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A Comparative study of immunohistochemical expression of MMP-9 and its inhibitor (TIMP-1), Ki-67 and erbB3 in polymorphous low grade adenocarcinoma and adenoid cystic carcinoma of the salivary glands

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Abstract : Background: A number of matching histological forms were seen in polymorphous low grade adenocarcinoma (PLGA) and adenoid cystic carcinoma (ACC) including three patterns which are solid, tubular and cribriform. The bisecting features of clinicopathology of PLGA and ACC may end in a challenging diagnostic. ACC has an abundant not as good as prognosis than PLGA, making the differentiation vital for the purposes of therapy and prognosis. The Aims of our study were to assess the immunohistochemical expression of MMP-9, TIMP-1, Ki-67 and erbB3 in the salivary gland tumors including adenoid cystic carcinoma and polymorphous low grade adenocarcinoma and immunoexpression equivalent of these proteins with the outcomes of clinicopathology.

Materials and Methods: Twenty five blocks of salivary polymorphous low grade adenocarcinoma and another twenty five of salivary adenoid cystic carcinoma attained from Al-Shaheed Ghazi hospital and the oral pathology department archives of Dentistry College in Baghdad University were retrospectively involved in our study which are inserted in archival formalin fixed paraffin. We gained and immunostained sections of four micrometer using monoclonal antibody against MMP-9, TIMP-1, Ki-67 and erbB3.

The detection of immunoexpression was by the presence of stain which are brown in color seen in the cytoplasm of cells of tumor. The clinicopathological documents of the patients associated with the quantity of cells that expressed the stain.

Results: MMP-9 expression was found positive in 21cases of PLGA and 22 cases of ACC restricted in tumor cells.

TIMP-1 expression was found positive in 20 cases of PLGA and 19 cases of ACC limited in tumor cells, Ki-67 expression was found positive in 23 cases of PLGA and 24 cases of ACC limited in tumor cells and ErbB3

expression was found positive in 20 cases of P L G A and 24 cases of ACC limited in tumor cells.

T h e relation was seen n o n - significant (P=0.357) in concerning MMP-9 appearance in both types of tumor , (P=0.937) was seen about TIMP-1 expression in two types of tumor, (p=0.798) was seen about Ki-67expression in two types of tumor and (p=0.006) was seen about erbB3 expression in two types of tumor.

The relation was seen non -significant (p> 0.05) in examples of gender, sites and stage of PLGA and ACC concerning MMP-9 expression. Non-Significant relation (p> 0.05) was seen with grade of ACC was detected regarding MMP-9.

The relation was seen non -significant(p> 0.05) in examples of gender, sites and stage of PLGA and ACC was seen concerning TIMP-1 expression.

Non-Significant relation (p> 0.05) was seen with grade of ACC was detected regarding TIMP-1 . The relation was seen non -significant(p> 0.05) in examples of gender, sites and stage of PLGA and ACC was seen concerning Ki-67 expression.

Non-Significant relation (p> 0.05) was seen with grade of ACC was detected regarding Ki-67 .The relation was seen non -significant(p> 0.05) in examples of gender, sites and stage of PLGA and ACC was seen concerning erbB3 expression.

Non-Significant relation (p> 0.05) was seen with grade of ACC was detected regarding erbB3.

Conclusion: We can understand an concerned balance between MMP-9 and TIMP-1 in malignant salivary gland neoplasm. Imbalanced of MMP-9/TIMP-1 expressions might offer the tumor cells a double growth benefit because uninhibited TIMP-1; deposition of ECM is joint with increase MMP-9; degradation of ECM. A multistep process created due to this communication which is capable to excite and possibly will display a portion in the genesis of salivary gland tumor; may chain forces to normalize invasive events related to these neoplasms. ACC greater than PLGA in Ki-67 mean of expression which shows advanced malignant potential. EBP1 expression is reduced in polymorphous low grade adenocarcinoma and adenoid cystic carcinoma, indicating unfavorable prognosis of PLGD and ACC patients.

Keywords: Polymorphous low grade adenocarcinoma, Adenoid cystic carcinoma, immunohistochemistry, MMP-9, TIMP-1, Ki-67and erbB3.

Introduction

Oral minor salivary gland provide growth to an infrequent type of malignant tumors, but establish an significant area in the field of oral pathology as they are commonly reflected to be better than squamous cell carcinoma in their clinical behavior (1). Of all malignant neoplasms of upper aerodigestive tract , oral salivary carcinomas account for 2-3% and up to 20% of all salivary gland tumors (2). Adenoid cystic carcinoma (ACC) denote 25% of oral carcinoma of salivary gland and polymorphous low-grade adenocarcinoma (PLGA) represent 20% (3-7).

Polymorphous low-grade adenocarcinoma term was announced in 1984 by Evans and Batsakis (10) and denotes the current nomenclature in the series of WHO (14). This following its first recognition by Freedman and Lumerman (8) and Batsakis et al. (9) in 1983and this occur as “lobular carcinoma” and “terminal duct carcinoma", respectively.

Much attention in the recent literature seen about PLGA with greatest studies directing on structures that may aid to distinguish PLGA from ACC as both entities are morphologically characterized by a variety of growth patterns and share an intercalated duct origin (11, 12). ACC is a somewhat infrequent epithelial tumor of the major and minor salivary glands and represent a relentless and unpredictable tumor (13-15). Nonetheless, ACCs with a perineural infiltration

predilection, which partially explains the tendency for local recurrence, are highly aggressive neoplasms even though causes for the invasiveness and aggressive metastatic dissemination of ACCs persist vague (13,14). So, it will be of abundant clinical value to recognize the molecular events linked with the ACC development and progression for initial detection and prognosis (16).

Histologically, ACC is with a tendency to invade perineural spaces, and is stubbornly recurrent. (14,15). We can categorized ACC into three types; cribriform ,tubular and solid. The much more aggressive pattern than the other two types is the solid pattern (17, 18).

Very significant characterising features of PLGA that could be a malignant neoplasm of salivary gland of epithelial origin seen as morphological diversity ,cytological uniformity, acquaintance infiltrative growth pattern , and slight metastatic potential (19,20).

The morphologic nonuniformity of PLGA is responsible for the diagnostic distress and mistake with ACC. So we have got a diagnostic problem and it is central and demanding because of its myoepithelial differentiation to distinguish it from tumors ACC (21,22).

The aggressive clinical manners is currently joined with papillary cystic pattern so that an relationship between clinical progression and histological characteristic has been recognized.

ACC arise from malignant change of the intercalated duct reverse cell soil it's malignant neoplasm of altered myoepithelial island ductal cells that form three important characteristic growth patterns that include cribriform, tubular and solid ,also contains perineural invasion tendency so it is a tumor of each the major and minor salivary glands. ACC is patented by regionally invasive growth with distant metastasis and resident recurrence shows great propensity (23,24).

Among many matrix metalloproteinase families (MMP); matrix metalloproteinase (MMP-9) is recognized to possess acute roles in neoplasm metastasis and invasion concluded their capability to destroy varied element of extracellular matrix and liberating many cytokines and triggering completely different growth factors. MMP-9 might show a very important role in angiogenesis and neovascularization.

The actions of all best-known MMPs are inhibited by tissue inhibitor matrix metalloproteinase (TIMP-1) and it is intrinsically plays a significant role to keep the equilibrium seen in numerous physiological processes between extracellular matrix deposition and degradation and prevents neoplasm growth, metastases and invasion, and this has been related to their matrix metalloproteinase repressing activity(25,26).

The balance between MMP-9 and TIMP-1 which denote their tissue inhibitors is alarmed within the histogenesis of normal salivary glands also within the mechanism of neoplasm invasion and metastases (27,28).

Evaluation of the rate of tumor growth and cellular proliferation associated with and may be need antigen Ki-67 (29,30).

In addition ribosomal RNA transcription is associated with this nuclear protein . Inhibition of ribosomal RNA synthesis occur due to inactivation of antigen Ki-67 (31).

During inter-phase, the Ki-67 protein is found specifically in the cell nucleus, whereas in mitosis most of the protein is transported to the surface of the chromosomes so that Ki-67 is used as a marker for cell proliferation . This protein is vague in resting cells (G0) but is existing in all active phases of the cell cycle (G1, S, G2 and mitosis) (32).

Ki-67 is very vital for determining prognosis of tumors and determine the recurrence rate after radiotherapy in patients with adenocarcinoma (33).

A membrane bound protein that is encoded in humans by the PA2G4 gene denotes receptor tyrosine-protein kinase erbB3, also known as HER3 (human epidermal growth factor receptor 3) (34), that is involved in growth regulation encoded by human erbB3 gene . This protein may be involved in ribosome gathering and is present in pre-ribosomal ribonucleoprotein complexes. This protein might contribute to transducing growth regulatory signals and can cooperate with the cytoplasmic domain of the erbB3 receptor . This protein has been concerned in growth inhibition and the induction of human cancer cells differentiation (35).

ErbB3 binding protein 1 (Ebp1) has been established to be a potent tumor suppressor and represents a downstream effector of the erbB3 signaling network in numerous human malignancies (36).

It is can be detected in several cell lines tested so it is an RNA-binding protein which, including primary and transformed cell lines. EBP1 has been implicated in growth inhibition and the induction of differentiation of human cancer cells. It appears to be tangled in growth regulation (37).

Ebp1, an erbB3 binding protein, was shown to be a potent tumor suppressor in adenoid epithelium carcinomas, (38, 39, 40) and if ectopically expressed ACC cells of salivary gland of epithelial origin ,the inhibitory properties of the ebp1 gene could be beneficial and keratinocyte growth factor receptor gene therapy inhibits growth of human salivary adenocarcinoma cells through induction of differentiation and apoptosis (41).

A member of the PA2G4 family and the human homologue of a previously identified proliferation regulated protein, Ebp1, was isolated as an erbB3 binding protein.(42) .

Expression of Ebp1 suppresses the growth of erbB positive cancer cells(39) and induces cellular differentiation (38) and results in dissociation of Ebp1 from erbB3 and translocation from the cytoplasm to the nucleus . Ebp1 gene transfer resulted in distinct antitumor activity in ACC cell line, illuminating a previously unrecognized therapeutic gene for treatment of another gland epithelial neoplasm. We also demonstrate for the first time that expression of Ebp1 results in reduced metastatic potential.

Blocking new DNA synthesis and Decreasing the cell growth rate under anchorage - independent conditions done by Ebp1 exerted strong antiproliferative effects. These actions may donate to a low efficiency of cell survival in the circulation. ErbB3 binding protein 1 (EBP1) is the human homologue of a previously identified cell cycle-regulated protein (43) and it is a conserved molecule across species with multiple roles in cell proliferation and differentiation (44,45,46,47,48). EBP1 gene transfer into human salivary ACC cells significantly inhibits cell proliferation and most importantly, reduces tumor metastatic potential (49) . EBP1 immunoreactivity inversely correlates with local invasion and distant spread of ACCs. Patients with lower EBP1 levels had poorer long-term survival than those with higher EBP1 expression.

Aims of the current study were to analyze immunohistochemical appearance pattern of MMP-9, TIMP-1 ,Ki-67 and erbB3 in PLGA and ACC and to use them as diagnostic marker for differentiation between these two tumors.

Material and Methods:

Sample

Fifty patients affected with malignancy of salivary glands haphazardly chosen from the pathologic specimens and file records from maxillofacial Center in Baghdad Hospital of Al-Shaheed Ghazi, and furthermore from the oral diagnosis department archives of Dentistry Collage in Baghdad University collected from the year 1973 to 2015.

Represent the clinical and demographic , data delivering was completed by the operating surgeon were calm from the patients case sheets given with the neoplasm specimens that land patient's

statistics regarding of the age , site, sex and neoplasm clinical staging were recognized and approving to the malignant neoplasms TNM classification, the staging was administrated. We studied all clinical and histopathologic knowledge existing to discard cases representing disease with secondary metastases to the salivary gland.

Control

Five normal salivary gland tissues were used as negative external controls. And for negative control and at a corresponding time we are neglecting of the primary antibody step but all altered reagents were added . Staining positivity requires an lack of specificity of the antibody, dependable with Abcam manufacturer's data sheets . Here in our study for MMP-9,TIMP-1 , Ki-67 and erbB3 , gastric adenocarcinoma , liver tissue ,tonsil tissue and breast carcinoma were taken here as positive control respectively.

Immunohistochemical procedure

Deparaffinized of four µm sections were done by using xylene and next the step of graded alcohol used for rehydrated procedure . To my slides the blocking by hydrogen peroxide in adequate drops were applied , following the slides incubated at 37C°in wet chamber for ten minutes .Next step after that our slides socked in buffer in order of a pair of times (5 minutes for each one). In tissue specimens masking of antigen sites by formalin by forming protein cross-links , tissue retrieving procedure used for our slides in order to disclose antigenicity. Subsequently adequate drops of protein used as block were applied to my slides and at 37°C for ten minutes protected , here the buffer used for washing two times (5 minutes for each one) ,to finish gently drained and blotted and primary antibody in dilution form inside the following step was added to every slide, incubated nightlong in wet chamber within the next day and at 37°C., then the slides were washed in buffer (4 times for each one) , last of all drained and blotted softly. In the subsequent step suitable drops of secondary antibody which used as a reagent were applied and incubated at 37°C for 30 minutes in humid chamber. In the afterward step we used the buffer for washing the slides in four times (5minutes for each one), formerly draining and blotting was completed, at this time in the next step we applied Streptavidine-HRP antibodies on tissue for 30 minutes and incubated at 37°C. in humid chamber at the moment tissue arranged with DAB which are diluted at that time kept in darkroom and next incubated for ten minutes. Now by using tap water the slides washed for 5minutes thoughtfully. Formerly Hematoxylin as a counter stain used for bathing the slides for 1-2 minutes after that tap water was used for ten minutes for rinsing the slides. At that time in the following step for the slides dehydration ethanol and xylene were used by immersing them in a jars containing these two fluids and next to that time DPX as a mounting medium were used to the xylene humid sections in one or two drops and coated with covers lips and let them to dry nightlong.

Evaluated our results were by the presence of our outcome as brown colored at the positioning of our target antigen (cytoplasm) was suggestive of immunoreactive positivity. IHC positive neoplasm cells percentage per hotspot was prearranged and also the mean per slide percentage was determined from assembly of ten high power field from the more demonstrative area of immunostaining fields. The concentration was ignored as a consequence of it's uncovered to individual discrepancy throughout testing.

The positive control of MMP-9 existing by gastric adenocarcinoma which was stated as brown diffuse cytoplasmatic immunoreactivity moreover to stromal cells of extracellular matrix of neoplasm cells.

Immunoreactivity of MMP-9 was shows:(score 0) or (-ve) seen as 0% of the neoplasm cells, (+) or low (score I) seen as 1-25% while moderate (score II) or (++) 26-50% and high (score III) or (+++) 51-100% of positive cells, depending on account(50). .

Immunoexpression of TIMP-1was brown diffuse cytoplasmic expression in liver tissue as the positive control. .

TIMP-1 immunoreactivity was shows: (-ve) or (score0) < 5%of the neoplasm cells, low (score I) or (+) 6-25% while moderate (score II) or (++) 26-50% ,high (score III) or (+++) 51-75% and very high (score IV) (++++)76%-100% of positive cells, depending on enumeration (51) .

The positive control of Ki-67 is tonsil tissue which was expressed as brown nuclear and nucleolar immunoreactivity.

The immunoreactivity of Ki-67 was categorized as follows: (score 0) (-ve) ≤ 5% of the tumor cells, low (score I) (+) 6-25%, moderate (score II) (++) 26-50%, high (score III) (+++) 51-100 % of positive cells, depending on counting(52).

Carcinoma of breast was the positive control of erbB3 which was expressed as brown diffuse cytoplasmatic immunoreactivity.

The immunoreactivity of erbB3 was ordered as follows: (score 0) (-ve) < 10 % of the tumor cells, low (score I) (+) 10 -25% while moderate (score II) (++) 26 -50% and high (score III) (+++) > 50% of positive cells, depending on counting (53).

Statistical Analysis

We have a tendency to tabulated and exposed all the immunohistochemical, histopathological and clinical relevant information to proper statistical analysis pursuit the SPSSv.20 software system. The calculated parameters were count up and given as percentage and count.

Fisher's exact test useful to squared the assembly concerning categories. Analysis of modification in form of ANOVA test was used to sight alterations for an remaining the markers. P value was well-thought-out to be statistically substantial if it is equal or less than 0.05 .

Immunohistochemical Results

MMP-9immunoexpression

Gastric adenocarcinoma indicate the positive control for MMP-9 which appreciated as brown color and its immunoreactivity show diffuse cytoplasmic furthermore to extracellular matrix and cellular membrane of neoplasm cells.

MMP-9 in normal salivary glands appearance appear as brown color and its immunoreactivity show cytoplasmic expression.

The omission step of primary antibody was used as negative control ,this is for antibody engaged in this study in order to test its specificity , an absence of antibody specificity shows positive staining.

MMP-9 immunoreactivity was branded as brown color staining limited within the cytoplasm of the PLGA and ACC and cells of tumour figures 1,2.

Current study shows the fascinating verdict of the nuclear MMP-9 expression in some ACC tumour cells and present in ductal epithelial cells rather than cytoplasmic localization.

Twenty one PLGA cases shows positive MMP-9 expression in addition twenty two cases of ACC shows this positivity and this seen in abundant modification scores, the larger percentage of MMP-9 expression (40%) (score III)was seen in ten cases of PLGA whereas in ACC twelve cases (48%) and the greater % was positioned in (score II).

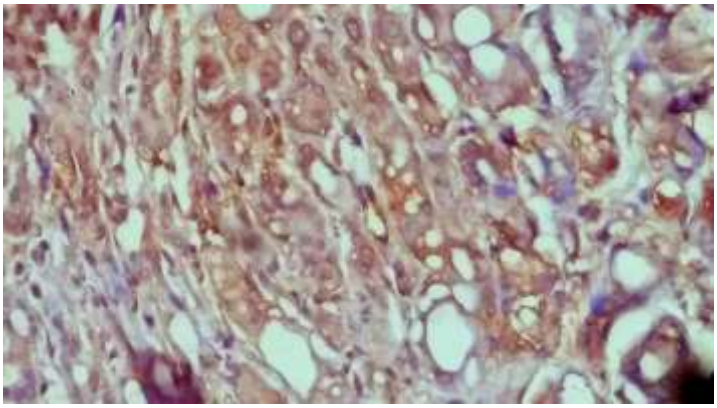

Figure 1 : MMP-9 positive staining expression in PLGA (X40) .

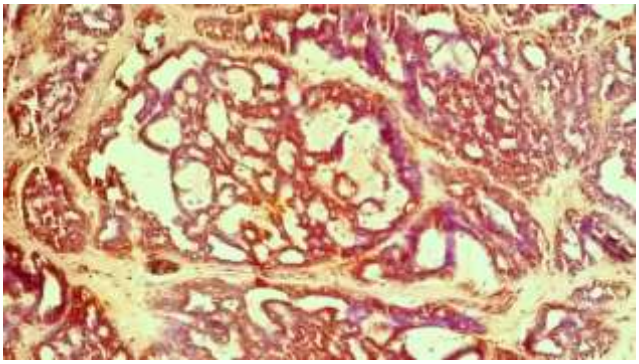

Figure2:MMP-9 positive staining expression in ACC(cribriform pattern)(X40).

Non- significant relation (P= 0.357) of MMP-9 attendance in each types of tumour shows as in table 1 .

Regarding MMP-9 expression with gender, site and stage of tumour (P > 0.05) in PLGA and ACC, non- significant relation seen as in tables 2. Concerning grade non- significant relation was resolute (P > 0.05) in ACC as in tables 2.

Table 1: MMP-9 scores in PLGA and ACC

MMP-9 score	**PLGA**	**ACC**
Score 0	4(16 %)	3 (12 %)
Score 1	4 (16 %)	5 (20 %)
Score 2	7 (28%)	12 (48 %)
Score 3	10 (40 %)	5 (20 %)
Total	25(100%)	25 (100%)
	NS P value= 0 .357	

Table 2: The clinicopathological finding of PLAC and ACC in relation to MMP-9 expression.

Variable		**PLGA No.25**	**ACC No.25**	**P value**
		MMP-9	MMP-9	
		+ve	+ve	
AGE mean$\pm SD$		53.3$\pm$16.0	44.5$\pm$12.0	
SEX	Male Female	10 11	13 9	NS
SITE	Palate Floor of mouth Upper lip Check Submandibular gland others	17 2 1 0 0 1	16 3 0 0 2 1	NS
STAGE	I II III IV	4 9 5 3	8 10 2 2	NS
GRADE	I(tubular) II(cribriform) III(solid)	- - -	4 12 5	NS
PERINEURAL INVATION		0	2	

TIMP-1immunoexpression

Liver tissue signify the positive control for TIMP-1 expression and stated as brown color with its immunoreactivity as diffuse cytoplasmic expression .

Brown cytoplasmic immunoreactivity was seen for TIMP-1 expression in normal salivary glands .

For analysis the specificity of antibody employed in our study, the negative control existing here by using of primary antibody omission , a scarceness of antibody specificity appear as positive staining .

Immunoreactivity of TIMP-1 was seen as staining which are brown color limited within the cytoplasm of the neoplasm and stromal cells of each PLGA and ACC figures 3 and 4. PLGA twenty cases and nineteen cases of ACC shows positive TIMP-1 expression, the greater percentage of TIMP-1 expression (score I) was found in twelve PLGA cases (48 %) and in ten (40%) ACC cases.

Concerning appearance of TIMP-1 in each types of neoplasm, the relation seen non-significant (P= 0.937) as in table 3. Non- significant statistical relation was seem about TIMP-1 expression with gender, site and stage of neoplasm (P>0.05) in PLGA and ACC as in tables 4.

Non- significant relation was resolute about grade (P> 0.05) in ACC as in tables 4.

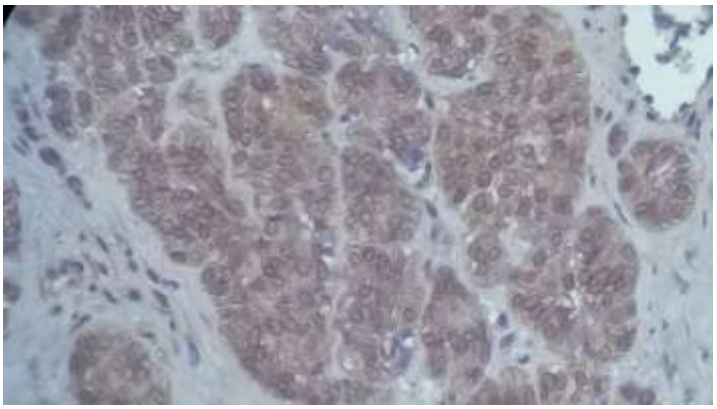

Figure 3 :TIMP-1 positive staining expression in PLGA(X40).

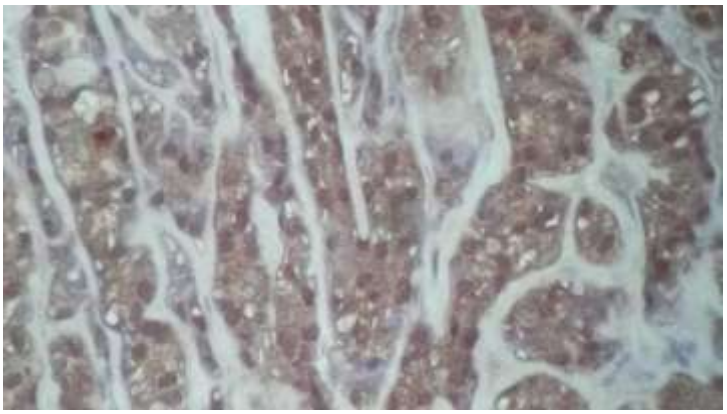

Figure 4 :TIMP-1 positive staining expression in ACC (X40).

Table 3: TIMP-1 scores in PLGA and ACC.

TIMP-1 score	**PLGA**	**ACC**
Score 0	5 (20 %)	6 (24 %)
Score 1	12 (48%)	10 (40 %)
Score 2	5 (20 %)	5 (20%)
Score 3	3 (12 %)	4 (16 %)
Total	**25 (100 %)**	**25 (100 %)**
	NS P value= 0.937	

Table 4: The clinicopathological finding of PLAC and ACC in relation to TIMP-1 expression.

Variable		**PLGA No.25**	**ACC No.25**	**P value**
		TIMP-1	TIMP-1	
		+ve	+ve	
AGE mean $\pm SD$		52.8±16.3???	45.4±12.5	
SEX	Male Female	10 10	12 7	NS
SITE	Palate Floor of mouth Upper lip Check Submandibular gland others	16 2 1 0 0 1	13 2 0 1 1 2	NS
STAGE	I II III IV	3 9 4 4	7 9 1 2	NS
GRADE	I(tubular) II(cribriform) III(solid)	- - -	4 11 4	NS
PERINEURAL INVATION		0	2	

Ki-67immunoexpression

The positive control for Ki-67 expression vacant by tonsil tissue and stated as brown color with its immunoreactivity as nuclear and nucleolar expression .

Brown nuclear and nucleolar immunoreactivity was realized for Ki-67 expression in normal salivary glands .

For trying the specificity of antibody employed in our study, the negative control existing here by using of primary antibody omission , a scarceness of antibody specificity appear as positive staining .

Immunoreactivity of Ki-67 was seen as staining which are brown color limited within the nuclear and nucleolar of the neoplastic cells of each PLGA and ACC figures 5-8.

Twenty three cases of PLGA and ACC twenty four cases shows positive Ki-67 expression , the greater percentage of Ki-67 expression (score I & III) was found in nine PLGA cases (36 %) but in twelve ACC cases(score I) (48%).

Non-significant relation seen (P= 0.852) regarding appearance of Ki-67 in each types of neoplasm as in table 5.

Non- significant statistical relation was appear about Ki-67 expression with gender, site and stage of neoplasm ($P>0.05$) in PLGA and ACC as in tables 6.

Non- significant relation was resolute concerning grade ($P>0.05$)in ACC as in tables 6.

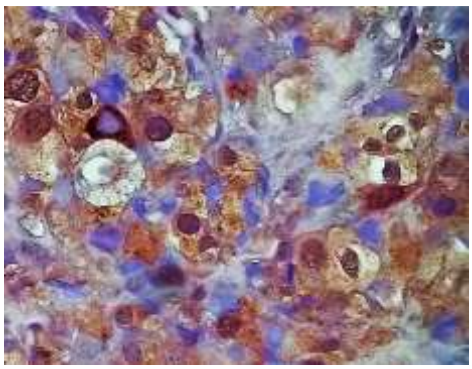

Figure 5 : Ki-67 positive staining expression in PLGA (X40).

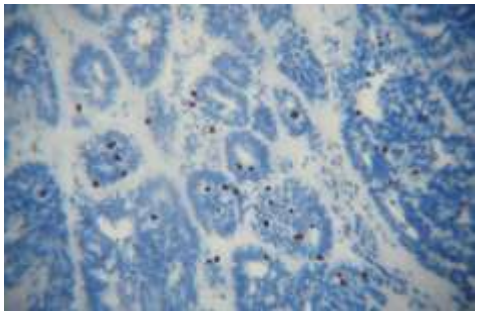

Figure 6 : Ki-67 positive staining expression in ACC (tubular pattern) (X40).

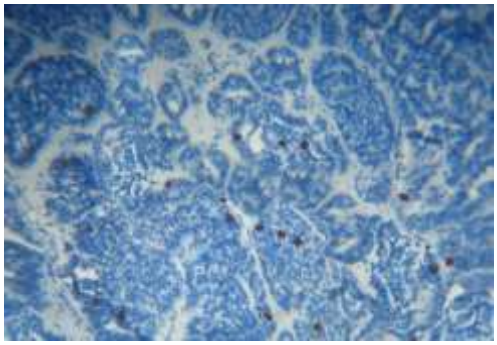

Figure 7 : Ki-67 positive staining expression in ACC (cribriform pattern) (X40).

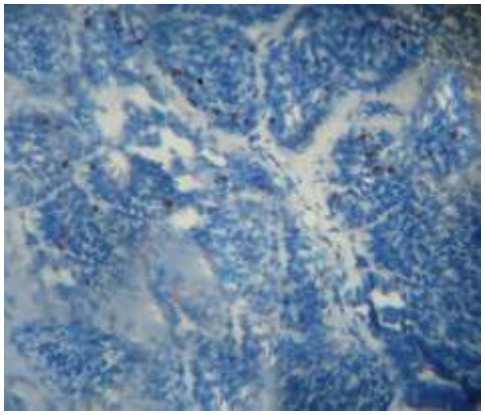

Figure 8 : Ki-67 positive staining expression in ACC (solid pattern) (X40).

Table 5: **Ki-67 scores in PLGA and ACC**

Ki-67 score	**PLGA**	**ACC**
Score 0	**2 (8 %)**	**1 (4 %)**
Score 1	**9 (36%)**	**12 (48 %)**
Score 2	**5 (20 %)**	**5 (20%)**
Score 3	**9 (36 %)**	**7 (28 %)**
Total	**25 (100 %)**	**25 (100 %)**
	NS P value=0.852	

Table 6: The clinicopathological finding of PLAC and ACC in relation to Ki-67 expression.

Variable		PLGA No.25 Ki-67	ACC No.25 Ki-67	P value
		+ve	+ve	
AGE mean $\pm SD$		57.6± 15.950	42.20± 2.533	
SEX	Male Female	9 14	15 9	NS
SITE	Palate Floor of mouth Upper lip Check Submandibular gland others	19 1 1 0 0 2	16 3 0 1 2 2	NS
STAGE	I II III IV	7 9 4 3	10 10 2 2	NS
GRADE	I(tubular) II(cribriform) III(solid)	- - -	5 14 5	NS
PERINEURAL INVATION		0	2	

Discussion:

The conclusion of immunohistochemical differences between PLGA and ACC is earlier the care of our study , essentially within the both tumors histology shared in cribriform design that has been tirelessly tried (54, 55).

Argument on this topic continues that some authors believe within the literature that immunohistochemistry doesn't require any definite diagnostic worth for recognizing PLGA (56).

However, we have a propensity to do not share this recognition as we have a affinity to creation an attempt to use immunohistochemistry to support a diagnostic marker for histologic similarity.

The balance of matrix metalloproteinases (MMPs) with their tissue inhibitors (TIMPs) is alarmed within the morphogenesis of normal salivary gland further and within the invasion and metastasis of the neoplasm. MMP-9 and TIMP-1 role in PLGA and ACC has not been confirmed effectively .

During this study TIMP-1and MMP-9 proteins appearance was matched in polymorphous low grade adenocarcinoma and adenoid cystic carcinoma.

The positivity of MMP-9 protein appearance was observed in 21cases (84%) of PLGA and twenty two cases (88%) of ACC and therefore the positive response of TIMP-1 protein appearance was observed in twenty cases (80%) of PLGA and nineteen cases (76%)of ACC. MMP-9 is tremendously controlled protein and this regulation includes a minimum of 3 totally diverse levels: reserve of MMPs ,transcriptional regulation and activation of hidden MMPs (57). On the extra hand,TIMP-1have been associated with different cellular functions, like angiogenesis, growth of cells and anti-apoptotic action (58,59).

Our study results shown that MMP-9 expression elevated when tissues changed from cribriform to solid and tubular pattern of growth of ACC direct that MMP-9 immunostaining will facilitate to evaluate the grade of histology of malignant morphological pattern of growth.

Additionally, of ACC shows 2 cases with neural invasion which are positive to MMP-9 expression.

Later the most important result of current study was that MMP-9 appearance levels were greater in myoepithelial cells of stroma compared with ductal epithelium in both tumors, it is appealing to invest that MMP-9 is principally not created by the epithelium but the stromal myoepithelium.

Neoplasm cells could also be the supply of gelatinases in some patients and this confirmed in preceding studies (60) and stroma with neoplastic features is one of the essential parts that stimulate the conversion to invasive cancer from carcinoma in situ , within the literature it has been obviously recognized that neoplasm stroma is in a straight line associated with biological manners of tumour (61).

Our results powerfully recommend the possibility that myoepithelial cells of stroma could also be the first source of gelatinases in PLGA an d ACC and stroma maybe plays a lot of acute role than epithelium within the progression of PLGA and ACC.

The amount of MMP-9 in both the ductal epithelium and the myoepithelia of stroma of PLGA and ACC was above in the cells of normal salivary gland.

This result could also be due to the low quantity of ductal cells in normal salivary gland tissues, as a product of our result and prior studies have shown that in normal salivary gland, the ductal cells identified great quantities of TIMP-1 and MMP-9 though acinar cells did not shows MMP-9 and TIMP-1(62).

Current result showed improved MMP-9 formation by neoplastic modification of salivary gland tissues, at this time TIMP-1and MMP-9 still unclear that one is more essential in neoplasm metastasis and progression.

It's moreover significance noting that MMP-9 appearance was not noticed (three and four cases) of neoplasm specimens of PLGA and ACC respectively. One possible explanation of this findings is that MMP-9 could also be seen in ACC and PLGA however typically its production is instable and conserved at closely undetectable levels.

MMP-9 and its inhibitor TIMP-1 levels shows increase protein expression causing delineated physiological plan to control the activity of MMP and preserve a constant ratio between the 2 proteins. It recognized that TIMPs antagonized MMPs in the gelatinolytic function during a stoichiometric manner (63), it is significant to maintenance of this balance and tissue injury by augmented proteolysis occur due to disruption of this balance . As matrix remodeling may be a outcomes of the balance between degradation and synthesis; it monitors that down regulation of TIMPs will support proteolysis. Some studies have established that overexpression of TIMP-1 resulted during a significant drop in neoplasm growth and elongated periods before the formation of tumors (64).

This current study, however, suggests the chance that myoepithelial cells could also be the first supply of gelatinases and possibly performance an vital role in progression and/or development of PLGA and ACC.

Reduction of protein expression of TIMP-1 occur within the two patterns which are cribriform and tubular . These results display that there is shut in association of TIMPs with growth patterns of ACC, and TIMP-1 might play vigorous roles in biological character and morphogenesis of adenoid cystic carcinoma. The ability of cells of human salivary gland cancer for metastasis was related closely to TIMP-1 expression in minimization or alteration (65).

Salivary gland cancer cells was strictly related to altered TIMP-1 appearance. The down regulation of TIMP-1 protein expression in adenoid cystic carcinoma tumoral cell might be able to regarding acquisition of capabilities of metastasis and recurrence by the special effects of TIMP-1.

Our study profiled EBP1 expression in PLGA and ACC patients and its linicopathological relevance. Mainly, our results demonstrated that EBP1 expression was inversely correlated with the progression of ACC. This data is consistent with previously published results showing that wild-type EBP1 gene transfer into human salivary ACC cell line significantly inhibits cell proliferation and reduces tumor metastatic potential. Non-significant trend was recorded between erbB3 levels and Ki67 immunostaining. These results clearly demonstrate that increased erbB3 expression appears to be associated with the favorable prognosis.

Conclusion:

Unbalanced between MMP-9 and TIMP-1 in malignant salivary gland tumors was detected. The high MMP-9 and TIMP-1 might justify the expected course of ACC and PLGA invasion and show that unbalanced of MMP-9/TIMP-1 expressions would possibly provide the neoplasm cells a double growth advantage as a result of uncontrolled TIMP; deposition of ECM is combined with rise MMP; degradation of ECM. The communication might activate a multistep method that is in a position to push and will play a job in salivary gland neoplasm genesis; may mix forces to manage invasive events associated with these neoplasms.

The tumor Ki-67 staining play a vital role to evaluate patients at high risk of tumor progression but cannot consider as independent prognostic factors. Ki-67 mean of expression was higher in ACC than PLGA which indicates higher malignant potential. EBP1 expression is reduced in adenoid cystic carcinoma, indicating unfavorable prognosis of ACC patients. Its regulation of MMP9 protein levels suggests a critical therapeutic potential.

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