Immunohistochemical expression of E-cadherin and CD44 adhesion molecules in oral squamous cell carcinoma

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ABSTRACT

Background: Head and neck squamous cell carcinoma is the sixth most common cancer world wide. Despite greater emphasis on multi-modality therapy including surgery, radiation and chemotherapy, advanced stage head and neck squamous cell carcinoma continues to have poor 5-year survival rates (0-40%) that have not significantly improved in the last (30) years. To improve outcomes for this deadly disease , It is required a better understanding of the mechanisms underlying head and neck squamous cell carcinoma tumor growth, metastasis, and treatment resistance. This study evaluates the Immunohistochemical expression of E-cadherin and CD44 adhesion molecules in OSCC and to correlate the expression of either marker with each other, with lymph node metastasis and with tumor grade.

Materials and methods: Thirty blocks of OSCC were included in this study. An immunohistochemical staining was performed using anti E-cadherinand anti CD44 monoclonal antibodies.

Results: Negative immunohistochemical expression of E-cadherin was found in(66.7%)of the cases and only (33.3%)revealed positive immunoexpression. Positive CD44 immunoreaction was seen in(86.7%)of the cases. There was no statistically significant correlation regarding either marker with respect to the tumor stage, grade and lymph node matastasis. Moreover anon-significant correlation was found between the expression of both markers.

Conclusions: this study revealed negative E-cadherin expression in two thirds of the cases, while positive CD44 was illustrated in most of them. Non- significant correlation was found regarding the expression of both markers with tumor stage, grade and lymph node status. Inverse significant correlation was found regarding CD44 expression with the clinical presentation of the study sample. In addition, non significant correlation was found between the E-cadherin and CD44 immunoexpression.

Key words: Oral squamous cell carcinoma, Adhesionmolecules, E-cadherin, CD44, Immunohistochemistry. (J Bagh Coll Dentistry 2013; 25(Special Issue 1):36-42).

الخلاصة

الخلفية:سرطان الخلايا الحرشفية للراس والرقبة هو السادس الاكثرشيوعا في كل العالم. بالرغم من التاكيدالشديدعلى العلاج المتعدد الاشكال الذي يشمل الجراحة،الاشعاع والعلاج الكيميائي،مراحل متقدمة من سرطان الخلايا الحرشفية للراس والرقبة يواصل امتلاكه مستوى (5) سنوات للبقاء هزيل(0-40%) والذي لم يتقدم بشكل مفيد في السنوات الثلاثين الاخيرة. ولتحسين النتائج لهذا المرض المميت يحتاج الى فهم افضل للالية وراء نمو ورم سرطان الخلايا الحرشفية للراس والرقبة،الانبثاث ومقاومة العلاج.

الاي- كادهرين ضروري للتكوين وللمحافظة على الظهاروهو واحد من اهم الجزيئات لالتصاق خلية مع خلية في الانسجة الظهارية0موقعه على سطح الخلايا الطلائية في مناطق تماس خلية مع خلية تعرف بالرباط الالتصاقي.

في اورام الانسان، فقدان الاي- كادهرين الذي يكون التصاق الخلايا يرتبط بفقدان تشكل الظهار مع اكتساب امكانية الانبثاث من قبل الخلايا السرطانية.

السي دي- (44) هوجزيئة في غشاء الخلية الذي وجد اولا في الخلية اللمفية وعرف ابتدائيا بامتلاكه لوظائف لصق وايواء الخلية. وجد مولد المضاد في معظم انسجة الانسان وعرف بامتلاكه وظائف متعددة منذ اكتشافه الاول. وقد درس فيما يتعلق بدوره في الاسهام بتقدم الورم في مختلف الاورام الصلبة بما في ذلك سرطان الخلايا الحرشفية للراس والعنق. اشارة حامض الهيالورونيك- سي دي- (44) ربطت بتقدم الورم بما في ذلك عملية الاختراق والانبثاث.

تهدف هذه الدراسة الى تقييم وربط الظهور الكيميائي النسيجي المناعي لجزيئات الاي-كادهرين والسي دي-(44) اللاصقة في سرطان الخلايا الحرشفية للفم وربط ظهور كل منهما بمرتبة الورم والانبثاث للعقد اللمفية.

المواد والطرق:تضمنت هذه الدراسة ثلاثين عينة استرجاعية لاشخاص مصابين بسرطان الخلايا الحرشفية للفم والتي استخرجت من المقاطع النسيجية المثبتة بالفورمالين والمطمورة بشمع البارافين. جرى صبغ كل عينة بالهيماتوكسلين والايوسين لاعادة تقييمها لغرض الفحص النسيجي المرضي. اجريت الصبغات الكيميائية النسيجية المناعية باستخدام مضاد الاي- كادهرين ومضاد السي دي- (44) على شرائح نسيجية دقيقة من العينات.

النتائج:ظهرت نتائج (30) عينة من سرطان الخلايا الحرشفية للفم كمايلي:

- سجلت اكثرالحالات في الاعمارمافوق (50) سنة ومعظم الحالات هم من الذكور(70%) مع نسبة الذكور الى الاناث 1:2.
- وجدت معظم الحالات في اللسان (36,7%) ومعظمها ظهرت سريريا بشكل تقرح (50%).
- الفحوصات النسيجية المرضية اظهرت ان (70%) من الحالات متوسطة التمايز وفقط (30%) واضحة التمايز.
- سلبية الظهورالكيميائي النسيجي المناعي للاي- كادهرين ظهرت في (66,7%) وفقط (33,3%) اظهرت ايجابية الظهورالمناعي للاي-كادهرين.
- لوحظ ايجابية الظهورالكيميائي النسيجي المناعي للسي دي (44) في (86,7%) من الحالات من بينها (46,7%) اظهرت الدرجة (3) ،(26,7%) اظهرت الدرجة (2) ،(6,7%) اظهرت الدرجة (1) وفقط (6,7%) اظهرت الدرجة (4) من ايجابية الاصطباغ المناعي للسي دي- (44).
- لم يكن هناك اي ارتباط احصائي معنوي بين أي من المؤشرتين الحيويتين ودرجة ومرحلة الورم والانبثاث للعقد اللمفية. علاوة على ذلك لم يوجد أي ارتباط معنوي بين الظهورلكلا المؤشرتين الحيويتين مع بعضهما البعض.

الاستنتاجات: اظهرت هذه الدراسة سلبية الظهورالمناعي للاي- كادهرين في ثلثي الحالات بينماوجدت ايجابية الظهور المناعي للسي دي - (44) في معظم الحالات لسرطان الخلايا الحرشفية للفم . وجد ارتباط لامعنوي فيما يخص ظهوركلا المؤشرتين الحيويتين مع درجة ومرحلة الورم وحالة العقداللمفية . وجدت علاقة معنوية عكسية فيما يخص ظهور السي دي- (44) مع الظهور السريري لعينات الدراسة. بالاضافة الى ذلك فأن الظهور المناعي للاي-كادهرين لم يؤثر معنويا في الظهور المناعي للسي دي- (44) في الحالات المدروسة.

INTRODUCTION

Over 90% of oral cancers (OCs) are squamous cell carcinomas (SCCs). They constitute a major health problem in developing countries, representing a leading cause of death. The survival index continues to be small (50%), as compared to the progress in diagnosis and treatment of other malignant tumors.

This is because patients continue to die from metastatic diseases at regional and distant sites [(1)]. Neoplasia or cancer is viewed as a cell cycle disease. Although this concept implies that every tumor is defective in one or more aspects of the cell cycle control, it clearly does not mean that oncogenesis targets only oncogenes and the cell cycle clock. Development of malignancy appears to require also aberrations in the cell death machinery and cell-cell and/or cell-matrix interactions that cooperate with cell cycle defects.

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Many of the processes in which adhesion molecules play central role – anchorage dependent growth, apoptosis, differentiation, and migration are those that are characteristically dysregulated in malignancy [2].

Adhesion molecules (AM) are transmembrane glycoproteins acting as a molecular link between the outside and inside of the cell.

The adhesion molecules are involved in the cell differentiation, migration and sorting. Broadly, these proteins can be classified into five families including immunoglobulin superfamily, integrins, cadherins, selectins, and CD44[3]. Alterations of these cell adhesion molecules are a common event in cancer. The disrupted cell-cell or cell-ECM adhesion significantly contributes to uncontrolled cell proliferation and progressive distortion of normal tissue architecture. More importantly, changes in cell adhesion molecules play a causal role in tumor dissemination. Loss of cell adhesion contacts allows malignant cells to detach and to escape from the primary mass [4,5]. E-cadherin, a calcium-dependent cell adhesion molecule,is a cell membrane-associated protein involved in cell–cell adhesion, and loss of expression of the cadherin/catenin complex has been described in various human malignancies [6]. Changes or alterations in the function and expression of this cell to cell adhesion molecule have been postulated to be an early event in the multiple step process of tumour metastasis and an important factor in tumour progression[7]. The loss of cadherines expression was observed in many types of carcinomas and usually it is associated to late stages of the disease and to the progression of malignant epithelial neoplasias [8]. CD44 was first described by Dalchau et al. as a molecule present on the surface of T-lymphocytes, granulocytes, and cortical thymocytes [9]. Human CD44 is a transmembrane hyaluronan-binding glycoprotein that can bind to hyaluronic acid, an extracellular matrix, and regulate a variety of cellular functions, such as cell migration, proliferation, cell–cell interaction, and apoptosis [10]. These cellular functions of CD44 imply that a disorder of CD44expression plays a crucial role in the behavior of a malignant tumor [11]. CD44 plays an important rolein metastases. In OSCC, decreased immnoexpression is associated with increased invasive potential of tumors and the presence of metastases [12, 13]. Since (AM) are involved in many fundamental processes of the cell involving normal physiological growth and development as well certain pathological conditions (wound heals, inflammation and neoplasia) and the loss of their expression or disordered expression plays important roles in the behavior of malignant tumors, therefore, this study concerned E-cadherin and CD44 adhesion molecules to elucidate their role in OSCC development and progression.

MATERIALS AND METHODS

Thirty formalin-fixed paraffin-embedded tissue blocks of OSCC were collected the archieves of the Department of Oral Diagnosis / College of Dentistry / Baghdad University;Al-Shaheed Ghazi hospital/Medical city/Baghdad; and private laboratories in Baghdad and Najaf, dated from(2000-2012). Four-micrometer-thick sections were cut from each paraffin tissue block and stained with hematoxylin(Mayer′s) and eosin for diagnostic confirmation and histological grading. Another two 4-µm section was cut from each tissue block and mounted on positively charged slides (Fisher super frost,USA) to be stained with monoclonal antibodies to E-cadherin and CD44 (ABCAM). Negative and positive tissue controls were included into each immunohistochemical run (according to the manufacturer).

Immunostaining

Five micrometer thick sections were cut and mounted on (Fisher super frost, USA) positively charged slides, then deparaffinized and rehydrated for immunohistochemical staining by E-cadherin and CD44(ABCAM) monoclonal antibodies;Heat mediating Antigene Retrieval was done for CD44 using phosphate buffer PH(6) then the sections were immersed in hydrogen peroxide (H2O2) to block the endogenous peroxidase activity, washed in phosphate-buffered saline (PBS), and then protein blocking reagent and incubated for 20 minutes at 37 c within humid chamber to reduce non specific staining. The tissue sections were incubated with mouse monoclonal [5H9] anti-human E-cadherin antibody (diluted 1:10) and CD44 (diluted 1:50) antibodies for one hour at 37 c.After that the slides were kept in the refrigerator at 4 c over night in humid chamber. The bounded antibodies were detected by the streptavidin-biotin complex method, after an immunoreaction, the sections were counterstained with Hematoxylin (Mayer's).

Scoring system

The scoring of the markers was done by examining of at least 1000 cells per section in five different representative fields. The membranous or membranous and cytoplasmic was considered positive for E-cadherin and membranous was considered positive for CD44 immunostaining. The percentage of positive cells was scored as

follows:score (0):<10% positive cells,score(1):10-25%positive cells,score(2):25-50%positive cells,score(3):50-75%positive cells and score(4):>75%positive cells [14].

Statistical analysis

The data was compiled into statistical software, statistical package of social sciences (SPSS) version 17. All variables were compared using Chi- square test. While Pearson correlation coefficient was applied to plot a correlation matrix among the different immunohistochemical markers expression values altogether. P values of less than 0.05 were considered statistically significant. Anova test was carried out to compare the numerical values of the study samples, Spearman′s rho was also applied in order to find any possible correlation between the categorical variables of the study sample.

RESULTS:

The results of (30) oral squamous cell carcinoma cases were designed as follows: most of the cases (62%) aged > 50 years; the majority of the cases were males (70%) with male to female ratio 2:1.The most common site was the tongue (36.7%). Most of the cases presented clinically as ulcer (50%). Histopathological examination showed that (70%) of the cases were moderately differentiated and only (30%) were well differentiated carcinomas.

Negative immunohistochemical expression of E-cadherin was found in(66.7%) of the cases and only (33.3%) revealed positive immunoexpression. Positive CD44 immunoreaction was seen in (86.7%) of the cases, of which (46.7%) presented score (3), (26.7%) score (2), (6.7%) score (1) and only (6.7%) presented score (4) CD44 positive immunostaining.

Table 1: Age and sex distribution of the study sample

Age	No.	%
50>	18	62
50≤	11	37
	29*	99
sex		
Male	21	70
female	9	30
	30	100
1 case the age was not recorded.		

Table 2: E-cadherin IHC expression in OSCC cases

E-cadherin Score*		Frequency	Valid Percent
Valid	0	20	66.7
	1	7	23.3
	2	2	6.7
	3	1	3.3
	Total	30	100.0

***score 0:<10% positive cells,score 1:10-25% positive cells,score 2:25-50%positive cells,score 3:50-75% positive cells,score 4:>75% positive cells.**

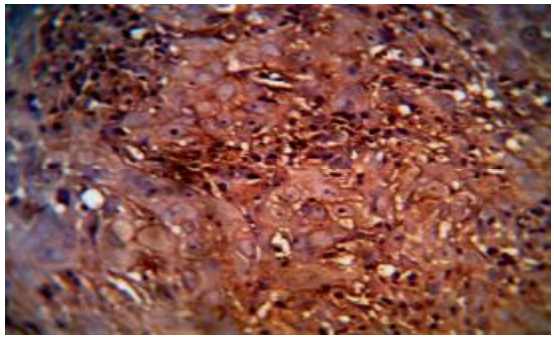

Figure 1: Positive brown membranous and /or cytoplasmic immunostaining of E-cadherin in well differentiated OSCC-Buccal mucosa(400X).

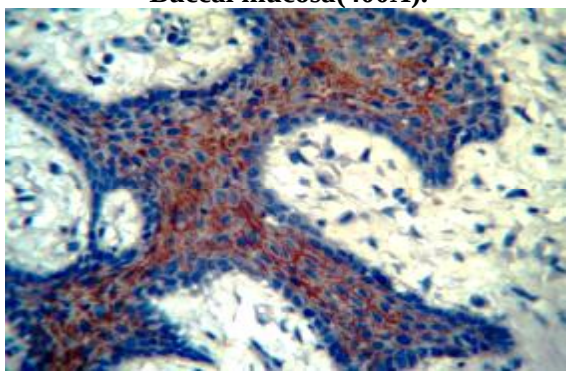

Figure 2: Positive brown membranous immunostaining of E-cadherin in Moderately-differentiated OSCC-Maxilla (400X).

Table 3: CD44 IHC expression in OSCC cases

CD44 score *		Frequency	Percent
Valid	0	4	13.3
	1	2	6.7
	2	8	26.7
	3	14	46.7
	4	2	6.7
Total		30	100.0

***score 0:<10% positive cells,score 1:10-25% positive cells,score 2:25-50% positive cells,score 3:50-75% positive cells,score 4:>75% positive cells.**

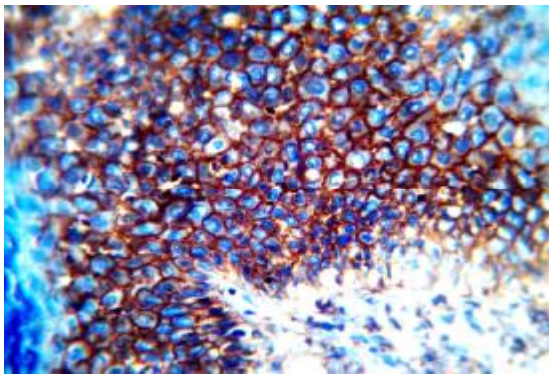

Figure 3: Positive brown membranous immunostaining of CD44 in well differentiated OSCC –Buccal mucosa (400X).

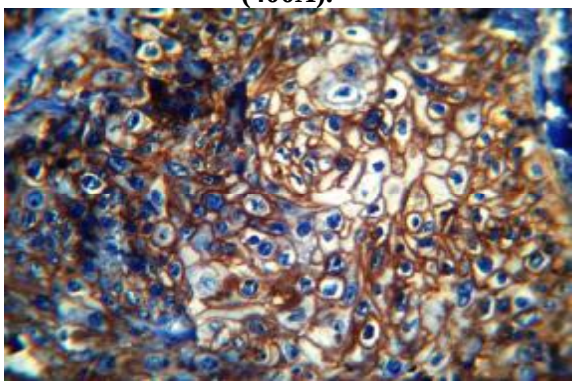

Figure 4: Positive brown membranous immunostaining of CD44 in Moderately differentiated OSCC –Buccal mucosa (400X).

Table 4: The correlation of E-cadherin & CD44 expressions.

		CD44 scores	E-cadherin scores
CD44 scores	Pearson Correlation	1	0.124
	Sig. (2-tailed)		*0.513
	N	30	30
E-cadherin scores	Pearson Correlation	0.124	1
	Sig. (2-tailed)	*0.513	
	N	30	30

***p value more than 0.05 is considered non- significant**

Table 5: Correlation of CD44 and E-adherin scores with the clinical presentation using Spearman's rho.

scores	Sperman's rho	clinical presentation
CD44 score	r Sig. (2-tailed) N	**-.419 0.033* 26
E-Cadherin score	r Sig. (2-tailed) N	-.277 0170 26

*** Correlation is Significant at the 0.05 level (2-tailed) .**
**** negative(-ve) indicates a reverse correlation .**

There was no statistically significant correlation regarding either markers with respect to the tumor grade, lymph node status and stage .Moreover a statistically non significant correlation was found between the expressions of both markers.

DISCUSSION

This study is not a large epidemiological one that expressed the incidence and prevalence of different clinicopathological features of OSCC. The clinicopathological information were evaluated and analyzed for only (30) OSCC surgical specimens, however, there is a close correlation between the present data and other published data concering the incidence of OSCC in previous foreign and Iraqi studies records.

Assessment of E-cadherin immunohistochemistry

The results of this study showed reduction in the immunoexpression of E-cadherin in (66.7%) of the cases, this is in agreement with the findings of Williams et al. who found E-cadherin underexpression in carcinoma in situ cases and infiltrative tumors and Santos et al. who recorded E-cadherin underexpression in (90%) of oral squamous cell carcinoma cases [15,16]. Generally E-cadherin is expressed as membranous immunostaining, but cytoplasmic expression (a translocation of this marker into the cytoplasim) was detected in some cases of the study sample such finding was also found by Massarelli et al. and Aguiar et al. who observed higher cytoplasmic expression of E-cadherin in OSCC with nodal metastasis[17,18].

In fact, a redistribution of the E-cadherin complex out of tight junctions can affect its functions in cell-cell adhesion and increase its degradation by cytoplasmic endocytosis resulting in cytoplasmic E-cadherin expression[19]**.** This study revealed a non-significant correlation regarding E-cadherin expression with any of the clinicopathological data including the tumor stage, grade and lymph node involvement. Generally, more aggressive oral carcinomas show loss of epithelial cell cohesion, and this is often associated with a reduction in E-cadherin expression. However, loss of cohesion may also be due to reduced E-cadherin function as a result of sequence mutation or by abnormalities in the cadherin-catenin complexes.

Thus, E-cadherin may still be detected by immunohistochemistry, even in non-functional form, and this possibly explains some of the apparently conflicting results produced by immunohistochemical studies on oral carcinoma.

Furthermore, Several factors such as the sample size, methods of histological grading of

malignancy, the visual judgment of pathologists ,type of antibody used, immunohistochemical techniques, the choice whether to use frozen or paraffin embedded material, relative subjectivity in interpreting and scoring the staining results and the cellular heterogeneity of OSCC may also be responsible for those conflicting results[20].

Assessment of CD44 immunohistochemistry

The expression of different CD44 isoforms in HNSCC has been studied, but their role remained controversial. Whereas some studies have found a correlation between increased CD44 expression and HNSCC progression, others have reported no such correlations or negative correlations [21].

In the present study isoform (10)epithelial isoform is used, but regardless to its type, the study focused on the tissue specificity of the marker namely epithelial cells in which CD44 expressed in carcinomas was indicated according to the manufacturer data sheet. The results of this study showed that most of the studied cases(86.7%) presented positive CD44 immunostaining ,of which (46.7%) revealed score (3)immuno expression, and only(13.3%) of the studied cases were negative.Similar results reported in other studies in different cancers including head and neck, breast ,lung ,gastrointestinal, bladder, cervical carcinomas by employing immunohistochemical staining,RT-PCR and Northern blotting techniques and using different isoforms of CD44[22,23,24]. In the present study no correlation was found between the histological grade of the tumors and the CD44 expression,this finding is in agreement with Herold-Mende et al and Van Hale who also found no correlation regarding CD44splice variants expression and any clinicopathological variables [25,26]. However, Ue et al. found that the reduction in the expression of certain variants of CD44 was correlated with tumor cell differentiation in primary OSCC cases[27]. Concerning the relation of CD44 expression with the tumor stage, A non –significant correlation was found . Similar results recorded by Van Hal et al. and Kanke et al. who found (96%) CD44v6 immunoexpression in HNSCC, with no correlation to the tumor stage [26,28]. While other investigators revealed that CD44 play crucial role in tumor progression and its expression is correlated well to the tumor stage [29-31]. Regarding the relation of CD44 immunoexpression with lymph node involvement, this study showed a non-significant correlation, As mentioned previously, this finding may be due to the small size of the node positive cases enrolled in this study (14 out of 30).

Another investigators found direct correlation between increased or decreased expression of CD44variant isoforms with lymph node involvement and development of metastasis within different kinds of tumors in different organs[32,33] . The present study revealed an inverse significant correlation between CD44 immunostianing and the clinical presentation of the studied cases. No previous studies highlighted such correlation to compare with; however, this inverse correlation suggests that reduced CD44expression is associated with increased tumor aggrasiveness (ulcer), while increased expression of CD44 is associated with decreased tumor aggressiveness (mass). There are some possible explanations for the discrepant results among different studies regarding the correlation between CD44expression and the clinicopathological presentation, these could be, the employment of different antibodies (CD44 different isoforms), which makes comparison between research groups difficult.Moreover, certian CD44 variant domain epitopes may become hidden and not regognized by some antibodies due to post -translational changes which alter the three-dimensional conformation of the protein.In addition, assessment of immunostaining positivity is dependant on what region of the tumor is examined, size of the study sample, method used for assessment of CD44 expression (RT-PCR, FISH, Immunohistochemistry ….etc), techniques of immunohistochemistry (manual or automated) [34,35,36].

Assessment of the correlation between E-cadherin and CD44 immunohistochemical expression:

Regarding the correlation between both markers, the results revealed a non-significant correlation between them, i.e,each marker acts independently(each marker works alone) . Furthermore, either markers showed no correlation to tumors stage, grade and lymph node status.Similar results revealed by (Carmen et al. and Vazifeh et al., IVSL) [36,37].However,different findings revealed by Simionescu et al.[14]. Indeed, the cellular and molecular processes involved in malignant neoplasms are complex. Further studies are required to clarify the role of E- cadherin and CD44 AMs in the development and progression of OSCCs.

Fortunately, the progress in the area of adhesion molecules is expected to be rapid in the following years. This may result in novel prognostic and therapeutic tools in the problematic field of head and neck cancer. It

seems that this interesting journey is long and we are just at the beginning.

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