ORIGINAL RESEARCH

Epithelial and Stromal Syndecan-1 roles on the biological behavior of Dentigerous cyst, Ameloblastoma and Ameloblastic Carcinoma.

Shaza Ismail Ahmed Hasan, Sherief Youssef Mohamed El Nagdy, Mona Mohsen Abdo Ibrahim

Abstract

Background: Dentigerous cyst diagnosis is simple but several case reports have documented neoplastic changes arising from them. Ameloblastoma is a common benign Odontogenic tumor with an aggressive manner and a high rate of recurrence. Ameloblastic Carcinoma is the malignant counterpart of Ameloblastoma but usually difficult to be distinguished from each other's. Hence, need for Immunohistochemical markers may help achievement of accurate diagnosis.

Objectives: Evaluation of Epithelial and Stromal Syn1 expressions and their roles in tumorigenesis and biological behavior of Dentigerous cyst, Ameloblastoma and Ameloblastic Carcinoma.

Methods: Tissue samples comprising of 54 archived histopathologically confirmed cases of (10 Dentigerous cysts, 29 Ameloblastomas and 15 Ameloblastic Carcinoma). The sections were subjected to Immunohistochemical staining according to a standard protocol using antibody to Syn1.

Results: Stromal Syn1 expression was higher in Desmolpastic Ameloblastoma than other Conventional Ameloblastoma subtypes. Unicystic Ameloblastoma showed higher Stromal Syn1 than Dentigerous cyst. Ameloblastic Carcinoma showed the highest immune-reactivity to Stromal Syn1 than Conventional Ameloblastoma. While, Epithelial Syn1 immune-reactivity was weak.

Conclusions: Desmoplastic Ameloblastoma behaves in a more aggressive manner than other subtypes.Stromal Syn1 are highly expressed in aggressive and malignant odontogenic tumors and could be used together as prognostic predictor tool for odontogenic tumors.

Keywords: Odontogenic tumors, Dentigerous cyst, Ameloblastoma, Ameloblastic Carcinoma Immunohistochemistry, Syndecan-1.

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Introduction

Odontogenic lesions are common; some of them represent hamartomas. While, others represent neoplasms with variable levels of aggressiveness and tendency to metastasize.[1] Dentigerous cyst (DC) is often asymptomatic and discovered on routine dental x-ray.[2] Previous studies reported a neoplastic transformation of DC into Unicystic Ameloblastoma (UAB) or conventional AB. [3–5] Hence, DCs histopathological diagnosis is critical.[2]

Ameloblastoma (AB) is a common benign odontogenic tumor (OT). It is has a local aggressive manner and high recurrence rate, and a considerable liability for malignancy changes as well as metastasis.[6,7] Ameloblastic Carcinoma (AC) is a rare aggressive malignant OT. It shows cytological atypia of the epithelial components within the benign histological features of AB even in the absence of metastasis.[8,9]

Syndecan-1 (Syn1) is a transmembrane proteoglycan that is expressed in epithelial and stromal cells, and has a significant role in the biological processes, such as cell to cell and cell to extracellular matrix (ECM) adhesion.[10] Syn1 expression is often altered in human tumors; it might be either tumor initiator or tumor suppressor in the same tumor type, as in colorectal Cancer and prostate cancer.[11-14] Stromal expression and loss of epithelial Syn1 expression is a sign for poor prognosis.[15] Similar Studies on its expression in OTs is limited and its actual role in the pathogenesis and biology of these tumors remains unknown.

Aim Of The Study: Evaluation of Epithelial and Stromal Syn1 expression and its roles on the biological behavior of DC, AB and AC.

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Conflict of Interest: None

Material and Methods

This study was retrospectively applied on 54 paraffin embedded tissue samples that were collected from archives of oral pathology labs, Dentistry Faculties; Mansoura and Alexandria Universities. Tooth germ tissues were used as a normal tissue control, and Tonsil tissues were used as a positive control. This Study was approved by the local Ethics Committee in Research, faculty of Dentistry, Mansoura University (Code Number A04260219).

A. Immunohistochemical markers:

"Universal Kit: Power Stain TM 1.0 Poly HRP DAB Kit for Mouse + Rabbit. Syndecan-1 / CD138 [EP201] antibody (AN837-5M; BioGenex, USA); Primary Rabbit Monoclonal antibody (ready to use)."

B. Methods:

Clinical and radio-graphical data were collected from the patient´s files when available. Two serial tissue sections (4µm thickness) were cut (one section for H and E to confirm the diagnosis according to the current WHO Histopathological Classification of OTs[16] and one section was mounted on positive charged coated slide for Immunohistochemical (IHC) evaluation of Syn1 antibody according to manufacturer's instructions).

Deparaffinization and rehydration of slides in xylene and alcohol respectively. Slides were immersed in buffered citrate PH6 (10 minutes), heated, blocked (30 minutes) with 1.5% Santa Cruz Biotechnology. Incubation of primary antibody at room temperature (45 minutes). 1:2 drops of anti- Syn1 was used, Slides were washed twice with PBS, then were treated with 4-5 drops of Ultra Vision biotinylated goat anti-polyvalent secondary antibody (10 minutes), rewashed in PBS (3 minutes). Then were treated with streptavidin–biotin enzyme (DAKO, Denmark) (10 minutes), rewashed in PBS (3 minutes). Application of 3.3- Diaminobenzidine tetrahydrochloride (DAB) drops as a chromogen, then washed with PBS (3 minutes). Slides were counterstained with Mayers hematoxyline and mounted using xylene-based mounting medium (3 minutes). Positive brown deposits were detected.

Evaluation was done at two different levels: epithelial (Syn1E) and stromal (Syn1S); according to the stained cells. Slides were examined under a light microscope; five selected non-overlapping fields were evaluated at 400X magnification. Dark brown cytoplasmic and/or membranous staining of the tumor cells was considered positive. The staining intensity was evaluated as following: Negative, Weak, Intermediate, and Strong. Percentage was semi-quantitatively scored using four grades scoring; (0) Negative expression = 0% positive cells, (1) Low expression = 1–10% positive cells, (2) Moderate expression = 11–50% positive cells, and (3) High expression = >50% positive cells. The cases were evaluated at two different levels: Epithelial (Syn1E) and Stromal (Syn1S)[17,18]

C. Statistical analysis:

Statistical analysis was performed using program of SPSS 16, IBM Corporation. Kruskal Wallis test and Mann Whitney test were used to compare groups of non-parametric data, Spearman's correlation coefficient test was used correlating different parameters, and A P value <0.05 was considered statistically significant.

Table 1: Syn1E and Syn1S Expression and Intensity as regard to (Conventional AB subtypes), (Unicystic ameloblastoma and DC) and (Conventional AB and Ameloblastic Carcinoma):

			Syn1E Expression		Syn1E Intensity		Syn1S Expression		Syn1S Intensity	
			Mean Rank	P	Mean Rank	P	Mean Rank	P	Mean Rank	P
A	Conv. AB Subtypes	Follicular	11.06	0.10	10.44	0.07	10.56	0.06	9.33	0.12
		Acanthomatous	11.50		14.00		14.50		13.67	
		Desmoplastic	3.17		9.50		17.50		16.00	
		Plexiform	11.50		11.33		4.00		7.00	
B	UAB		9.27	0.3	8.32	0.06	14.00	.005**	13.00	0.02*
	DC		11.90		12.75		7.00		8.00	
C	Conv.AB		25.59	0.02	24.90	0.07	19.76	0.04	20.34	0.09
	AC		16.53		17.87		27.80		26.67	

A-Test Used: Kruskal Wallis.
B &C- Test Used: Mann-Whitney test.
P: significant <0.05.

Results

The studied 54 cases were histologically classified into10 DC (Fig.1A), 10 UAB (Fig.1B), 19 Conventional AB (Fig.1C), and 15 AC (Fig.1D). Conventional AB showed 4 different histological subtypes as the following percentages; 8 Follicular (42.10%), 6 Plexiform (31.50 %), 3 Desmoplastic (15.7%) and 2 Acanthomatous (10.5%).

Ameloblastic Carcinoma cases were histologically formed of benign ameloblastomatous components which was invaded with malignant features like; loss of normal cellular architecture, excessive hemorrhage, cellular atypia, mitotic activity, nuclear hyper chromatism, basilar hyperplasia, vascular invasion, clear cells and focal areas of necrosis.

All the studied DC cases showed positive Syn1E immune reaction that appeared along the cell membranes together with little or no cytoplasmic staining. But, Syn1S was detected in 40% (n=4) of cases and appeared in the cytoplasm of stromal fibroblasts and some inflammatory cells (Fig 2A).

Syn1E expression was detected in 89.74% (n=17) of the 19 studied Conventional AB cases and in 90% (n=9) out of the 10 studied UAB cases. While, Syn1S expression was detected in 94.73% (n=18) of Conventional AB cases and in 90% (n=9) of UAB cases. In UAB, the expression of Syn1E was noticed in the membrane and/or the cytoplasm of the neoplastic cystic epithelial cells, in the intraluminal projections and in the tumor islands contained within the cyst wall. In addition, Syn1S expression was found in the stroma close to the epithelial neoplastic cells). (Fig. 2B).

In Conventional AB, Syn1E was mainly expressed in the cytoplasm and/or membranes of the tumor epithelial cells. The highest expression was detected in basal and suprabasal cells with weak positivity in the central areas. Moreover, Syn1S expression exhibited the same pattern as was in UAB cases. (Fig.2C). In AC expression was membranous and/or cytoplasmic. It was observed in the tumor epithelial cells except in undifferentiated areas where the expression was weak or lost. Furthermore, Syn1S was expressed in all of the studied AC cases (Fig.2D).

Statistical analysis revealed that Desmoplastic AB had the lowest levels of Syn1E, and the highest levels of Syn1S than other subtypes without significant difference (Table1A). Moreover, high Syn1E levels in DC than UAB with no significant differences (Table 1B), and was higher in AC than Conventional AB with significant difference in the expression (Table 1C). Syn1S levels were higher in UAB than DC with statistical significant differences (Table1B), and in (AC than Conventional AB) with significant differences in the expression (Table 1C). No correlation was found between Syn1E and syn1S expression and intensity either in DC, AB or AC (r=0.08, 0.21 & 0.09) respectively without significant differences (p = 0.53, 0.11 & 0.31) respectively.

Discussion

Odontogenic tumors originate from the tissues of tooth forming apparatus due to altered degrees of inter tissue interaction and various growth patterns.[19] Neoplastic transformations from DC have been documented in several case reports; so, submitting

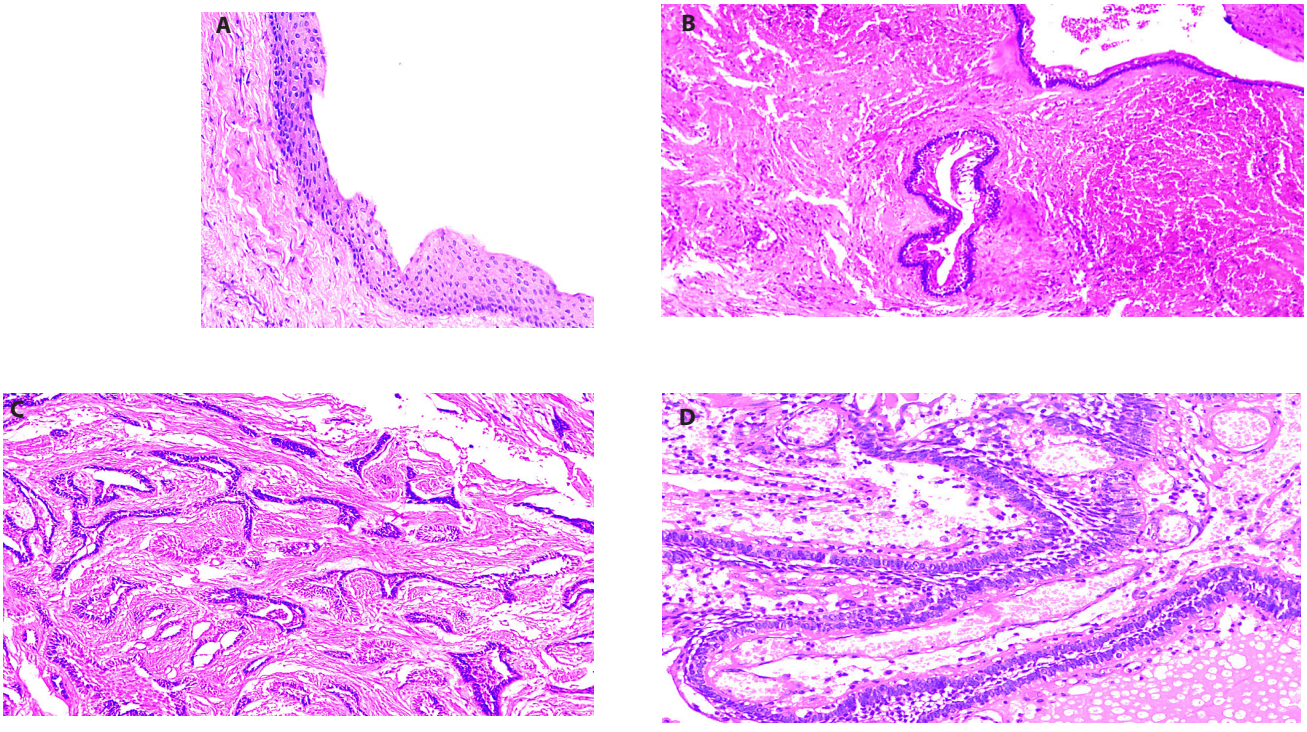

Fig 1: (A) Dentigerous cyst showing thick epithelial cystic lining and dense fibrous CT (H and E, 200X). **(B)** Mural variant of UAB. **(C)** Desmoplastic Ameloblastoma case showing, islands of odontogenic epithelium with variable shapes and sizes that proliferates within a highly collagenous stroma (H and E, 100X). **(D)** Ameloblastic carcinoma case showing; area of Stromal necrosis, angiogenesis and hyperplasia (Hand E, 400X).

samples for pathology examination even when cysts exist clinically as a conventional DC is required.[20] Clinically and radiographically, AB and AC are similar, but AC can be expected if there are more uncommon aggressive features.[21]

Syn1 has a role in cellular proliferation, passage, and cell to ECM interactions.[22] Epithelial Mesenchymal Interaction (EMI) for cancer cells increase cellular motility, which helps tumor cells to spread.[23] Syn1 is expressed predominantly in epithelial cells and has a significant role in maintaining their nature and morphology. Loss Syn1 induces cells to acquire a fibroblast like phenotype.[24] Stromal expression of Syn1 and loss of epithelial expression is considered as an indicator for poor prognosis.[15] Epithelial and Stromal syn1 were evaluated separately previously in different types of tumors[25–27]. But, similar studies on OTs are still so limited.

According to our findings, the lining epithelium of DCs revealed a high membranous Syn1E expression with little or no cytoplasmic staining when compared with UAB without significant difference. While, Syn1S showed higher levels in UAB than was in DC; this could be due to the non-invasive nature of DCs when compared to UAB. Similar observations were reported by Al-Otaibi et al., 2013 and Hammad et al., 2020,[17,28] who found Syn1 in the epithelial lining of DC and Odontogenic Keratocyst (OKC), and explained lower Syn1 expression in OKC compared with DC might be related to the local aggressiveness and high potential rate for recurrence of OKC when compared with clinical behavior of DCs.

According to the findings of this study, Syn1E was generally expressed in the peripheral epithelial cells of AB, with a weak reactivity in the center. This may refer to the active proliferating nature of the peripheral cells which helps the tumor to increase in size. Also in AC, Syn1E was expressed in most parts except in less differentiated areas, where the expression was diminished or absent. Martínez et al., 2017[29] found the same pattern of Syn1 expression in AB and AC. In the current work, AB showed higher levels of Syn1E expression and intensity than was in AC. This was supported by Urvashi et al., 2019 and Carreón et al., 2018[30,31] findings who related the decrease of Syn1E expression to increase tumor progression and more invasive manner.

Ahmed Haji., 2013[27] suggested that Syn1 may have a role in tumor stroma of some neoplasms. In the current research, Syn1S expression and intensity showed higher levels in AC than AB. Hence, the increase of Syn1S expression in AC than in AB could be suggestive for greater invasive and more destructive biological behavior. This was supported by the findings of a previous study on AB[31] which related high Syn1S expression to cell invasion, tumor

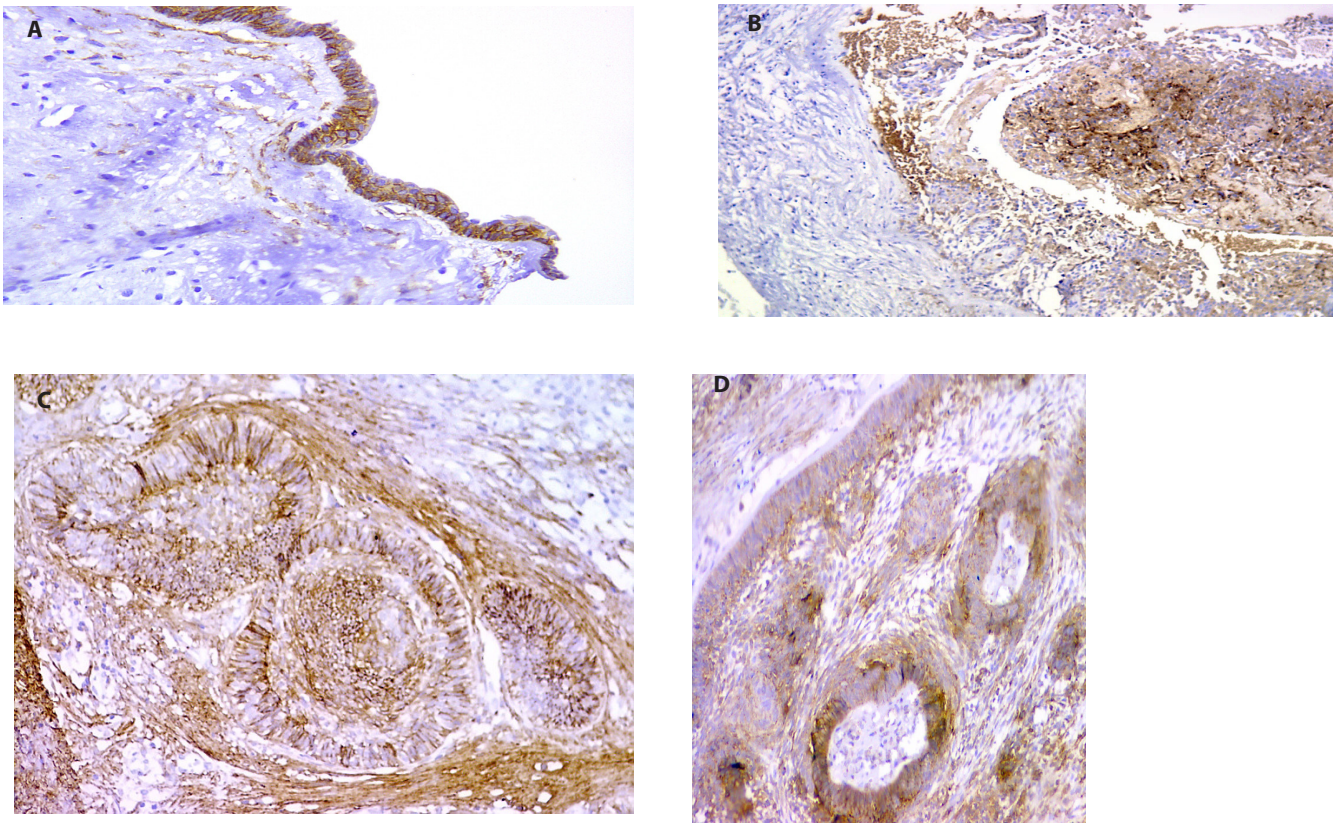

Fig. 2: (A) Dentigerous cyst case showing; membranous and cytoplasmic immuone positivity to Syn1E in the cyst lining epithelium and low Syn1S expression in the cytoplasm of fibroblasts in the CT (Syn1, PAP-DAB, 200X). **(B)** Unicytic- L/I Ameloblastoma showing the expression of Syn1 restricted to the lumial and intaluminal cystic epithelial contents with negative stromal reaction in the cyst wall (Syn1, PAP-DAB,100X). **(C)** Follicular Ameloblastoma with Strong diffuse membranous and cytoplasmic Syn1 expression (Syn1, PAP-DAB, 100X). **(D)** Ameloblastic Carcinoma cases showing: Diffuse cytoplasmic expression of Syn1 in the epithelium and CT (Syn1, PAP-DAB, 100X).

progression, and metastasis.

The current work has evaluated the expression of Epithelial and Stromal Syn1 markers according to the histological patterns of Conventional AB; we found that the Desmoplastic subtype of Conventional AB had the highest levels of Syn1S expressions than the other subtypes without significant differences. Moreover, it had the lowest levels of Syn1E. These findings could be due to the more aggressive nature of the Desmoplastic type as was mentioned by Zhi-Jun et al., 2009.[32] However, the published data about Conventional AB subtypes regarding to the proliferative and invasiveness indexes still very controversial. No correlations were detected between epithelial and stromal Syn1in DC, AB, or AC. From this context, epithelial and stromal Syn1 may act as two separate entities and better to be evaluated individually in future studies on OTs. These findings were supported by the findings of Alaeddini et al., 2019[26] who found no association between the expression of stromal and epithelial Syn1 in Salivary Gland Tumors.

Conclusion

Syn1S is highly expressed in ameloblastic carcinoma than ameloblastoma so, it can be used to differentiate ameloblastic carcinoma from aggressive ameloblastoma. Desmoplastic ameloblastoma is the most aggressive than other subtypes. Epethelial and Stromal Syn1 are acting as two separate entities.

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