Role Of Collagen Fibres In The Expansion Of Odontogenic Cysts – A Histochemical Study.
S Viswanathan, R Venkatapathy, B Danasekaran

Citation

Abstract
Objective: To assess the thickness and color of collagen fibres in various odontogenic cysts for its possible role in expansion.
Study design: 49 cases of 4 types of odontogenic cysts and 5 normal mucosal tissues were selected as the study and the control group respectively. All slides were stained with both Hemotoxylin and Eosin and the picrosirius red stain. The thickness of collagen fibres were assessed using polarizing microscopy under oil immersion. Results: Irrespective of the control and the study groups, the overall distributions of the thin fibres were predominantly greenish yellow in color and the thick fibres were orange red. Variations were noticed in the fibres of radicular cyst and odontogenic keratocysts where in radicular cyst even the thin fibres expressed more of orange red color and in odontogenic keratocyst the thick fibres also expressed more of greenish yellow color. Conclusion: There is a definite altered staining reaction noticed in the fibres of radicular and the odontogenic keratocyst which suggests that there is a possibility of a biochemical and morphological change in the constituent of the fibres in these cyst groups which could be responsible for their behavior.

INTRODUCTION
The jaws are a host to wide variety of cysts and neoplasm due to the complex distribution of tissues involved in tooth formation. The tumors and cysts of both odontogenic and nonodontogenic origin exhibit a biologically aggressive course which can result in destruction of bone. Cysts like odontogenic keratocysts with different behavioral patterns, aggressive growth with increased recurrence and its association with other syndromes had been the centre of many studies. Various theories have been put forward to explain the expansion of the cysts including active cell division of the epithelial lining, expansion from the hydrostatic pressure of cystic contents, Prostaglandins, IL-1, leucotrins in the cyst wall which allows bone resorption during cyst expansion, and also the collagenolytic activity of the cyst wall which is more evident in the odontogenic keratocyst which is considered to be one of the reason for its incomplete removal of the cyst and also for a possible role in recurrence. Recent studies have also tried to focus on the importance of the epithelial - mesenchymal interaction in the expansion of the odontogenic cysts. Even though immunohistochemistry and molecular techniques have been widely used for investigative procedures, they have had a very little impact in demonstrating the cystic fibres and traditional histopathology continues to be the mainstay for the diagnosis together with the histochemical stains.

Collagen, the most abundant protein in the body is responsible for maintaining the functional integrity of tissues including the odontogenic apparatus and was thought that it might also play a role in pathogenesis and expansion of odontogenic cyst. Many histochemical stains have been used to demonstrate collagen fibres like Van Gieson, Masson’s trichrome, Weigert’s Resorcin Fushsin, modified Movat’s stain, Goldner’s Trichrome method, Wilder Modification of Bielschowsky’s method etc., but the picrosirius red stain under the polarizing microscope is the most widely used because of the inherent nature of birefringence of collagen.

The Picrosirius red stained slides, under the polaroid microscopy had been used to demonstrate various dental structures including mandible, teeth, dentinal tubules, and the collagen fibres. It was also used to assess various pathologic lesions including oral mucosal irritation fibroma, thyroid follicular carcinoma, ameloblastic fibroma, odontogenic fibroma, hyperplastic dental follicle etc. Keeping this in view, a study was taken up to show the role of the picrosirius red stain to demonstrate the fibres and to...
assess the difference in the nature of the fibres among the odontogenic cysts and to find out the role of it, if any in the pathogenesis of the cysts. Further, the effect of inflammation on the thickness of the collagen fibres was also assessed.

MATERIALS & METHODS
The study was conducted in the department of Oral Pathology and Microbiology of Mahatma Gandhi Post Graduate Institute of Dental Sciences, Puducherry, India. Histopathologically confirmed 49 cases of Odontogenic cysts were retrieved: 15 Radicular cysts (RC), 15 Dentigerous cysts (DC), 14 Odontogenic Keratocysts (OKC) and 05 Calcifying Odontogenic Cysts (COC) were included as the study material. For the standardization purpose, five mucosal tissues were included to assess the color taken up by the normal fibres. Sections of 5 µm thickness were made from each block for both the study and control groups. They were stained with both Hematoxylin and Eosin and by modified Picrosirius red procedure. H& E stained section was assessed for the diagnosis of the cyst and degree of inflammation which was categorized as mild, moderate and severe. The picrosirius red stained section was assessed using the ocular micrometer under oil immersion; the thickness of the fibres along with the color exhibited by them were noted. Around 50 fibres were measured in each of the control and study groups and was categorized as thin (< 0.8 µm) and thick fibres (1.2 µm - 2.4 µm) and the color of fibres were categorized as greenish yellow or orange red. Overlapping of the colors or the thickness of fibres was not taken into account.

Statistical analysis was done to determine the significance of these findings observed between the thickness of the fibres among the cyst groups and to see the effect of inflammation on the color of the fibres. Mean and standard deviation were calculated for the individual groups. They were compared using the following test of significance: T test, F test, Chi-square test.

RESULTS
The sections stained with picrosirius red were examined under 100x in oil immersion and the following findings were observed: the effectiveness of picrosirius red stain in demonstrating the collagen fibres, the thickness of collagen and its corresponding color in both the study and the control groups and the effect of inflammation on the color of the fibres in the study groups.

The normal mucosa showed that almost 83.2% of the thin fibres showed greenish yellow color and among the thick fibres 84% showed orange red color.

In the four study groups compared, overall majority of the thin fibres showed generalized distribution of greenish yellow color and orange red color for the thick fibres - Graph 1 & 2.

In the Radicular cysts, 80.27% the thin fibres were Greenish yellow in color and 78.4% of the thick fibres were predominantly Orange red in color.
Role Of Collagen Fibres In The Expansion Of Odontogenic Cysts – A Histochemical Study.

Figure 3

Note the generalized distribution of collagen fibres with orange red color in the radicular cyst. (10x)

In Dentigerous cysts 90.93% of the thin fibres showed greenish yellow color and of the thick fibres, 69.87% showed orange-red color.

Figure 4

In Odontogenic Keratocysts, 91.43% of the thin fibres had taken up greenish yellow color and among the thick fibres, only 58.58% of the fibres showed orange red color.

Figure 5

Note the generalized distribution of fibres with greenish yellow in odontogenic keratocyst (10x)

In calcifying odontogenic cysts, among the thin fibres, 91.2% showed greenish yellow color and in the thick fibres, 66.4% of the fibres showed orange red color.

Figure 6

It was noticed that in the radicular cysts, the generalized distribution of fibres were more of orange red and in odontogenic keratocyst it was more of greenish yellow when compared with other odontogenic cysts.

Results were compared for the colors expressed by the thin and thick fibres among all the cyst groups (Tables 1&2).
TABLE 1: RESULTS WITH RESPECT TO THIN FIBRES:
Comparison of mean thin fibres among the four cyst groups based on colors

<table>
<thead>
<tr>
<th>FIBRE COLOR</th>
<th>RC</th>
<th>OKC</th>
<th>DC</th>
<th>COC</th>
<th>F-VALUE</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GREENISH YELLOW</td>
<td>20.07±3.87</td>
<td>22.86±1.92</td>
<td>22.73±2.86</td>
<td>22.80±1.64</td>
<td>3.52</td>
<td>0.0224</td>
</tr>
<tr>
<td>ORANGE RED</td>
<td>4.93±3.37</td>
<td>2.34±1.92</td>
<td>2.77±2.84</td>
<td>2.26±1.64</td>
<td>3.52</td>
<td>0.0224</td>
</tr>
</tbody>
</table>

Comparison was also made to check for any significant differences among two cyst groups. In relation to the thin fibres it was found that significant differences were noted only when radicular cyst was compared with dentigerous and odontogenic keratocysts; and among the thick fibres significant difference was seen between radicular cysts and odontogenic keratocysts.

The influence of inflammation on the color of the fibres was also assessed for all the study groups and it was seen that the inflammation within the cyst wall did not show much of significant difference among the cyst groups (Table 3-6).
Role Of Collagen Fibres In The Expansion Of Odontogenic Cysts – A Histochemical Study.

Figure 11
TABLE 5- ASSOCIATION BETWEEN FIBRE COLOR AND LEVEL OF INFLAMMATION IN DENTIGEROUS CYSTS

<table>
<thead>
<tr>
<th>INFLAMMATION</th>
<th>AVERAGE THIN FIBRES</th>
<th>AVERAGE THICK FIBRES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Greenish yellow</td>
<td>Orange red</td>
</tr>
<tr>
<td>Mild</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>Moderate</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>Severe</td>
<td>24</td>
<td>1</td>
</tr>
</tbody>
</table>

Chi square value: 1.36, 2.05
P value: 0.5117, 0.0588

Figure 12
TABLE 6- ASSOCIATION BETWEEN FIBRE COLOR AND LEVEL OF INFLAMMATION IN CALCIFYING ODONTOGENIC CYSTS

<table>
<thead>
<tr>
<th>INFLAMMATION</th>
<th>AVERAGE THIN FIBRES</th>
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</tr>
<tr>
<td>Moderate</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>Severe</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Chi square value: 1.53, 0.41
P value: 0.6653, 0.8146

DISCUSSION

Earlier, extensive research works were done on the epithelial component of the odontogenic cyst to explain its role in the expansion of these cysts without the involvement of its non epithelial component. It was Vedtofte et al who showed the importance of stromal component from a study on the 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 interaction. The possibility of primary defect in OKC in the mesenchymal capsule rather in epithelial cells was mooted by Browne.

Picrosirus red stain with polarizing microscopy had been used to study the individual collagen fibres and the color exhibited by these fibres depends on fibre size, alignment and packing of fibres, molecular organization, ground substances and water content. Normally, green to greenish yellow corresponds poorly packed fibres, whereas orange red represents well packed fibres.

Junqueira et al stated that under pathological conditions birefringence shows a different pattern in comparison with collagen in normal tissue and proved that type I collagen were thick, strongly birefringent red fibres while type III collagen appeared as thin weakly birefringent green fibres. Studies done in Sub mucous fibrosis also revealed that there was a gradual decrease in the greenish yellow color of the fibers and a shift to orange red color with increase in severity of the disease which appeared that the tight packing of collagen fibers in OSMF progressively increased as the disease progressed from early to advanced stages.

The predominance of the greenish yellow color in odontogenic keratocyst in our study show that the fibres are loosely packed and could be composed of procollagen, intermediate or pathological collagen when compared to the other odontogenic cysts; this result goes in accordance to the study by Hirshberg et al. Several authors have attempted to correlate the behavior the OKC with production of collagenase, prostaglandins and highly oxidative enzymes which probably explains the defective collagen which are at the process of degradation and also to the high level of amino peptidase which degrade the fibres.

Generally, the radicular cyst being inflammatory in origin shows dense fibrosis compared to other odontogenic cysts which could be because of the release of cytokines and growth factors that promotes fibroblastic proliferation and its functional outcome namely the extracellular matrix and possibly the fibres as well, which occurs as a result of inflammation.

With regard to inflammation our study showed that inflammation in the cyst wall did not have any significant effect on the color of the fibres in the study groups which could be due to the fact that baring the radicular cyst other odontogenic cyst groups did not show active inflammatory features. Whereas, the study by Hirshberg et al showed that inflammation had a direct influence on the color of the fibres.
Role Of Collagen Fibres In The Expansion Of Odontogenic Cysts – A Histochemical Study.

in the cysts.

From previous studies as well as from our present study, we can suggest that the connective tissue stroma of odontogenic keratocysts seem to have an altered quality of collagen fibres, which directly or indirectly might influence the expansion of the cyst. To summarize, the following findings were observed in our study: the Picrosirius – Polarization method is useful in assessing the thickness and orientation of collagen fibres in a cyst, the thin fibres were predominantly greenish yellow whereas the thick fibres were orange red in color both in the study and control groups, in the radicular cysts, even the thin fibres expressed more of orange red color and in odontogenic keratocyst, the thick fibres expressed more of greenish yellow color which only indicates that there is a certain amount of alteration in constituent and make up collagen fibres and the inflammation did not seem to have an effect on the color of the fibres.

But 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
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