Pathol. Oncol. Res. (2014) 20:625–633
DOI 10.1007/s12253-014-9741-6

RESEARCH

Prognostic Significance of Cyclin D1 and E-cadherin Expression in Laryngeal Squamous Cell Carcinoma

Rehab Allah Ahmed · Abd El-Aty Shawky · Rasha Hamdy Hamed

Received: 12 March 2013 / Accepted: 3 January 2014 / Published online: 29 January 2014

Abstract Cyclin D1 and E-cadherin are important factors in the progression and metastasis of cancers. Their role in laryngeal carcinoma has been studied with conflicting results. To define the frequency of cyclin D1 and E-cadherin expression and its correlation with both the clinicopathological characteristics and prognosis of patients with laryngeal squamous cell carcinoma (LSCC). Tumor tissue samples from 75 patients with laryngeal squamous cell carcinoma were examined for cyclin D1 and E-cadherin expression by immunohistochemistry. The relationship between the expression of both molecules and the age and sex of the patient, tumor site, tumor differentiation, lymph node metastasis, tumor invasiveness, TNM stages, tumor recurrence and overall survival was analyzed. Cyclin D1 was found to be a significant independent prognostic factor of lymph node metastasis (p=0.000). The multivariate analysis revealed that cyclin D1 and E-cadherin expression wasn't an independent prognostic factor of local recurrence free survival (LRFS) in patients with LSCC (P=0.56 and 0.28) respectively. However, the univariate analysis revealed a significant association between them and LRFS (p=0.003 and 0.000) respectively. Also, the group of high cyclin D1 /low E-cadherin expression had the poorest prognosis, so they might serve as potential predictors of the prognosis of the patients with LSCC. E-cadherin was found to affect the overall survival (OAS) significantly by the univariate analysis (p=0.01). However, by the multivariate analysis the TNM stage was the only independent prognostic factor of OAS (p<0.05). Cyclin D1 can be used as an independent prognostic marker of lymph node metastasis in patients with LSCC and can help to identify those patients with clinically negative lymph nodes but with considerable risk for occult metastasis. Detection of cyclin D1 and E-cadherin status in LSCC may contribute to the identification of patients with high risk factors of local recurrence. However, they don't appear to be better prognostic predictors than other established markers in LSCC.

Keywords E-cadherin · Cyclin D1 · Laryngeal squamous cell carcinoma · Prognosis

R. A. Ahmed · A. E.-. Shawky
Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt

R. H. Hamed (✉)
Department of Clinical Oncology and Nuclear Medicine, Faculty of Medicine, Mansoura University, Mansoura, Egypt
e-mail: rashahamdy532@yahoo.com

Introduction

Laryngeal cancer is one of the most common malignant neoplasias of the head and neck, accounting for 11–22 % of head and neck cancers. Moreover, it remains the second most common respiratory tract cancer after lung cancer. The predicted mortality is 32 % with an overall 5 year-survival rate of approximately 70 % [1]. In Egypt, it accounts for 1.4 % of cancers [2]. The vast majority of laryngeal cancers are of squamous cell histology and it includes keratinizing and nonkeratinizing varieties [3].

Among many factors deciding the prognosis of various cancers, TNM stage and grade are the most important independent factors. However, these factors seem to be insufficient to detect the outcome of laryngeal cancer and may be influenced by many host and tumor factors. Therefore, there is a strong need to determine biomarkers that can help clinicians to decide how to manage individual patients appropriately [4].

Cyclin D1 is an oncogene. It is expressed during the G1 phase of the cell cycle and becomes associated with the catalytic partner CDK4 or CDK6, which participate in the transit through G1 phase of the cell cycle [4]. Dysregulation of cyclin D1 expression is likely to contribute to tumor development and has been reported in parathyroid adenoma, some

breast carcinomas, esophageal, colorectal and hepatic carcinomas [5–9]. It was found that there is an amplification of gene 11q13 where cyclin D1 and EMS-1 are found in HNSCC (head and neck squamous cell carcinoma) and there is a relation between this amplification and nodal metastasis [10].

E-cadherin is a 97-kD transmembrane glycoprotein and its gene CDH1 maps to chromosome 16q22.1. It functions as adhesion protein of epithelial cells and is involved in cell attachment through the adherens junction composed of E-cadherin and catenin [11, 12]. In a variety of epithelial neoplasms, a loss or reduction of E-cadherin expression has been associated with advanced stages of tumor growth, increased metastatic potential, a shortened disease-free period and a lowered overall survival rate, with a concomitant poor prognosis [13].

To our knowledge, there is only one study that studied both cyclin D1 and E-cadherin in head and neck squamous cell carcinoma [11]. In our study, we examined the expression of cyclin D1 and E-cadherin immunohistochemically in Egyptian patients with LSCC and evaluated the relation between the two markers; as it is well known that increased cell proliferation is associated with genomic instability and loss of differentiation, so more mutations will result in some genes [14]. We also studied the importance of the two proteins, in terms of their relation to the clinicopathological parameters including lymph node metastasis, local recurrence free survival and overall survival.

Materials and Methods

The records of selected 75 patients (those with full clinical and follow up data and with available paraffin blocks) with histologically proven laryngeal squamous cell carcinoma were reviewed. Of these patients, 70 were males and 5 were females with an age range from 42 to 85 years and a mean of 59.6 years. The other patients' characteristics (primary tumor location, histological grade, lymph node metastasis, T stage and TNM stage) are shown in Table 1. These cases had undergone complete resection and neck dissection with variable extension in the period between June 2007 and June 2011. None of the patients had distant metastasis. All patients received postoperative radiotherapy with or without cisplatin based chemotherapy in Clinical Oncology and Nuclear Medicine Department, Mansoura University Hospital.

Histological types of laryngeal squamous cell carcinoma were determined according to the system of World Health Organization. Grading of the tumor was done using the three-tiered system G1, 2 and 3 [15]. TNM staging system was performed for these cases according to The American Joint Committee on Cancer [16]. Follow up of patients was done by means of 3-monthly outpatients returns. Their overall survival, local recurrence-free survival data were retrieved from the archive of Clinical Oncology and Nuclear Medicine Department, Faculty of medicine Mansoura University.

These cases were retrieved from the archives of the pathology department, faculty of medicine, Mansoura University. For each of these cases, the pathology reports were revised to obtain, demographic and clinical data including the tumor site. The original H & E sections were retrieved and reassessed regarding the histological grade, depth of invasion and lymph node metastasis as well as the adequacy of the specimen. The selected sections contained normal laryngeal epithelium to compare the normal tissue with the tumorous tissue. Thereafter, the available paraffin blocks were retrieved from the archive. Sections of 3–4 μm thickness were cut onto coated slides. The slides were then submitted for immunohistochemistry for cyclin D1 and E-cadherin.

All specimens were fixed in 10 % formalin and routinely processed for paraffin embedding for Immunohistochemistry. Xylene deparaffinization and rehydration in descending grades of alcohol into water were performed. Antigen retrieval procedure was performed using citrate buffer at PH 6 and heating in microwave for 10 min. The sections were incubated in 3 % H2O2 blocking medium for 5 min then washed with distilled water. After that, monoclonal rabbit anti human antibodies against cyclin D1 (Clone:EP12; 1:150, Epitomics, Burlingame, CA 94010, USA) and E- cadherin (Clone: EP6; ready to use, Epitomics, Burlingame, CA 94010, USA) antigens were used and incubated for 60 min at room temperature. A slide from a case of mantle cell lymphoma was used as a positive control for cyclin D1. A slide from normal colon was used as a positive control for E-cadherin.

Immunodetection was performed with Dako REAL™ EnVision™system, peroxidase/DAB+, Rabbit/Mouse (Code: K5007, DAKO, Glostrup, Denmark) for use with Dako automated immunostaining instruments. The staining was performed according to the manufacturer instructions. The visualization of the immunoreaction was done by incubating in a DAB chromogen substrate kit for 3 min. The slides were counterstained with Dako REAL hematoxylin for 1 min and cover slipped with the mounting media. Negative controls were assessed by replacing the primary antibody by PBS.

The intensity of cyclin D1 nuclear immune staining was scored on a three-tiered scale as follow: (−) absent staining, or very weak-10–30 % cancer cells stained, (+) moderate-30–50 % cancer cells stained, (++) strong-above 50 % cancer cells stained (Fig. 1). Scores recorded as negative or moderate expression were regarded as low expression, whereas score recorded as strong was regarded as over expression [4].

For E-cadherin, the pattern of immune staining was described as normal when complete membranous staining was observed but in some cells there was cytoplasmic staining near the cell membrane. No staining included absent membranous staining with or without cytoplasmic staining. The following scoring system was used to describe the staining pattern;

Table 1 Clinicopathological association of E-cadherin and cyclin D1 in laryngeal squamous cell carcinoma

		E-cadherin			Cyclin D1		
Characteristics	NO (%)	Reduced no (%)	Retained no (%)	*p*-value	Reduced n0 (%)	Overexpressed no (%)	*p*-value
Age category							
• <60	45(60 %)	26 (57.8)	19 (42.2)	0.16	27 (60)	18 (40)	0.019
• >60	30(40 %)	12 (40)	18 (60)		26 (86.7)	4 (13.3)	
Sex							
• Male	70(93.3 %)	36 (51.4)	34 (48.6)	0.67	48 (68.6)	22 (31.4)	0.31
• Female	5(6.7 %)	2 (40)	3 (60)		5 (100)	0 (0)	
Primary site							
• Supraglottic	22(29.3 %)	16(72.7)	6(27.3)	0.02	12 (54.5)	10 (45.5)	0.57
• Glottic-Infraglottic	53(70.7 %)	22(41.5)	31 (58.5)		41 (77.4)	12 (22.6)	
Histological grade							
• GI-GII	55(73.3 %)	22 (40)	33 (60)	0.004	46 (83.6)	9 (16.4)	0.000
• GIII	20(26.7 %)	16 (80)	4 (20)		7 (35)	13 (65)	
Lymph node metastasis							
• Negative	51(68 %)	20 (39.2)	31 (60.8)	0.006	46 (90.2)	5 (9.8)	0.000
• Positive	24(32 %)	18(75)	6(25)		7 (29.2)	17 (70.8)	
T-stage							
• T1–T2	46(61.3 %)	16(34.8)	30 (65.2)	0.001	36 (78.3)	10 (21.7)	0.11
• T3–T4a	29(38.7 %)	22(75.9)	7(24.1)		17 (58.6)	12 (41.7)	
TNM stage							
• I	19(25.3 %)	2 (10.5)	17 (89.5)	0.001	19 (100)	0 (0)	0.000
• II	12(16 %)	3 (25)	9 (75)		12 (100)	0 (0)	
• III	25(33.3 %)	18 (72)	7 (28)		11 (44)	14 (56)	
• IV	19(25.3 %)	15 (78.9)	4 (21.1)		11 (57.9)	8 (42.1)	
Recurrence							
• Recurrence	46(61.3)	34 (73.9)	12 (26.1)	0.000	20 (43.5)	26 (56.5)	0.001
• NO recurrence	29(38.7)	4 (13.8)	25 (86.2)		27 (93.1)	2 (6.9)	

3=normal staining of more than 50 % of cancer cells, 2= staining between 10 and 50 % of cancer cells, 1= staining of less than 10 % of cancer cells, 0=no staining of cancer cells (Fig. 2). The samples with score 3 were categorized as retained expression and those with score 1 and 2 were defined as reduced expression [17].

Statistical Analysis

Statistical analysis of the data was done by using Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA) program version 17.0. Qualitative data were presented as number and percent. Chi-square test and Fisher's exact

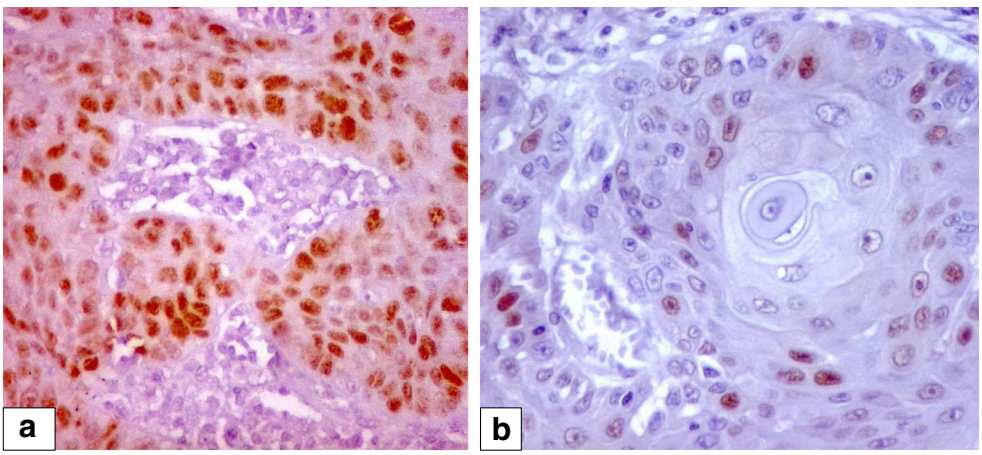

Fig. 1 Cyclin D1 nuclear expression by immunohistochemical analysis in LSCC tissue. **a** Strong expression (more than 50 % of tumor cells) in poorly differentiated LSCC ×400 **b** weak expression (less than 10 % of tumor cells) in well differentiated LSCC ×400

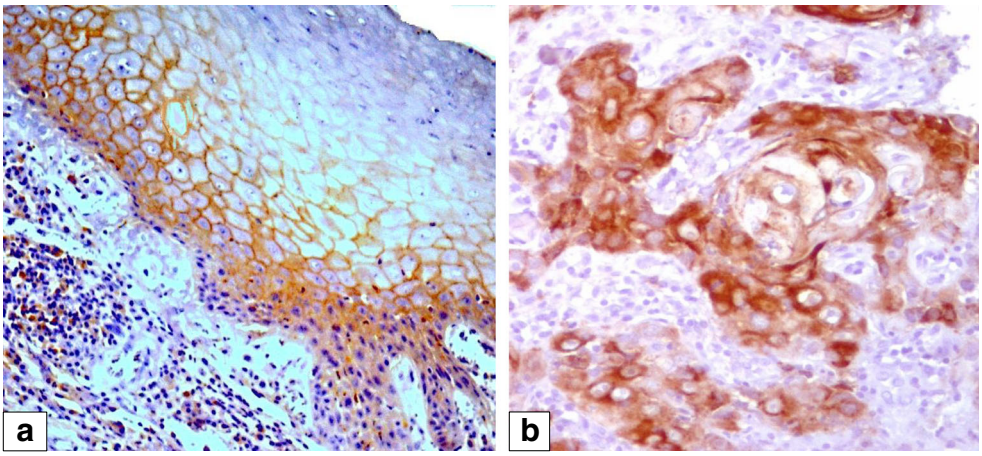

Fig. 2 E-cadherin membranous expression by immunohistochemical analysis **a** In normal laryngeal mucosa; Strong staining is seen in the parabasal and low spinous layers and weak to absent in the basal and upper spinous layers ×100 **b** strong E-cadherin membranous expression with cytoplasmic staining near the cell membrane (more than 50 % of tumor cells) in moderately differentiated LSCC ×200

probability test were used for comparisons of categorical data. Spearman rank correlation test was done to assess the association between cyclin D1 and E-cadherin expression. Association of clinicopathological parameters, E-cadherin and cyclin D1 expression were analyzed using the logistic regression model with the presence of lymph node metastases serving as the dependent variable. Multivariate survival analyses were performed with the Cox proportional hazards model. Survival curves were constructed according to the Kaplan–Meier method. Finally, a log-rank test was performed to evaluate the statistical significance of differences in survival; a P value less than 0.05 was considered to be statistically significant.

Results

Clinicopathological Associations of E-cadherin and Cyclin D1 Expression

From this study 29.3 % of cases had cyclin D1 overexpression, whereas 50.7 % of cases had reduced E-cadherin expression. From Table 1, there was a significant association between cyclin D1 and the age category, tumor differentiation, lymph node metastasis, TNM stages and tumor recurrence with p values of (0.019, 0.000, 0.000, 0.000, 0.001) respectively. On the other hand, there wasn't a significant association between cyclin D1 expression and the patient sex, primary tumor site and tumor invasiveness with p values 0.31, 0.57 and 0.11 respectively.

Regarding E- cadherin expression, Table 1 also shows that, there was a significant association between E- cadherin expression and primary tumor site, tumor differentiation, lymph node metastasis, tumor invasiveness, TNM stages and tumor recurrence with p values of 0.02, 0.004, 0.006, 0.001, 0.001 and 0.000 respectively. Furthermore, when E-cadherin and cyclin D1 were stratified into subgroups, advanced T stages were mostly seen in the group of low E-cadherin level/over expressed cyclin D1 (60 %), while the lowest rate of advanced T stages was within the group of low cyclin D1 level/ retained E-cadherin (3.4 %) (p=0.002; data not shown).

This study showed that there was a significant negative relationship between E-cadherin and cyclin D1 (p=0.002 and r=−0.36 ; data not shown), where 89.2 % of cases that retained E-cadherin expression were associated with reduced cyclin D1 levels and 81.1 % of cases with cyclin D1 over expression were associated with reduced E- cadherin levels.

Association of Lymph Node Metastasis with the Clinicopathological Parameters and the Expression of E-cadherin and Cyclin D1

The univariate analysis revealed a significant association between lymph node metastasis and tumor differentiation, primary tumor site, reduced E-cadherin levels and cyclin D1overexpression with p values of 0.000, 0.035, 0.005 and 0.000 respectively (Table 2). When analyzed by subgroup, the highest rate of lymph node metastasis was within the group of reduced E-cadherin/cyclin D1 overexpression with a rate of 73.7 %, while the lowest rate (6.1 %) was within the subgroup of retained E-cadherin/low cyclin D1 expression (p=0.000). A multivariate logistic regression analysis was performed for each predictor of lymph node metastasis as shown in Table 2 and revealed that only cyclin D1 remained statistically significant independent prognostic factor of lymph node metastasis with p=0.000.

Follow Up Outcome and Survival Analysis

Recurrences were confirmed by histopathology and were found to have occurred in 61.3 % of the patients. The mean time of recurrence was 22±1.6 months ranged from 3 to 38 months. The Kaplan Meier survival analysis revealed that the mean time of recurrence was 24.9 months in patients with low level of cyclin D1 versus 16.2 months in patients with cyclin D1 over expression (p=0.002), with Kaplan Meier

Table 2 Association of clinicopathological parameters, E-cadherin and cyclin D1 expression with lymph node metastases

		Univariate			Multivariate		
		P value	95%CI	Odd's ratio	*P* value	95%CI	Odd's ratio
Tumor site	Supraglottic Glottic-subglottic	0.03	1.08–8.7	3.07	0.65	0.16–3.7	0.7
Grade	Low grade High grade	0.000	0.02–0.3	0.09	0.05	0.95–17.9	4.15
Cyclin D1 expression	Reduced Overexpression	0.000	0.01–0.16	0.04	0.000	3.08–50.9	12.534
E-cadherin expression	Reduced Retained	0.005	1.5–13.7	4.6	0.63	0.15–3.09	0.7

plots are shown in (Fig. 3a). It also shows that the mean time for local recurrence was 14.7 months in patients with low levels of E-cadherin versus 30.4 months in patients with retained E-cadherin (p=0.000), with Kaplan Meier plots are shown in (Fig. 3b). When analyzed by subgroup, the mean time for LRFS of the subgroup of reduced E-cadherin /cyclin D1 overexpression was 10 months versus 32 months for the subgroup of retained E-cadherin/low cyclin D1 (P=0.000) with Kaplan Meier plots are shown in (Fig. 3c).

The univariate analysis in the Cox Proportional Hazards model as shown in (Table 3) revealed that in addition to cyclin D1 and E-cadherin, there was a significant association between the local recurrence free survival and the age category, primary tumor site, tumor differentiation, lymph node

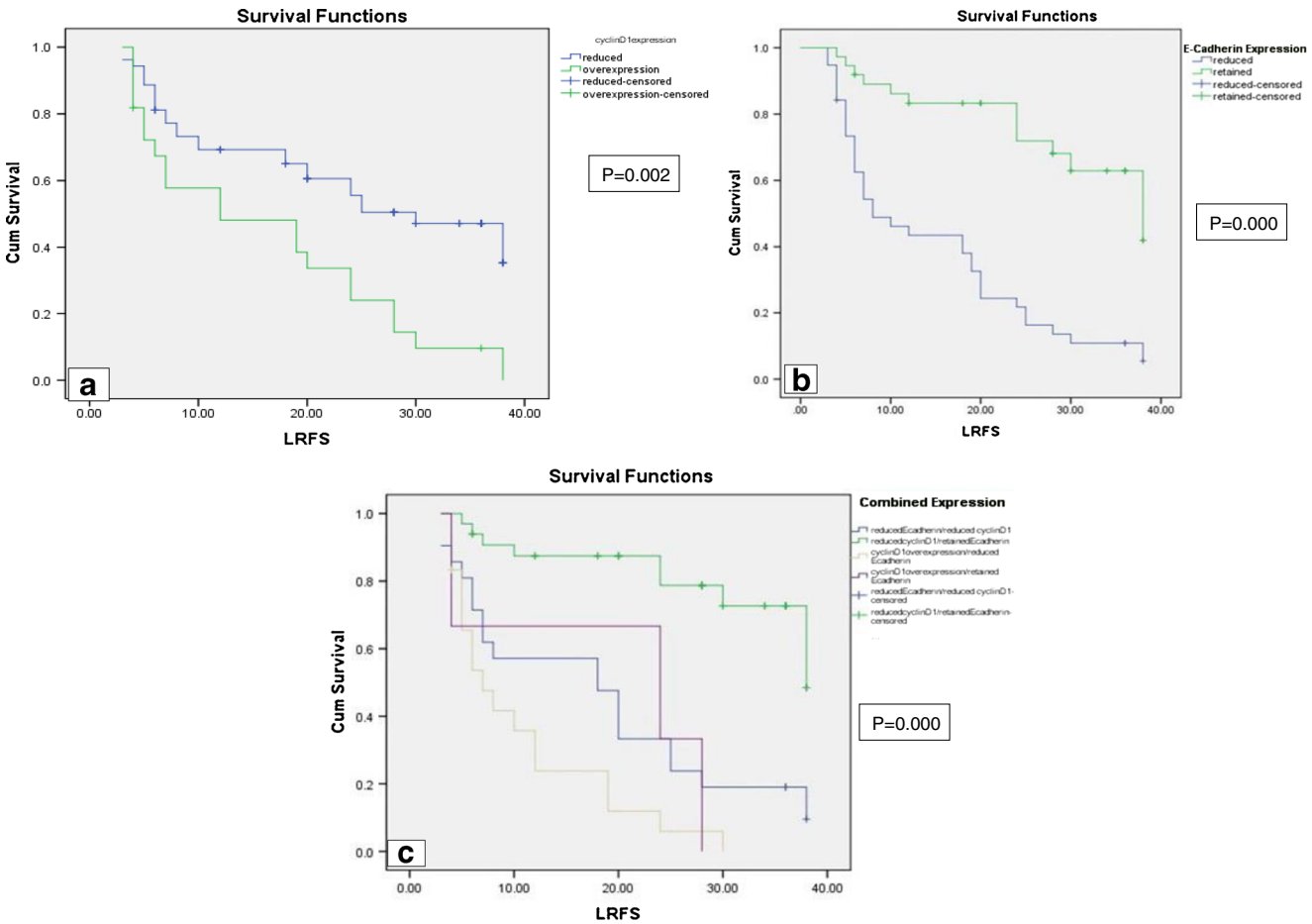

Fig. 3 Local recurrence free survival rates (in months) according to: **a** cyclin D1 expression status **b** E-cadherin expression status, and **c** combined expression status of cyclin D1/E-cadherin in laryngeal squamous cell carcinoma. Statistical differences in univariate Kaplan–Meier curves were calculated through log-rank comparisons

Table 3 Cox proportional hazard models showing prognostic factors of local recurrence free survival

		Univariate			Multivariate		
		P value	95%CI	HR	*P* value	95%CI	HR
Age category	<60 >60	0.004	1.3–4.7	2.5	0.05	0.99–5.1	2.25
Sex	Male Female	0.3	0.46–8.04	1.9	–	–	–
Site as a whole	Supraglottic Glottic-subglottic	0.01	1.17–3.9	2.2	0.03	1.07–6.1	2.6
Lymph nodes	Negative Positive	0.003	0.2–0.7	0.4	0.04	0.12–0.99	0.35
T-stage	Low invasive High invasive	0.000	0.07–0.27	0.14	0.04	0.09–0.98	0.3
Grade	Low grade High grade	0.006	0.24–0.79	0.4	0.56	0.57–2.7	1.26
TNM stage	I	0.000			0.000		
	II	0.000	0.001–0.03	0.005	0.000	0.002–0.15	0.01
	III	0.000	0.006–0.11	0.026	0.004	0.005–0.35	0.04
	IV	0.000	0.02–0.2	0.07	0.000	0.017–2.33	0.06
Cyclin D1 expression	Reduced Overexpression	0.003	0.22–0.73	0.41	0.56	0.58–2.66	1.24
E-cadherin expression	Reduced Retained	0.000	2.37–8.9	4.6	0.28	0.69–3.4	1.55

metastasis, T- stage and TNM stage, with p values (0.003, 0.000, 0.004, 0.01, 0.006, 0.003, 0.000 and 0.000) respectively. The multivariate Cox proportional hazards model analysis as seen in (Table 3) revealed that, only tumor site, lymph node metastasis, tumor invasiveness, and TNM stages retained the independent prognostic features (p=0.03, 0.04, 0.04 and 0.000) respectively. On the other hand, cyclin D1 and E-cadherin weren't independent prognostic factors on the multivariate analysis (p=0.56 and 0.28) respectively.

Thirty deaths (40 %) were reported in our study. 26 (86.7 %) cases were related to local recurrence of the tumor. The mean time to death was 30 ±1.4 months ranging from 6 to 40 months. The univariate analysis in the Cox proportional hazards model as shown in Table 4 revealed that, the factors that affect the overall survival (OAS) were tumor invasiveness, TNM stages (stage I, II and IV) and E-cadherin expression (with p values=0.000, 0.000 and 0.01) respectively. The Kaplan Meier survival analysis showed that, the mean overall survival was 26 months in reduced E-cadherin group versus 35 months in retained E-cadherin group (p=0.005). When analyzed by subgroup, the lowest mean to overall survival was within the subgroup of overexpressed cyclin D1 /reduced E-cadherin accounting for 23 months and the highest survival in subgroup of low cyclin retained E-cadherin accounting for 35 months but with no statistical significance (p=0.067). The multivariate analysis revealed that only the TNM stages remained as significant independent prognostic factors of OAS (P=0.000).On the other hand, there was no significant

Table 4 Cox proportional hazard models showing prognostic factors of overall survival

		Univariate			Multivariate		
		P value	95%CI	HR	*P* value	95%CI	HR
T-stage	Low invasive High invasive	0.000	0.07–0.38	0.17	0.39	0.2–1.8	0.61
TNM stage	I	0.000	–	–	0.007		
	II	0.000	0.01–0.2	0.062	0.012	0.01–0.6	0.109
	III	0.969	0	0.000	0.970	0	0.000
	IV	0.000	0.03–0.29	0.102	0.001	0.04–0.4	0.131
E-cadherin expression	Reduced Retained	0.01	1.26–6.03	2.7	0.65	0.5–3.003	1.22

association between age and sex of the patient, tumor site, lymph node metastasis, tumor differentiation and cyclin D1 expression (p=0.16, 0.3, 0.59, 0.74, 0.19, 0.78) respectively.

Discussion

Lymph node metastasis and local tumor invasion predispose to tumor recurrence in patients with LSCC, which often points to a failure in treatment and adversely affects quality of life [18]. It is hypothesized that risk of recurrence and nodal metastasis may be because of an aggressiveness within tumor cells that remains undetected by the conventional methods of investigation. To improve the detection of tumor aggressiveness, biological markers have been proposed and are now used to observe and predict local recurrence of head and neck cancer, and so patients with poor prognosis may benefit from comprehensive multimodality treatments, such as those comprising radiotherapy and chemotherapy. However, there are presently few precise biological markers for predicting the prognosis of patients with LSCC such as Her-2/new, p53, cell adhesion molecules, cell cycle regulatory genes and tumor suppressor genes [4, 11, 19]. In this study, we investigated, whether the abnormal expression of cyclin D1 or E-cadherin could serve as potential biological markers for LSCC.

Cyclin-D1 overexpression was present in 29.3 % of our cases versus 65.22 % of cases in another study and this difference is due to the different scoring systems used where they considered tumors with more than 20 % of stained nuclei as high expression [20].

In our study, cyclin D1 overexpression was significantly higher in advanced TNM stages and in cases with lymph node metastases, which is consistent with other studies [20, 21]. Other literatures reported that there is a significant association between cyclin D1 overexpression and T-stage, which disagrees with us [4, 21, 22]. It was found that there was a significant association between cyclin D1 overexpression and the age category older than 60 years and poor tumor differentiation which disagrees with other studies [20, 21]. Our study and others didn't find a significant association between cyclin D1 and the sex of the patient or primary tumor site [20, 21]. The controversy between the different studies can be explained according to Loddo et al. by that the cell cycle can be in a G1-delayed/arrested state, although the tumor is expressing a higher level of the phenotypes of this phase and that high grade tumors with a G1-delayed/arrested phenotype showed an identical low risk of relapse compared with well differentiated out-of-cycle tumors [14].

The low expression rate of E-cadherin in our study was 50.7 % versus 83.2 % reported by Larizadeh et al. [17]. Meanwhile, there was a significant association between reduced E-cadherin and tumors of the supraglottic region, poor tumor differentiation, lymph node metastasis, advanced T-stage and TNM stage, which agree with the previous literatures [17, 23–27].

Our study revealed that there was a significant inverse relationship between cyclin D1 and E-cadherin expression. Also, the group of low E-cadherin/ overexpressed cyclin D1 was mostly seen in patients with advanced T stages (p=0.000). These results imply that cyclin D1 overexpression with increased cell proliferation might lead to further gene mutations, which may include the gene of E-cadherin protein and that their expression might serve as a sign of aggressiveness in LSCC. However, Do et al. [11] found that, there is no correlation between cyclin D1 overexpression and E-cadherin. This difference could be attributed to difference in the scoring system between the two studies or the number of cases. They also studied them in all cancers of the head and neck region not laryngeal carcinomas only.

In the present study, cyclin D1 was an independent prognostic factor of lymph node metastasis and this suggests that the immunohistochemical evaluation of cyclin D1 in primary tumors might help to identify those patients with clinically negative lymph nodes, but with considerable risk for occult metastases, so the patients may benefit from treatment such as neck dissection or postoperative radiation and chemotherapy. Others found no relation between cyclin D1 overexpression and nodal metastasis [28]. One study found that, neither the non-neoplastic nor the neoplastic samples expressed any cyclin D1. They explained their results by that cyclin D1 protein expression has been associated with a high frequency of nodal metastases and its absence in their series could be related to the rarity of nodal involvement in the early glottic LSCCs [29].

Although E-cadherin wasn't an independent prognostic factor of nodal metastasis in the present study, it was a significant factor by the univariate analysis. Also, the group of cyclin D1 overexpression /reduced E-cadherin showed the highest lymph node metastasis with statistical significance, so E-cadherin may play a role, but it needs further analysis on a wider scale. Previous studies found that E-cadherin is an independent predictor of lymph node metastasis [17, 24, 26, 27]. This difference may be contributed to other host, therapeutic, environmental or tumor biological factors. Tumors of the supraglottic region had the higher incidence of nodal metastasis with a statistical significance. However, it wasn't an independent prognostic factor of lymph node metastasis. This can be attributed to the richer blood supply in the supraglottic region, which makes the supraglottic LSCC metastasize easier [30].

Pigantaro et al. [21] noticed that the shorter disease-free survival was significantly associated with the anatomical site, tumor extension, clinical stage and cyclin D1 overexpression, which agrees with our results and the results reported by Zhang et al. [20] but the multivariate analysis revealed that only tumor extension, which is consistent with ours and cyclin

D1 overexpression, which isn't consistent with us were independent prognostic factors of disease-free survival. We also found that tumor site, T stage, TNM stages, and lymph node metastasis were independent prognostic factors of LRFS. Li et al., also detected the same results except that for tumor site and T-stage [24].

E-cadherin expression wasn't an independent prognostic factor of LRFS in the present study and this agrees with other literatures [24, 31]. However, it was a significant prognostic factor on the univariate analysis. In addition, the group of low E-cadherin expression/high cyclin D1expression had the poorest LRFS, which suggests that E-cadherin and cyclin D1 may play a role in tumor recurrence but need further studies on a large scale.

Regarding the OAS in our study, the univariate analysis revealed that the T-stage and TNM stages were significant prognostic factors, which agrees with other literatures [21, 32]. In addition, E-cadherin was a significant one. However, only the TNM stage remained as an independent prognostic factor by the multivariate analysis. Others noticed that tumour extension was an independent prognostic factor, whereas the TNM stage wasn't [21, 32]. Cyclin D1 expression showed no significant association with OAS, which disagrees with other literatures who reported that cyclin D1 is an independent prognostic factor [21, 32]. The age and sex of the patient, lymph node metastasis and tumor differentiation showed no significant association with the OAS. Li et al. also reported that the prognosis in patients >60 years wasn't significantly different from that in patients <60 years [24]. Other studies showed that anatomical site was an independent prognostic factor for OAS, which disagrees with us [21]. Our results indicate that the TNM stage is very important in predicting the OAS in LSCC and that there are other factors which may be social, economic and psychic factors that affect the patient OAS.

Conclusion

This study is the first to evaluate cyclin D1 and E-cadherin expression in LSCC simultaneously. Cyclin D1 was a significant independent prognostic factor of lymph node metastasis, so its immunohistochemical evaluation in primary tumors might help to identify those patients with clinically negative lymph nodes but with considerable risk for occult metastases. Cyclin D1 and E-cadherin weren't independent prognostic factors of LRFS but the significant results by the univariate analysis and when combined together suggest that they may play a role in tumor recurrence. The inconsistent results between the different studies hamper the actual introduction of these markers for clinical purposes, so uniform standards and further investigations on wider scales are required to make the results of studies comparable. In addition, we suggest more studies on the cell cycle regulatory proteins, which may reveal cells arrested or delayed at early phase (G1) of the cell cycle.

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