Comparative Evaluation of CD138 Expression in Pleomorphic Adenoma and Mucoepidermoid Carcinoma: An Immunohistochemical Study

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Abstract- Salivary gland neoplasms are significant lesions in oral and maxillofacial pathology that exhibit diverse clinical and biological behaviors. Therefore, identifying novel markers capable of predicting the behavior of salivary gland tumors is crucial. CD138 or syndecan-1 is the primary syndecan expressed in epithelial cells and plays a critical role in tumor progression. The aim of this study was to evaluate the expression of this marker in two common salivary gland tumors. In this cross-sectional study, 20 pleomorphic adenomas (PAs) and 20 mucoepidermoid carcinomas (MECs) obtained via excisional biopsy and archived in the Pathology Departments of Al-Zahra and Ayatollah Kashani Hospitals of Isfahan University of Medical Sciences were evaluated. Clinical data including age gender of patients and anatomical location were collected. All specimens were immunohistochemically stained for syndecan-1 and the frequency and intensity of CD138 expression were calculated using the SID staining index and compared between the two tumor types. All statistical analyses were performed using SPSS version 28 employing the Mann-Whitney, Kruskal-Wallis, Chi-square, Fisher's exact and independent t-tests. A *P* of<0.05 was considered statistically significant. Statistical analysis of the lesions revealed a significant difference in staining intensity between the two tumor groups (*P*=0.033). A statistically significant difference was observed between the PA and MEC groups based on the SID index (*P*=0.002). Spearman's correlation analysis demonstrated a significant correlation between the SID index and age (*P*=0.007). Intermediate- and high-grade MECs showed significantly higher expression rates of CD138 (*P*<0.05). Increased marker expression correlated with a higher histological grade and advanced clinical stage in MECs. The use of this marker should be considered for determining the prognosis of PAs and MECs; specifically, CD138 serves as a useful prognostic predictor for MECs.

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Introduction

Salivary gland tumors constitute a significant proportion of oral and maxillofacial lesions. The annual incidence of salivary gland tumors is approximately 1-6.5 per 100,000 populations (1). Mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland tumor, accounting for 10%-15% of all salivary gland neoplasms and 30% of malignant salivary gland tumors, and it occurs most frequently after the age of 55 years, with a slight female predilection (2). It accounts for 4%-10% of all primary gland tumors and 13%-23% of

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accessory gland tumors (1). Low-grade MEC is most often seen in the minor salivary glands and is usually diagnosed in the early clinical stages, whereas high-grade MEC often develops in the major salivary glands especially the parotid glands (2). Histopathologically, this tumor is composed of mucous, interstitial, and epidermoid cells and sometimes cylindrical, clear, and oncocytic cells. Some tumors also show marked sclerosis of the stromal tissue. It is graded based on the proportion of different cell types, the ratio of solid to cystic components, and the degree of cytomorphologic atypia (1,3).

Pleomorphic adenoma is the most common salivary tumor in the major and minor glands (40%-70%) and is also the most common benign tumor. This tumor develops in the parotid gland and then in the hard palate and upper lip (4). The patient may be aware of the presence of the lesion for months or even years before presentation, as it usually presents clinically as a hard, painless, and slow-growing mass in young and middle-aged patients and occurs more often in females. Histopathologically, this lesion consists of a mixture of ductal and myoepithelial cells with round, spindle, or plasmacytoid cells in a mesenchymal stroma that can undergo myxomatous, chondroid, hyalinized, and other stromal changes depending on the activity of the myoepithelial cells. The tumor often has well-defined borders and it is encapsulated (5).

Syndecan-1 (SDC1) or CD138 is a member of the heparan sulfate proteoglycan family and is involved in cellular events by binding to various growth factors and extracellular matrix components. This protein plays an essential role in epithelial homeostasis, cell morphology, differentiation, proliferation and migration (6). This marker consists of 288 amino acids and has three domains: a C-terminal cytoplasmic domain, an extracellular N-terminal domain and a transmembrane region. CD138 is physiologically expressed on epithelial and leukocyte cells (7). The syndecan family is involved in cell adhesion, cytoskeletal organization, infiltration, and angiogenesis (8). CD138 expression has been investigated in various cancers of the body including lung, colorectal, gastric, prostate and others (9). However, insufficient studies have been conducted on this marker in salivary tumors (6). Identifying the marker expression level in tumors could be very helpful as a prognostic factor and determinant of tumor properties including type and could guide a more appropriate therapeutic approach for the patient. The purpose of this research was to compare the expression of CD138 in MEC and PA and to correlate its expression with clinicopathological parameters.

Materials and Methods

In this cross-sectional study, 40 formalin-fixed, paraffin-embedded tissue blocks including 20 pleomorphic adenomas and 20 mucoepidermoid carcinomas obtained through excisional biopsy were retrieved from the archives of the Pathology Departments of Al-Zahra Hospital and Ayatollah Kashani Hospital, of Isfahan University of Medical Sciences. Two oral pathologists reviewed the slides and the diagnoses of all tumors were confirmed. Specimens obtained through incisional biopsy, those lacking the necessary clinical information and those with inadequate block quality were excluded from the study. Clinicopathological data including age, gender of pateints, location of lesion, size, histopathological grade and clinical stage were extracted from the medical records.

After samples selection, immunohistochemical staining for CD138 was performed using a rabbit anti-human CD138 monoclonal antibody (clone EP201; Master Diagnostica) according to the following steps. Immunohistochemistry was performed on 3-4-µm-thick sections of paraffin-embedded tissue mounted on slides for CD138 staining. For antigen retrieval, the tissue sections were deparaffinized and rehydrated with distilled water. To block endogenous activity, the sections were immersed in 3% H_2O_2 for 3 minutes and washed with running tap water. Antigen retrieval was performed by heating 1,500 mL of the recommended retrieval solution (0.01 M citrate buffer, pH 6.0) to boiling in a pressure cooker. After the boiling temperature was reached, the sections were maintained at that temperature for 10 minutes and then immediately placed in cold water. The sections were washed in Tris-buffered saline (TBS) for 15 minutes and incubated in normal serum for 10 minutes. The sections were incubated overnight at 4° C with the primary CD138 antibody at a dilution of 1:25. They were then washed with TBS for 2-5 minutes and incubated with the appropriate biotinylated secondary antibody for 1 hour at room temperature (Mouse EnVision System HRP; Dako Cytomation).

Visualization was performed using freshly prepared 3,3′-diaminobenzidine (DAB) chromogen for 10 minutes, after which the slides were counterstained with Hematoxylin (Merck KGaA, Darmstadt, Germany). A sample of human tonsillar tissue was used as a positive control, whereas a sample prepared without the primary antibody served as the negative control.

The immunohistochemically stained slides were

examined simultaneously using a light microscope (Olympus BX41TF, Tokyo, Japan)with the student andtwo pathologists. The frequency and intensity of CD138 expression in the tumor cells were then determined. Stained cells were randomly counted at 400× magnification in 10 microscopic fields. Tissue samples showing brown cytoplasmic, membranous or both cytoplasmic and membranous staining were considered positive. The frequency of stained tumor cells was scored as follows: 0, negative; 1, 1%-10%; 2, 11%-50%; and 3, >50%. In addition, staining intensity was reported as mild, moderate, or severe (6). Staining was evaluated according to age and gender of pateints, tumor location and the microscopic grade of MEC. To determine the patients' clinical status, they were contacted by telephone and their medical records were reviewed. The 3-year prognosis was evaluated in terms of recurrence, metastasis or death.

All data were entered into SPSS version 28 and statistically analyzed using Fisher's exact, the Mann-Whitney, and Kruskal-Wallis tests. $P<0.05$ were considered statistically significant.

Results

This study included 40 samples of salivary gland tumors. In the present study, the mean age of patients with MECs was higher than PAs. PAs were more common in men, whereas MECs were more common in females. The parotid gland was the most common location in both lesions (Table 1).

In this study, there was not statistically significant between the two studied groups based on frequency of staining cells ($P=0.064$). A significant difference was found in CD138 staining intensity between the two salivary gland tumors ($P=0.033$). According to the Mann-Whitney test, the SID index in the MEC group (4.25±2.77) was higher than that in the PA group (1.8±1.609) which was statistically significant ($P=0.002$) (Tables 2 and 3).

The frequency of stained cells with CD138 was increased with increasing of histopathological grade of MECs, and this difference was significant based on Fisher's exact test ($P=0.01$). On the other hand, with increasing stage and worsening prognosis, as well as in patients who died, the percentage of tumor-stained cells was also higher, but this difference was not significant ($P>0.05$). Similarly, as the grade of malignancy of MEC increased, the intensity of stained cells was higher and this difference was significant based on Fisher's exact test ($P=0.001$). On the other hand, with increasing stage and worsening prognosis, as well as in patients who died, the intensity of tumor-stained cells was also higher, but this difference was not significant ($P>0.05$). Based on the SID index, CD138 expression in MEC increased significantly with worsening prognosis and patient death ($P<0.05$) (Table 4).

CD138 expression in MEC and PA increased with age and was also higher in females, although this was not statistically significant ($P>0.05$). Among patients with MEC, the highest expression was observed in the parotid gland tumors and the lowest expression in the palate tumors, with no significant difference based on location ($P=0.226$). In the PA group, the highest expression was in the palatal salivary glands tumors and the lowest in the submandibular gland tumors, which was statistically significant ($P=0.023$).

In this study, we also analyzed the association between CD138 expression and clinicopathological factors in both studied tumors. The Spearman test showed a statistically significant correlation between the SID index and age ($P=0.007$), as well as between staining intensity and age ($P=0.033$), but there was no significant correlation between staining frequency and age ($P=0.064$). The t-test showed that the mean age differed significantly between MEC and PA ($P=0.019$). There was no significant difference between the studied groups based on gender according to the Chi-square test ($P=0.11$), or based on location, according to Fisher's exact test ($P=0.248$). The SID index also did not show a significant difference in terms of gender or location ($P>0.05$). The percentage of stained cells for the CD138 did not show any significant difference based on age, gender or location ($P>0.05$). There was no significant difference between staining intensity and gender, according to the Chi-square test ($P=0.999$), or location, according to Fisher's exact test ($P=0.149$) (Table 5; Figures 1A-D).

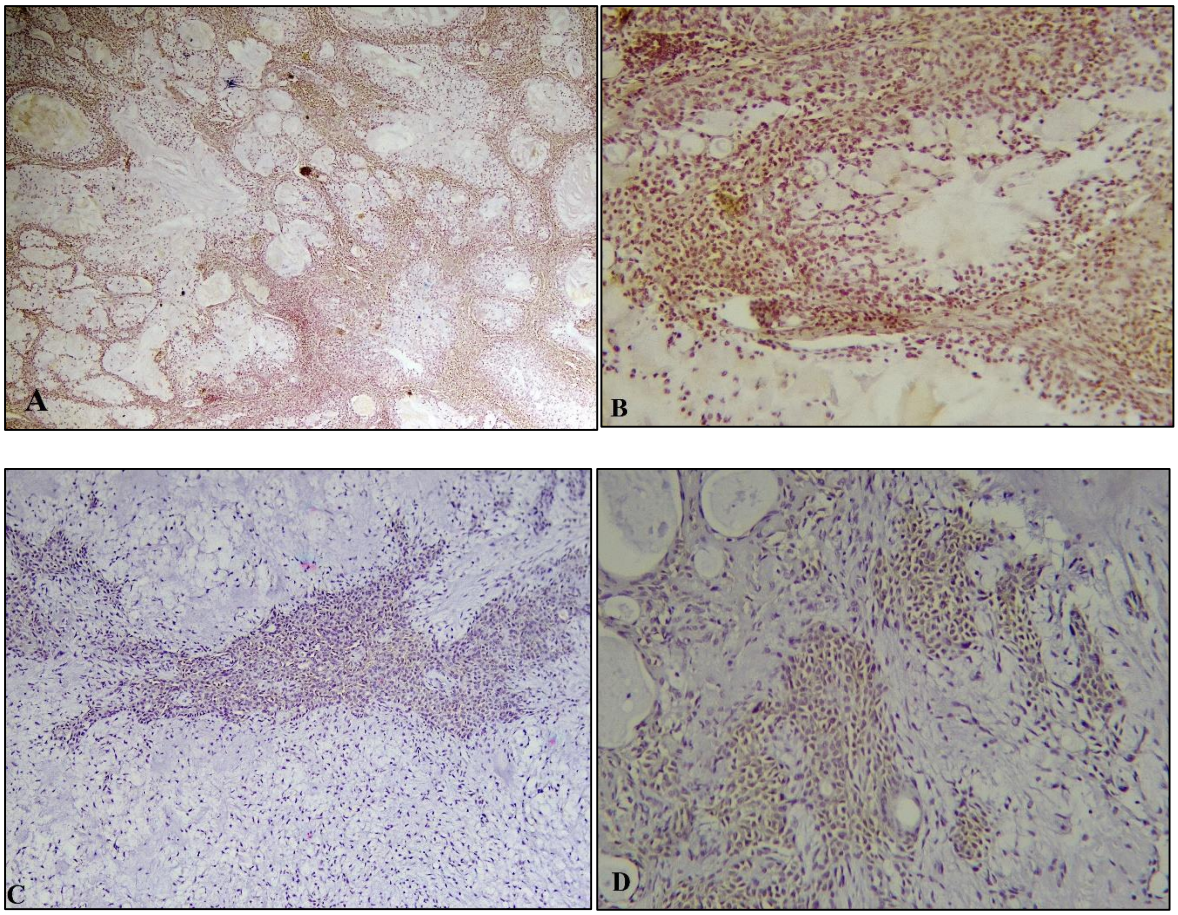

Figure 1. (A) Immunohistochemical staining for CD138 in MEC (IHC staining, ×100), (B) Strong positive staining for CD138 in MEC (IHC staining,×400), Immunohistochemical staining for CD138 in PA (IHC staining,×100), Mild positive staining for CD138 in PA (IHC staining,×400)

Table 1. Frequency of samples based on demographic variables

Variable		PA	MEC	Total
Age (Standard deviation±mean)		42.7±13.00	53.4±14.58	48.05±14.67
Gender	**Male**	11(55%)	6(30%)	17(42.5%)
N (%)	**Female**	9(45%)	14(70%)	23(57.5%)
	Parotid Gland	11(55%)	15(75%)	26(65%)
Location	**Submandibular Gland**	5(25%)	1(5%)	6(15%)
	Sublingual Gland	4(20%)	4(20%)	8(20%)

Table 2. Frequency of samples based on the mean of SID index for CD138

Variable		SD±mean	*P*
Groups	**PA**	1.8±1.609	*P*=0.002
	MEC	4.25±2.77	Mann-Whitney
Gender	**Male**	2.82±2.48	0.623
	Female	3.17±2.65	Mann-Whitney
	Parotid Gland	3.5±2.804	
Location	**Submandibular Gland**	1.17±1.602	0.072
	Sublingual Gland	2.88±1.642	Kruskal-Wallis

Table 3. Frequency of samples based on stained cells frequency and intensity for CD138

Lesion	Frequency {Number (%)}				Intensity {Number (%)}			
	Negative	1%-10% (+1)	11%-50% (+2)	More than 50% (+3)	No stained cells (0)	Mild (+1)	Medium (+2)	Intense (+3)
PA	4(20%)	9(45%)	7(35%)	0(0%)	4(20%)	9(45%)	6(30%)	1(15%)
MEC	0(0%)	3(15%)	12(60%)	5(25%)	0(20%)	7(35%)	9(45%)	4(20%)
Total	4(10%)	12(30%)	19(47.5%)	5(12.5%)	4(10%)	16(40%)	15(37.5%)	5(12.5%)

Table 4. Frequency of MECs based on SID index

Variable		Number	SD±mean	*P*
Age	**≤50**	8	3.5±2.5	0.314
	>50	12	4.75±2.92	
Gender	**Male**	6	4.33±2.87	0.86
	Female	14	4.21±2.83	
Salivary gland	**Parotid**	15	4.8±2.93	0.226
	Submandibular	1	4.00±0.00	
	Sublingual	4	2.25±1.25	
degree of malignancy (Grade)	**Low**	5	1.4±0.54	0.002
	Intermediate	10	4.3±2.00	
	High	5	7.00±2.73	
Stage of the disease	**I**	7	3.71±2.68	0.04
	Ⅱ	7	2.57±1.39	
	Ⅲ	5	6.4±2.51	
	Ⅳ	1	9.00±0.00	
Prognosis	**Without disease**	10	3.2±2.44	0.037
	Recurrent	6	3.67±1.50	
	death	4	7.75±2.50	

Table 5. Frequency of PAs based on SID index

Variable		Number	SD±mean	*P*
Age	**≤50**	10	1.20±1.29	0.079
	>50	10	2.40±1.77	
Gender	**Male**	11	2.00±1.89	0.844
	Female	9	1.56±1.23	
Salivary gland	**Parotid**	11	1.73±1.27	0.023
	Submandibular	5	0.6±0.89	
	Sublingual	4	3.5±1.91	

Discussion

In the current study, the mean age was 42.7±13 years in PAs and 53.4±14.58 years in MECs, representing a significant difference (*P*=0.019). In contrast to the present findings, Mayer reported a mean age of 44.6±18.3 years for MECs (7), and all patients in the study by Mohammad *et al*., were under 50 years of age (8). In a study by Alaeddini *et al*., 51.7% of MECs and 68.9% of PAs occurred in patients under 50 years of age (6). Nevertheless, most studies indicate that the mean age of patients with malignant salivary gland tumors is higher than benign tumors, which is consistent with our results (10,11). Variations in sample size and geographic regions may account for these discrepancies in studies results.

In the present study, no statistically significant difference was observed between the MEC and PA groups regarding gender (*P*=0.11). Although, most PAs were observed in males and most MECs occurred in females, the overall number of female patients exceeded that of males across the entire sample. Contrary to our findings, MECs were more predominant in males in the studies by Alaeddini *et al*., and Mayer *et al*., (6,7). Similar to our study, Mohammad *et al*., reported that 71.4% of PAs and 100% of MECs occurred in females (8). Likewise, Ghaderi *et al*., found that most patients were female (11), and Chloupek et al., observed that malignant salivary gland tumors were more common in females (12). These variations can likely be attributed to differences in sample size and location of research.

In the present study, the parotid gland was the most commonly affected site; however, Mohammad *et al*., found that while PAs most frequently involved the parotid gland, MECs were most common in the buccal mucosa (8). All MEC cases were located in the parotid gland in Mayer's study (7), while Alaeddini *et al*.,

reported that the major salivary glands were the most common site for both MECs and PAs (6). Consistent with our findings, the parotid gland is cited as the most common site of tumor involvement in most studies (10-13).

In the present study, the frequency of CD138-stained cells did not differ significantly between the two groups (P=0.064). The percentage of stained cells was 1%-10% in 45% of PAs, while 60% of MECs showed a rate of 11%-50%. However, staining intensity differed significantly between the groups (P=0.033); 45% of PAs exhibited mild staining intensity, whereas 45% of MECs showed moderate intensity. Furthermore, the mean SID index was significantly higher in MEC than in PA (P=0.002). Consistent with our results, Alaeddini *et al*., reported a higher percentage and intensity of CD138 expression in malignant lesions, such as MEC and adenoid cystic carcinoma, compared to benign tumors like PA and Warthin's tumor (6). In Mayer's study, the percentage of stained tumor cells in MECs was higher than in all other malignant tumors, particularly in those with moderate and high grades of malignancy (7). Similarly, Mohammad et al., observed higher CD138 expression in malignant tumors, especially adenoid cystic carcinoma and MEC than in benign tumors. Although, the difference between these two malignant lesions was not significant (8). Among benign tumors, PA exhibited the highest CD138 staining, although this was not statistically significant (8). Garbaran *et al*., also reported higher syndecan-1 expression in malignant tumors (14). Overall, these studies have demonstrated a significant increase in the expression of this marker, resemble to our findings.

Syndecan-1 (CD138) is a member of the proteoglycan family that plays an essential role in epithelial homeostasis, cell morphology, differentiation, proliferation, angiogenesis, and cell adhesion; it is involved in multiple cellular events by binding to various growth factors and extracellular matrix components (6). Consequently, various studies on tumors in different anatomical sites have implicated this marker in pathological processes involving both benign and more significantly malignant tumors (18).

In the current study, a significant correlation was found between age and the staining of salivary tumor cells (SID index); specifically, CD138 staining increased with advancing age. Furthermore, a significant difference was observed between staining intensity and patient age (P=0.033). However, no significant correlation was found between the percentage of stained cells and age (P=0.064). In contrast to our results, Alaeddini *et al*., reported a significant negative correlation between tumor cell staining and age (6). Mohammad *et al*., also observed higher staining intensity in tumors in patients under 50 years of age (8). Discrepancies between our findings and those of other studies may be attributed to differences in the types of tumors investigated, sample sizes, and geographical locations. In our study, CD138 expression was higher in females than males, though this difference was not significant. Alaeddini *et al*., reported a gender-based difference in the expression of this marker specifically in adenoid cystic carcinoma (6). Similar to the study by Alaeddini *et al*., there was no significant difference in CD138 expression across different lesion locations.

In the present study, CD138 expression increased significantly with the histological grade of MEC. Moreover, expression levels increased significantly with advanced clinical stages and were associated with a poorer prognosis and patient mortality. Mayer et al., reported higher CD138 expression in intermediate- and high-grade MECs than in low-grade cases, and expression levels were significantly elevated in tumors with lymph node metastasis (7). Garbaran *et al*., noted that high CD138 expression in malignant tumors serves as a prognostic factor and is often associated with a poor clinical course and increased invasiveness (14). The association between high CD138 expression and poor prognosis in malignant tumors stems from several pro-tumorigenic processes, including cell growth, proliferation, inhibition of apoptosis, angiogenesis, metastasis, and invasion. CD138 expression may directly or indirectly modulate the expression of other genes and can stimulate cancer stem cells and tumor-initiating cells, potentially influencing disease recurrence and treatment resistance (4).

CD138 (syndecan-1) and other syndecan proteins are present in various components of the tumor microenvironment including fibroblasts, inflammatory immune cells, vessels and extracellular matrix. Both epithelial and non-epithelial tumors may express stromal syndecans, and a significant link has been established between syndecans as biomarkers and tumor characteristics such as type, differentiation, and prognosis (15). Limitations of this study include the small sample size, limited patient follow-up, the focus on a single geographical area, and the occasional lack of statistical significance in some subgroup analyses. Given the limited research on CD138 expression in salivary gland tumors, further large-scale studies are warranted to validate these findings.

CD138 expression was significantly higher in MEC than in PA, and this difference may play a role in the biological behavior of these two lesions. Increased expression of this marker was associated with a poorer prognosis in MECs and should be considered when developing improved treatment strategies.

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