

Association Of Caveolin-1 With Tumor Angiogenesis And Histological Aggressiveness In Salivary Mucoepidermoid And Adenoid Cystic Carcinomas

Reham AA Morsy¹, Effat Ahmed Abbas¹, Mona M El-Batran¹, Adel A Shoman² and Salwa M Sarhan¹

¹Basic and Dental Science Department, Oral and Dental Medicine Research Division, National Research Center, Cairo, Egypt

²Faculty of Dental Medicine for Girls, Oral and Dental Pathology, Oral Pathology Department, Al-Azhar University, Cairo, Egypt

E-mails: Rehammorsy77@yahoo.com

Abstract: Background: Angiogenesis is a critical aspect of cancer biology and considered as crucial step in the successful growth, invasion and metastasis of many solid tumors. The role of caveolin-1(CAV-1) in cancers remains controversial, and has been a subject for numerous investigations. In addition to its role in tumor cell biology, there is increasing evidence that caveolin-1 may also play an important role in angiogenesis. However CAV-1 has been implicated in the pathogenesis of oncogenic cell transformation, tumorigenesis, and metastasis of different tumors, the histopathologic significance of CAV-1 expression and its correlation with angiogenesis remains unknown in salivary mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (ACC) **Methods:** Based on an immunohistochemical study, the expression levels of caveolin-1 and vascular permeability factor (VPF) and the intratumoral microvessel density (MVD) in 20 samples of MEC and 20 samples of ACC were investigated, and correlations with histopathologic parameters (different grades and patterns of MEC and ACC) were evaluated statistically. **Results:** The overall percentages of the 40 specimens expressing CAV-1 in MEC and ACC were 80% and 60% respectively. Most of the cases of MEC and ACC were positive for antibodies of VPF (95%). Immunoreactivities of these biomarkers in salivary MEC and ACC were higher than those in normal salivary gland with a statistical significant difference ($P < 0.00$). Furthermore, CAV-1 immuno-reactivity was higher in low grade MEC than in high grade MEC and also, CAV-1 expression was higher in MEC than in ACC but with no statistical significance. VPF immuno-reactivity was higher in high grade MEC than in low grade MEC and was higher in the solid form of ACC, followed by tubular-trabecular pattern and finally the cribriform pattern showed the least values with a statistical significant difference ($P < 0.05$). Microvessel density of salivary MEC and ACC was higher than that of normal salivary gland ($P < 0.00$). MVD was significantly higher in the tumors with low caveolin-1 expression and in the tumors with high VPF expression in the different salivary gland carcinomas. **Conclusions:** The current results suggest that caveolin-1 may function as a tumor suppressor in MEC and ACC of the salivary glands. Reduced expression of caveolin-1 and increased VPF expression and MVD may indicate a poor prognosis for patients with salivary MEC and ACC. Therefore, these biomarkers can be molecular targets for therapy of metastasis of salivary gland tumors.

[Reham AA Morsy, Effat Ahmed Abbas, Mona M El-Batran, Adel A Shoman and Salwa M Sarhan. **Association of Caveolin-1 With Tumor Angiogenesis And Histological Aggressiveness In Salivary Mucoepidermoid And Adenoid Cystic Carcinomas.** *Life Sci. J* 2014; 11(6):646-654] (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 99

Keywords: Caveolin-1, Vascular permeability factor, Microvessel density, Mucoepidermoid carcinoma, Adenoid cystic carcinoma, Immunohistochemistry.

1. Introduction

Malignant salivary gland tumors constitute a major challenge in head and neck oncology because of its frequency, varied histological typing, and poor response to other therapies. It is further complicated by the absence of clear parameters to preview biological behavior, in particular to predict development of metastasis (Chen *et al.*, 2008 and Sergio *et al.*, 2009). In this sense, there is few information regarding pathways implicated in tumor dissemination of salivary gland cancer. So, knowledge of cellular and molecular properties that influence the

dissemination of metastatic tumor cells is important for new treatment strategies of salivary gland cancer.

Caviolin proteins (Caviolin 1, Caviolin 2, Caviolin 3) serve as the structural components of caveolae and also functioning as scaffolding proteins, which are capable of recruiting numerous signaling molecules to Caveolae and regulating their activity (Cohen *et al.*, 2004 and Parton *et al.*, 2006). Caveolin 1 (CAV-1) (a 22 kDa protein) has been implicated in the pathogenesis of oncogenic cell transformation, tumorigenesis, and metastasis of many solid tumors (Navarro *et al.*, 2004 and Williams and Lisanti, 2005). Caveolin-1 has been reported to be

dysregulated in various cancers, as caveolin-1 expression is decreased in several cancers such as ovarian carcinoma, pulmonary adenocarcinoma, and breast cancer and thought to function as a tumor suppressor gene (*Hino et al., 2003 and El-Sheikh et al., 2008*). On the other hand, the CAV-1 expression is upregulated in the other cancers such as renal cell carcinoma, prostate cancer, and breast carcinoma and associated with higher stage, cancer progression, and poor prognosis (*Savage et al., 2007, Cho et al., 2008 and Sloan et al., 2009*). In addition to its role in tumor cell biology, there is increasing evidence that caveolin-1 may also play an important role in angiogenesis.

Angiogenesis is regulated by the balance of positive and negative angiogenic factors. Among these factors, Vascular permeability factor (VPF) which is regarded as the most potent candidate for the induction of tumor angiogenesis by acting as specific stimulatory mitogenic molecule for endothelial cells (*Tonini et al., 2004, Hillen and Griffioen, 2007 and Lorusso and Ruegg, 2008,*). The assessment of the angiogenic capacity of a tumor could be performed via measuring the density of the microvessel in histological sections of the tumor using pan endothelial markers such as (CD34) which is an immunoglobulin vascular adhesion molecule that is involved in endothelial cell migration and angiogenesis (*Gratzinger et al., 2003*). Individual tumor may demonstrate a great deal of heterogeneity in MVD values where the MVD value were significantly higher in group with worse pathologic types, metastases or advanced TNM stage than their counterpart (*Chou et al., 2005*).

The correlation between the expression of caveolin-1, VPF and the MVD may be a reliable indicator of disease progression and clinical outcome of several tumors. In addition, few reports are available on angiogenesis and its histological relevance in salivary gland tumors. Thus, the objectives of the current study were to investigate the expression levels of caveolin-1, VPF and the intratumoral microvessel density (MVD) (labeled by CD34) in MEC and ACC and their correlation with each other and with different histological grades and patterns of the salivary gland carcinomas (MEC, ACC).

2. Methodology

1). Tissue Specimens

Formalin-fixed, paraffin-embedded tissue blocks were obtained from 40 patients with salivary gland carcinomas (20MEC, 20ACC) of SGT. Sections were retrieved from files of National Cancer Institute, Cairo University. Diagnoses of the carcinomas were based on histological examination of H&E stained tissue.

Serial sections of 4 mm were cut from the paraffin embedded blocks. Another 10 paraffin embedded tissue blocks of normal non-neoplastic salivary gland tissue at the periphery of the sample were also selected as control group. The specimens are mounted on positively charged glass slides (Optiplus, Biogenex, USA). Histopathologic grading was determined according to the World Health Organization grading scheme (*Seifert et al., 1991*). Of the 20 MECs, 10 were classified as low grade and 10 as high grade MEC. Of the 20 ACCs, 10 were classified as cribriform, 5 were classified as tubular-trabecular and 5 as solid patterns.

2)- Immunohistochemistry

The following primary antibodies were used: monoclonal mouse antihuman caveolin-1 (Clone 4D6 Cat. No. L155908 1ml, Novocastra, UK, dilution, 1:10). Monoclonal mouse antihuman VPF (dilution, 1:50; Santa Cruz Biotechnology, Santa Cruz, Calif). Monoclonal mouse antihuman CD34 (ready to use; Santa Cruz Biotechnology, Santa Cruz, Calif). Tissue sections were deparaffinized, rehydrated and treated with endogenous peroxidase in 0.3% H₂O₂ for 30 min to block the endogenous peroxidase activity. For antigen retrieval, the slides were placed in a container filled with target retrieval solution (code no. S3308 Dako), and heated in a microwave oven at 100°C for 15 min. The positive test slides were incubated with the mouse monoclonal primary antibodies in their appropriate dilution ranges overnight at room temperature in a humidified chamber.

After washing with phosphate buffer solution (PBS), the slides were treated with the biotin labeled link antibody, then the streptavidin conjugated to horseradish peroxidase was used. The diaminobenzidine (DAB) chromogen was applied to visualize the antigen antibody reaction. All these reagents belong to the universal Labeled Streptavidin-Biotin 2 System, Horseradish Peroxidase (code no.K0673 DakoCytomation, Denmark). All the slides were immersed in Mayer's hematoxylin for counterstaining. Finally, the sections were covered by cover slips using aqueous mounting medium.

3). Immunohistochemical Evaluation:

The ordinary light microscope was used to detect and localize the immunostaining of Caveolin-1 and VPF proteins. Cells with cytoplasmic or membranous brown staining were considered positive. Then, all the sections were examined by an image analyzer computer system using the software Leica Queen 500. Five random fields in each specimen were captured using a magnification (X400) to determine the area percentage and immunostaining intensity of the positive tumor cells. The area percentage and

immunostaining intensity were scored from 1 to 3 according to the difference between the largest and smallest mean value of each parameter in the studied cases. The scores of both area percentage and immunostaining intensity were then summed to obtain a single total score. The overall reaction was considered mild (Score 1 & 2), moderate (Score 3 & 4) or strong (Score 5 & 6) according to the single total score (*Lim et al., 2003*).

4)- Microvessel Density Determination

To determine the MVD, the stained normal and tumor tissue sections were initially screened microscopically at low power (100×) to identify the areas of highest vascularization ("hot spots"). Five high-power (400×) fields were then chosen randomly, and the number of microvessels in each high-power field (0.09 mm²) was counted for each sample with the use of an ocular grid. MVD for each sample was taken as the mean count of CD34 immuno-stained vessels of the five values obtained. MVDs in the NSG adjacent to tumor tissue were also counted for comparison.

5)- Statistical Analysis

All the obtained total scores of the examined sections were given as mean values \pm standard deviation (SD) for statistical evaluation. The ANOVA test was used to compare the overall expression of different proteins among the NSG tissue and different grades and patterns of SG tumors. Paired Student's t-test was used to compare each two grades and patterns together. Correlation Matrix test was done to examine if there is significant correlation between the Caviolin-1 expression, VPF expression and MVD in the different salivary gland carcinomas. Statistical analysis was calculated using the SPSS version 11.5 software package. A statistical difference of $P < 0.05$ was considered significant.

3.Results

Immunohistochemical Findings

Normal salivary gland (Control group):

Immunohistochemical analysis revealed a positive, diffuse, cytoplasmic and membranous staining pattern of CAV-1 expression in the ductal epithelial cells and myoepithelial cells in all 10 normal salivary gland samples (Fig. 1C). Measured by the image analyzer computer system, the mean value of area % of CAV-1 immunoreactivity was 5.04 ± 1.67 .

Immunohistochemical staining of VPF was detected in the cytoplasm of both normal ductal epithelial cells and serous acini, as evidenced by the presence of brown stained, granular, immunoreaction products, while the mucous acini were devoid of VPF expression (6.05 ± 0.35) (Fig. 1A). Regarding MVD,

Most of the normal salivary gland control cases showed mild immunostaining for CD34 in the endothelial lining of BV and only very few cases showed moderate degree of MVD. The mean value of no of BV was 2.10 ± 0.28 (Fig. 1B).

Mucoepidermoid carcinoma (MEC):

Sixteen out of the twenty examined cases of MEC revealed CAV-1 immunopositivity (60%). The CAV-1 immunostaining was seen in (60%) of low grade and (60%) of high grade MEC. Eight cases of MEC showed marked fine, granular pattern at the surface membrane and the cytoplasm of cancer cells, eight cases showed reduced expression, while four cases were negative. The CAV-1 was seen in cytoplasm of both epidermoid and mucous secreting cells. The mean value of area % of caviolin-1 immunoreactivity in high and low grade MEC was 10.22 ± 3.13 and 10.57 ± 4.51 respectively (Fig. 2A and 2D).

The studied cases of low grade MEC showed positive VPF immuno-reaction which ranged from moderate to strong. The mean value of area % was 28.04 ± 0.85 . Regarding MVD, Most of the studied cases showed moderate MVD while few cases showed marked MVD and other showed mild MVD. The mean value of no of BV was $9.85 \pm .715$ (Figs. 2B&2C). The studied cases of high grade MEC showed positive VPF immuno-reaction and the positive expression was diffuse in all the eopidermoid cell nests or sheets with a strong intensity. The mean value of area % was 47.8 ± 1.33 . Regarding MVD, in contrast to the low grade MEC, the high grade MEC showed marked MVD and few cases showed moderate MVD. Most of the B.V. showed sever elongation and marked increase in the diameter of B.V. The mean value of no of BV was 19.01 ± 2.04 (Figs. 2E&2F).

Adenoid cystic carcinoma:

Fifteen out of the twenty cases of ACC examined revealed CAV-1 immunopositivity (8 cases of cribriform, 4 cases of tubular-trabecular pattern while the five cases of solid pattern were negative). Seven cases of ACC showed marked diffuse, granular pattern at the surface membrane and the cytoplasm of the uniform neoplastic and myoepithelial cells lining the duct and cystic spaces, five cases showed reduced expression, while eight cases were negatively stained. The mean value of area % of CAV-1 immunoreactivity in cribriform and tubular patterns of ACC was 9.17 ± 2.63 and 9.95 ± 2.46 respectively (Figs. 3A&3B).

In cribriform pattern of ACC, the examined cases showed mild to moderate diffuse VPF immuno-reaction which detected mainly in the tumor cells but

not in the stromal cells. The mucinous substances as well as hyalinization material showed negative VPF immunoreactivity. Few cases showed strong VPF immunoreaction. The mean value of area % was 38.0 ± 2.74 (Fig. 4A). Regarding MVD, Most of the cribriform cases of ACC showed moderate MVD, while some cases demonstrated mild MVD and very few cases showed marked MVD. Some cases showed little elongation of B.V and slight increase in the diameter of B.V. The mean value of no of BV was 13.37 ± 0.35 .

Most of the tubular-trabecular cases of ACC revealed positive expression of VPF ranged from moderate to strong expression while few cases showed mild immunoreaction. The mean value of area % was

44.65 ± 0.07 (Fig. 4B). Regarding MVD, Most of the studied tubular-trabecular cases of ACC showed moderate MVD, while other cases demonstrated marked MVD. The mean value of no of BV was 14.35 ± 0.21 .

Most of the solid cases of ACC showed a strong positive VPF expression in the tumor cells of solid sheets while C.T stroma showed negative immunoreaction. Few cases revealed a moderate immunostaining for VPF. The mean value of area % was 46.3 ± 1.05 (Fig. 4C). Regarding MVD, Most of the solid cases of ACC showed marked MVD and few cases showed moderate MVD with marked increase in B.V. diameter. The mean value of no of BV was 15.7 ± 0.23 (Fig. 4D).

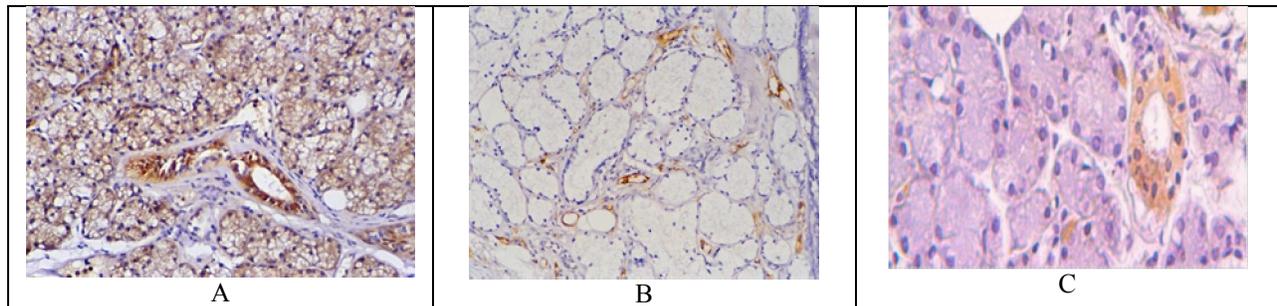


Fig (1): A photomicrograph of NSG stained immunohistochemically with VPF (A), CD34 (B) and CAV-1 (C).

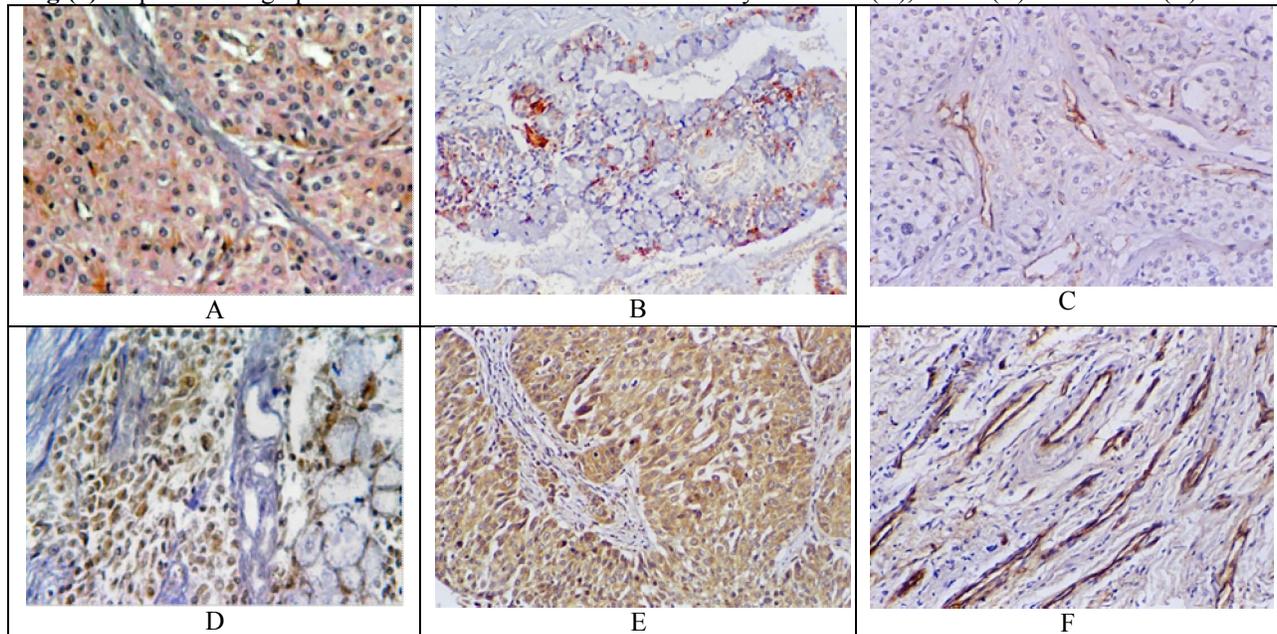


Fig (2): A photomicrograph of low grade MEC showing marked immunostaining for CAV-1(A), moderate immunoreaction for VPF (B) and mild-moderate MVD (C), While in high grade MEC moderate immunostaining for CAV-1 was detected (D), strong positive immune-reaction for VPF (E) and marked MVD and sever elongation of blood vessels were showed (F).

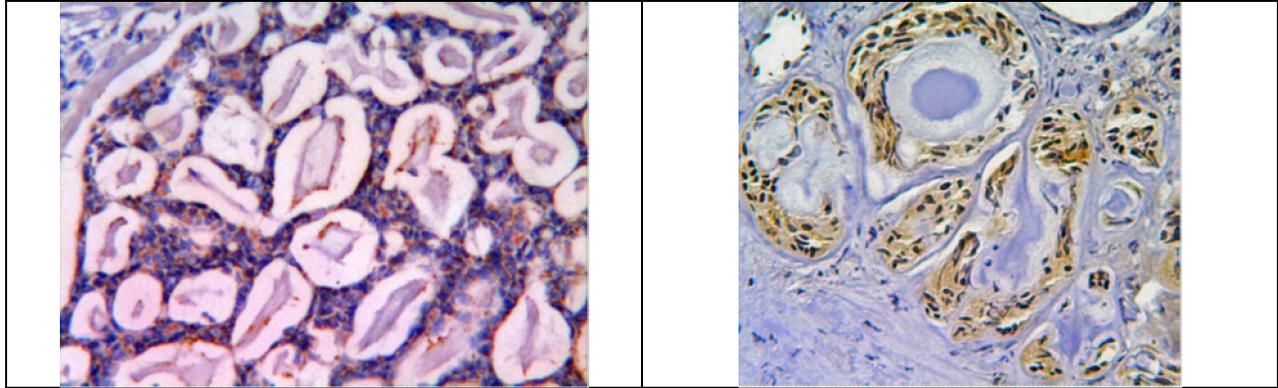
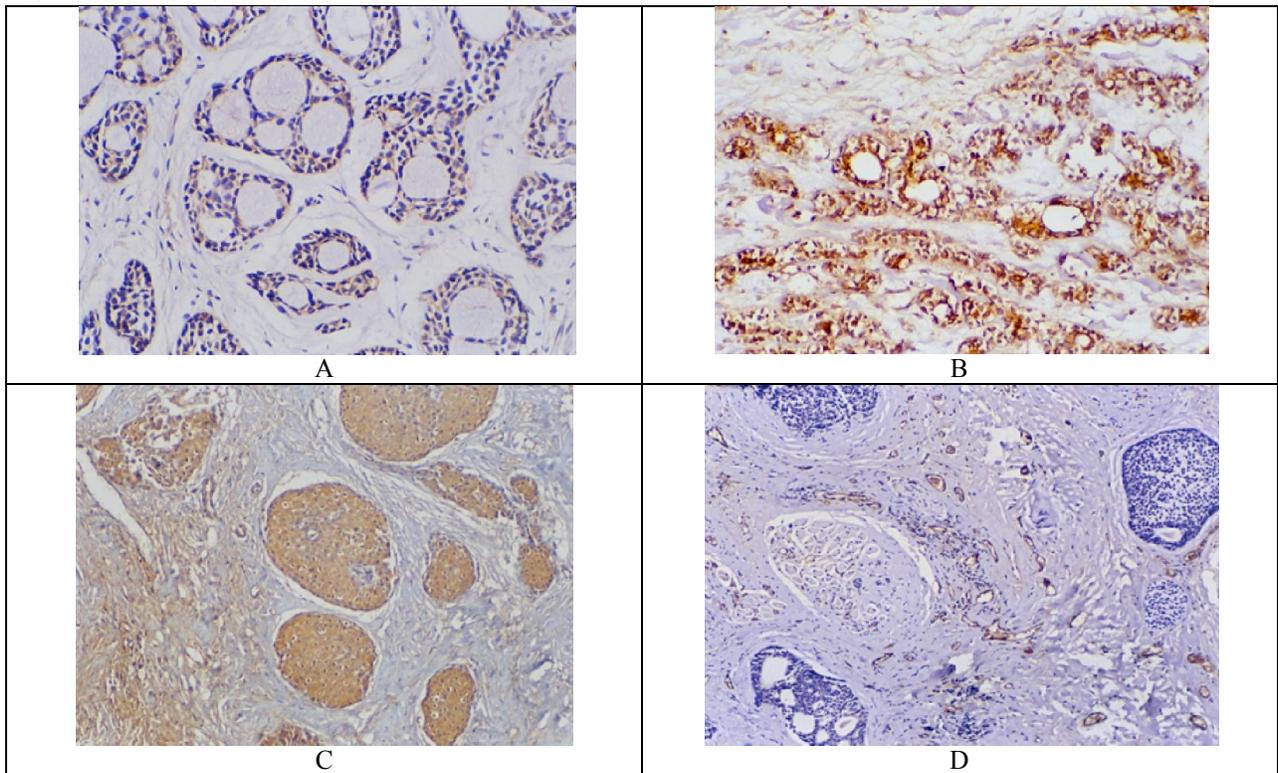


Fig (3): A photomicrograph of cribriform pattern of ACC showing marked immunostaining for CAV-1(A) and moderate immune-reaction for CAV-1 in tubular-trabecular pattern of ACC(B), Note the negative immunoreaction in connective tissue stroma.



Fig(4): A photomicrograph of cribriform pattern of ACC showing mild immunostaining for VPF(A) and moderate-strong immune-reaction in tubular-trabecular pattern of ACC(B), while, solid pattern of ACC showed strong positive immune-reaction in tumor cells (C) with negative CT stroma. Note the moderate to strong MVD in ACC (D).

Table (1): Correlation between CAV-1 expression, VPF expression and MVD

Group	Mean \pm SD			P-value
	Caveolin %	VPF	MVD	
Normal	5.04 \pm 1.67	6.05 \pm 0.35	2.10 \pm 0.28	
ACC cribr	9.17 \pm 2.63	38.0 \pm 2.74	13.38 \pm 0.35	
ACC Tub	9.59 \pm 2.46	41.63 \pm 0.68	14.35 \pm 0.21	
ACC solid	- Ve	44.06 \pm 0.125	15.73 \pm 0.23	<0.001*
MEC low	10.57 \pm 4.51	28.04 \pm 0.85	9.85 \pm 0.72	
MEC high	10.22 \pm 3.13	47.86 \pm 1.33	19.01 \pm 2.04	

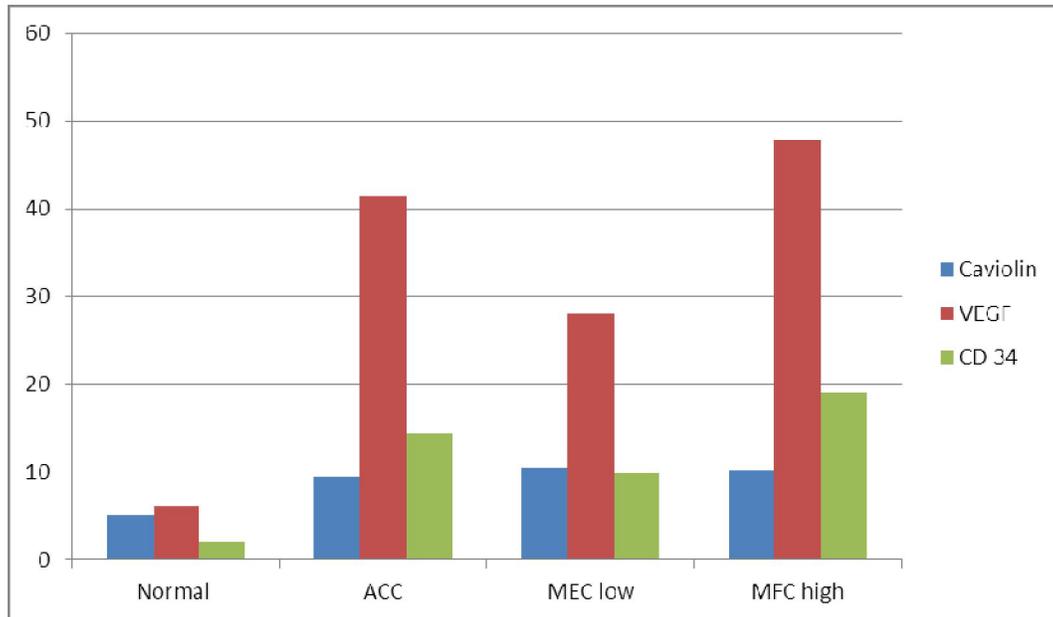


Fig (5): Bar chart illustrating the overall expression of CAV-1, VPF and MVD in NSG, MEC (different grades) and ACC.

Statistical Results

The mean value of area % of CAV-1 immuno-reactivity was higher in the S.G carcinomas than in the normal S.G. tissue with a statistical significant difference ($P < 0.00$). The mean value of area % of CAV-1 immuno-reactivity was higher in low grade MECs than in high grade MECs but with no statistical significant difference. In addition, there was no statistically significant difference between cribriform and tubular patterns of ACC as well as there was no statistically significant difference between ACC and MEC.

The mean value of area % of VPF and CD34 immuno-reactivity was higher in the S.G carcinomas than in the normal S.G. tissue with a statistical significant difference ($P < 0.00$). The mean value of area % of VPF and CD34 immuno-reactivity was higher in high grade MECs than in low grade MECs with a statistical significant difference ($P < 0.05$). The mean value of area % of VPF and CD34 immuno-reactivity was higher in the solid form of ACC, followed by tubular-trabecular pattern and finally the cribriform pattern showed the least values with a statistical significant difference ($P < 0.05$).

In addition, The multiple comparison tests revealed that in all subtypes of ACC, the cribriform pattern has no statistical significant difference with the tub-trabecular pattern (regarding area % of VPF expression) ($P < 0.05$). While, the multiple comparison tests revealed that all subtypes of ACC have statistical significant difference with each other's (regarding no of BV) ($P < 0.00$).

Correlation between CAV-1 expression, VPF expression and CD34 in salivary gland carcinomas:

Correlation Matrix test revealed that there was positive correlation between the VPF expression and MVD (measured by CD34 expression) ($P = 0.000$) in the different grades and patterns of SGT. In addition, the analysis revealed that caviolin-1 expression was correlated inversely with MVD and VPF expression (coefficient [rs] = -0.259; $P = .025$) where, MVD was significantly higher in the tumors with low caveolin-1 expression and in the tumors with high VPF expression ($P < 0.00$, $P < 0.05$ respectively). The results of the analysis are listed in Table (1) and Fig (5).

4. Discussion

In this study, caveolin-1 expression in samples of normal salivary glands and in samples of MEC and ACC of the salivary glands were evaluated immunohistochemically. Immunolocalization revealed positive immuno-expression of caveolin-1 in ductal epithelial cells and myoepithelial cells surrounding the mucous and serous acini of normal salivary glands which support the results of *Tajika et al. (2002)*, *Cao et al. (2003)* and *Shi et al. (2007)* who revealed the physiologic function of caveolin-1 in NSG as water and calcium reabsorption, thus regulating the saliva production. A noteworthy finding in the current study was that the frequency of caveolin-1 expression was higher in the tumors tissue than that of NSG tissue. These were in accordance with the results of *Furuse et al. (2005)*.

In the current study, MEC showed positive immunostaining of CAV-1 in the cytoplasm of both epidermoid and mucous secreting cells of 80% of cases, this is in accordance to *Frid et al. (1992)*, who showed diffusely positive staining for alpha-SMA and focally positive staining for calponin. The current study, some cases showed absence of immunoreactivity for CAV-1 in MEC, this would appear to rule out myoepithelial differentiation. In addition, high grade MEC showed less frequently staining intensity of Caviolin-1 than that in low grade MEC, this is accordance to *Shi et al. (2007)* who suggested that CAV-1 may serve as a tumor suppressor in MEC.

Regarding ACC, CAV-1 showed positive immunostaining in 60% of cases being noted in cribriform and tubular areas but not in solid ACC. This is in agreement with the findings of *Furuse et al. (2005)* who demonstrated negative immunostaining for alpha-SMA in cells of the solid type of ACC. These findings are expected considering that the cells of solid ACC are poorly differentiated.

In this study, down-regulated caveolin-1 expression was associated with high grade malignancy of MEC, and ACC. These was similar to that findings reported in studies of lung carcinoma, and ovarian carcinoma (*Wiechen et al., 2001 a & b and Shi et al., 2007*) who found that frequency of CAV-1 expression was higher in benign tumors compared with malignant tumors indicating that the tumor suppressor characteristics of CAV-1 retarded the progression of tumors and prolonged their duration.

In this study, the mean value of VPF expression and MVD are higher in salivary gland carcinomas group than in the control group and this difference was statistically significant ($P < 0.05$). This agrees with the finding of *Oliveira et al. (2002)* and *El-Rouby and Siam (2003)* who suggest that disease progression in salivary glands is associated with angiogenesis.

The significantly higher value of VPF immunoexpression in the higher grades of MECs is in accordance with the finding of *Lim et al. (2003)* who concluded that VPF over-expression in MEC is correlated with a less favorable outcome; as the proportion of proliferating tumor cells progressively increased from low to high grade tumors. In addition, VPF were significantly higher in solid type than in the cribriform pattern and tubular-trabecular patterns. This finding is in agreement with *El-Rouby & Siam, (2003)* and *Zhang et al. (2005)*, since the solid type is known to be the most aggressive pattern with early recurrence, early metastases and high mortality (*El-Naggar et al., 2005*). This finding confirmed the

hypothesis that VPF over-expression is associated with more tumor aggressiveness and may be served as an important prognostic factor.

A higher microvessel count was noted in the high grade MEC compared to the low grade MEC as well as marked MVD was associated with solid pattern followed by both cribriform and tubular trabecular of ACCs with statistical significant difference between them. This finding is in accordance with the finding of *In et al. (2007)* who reported that more aggressive tumors are associated with high levels of angiogenic activity. These findings confirm the opinion of *Park and In (2006)* that angiogenesis may promote metastatic disease by exposing tumors to a greater endothelial surface area, thus increasing the likelihood of haematogenous dissemination. Further, increased angiogenesis was also higher in MEC than in ACC; this finding is in accordance with the finding of (*Costa et al. 2008 and Sergio et al. (2009)*) that supporting a likely role of myoepithelial cells as regulators of the formation of new blood vessels.

Finally, the present study showed a statistically positive correlation between the expression of VPF, intratumoral MVD (measured by CD34) and histological grades of salivary gland carcinomas. Also expression of caveolin-1 was correlated inversely with VPF expression, MVD and histological grades and patterns of MEC and ACC. Based on previous studies, *Hoffman (2004)* has hypothesized that, in the quiescent vasculature, many factors that regulate angiogenesis normally are held together as part of an inactive modular unit (termed as angosome); and, when angiogenesis is stimulated, the angosome dissociates, thus enabling angiogenic regulators to become active. It is proposed that the angosome is present in the caveolae of capillary endothelial cells. The presence of caveolin-1 can inhibit proangiogenic factors: it may act as a master-switch, coordinating events during angiogenesis. *Philippe et al (2003)* reported that angiogenesis activators, such as VPF down-regulate caveolin-1 in human endothelial cells, and the down-regulation of caveolin-1 may be an important step along the pathway toward endothelial cell proliferation. **In conclusion**, the results of the present study highlight the role played by caveolin-1 that may serve as a tumor suppressor in MEC and ACC of the salivary glands. In addition, the increase in the aggressive biologic potential of tumor cells was accompanied by enhancement of angiogenesis and reduction of caveolin-1 expression. Modulation of Caveolin-1 could provide a novel therapeutic target for salivary gland carcinomas. However, the mechanisms by which caveolin-1 regulates VPF-induced angiogenesis remain largely unknown so these

studies provide a basis for further investigation of the role of caveolin-1 in tumor angiogenesis.

References

- Bailey K.M. and Liu J.: Caveolin-1 up-regulation during epithelial to mesenchymal transition is mediated by focal adhesion kinase. *J. Biol. Chemist.* 28: 13714-24, 2008.
- Cao G.; Yang G.; Timme T.L.; Saika T.; Truong L.D.; Satoh T.; Goltsov A.; Park S.H.; Men T.; Kusaka N.; Tian W.; Ren C.; Wang H.; Kadmon D.; Cai W.W.; Chinault A.C.; Boone T.B.; Bradley A. And Thompson T.C.: Disruption of the caveolin-1 gene impairs renal calcium reabsorption and leads to hypercalciuria and urolithiasis. *Am J Pathol.* 162: 1241–48, 2003.
- Chen AM, Garcia J, Granchi PJ, Johnson J, Eisele DW: Late recurrence from salivary gland cancer: when does "cure" mean cure. *Cancer*, 112(2):340-4, 2008.
- Cho D.S.; Yim H.; Cho K.S.; Hong S.J.; Cho N.H.; Kim S., Ahn H.S. and Kim S. J.: Impact of Caveolin-1 expression on the prognosis of transitional cell carcinoma of the upper urinary tract. *J. of the Korean Academy Med. Sci.*, 23: 296- 301, 2008.
- Chou K C, Chang L C, Su H C, Shu-Hui Lee S H and Heng-Sheng Lee H S: Immunohistochemical Study of Tumor Angiogenesis in Mucoepidermoid Carcinoma. *J Med Sci* ; 25(6):285-290, 2005.
- Cohen A.W; Hnasko R.; Schubert W. and Lisanti M.P.: Role of caveolae and caveolins in health and disease. *Physiol. Rev.*, 84: 1341-79, 2004.
- Costa C, Soares R and Schmitt F: Angiogenesis now and then. *APMIS*; 112:402-12, 2004.
- Costa AF, Demasi AP, Bonfitto VL, Bonfitto JF, Furuse C, Araújo VC, Metze K, Altemani A: Angiogenesis in salivary carcinomas with and without myoepithelial differentiation. *Virchows Arch*, 453(4):359-6, 2008.
- El-Naggar EK, Huvos AG: Adenoid cystic carcinoma. I. In *World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours*. Edited by: Barnes L, Eveson JW, Reichart P, Sidransky D. Lyon: IARC Press, 263-265, 2005.
- El-Rouby DH and Siam MKA: Vascular endothelial growth factor (VEGF) expression and microvessel density in benign and malignant salivary gland neoplasms. *Egyptian Dental J*; 49: 1671-86, 2003.
- El-Sheikh S.E.; Green A.R.; Rakha E.A.; Samaka R.M.; Ammar A.A.; Powe D.; Reis-Filho j. and Ellis I.O.: Caveolin-1 and caveolin-2 are associated with breast cancer basal-like and triple-negative immunophenotype. *Br. J. Cancer.* 99: 327-34, 2008.
- Fong A.; Garcia E.; Gwynn L.; Lisanti M.P.; Fazzari M.J. and Li M.: Expression of caveolin-1 and caveolin-2 in urothelial carcinoma of the urinary bladder correlates with tumor grade and squamous differentiation. *Am. J. Clin. Pathol.* 120: 93–100, 2003.
- Frid M.G.; Shekhonin B.V.; Koteliansky V.E. and Glukgova M.A.: Phenotypic change of human smooth muscle cells during development: late expression of heavy caldesmon and calponin. *Devel. Biol.* 153:185-93, 1992.
- Furuse C.; Machado S.S.O.; Nunes F.D.; Gallottini M.M.H. and Araujo V.C.: Myoepithelial cell markers in salivary gland neoplasms. *Int. J. Surg. Pathol.* 13: 57-65, 2005.
- Gratzinger D, Canosa S, Engelhardt B and Tladri JA.: Platelet endothelial cell adhesion molecule-1 modulates endothelial cell motility through the small G. protein Rho. *FASEB*; 17:1458, 2003.
- Hillen F, Griffioen AW: Tumour vascularization: sprouting angiogenesis and beyond. *Cancer Metastasis Rev.*, 26(3-4):489-502, 2007.
- Hino M.; Doihara H.; Kobayashi K.; Aoe M. and Shimizu N.: Caveolin-1 as tumor suppressor gene in breast cancer. *Surg. Today.* 33: 486–90, 2003.
- Hoffman R. Do the signalling proteins for angiogenesis exist as a modular complex? The case for the angosome. *Med Hypotheses.* 2004; 63:675–680.
- In YS, Kim SM, Park YW: Comparative immunohistochemical assays for the expression of angiogenic factors in tumors of human salivary glands. *J Korean Assoc Maxillofac Plast Reconstr Surg*, Jan 29(1):10-23, 2007.
- Joo H.J.; Oh D.K.; Kim Y.S.; Lee K.B. and Kim S.J.: Increased expression of caveolin-1 and micro-vessel density correlates with metastasis and poor prognosis in clear cell renal cell carcinoma. *BJU Int.* 93: 291- 96, 2004.
- Lim JJ., Kang S., Lee MR., Pai HK., Yoon HJ., Lee JI., Hong SP. And Lim CY.: Expression of vascular endothelial growth factor in salivary gland carcinomas and its relation to p53, Ki-67 and prognosis. *J Oral Pathol Med*; 32: 552-61, 2003.
- Lorusso G, Rüegg C: The tumor microenvironment and its contribution to tumor evolution toward metastasis. *Histochem Cell Biol.*, 130(6):1091-103, 2008.
- Navarro A.; Anand-Apte B. and Parat M.O.: A role for caveolae in cell migration. *FASEB J.* 18: 1801-11, 2004.
- Oliveira LA, de Oliveira VF and Comez RS: Vascular endothelial growth factor in minor

- salivary glands; effect of ageing J. Oral Rehabil; 29: 105-107, 2002.
25. Park S.S.; Kim J.E.; Kim Y.A.; Kim Y.C. and Kim S.W.: Caveolin-1 is down-regulated and inversely correlated with HER2 and EGFR expression status in invasive ductal carcinoma of the breast. *Histopathology*. 47:625–30, 2005.
 26. Park YW, In YS: Immunohistochemical assays for the expression of angiogenic signaling molecules and microvessel density in adenoid cystic carcinomas of human salivary glands. *J Korean Assoc Oral Maxillofac Surg*; 32(6):530-543, 2006.
 27. Parton R.G.; Hanzal-Bayer M. and Hancock J.F.: Biogenesis of caveolae: a structural model for caveolin-induced domain formation. *J. Cell Sci*. 119: 787 -96, 2006.
 28. Philippe G. Frank, Scott E. Woodman, David S. Park, Michael P. Lisanti: Caveolin, Caveolae, and Endothelial Cell Function. *Arterioscler Thromb Vasc Biol*; 23:1161-1168, 2003.
 29. Savage K.; Lambros M.B.K.; Robertson D.; Robin L. Jones R.L.; Jones C.; Mackay A.; James M.; Hornick J.L.; Pereira E.M.; Milanezi F.; Fletcher C.D.M.; Schmitt F.C.; Ashworth A. and Reis-Filho J.S.: Caveolin-1 is overexpressed and amplified in a subset of basal-like and metaplastic breast carcinomas: a morphologic, ultrastructural, immunohistochemical, and in situ hybridization analysis. *Clin. Cancer Res*. 13: 90-101, 2007.
 30. Seifert G, Sobin LH, Batsakis JG, et al. WHO: *Histological Typing of Salivary Gland Tumors*. Heidelberg: Springer- Verlag; 1991.
 31. Sergio V Cardoso, Kelen Christine N Souza, Paulo R Faria, Ana Lucia A Eisenberg, Fernando L Dias and Adriano M Loyola: Assessment of angiogenesis by CD105 antigen in epithelial salivary gland neoplasms with diverse metastatic behavior. *BMC Cancer* 9:391doi:10.1186/1471-2407-9-391, 2009.
 32. Shi L.; Chen X.M.; Wang L.; Zhang L. and Chen Z.: Expression of caveolin-1 in mucoepidermoid carcinoma of salivary glands: correlation with vascular endothelial growth factor, micro-vessel density and clinical outcome. *Am. Cancer Society*. 109: 1523-31, 2007.
 33. Sloan E.K.; Ciocca D.R.; Pouliot N.; Natoli A.; Restall C.; Henderson M.A.; Fanelli M.A.; Cuello-Carrión F.D.; Gago F.E. and Anderson R.L.: Stromal cell expression of caveolin-1 predicts outcome in breast cancer. *Am. J. Pathol*. 174: 2035- 43, 2009.
 34. Tajika Y.; Matsuzaki T.; Suzuki T.; Aoki T.; Hagiwara H.; Tanaka S.; Kominami E.; Takata K.: Immunohistochemical characterization of the intracellular pool of water channel aquaporin-2 in the rat kidney. *Anat. Sci. Int*. 77: 189–95, 2002.
 35. Tonini T, Rossi F and Claudio PP: Molecular basis of angiogenesis and cancer. *Oncogene*; 22: 6549-56, 2004.
 36. Wiechen K.; Diatchenko L.; Agoulnik A.; Scharff K.M.; Schober H. Arlt K.; Zhumabayeva B.; Siebert P.D.; Dietel M.; Schäfer R. and Sers C.: Caveolin-1 is down-regulated in human ovarian carcinoma and acts as a candidate tumor suppressor gene. *Am. J. Pathol*. 159: 1635–43, 2001(a).
 37. Wiechen K.; Sers C.; Agoulnik A.; Arlt K.; Dietel M.; Schlag P.M. and Schneider U.: Down-regulation of caveolin-1, a candidate tumor suppressor gene, in sarcomas. *Am. J. Pathol*. 158: 833–39, 2001(b).
 38. Williams T.M. and Lisanti M.P.: Protein family review. The caveolin proteins. *Genome Biol*. 5: 214–22, 2004(a).
 39. Williams T.M. and Lisanti M.P.: The caveolin genes: from cell biology to medicine. *Ann. Med*. 36: 584–95, 2004(b).
 40. Zhang J, Peng B and Chen X: Expression of nuclear factor kappa B, inducible nitric oxide synthase and vascular endothelial growth factor in adenoid cystic carcinoma of salivary glands; correlation with the angiogenesis and clinical outcome. *Clin. Cancer Res.*; 11: 7334-43, 2005.

5/12/2014