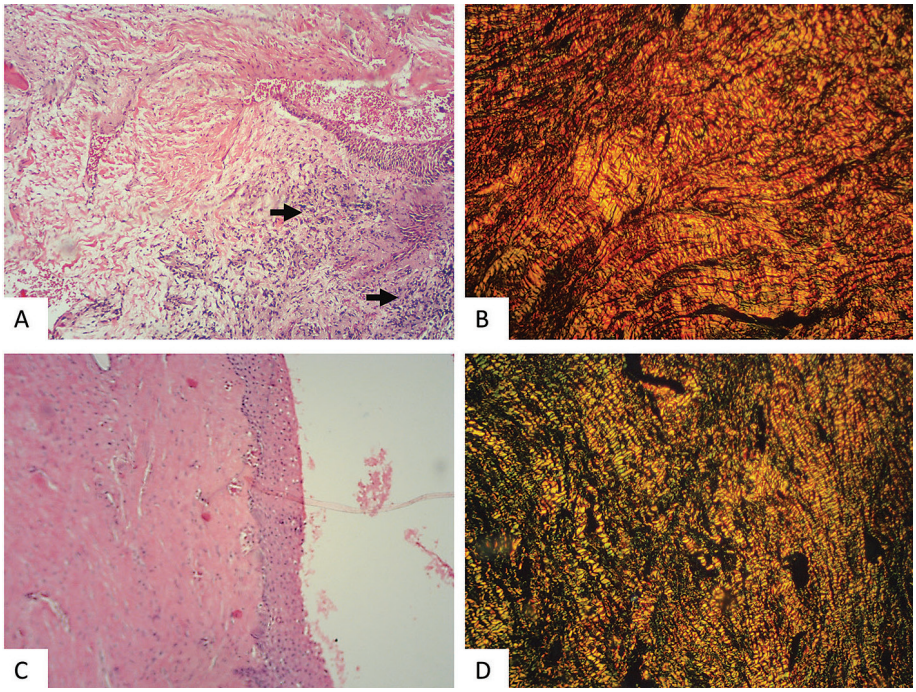

Figure 1: Polarization colors of picrosirius red staining in radicular cyst (RC) and dentigerous cyst (DC) with the corresponding area of hematoxylin and eosin staining. (A) RC sample shows moderate inflammation (arrow), (B) photomicrograph showing RC with predominant orange-red birefringence exhibited by collagen fibers, (C) DC sample shows mild inflammation, (D) photomicrograph showing DC with predominant orange birefringence exhibited by collagen fibers. Original magnification: ×100

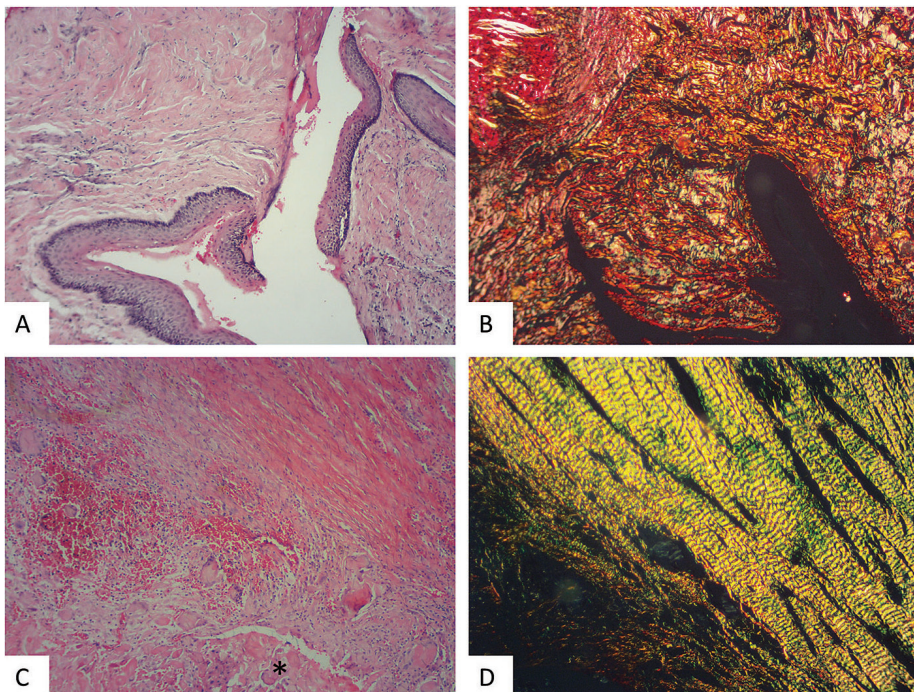

Figure 2: Polarization colors of picrosirius red staining in odontogenic keratocyst (OKC) and calcifying odontogenic cyst (COC) with the corresponding area of hematoxylin and eosin staining. (A) OKC sample shows mild inflammation, (B) photomicrograph showing OKC with predominant orange-red birefringence exhibited by collagen fibers, (C) COC sample shows mild inflammation with areas of the ghost cells (asterisk), (D) photomicrograph showing COC with predominant greenish-yellow birefringence exhibited by collagen fibers. Original magnification: ×100

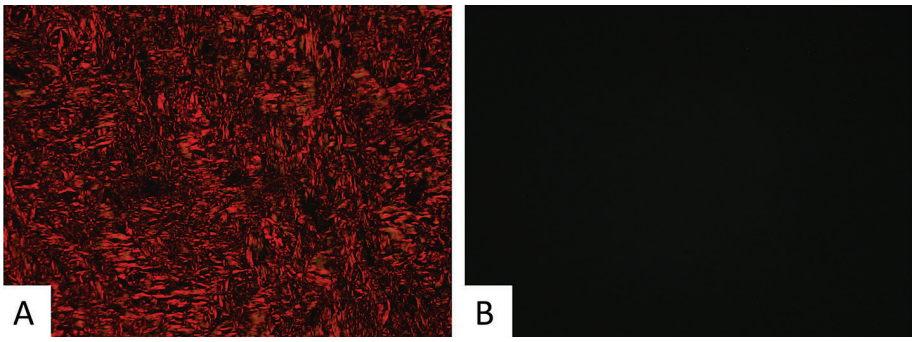

Figure 3: Polarization colors of picrosirius red staining in tongue irritation fibroma (positive control) and tongue irritation fibroma omitted picrosirius red staining (negative control). (A) photomicrograph showing positive control with red birefringence exhibited by collagen fibers, (B) photomicrograph showing negative control without birefringence polarization color exhibited by collagen fibers.

however, no statistically significant difference between the OCs was observed (p>0.05).

For this study, 70.0% of RC cases showed a predominant orange-red birefringence (mean LI score of 1.93±0.73), which was in concordance with some previous studies.[7,18,19] RC is an inflammatory cystic lesion of the jaw originating from epithelial rests of Malassez resulting from the pulp necrosis.[2,20] Inflammation can lead to the increases in cytokines and growth factors which promote fibroblastic and collagen fiber proliferation.[18] Martin R *et al*.[21] reported that transforming growth factor beta 1 (TGF-ß1) was an important cytokine in RC which involved several biological mechanisms, including being a chemoattractant for fibroblast that synthesizes the extracellular matrix and collagen in tissue repair and angiogenesis processes. The results in this study disagreed with some previous studies which suggested that chronic inflammation may destroy the structure and arrangement of collagen fibers resulting in greenish-yellow polarizing color found in RCs.[22,23] It can be seen that the roles of inflammation in CNT of RC are not clearly understood and are required further investigation.

The predominant polarization color of collagen fibers in DC was orange-red (90.0% cases, mean LI score of 1.90±0.44), which was in line with previous studies.[7,19,20-24] The orange-red birefringence of collagen fibers observed in DC is considered to be the mature, coarsely packed collagen suggesting less aggressive behavior and long-standing nature of the DC.[22-24]

OKC is still a controversy of OC that has been re-classified the terminology between cyst and tumor in recent decades.[4] This study found that the predominant color of collagen fibers in CNT of OKC was also orange-red (60.0% samples, mean LI score of 2.03±0.93). These results were in accordance with the studies of Aggarwal *et al*.[22] and Jahagirdar *et al*.[23] However, they disagreed with several previous researches which reported that the color of collagen fibers in OKC was greenish-yellow birefringence.[6,7,18,19,24,25] Recently (2017), the WHO has categorized the OKC as non-neoplastic cystic lesion regarding to its response to marsupialization followed by enucleation technique.[2,26] Moreover, Portes *et al*.[27] reported that no statistically significant difference was observed in the Ki-67 (proliferation marker) immunoexpression and immunostaining intensity between DC and OKC using automated method, suggesting that they belonged to the same developmental cystic lesion.[27] This finding could be an evidence supporting that OKC might be regarded as a cystic lesion rather than neoplasm.

To the best of our knowledge, this work is the first study determining the packing nature of the collagen in CNT of the COCs. The results demonstrated that the predominance of greenish-yellow color, which originates from loosely packed, pathological collagen fibers, was found in 50.0% of COC samples (mean LI score of 1.40±0.59). Due to the lower mean LI score compared to other OCs, despite insignificance, the mesenchymal composition of COC might differ from other OCs. Although, in 2017, the WHO has reclassified COC as a cystic lesion, there has been disagreement regarding its concept and terminology for several years.[2,5] Previous studies reported that the major polarization color of solid multicystic and unicystic ameloblastoma was greenish yellow[24,25], which was similar to the major areas of COC samples presented in this study. Moreover, it is widely accepted that the genetic alteration involving COC

is ß-catenin mutation, which will be the basis of tumorigenesis in COC.[28] Thus, the finding is this work might further support that some of COCs might be categorized as a cystic neoplasm rather than a cyst lesion.

Again, this research is the first study that investigates the association between nature of collagen fibers and various clinicopathological variables including sex, age, location, size, and histology (for OKC). The results demonstrated that there was no significant correlation between nature of collagen fibers and these clinicopathological data. This might be attributable to the small sample size in this study.

Finally, the main limitation of this study is the small sample size. Future studies with larger sample sizes (at least 20 samples for each OCs) and the use of molecular biology technique are required to verify the results in this study. Moreover, in-depth study of the effect of mesenchymal component in the pathogenesis of odontogenic lesions is needed.

Conclusions

The use of PSR staining with polarizing microscopy proved to be an effective method to evaluate the nature of the collagen fibers in different OCs. Determination of the collagen fibers in OCs could provide the biological behaviors of the lesions leading to the precise treatment planning. Based on the frequency of polarization colors and mean LI of the lesions, a predominant orange-red birefringence was observed in the majority cases of RC, DC, and OKC, whereas a greenish-yellow birefringence was found in most areas of COC samples, which might reflect the cystic neoplasm behavior of some COCs. Further studies using molecular biology are needed to confirm the roles of CNT in pathogenesis of these lesions.

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Conflicts of interest

The authors declare no conflicts of interest.

References

1. Santosh ABR. Odontogenic cysts. Dent Clin North Am. 2020;64(1):105-19.
2. El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ. WHO classification of head and neck tumours. 4th ed. Lyon: IARC;2017.
3. Johnson NR, Gannon OM, Savage NW, Batstone MD. Frequency of odontogenic cysts and tumors: a systematic review. J Investig Clin Dent. 2014;5(1):9-14.
4. Stoelinga PJW. Keratocystic odontogenic tumour (KCOT) has again been renamed odontogenic keratocyst (OKC). Int J Oral Maxillofac Surg. 2019;48(3):415-416.
5. de Arruda JAA, Monteiro JLGC, Abreu LG, de Olivera Silva LV, Schuch LF, de Noronha MS, *et al*. Calcifying odontogenic cyst, dentinogenic ghost cell tumor, and ghost cell odontogenic carcinoma: a systematic review. J Oral Pathol Med. 2018;47(8):721-30.
6. Peddapelli K, Rao V, Kumar MP, Sravya T, Rakesh D. Analaysis of collagen fibers in keratocystic odontogenic tumor and ameloblastoma: a polarizing microscopic study. Indian J Dent Res. 2019;30(5):731-5.
7. 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(9):410-2.
8. Devendra A, Niranjan KC, Swetha A, Kaveri H. Histochemical analysis of collagen reorganization at the invasive front of oral squamous cell carcinoma tumors. J Invest Clin Dent. 2018;9(1):e12283.
9. Lattouf R, Younes R, Lutomski D, Naaman N, Godeau G, Senni K, *et al*. Picrosirius red staining: a useful tool to appraise collagen networks in normal and pathological tissue. J Histochem Cytochem. 2014;62(10):751-8.
10. Kumari K, Ghosh S, Patil S, Augustine D, Venkatesiah SS, Rao RS. Expression of type III collagen correlates with poor prognosis in oral squamous cell carcinoma. J Invest Clin Dent. 2017;8(4):e12253.
11. Arora KS, Nayyar A, Kaur P, Arora KS, Goel A, Singh S. Evaluation of collagen in leukoplakia, oral submucous fibrosis and oral squamous cell carcinomas using polarizing microscopy and immunohistochemistry. Asian Pac J Cancer Prev. 2018;19(4):1075-80.
12. Rittie L. Method for picrosirius red-polarization detection of collagen fibers in tissue sections. Methods Mol Biol. 2017;1627:395-407.
13. Tisty TD, Hein PW. Know the neighbor: stromal cells can contribute oncogenic signals. Curr Opin Genet Dev. 2001;11(1):54-9.

14. High AS, Robinson PA. Differences in adhesion and collagen gel contraction between fibroblasts from various types of odontogenic cysts. Arch Oral Biol. 1994;39(5): 387-93.
15. Nissen NI, Karsdal M, Willumsen N. Collagens and cancer associated fibroblasts in the reactive stroma and its relation to cancer biology. J Exp Clin Cancer Res. 2019;38:115.
16. Manjunatha BS, Agrawal A, Shah V. Histopathological evaluation of collagen fibers using picrosirius red stain and polarizing microscopy in oral squamous cell carcinoma. J Cancer Res Ther. 2015;11(2):272-6
17. Sharma R, Rehani S, Mehendiratta M, Kardam P, Kumra M, Mathias Y, *et al*. Architectural analysis of picrosirius red stained collagen in oral epithelial dysplasia and oral squamous cell carcinoma using polarization microscopy. J Clin Diagn Res. 2015;9(12):13-6.
18. 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(3):257-62.
19. Mahajan AM, Mahajan MC, Ganvir SM, Hazarey VK. The role of stroma in the expansion of odontogenic cysts and adenomatoid odontogenic tumor: a polarized microscopy study. J Nat Sc Biol Med. 2013;4(2):316-20.
20. Bilodeau EA, Collins BM. Odontogenic cysts and neoplasms. Surg Pathol Clin. 2017;10(1):177-222.
21. Martins R, Armada L, dos Santos TC, Pires FR. Comparative immunoexpression of ICAM-1, TGF-β1 and ki-67 in periapical and residual cysts. Med Oral Patol Oral Cir Bucal. 2017;22(1):e24-30.
22. 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(2):211-5.
23. Jahagirdar PB, Kale AD, Hallikerimath S. Stromal characterization and comparison of odontogenic cysts and odontogenic tumors using picrosirius red stain and polarizing microscopy: a retrospective and histochemical study. Indian J Cancer. 2015;52(3):408-12.
24. Raj Y, Sekhar MSM, Shylaja S, Bhavani SN, Ramanand OV, Patha S, *et al*. Evaluation of the nature of collagen fibers in KCOT, dentigerous cyst and ameloblastoma using picrosirius red stain-a comparative study. J Clin Diagn Res. 2015;9(11):ZC01-4.
25. Zhang JY, Dong Q, Li TJ. Differences in collagen fibers in the capsule walls of parakeratinized and orthokeratinized odontogenic cysts. Int J Oral Maxillofac Surg. 2011;40(11):1296-1300.
26. Ribeiro-Junior O, Borba AM, Alves CAF, de Gouveia MM, Deboni MCZ, Naclerio-Homem MG. Reclassification and treatment of odontogenic keratocysts: a cohort study. Braz Oral Res. 2017;31:e98.
27. Portes J, Cunha KSG, da Silva LE, da Silva AKF, Conde DC, Silva Junior A. Computerized evaluation of the immunoexpression of Ki 67 protein in odontogenic keratocyst and dentigerous cyst. Head Neck Pathol. 2020;14(3):598-605.
28. de Sousa SF, Moreira RG, Gomez RS, Gomes CC. Interrogation of cancer hot spot mutations in tumor suppressor and oncogenic genes in the calculation of cystic odontogenic tumor. Oral Oncol. 2016;57:e1-3.