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Expression of E-Cadherin in Squamous Cell Carcinoma of the Larynx and its Correlation with Clinicopathological Features

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The purpose of this study was to define the frequency of E-cadherin underexpression and its correlation with clinicopathological behaviors of the tumor in Iranian patients with laryngeal carcinoma. In 95 paraffin-embedded specimens of patients with squamous cell carcinoma of larynx at our hospital from 2002 to 2007, the expression of E-cadherin was examined by immunohistochemical staining. To evaluate the correlation between E-cadherin expression and clinicopathological behavior, Chi-squared or Fisher exact test was used. Analysis of these samples showed reduced E-cadherin expression (Staining of less than 50% of the cells) in 79 (83.2%) patients. Reduced E-cadherin expression had correlation with tumor differentiation ($p = 0.03$). Also it had significant association with node involvement ($p = 0.02$) and tumor recurrence ($p = 0.01$). Determination of E-Cadherin expression might be useful in prognostic assessment. Whoever, to use it in clinical practice more studies are necessary.

Key words: Immunohistochemistry, head and neck, cancer, grade, tumor progression, prognosis

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INTRODUCTION

Normal E-cadherin function is necessary to maintain cell-cell adhesion (Takeichi, 1991; Suzuki, 1996; Rodrigo *et al*., 2002). It has been defined that abnormal E-cadherin function has relation to tumor progression in many human cancers, including squamous cell carcinoma of the head and neck (Birchmeier and Behrens, 1994; Hirohashi, 1998; Gloushankova, 2008). In squamous cell carcinoma of the head and neck, several studies have shown that reduction or loss of E-cadherin expression has significant correlation with high incidence of lymph node metastasis and poor prognosis (Schipper *et al*., 1994; Frixen *et al*., 1991; Franchi *et al*., 1996; Mattijssen *et al*., 1993; Goulioumis *et al*., 2008). However, a number of other studies have failed to show this association (Bowie *et al*., 1993; Takes *et al*., 1997). The different methods and definitions, which were used for description of E-cadherin underexpression is one explanation for this discrepancy.

The aim in this study was to investigate E-cadherin expression in Iranian patient population with laryngeal carcinoma. Also, an attempt was made to define the correlation between tumor grade, nodal metastases and recurrence with E-cadherin expression. There are relatively few studies that have evaluated the E-cadherin expression and its role exclusively in laryngeal carcinoma.

MATERIALS AND METHODS

The cases were retrieved from the archived pathology files at Shafa Hospital and consisted of laryngeal carcinoma diagnosed between 2002 and 2007. These cases were not consecutive but were selected on the basis of availability of at least one suitable paraffin block. We obtained 95 samples for the present study. The mean age of patients was 58 year (minimum: 31, maximum: 81). The other characteristics (i.e., sex, subsite, differentiation, pN and pT stages) are shown in Table 1. All of the patients included in present study had undergone laryngectomy and neck dissection with variable extension. None of the patients had distant metastases at the time of surgery. Most of them (83 patients) received postoperative radiotherapy.

Four-micrometer paraffin embedded sections were used for immunohistochemical examination. The sections were deparaffinized with standard xylen and hydrated through graded alcohol into water. Antigen retrieval procedure was performed using citrate buffer and heating for 10 min in a pressure cooker. Slides were placed for 15 min into a 3% hydrogen peroxide blocking medium and then allowed to react with the anti-E-cadherin antibody at a concentration of 1:2000 for 30 min. Immunodetection was performed with the Envision system and diaminobenzidine (DAB) as chromogen. Counterstaining with hematoxylin for 1 min was the final step. After staining, the slides were dehydrated through graded alcohol.

Two pathologists without knowledge of clinicopathological data viewed the slides. The pattern of immunostaining was described as normal when complete membranous staining was observed. No staining included absent membranous staining with or without cytoplasmic staining. To describe staining pattern, the following scoring was used: 3 = normal staining of more than 50% of cells, 2 = staining between 10 and 50% of cells, 1 = staining of less than 10%, 0 = no staining (Fig. 1a-d). The samples with scoring of 3 were categorized as retained expression and those with lower scoring were defined as reduced expression. Correlation between N stage, histological grade, recurrence and E-cadherin expression were computed using the Chi-squared or Fisher exact test. Correlation between histological grade and scoring was tested by Kruskal-wallis test. For all analysis, $p<0.05$ was considered significant.

Table 1: Population characteristics of patients with laryngeal cancer

Characteristic	No. of patients (%)
Sex	
Male	86(90.5)
Female	9(9.5)
Sites	
Supraglottic	27(28.42)
Glottic	57(60.0)
Infraglottic	11(11.58)
pT stage	
T_3	54(56.84)
T_4	41(43.16)
pN stage	
N_0	57(60.0)
N_1-N_3	38(40.0)
Histological grade	
Well differentiated	43(45.3)
Moderately differentiated	33(34.7)
Poorly differentiated	19(20.0)

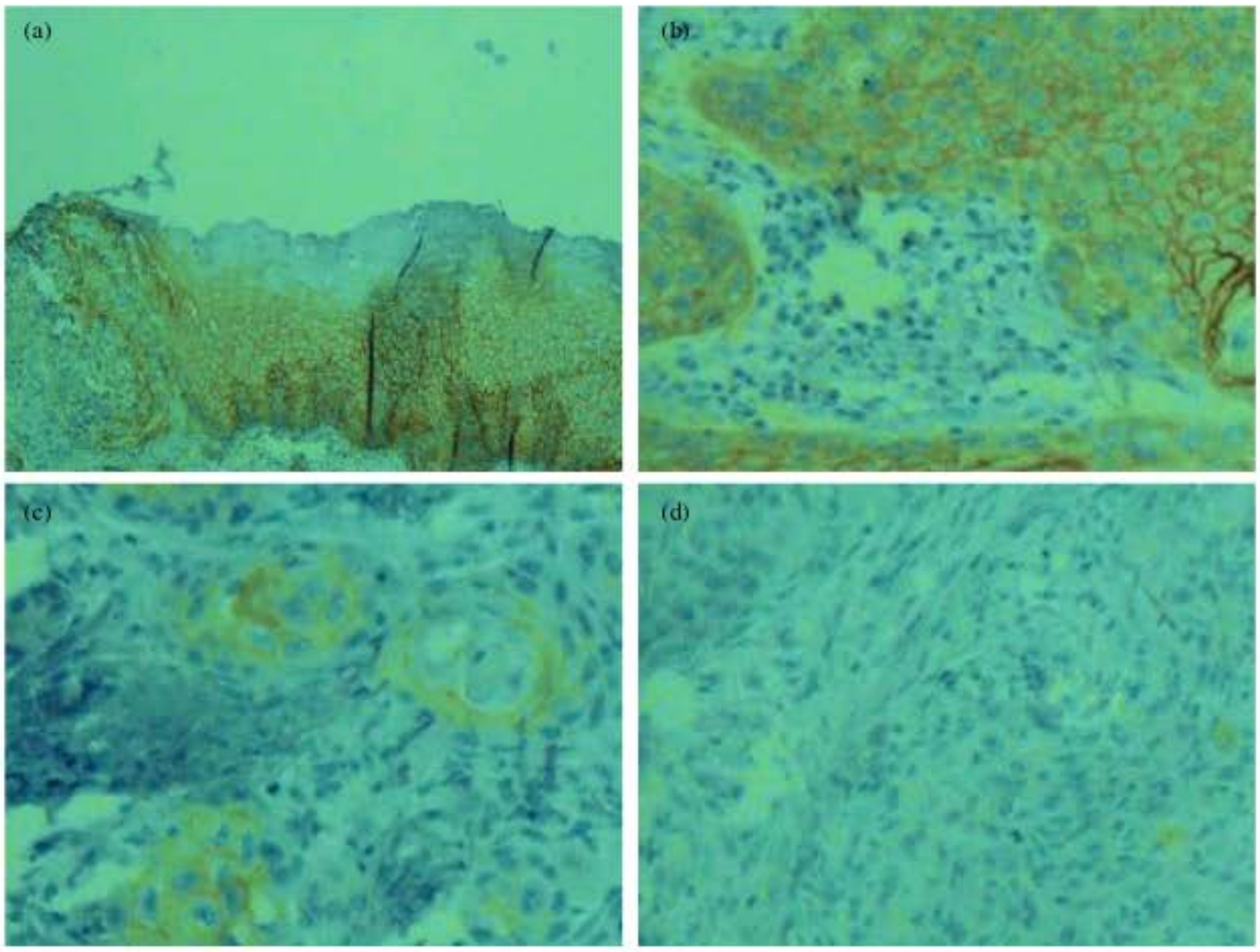

Fig. 1: (a) E-cadherin immunohistochemical staining in normal mucosa. Strong staining is seen in the parabasal and lower spinous layers. Staining is low to absent in upper spinous and basal layers (x100), (b) E-cadherin staining in more than 50% of the cells (Retained expression x400), (c) E-cadherin staining in fewer than 50% of the cells (Reduced expression x400) and (d) Absent E-cadherin staining (x400)

RESULTS

Reduced E-cadherin expression was seen in 79 (83.2%) patients. Immunohistochemistry scoring of the samples with different grading have been shown in Table 2. The numbers (%) of the patients with staining scores of 0, 1, 2 and 3 were 12(12.6), 43 (42.1), 24 (29.5) and 16 (15.8), respectively. The differences of the scoring were not significant between the patients with variable degree of differentiation.

Table 3 shows the correlation of E-cadherin expression with nodal metastases, pathological grading and recurrence. Reduced E-cadherin expression was seen in 30 (69.8%), 30 (90.9%) and 19 (100%) of the patients with well, moderately and poorly differentiated tumor, respectively ($p = 0.03$).

Also, Correlation between E-cadherin expression and nodal metastases was significant: E-cadherin underexpresion was seen more frequently in the presence of nodal metastases ($p = 0.02$).

With a mean follow up of 38 months, 32 patients (33.68%) developed recurrence (6 local recurrence, 22 regional recurrence and 4 distant metastases). There was significant association between E-cadherin expression and recurrence ($p = 0.01$).

Table 2: Immunohistochemical scoring of the samples with variable grading

	Staining scores, No. of patients (%)				
Differentiation	0	1	2	3	Total
Well differentiated	3(7)	22(51.2)	5(11.6)	13(30.2)	43(100)
Moderately differentiated	4(12.1)	12(36.4)	14(42.4)	3(9.1)	33(100)
Poorly differentiated	5(26.3)	9(47.4)	5(26.3)	0(0.0)	19(100)

$p = 0.06$

Table 3: E-Cadherin expression by clinicopathological findings

Characteristic	Reduced E-cadherin	Retained E-cadherin	p-value
T classification			
T_3	43(79.6)	11(20.4)	0.40
T_4	36(87.8)	5(12.2)	
N classification			
N_0	43(75.4)	14(24.6)	0.02
N_1-N_3	36(94.7)	2(5.3)	
Histological grade			
Well differentiated	30(69.8)	13(30.2)	0.03
Moderately differentiated	30(90.9)	3(9.1)	
Poorly differentiated	19(100.0)	0(0.0)	
Recurrence			
Yes	31(96.9)	1(3.1)	0.01
No	48(76.2)	15(23.8)	

DISCUSSION

In several human cancers, it has been shown that E-cadherin expression has correlation with the clinicopathological characteristics of the tumor, such as tumor stage, grade of differentiation, lymph node involvement, distant metastases and treatment outcome (Bukholm *et al*., 1998; Pignatelli *et al*., 1994; Shun *et al*., 1998; De Marzo *et al*., 1999).

However, studies of E-cadherin expression in squamous cell carcinoma of the head and neck have yielded conflicting results. Generally, E-cadherin has been preserved more frequently in well differentiated squamous cell carcinoma of the head and neck, when it has been compared with the undifferentiated types of these tumors. The correlation between reduced E-cadherin expression and nodal metastases has been significant in some studies (Schipper *et al*., 1994; Frixen *et al*., 1991; Franchi *et al*., 1996) and has not been significant in some another studies (Bowie *et al*., 1993; Takes *et al*., 1997). Similarly, there are no consistent results about the correlation between E-cadherin expression and recurrence rate or survival outcome (Mattijssen *et al*., 1993; Bowie *et al*., 1993). Several factors may account for this discrepancy (Rodrigo *et al*., 2002). Selection of patients entering the study, tumor site, type of treatment and tumor heterogeneity may individually or in combination be responsible. Another explanation may be the different methods and definitions, which were used for description of E-cadherin expression (Rodrigo *et al*., 2002; Takes *et al*., 2002). The problem of tumor heterogeneity in determining biological markers is well known. Protein expression is different in some parts of the tumor compared with the other parts. In this manner the result of the expression study is dependent largely to the part of tumor, which is under study. This problem makes the choice of biologically cutoff points arbitrary. It is not clear that what percentage of stained cells should be considered as positive expression. For this reason, different definitions have been used for E-cadherin expression in studies (Takes *et al*., 2002). The problem of heterogeneity, particularly is seen in tissue microarray technology (Boon *et al*., 2008). To optimize this problem, in this study we used full tissue sections and routine tissue processing instead of tissue microarray. Rodrigo *et al*. (2002) showed that there was a significant correlation between decreased E-cadherin expression and nodal metastases and recurrence rate in the patients with supraglottic larynx. In this study a semi quantitative measurement was used. The intensity of the membranous stain (0-4) and the percentage of cells stained (0-100%) were multiplied for an overall staining score between 0 and 400. Then staining scores were averaged within variable groups stratified with respect to clinicopathological parameters. Mahomed *et al*. (2007) reported no correlation between E-cadherin expression and nodal metastases in a series of the patients with oral carcinoma. Whoever, the correlation with differentiation was significant. In this study, staining of less than 50% of the cells was defined as loss of E-cadherin expression. In the present study, we used the scoring. Using this scoring, the distribution of staining scores had no significant differences among the variable grades. But when we subclassified score of 3 as retained expression and the other scores as reduced expression, the correlation between E-cadherin expression and grade was significant. This is not consistent with one study on laryngeal carcinoma (Rodrigo *et al*., 2002). But the significant relation between E-cadherin expression and node metastases or recurrence was consistent with this study and the one (Rodrigo *et al*., 2007) obtained in laryngeal cancer.

It has been shown that radiation can induce expression and secretion of some factors that are responsible for patient's outcome. One example is plasminogen activator inhibitor type-1 (PAI-1). This effect of radiation should be considered during interpretation of the results (Schilling *et al*., 2007). As mentioned earlier most of patients enrolled in this study received post-operation radiotherapy. Therefore, the effect of treatment modality on the results is less important in the present study.

CONCLUSION

E-cadherin is abnormally expressed in laryngeal carcinoma. Reduction of E-cadherin expression has correlation with the degree of tumor differentiation, node metastases and tumor recurrence. But the inconsistent results between studies in the literature show that uniform standards are required to make the results of the studies comparable. After this the future studies with more patients and with more balanced characteristics can reveal the clinical role of this marker.

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REFERENCES

Birchmeier, W. and J. Behrens, 1994. Cadherin expression in carcinomas: Role in the formation of cell junctions and the prevention of invasiveness. Biochim. Biophys. Acta, 1198: 11-26.

Boone, J., R. Van Hillegersberg, P.J. Van Diest, G.J. Offerhaus, I.H. Rinkes and F.J. Kate, 2008. Validation of tissue microarray technology in squamous cell carcinoma of the esophagus. Virchows Arch., 425: 507-514.

Bowie, G.L., A.W. Caslin, N.J. Ronald, J.K. Field, A.S. Jones and A.R. Kinsella, 1993. Expression of the cell-cell adhesion molecule E-cadherin in squamous cell carcinoma of the head and neck. Clin. Otolaryngol., 18: 196-201.

Bukholm, I.K., J.M. Nesland, R. Karesen, U.J. Acobsen and A.L. Borresen-Dale, 1998. E-cadherin and alpha-beta-and gamma-catenin protein expression in relation to metastasis in human breast carcinoma. J. Pathol., 185: 262-266.

De Marzo, A.M., B. Kundsen, K. Chan-Tack and J.I. Epstein, 1999. E-cadherin expression as a marker of tumor aggressiveness in routinely processed radical prostatectomy specimens. Urology, 53: 707-713.

Franchi, A., O. Gallo, V. Boddi and M. Santucci, 1996. Prediction of occult metastases in laryngeal carcinoma: Role of proliferating cell nuclear antigen, MIB-1 and E-cadherin immunohistochemical determination. Clin. Cancer Res., 2: 1801-1808.

Frixen, U.H., J. Behrens, M. Sachs, G. Eberle and B. Voss *et al*., 1991. E-cadherin expression in squamous cellcarcinoimas of the head and neck: Inverse correlation with tumor differentiation and lumph node metastasis. Cancer Res., 51: 6328-6337.

Gloushankova, N.A., 2008. Changes in regulation of cell-cell adhesion during tumor transformation. Biochemistry, 73: 742-750.

Goulioumis, A.K., V. Bravou, J. Varakis, P. Goumas and H. Papadaki, 2008. Integrin-linked kinase cytoplasmic and nuclear expression in laryngeal carcinomas. Virchows Arch., 453: 511-519.

Hirohashi, S., 1998. Inactivation of the E-cadherin-mediated cell adhesion system in human cancers. Am. J. Pathol., 153: 333-339.

Mahomed, F., M. Altini and S. Meer, 2007. Altered E-cadherin/B-catenin expression in ral squamous carcinoma with and without nodal metastasis. Oral. Dis., 13: 386-392.

Mattijssen, V., H.M. Peter, L. Schalkwijk, J.J. Manni, B. Vant Hof-Grootenboer and P.H. De Mulder, 1993. E-cadherin expression in head and neck squamous cell carcinoma is associated with clinical outcome. Int. J. Cancer, 55: 580-585.

Pignatelli, M., T.W. Ansari, P. Gunter, D. Liu and S. Hirano *et al*., 1994. Loss of membranous E-cadherin expression in pancreatic cancer: correlation with lymph node metastasis, high grade and advanced stage. J. Pathol., 174: 243-248.

Rodrigo, J.P., F. Dominguez, C. Alvarez, C. Manrique, A. Herrero and C. Suárez, 2002. Expression of E-cadherin in squamous cell carcinomas of the supraglottic larynx with correlations to clinicopathological features. Eur. J. Cancer, 38: 1059-1064.

Rodrigo, J.P., F. Dominguez, V. Suarez, M. Canel, P. Secades and M.D. Chiara, 2007. Focal adhesion kinase and E-cadherin as markers for nodal metastasis in laryngeal cancer. Arch. Otolaryngol. Head Neck Surg., 133: 145-150.

Schilling, D., C. Bayer, A. Geurts-Moespot, F.C. Sweep and M. Pruschy *et al*., 2007. Induction of plasminogen activator inhibitor type-1 (PAI-1) by hypoxia and irradiation in human head and neck carcinoma cell lines. BMC Cancer, 7: 143-143.

Schipper, J.H., A. Unger and K. Jahnke, 1994. E-cadherin as a functional marker of the differentiated and invasiveness of squamous cell carcinoma of the head and neck. Clin. Otolaryngol., 19: 381-384.

Shun, C.T., M.S. Wu, J.T. Lin, H.P. Wang and R.L. Houng *et al*., 1998. An immunohistochemical study of E-cadherin expression with correlations to clinicopathological features in gastric cancer. Hepatogastroenterology, 45: 944-949.

Suzuki, S.T., 1996. Protocadherins and diversity of the cadherin superfamily. J. Cell Sci., 109: 2609-2609.

Takeichi, M., 1991. Cadherin cell adhesion receptors as a morphogenetic regulator. Science, 251: 1451-1455.

Takes, R.P., R.J. Baatenburg de Jong, E. Schuuring, J. Hermans, A.A. Vis, S.V. Litvinov and J.H. Van Krieken, 1997. Markers for assessment of nodal metastasis in laryngeal arcinoma. Arch. Otolaryngol. Head Neck Surg., 123: 412-419.

Takes, R.P., R.J. Baatenburg de Jong, M.J. Alles, C.A. Meeuwis and H.A. Marres *et al*., 2002. Markers for nodal metastasis in head and neck squamous cell cancer. Arch. Otolaryngol. Head Neck Surg., 128: 512-518.