Original Research
Medical Journal of the Islamic Republic of Iran. Vol. 24, No. 3, November, 2010. pp. 146-150

Predictive value of epidermal growth factor (EGF) and laminin-5 for clinicopathologic oral squamous cell carcinoma (OSCC) staging and grading in Iranian population

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Received: 5 May 2010 Revised: 1 June 1010 Accepted: 24 July 2010

Abstract

Background: Squamous cell carcinoma (SCC) constitutes the main oral malignancy. Parallel to better understanding of molecular and genetic patterns of tumor behavior, more precise correlation of tumor markers such as Epidermal Growth Factor (EGF) and Laminin-5 are sought to estimate macroscopic and microscopic tumor status.

Methods: We conducted a cross-sectional study collecting oral SCC samples during 2006-2007 from Pathology Department of Shahid Beheshti Dental School. Immunohistochemical staining with antibodies against EGFR and laminin-5 along with staining degree were reported by two experienced pathologist including degree of staining (low, medium, high), and pathological grading and clinical staging obtained from medical records.

Results: Forty two patients' paraffin blocks of SCC examined with mean age 58(18.72) yrs ranged between 21-88, female to male ratio of 1.33:1 was observed. The study analyses revealed a significant correlation between the expression of laminin-5 with tumor stage and grade (P< 0.001 r=0.547 and r=0.545 respectively), yet no significant correlation between expression of EGFR and tumor stage or grade (P=0.894 r=-0.018 and P=0.543 r=0.86 respectively). Considering high degree of staining and stage IV; sensitivity, specifity, positive predictive value (PPV) and negative predictive value (NPV) of 44%, 54%, 44% and 78% calculated for ERGF and 55%, 78%, 58% and 86% for laminin-5 respectively. Considering high degree of staining and grade 3; sensitivity, specifity, positive predictive value (PPV) and negative predictive value (NPV) of 57%, 57%,17% and 86% calculated for EGFR and 85%, %82, 50% and 96% for Laminin-5 respectively.

Conclusion: We concluded that laminin-5 has a better prediction for developing higher tumor stage or grade but further research needed for identifying the precise role of EGFR.

Keywords: Epidermal growth factor, laminin-5, SCC, stage, grade, negative predictive value.

Introduction

The Squamous cell carcinoma (SCC) comprises approximately 94% of all oral malignancies [1]. It is the 8th most common cancer in male and the 15th most common in female. Overly cancer causes more than 20% of all deaths in the United States annually [2]. It may even reach 50% of all cancers as the overall most frequent cancer in some parts of Asia, es-

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pecially in India. The Oral cancer incidence reported as 1.13-1.75/100000 in Iran which encompasses most commonly for oral SCC with male preponderance [3, 4].

Genetic and molecular changes are important factors in the outcome of disease and may help in target therapy [5]. Epidermal Growth Factor Receptor (EGFR) is a transmembrane protein which binds to Epidermal Growth Factor (EGF) and TGFα thus regulates cellular growth [6]. The relation between expression of EGFR and clinicopathological parameters in oral squamous cell carcinoma have been evaluated in several researches; and over expression of EGFR was correlated with poor prognosis in head and neck malignancies [7-9]. Previously shown that Laminin-5 is a main molecule of basement membrane which helps the arrangement of normal cells but in tumors it helps the attachment of cells to their surrounding stroma and accelerates their migration [10]. Many studies suggested that laminin-5 γ^2 is relevant in tumor invasion in oral cavity [11, 12] . It has been shown that laminin-5 increases the risk of transforming premalignant lesions to in situ or invasive malignancies [6.] We aimed to investigate the correlation between expression of EGFR and laminin-5 with clinical staging and microscopic grading in Iranian patients suffering from oral SCC (OSCC).

Methods

This cross-sectional study conducted during 2006-2007 was based on patients with OSCC referred to Oral and Maxillofacial Pathology Department of Shahid Beheshti Dental School, Tehran, Iran. Tumor samples were formalin - fixed, embedded in paraffin wax and stored for varying periods of time. Hematoxylin and Eosin stains were prepared for each sample. The tumor grade and clinical stage were assessed by two expert pathologists in a blinded manner using standard pathologic criteria. Other demographic and clinical data were obtained from medical records.

Immunohistochemistry: The expression of EGFR (clone: M 7262) and laminin-5 (clone: M 7232) were assessed by immunohistochemistry using murine monoclonal antibody (Dako cytomation, Denmark). For each, a 5μm section was cut from paraffin blocks and mounted on salinized slides. Deparaffination and rehydration performed and Methanol instilled to block endogenous peroxidase. Diaminobenzidine solution was also used as a chromogen. Positive and negative controls were included in each run [13].

*Evaluation of slide*s: To quantify EGFR expression a total score calculated using the product of a proportion score and intensity score. The proportion score showed the fraction of positive stained tumor cells. (0 = none 1= < 10% 2= 10-50% 3=50-80% 4=>80%). The intensity score represented the estimated staining intensity (0= no staining 1 = weak 2 = moderate 3 = strong), the total score ranged from 0 to 12. Tumors were classified to high, moderate, low and no EGFR expression using a cut- off value: 0=0, 1-3=low, 4-8=moderate, 8-12=high [13].

For counting ?2-positive cells, 4 optical fields of 200X observed. The γ^2-positive cells were divided into the three groups: first group included counts up to 20-postive cells within all the 4 fields, second 21-50 cells and the third included the number of positive cells over than 50 [14].

For the statistical analysis, categorical variables demonstrated with frequencies and numeric data displayed as mean (± standard deviation). The correlation between ordinal variables was also tested by Kendall's tau rank correlation. Positive predictive values (PPV) and negative predictive values (NPV) were adjusted with previously reported annual incidence of Oral SCC from Iran. The two-tailed ? and P value <0.05 were considered statistically significant.

Results

This study performed on 42 patients' paraffin blocks. The mean age of the patients was 58(18.72) yrs ranged 21-88. Twenty four (57.1%) patients were female and 18 (42.9%) male. The OSCC was seen most frequently in specimens from tongue (%47.6) followed by buccal mucosa (%16.7, gingiva (%16.6),), palate (7.1%), retro molar mucosa (4.8%), mouth floor (4.8%) and alveolar mucosa (2.4%). Eleven patients (26.19%) fall into each stage I-III and 9 (21.42%) patients were diagnosed as stage IV. Pathologic study clarified 18(42.85%), 17 (40.47%) and 7 (16.66%) patients had microscopic grading I, II and III respectively (Table 1, 2). Significant correlation was found between laminin-5 expression and clinical stage and tumor grade (P< 0.001 r=0.547 and r=0.545 respectively). No significant correlation was found between EGFR expression and clinical stage and tumor grade (P=0.894 r= -0.018 and P=0.543 r=0.86 respectively). Degree of EGFR and Laminin-5 expression is shown in Table 1 and 2. Considering high degree of staining and stage IV; sensitivity, specifity, positive predictive value (PPV) and negative predictive value (NPV) of 44%, 54%,44% and 78% calculated for EGFR and 55%, %78, 58% and 86% for Laminin-5 respectively. Considering high degree of staining and grade 3; sensitivity, specifity, positive predictive value (PPV) and negative predictive value (NPV) of 57%, 57%,17% and 86% calculated for EGFR and 85%, %82, 50% and 96% for Laminin-5 respectively. Moreover there was a significant correlation between Degree of laminin-5 expression regarding degree of Epidermal Growth Factor Receptor (EGFR) expression (P=0.047, r=0.27) (Table 3).

Table1. Epidermal growth factor receptor (EGFR) expression in relation to clinical stage and microscopic grade

Clinical stage	EGFR expression			Total
	Low	Moderate	High	
I(n=)	2	4	5	11
II(n=)	4	3	4	11
III(n=)	1	4	6	11
IV(n=)	4	1	4	9
Total	11	12	19	42
Microscopic grade				
I(n=)	5	6	7	18
II(n=)	4	5	8	17
III(n=)	2	1	4	7
Total	11	12	19	42

Table2. Laminin-5 expression in relation to clinical stage and microscopic grade

Clinical stage	Laminin -5 expression			Total
	Low	Moderate	High	
I(n=)	8	2	1	11
II(n=)	2	8	1	11
III(n=)	1	5	5	11
IV(n=)	1	3	5	9
Total	12	18	12	42
Microscopic grade				
I(n=)	11	4	3	18
II(n=)	0	14	3	17
III(n=)	1	0	6	7
Total	12	18	12	42

Table 3. Degree of laminin-5 expression in comparison with the degree of Epidermal Growth Factor Receptor (EGFR) expression.

Expression of EGFR	Laminin-5 expression			Total
	Low	Moderate	High	
Low	8	2	1	11
Moderate	4	6	2	12
High	5	11	3	19
Total	6	19	17	42

Discussion

We analyzed the expression of EGFR and laminin-5 in oral squamous cell carcinoma. Relative even distribution into different clinical stage clarified with the evidence of benign trend in microscopic examinations. Mean age of patients was similar to previous studies, though older lower range obtained (21 vs. 4) [3, 15]. A somewhat female predominance (female/male ratio 1.33:1) signified changing epidemiologic patterns of the disease probably due to higher tendency of smoking in women. The most commonly affected site was tongue, although a recent study from Iran illustrated the lip as the most frequent site besides male preponderance. We did not calculate annual inci-

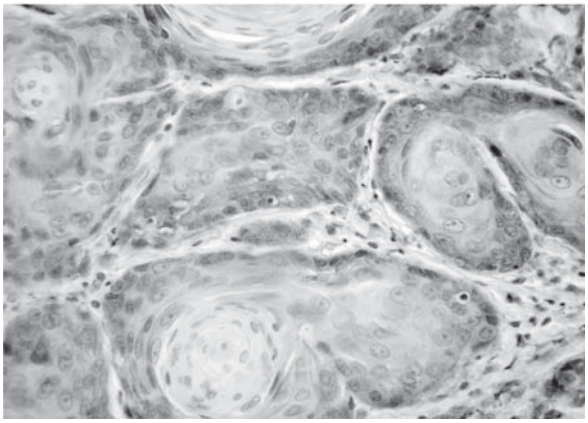

Fig. 1. EGFR expression in a tumor with grade I and stage I.

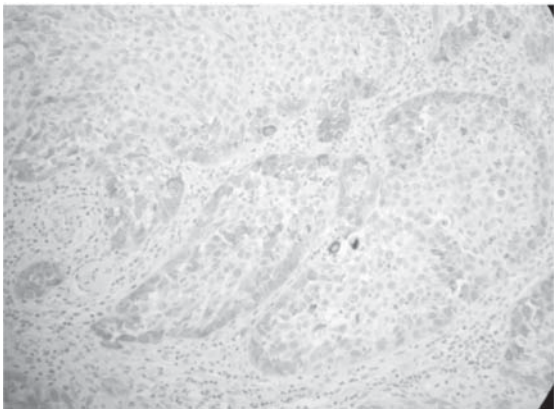

Fig. 2. Laminin-5 expression in a tumor with grade II and stage II.

dence of oral SCC, yet based on previously reported incidence of less than 2% in comparison to other reports from the western countries, it seems that the notable lower incidence caused by lower alcohol consumption, a well known risk factor, presumably due to Islamic religious customs [4]. Nonetheless since we received patients from the all over country as a referral, female predominance should be negotiated better and socially searched and scheduled by the assumption of plausible increased tendency to smoking. Our study displayed significant correlation between laminin-5 expression and clinical stage or grade but no significant correlation was seen between EGFR expression and clinical stage or grading. Although EGFR found to be a non-sensitive non-specific biomarker, it had a considerable NPV for high clinical staging and microscopic grading. Besides, it was revealed that laminin-5 may be specific with strong NPV to predict high OSCC clinical stage (versus stage I-III) and being specific and sensitive enough with significant NPV for grade 3 microscopic with a given degree of staining, hense the proper evaluation of the immunohistochemical EGFR expression is still a matter of debate. Pertaining to Laminin-5, our findings in this study were in commitment with results of Nordermar et al and Kawano et al studies [11,12]. Lomuzio et al speculated that there was no relation between degree of staining and tumor stage [13]. This incontinency may be due to uneven distribution of samples into various tumor stages. Considering EGFR expression and tumor stage and grade, we found similar results with Klaus et al, Ekberg et al and Shiratsuchi et al studies[12,16,17], even though, Yamada et al, found a significant correlation between EGFR and tumor grade which could be resulted from higher proportion of samples from oral floor and different antigen retrival heating system[9]. More In-Vivo and in-Vitro experiments are yet to be accomplished to demonstrate conclusive evidence-based facts.

Conclusion

Our study confirmed laminin-5 to be a valuable prognostic biomarker in patients with oral squamous cell carcinoma and further researches is required to clarify the role of EGFR more precisely.

Acknowledgment

The authors would like to thank the Deputy of research, Dental school, Shahid Beheshti University of Medical Sciences for supporting this study.

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