Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 06, pp.33513-33519, June, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

COLLAGEN FIBRE ORIENTATION AND BEHAVIOUR OF ODONTOGENIC CYSTS; A PICROSIRIUS RED STAINING AND POLARISING MICROSCOPIC STUDY

[1]Yogish, P., [2]Girish, H. C., [3]Sanjay Murgod, [4]Sandesh, M., [5]Asha Yogish and [6]Kavitha, M.

[1]Assistant Professor, Department of Oral Pathology & Microbiology, Sharavathi Dental College and Hospital, Shimoga, Karnataka, India
[2]Head of the Department, Department of Oral Pathology & Microbiology, Rajarajeswari Dental College and Hospital, Bangalore, Karnataka, India
[3]Professor, Department of Oral Pathology & Microbiology, Rajarajeswari Dental College and Hospital, Bangalore, Karnataka, India
4 Assistant Professor, Department of Oral Pathology & Microbiology, VS Dental College & Hospital, Bangalore, Karnataka, India
[5]Post Graduate Student, Department of Oral Medicine & Radiology, Coorg Institute of Dental Sciences, Virajpet, Karnataka, India
[6] Post Graduate Student, Department of Paediatric Dentistry, V.S. Dental College and hospital, Bangalore, Karnataka, India

ARTICLE INFO

Article History:
Received 04th March, 2016
Received in revised form
07th April, 2016
Accepted 15th May, 2016
Published online 30th June, 2016

Key words:

Collagen,
Stroma,
Mesenchyme,
Inflammation.

Corresponding author: Yogish, P.
Department of Oral Pathology & Microbiology, Sharavathi Dental College and Hospital, Shimoga, Karnataka, India.

ABSTRACT

Introduction: Epithelial–mesenchymal interactions are thought to play an important role in the pathogenesis of odontogenic cysts. Odontogenic Keratocyst (OKC) is a benign cystic neoplasm with a characteristic parakeratinized epithelial lining, which differs histologically and behaviourally from the Dentigerous cyst (DC) and Radicular cyst (RC). Biological behaviour of OKC is dependent on both epithelium and the underlying stroma. Collagen plays an important role in maintaining structural integrity and in determination of tissue functions. Collagen has natural birefringence which is attributed to arrangement of its fibres; this property is enhanced by Picrosirius Red staining.

Aim: The purpose of this study will be to investigate the differences in collagen fibres within the fibrous tissue walls of OKC, DC and RC.

Study design: Formalin-fixed paraffin-embedded tissue samples of OKC, DC, RC was segregated from the department of Oral Pathology & Microbiology, RRDCH and cut sections was subjected to staining with Picrosirius red and was observed under polarising light microscope. Polarising colours of the collagen fibres was recorded and statistically analysed.

Materials and Methods: A group of 30 histopathologically diagnosed cases of Odontogenic cysts, 10 cases of Radicular cysts, 10 cases of Dentigerous cyst, 10 cases of Odontogenic Keratocyst and 2 cases of Dental follicular tissue as controls were retrieved from files of Department of Oral Pathology and Microbiology, Rajarajeswari Dental College and Hospital. Haematoxylin and Eosin stained slides were evaluated for the existence and degree of inflammation in all the slides. Paraffin embedded tissue blocks were sectioned at 5µm thickness. Sections were deparaffinised, hydrated and stained with Picro Sirius Red stain for collagen.

Statistical analysis:
Level of Significance: α=0.05
Statistical test used: Chi-squared (χ^2) test

Results: Our study showed that in, RC and DC - yellowish orange and orange color was seen indicating dense fibrosis in case of mild and moderate inflammation. Whereas in case of OKC , greenish yellow color was dominant in subepithelial, intermediate layer in absence and mild inflammation, whereas in periphery yellowish orange was seen in mild inflammation

Conclusion: This method can be used to analyze the orientation of collagen fibers and also how inflammation can induce a change in the collagen from loosely packed thin fibrils to closely packed mature collagen. It can also be used as a diagnostic tool to differentiate two lesions and to predict their nature in terms of biologic behaviour and prognosis.

Citation: Yogish, P., Girish, H. C., Sanjay Murgod, Sandesh, M., Asha and Kavitha, M. 2016. "Collagen Fibre orientation and behaviour of odontogenic cysts; A picrosirius red staining and polarising microscopic study", *International Journal of Current Research*, 8, (06), 33513-33519.

INTRODUCTION

The term odontogenic keratocyst (OKC) was first used by Philipsen in 1956 to designate an odontogenic cyst that demonstrated keratinization of its lining epithelium. (Angelopoulou and Nicolatou, 1990) OKC is a clinicopathologically distinct form of odontogenic cyst, known for its pathognomonic microscopic features, aggressiveness and high recurrence rate. (De Paula *et al*., 2000) Many studies have focused on intrinsic growth potential of epithelial lining of OKC6-9 and it has been reclassified as keratocystic

odontogenic tumor by the WHO. (Barnes *et al*., 2005) The inflammation in the connective tissue wall of OKC has been observed in many of the reported cases in the literature. The studies have shown that when inflammation is present within the cyst wall of an OKC, the character of the cyst lining changes from the classical parakeratinized epithelium to nonkeratinized stratified squamous epithelium similar to other inflammatory cysts. (Rodu *et al*., 1987) Constantine and Mowry (1968) described staining with Picrosirius red followed by polarization microscopy for selective demonstration of collagen. Junqueira *et al*. (1979) suggested that this procedure be considered specific for collagen. In 1978, Junqueira *et al*. proposed that the different polarization colours obtained after Picrosirius red staining could be used to differentiate between collagens of types I, II and III. However, it was later shown that the differences in polarization colours were due to fibre thickness rather than to the nature of the stained collagens (Junqueira *et al*. 1982). (Dayan *et al*., 1989) When Sirius Red, an elongated strongly acidic dye, reacts with collagen its normal birefringence is enhanced due to the fact that the dye molecules are attached to the collagen fibrils in such a way that their long axes are parallel. The mechanism of staining and the quantitation of the increase in birefringence, as well as the conditions for optimal staining (influence of fixation, dye concentration, picric acid concentration, time of staining, pH, and washing) have been recently studied by Junqueira *et al*. (1979 a) showing that the enhancement in birefringence observed when studying tissue sections by the Picrosirius-polarization method is specific for collagen detection. (Junqueira *et al*., 1982) This study was undertaken to analyse the nature of collagen fibres in odontogenic cyst walls. Biologic behaviour, histogenesis and role of inflammation can be predicted by the birefringence pattern of collagen fibres in these cysts.

MATERIALS AND METHODS

A group of 30 histopathologically diagnosed cases of Odontogenic cysts- 10 cases of Radicular cysts, 10 cases of Dentigerous cyst, 10 cases of Odontogenic Keratocyst and 2 cases of Dental follicular tissue as controls were retrieved from files of Department of Oral Pathology and Microbiology, Rajarajeswari Dental College and Hospital. Haematoxylin and Eosin stained slides were evaluated for the existence and degree of inflammation in all the slides. Paraffin embedded tissue blocks were sectioned at 5µm thickness. Sections were deparaffinised, hydrated and stained with Picro Sirius Red stain for collagen. Picrosirius red stained slides of the above mentioned sections were used to evaluate the nature of collagen fibres. The connective tissue in theses slides showed polarization colours varying from greenish yellow to yellowish orange to orange. The polarization colours of the connective tissue were assessed in three zones in each section: subepithelial zone, intermediate zone and peripheral zone. At the same time the slides were also assessed under light microscopy and the inflammatory cell infiltrate was evaluated in the above zone as 0-absent, 1- mild, 2- moderate and 3-intense. Inflammatory density were graded on a 4grade scale, Grade 0- no inflammation, Grade 1- <15 cells/field, Grade 2 – 15-50 cells/field, and Grade 3 - > 50 cells / field.

RESULTS

Statistical analysis

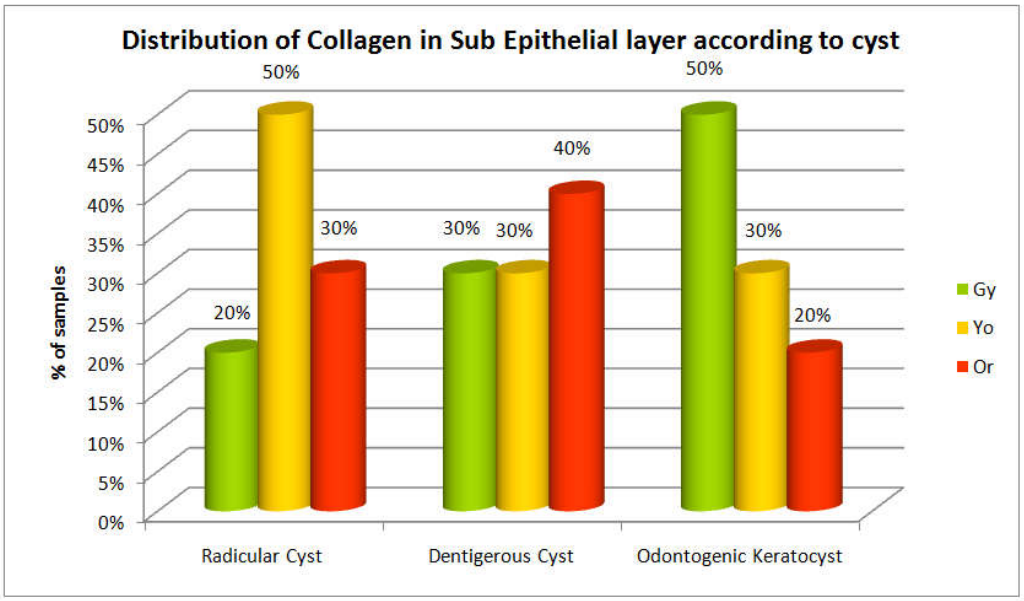
Level of Significance: α=0.05

Statistical test used: Chi-squared (χ^2) test

Computations: The tables below give us the various computations and the P-Value.

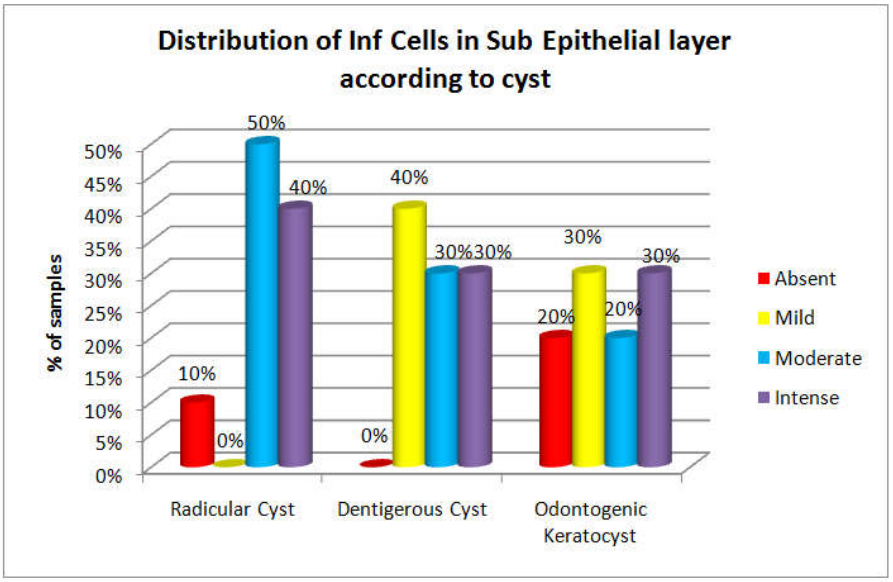
Sub epithelial

Collagen	Radicular cyst		Dentigerous cyst		Odontogenic Keratocyst		χ^2	P-Value
	n	%	n	%	n	%		
Greenish Yellow	2	20%	3	30%	5	50%	2.79	0.593
Yellowish Orange	5	50%	3	30%	3	30%	4	
Orange	3	30%	4	40%	2	20%		



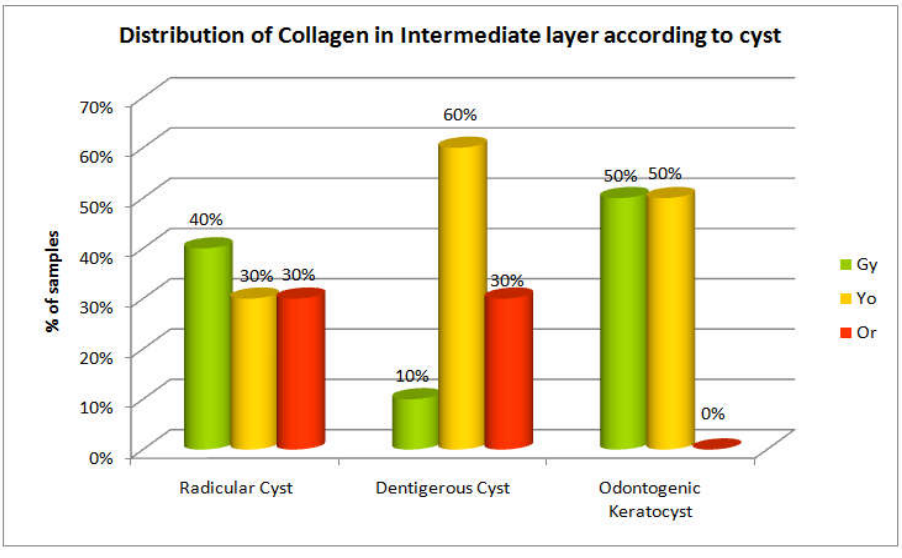
Inflammatory cells

Collagen	Radicular cyst		Dentigerous cyst		Odontogenic Keratocyst		χ^2	P-Value
	n	%	n	%	n	%		
Absent	1	10%	0	0%	2	20%	7.31	0.293
Mild	0	0%	4	40%	3	30%	4	
Moderate	5	50%	3	30%	2	20%		
Intense	4	40%	3	30%	3	30%		



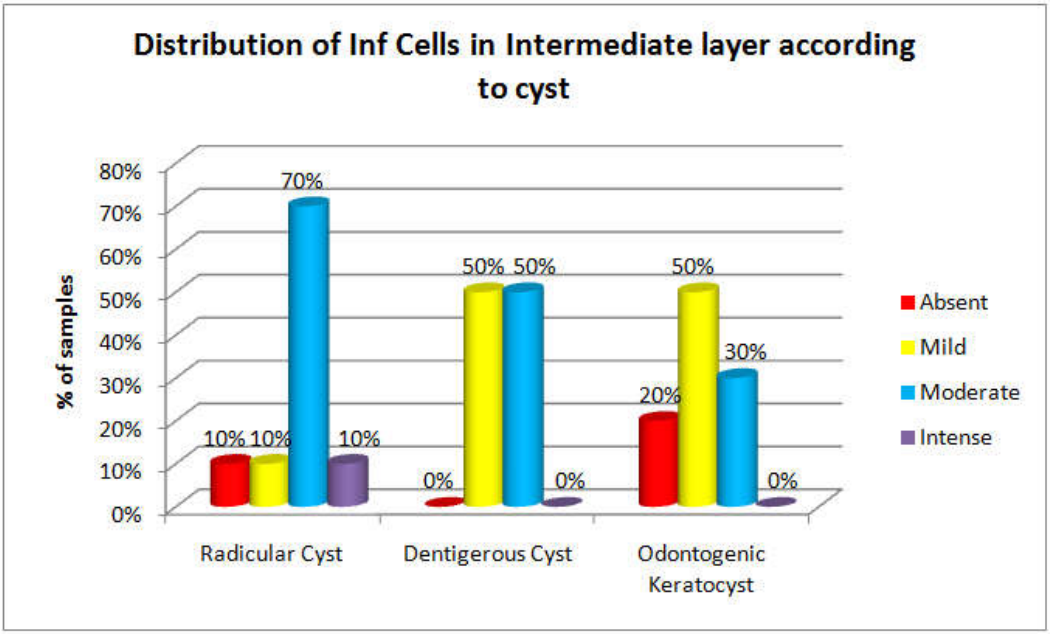
Intermediate

Collagen	Radicular cyst		Dentigerous cyst		Odontogenic Keratocyst		χ^2	P-Value
	n	%	n	%	n	%		
Greenish Yellow	4	40%	1	10%	5	50%	6.60	0.159
Yellowish Orange	3	30%	6	60%	5	50%	0	
Orange	3	30%	3	30%	0	0%		



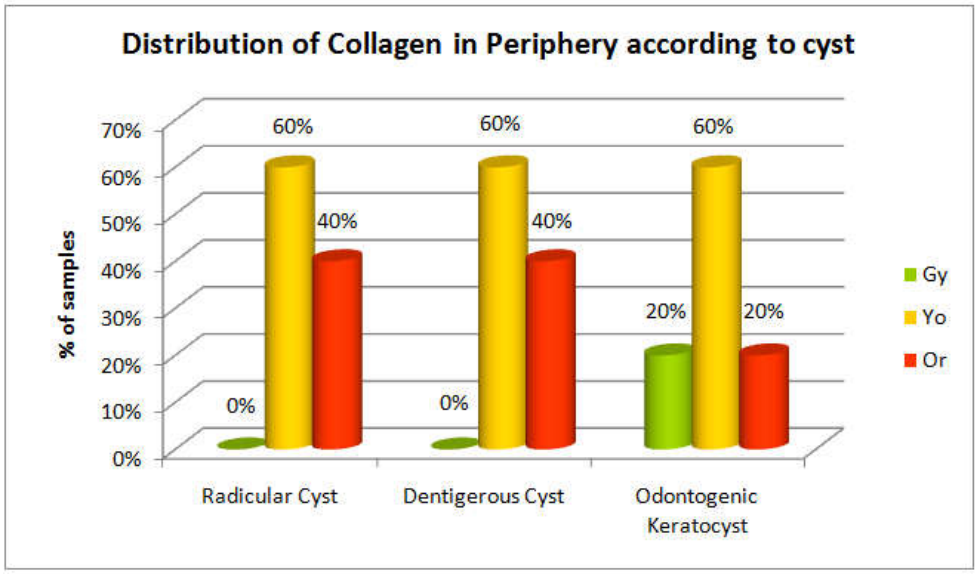
Inflammatory cells

Collagen	Radicular cyst		Dentigerous cyst		Odontogenic Keratocyst		χ^2	P-Value
	n	%	n	%	n	%		
Absent	1	10%	0	0%	2	20%	8.50	0.203
Mild	1	10%	5	50%	5	50%	9	
Moderate	7	70%	5	50%	3	30%		
Intense	1	10%	0	0%	0	0%		



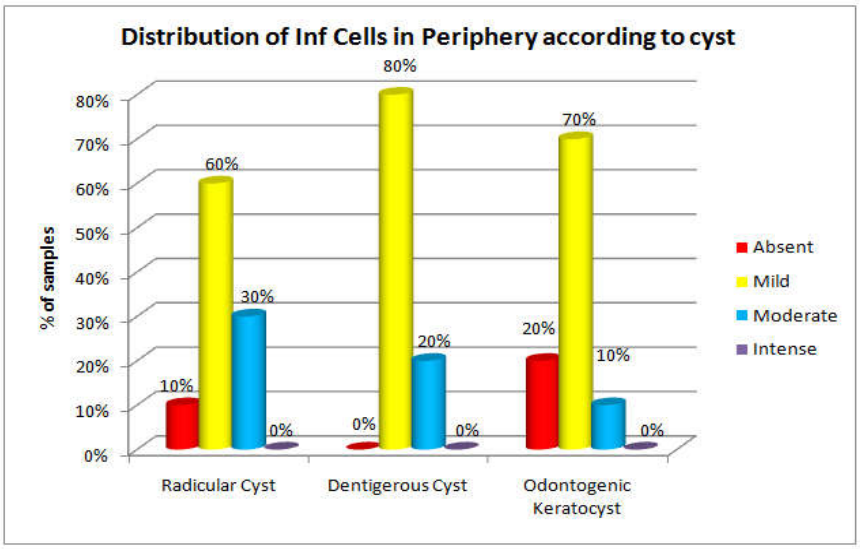
Periphery:

Collagen	Radicular cyst		Dentigerous cyst		Odontogenic Keratocyst		χ^2	P-Value
	n	%	n	%	n	%		
Greenish Yellow	0	0%	0	0%	2	20%	5.400	0.249
Yellowish Orange	6	60%	6	60%	6	60%		
Orange	4	40%	4	40%	2	20%		



Inflammatory cells

Collagen	Radicular cyst		Dentigerous cyst		Odontogenic Keratocyst		χ^2	P-Value
	n	**%**	**n**	**%**	**n**	**%**		
Absent	1	10%	0	0%	2	20%	3.286	0.511
Mild	6	**60%**	8	**80%**	7	**70%**		
Moderate	3	30%	2	20%	1	10%		
Intense	0	0%	0	0%	0	0%		



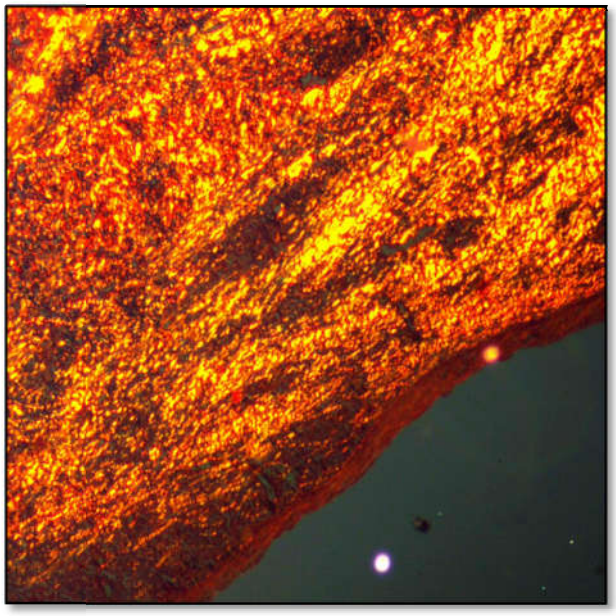


Figure 1. Photomicrograph of Dentigerous cyst with predominantly Yellowish birefringence in Picrosirius red stain. (40X)

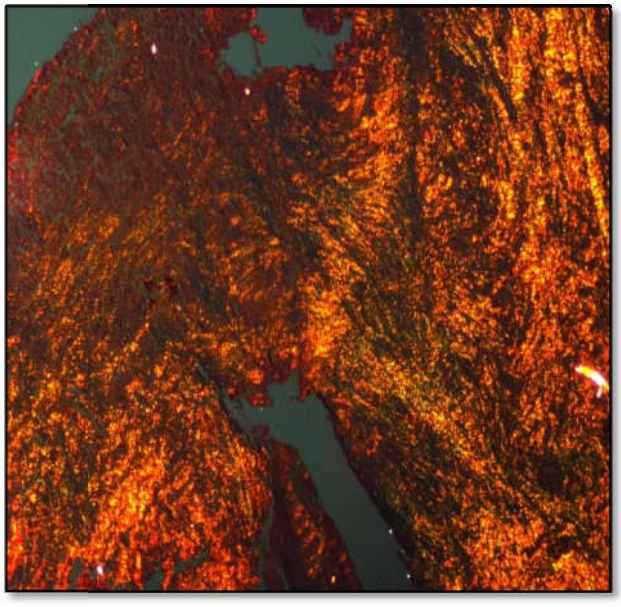


Figure 2. Photomicrograph of Odontogenic keratocyst with predominantly Greenish yellow birefringence in Picrosirius red stain. (40X)

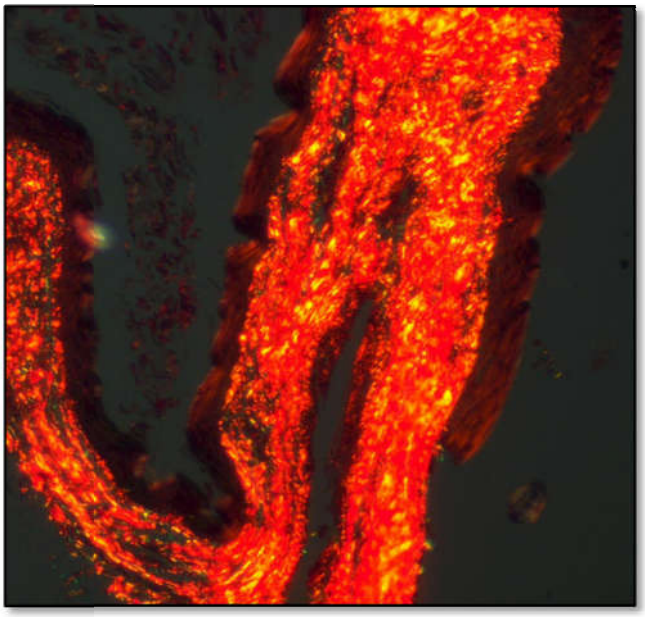


Figure 3. Photomicrograph of Odontogenic keratocyst with predominantly orange birefringence in Picrosirius red stain. (40X)

DISCUSSION

The term collagen relates to a family of glycoproteins whioh are contained in different histological entities such as collagen fibres, reticulin fibres, basement membrane etc. Since the bulk of the collagen molecules are orderly disposed in a parallel orientation, a normal birefringence is one of the classic characteristics of those collagen entities. (Montes and Junqueira, 1991) Sirius red, a strong anionic dye, stains collagen by reacting, via its sulphonic acid groups, with basic groups present in the collagen molecule. (Kaplan and Hirshberg, 2004) Sirius red is an elongated dye molecule which reacts with collagen and promotes an enhancement of its normal birefringence due to the fact that many dye molecules are aligned parallel with the long axis of each collagen molecule. (Montes and Junqueira, 1991)

- Collagen type 1 presented as yellow, orange or red colour while collagen type 3 appeared green.
- Greenish yellow color represents loosely packed collagen – procollagen, intermediate, pathological collagen. (Vishwanathan and Venkatapathy, 2011)

Growth and expansion of odontogenic cysts has been believed to be the result of osmotic pressure exerted by the cystic contents, but recent studies have focused on the importance of epithelial- mesenchymal interactions for the same. It has been suggested that stroma is essential for maintaining the epithelial tissues and both these make up an ecosystem with continuous molecular interactions. (Agarwal and Saxena, 2011) It was Vedtofte *et al* who showed the importance of stromal component from a study on a transplanted keratocyst epithelium in nude mice which led the authors to believe that the differentiation of the cystic epithelium is not independent of the stroma and suggested that the stromal component has a role to play in the biological behavior by establishing ectomesenchymal interactions. (Vij and Vij, 2011) Polarization color of green to greenish yellow corresponds to poorly packed collagen fibres whereas orange red originates from well packed fibres. (Dayan *et al*., 1993) The polarization color is a result of fiber thickness, as well as the arrangement and packing of the collagen molecules. Hirshberg et.al has demonstrated thick collagen fibres with green birefringence in the wall of OKC in contrast to the orange to red spectrum in radicular and dentigerous cysts. It suggests that collagen found in OKC is loosely arranged and might be composed of procollagen, intermediate or pathologic collagens rather than the normal tightly packed fibres seen in dentigerous and radicular cysts. (Hirshberg *et al*., 19999) The presence of green to greenish yellow color of thick fibres was found in different pathologic conditions like odontogenic tumors in humans (Hirshberg *et al*., 1996) and in an ameloblastic fibroma in a cat. (Nyska and Dayan, 1995) Our study showed that in case of RC and DC, yellowish orange and orange colour was seen indicating dense fibrosis in case of mild and moderate inflammation. Whereas in case of OKC, greenish yellow color was dominant in subepithelial, intermediate layer in absence and mild inflammation, whereas in periphery yellowish orange was seen in mild inflammation. Our study did not show any significant value on the color of the fibres w.r.t. inflammation. These findings are consistent with the study done by Hirshberg *et al*. The results of the present study demonstrated that the inflammation has a direct influence on the polarizing colors of collagen fibres in RC and DC. Polarization colours of the thin fibres mostly ranged from green to yellow without significant differences between different groups. These findings are consistent with the study done by Hirshberg *et al*. (2007) In cysts with mild to moderate inflammation, most of the thick fibres were showing green to yellow polarization colours. Studies done in oral sub mucous fibrosis revealed that there was a gradual decrease in the greenish yellow color of the fibres and a shift to orange red color with increase in severity of the disease which appeared that the tight packing of collagen fibres in OSMF progressively increased as the disease

progressed from early to advanced stages. (Ganganna *et al*., 2012) Similarly in a study done with respect to the relationship between the collagenous components in the stroma and the invading tumor cells, there have been some observable changes in different histological grades of Oral squamous cell carcinoma. In well differentiated squamous cell carcinoma and moderately differentiated squamous cell carcinoma, distinct deposits of collagen showed reddish orange to yellowish orange birefringence, which was mainly concentrated around the tumor islands. This may be due to the deposition of collagen fibres which were in the form of thick bands and composed of closely packed fibrils, this feature being consistent with the concept of Junqueira *et al* and Montes *et al*. They stated that the thick fibres were Type I collagen fibres and exhibited an intense birefringence of red, orange and yellow colour by polarizing microscopy and a weak birefringence of green when the fibres were thin fibrillar, thus constituting Type III collagen (Venigella and Charu, 2010).

Conclusion

This method can be used to study to analyze the orientation of collagen fibers and the role of inflammation on collagen. Inflammation induces a change in the collagen from loosely packed thin fibrils to closely packed mature collagen. It can also be used as a diagnostic tool to differentiate two lesions and to predict their nature in terms of biologic behavior and prognosis. Further investigations on the biochemical and molecular studies are required to know the major role of collagen fibres in the pathogenesis of odontogenic cysts and also the influence of mesenchyme in the behavior of the odontogenic cysts.

REFERENCES

Agarwal P, Saxena S. 2011. 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*, vol 48; issue 2.

Angelopoulou EK, Nicolatou O. 1990. Odontogenic keratocyst: Clinicopathologic study of 87 cases. *J Oral Maxillofac Surg*., 48:593- 9.

Barnes L, Eveson JW, Reichart P, Sidransky D. 2005. World Health Organization of tumours. Pathology and genetics of head and neck tumours. Lyon: IARC Press.

Dayan D, Waner T, Tal H, *et al*. 1993. Polarization microscopy of picrosirius red stained collagen from oxipidine-induced hyperplastic gingiva of beagle dogs. Int *J Exp pathol*., 74:225-8.

Dayan D., Hiss, Hirshberg, Bubis, M. Woiman. 1989. Are the polarization colors of Picrosirius red-stained collagen determined only by the diameter of the fibers ? *Histochemistry*, 93: 27 29

De Paula AMB, Carvalhais JN, Domingues MG, Barreto DC, Mesquita RA. 2000. Cell proliferation markers in the odontogenic keratocyst; effect of inflammation. *J Oral Pathol Med*., 29: 477-2

Ganganna K, Pushparaja shetty, Shroff SE. 2012. Collagen in histologic stages of oral submucous fibrosis: a polarising microscopic study. *J Oral Maxillofac Pathol*., May-Aug; 16(2): 162–166.

Hirshberg A, Buchner A, Dayan D. 1996. The central odontogenic fibroma and the hyperplastic dental follicle. Study with picrosirius red and polarization microscopy. *J Oral Pathol Med*., 25;125-7.

Hirshberg A, Lib M, Kozlovsky A. *et al*. 2007. The influence of inflammation on the polarization colors of collagen fibres in the wall of odontogenic keratocysts. *Oral Oncology*, 43:278-82.

Hirshberg A, Sherman S, Buchner A, *et al*. 1999. Collagen fibres in the wall of odontogenic keratocyst: a study with picrosirius red and polarization microscopy. *J Oral Pathol Med*., 28:410-12.

Junqueira LC, Montes GS, Sanchez EM. 1982. The influence of tissue section thickness on the study of collagen by the Picrosirius-polarization method. *Histochemistry*., 74(1):153-6.

Kaplan I, Hirshberg A. 2004. The correlation between epithelial cell proliferation and inflammation in OKC; *Oral Oncology*, 40;985-991.

Montes.GS. and Junqueira.LCU. 1991. The use of the picrosirius polarization method for the study of the biopathology of collagen; Mem inst. Oswako Cruz, Rio de Janeiro, vol 86.

Nyska A, Dayan D. 1995. Ameloblastic fibroma in a young cat. *J Oral Pathol Med*., 24:233-6.

Rodu B, Tate AL, Martinez MG. 1987. The implications of inflammation in odontogenic keratocysts. *J Oral Pathol Med.*, 16:518-21.

Venigella A, Charu S. 2010. Evaluation of collagen in different grades of oral squamous cell carcinoma by using the Picrosirius red stain- a histochemical study. *Journal of Clinical and Diagnostic Research*, (4):3444-3449

Vij R. and Vij H. 2011. Evaluation of collagen in connective tissue walls of odontogenic cyst a histochemical study; *J Oral Pathol Med*., 40:257-262.

Vishwanathan S. and Venkatapathy BP. 2011. Role of collagen fibres in the expansion of odontogenic cyst- a histochemical study. *Internal Journal of Pathology*; Vol 11; 2.

****