Value of Matrix metalloproteinase-7and Vimentin as Prognostic Biomarkers in Adenoid Cystic Carcinoma (Immunohistochemical Study)

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Abstract

Background: matrix metalloproteinase-7 (MMP7), its primary role is to break down extracellular matrix including casein, type I, II, IV, and V gelatins, fibronectin, and proteoglycan. The upregulation of MMP7 is associated with many malignant tumors. Vimentin (Vim) plays an important role in malignant tumors with regard to cellular adhesion, migration and signaling. In our current study, we have attempted to evaluate the expression of MMP7 and Vim as markers for aggressive biological behavior of AdCC. **Materials and Methods**: Nineteen formalin fixed paraffin embedded blocks of AdCC were collected retrospectively were included in this study. An immunohistochemical (IHC) staining was performed using anti MMP7 and anti Vim monoclonal antibodies. **Results:** MMP7 showed significant relation with larger size tumor (P=0.003), higher grade (P=0.006). The Vim was significantly correlated with minor salivary gland (P=0.041), larger size tumor (P=0.021), high grade (P=0.040). Both markers revealed highly significant correlation with advanced stage (P<0.001), and positive lymph node (LN) (P<0.001). There was a significant positive correlation between Vim and MMP7 (P=0.001).**Conclusion:** Both MMP7 and Vim are positively correlated with tumors of poor prognosis.

Keywords: Adenoid cystic carcinoma, MMP7, Vimentin, immunohistochemistry.

الخلاصة

خلفية:MMP7 دورها الأساسي هو كسر المصفوفة خارج الخلية بما في ذلك الكازين، النوع الأول، الجيلاتين الثاني والرابع، والخامس. ويرتبط زيادة التعبير النسيجي ل MMP7 مع العديد من الأورام الخبيثة. Vim لها دور مهم في الأورام فيما يتعلق بالتصاق الخلية والهجرة. في الدراسة الحالية، نحاول تقييم تعبير MMP7 و Vim كعلامات للسلوك العدواني البيولوجي ل AdCC.

المواد والطرق: تسعة عشر نسيج مثبت بالفورمالين ومطمورة بشمع البارافيين مشخصة نسيجيا كسرطان كيسي شبيه بالغدة. استخدم تقنية المناعة الكيميائية النسيجية لتقيم تعبير MMP7 و Vim كمضادات احادية النسل. **النتائج:** أظهرت MMP7 علاقة ذات دلالة مع الاورام الكبيرة (P = 0.003) ، والمتباينة للغاية نسيجيا (P = 0.006). ارتبط Vim بشكل ملحوظ مع الغدة اللعابية الصغيرة (P = 0.041) ، الاورام الكبيرة (P = 0.021)، والمتباينة للغاية نسيجيا (P = 0.040). كشفت كل من MMP7 و Vimعلامات ارتباط كبير للغاية مع المراحل المتقدمة نسيجيا (P <0.001) ، والعقدة الليمفاوية (P <0.001). وعلاوة على ذلك، كان هناك علاقة طردية ذات دلالة إحصائية بين MMP7وVim (P = 0.001).

الخلاصة: كل MMP 7 و Vim مرتبطة بشكل إيجابي مع أورام ذات مستقبل مرضي سيء.

الكلمات المفتاحية: سرطان كيسي شبيه بالغدة، ميتراسين، فامينتين، المناعة الكيميائية النسيجية.

1. Introduction

Adenoid cystic carcinoma (AdCC) was initially described by Bilroth in 1856 and named as cylindroma for its classic histologic appearance; this neoplasm underwent numerous name changes before being given its current name by Spies in 1930 (Jaso and Malhotra 2011). Is accounts for 1% of all head and neck cancers and about 10-22% of all malignant tumors of the major and minor salivary glands.Minor salivary glands (65%) are more frequently involved than major salivary glands (submandibular

- 19%, parotid - 16%). Occasionally, they arise from sites other than salivary glands, such as the lacrimal glands, the ceruminal glands of external auditory canal, nose, paranasal sinus, palate, nasophaynx, and larynx (Gandhi *et al.,* 2015; Spiro *et al.*, 1974). It is most common in the fifth and sixth decades of life; however, it can appear at virtually any age. The patient population review was reported to range from 10 to 96 years (Da Cruz *et al.,* 2006). Gender predilection is an inconsistent feature in the literature with some authors reporting a male predominance and others finding a female or no gender predilection (Khan *et al.,* 2001).The clinical course is characterized by an initial period of slow and indolent growth that is usually asymptomatic. In most cases the tumor goes unnoticed until it has invaded local nerves and structures causing varying symptoms depending on location (Da Cruz *et al.,* 2006). Thus, most patients will present with locally invasive disease. In a recent review of AdCC of the nasopharynx, 74.3% of patients showed advanced disease at the time of initial evaluation (Wiseman *et al.,* 2002). Cervical LN metastasis is a rare event; instead, the tumor spreads through a hematogenous route with distant metastasis appearing years, even decades, after initial diagnosis (Da Cruz *et al.,* 2006; Spiro, 1997). In one recent series, rate of distant metastasis was reported as 47.8% with mean time to distant metastasis reported as approximately 5 years (Rapidis *et al.,* 2005).

Matrilysin, also known as matrix metalloproteinase-7 (MMP7), pump-1 protease (PUMP-1), or uterine metalloproteinase is an enzyme in humans that is encoded by the *MMP7*gene (Quondamatteo *et al.,* 1999). MMP7 is a member of the matrix metalloproteinase family consisting of structural-related zinc-dependent endopeptidases. The primary role of cleaved/activated MMP7 is to break down extracellular matrix by degrading macromolecules including casein, type I, II, IV, and V gelatins, fibronectin, and proteoglycan (Yokoyama *et al.,* 2008). MMP7 found to potentially involve in tumor metastasis and inflammatory processes (Edman *et al.,* 2011). The upregulation of MMP7 is associated with many malignant tumors including esophagus, stomach, colon, liver, pancreas, and renal cell carcinomas. High MMP7 expression facilitates cancer invasion and angiogenesis by degrading extracellular matrix macromolecules and connective tissues. Theses degradations are associated with many mechanisms including embryogenesis, postpartum uterine involution, tissue repair, angiogenesis, bone remodeling, arthritis, decubitus ulcer, and tumor metastasis/invasion (Yokoyama *et al.,* 2008).

Vimentin (Vim) is a protein that in humans is encoded by the *VIM*gene. Is a type III intermediate filament protein that is expressed in mesenchymal cells (Eriksson *et al.,* 2009). Vim is reported as an important mesenchymal marker, and plays an important role in epithelial-mesenchymal transition in malignant tumors with regard to cellular adhesion, migration and signaling (Ivaska *et al.,* 2007). Several investigators have previously shown that Vim is an important marker for the early detection of cancer, such as bladder cancer, hepatocellular carcinoma and colorectal cancer (Costa *et al.,* 2010; Wong and Luk 2012). In addition, methylation of the *VIM* gene is described as a marker in several malignant tumors, including gastric carcinoma, colorectal carcinoma, cervical cancer and bladder cancer (Costa *et al.,* 2010; Shirahata *et al.,* 2009; Jung *et al.,* 2011). In our current study, we have attempted to evaluate the expression of MMP7 and Vim as markers for aggressive biological behavior of AdCC .

2. Materials and Methods

A total of Nineteen retrospective formalin fixed paraffin embedded blocks of AdCC were collected pro- and retrospectively from the archives of Oral and Maxillofacial Pathology Department, College of Dentistry, University of Baghdad; Al-Shaheed Ghazi Hospital; Al-Yarmok Hospital, and Al-Diwaniya Hospital from (2010 – 2016). The diagnosis of each case was confirmed by the histological examination of the Hematoxylin and Eosin stained sections (Figure 1) by two experienced pathologists. Tumor Stages were carried out according to WHO classification schema (Seifert and Sobin, 1991), while tumor grades were established as follows: Grade I (well differentiated), tumors with tubular and cribriform areas but without solid components; Grade II (moderately differentiated), cribriform tumors that were either pure or mixed with less than 30% of solid areas; and Grade III (poorly differentiated), tumors with a predominantly solid pattern (Gnepp, 2009). Four μm thick sections were cut for IHC staining with anti MMP7 and Vim Rabbit monoclonal antibodies (Abcam UK). Negative and positive controls were included in each IHC run. Four normal salivary gland tissues were used as negative external controls, while tissue blocks of prostatic carcinoma was used for MMP7, and tissue blocks of breast adenocarcinoma was used for Vim (according to antibodies manufacturer).

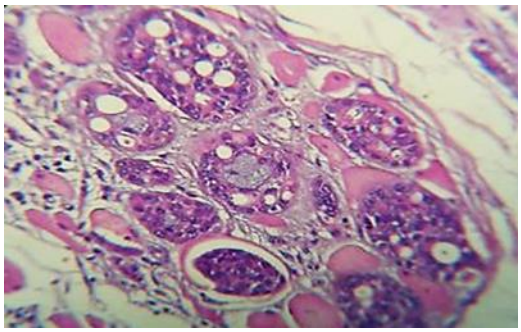

Figure1: Adenoid Cystic Carcinoma (40X). (H&E)

2.1 Evaluation of IHC Results:

The immunoreactions evaluation analyzed according to the presence or absence of brown immunostaining in the cytoplasm, however,the intensity of stain was ignored because its subjected to individual difference during checking.The expression of both markers was evaluated semi-quantitatively. It was obtained by counting the number of tumor cells by light microscope in 5 fields (using 40Xobjective in most represented areas of sections). Labeling index for each field was calculated using the following equation: (number of positive cells/ number of total cells); the mean value of labeling indices for the five fields was considered to be the label index for the case. For the MMP7 the percentage of positive cells was scored as follows:0 = 0%, score 1= 1-25%, score2= 26-50%, score 3= 51-75% and score 4= 76-100% (Sugita et al., 2004). While for Vim positive cells was scored as follows: 0= negative, score 1=<10%, score 2= 11–50%, score 3=51–80%, and score 4=>80% positive cells (Liu *et al.*, 2010).

2.2 Statistical analysis

Data were analyzed using SPSS (Statistical Package for Social Science; version 16) and Microsoft Office Excel 2007. Numeric variables were expressed as mean±SD (standard deviation) whereas nominal variables were expressed as number and percentage. Pearson's Chi-Square test was used to evaluate nominal variable frequency difference between groups.Spearman Rank correlation coefficient would be used to study correlation between ordinal variables. P-value was considered

significant when it was (≤0.05), while the level of (<0.001) was considered highly significant.

3. Results

The current work included 19 patients with AdCC with a mean age of 43.11±20.11 years and age range of 25-80 years. Thirteen patients (68.4%) were less than 45 years and 6 patients (31.6%) were more than 45 years of age , the majority of patients were female (12= 63.2%) whereas male patients accounted for (7= 36.8%). The tumor was mostly seen in minor salivary glands (12= 63.2%), and mass lesion was the dominant presentation (14= 73.7%). Mean size of tumors was 2.66±1.07 cm with a range of (1- 5.5 cm). Table: 1.

Table 1: Clinical characteristics

Characteristic		N (%)	Mean±SD (range)
Age (years)	≤45 years	13 (68.4%)	43.11±20.11 (25-80)
	>45 years	6 (31.6%)	
Gender	Male	7 (36.8%)	
	Female	12 (63.2%)	
Site	Major	7 (36.8%)	
	Minor	12 (63.2%)	
Clinical presentation	Mass	14 (73.7%)	
	Ulcer	5 (26.3%)	
Size (cm)			2.66+1.07 (1- 5.5)

N: Number of cases; SD: Standard deviation

Histological sections revealed that the majority had moderately differentiated morphology (14= 73.7%), followed by well differentiated (3= 15.8%) then poorly differentiated morphology (2=10.5%). Positive LN metastasis was seen in 10 patients (52.6%) and the majority of patients had stage II disease (6= 31.6%); stage I disease was seen in 3 patients (15.8%), stage III was seen in 5 patients (26.3%) and stage IV was seen in 5 patients (26.3%). Table: 2.

Table 2: Stage, grade and LN involvement

Characteristic		N (%)
Grade	Well	3 (15.8%)
	Moderate	14 (73.7%)
	Poor	2 (10.5%)
LN	Positive	10 (52.6%)
	Negative	9 (47.4%)
Stage	I	3 (15.8%)
	II	6 (31.6%)
	III	5 (26.3%)
	IV	5 (26.3%)

Immunohistochemical expression of MMP7, both score 2 and 3 reveled the same number and percentage (8 patients =42.1%), whereas 3 patients (15.8%) had score 1. The immunohistochemical expression of Vim was distributed as follows: The majority of the cases expressed Vim in score 2 (10 patients =52.6%), followed by score 1 (6 patients =31.6%) whereas 3 patients (15.8%) had score 3. Table: 3 and Figure: 2, 3 and 4.

Table 3: Immunohistochemical expression score of Vim and MMP7

Score	MMP7		Vim	
	N	%	N	%
1	3	15.8%	6	31.6%
2	8	42.1%	10	52.6%
3	8	42.1%	3	15.8%
Total	19	100%	19	100%

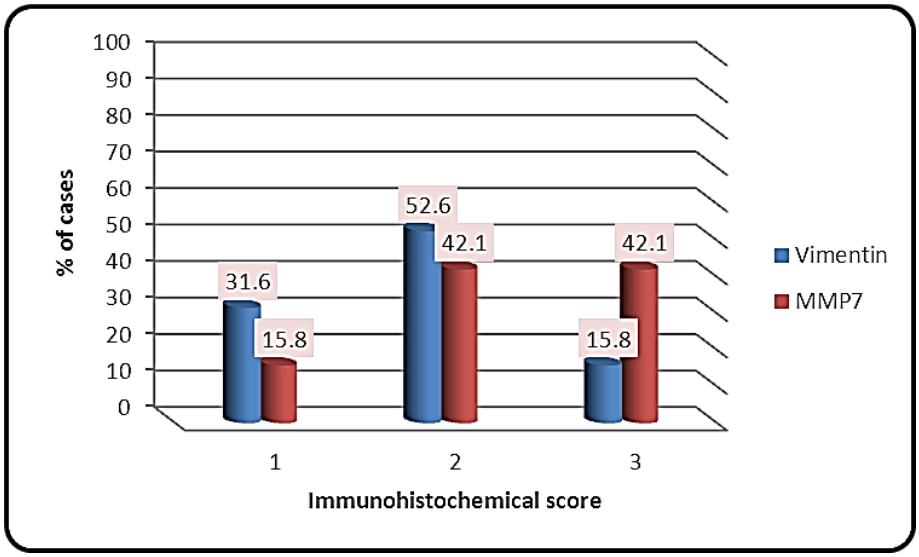

Figure 2: Immunohistochemical expression score of MMP7 and Vim

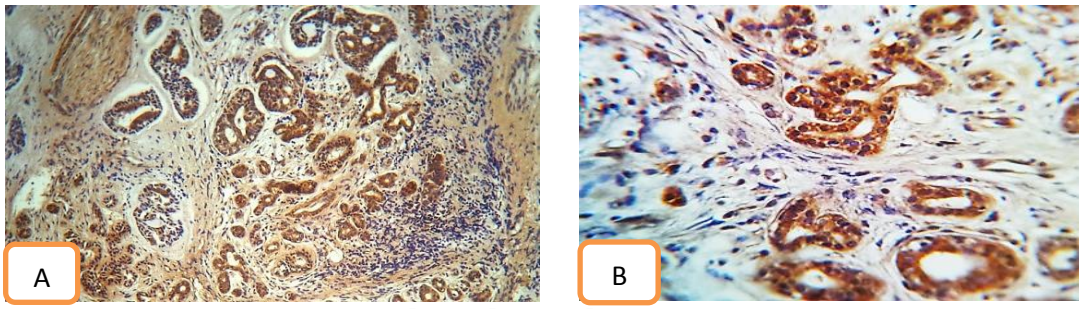

Figure 3: MMP7 immunohistochemical expression (A-40X) (B-100X)

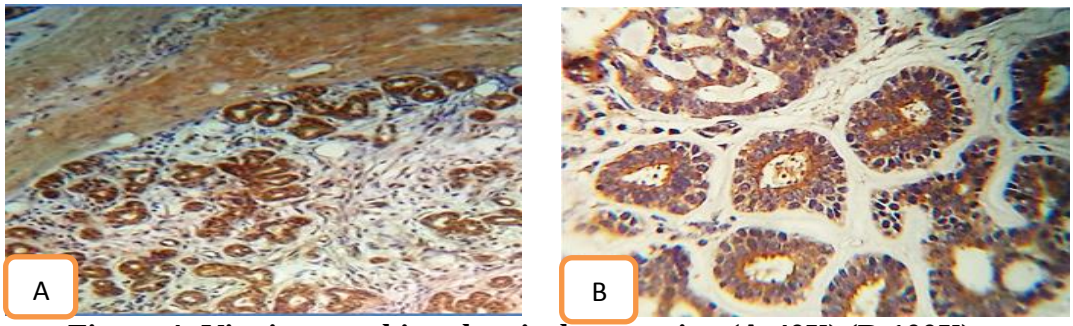

Figure 4: Vim immunohistochemical expression (A-40X) (B-100X)

According to Chi-Square test, the immunohistochemical expression of MMP7 was positively correlated with larger size tumor (r=0.649, P=0.003), higher grade (r=0.506, P=0.006), while highly correlation were registered with advanced stage (r=0.780, P<0.001) and positive LN metastasis (r=0.815, P<0.001). The Vim was positively correlated with minor salivary gland (r=0.473, P=0.041), larger size tumor (r=0.526, P=0.021), high grade (r=0.476, P=0.040), whereas highly significant relation recorded with advanced stage (r=0.763, P<0.001), and positive LN involvement (r=0.733, P<0.001). Moreover, There was a significant positive correlation between Vim and MMP7 (r=0.709, P=0.001). Table: 4.

Table 4: Correlation between score of Vim and MMP7 and clinicopathological parameters

	Correlation	Site	Size	Stage	Grade	LN	Clinically	Vim
Vim	r	0.473	0.526	0.763	0.476	0.733	0.494	
	P	0.041	0.021	<0.001	0.040	<0.001	0.232	
MMP7	r	0.389	0.649	0.780	0.605	0.815	0.272	0.709
	P	0.100	0.003	<0.001	0.006	<0.001	0.259	0.001

***r:** Correlation coefficient**; P:** level of significance

4. Discussion

This study is not a large epidemiological one that expressed the incidence and prevalence of different clinico-pathological features of AdCC, therefore the limited number and the random selection of the cases according to what is available preclude for definitive clinical findings.

The primary role of MMP7 is to break down extracellular matrix by degrading macromolecules including casein, type I, II, IV, and V gelatins, fibronectin, and proteoglycan, and the upregulation of MMP7 was found to associate with many malignant tumors (Yokoyama *et al.*, 2008). In our study sample, the tumor cells mostly showed MMP7 overexpression in score 2 and 3 (42.1%) respectively, while the lowest expression was noted in score 1(15.8%). This is in agreement with the finding of Lu *et al.*, 2006. However, in study done by Saarialho-Kere *et al.*, 1995 found reduced expression of MMP7 in cancer cells and concluded that this may be caused by incomplete differentiation, since healthy salivary gland tissue also expresses MMP7.

The present study showed that MMP7 is positively correlated with larger size tumor and this may be an indication of poor prognosis. This correlation with larger size may be explained by the ability of a tumor cell expressing this enzyme for rapid degradation of surrounding tissues. This is in harmony with the result of Tanioka *et al.*, 2003 which found that overexpression of MMP7 may result in the destruction of

physical barriers surrounding tumor cells and promote the progress of tumor invasion. Spiro *et al.,* 1974 reported that tumor size in excess of 3 cm and cervical node involvements were highly predictive of distant metastases in 196 AdCC patients.

The MMP7 also showed a significant positive correlation with higher grade tumor which is probably due to the acquisition of further cellular potential with advancing grade due to accumulation of multiple mutation and enhancement of dedifferentiation of tumors, which is consistent with previous study (Han *et al.,* 2015), and indicates that MMP7 expression is closely related to advanced histological grades.

MMP7 was proved, in the present study, to be highly correlated with advanced stage and positive LN metastasis and this is a poor marker prognosis. The proposed explanation for these correlations is that MMP7 facilitates invasion and angiogenesis mechanism which leads to the ability of a cell expressing metalloproteinase to invade vascular and lymphatic channels. Similar results were recorded in previous studies (Yokoyama *et al.,* 2008; Lu *et al.,* 2006).

In the present study, high Vim overexpression was registered in score 2(52.6%), while lowest expression was registered in score 3(15.8%). This result is consistent with a literature review (Darling *et al.,* 2002); similar findings have been reported by Cavalcante *et al.,* 2007 who concluded that Vim is sensitive immunohistochemical marker of myoepithelial cells in salivary gland tumors.

Concerning the positive correlation of Vim with minor salivary glands, might be explained by the more aggressive behavior of minor salivary gland tumors in comparison with major salivary tumors and this may be related to differences in anatomical patterns of major and minor salivary glands since minor salivary glands are devoid of well-developed connective tissue capsule and subsequently malignant tumors will have easier access to the surrounding tissue than the case in well capsulated major salivary gland tumors. In other words Vim may be regarded as a marker of the invasive capability of the salivary gland AdCC. This is in harmony with the result of Chomette *et al.,* 1982, in which 86 out of 117 cases were located in minor salivary glands. Contrarily, Eisele and Johns 2001 pointed out that AdCC is most common in the submandibular and sublingual glands.

The significant positive correlation of Vim with higher grade may be an indication of poor prognosis; similarly the highly significant correlations with advanced stage and positive LN are markers of poor prognosis. This indicates that Vim plays a role in epithelial-mesenchymal transition, in which an epithelial acquires mesenchymal properties. This was also observed by Mani et al., 2008 who concluded that epithelial-mesenchymal transition transformed malignant cells are more motile and can be more efficient in invading the surrounding tissues and as a result metastasize to distant organs. However, Abdulhussain et al., 2013 did not find any significant association between Vim expression and the tumor grade or stage, suggested that Vim expression may be important in oncogenesis but not in determining the tumor grade or stage. Similarly, Spiro, 1997 concluded that cervical LN metastasis is a rare event. Instead, the tumor spreads through a hematogenous route with a distant metastasis appearing years, even decades, after initial diagnosis.

Increased tumor size has been linked to cervical involvement, high recurrence rate, and poor prognosis (Woolgar, 2006). In our study the significant positive correlation between Vim expression and size of tumor is also an indirect evidence of invasive capacity of the tumor; since the larger the tumor, the greater the possibility of invasion. However, recent studies suggested that tumor size did not predict nodal disease, and it is now widely accepted that tumor depth is more accurate predictor of sub-clinical nodal metastasis, local recurrence and survival than tumor size (Woolgar, 2006; Larsen *et al.,* 2009).

MMP7 score was proved to be positively correlated with Vim. This is an indication of the combined ability of a malignant cell to express both markers when they undergo down differentiation. These results suggest that MMP7 and Vim may contribute in epithelial acquires mesenchymal properties and making the cell more powerful in invasion and metastasis. This is in agreement with the findings of Ding et al., 2016. In accordance with these figures, Zhu, 2010 found that Vim and MMP7 expression showed a positive correlation ($rs = 0.789$, $P<0.01$), and concluded that they can be used as a reference indicator of early diagnosis and prognosis. Our study supports these findings and concluded that VIM and MMP7 are positively correlated with tumors of poor prognosis.

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