# Association of E-cadherin Expression with Histopathological Grades and Clinical Stages of Laryngeal Squamous Cell Carcinoma

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**Abstract**

**Background:** E-cadherin gene plays an important role in the carcinogenesis of laryngeal squamous cell carcinoma (LSCC). This study aimed to evaluate the immunohistochemical expression of E-cadherin in different grades and clinical stages of LSCC and to determine the association of E-cadherin with clinical stages and histopathological grades.

**Methods:** This cross-sectional study was conducted in the Department of Pathology, Sylhet MAG Osmani Medical College, from March 2021 to January 2023. Immunohistochemistry was performed in 50 histopathologically diagnosed cases of LSCC using a commercially available anti-E-cadherin antibody. The total score of immunoreaction was calculated by multiplying the expression score and intensity score.

**Results:** The mean age of the LSCC patients was 62.8 years, and 66% were male. Grade I is the most frequent histopathological grade (48%), followed by grade II (42%) and grade III (10%). The most frequent clinical T stage was T3 (56%), and the N stage was N2 (58.06%). E-cadherin expression was positive in 72% of cases; the rest, 28%, showed reduced expression. A significant association was found between E-cadherin expression with histopathological grades (p=0.007) and clinical N stage (p=0.009) but not significant with clinical T stage (p=0.502), anatomic site (P=0.132), and the habit of smoking (0.276).

**Conclusion:** LSCC patients with reduced E-cadherin expression are at the risk of high-grade carcinoma and nodal metastasis. So, the expression of E-cadherin in pre-treatment biopsy samples can be utilized as one of the prognostic factors and advocated to anti-E-cadherin targeted therapy in LSCC.

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## Introduction

LSCC represents the second most common malignancy of the head and neck region in adults, accounting for about 1.5% of all cancers. South and East Asia carried the heaviest burden of larynx cancer worldwide.[1] The usual treatments for LSCC are surgery, radiotherapy, and chemotherapy. Despite advances in the diagnosis and treatment of LSCC in recent decades, the prognosis of patients with LSCC remains unsatisfactory due to different subtypes and stages of laryngeal cancer.[2] So, the identification of a sensitive and specific disease marker is important for prognostication and appropriate treatment.

Known prognostic factors for survival and prognosis in LSCC are histopathological grading, involved subsite, and tumor stage according to the TNM classification. Of these, the TNM system is the most widely used. Still, its weakness in terms of prognostic accuracy is well recognized, and patients with identical clinicopathologic features can show different clinical courses or responses to the same therapy.[3] For subjects affected by LSCC, poor survival can be mainly attributed to the high frequency of local recurrence. The presence of lymph node metastases represents the most important adverse independent prognostic factor in this malignancy and decreases overall survival by more than 50% if extra-nodal extension exists.[4]

During the last years, many studies have been planned to investigate the role played by different cellular biomarkers, which could help potentiate our diagnostic and therapeutic capability.[5]

An important step in tumor metastases is the detachment of malignant cells from their original site. In normal epithelial tissues, cell-cell adhesion is mediated by many cell adhesion molecules.[6] Adhesion molecules are crucial to maintain cell-cell cohesiveness. E-cadherin is a transmembrane glycoprotein of the type 1 cadherin superfamily, which is calcium-dependent and is encoded by the CDH1 gene, whose location is on chromosome 16q21 and is expressed in most of the epithelial cells.[7]

E-cadherin is involved in cell adhesion, morphogenesis, and cellular signal transduction. In a variety of epithelial neoplasms, loss or reduction of E-cadherin expression has been associated with advanced stages of tumor growth, increased metastatic potential, shortened disease-free period, and lowered overall survival, with a concomitant poor prognosis.[8,9] Several studies were published describing the role of E-cadherin in LSCC, but many inconsistencies were present between the studies analyzed, especially regarding the patient populations and IHC scoring system.[10-12] The present study evaluated the immunohistochemical expression of E-cadherin in LSCC. The study also assessed the relationship between E-cadherin expression and histological grades and clinical stages of laryngeal carcinoma in our population. The study results would add to the growing body of evidence regarding the utility of E-cadherin in determining the prognosis of LSCC.

Several study showed that Histone Deacytylase inhibitors (HDACI) are associated with suppression of EMT in various tumor. HDACI reverse or attenuate EMT through the upregulation of E-cadherin in different solid tumor such as Head & neck SCC, Hepatocellular carcioma, Breast cancer, and Esophageal cancer.[11]

So, reconstitution of E-cadherin expression in Laryngeal SCC are being targeted in cancer therapies.

*Objectives*

The present study was conducted to evaluate the immunohistochemical expression of E-cadherin in pretreatment laryngoscopic biopsy cases and to determine the association of E-cadherin expression with histopathological grades and clinical stages.

**Methods**

This was a cross-sectional study conducted in the Department of Pathology, Sylhet MAG Osmani Medical College, Sylhetin collaboration with the department of Otorhinology and Head-Neck surgery, Sylhet MAG Osmani Medical College Hospital, Sylhet. from March 2021 to January 2023 after taking institutional review board clearance.The immunostainig for E-cadherin was carried out in the Armed Forces Institute of Pathology (AFIP), Dhaka Cantonment.

Histopathologically confirmed 50 cases of LSCC were included in this study. Patients having history of previous chemotherapy and/or radiotherapy were excluded.

Clinical staging was done according to AJCC cancer staging manual. Nodal stage was found in 31 study cases. For immunohistochemistry staining 4µm thick tissue sections was cut from formalin fixed paraffin embedded tissue blocks by using a rotary microtome and transferred to poly-L-lysine coated slide. FLEX Monoclonal Mouse Anti-Human E-cadherin Clone NCH- 38 Ready-to-use (Link) was used as primary antibody and DAKO REALTM EnVision TM (HRP RABBIT/MOUSE) (ENV) was used as secondary antibody. Normal breast tissue was used as control.

Evaluation of immunostaining and scoring was performed by light microscopy based on the staining-intensity and proportion. E-cadherin expression was scored (ES) by semi-quantitative optical analysis according to a five-tiered system as 0 (0% positive), 1 (≤10% positive), 2 (11%-50% positive), 3 (51%-80% positive), and 4 (>80% positive).

Intensity score of E-cadherin expression is graded as 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining). Total score (TS) was calculated by multiplying ES and IS. Positive E-cadherin expression is defined as an TS value of 5-12. Samples having a final score of 0 are considered to be negative and those with score of 1-4 are considered to be reduced.[12]

The results of the study were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) version 25.0. Data were expressed as mean ± SD for the quantitative variables, numbers, and percentage for qualitative variables. Comparison between groups were made using Chi-square test and Fisher's Exact test for qualitative data. A value of $p< 0.05$ was considered statistically significant.

**Results**

A total of 50 cases showed age variations ranging in between 38 years to 100 years. Most of the cases (40%) were found in 61 to 70 years. Mean age of patients was 62.82 (±13.26) years. There were 33 (66%) male patients and 17 (34%) cases were female with male to female ratio 1.9:1. Out of 50 patients, 31 (62%) reported to smoke tobacco and 42 (82%) of them reported to chew tobacco.

Majority of the tumours were supraglottic in origin (62%), others were glottic. Most frequent histopathological grade was grade I (48%), in the present study followed by Grade II (42%), and Grade III (10%). Out of Fifty (50) study cases, most frequent clinical T stage was T3 (56%) followed by T2 (30%) and T1 (14%).In this study, most frequent clinical N stage was N2 (58.06%) followed by

N1 (41.94%). E-cadherin expression was positive in 36 (72%) cases and reduced in 14 (28%) cases (Figure 1-6). Negative expression was not found.

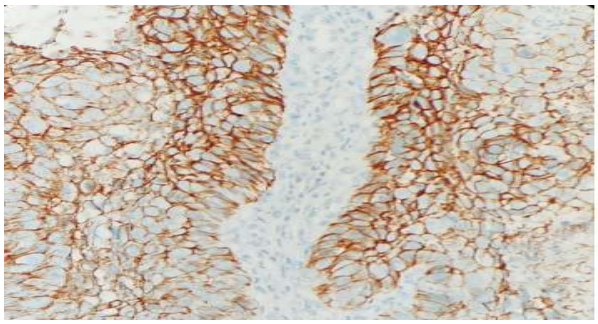

Figure 1. Photomicrograph showing Grade-1 LSCC with positive E-cadherin expression (IHC 400X magnification)

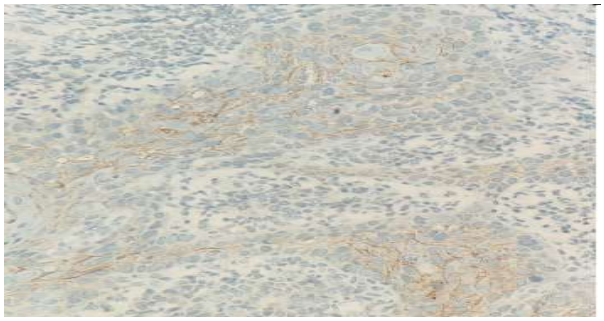

Figure 2. Photomicrograph showing Grade-1 LSCC with reduced E-cadherin expression (IHC 400X magnification)

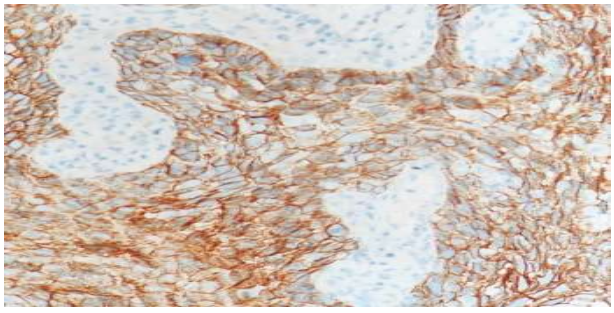

Figure 3. Photomicrograph showing Grade-2 LSCC with positive E-cadherin expression (IHC 400X magnification)

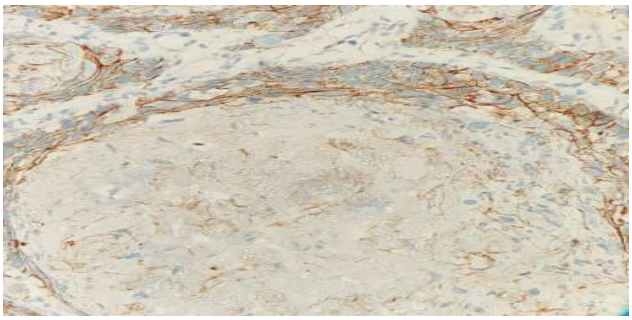

Figure 4. Photomicrograph showing Grade-2 LSCC with reduced E-cadherin expression (IHC 400X magnification)

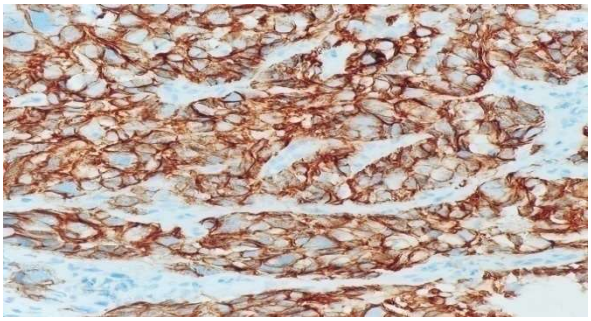

Figure 5. Photomicrograph showing Grade-3 LSCC with positive E-cadherin expression (IHC 400X magnification)

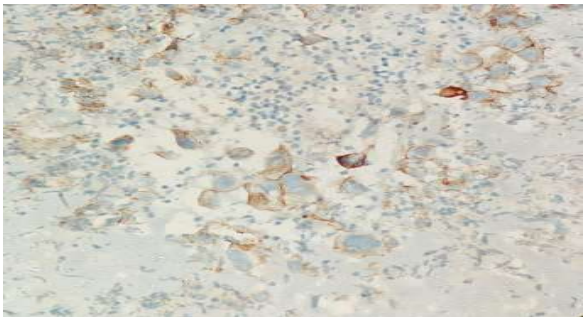

Figure 6: Photomicrograph showing Grade-3 LSCC with reduced E-cadherin expression (IHC 400X magnification)

Table I: Clinicopathological association of E-cadherin in laryngeal squamous cell carcinoma

| Characteristics | N (%) | E-cadherin immunostaining | | p value* |
|---|---|---|---|---|
| | | Positive | Reduced | |
| Age | | | | |
| ≤60 years | 23 (46.0) | 19 (82.6) | 4 (17.4) | 0.127 |
| >60 years | 27 (54.0) | 17 (63.0) | 10 (17.0) | |
| Sex | | | | |
| Male | 33 (66.0) | 25 (75.8) | 8 (24.2) | 0.410 |
| Female | 17 (34.0) | 11 (64.7) | 6 (35.3) | |
| Smoking status | | | | |
| Smoker | 31 (62.0) | 24 (77.4) | 7 (22.6) | 0.276 |
| Non-smoker | 19 (38.0) | 12 (63.2) | 7 (36.8) | |
| Anatomical site | | | | |
| Supraglottic | 31 (62.0) | 20 (64.5) | 11 (35.5) | 0.132 |
| Glottic | 19 (38.0) | 16 (84.2) | 3 (15.8) | |
| Histological grade | | | | |
| G I | 24 (48.0) | 21 (87.5) | 3 (12.5) | |
| G II | 21 (42.0) | 14 (66.7) | 7 (33.3) | 0.007 |
| G III | 5 (10.0) | 1 (20.0) | 4 (80.0) | |
| Clinical T stage | | | | |
| T1 | 7 (14.0) | 6 (85.7) | 1 (14.3) | |
| T2 | 15 (30.0) | 12 (80.0) | 3 (20.0) | 0.502 |
| T3 | 28 (56.0) | 18 (64.3) | 10 (35.7) | |
| Clinical N stage | | | | |
| N1 | 13 (41.9) | 11 (84.6) | 2 (15.4) | 0.009 |
| N2 | 18 (58.1) | 6 (33.3) | 12 (66.7) | |

Figure within parenthesis indicates in percentage. *Chi-square test

Table Ishows that, there was a significant association between E-cadherin expression and tumor grade and clinical N stage with p values of 0.007 and 0.009, respectively.

**Discussion**

Our study results demonstrate that demographic characteristics like age and sex had no association with E-cadherin expression which is similar to the study of Ahmed et al.[10] In the current study association between tumor site and E-cadherin expression was not statistically significant which is consistent with other studies[13-19] and is different from a study that showed a significant association with tumour site with less expression of E-cadherin (p = 0.002) at the supraglottic site.[10]

In the present study, significant association between tumor grade and E-cadherin expression status was detected. Reduced expression for E-cadherin was observed in 12.5%, 33.3%, and 20% of the Grade I, II, and III tumor, respectively. Most of the Grade III tumor (80%) were giving reduced expression for E-cadherin.The present study agreed to the study of Ghosh et al. which found that, all tumors of poorly differentiated carcinoma showed negative expression for E-cadherin (100% negative expression), which indicated an inverse correlation between expressions of E-cadherin with degree of differentiation. These findings were concordant with previous studies [15,21] but discordant with the study of Rodrigo et al. and Zvrko et al.[22,23]

The association between clinical T stage and E-cadherin expression was not statistically significant (p=0.502) in the present study which was concordant with previous studies [16,17,22]; but discordant with the study of Zou et al.[24] On the other hand, the association

between clinical N stage and E-cadherin expression was statistically significant (p=0.009) with higher rate of positive expression in N1 stage than the N2 stage (84.6% vs. 33.3%), These findings were concordant with previous studies;[10,21,22,24] but discordant with the study of Pakosy et al.[14] Psyrri et al.,[16] and Qian et al.[17]

*Conclusion*

Our study found an association between E-cadherin expression and histological grades and clinical N stage of LSCC. LSCC patients with reduced E-cadherin expression are at the risk of high-grade carcinoma and nodal metastasis. So, the E-cadherin expression in pre-treatment biopsy samples can be utilized as one of the prognostic factors and advocated to anti E-cadherin targeted therapy in LSCC. Nevertheless, as the sample size was small and study was conducted in a single center, a large-scale prospective study with standardized techniques is desirable to validate the findings of the present study and to determine the clinical role of E-cadherin in LSCC.

## References


1. Deng Y, Wang M, Zhou L, Zheng Y, Li N, Tian T, et al. Global burden of larynx cancer, 1990-2017: estimates from the global burden of disease 2017 study. Aging (Albany NY). 2020;12(3):2545-83.
2. Steuer CE, El□Deiry M, Parks JR, Higgins KA, Saba NF. An update on larynx cancer. CA: a cancer journal for clinicians. 2017;67(1):31-50.
3. Bradford CR, Ferlito A, Devaney KO, Mäkitie AA, Rinaldo A. Prognostic factors in laryngeal squamous cell carcinoma. Laryngoscope Investigative Otolaryngology. 2020;5(1):74-81.
4. Zhang Q, Wang H, Zhao Q, Zhang Y, Zheng Z, Liu S, et al. Evaluation of risk factors for laryngeal squamous cell carcinoma: a single-center retrospective study. Frontiers in oncology. 2021:606010.
5. Ludwig JA, Weinstein JN. Biomarkers in cancer staging, prognosis and treatment selection. Nature Reviews Cancer. 2005;5(11):845-56.
6. Cavallaro U, Schaffhauser B, Christofori G. Cadherins and the tumour progression: is it all in a switch?. Cancer letters. 2002;176(2):123-8.
7. Chow V, Wing Yuen AP, Lam KY, Wah Tsao GS, et al. A comparative study of the clinicopathological significance of E-cadherin and catenins (α, β, γ) expression in the surgical management of oral tongue carcinoma. Journal of cancer research and clinical oncology. 2001;127:59-63.
8. Diniz-Freitas M, García-Caballero T, Antúnez-López J, Gándara-Rey JM, García-García A. Reduced E-cadherin expression is an indicator of unfavourable prognosis in oral squamous cell carcinoma. Oral oncology. 2006;42(2):190-200.
9. Gould Rothberg BE, Bracken MB. E-cadherin immunohistochemical expression as a prognostic factor in infiltrating ductal carcinoma of the breast: a systematic review and meta-analysis. Breast cancer research and treatment. 2006;100:139-48.
10. Ahmed RA, Shawky AE, Hamed RH. Prognostic significance of cyclin D1 and E-cadherin expression in laryngeal squamous cell carcinoma. Pathology & Oncology Research. 2014; 20:625-33.
11. Wong, S.H.M., Fang, C.M., Chuah, L.H., Leong, C.O. and Ngai, S.C., 2018. E-cadherin: Its dysregulation in carcinogenesis and clinical implications. Critical reviews in oncology/hematology, 121, pp. 11-22.
12. Ghosh U, Ghosh S, Roy AK. Expression of E-cadherin and its implication in laryngeal squamous cell carcinoma. J. Evolution Med. Dent. Sci. 2019;8(50): 3790-3795,

13. Li JJ, Zhang GH, Yang XM, Li SS, Liu X, Yang QT, et al. Reduced E-cadherin expression is associated with lymph node metastases in laryngeal squamous cell carcinoma. Ear Nose Larynx. 2012; 39(2):186-92.
14. Paksoy M, Hardal U, Caglar C. Expression of cathepsin D and E-cadherin in primary laryngeal cancers correlation with neck lymph node involvement. Journal of cancer research and clinical oncology. 2011;137:1371-7.
15. Moussa LA, Al Kholy MA, Taha AK. Immunohistochemical Study of Epithelial Mesenchymal Transition Markers Slug and E-Cadherin in Laryngeal Squamous Cell Carcinoma. The Egyptian Journal of Hospital Medicine. 2018;73(9):7435-43.
16. Psyrri A, Kotoula V, Fountzilas E, Alexopoulou Z, Bobos M, Televantou D, et al. Prognostic significance of the Wnt pathway in squamous cell laryngeal cancer. Oral oncology. 2014;50(4):298-305.
17. Qian X, Ma X, Zhou H, Yu C, Zhang Y, Yang X, Shen X, Gao X. Expression and prognostic value of E-cadherin in laryngeal cancer. Acta Oto-Laryngologica. 2016; 136(7):722-8.
18. Larizadeh MH, Damghani MA, Tabrizchi H, Mirshekari TR. Expression of E-cadherin in squamous cell carcinoma of the larynx and its correlation with clinicopathological features. J. Med. Sci. 2009;*9*(1):41-5.
19. Zhu GJ, Song PP, Zhou H, Shen XH, Wang JG, Ma XF, et al. Role of epithelial-mesenchymal transition markers E-cadherin, N-cadherin, β-catenin and ZEB2 in laryngeal squamous cell carcinoma. Oncology letters. 2018;15(3):3472-81.
20. García-Cabo P, García-Pedrero JM, Villarong MA, Hermida-Prado F, Granda-Díaz R, et al. Expression of E-cadherin and -catenin in Laryngeal and Hypopharyngeal Squamous Cell Carcinomas. Acta Otorrinolaringol Esp. 2020;71(6):358-66.
21. Akdeniz O, Akduman D, Haksever M, Ozkarakas H, Muezzinoglu B. Relationships between clinical behavior of laryngeal squamous cell carcinomas and expression of VEGF, MMP-9 and E-cadherin. Asian Pacific journal of cancer prevention. 2013;14(9):5301-10.
22. Rodrigo JP, Domınguez F, Alvarez C, Manrique C, Herrero A, Suárez C. Expression of E-cadherin in squamous cell carcinomas of the supraglottic larynx with correlations to clinicopathological features. European journal of cancer. 2002;38(8):1059-64.
23. Zvrko E, Mikić A, Jančić S. Relationship of E-cadherin with cervical lymph node metastasis in laryngeal cancer. Collegium antropologicum. 2012;36(2):119-24.
24. Zou J, Yang H, Chen F, Zhao H, Lin P, Zhang J, et al. Prognostic significance of fascin-1 and E-cadherin expression in laryngeal squamous cell carcinoma. European Journal of Cancer Prevention. 2010 Jan 1;19(1):11-7.