The Capsular Connective Tissue Stroma Plays a Vital Role in the Behaviour of Odontogenic Cysts: A Histochemical Study

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ABSTRACT

Introduction: Odontogenic cysts are among the most frequent destructive benign lesions of jaws. The most common odontogenic cysts are radicular cysts, dentigerous cysts and odontogenic keratocyst. Many efforts have been carried out to understand the pathogenesis of jaw cysts, but many of those have been unsuccessful. Also, WHO has classified odontogenic keratocyst as an odontogenic tumor because of its growth potential. There is this hypothesis that the more aggressive behavior of odontogenic keratocysts is related at least, partly, to distribution of mast cells. The present study was undertaken to correlate and compare the pattern of collagen fibers and glycosaminoglycans in odontogenic cyst and also to find out if this approach could be used to predict the aggressive nature and its role in expansion of cyst. Material and Methods: Ten formalin-fixed paraffin embedded tissue blocks each of odontogenic keratocyst, dentigerous and radicular cysts were selected. Tissue sections were cut and were stained with Alcian Blue-PAS stain was for staining glycosaminoglycans and Verhoeff Van Gieson stain to detect collagen fibers. The stained sections were evaluated for variations in the structure of the ground substance and the collagen fibers in the different cysts. Results: The connective tissue capsule of the odontogenic keratocyst showed a A variation in the connective tissue capsule of different odontogenic cysts is expected. Conclusions: Increased glycosaminoglycans content and more loose collagen fibers was found in case of OKC as compared to other two entities. Thus, the OKC exhibits differing collegenous and ground substance patterns which probably relate to the aggressiveness of this cysts.

KEYWORDS: OKC, Dentigerous cyst, Radicular cyst, Verhoeff Van Gieson, Collagen fibers, Glycosaminoglycans

INTRODUCTION

Cysts constitute about 17% of all the tissue specimens submitted to oral pathology biopsies service. The most common odontogenic cyst is the radicular cyst (52.3–70.7%) followed by the dentigerous cyst (16.6–21.3%) and odontogenic keratocyst (5.4–17.4%).

Degranulating mast cells release heparin and other hydro discative enzymes which facilitate breakdown of glycosaminoglycans and proteoglycans. The release of glycosaminoglycans and proteoglycans into the luminal fluid contributes significantly to osmotic and hydrostatic pressure by increasing the osmolality of the cyst fluid thereby raising the internal hydrostatic pressure. Collagen, the most abundant protein in the body is responsible for maintaining the functional integrity of tissues including the odontogenic apparatus and thought was to play a role in pathogenesis and expansion of odontogenic cysts.

A radicular cyst is generally defined as a cyst arising from epithelial residues (cell rests of Malassez) present in the periodontal ligament. This death of the dental pulp stimulates an inflammatory response in the periodontal tissue which results in the formation of this. OKCs are relatively common developmental odontogenic cysts and account for 10–12% of all jaw cysts. OKCs usually occur in the second and the third decades of life. This cyst is more common in males than in females (1.3:1). A majority of OKCs occur in the body of the mandible. The most common site of occurrence for this cyst is molar region of the mandible and the vertical ramus. The dentigerous cyst is one of the second most common jaw bone cyst (15.7%), this finding is in agreement with that conducted by Mourshed and Daley et al. The lesion occurs most often in the second and third decades of life.

The present study will be undertaken to correlate and compare the pattern of collagen fibers and glycosamino-
glycans in odontogenic cysts and also to find out if this approach could be used to predict the aggressive nature and its role in expansion of cyst.

**MATERIALS & METHODS**

The study was approved by the institutional ethical committee. Formalin fixed paraffin embedded tissue blocks of histological confirmed cases of dentigerous cyst (n=10) odontogenic keratocyst (n=10) and radicular cyst (n=10) were retrieved from the departmental archives. Two sections of normal buccal mucosa served as controls for the staining procedures.

**Alcian Blue Staining Protocol for Glycosaminoglycans**

**Fixation:** 10% Formalin. Paraffin sections at 5 um.

**Procedure:** Deparaffinize slides and hydrate to distilled water. Stain in alcian blue solution for 30 minutes. Wash in running tap water for 2 minutes. Rinse in distilled water. Counterstain in nuclear fast red solution for 5 minutes. Wash in running tap water for 1 minute. Dehydrate and through 95% alcohol, 2 changes of absolute alcohol, 3 minutes each. Clear in xylene or xylene substitute. Mount the sections with resinous mounting medium.

**Results:** The tissues containing acidic sulfated mucosubstances – Blue, Nuclei - pink to red, Cytoplasm - pale pink.

**Evaluation of Glycosaminoglycans:** The location, type and staining intensity of glycosaminoglycans in the subepithelial, intermediate and the outer (peripheral) zones of the capsule is examined. To eliminate the subjective bias 2 observers independently evaluate all the slides.

**Verhoeff Van Gieson Staining Protocol for Collagen Fiber**

**Fixation:** 10% formalin. Paraffin sections at 5 um.

**Procedure:** Deparaffinize and hydrate slides to distilled water. Stain in Verhoeff’s solution for 1 hour. Tissue should be completely black. Rinse in tap water with 2-3 changes. Differentiate in 2% ferri chloride for 1-2 minutes. Stop differentiation with several changes of tap water and check microscopically for black elastic fiber staining and gray background. Wash slides in tap water. Treat with 5% sodium thiosulfate for 1 minute. Discard solution. Wash in running tap water for 5 minutes. Counterstain in Van Gieson’s solution for 3-5 minutes. Dehydrate quickly through 95% alcohol, 2 changes of 100% alcohol. Clear in 2 changes of xylene for 3 minutes each. The sections should be mounted with a resinous mounting media and a coverslip should be placed on the tissue sections.

**Evaluation of collagen fibers:** The location, type and staining intensity of collagen fibers in the subepithelial, intermediate and the outer (peripheral) zones of the capsule is examined. To eliminate the subjective bias 2 observers independently evaluate all the slides.

**Results:** Elastic fibers – blue-black to black, Nuclei – blue to black, Collagen – red, Other tissue elements – yellow. Also assessment for Alcian Blue-PAS stain done:

0-Negative Staining; 1-Faint Staining; 2-Strong Staining

**Statistical analysis:**

Statistical analysis was carried out using SPSS package version 16. Kruskal Wallis Test was carried out where P value<0.05 is significant, P<0.005 is highly significant.

**MATERIALS & METHODS**

In dentigerous cysts, among 10 cases which were taken, staining intensity for glycosaminoglycans (Alcian blue-PAS) (Figure 1a and 1b) was faint in subepithelial, intermediate and outer zone. Collagen fibers (Verhoeff Van Gieson) were mostly dense in subepithelial, intermediate & outer zone. Comparison of staining intensity of the Alcian blue – PAS stain and the collagen structure as seen in Verhoff’s Van Gieson was done, in which the association of the subepithelial and intermediate zone was not significant but that of the outer zone was significant (Table 1).

In radicular cysts, among 10 case which were taken staining intensity for glycosaminoglycans (Alcian blue-PAS) (Figure 2a and 2b) was faint in subepithelial, intermediate and outer zone. Collagen fibers (Verhoeff Van Gieson), were loose in subepithelial, dense in intermediate & loose in outer zone. Comparison of staining intensity of the Alcian blue – PAS stain and the collagen structure as seen in Verhoff’s Van Gieson was
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In odontogenic keratocysts, staining intensity for glycosaminoglycans (Alcian blue-PAS) (Figure 3a and 3b). done, in which the changes in the subepithelial and intermediate zone was not significant but that of the outer zone was significant (Table 2).

Table 1: Comparison of staining intensity of the Alcian blue – PAS stain and the collagen structure as seen in Verhoff’s Van Gieson stain among the dentigerous cysts

<table>
<thead>
<tr>
<th>Cyst</th>
<th>Sample Size</th>
<th>Zones</th>
<th>Alcian Blue-PAS Stain</th>
<th>Verhoeff Van Gieson</th>
<th>Total</th>
<th>Chi Square</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dentigerous Cyst</td>
<td>10</td>
<td>Subepithelial</td>
<td>0 1 1 2</td>
<td>1 4 2 6</td>
<td>10</td>
<td>4.002</td>
<td>0.444</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intermediate</td>
<td>0 2 1 3</td>
<td>1 5 1 6</td>
<td>10</td>
<td>0.432</td>
<td>0.3</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Outer</td>
<td>0 1 0 1</td>
<td>1 4 4 8</td>
<td>10</td>
<td>1.001</td>
<td>0.04</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Assessment for Alcian Blue-PAS: 0-Negative Staining; 1-Faint Staining; 2-Strong Staining. Kruskal Wallis Test: P<0.05 is significant, P<0.005 is highly significant. Dense – Dense collagen fibers; Loose – Loose collagen fiber

Table 2: Comparison of staining intensity of the Alcian blue – PAS stain and the collagen structure as seen in Verhoff’s Van Gieson stain among the Radicular cysts

<table>
<thead>
<tr>
<th>Cyst</th>
<th>Sample Size</th>
<th>Zones</th>
<th>Alcian Blue-PAS Stain</th>
<th>Verhoeff Van Gieson</th>
<th>Total</th>
<th>Chi Square</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radicular Cyst</td>
<td>10</td>
<td>Subepithelial</td>
<td>0 1 0 1</td>
<td>1 0 7 7</td>
<td>10</td>
<td>3.554</td>
<td>0.3</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intermediate</td>
<td>0 1 0 1</td>
<td>1 4 3 7</td>
<td>10</td>
<td>2.444</td>
<td>0.23</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Outer</td>
<td>0 0 0 0</td>
<td>1 4 4 8</td>
<td>10</td>
<td>0.999</td>
<td>0.031</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Assessment for Alcian Blue-PAS: 0-Negative Staining; 1-Faint Staining; 2-Strong Staining. Kruskal Wallis Test: P<0.05 is significant, P<0.005 is highly significant. Dense – Dense collagen fibers; Loose – Loose collagen fiber
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3b) was faint in subepithelial & intermediate zone and strong in the outer zone. Collagen fibers (Verhoeff Van Gieson) were mostly loose in subepithelial, intermediate and outer zone. Comparison of staining intensity of the Alcian blue – PAS stain and the collagen structure as seen in Verhoff’s Van Gieson was done, in which association at subepithelial zone was not significant, intermediate zone was significant and outer zone was highly significant (Table 3).

### DISCUSSION

Radicular cysts are the most common odontogenic cystic lesions of inflammatory origin affecting the jaws. They are frequently found at the apices of the involved teeth. However, they may also be found on the lateral aspects of the roots in relation to lateral accessory root canals. In our study we glycosaminoglycans took up a weak stain while the collagen fibers were dense fibers in this cyst. The odontogenic keratocyst (OKC) is a developmental lesion of that arises from the dental lamina. This lesion was first described in 1956 by Phillipsen. It is one of the most aggressive odontogenic cysts of the oral cavity. OKC is known for its rapid growth and its tendency to invade the adjacent tissues including bone. The recurrence rate for this cyst is high and it is frequently associated with the basal cell nevus syndrome. The cyst usually presents in males with a male to female ratio of 1.6:1. OKCs may occur in any part of the upper or lower jaw.

#### Table 3 – Comparison of staining intensity of the Alcian blue – PAS stain and the collagen structure as seen in Verhoff’s Van Gieson stain among the Odontogenic Keratocysts

<table>
<thead>
<tr>
<th>Cyst</th>
<th>Sample Size</th>
<th>Zones</th>
<th>Alcian Blue-PAS Stain</th>
<th>Verhoeff Van Gieson</th>
<th>Total</th>
<th>Chi-Square</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odontogenic Keratocyst</td>
<td>10</td>
<td>Subepithelial</td>
<td>0 1 1 2</td>
<td></td>
<td>10</td>
<td>3.114</td>
<td>0.2</td>
<td>Not significant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intermediate</td>
<td>0 2 1 3</td>
<td></td>
<td>10</td>
<td>0.333</td>
<td>0.001</td>
<td>Significant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Outer</td>
<td>0 0 1 1</td>
<td></td>
<td>10</td>
<td>0.771</td>
<td>0.002</td>
<td>Highly Significant</td>
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</tr>
</tbody>
</table>

Assessment for Alcian Blue-PAS: 0-Negative Staining; 1-Faint Staining; 2-Strong Staining. Kruskal Wallis Test: P<0.05 is significant, P<0.005 is highly significant. Dense – Dense collagen fibers; Loose – Loose collagen fibers.

Figure 3a – Odontogenic Keratocyst shows strong staining with Alcian Blue-PAS stain (20X)

Figure 3b – Odontogenic Keratocyst shows loose collagen fibers with Verhoeff Van Gieson (10X)
A majority of them occur in the mandible, mostly in the angle of the mandible and ramus. The OKC involves approximately 11% of all cysts in the jaws and is most often located in the mandibular ramus and angle. This lesion is frequently associated with an impacted third molar. In our study, OKC exhibits differing collagenous and ground substance patterns which probably relates to the aggressiveness of this cysts. Dentigerous cysts usually occur in young patient, under the age of 30 years. However, Brown conducted a study on 81 diagnosed dentigerous cysts and found a higher prevalence in the fifth decade of life. Main, and Angela and Mario reported that the common site for the dentigerous cyst was the mandibular third molar area.

Clinically, dentigerous cyst occur most often as a painless intra-oral alveolar a swelling, sometimes the cyst is associated with pain. Tooth mobility and displacement were occasionally observed. Recurrence of dentigerous cyst is rare. According to Main, only one case was recurred within a period of 12 years after treatment. The histological features of our dentigerous cyst are similar to those seen elsewhere.

Kim and Ellis showed that the dentigerous cyst may be lined by a stratified squamous epithelium especially in older patient. Stanley et al in their study, found that all follicles of patients older than 26 years were lined by squamous epithelium rather than cuboidal to columnar cell or reduced enamel epithelium. This confirm by our finding in which the lining epithelium shows squamous metaplasia in the older patient. Furthermore, the dentigerous cyst may give rise to a variety of tumors, notably ameloblastoma, squamous cell carcinoma, mucoepidermoid carcinoma, and rarely other tumors. This confirm by our finding in which we found less staining of glycosaminoglycans and more dense collagen fibers which states less aggressiveness of the cyst.

The ground substance of connective tissue is rich in proteoglycans and glycosaminoglycans (acid mucopolysaccharides) and these molecules have been demonstrated in odontogenic cyst capsules both by histochemistry with Alcian blue under controlled conditions of pH and critical electrolyte concentration and also biochemically by their extraction from the tissue and subsequent electrophoretic examination. Degradation of this connective tissue will occur during normal metabolic turnover. Further breakdown will arise due to enzymes released as part of the process of inflammation and possibly from bacteria in the cyst lumen. As a consequence glycosaminoglycans and proteoglycans maybe released and subsequently diffuse into the cyst lumen which is also shown in our study. Skaug and Hofstadm were the first to report the presence of glycosaminoglycans in the fluids of non-keratinizing odontogenic cysts, which were observed to distort the protein patterns in cellulose acetalde electrophoresis. Further study of these molecules has revealed that both keratinizing and non-keratinizing odontogenic cyst fluids contain appreciable amounts of hyaluronic acid and chondroitin-4-sulfate with lesser amounts of the other glycosaminoglycans. A considerable proportion of the glycosaminoglycans of the fluids appeared to be complexed with protein as proteoglycan and work in our laboratory has shown that some of this material is of high molecular weight, of the order of 350,000 Da or greater. Since the size of glycosaminoglycans and proteoglycans in connective tissues is generally between 100,000 and several million Da, it is possible that some of those in cyst fluids may have arisen from sources other than the degradation of the connective tissue. Mucous metaplasia of the epithelial lining is common in some types of odontogenic cysts. Alcian blue histochemistry suggests that these cells could contribute to the glycosaminoglycans present in the fluid. The levels of glycosaminoglycan are not as high in odontogenic keratocyst as radicular and dentigerous cyst fluids. Inflammation is not generally regarded as a prominent feature of odontogenic keratocysts, although foci of inflammation can be seen in their capsules. Thus the release of glycosaminoglycan from the fibrous tissue as a result of inflammation and their diffusion into the luminal fluid would not be expected to occur to such an extent in these cysts. However, lactoferrin, a constituent of neutrophils, is present in higher quantities in odontogenic keratocyst fluids than in those of other types of odontogenic cyst. The co-presence of lactoferrin with elastase in cyst fluids and its immunocytotoxic detection only in neutrophils in cyst capsules is interesting in view of the developmental origin of odontogenic keratocysts. The greater concentration of lactoferrin in the fluids of odontogenic keratocysts may, however, be due to factors other than a greater release from neutrophils. For example, lactoferrin may be less able to diffuse out through the linings of odontogenic keratocysts, its half-life may be prolonged by factors in their cyst fluids and or the antigenic sites on lactoferrin molecules may be less masked by the lower concentrations of proteins present in these fluids.

A higher incidence and abundance of heparin has also been observed in the fluids of odontogenic keratocyst compared with those from other types of odontogenic cyst. Its origin is probably from the degranulation of mast cells releasing heparin, among other components, into the cyst capsule and lumen. Mast cells are present in appreciable numbers in the capsules of odontogenic cysts and appear to be equally prevalent in all types of odontogenic cyst. These cells appear to be more prevalent beneath the epithelium compared with the outer parts of the capsule and the enhanced enzymic degradative activity observed in this area of the odontogenic keratocyst capsule could promote mast cell degranulation and the appearance of heparin in the luminal fluid. On degranulation of mast cells, dissociation of heparin proteoglycan may provide a mechanism for activation of granule associated proteases. A variety of hydrolytic enzymes, including trypstat, arylsulfatase, hexosaminidase, B-glucuronidase and B—galactosidase, are released on degranulation of...
Such enzyme activities are part of the inflammatory response, thereby facilitating connective tissue degradation and release of components such as glycosaminoglycans and proteoglycans. Histamine release would also increase vascular permeability, thus permitting the emigration of greater numbers of white blood cells and the exudation of more serum proteins into the luminal fluid. The presence of glycosaminoglycans and proteoglycans together with serum proteins in cyst fluids would be expected to contribute significantly to the osmolality. Glycosaminoglycans such as hyaluronic acid have been demonstrated to increase the osmotic pressure in other fluids, e.g. synovial fluid. The effect of glycosaminoglycans would be enhanced by the presence of albumin in cyst fluids, which has been demonstrated to have a synergistic effect with hyaluronic acid on osmotic pressure. There is considerable variation in the glycosaminoglycan levels of cyst fluids and it is possible that the concentration increases with the age of the cyst. In view of the limited lymphatic drainage from odontogenic cysts, an accumulation of molecular components such as glycosaminoglycans might occur with time and thus long-standing cysts contain higher levels of glycosaminoglycans in the fluid. Clearly, any tendency to an increase in concentration with time would be counter balanced by the presence of enzymes which might lead to the breakdown of these molecules.

Collagenolytic activity has been demonstrated in explants and tissue cultures of odontogenic keratocyst walls. Another study has shown this activity in homogenates of the cyst walls from both odontogenic keratocysts and radicular cysts. Collagenase in the extract of radicular cyst capsu...
18. Investigative Pathology of Odontogenic Cysts By Roger M. Browne

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Conflict of Interest: Nil