Comparative Study of CD105 and Ki-67 in Adenoid Cystic Carcinoma and Polymorphous Low Grade Adenocarcinoma of the Salivary Glands

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Abstract

Background: The intersecting clinicopathological features and histological patterns, including cribriform, tubular and solid patterns of adenoid cystic carcinoma (ACC) and polymorphous low-grade adenocarcinoma (PLGA) may end in a problematic diagnosis .ACC has a worse prognosis than PLGA making distinction important for therapeutic and prognostic purposes . The Aims of this study were to evaluate the immunohistochemical expression of CD105 and Ki-67 in adenoid cystic carcinoma and polymorphous low grade adeoncarcinoma of the salivary glands and to correlate the immunoexpression of these proteins with the clinicopathological findings.

Materials and methods
In this retrospective study , fifty of archival formalin fixed paraffin embedded tissue samples of salivary gland malignancies were used, twenty five blocks of adenoid cystic carcinomas and twenty five blocks of polymorphous low grade adenocarcinoma obtained from the archives of the department of oral pathology / college of dentistry / Baghdad university, Al-Shaheed Ghazi hospital, were included in our study. Four micrometer sections gained and immunostained using monoclonal antibody against CD105 and Ki-67. The immunoexpression was identified by the presence of brown stain in the cytoplasm of tumor cell in CD105 and the presence of brown stain in the nucleus of tumor cell in Ki-67. The proportion of cells that expressed the stain was correlated with the clinicopathological data of the patients.
Results: CD105 expression was found positive in 21 cases of ACC and 20cases of PLGA localized in tumor cells.
andKi-67 expression was found positive in 24 cases of ACC and 23 casesof PLGA localized in tumor cells.
Non- significant statistical relation (P=0.801) was detected regarding CD105 expression in both types of tumor and non- significant statistical relation (P= 0.852) was detected regarding Ki-67 expression in both types of tumor .
Non- significant statistical relation (P=>0.05) was detected regarding CD105 and Ki-67 expression in relation to sex, site and stage in both types of tumor.
Conclusion: Weak expression CD105 and Ki-67 in ACC and PLGA might be explained by CD105 and Ki-67 did not represent an exclusive factors consequently; other factors might be involved in the proliferation, progression and metastasis of both tumor types.
Key words: Adenoid cystic carcinoma**,** Polymorphous low grade adenocarcinoma , immunohistochemistry, CD105 and Ki-67.

Introduction

Adenoid cystic carcinoma (ACC) is a malignant neoplasm of modified myoepithelial and ductal cells that affect both the major and minor salivary glands. Distinctive cribriform, tubular, and solid growth patterns are the most important

characteristic features of ACC. Malignant transformation of the intercalated duct reverse cells leading to ACC occurrence and ACC has a tendency for locally invasive growth and perineural invasion with high predilection for local recurrence and distant metastasis (1).

Morphological diversity, cytological uniformity , an infiltrative growth pattern and low metastatic potential are essential characteristic features of polymorphous low grade adenocarcinoma (PLGA) which is a malignant epithelial tumour of salivary gland(2). The confusion with adenoid cystic carcinoma and diagnostic trouble are due to morphologic heterogeneity. It is important and crucial to differentiate it from tumours with myoepithelial differentiation; adenoid cystic carcinoma (ACC) which consequently has this diagnostic challenge(3,4). A correlation between clinical progression and histologic characteristic has been documented; the papillary cystic pattern is now associated with aggressive clinical behavior. It has also been noted that this transformation phenomenon, like the clinical progression follows the course of time, and local recurrences (5).

Type I membrane glycoprotein ,CD105 (Endoglin), situated on cell surfaces and is part of the TGF beta receptor complex. CD105 is an important protein for tumor growth, survival and metastasis of cancer cells to other locations in the body due to its crucial role in angiogenesis (6).

Nuclear protein, Ki-67, that is related with and may be essential for proliferation of cells and provided a reliable method for tumor growth rate evaluation(7,8).

Additionally it is connected with ribosomal RNA transcription. Inactivation of antigen Ki-67 leads to inhibition of ribosomal RNA synthesis (9).

Aims of the our study were to evaluate the immunohistochemical expression of CD105 and Ki-67 in adenoid cystic carcinoma and polymorphous low grade adenocarcinoma of the salivary glands and to correlate the immunoexpression of these proteins with the clinicopathological findings.

Materials and Methods

Sample

Salivary gland malignancies of fifty patients haphazardly selected from the file records and pathologic specimens from the archives of the department of oral diagnosis/ Collage of Dentistry/Baghdad University and the Maxillofacial Center in Al-Shaheed Ghazi Hospital in Baghdad dated from 1973 to 2015.

Patient's information concerning age, sex, site and clinical staging of the tumor that represent the clinical data were collected from the case sheets presented with the tumor specimens. We are evaluated all the clinical and histopathologic data to exclude cases representing secondary metastatic disease to the salivary gland .

Control

Negative control was done by primary antibody omitting step and addition of all other reagents . Five normal salivary gland tissues were used as negative external control at the same period of time. Nonexistence of specificity of the antibody presented as positive staining; according to ab cam manufacturer’s data sheets. Tonsil tissue were used as positive control for CD105 and Ki-67

Immunohistochemical procedure

First of all was deparaffinization of 4µm sections in xylene and then rehydration of them in graded alcohol. Then take the slides and add hydrogen

peroxide block drops and then incubation in humid chamber at 37C° for 10 minutes was done, after that and for 5 minutes for each socked 2 times in buffer. Protein cross-links that mask antigenic places in tissue specimens occur due to formalin or other aldehyde fixation so that antigenicity reveal is very important so that tissue retrieving is done to the slides. After that the slides incubated at 37°C for 10 minutes after the adding of enough drops of protein block. Then washed 2 times in buffer this done by 5 minutes for each, draining and blotting gently were done lastly. After that each slide exposed to diluted primary antibody , incubated in humid chamber at 37°C. overnight. Next day, and in 4 times for each the slides were washed in buffer, lastly and as before the slides drained and blotted gently. Now secondary antibody reagent drops were added and for 30 minutes at 37°C incubated in humid chamber .Next, and in 5 minutes for each one the slides were washed 4 times in buffer, finally drained and blotted gently. After that Streptavidine-HRP antibodies were applied on tissue and incubated for 30 minutes at 37°C. Later in dark room diluted DAB was applied on tissue and for 10 minutes at 37°C incubated in humid chamber. Next slides washed carefully in tap water for 5 minutes. Now the slides were bathed in Hematoxylin counter stain for 1-2 minutes then they were rinsed with tap water for 10 minutes. Then the slides were dehydrated by immersing them in ethanol and xylene containing jars. One to two drops of DPX mounting medium were applied next to the xylene wet sections and covered with cover slips and left overnight to dry.

The results were evaluated by the presence of brown colored end product at the site of target antigen (cytoplasm) in CD 105 and the presence of brown colored end product at the site of target antigen (nucleus) in Ki-67 were indicative of positive immunoreactivity. Percentage of IHC positive tumor cells per hotspot was calculated and the mean percentage per slide was determined. Collection 10 high power field from the more representative area of immunostaining fields.

The intensity of stain because it's subjected to individual variance during checking was ignored.

The immunoreactivity of CD105 was classified as follows: (score 0) (-ve) <10 % of the tumor cells, low (score I) (+) 10 -25%, moderate (score II) (++) 26-50%, high (score III) (+++) 51-100% of positive cells, depending on counting(10,11)

The immunoreactivity of Ki-67 was classified 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 (12).

Statistical analysis

In our study we tabulated and subjected the clinical, histopathological and immunohistochemical relevant data to appropriate statistical analysis using the SPSS v 20 software. We are scored and presented the studied parameters as count and percentage.

The relationship between categories was tested by fisher's exact test. ANOVA test (analysis of variance) was used to detect differences for age and the marker. *P* value equal or less than 0.05 was considered to be statistically significant.

Clinicopathological Finding

CD105 immunoexpression

Tonsil tissue was used as positive control of CD105 which expressed as brown diffuse cytoplasmic immunoreactivity.

Negative control was detected by omitting of primary antibody ,this is for testing the specificity of antibody used in our study , if positive staining occur this indicates a lack of specificity of the antibody .

Brown staining localized in the cytoplasm of the tumor cells mean CD105 immunoreactivity .CD105 expression was found positive in 21 cases of ACC and 20 cases of PLGA in different scores .The higher percentage of CD105 expression (score I) was found in 12 cases of ACC (48 %) and in 14 cases of PLGA (56 %).

Non- significant statistical relation (P= 0.802) was detected regarding CD105 expression in both types of tumor as in table 1.

Non- significant statistical relation (P=>0.05) was detected regarding CD105 expression in relation to sex, site and stage in both types of tumor as in table 2.

Non- significant statistical relation (P=>0.05) was detected regarding CD105 expression in relation grade in ACC as in table 2.

Table 1: CD105 scores in ACC and PLGA

CD105 score	**ACC**	**PLGA**
Score 0	**4 (16 %)**	**5 (20%)**
Score 1	12 (48 %)	14 (56 %)
Score 2	8(32 %)	6 (24 %)
Score 3	1 (4%)	0 (0 %)
Total	25(100%)	25 (100%)
	NS P value= 0.802	

Table 2: The clinicopathological finding of ACC and PLGA in relation to CD105 expression

Variable		ACC No.25		PLGA No.25		P value
		CD105		CD105		
		-ve	+ve	-ve	+ve	
AGE mean $\pm SD$		42.20± 2.533		57.6±15.950		
SEX	Male Female	2 2	14 7	0 5	10 10	NS > 0.05
SITE	Palate Floor of mouth Upper lip Check Submandibular gland others	2 0 0 0 1 1	15 3 0 1 1 1	4 1 0 0 0 0	15 1 1 0 0 3	NS > 0.05
STAGE	I II III IV	3 1 0 0	8 9 2 2	4 0 0 0	4 8 4 4	NS > 0.05
GRADE	I(tubular) II(cribriform) III(solid)	2 3 2	5 12 4	- - -		NS > 0.05
PERINEURAL INVATION		0	2	0	0	

Ki-67 immunoexpression

Brown nuclear and nucleolar immunoreactivity of Ki-67 was expressed in tonsil tissue which is positive control in our study.

Omitting of primary antibody were used as negative control ,this is for testing the specificity of antibody used in our study ,positive staining indicates a lack of specificity of the antibody .

Ki-67 immunoreactivity was noticed as brown staining localized in the uncles and nucleolus of the tumor cells .Ki-67 expression was found positive in 24 cases of ACC and 23 cases of PLGA in different scores.The higher percentage of Ki-67 expression and (score I) was found in 12 cases of ACC (48%) and (scores I & III) was found in 9 cases of PLGA (36%).

Non- significant statistical relation (P=0.852) was detected regarding Ki-67 expression in both types of tumor as in table 3 .

Non- significant statistical relation (P=>0.05) was detected regarding Ki-67expression in relation to sex, site and stage in both types of tumor as in table 4.

Non- significant statistical relation (P=>0.05) was detected regarding Ki-67 expression in relation grade in ACC as in table 4.

Table 3: Ki-67 scores in ACC and PLGA

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

Table 4: The clinicopathological finding of ACC and PLGA in relation to Ki-67 expression

Variable		ACC No.25 Ki-67		PLGA No.25 Ki-67		P value
		-ve	+ve	-ve	+ve	
AGE mean $\pm SD$		42.20± 2.533		57.6±15.950		
SEX	Male Female	1 0	15 9	1 1	9 14	NS > 0.05
SITE	Palate Floor of mouth Upper lip Check Submandibular gland others	1 0 0 0 0 0	16 3 0 1 2 2	0 1 0 0 0 1	19 1 1 0 0 2	 NS > 0.05
STAGE	I II III IV	1 0 0 0	10 10 2 2	1 0 0 1	7 9 4 3	NS > 0.05
GRADE	I(tubular) II(cribriform) III(solid)	1 0 0	5 14 5	- - -		NS > 0.05
PERINEURAL INVATION		0	2	0	0	

Discussion

Our study is constantly attempted to emphasis largely on finding immunohistochemical differences between ACC and PLGA, mostly in the cribriform histology, common to both tumours, we confirm a diagnostic marker for histological resemblance by trying to use immunohistochemistry. In this study we compared Ki-67 protein expression in adenoid cystic carcinoma and polymorphous low grade adenocarcinoma.

Ki-67 marker play an important role in tumor development ,progression and in the overall prognosis . However the action of this molecule functioning has yet to be defined. The role of Ki-67 in ACC and PLGA has not been revealed adequately.

The results of this study showed that high - grade tumors characterized by overexpression of Ki-67 was overexpressed in the advanced - stage disease .These finding led us to believe that Ki-67 might be used as biomarkers for aggressive disease manners and as predictor of poor overall survival.

It is also worth noting that Ki-67 expression was not detected (1 and 2 cases) of tumor specimens of ACC and PLGA respectively . One possible explanation of this findings is that expression of Ki-67 may possibly be occur in ACC and PLGA but occasionally its production is unsteady and preserved at almost unnoticeable levels.

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On the other hand, Ki-67 examination indicated that its expression was useful for differentiation of ACC from PLGA . Ki-67 was expressed around the duct more intense than other markers. An increase in Ki-67 expression indicates an increase in

mitotic cell activity and proliferation . The growth fraction determination of cell population was done by Ki-67 which is an excellent marker .

In our study ,due to unavailability of information associated with prognosis ,survival and follow up of the patients ,it was impossible to assess the relationship between Ki-67 marker and factors associated with prognosis.

Ki-67expression in different studies show variance, this may be related to the type of antibody used (monoclonal and polyclonal) , and the differences in how the cells are counted

The nuclear distribution of the immunohistochemical staining product was found to be granular, diffuse, or a combination of both. This finding is in agreement with previous reports in which the staining sites have been accurately determined at an ultrastructural level and which have also demonstrated variations of the granular and diffuse staining patterns in the various phases of proliferation (13,14,15,16,17) We found that the diffuse staining pattern was generally strongly or moderately intense, whereas the granular pattern could show the whole spectrum of intensities.

Conclusion

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 .

CONFLICT OF INTERESTS.
There are non-conflicts of interest .

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الخلاصة

تمهيد

السرطان الكيسي الغدي والسرطان الغدي المتعدد الاشكال ذو الدرجة الواطئة ولهما تداخل في عدد من الانماط النسيجية والتي تشمل المثقب، الانبوبي والاصم. ان تداخل الصفات السريرية والمرضية للسرطان الكيسي الغدي والسرطان الغدي المتعدد الاشكال ذو الدرجة الواطئة يادي الى جعل التشخيص في مازق. ان السرطان الكيسي الغدي يمتاز بانه اسوا في تطور المرض من السرطان الغدي المتعدد الاشكال ذو الدرجة الواطئة وهذا يجعل التمييز بينهما ضروري لا جل العلاج ومتابعة تطور المرض.

الاهداف

تقييم الظهور الكيميائي النسيجي المناعي لل CD105. ومؤشر التكاثر.Ki-67. للسرطان الكيسي الغدي والسرطان الغدي المتعدد الاشكال ذو الدرجة الواطئة للغدد اللعابية، ومقارنة درجة ظهور هذه العوامل مع الخصائص السريرية والمرضية.

المواد وطرائق العمل

تضمنت الدراسة ٥٠ عينة لاشخاص مصابين بسرطان الغدد اللعابية، ٢٥ عينه لا شخاص مصابين بالسرطان الكيسي الغدي و٢٥ عينة لا شخاص مصابين بالسرطان الغدي المتعدد الاشكال ذو الدرجة الواطئة، جمعت هذه العينات من ارشيف قسم امراض الفم والوجه والفكين/ كلية طب الاسنان/ جامعة بغداد ومختبر الامراض العامة/ مستشفى الشهيد غازي الحريري للجراحات التخصصية،

وباستخدام قوالب شمعيه حاوية على النسيج المحفوظ في الفور مالين واجري لها الفحص النسيجي للتأكد من التشخيص بعد تقطيعها الى شرائح دقيقة وبسمك ٤ ما يكرو متر.

بعدها تم اجراء الفحوصات المناعية النسيجية الكيميائية لل. CD 105 ومؤشر التكاثر Ki-67 .للشرائح النسيجية بنفس السمك المذكور سابقا مع اجراء اختبار السيطرة السالبة والموجبة ثم تقييم النتائج الى بعضها البعض والى الخصائص السريرية والمرضية ايضا.

النتائج

اظهرت النتائج ان الظهور الكيميائي النسيجي المناعي لل.CD105 كان موجبا في ٢١ عينة للسرطان الكيسي الغدي و ٢٠ عينة للسرطان الغدي المتعدد الاشكال ذو الدرجة الواطئة كما اظهرت النتائج ان الظهور الكيميائي النسيجي المناعي لمؤشر التكاثر Ki-67 كان موجبا في ٢٤ عينة للسرطان الكيسي الغدي٢٣ عينة للسرطان الغدي المتعدد الاشكال ذو الدرجة الواطئة .

لوحظ عدم وجود علاقة معنوية لل.CD105 في السرطان الكيسي الغدي والسرطان الغدي المتعدد الاشكال ذو الدرجة الواطئة.

لوحظ عدم وجود علاقة معنوية لمؤشر التكاثر Ki-67. في السرطان الكيسي الغدي والسرطان الغدي المتعدد الاشكال ذو الدرجة الواطئة.

لوحظ عدم وجود علاقة معنوية لل CD105. ومؤشر التكاثر Ki-67 مع جنس المريض والموقع التشريحي ومكان تحديد الانتشار في السرطان الكيسي الغدي و السرطان الغدي المتعدد الاشكال ذو الدرجة الواطئة.

لوحظ عدم وجود علاقة معنوية لل CD105. ومؤشر التكاثر Ki-67 مع درجة التمايز في السرطان الكيسي الغدي.

الاستنتاجات

في هذه الدراسة ظهر ان ال CD105 ومؤشر التكاثر Ki-67 تلعب دور مهم في تقييم المرض ولا يمكن اعتبارها غير مهمة في مجال تقييم المرض.

ان الاختلاف في الميل البيولوجي ليس له علاقه مع تكوين اوعيه دموية جديدة، ان زياده تكوين الأوعية الدموية يتناسب طرديا مع تكون السرطانات الخبيثة ان ظهور ال CD105 في سرطانات الغدد اللعابية يظهر دوره في تطور السرطانات الخبيثة ودور خلايا الMyoepithelial cell في السيطرة على تكوين اوعية دموية جديدة .

ان نسبة ظهور مؤشر التكاثر Ki-67 يكون في السرطان الكيسي الغدي اكثر من السرطان الغدي المتعدد الاشكال ذو الدرجة الواطئة.

هذا التداخل والتفاعل يحفز عمليات بخطوات متعددة تساعد على حدوث سرطان الغدد اللعابية وتساهم في تنظيم عمليات الهجوم والنزعة الخبيثة لهذه السرطانات.

الكلمات الدالة: السرطان الكيسي الغدي، السرطان الغدي المتعدد الاشكال ذو الدرجة الواطئة، الظهور الكيميائي النسيجي المناعي،CD105. مؤشر التكاثر Ki-67 .