Comparative evaluation and Immunohistochemical expression of Syndecan-1 in Ameloblastoma and Dentigerous cyst

Dr. Shraddha Walekar Ghaisas  
Assistant Professor, Dept. of Oral & Maxillofacial Pathology, MGM’s Dental College & Hospital, Navi-Mumbai  
Email: shraddhawalekar7@gmail.com

Dr. Kishor K Patil  
Assistant professor Dept. of Oral & Maxillofacial Pathology, SMBT Dental College & Hospital, Sangamner  
Email: drpatilkishor1@gmail.com

Dr. Ashok V Patil  
Professor, HOD and Dean, Dept. of Oral & Maxillofacial Pathology, SMBT Dental College & Hospital, Sangamner

Dr. Suyog D Tupsakhare  
Professor, Dept. of Oral & Maxillofacial Pathology, SMBT Dental College & Hospital, Sangamner  
Email: drsuyesh1310@gmail.com

Dr. Ketan U Saraf  
Assistant Professor, Dept. of Oral & Maxillofacial Pathology, SMBT Dental College & Hospital, Sangamner  
Email: drketansaraf@gmail.com

Dr. Prasad P Karande  
Professor and Head Dept of Oral Pathology and microbiology, D.Y. Patil Dental School, Lohegaon, Pune  
Email: prasad352627@rediffmail.com

Dr. Pooja Bhagwat  
Assistant Professor, Dept. of Oral & Maxillofacial Pathology, Vyws Dental College and Hospital, Amravati  
Email: Poojabhagwat1985@gmail.com

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.  
Corresponding author: Ghaisas, S.W.; Email: shraddhawalekar7@gmail.com  
Manuscript submitted: 09 Feb 2022, Manuscript revised: 27 March 2022, Accepted for publication: 18 April 2022  
5614
**Abstract**---Background: Syndecans are type-1 heparan sulphate proteoglycans which play significant role in cell-cell and cell-extracellular matrix interaction. Syndecans are involved in tooth development and differentiation of mesenchymal cells. Amongst odontogenic lesions, ameloblastomas and dentigerous cysts are routinely encountered lesions with difference in treatment modality based on its aggressiveness. The objective of the present research was to study and compare immunohistochemical expression of syndecan-1 in ameloblastoma and dentigerous cyst.

Method: 40 retrospectively diagnosed cases of ameloblastomas and dentigerous cysts were immunohistochemically stained against syndecan-1. The intensity of immunostaining and percentage of positive cells was assessed by three independent blind observers. Weighted kappa test was used to find out inter-observer reliability. Comparative evaluation of syndecan-1 expression between the two lesions was done using student t-test. Results: There was statistically significant difference between the mean of score for intensity, mean of score for percentage of positive cells and total mean score of syndecan-1 between ameloblastoma and dentigerous cyst. Conclusion: Syndecan-1 may be involved in aetiopathogenesis of odontogenic lesions like ameloblastoma and dentigerous cyst. Also, weak expression in ameloblastoma indicates that tumor invasion and aggressiveness is related to cell adhesion molecule like syndecan-1. Additional studies are required with recurrent cases of ameloblastomas along with clinical follow-up.

**Keywords**---Ameloblastoma, Dentigerous cyst, Immunohistochemistry, Odontogenic tumors, Syndecans.

**Introduction**

Syndecans (CD138) are type-I transmembrane heparan sulphate proteoglycans consisting of four family members in vertebrates. Syndecan-1 is expressed in epithelial cells and fibroblasts, Syndecan-2 in cells of mesenchymal origin, syndecan-3 in neuronal tissue whereas type 4 exhibits much broader distribution. Syndecans are involved in inflammation, wound healing, development and progression of tumor.[1] Syndecans play significant role during tooth development and are involved in differentiation of mesenchymal cells.[2] (vainio 1989).

Odontogenic tumors includes malignant and benign neoplasms to dental hamartomas arising from odontogenic epithelia and/or ectomesenchyme. Ameloblastoma is a slowly growing, locally infiltrating, second most common epithelial odontogenic tumor of the jaw with high recurrence rate.[3] Similar to ameloblastoma, dentigerous cyst is the most common developmental odontogenic cyst with the potential to become aggressive.[4] Though dentigerous cyst is asymptomatic, at times, it causes cortical expansion and erosion. In tumors of epithelial origin like ameloblastoma, loss of cell adhesion molecules like syndecan results in invasion and growth of tumor cells. Thus, the main purpose of the
research was to evaluate and compare immunohistochemical expression of syndecan-1 in ameloblastoma and dentigerous cyst and its role in aggressiveness of both the lesions.

**Materials and Methods**

Forty samples of optimally formalin fixed, paraffin embedded, histopathologically proven cases of ameloblastomas (n=40) and dentigerous cysts (n=40) were selected from the archives of the institution. Ameloblastoma was not subclassified histologically to avoid confusion. Out of forty samples of ameloblastomas, four samples comprised of recurrent cases.

**Immunohistochemical procedure**

3µm thick sections each of forty samples of ameloblastomas and dentigerous cysts were mounted on aminopropyl tri-ethoxy silane coated microscopic slides after drying at room temperature, sections were incubated at 60°C for 1 hour. The sections were deparaffinised, cleared in xylene and rehydrated through descending grades of alcohol. Antigen retrieval procedure was carried out using EDTA solution in conventional pressure cooker at 100°C for one hour. For every cycle of antigen retrieval, one section of normal oral mucosa (associated with impacted third molar during surgical removal) was used as a positive control and treated similarly as the test sample. The slides were transferred to humidifying chamber and treated with peroxidase block for 5 mins, protein block for 5 mins. The sections were then incubated for 60 mins at room temperature with primary monoclonal antibody syndecan-1(Product code: RTU-CD138-MI15). A negative control was performed by omitting the step of primary antibody during the staining.

**Evaluation of tissue samples**

For the evaluation of Syndecan-1, three areas rich in lesional cells of ameloblastoma and cystic lining of dentigerous cyst were selected. Syndecan-1 expression was considered positive when immunoreaction was observed in the cell membrane of epithelial cells of normal oral mucosal membrane and odontogenic epithelial cells. (Figure.1) The immunoreactivity was scored using 4-grade scoring criteria:

- a) the intensity of the immunostaining in the epithelial odontogenic cells (0=absent, 1=weak, 2=moderate, 3=strong, and 4=very strong).
- b) the percentage of positive odontogenic cells (0=0% positive cells, 1=less than 25% positive cells, 2=25-50% positive cells, 3=50-75% positive cells, and 4=greater than 75% positive cells). The final immunostaining score was determined by the sum of (a) + (b). Final scores ranged from 0 to 8 (0=absent, 1-4=weak, and 5-8=strong). Mean of the three fields was estimated for each sample and considered as the final score for that sample. The scoring criteria was according to TjoeKC.[5]
**Statistical analysis**

Evaluation of syndecan-1 expression by epithelial odontogenic cells, in three fields in each slide of dentigerous cyst and ameloblastoma, was done by three blind independent observers. Inter-observer reliability of three observers for expression of syndecan-1 was found using weighted kappa test. The guidelines for interpreting kappa value were based on Landis and Koch criteria.[6] (Landis and Koch who characterized values <0 as indicating no agreement and 0-0.20 as slight, 0.21-0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1 as almost perfect agreement). Comparison of mean scores between study groups (Dentigerous cyst and Ameloblastoma) was done using student t test.

**Results**

Expression of syndecan-1 in dentigerous cyst: Out of forty dentigerous cysts, twenty-six showed strong expression of syndecan-1 in both basal and suprabasal layers (Figure.2). Weak expression was observed in remaining cases.

Expression of syndecan-1 in ameloblastoma: On the contrary, thirty-six ameloblastomas showed weak expression of syndecan-1 whereas no expression was observed in four recurrent cases of ameloblastoma. Expression was low in peripheral or tall columnar cells as compared to central stellate reticulum like cells (Figure 3 and Figure .4). Stromal cells were positive for syndecan-1 in few cases of both the lesions.

**Evaluation of syndecan-1 expression in ameloblastoma and dentigerous cyst**

According to Landis and Koch criteria, there was substantial agreement for mean intensity, moderate for mean percentage of positive cells and total mean for syndecan-1 in ameloblastoma. The representation of corresponding data is shown in graph.1, table.1. There was substantial agreement for mean intensity, mean percentage of positive cells and total mean score for syndecan-1 expression in dentigerous cyst. The representation of the corresponding data is shown in graph. 2 and table.2.

**Comparison between expression of syndecan-1 in ameloblastoma and dentigerous cyst**

The total final score of syndecan-1 in ameloblastoma was less as compared to syndecan-1 in dentigerous cyst. On comparison of expression between syndecan-1 in ameloblastoma and dentigerous cyst, using student t-test, it was found to be statistically significant different between the respective lesions. (P=0.022 for intensity, P=0.024 for percentage of positive cells, P= 0.031 for total final score). The representation of the corresponding data is shown in graph 3, table 3. *p <0.05 significant †Student t-test

**Discussion**

Various factors like clonality, apoptosis, cell cycle proliferation, osteoclastic mechanism, matrix metalloproteinases, ameloblastin, enamel matrix proteins,
tumor suppressor genes, dysregulation of Sonic hedgehog, smoothened and PTCH1 gene, BRAF V600 play role in pathogenesis of ameloblastoma. [7,8] Similarly, loss of cell adhesion molecules like cadherin and syndecan results in invasion and growth of epithelial tumors.[9] Syndecan-1 consists of 310 amino acids long core protein, transmembrane domain, extracellular domain with attached GAG (glycosaminoglycans) side chains. They exert main action through GAG side chains. Syndecan -1 is present mostly on basolateral surfaces of epithelial cells in adults. [10]

Syndecans acts as matrix receptor by binding to various ECM (Extracellular matrix) molecules through heparan sulphate chains and connects the cytoskeleton through cytoplasmic domain. [11] Syndecans in each tissue can bind different ligands. It is known that proteoglycans play important role in epithelial-mesenchymal interaction and expressed during tooth morphogenesis. [12] Taking this into consideration, we analysed IHC expression of syndecan-1 in odontogenic lesions like ameloblastoma and dentigerous cyst.

Dentigerous cyst is odontogenic cyst derived from reduced enamel epithelium which grows by accumulation of fluid and epithelial proliferation.[4] In the present study, strong expression of syndecan-1 was observed in both basal and suprabasal layers. High expression of syndecan-1 results in increased cell-ECM adhesion and decreased cell migration.[13] On the other hand, loss of syndecan-1 increases migratory capacity of cells. Strong expression of syndecan-1 in the present study suggests non-invasive behaviour of dentigerous cyst.

As the expression of syndecan-1 becomes weak, invasion of tumor cells increases.[14] Also epithelial cells with low syndecan-1 expression undergo molecular changes and acquire fusiform morphology having capacity to invade with anchorage independent growth.[15] Decreased syndecan-1 expression in head and neck squamous cell carcinoma is associated with tumor aggressiveness and poor survival outcome.[16] It is well-known that despite of being a benign epithelial odontogenic tumor, ameloblastoma grows in the form of islands infiltrating within the connective tissue. Thus, in the present study weak syndecan-1 expression in ameloblastomas as compared to dentigerous cysts is due to its infiltrating nature.

Expression of the cell surface proteoglycan antigen changes with the reciprocal epithelial-mesenchymal interactions during odontogenesis. When the cells undergo terminal differentiation, there is loss of expression which is seen as absence of stain in ameloblasts and presence in stellate reticulum and stratum intermedium. According to Vainio et al and Thesleff et al, peripheral or ameloblast like cells are fully differentiated and have reduced syndecan-1 expression during tooth formation. [2,12] Similarly, in our study, syndecan-1 expression was high in central stellate reticulum-like cells as compared to peripheral or tall columnar cells.

In the present study, we found stromal cells were positive for syndecan-1 in majority cases of both the lesions. According to Mennerich et al, stromal cells with positive syndecan-1 expression are spindle cells with myofibroblastic differentiation.[17] Previous literature suggested that stromal cells were positive
for syndecan-1 expression as it was shed by tumor cells and are in soluble form. However, recent studies have suggested that syndecan-specific monoclonal antibody B-B4 recognise only membrane bound form and not soluble form.[18] Also in situ hybridisation showed positivity at the mRNA level indicating that the protein does not originate from syndecan shed by other cells but is generated by the stromal cells themselves.[17] This issue of stromal syndecan expression can be addressed by studying the expression profiles of genes coding for above protein. Stromal syndecan-1 expression is regarded as poor prognostic indicator in breast and endometrial cancer. [19,20]

Nadalin et al analysed syndecan-1 and Ki-67 expression in dentigerous cyst, radicular cyst and odontogenic keratocyst. They observed intense expression in epithelial lining of all cystic lesions.[21] Similarly, Ohoud Al-Otaibi et al evaluated syndecan-1 expression in ameloblastoma, dentigerous cyst, odontogenic keratocyst and observed high syndecan-1 expression in lining epithelium of dentigerous cyst as compared to ameloblast-like cells in ameloblastoma. They concluded increased expression in dentigerous cyst and odontogenic keratocyst as compared to ameloblastoma accounts for its non-invasive behavior.[22] Alaeddini M et al evaluated syndecan-1 expression in adenomatoid odontogenic tumor, ameloblasticfibroma, ameloblastoma, keratocystic odontogenic tumor and odontogenic myxoma concluding that syndecan-1 is involved in pathogenesis of odontogenic tumors. [23] Findings in our study are in accordance with Nadalin et al and Ohoud AL-Otaibi et al.

In the present study, no syndecan-1 expression was observed in four recurrent cases of ameloblastomas. This indicates that tumor invasion and aggressiveness is related to cell adhesion molecules like syndecan-1 which maintains cell-cell and cell-extracellular matrix interaction. Additional studies with more recurrent cases of ameloblastomas along with clinical follow-up are required to determine role of syndecan-1 in its etiopathogenesis. Also, further in-depth research targeting syndecan-1 is required in ameloblastoma to predict the recurrence and malignant transformation. In addition, its role as an adjuvant in disputed histopathological diagnosis of ameloblastic carcinoma can also be explore.

CONFLICT OF INTEREST: None
ACKNOWLEDGEMENT: -
ETHICAL CLEARANCE: Not required

| Table I |
| Inter-observer reliability of 3 independent observers for expression of Syndecan-1 in ameloblastoma |

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WEIGHTED KAPPA</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean intensity</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Mean % of positive cell</td>
<td>0.57</td>
<td>&lt;0.024*</td>
</tr>
<tr>
<td>Total mean</td>
<td>0.59</td>
<td></td>
</tr>
</tbody>
</table>
Table II
Inter-observer reliability of 3 independent observers for expression of Syndecan-1 in dentigerous cyst

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WEIGHTED KAPPA</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean intensity</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Mean % of positive cell</td>
<td>0.64</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Total mean</td>
<td>0.72</td>
<td></td>
</tr>
</tbody>
</table>

Table III
Comparison between expression of syndecan-1 in ameloblastoma and dentigerous cyst

<table>
<thead>
<tr>
<th>Score</th>
<th>AMELOBLASTOMA (n=20)</th>
<th>DENTIGEROUS CYST (n=20)</th>
<th>&quot;t&quot; test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score for intensity</td>
<td>1.58(0.924)</td>
<td>2.53 (1.535)</td>
<td>-2.38</td>
</tr>
<tr>
<td>Score for % positive cell</td>
<td>1.56(0.873)</td>
<td>2.5(1.552)</td>
<td>-2.34</td>
</tr>
<tr>
<td>Total final score</td>
<td>3.16(1.789)</td>
<td>4.88(2.933)</td>
<td>-2.245</td>
</tr>
</tbody>
</table>

LEGENDS:
Figure 1: Photomicrograph of Syndecan-1(CD138) expression in normal oral mucous membrane. (100x)
Figure 2: Photomicrograph of Syndecan-1(CD138) expression in dentigerous cyst. (100x)
Figure 3: Photomicrograph of Syndecan-1(CD138) expression in ameloblastoma. (100x)
Figure 4: Photomicrograph of Syndecan-1(CD138) expression in stellate reticulum cells of ameloblastoma. (400x)

Table 1: Inter-observer reliability of 3 independent observers for expression of Syndecan-1 in ameloblastoma.
Table 2: Inter-observer reliability of 3 independent observers for expression of Syndecan-1 in dentigerous cyst.
Table 3: Comparison between expression of syndecan-1 in ameloblastoma and dentigerous cyst.
Graph 1 (Figure 5): Inter-observer reliability of 3 independent observers for expression of Syndecan-1 in ameloblastoma.
Graph 2 (Figure 6): Inter-observer reliability of 3 independent observers for expression of Syndecan-1 in dentigerous cyst. 
Graph 3 (Figure 7): Comparison between expression of syndecan-1 in ameloblastoma and dentigerous cyst. 

References

Fig1 Syndecan-1 (CD138) expression in normal oral mucous membrane
Fig3 Syndecan-1 (CD138) expression in ameloblastoma
Fig4 Syndecan-1 (CD138) expression in stellate reticulum cells of ameloblastoma
Evaluation of Syndecan 1 in ameloblastoma
Fig 6 Graph 2
Evaluation of Syndecan-1 in dentigerous cyst
Comparison between expression of Syndecan-1 between ameloblastoma and dentigerous cyst

**Fig 7 Graph 3**