

## **SYNDECAN-1 EXPRESSION AND RELATION WITH THE BIOLOGICAL BEHAVIOR OF VARIOUS TYPES OF AMELOBLASTOMAS (AN IMMUNOHISTOCHEMICAL STUDY)**

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### **ABSTRACT**

Ameloblastoma is the most frequent odontogenic tumor and is considered a benign, but locally invasive neoplasm with variable clinico-pathological expression. Syndecan-1 is a cell surface proteoglycan that binds cells to the extracellular matrix and is expressed in various types of ameloblastoma. The aims of this study were to evaluate and compare syndecan-1 expression within the various types of ameloblastomas and to find a correlation between this expression and the biological behavior of variants. Immunohistochemical studies were performed for syndecan-1 in 30 ameloblastomas. Follicular ameloblastoma showed the statistically significantly highest mean area percent (32.10%). This was followed by plexiform ameloblastoma which showed lower mean area percentage value (22.02%). Ameloplastic carcinoma showed the statistically significantly lowest mean area percent (11.59%). The present results suggested the down regulation of syndecan-1 expression indicated decreased cell adhesion and higher cell proliferation.

### **INTRODUCTION**

Syndecans are a four member family of transmembrane adhesion molecules with diverse expression and functionality. <sup>[1]</sup> Syndecan-1 (SDC1) is a member of a family formed by four proteoglycans (PGs) containing a C-terminal cytoplasmic domain, a well-conserved single-pass transmembrane domain, and a large N-terminal extracellular domain. The extracellular domain contains motifs for glycosaminoglycan attachment, proteolytic cleavage, and cellular interactions. <sup>[2]</sup>

SDC1 is mainly located on the basolateral surfaces of simple epithelial and surrounding stratified epithelial cells. Although SDC-1 is not present on the majority of mesenchymal cells in mature tissues, its expression is observed in small quantities within mesenchymatous cells in culture. <sup>[3]</sup> SDC1 participates in odontogenesis and regulates many biological processes, including cytoskeletal organization, growth factor signaling, cell-cell adhesion, and extra cellular matrix attachment. <sup>[4]</sup>

SDC1 gene expression, ranging from overexpression to complete absence, has been

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studied in various types of carcinomas.<sup>[5]</sup> The loss of expression of SDC1 in malignant epithelial neoplasms is associated with tissue invasion, metastasis, and poor prognosis<sup>[4]</sup>, and appeared to correlate with poor prognosis in stomach, breast, and head and neck neoplasms.<sup>[6,7,8]</sup>

The altered expression of SDC1 in ameloblastomas suggests that this cell surface PG could have prognostic value in the determination of the clinical outcome of these lesions, positive SDC-1 expression was associated with cell type and histological differentiation, which acted as a prognostic marker in those lesions.<sup>[9]</sup> Ameloblastoma is the second most common odontogenic tumor that mostly involves the mandible and maxilla. It is a slow-growing locally invasive epithelial tumor with a high recurrence rate (50%–72%) and rare metastasis (<2).<sup>[10,11]</sup>

The World Health Organization (WHO) has classified ameloblastoma into the following variants: solid/multicystic ameloblastoma, unicystic ameloblastoma, desmoplastic ameloblastoma and peripheral ameloblastoma, and also divided into follicular, plexiform, acanthomatous, granular types, etc., based on their histological features. The malignant transformation of ameloblastomas results in the formation of ameloblastic carcinomas and malignant ameloblastomas depending on cytological dysplasia and metastasis.<sup>[12]</sup>

The aims of this study were to evaluate and compare SDC1 expression within the various types of ameloblastomas and to find a correlation between this expression and the biological behavior of variants.

## MATERIAL AND METHODS

### I- Case Selection

Thirty formalin-fixed and paraffin-embedded of specimens various types of ameloblastomas were collected from the archives of the Oral and Dental

Pathology Department Faculty of Dentistry, Minia University, Clinical Pathology Department of the National Institute of Cancer, Cairo University. Normal mucous membrane specimens were used as normal control for this antibody from patients indicated for operculectomy over wisdom tooth.

### II- Histopathological Examination:

Specimens were routinely processed, sectioned and stained Using Hematoxylin and Eosin stain, according to the histopathological criteria, these cases were divided into three groups: group 1: 12 samples diagnosed as follicular ameloblastoma with its variants, group 2: 10 samples diagnosed as plexiform ameloblastoma and group 3: 8 samples diagnosed as ameloblastic carcinoma.

### Immunohistochemical staining:

#### A-Reagents

##### 1-Primary Antibody

The antibody used in this study was SDC1 (CD138) mouse monoclonal Ab-2 [clone MI15, Cat. #MS-1793- R7] [LAB VISION cooperation, USA].

##### 2-Detection System [LAB VISION cooperation, USA]

A streptavidin-biotin immune-peroxidase staining system was used for immune-detection. It included the following reagents: Hydrogen Peroxide Block, Ultra V Block, Biotinylated Goat Anti-Polyvalent Streptavidin Peroxidase, Di-Amino Benzidine (DAB) Plus Chromogen Di-Amino Benzidine (DAB) Plus Substrate, Citrate Buffer and Phosphate Buffered Saline (PBS).

#### B-Immunostaining Procedures:

For all specimens, paraffin sections 4  $\mu$ m thick were prepared. Sections were mounted on positively charged glass slides then put in oven to 56 °C for 20 minutes. Sections were deparaffinized by 2 change

of xylene for 15 minutes for each. Rehydration of slides of descending series concentrations of alcohol, 100%, 90% then 70%, 5 minutes each. Blocking of the endogenous peroxidase activity was performed by applying 3% hydrogen peroxide for 10 minutes at room temperature. The slides were completely immersed in antigen retrieval solution for 20 minutes in microwave oven (600-800). The retrieval solution was left to cool at room temperature for 30 minutes. Then the primary antibody (mouse monoclonal antibody) was applied to cover the sections completely followed by incubation for overnight at 4°C in refrigerator. The slides were completely covered with Biotinylated Goat Anti-Polyvalent (secondary antibody). The slides were completely covered with Streptavidin Peroxidase for 10 minutes at room temperature. The chromogen was prepared by adding 1-2 drops (40-100ul) DAB Plus Chromogen to 1 ml of DAB Plus Substrate, mixed by swirling. The slides were counterstained with Mayer's Hematoxylin, ready-to-use, for 2 minutes. Then rinsing of the slides with tap water for 10 minutes.

### Image analysis

The immune-stained sections were examined using light microscope to assess the prevalence of positive cases and location of immune-staining within the tissue. Tumor cells were considered to be SDC1 positive if there was membranous staining (brown color). Immune-reactivity was evaluated by estimating the percentage of positive immune-stained areas in relation to the total areas examined in each field, at x 100 magnification using computed image analysis. The image analysis was performed using a computer system, [Germany (Software Leica Qwin 500)].

### Statistical analysis

Data were represented as means and standard deviation (SD) values, one way ANOVA test used to compare means of the three groups.

Paired sample t-test was used for comparison of the means of two groups (compare all pairs of columns). The P value is significant if less than or equal 0.05 ( $P \leq 0.05$ ). The statistical analysis was performed by using STATA intercooled version 9.2 and Microsoft® excel 2007.

## RESULTS

### Histopathological Findings

Hematoxylin and Eosin stain of 12 cases of follicular ameloblastoma showed follicles of odontogenic epithelium within fibrous connective tissues, the basal cells of these islands are columnar and lined in palisaded pattern. The nuclei are polarized away from the basement membrane; the central core consists of loosely arranged cells that resemble the stellate reticulum of enamel organ (Fig. 1). H&E stain of ten cases of plexiform ameloblastoma showed plexus or anastomosing strands of odontogenic epithelium in connective tissue stroma (Fig. 2).

H&E stain of 8 cases of ameloblastic carcinoma showed cytological atypia; including cellular pleomorphism, nuclear hyperchromasia and abnormal mitosis (Fig. 3).

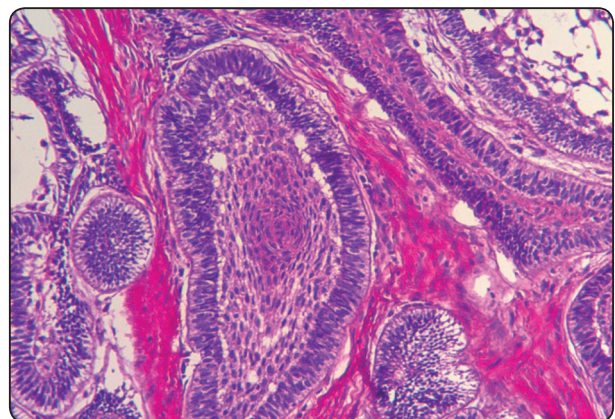


Fig. (1) Photomicrograph of Follicular Ameloblastoma showing follicles of odontogenic epithelium within fibrous connective tissues (H&E X100).

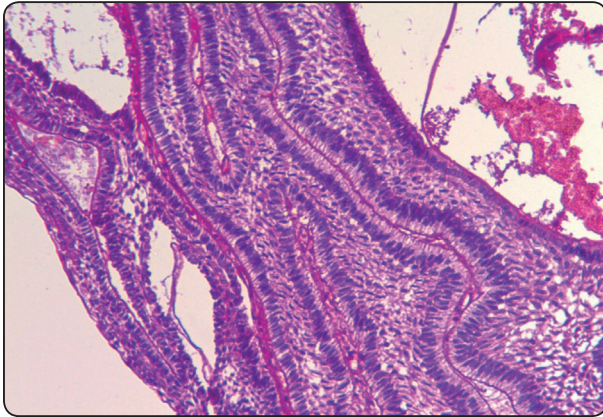


Fig. (2) Photomicrograph of Plexiform Ameloblastoma showing plexus or anastomosing strands of odontogenic epithelium in connective tissue stroma (H&E X100).

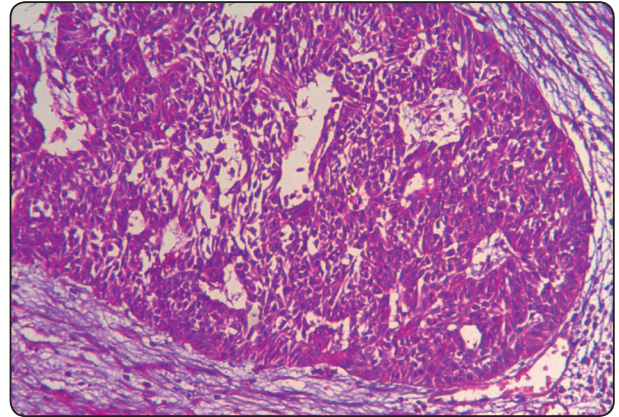


Fig. (3) Photomicrograph of Ameloblastic Carcinoma showing cytological atypia and cellular pleomorphism (H&E X100).

### Immunohistochemical Findings

Immunohistochemical staining of SDC1 in follicular subtype was found in all 12 cases studied. The positive cases showed membranous SDC1 immunohistochemical staining in the epithelial and stromal components of the studied lesions (Fig. 4). Immunohistochemical staining of SDC1 in plexiform subtype was found in all cases studied, membranous immunostaining of SDC1 is mostly seen in peripheral cells than central cells (Fig. 5). While immunohistochemical staining of SDC1 of ameloblastic carcinoma was negative (Fig. 6).

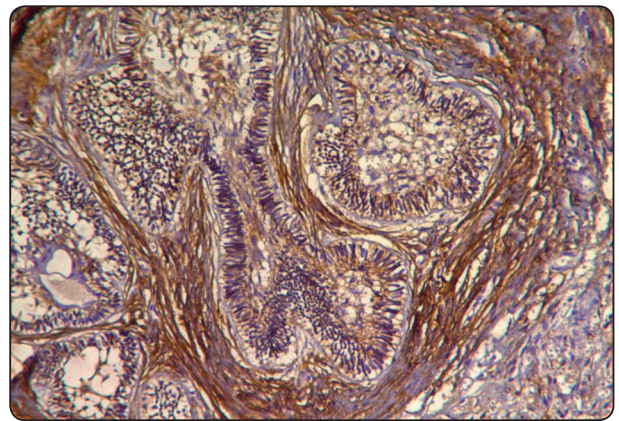


Fig. (4) Photomicrograph of Follicular Ameloblastoma showing positive SDC1 expression in epithelial (a) and stromal cells (b) (Anti-SDC1 X100)

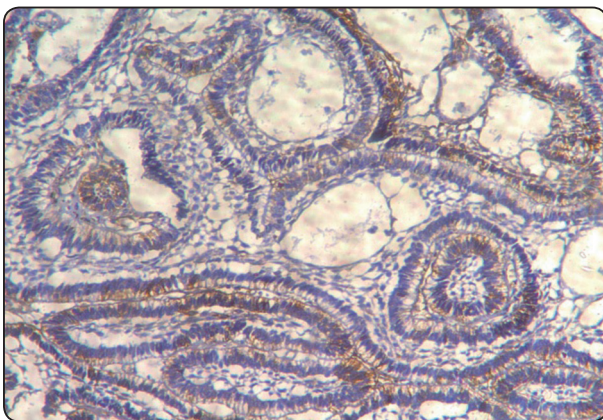


Fig. (5) Photomicrograph of Plexiform Ameloblastoma showing positive SDC1 expression in membranous areas of epithelial cells (Anti-SDC1 X100).

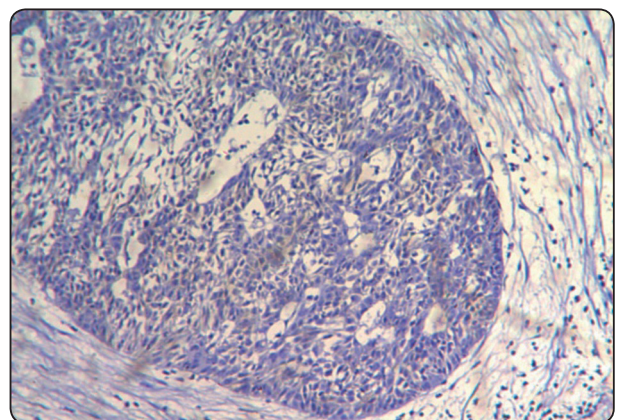


Fig. (6) Photomicrograph of Ameloblastic Carcinoma showing negative SDC1 expression (Anti-SDC1 X100).

TABLE (1) Means, SD values, median and range and results of ANOVA test for comparison between area percentage in follicular, plexiform and ameloblastic carcinoma:

Group	Mean	SD	Median	Range	P-value
Follicular ameloblastoma	32.10	3.57	32.26	25.08-37.75	<0.0001*
Plexiform ameloblastoma	22.02	3.03	21.19	16.54-26.89	
Ameloblastic carcinoma	11.59	5.48	10.35	5.71-20.5	

Follicular ameloblastoma showed the statistically significantly highest mean area percent (32.10%). This was followed by plexiform ameloblastoma which showed lower mean area percentage value (22.02%). Ameloblastic carcinoma showed the statistically significantly lowest mean area percent (11.59%) (Table 1 & Fig. 7).

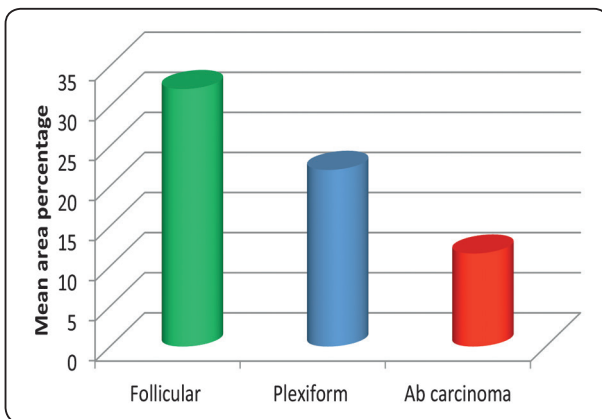


Fig. (7) Bar chart representing mean of area percentage in follicular ameloblastoma, plexiform ameloblastoma and ameloblastic carcinoma.

## DISCUSSION

Ameloblastomas are the most frequently encountered tumors arising from odontogenic epithelium with different clinicopathologic expressions, characterized by invasive behavior with high risk of recurrence.<sup>[13]</sup> Identification of invasive activities in ameloblastomas may be useful to predict their biological behavior. However, the exact molecular mechanism of invasion in ameloblastomas has not been well elucidated.<sup>[14]</sup>

Cell surface PGs play an important role in the functional and metabolic behavior of many cell types. These molecules are involved in cell differentiation, proliferation, and migration and are essential for the maintenance of normal cellular function. Syndecan molecules belong to the heparan sulfate PG family found as components of cell surfaces which are involved in cell-cell, cell-matrix adhesion, and growth factor binding.<sup>[15]</sup>

Syndecan-1 (SDC1) also known as CD138 is transmembrane heparan sulfate and best described member of SDC family. SDC1 is known to bind with cell to cell and cell to interstitial matrix<sup>[16]</sup>, it has role in the regulation of cell morphology, adhesion and differentiation.<sup>[17]</sup> Hence, loss of SDC1 can be associated with uncontrolled proliferation, decreased adhesion and the disordered differentiation of tumor cells.<sup>[18]</sup>

This study was conducted to examine the immunohistochemical expression of SDC1 in various types of ameloblastomas and to correlate the expression of SDC1 with their biological behavior, as reported in previous studies which utilized SDC1 to study ameloblastoma cases.<sup>[5,9,19]</sup> The immunohistochemical results of the present study revealed that all cases of follicular ameloblastomas under study showed immunopositive reaction. Immunoreactivity of SDC1 was located in membranous areas of peripheral and central epithelial cells of ameloblastic follicles; some cases showed immunoreactivity of SDC1 was also located in stromal cells. This finding agrees with

that of Regina et al.,<sup>[20]</sup> who found expression of SDC1 in the areas of typical epithelial lining of keratocystic odontogenic tumor. In the present study, some areas of ameloblastic follicle showed a decreased expression of SDC1 in the peripheral epithelial cells when compared to the central cells in accordance to the findings of SDC1 studied in tooth development.<sup>[21]</sup> They explained that post mitotic terminally differentiated ameloblasts have decreased SDC1 expression during amelogenesis and restricted localization of SDC1 in immature zone may enable the maturation of ameloblasts.

The acanthomatous variants showed decreased SDC1 immunoreactivity in the present cases, also the granular variants in the present cases demonstrated no cell surface reactivity. This may be explained by the fact that when cells undergo metaplastic changes, important functions of the original cells are lost.<sup>[22]</sup> Also, alteration in expression of SDC1 can result in alteration in cell adhesion which affects cellular shape and functional properties.<sup>[23]</sup> Another possible clarification is that the acanthomatous areas and the granular cells in Ameloblastoma are found to be strongly positive for some degrading enzymes. Nagatsuka et al.<sup>[24]</sup> revealed strong immunoreactivity for heparanase in the Acanthomatous Ameloblastoma. Additionally, Pinheiro et al.<sup>[25]</sup> found a strong immunoreaction for MMP 2 and 9 in granular cells. These findings suggest that degrading enzymes may have a role in cleaving SDC1 and thus decreased its expression as occurred in the present study.

In the present study, the immunohistochemical results revealed that all cases of plexiform ameloblastomas under study showed immunopositive reaction. Immunoreactivity of SDC1 was located in membranous areas of peripheral and central epithelial cells. On the contrary, to that reported by Leocata et al.<sup>[4]</sup> found that the immunoreactivity of SDC1 was expressed on the stromal cells and ECM

in plexiform ameloblastomas. In this study, it was found that there is statistically significant relation in the mean area percent between follicular and plexiform subtypes of ameloblastomas. However, in the previous studies showed no significant differences in expression of SDC1.<sup>[9,19]</sup> But this difference that found in this study may be attributed to the fewer cases of plexiform subtype compared to follicular subtype.

In the present study, the immunohistochemical results revealed that all cases of Ameloblastic Carcinoma (AC) under study showed negative or lower expression levels of SDC1, as reported in previous studies.<sup>[5,9]</sup> They explained that the down regulation of SDC1 expression indicates decreased cell adhesion and greater proliferation in ameloblastic carcinoma than other subtypes of ameloblastomas. Additionally, Muramatsu *et al*<sup>[26]</sup> studied expression levels and functions of SDC1 in oral cancer cell lines using siRNA (small interfering RNA), and found that reduction of SDC1 led to higher levels of cell proliferation. Furthermore, their results showed that the invasiveness increased when SDC1 function was blocked in these cell lines. Also, *in vitro* studies have indicated that SDC1 play a role in inhibiting cell invasion and suppressing the growth of carcinoma cell lines.<sup>[27]</sup> Therefore, the reduced SDC1 expression is an interpretation for diminished cell-to-cell adhesion giving the cell ability to detach and in turn to invade.<sup>[28]</sup> Regarding SDC1 expression, the current study revealed that a statistically highly significant relation ( $p < 0.0001$ ) between follicular, plexiform and AC which ACs showed the statistically significantly lowest mean area percent (11.59%) in accordance to follicular (32.10%) and plexiform (22.02%). These results were found to be in agreement with that reported by Bologna-Molina et al<sup>[5]</sup> who found that AC had greater loss of SDC1 expression than all other subtypes of benign ameloblastomas.

## CONCLUSIONS

In conclusion, AC had a greater loss of SDC1 expression than all other subtypes of benign ameloblastoma and suggested the down regulation of SDC1 expression indicated decreased cell adhesion and higher cell proliferation. This study might provide evidence for the valuable use of SDC1 as prognostic marker in patients with ameloblastic lesions. Therefore, more studies are necessary to better understand the role of SDC1 in the biological behavior of ameloblastomas in different stages of the lesion.

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