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ORIGINAL ARTICLE

Epithelial–Mesenchymal Transition Predicts Survival in Oral Squamous Cell Carcinoma

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Abstract

Synergistic loss of E-cadherin and acquisition of vimentin are characteristic feature of epithelial–mesenchymal transition (EMT) which confers an invasive phenotype of epithelial cancer cells. The aim of the study was to evaluate the prognostic significance of E-cadherin and vimentin expression individually and in combination as a measure of epithelial–mesenchymal transition (EMT) in oral squamous cell carcinoma (OSCC). Expression of E-cadherin and vimentin through immunohistochemical analysis was examined in 200 patients with surgically resected OSCC. Combined E-cadherin and vimentin expression was evaluated to determine the EMT status. Kaplan–Meier curves and log-rank test were used to compare differences in survival. Cox regression analysis was performed to identify independent prognostic factors. E-cadherin expression was negative in 28 (14%) tumors, and vimentin expression was positive in 87 (43.5%) tumors. Moreover, 99 (49.5%), 87 (43.5%), and 14 (7.5%) tumors exhibited no, partial, and complete EMT, respectively. Both individual protein expression were significant prognostic factors [Negative E-cadherin, hazard ratio (HR) = 1.74, 95% confidence interval (CI) = 1.04–2.93; positive vimentin, HR = 1.64, 95% CI = 1.12–2.41]. For EMT status, the HR increased with EMT progression [partial EMT, HR = 1.64, 95% CI = 1.09–2.49; complete EMT, HR = 2.88, 95% CI = 1.44–5.79], of which, the complete EMT had higher HR than was individual protein expression. Combined E-cadherin and vimentin expression as a measure of EMT showed a superior prognostic significance compared with individual protein expression.

Keywords Epithelial–mesenchymal transition · E-cadherin · Vimentin · p16 · Oral squamous cell carcinoma · Prognosis · Immunohistochemistry

Introduction

Oral cancer is a common malignancy worldwide, with an estimated 354,864 new cases having been reported in 2018 [1]. Oral cancer is more common in developing regions and shows an age-standardized incidence rate (ASR) of 8.7 per 100,000 males. In Thailand, the incidence is higher in the southern region (ASR, 9.1 per 100,000) than in other regions [2]. Oral squamous cell carcinoma (OSCC), which accounts for more than 90% of all oral cancers, is treated via surgery, radiation, and adjunct chemotherapy, either alone or in combination. Treatment selection depends upon the severity of disease. Nonetheless, despite improvements in surgical techniques and multimodal therapies, OSCC prognosis remains poor, with an overall 5-year survival rate of 15%–60%, depending on stage of disease [3, 4]. Therefore, identification of new prognostic biomarkers is valuable to obtain information for effective patient monitoring and treatment management.

Epithelial–mesenchymal transition (EMT) enables polarized, immotile epithelial cells to become motile mesenchymal cells [5]. EMT is characterized by the synergistic loss of epithelial cell junction proteins, such as E-cadherin, and acquisition of mesenchymal proteins, such as vimentin [6]. EMT plays crucial roles in embryonic development by enabling organ differentiation; however, during carcinogenesis, EMT confers an invasive phenotype in cancer cells and acts as a

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crucial enhancer of invasion and metastasis [5, 7]. Inaddition, EMT confers cancer stem cell properties as well as resistance to chemotherapy and immunotherapy [8, 9].

E-cadherin is the primary molecule in adhesive epithelial cell junctions, whereas vimentin is a major mesenchymal protein associated with migratory phenotypes [6]. Although previous studies have reported the expression of E-cadherin and/or vimentin in OSCC, results regarding their prognostic significance remain controversial [10–15]. Recently, evaluation of combined E-cadherin and vimentin expression as a measure of the EMT status of tumors was found to have strong prognostic significance in lung squamous cell carcinoma [16] and penile cancer [17]. However, no previous study has evaluated the prognostic significance of a combined evaluation of E-cadherin and vimentin expression as a measure of EMT in association with survival outcomes of patients with OSCC. Therefore, in this study, we aimed to evaluate the expression of E-cadherin and vimentin individually and in combination (i.e., the EMT status) to assess their association with clinicopathological characteristics and prognosis of patients with OSCC. Since HPV-related oropharyngeal squamous cell carcinoma (OPSCC) has been found to have different prognosis compared to the non HPV-related ones [18], we also evaluated the prognostic significance p16 expression, a surrogate maker of HPV infection, in the present study.

Material and Methods

Patients and Clinical Data

We included consecutive patients with primary OSCC who were diagnosed and treated at Songklanagarind Hospital between January 2008 and December 2011. Songklanagarind Hospital is an 800-bed university hospital in Southern Thailand, which provides tertiary care, including cancer care. All patients were treated via surgical resection with or without postoperative radiotherapy and/or chemotherapy. Clinical data including age, sex, tumor site, tumor size, nodal status, tumor–node–metastasis (TNM) stage, and tumor recurrence status were obtained from electronic medical records. Clinical staging was based on the TNM staging system in accordance with the American Joint Committee on Cancer, 7th edition, 2010. Pathological information was obtained from medical reports. Information on mortality was obtained from the national civil registration system. The study was approved by the Human Research Ethics Committee of the Faculty of Medicine, Prince of Songkla University (REC 61–132–5-1).

Tissue Microarray (TMA) Construction

Formalin-fixed, paraffin-embedded tissue samples and slides for the corresponding patients were retrieved from the archives of the Department of Pathology. Quick Ray® manual tissue microarrayer (Unitma, Seoul, Korea) was used for TMA construction. All hematoxylin and eosin-stained slides were reviewed, and two slides with representative tumors were selected for each patient. An area of the tumor–stromal interface was circled, and the corresponding area on the formalin-fixed, paraffin-embedded block was marked using a felt marker. Two cores from each patient were excised using a needle (diameter, 2 mm) and transferred to a recipient paraffin block for TMA construction. Following construction, 3-μm-thick sections of TMA blocks were cut and stained with hematoxylin and eosin to assess adequacy and to ensure that the cores represented the tumors. A core was excluded from evaluation if less than 30% of tissue was present or less than 30% of cells contained tumor.

Immunohistochemistry

TMA sections were deparaffinized with xylene and rehydrated through a series of graded alcohol. Staining was performed in an automated immunostainer (Leica BOND-MAX, Melbourne, Australia). Antigens were retrieved using Bond Epitope Retrieval Solution 2 (Leica Biosystems, Newcastle, UK). The sections were incubated with bond peroxidase-blocking reagent, followed monoclonal mouse anti-human E-cadherin (dilution 1:500, clone NCH-38, Dako, Denmark) or anti-vimentin (dilution 1:100, clone V9, Dako) antibody or a mouse monoclonal antibody $P16^{INK4a}$ (dilution 1:100, clone G175–405, Zeta Corporation, Arcadia, CA, USA). Reactions were detected using a bond polymer refine detection kit (Leica), followed by assessment of color development using 3,3′-diaminobenzidine as a chromogen and Mayer's hematoxylin as a counterstain. All sections were separately examined by two observers (TP and WC) who were blinded to the clinical data and outcomes. Then, all cases were jointly reassessed using a multi-headed microscope, and discrepancies were resolved by consensus.

Protein expression was quantified via visual assessment under a light microscope. Staining intensity and proportion of reactive tumors cells were assessed. The intensity was graded as follows: 0 = negative; 1 = weak; 2 = moderate; and 3 = strong. The proportion of positively stained tumor cells was quantified as a percentage (0%–100%). The final immunoreactivity score was calculated by multiplying the intensity score by the percentage of positively stained cells. Each core was evaluated separately, and a final score was calculated for each patient by averaging the total immunoreactivity scores of both cores.

Statistical Analysis

Clinicopathological characteristics of the patients were presented as percentages, means, or medians. The

association between clinicopathological characteristics and protein expression was examined using chi-squared or Fisher's exact test, as appropriate. The association between E-cadherin and vimentin expression was analyzed using Spearman's correlation coefficient. Disease-specific survival (DSS) time was calculated from the date of pathological diagnosis until the date of death or last follow-up (June 2016). Patients who were alive at the time of last follow-up and those who died of causes other than cancer were censored. The Kaplan–Meier method was used to estimate survival distributions, and log-rank test was performed to compare difference in survival between the groups. Univariate analysis was performed using log-rank test and Cox regression analysis. Multivariate Cox regression was used to evaluate independent prognostic factors. Differences were considered significant when the P value was <0.05. Statistical analyses were performed using R program Version 1.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Clinicopathological Characteristics of Patients

A total of 202 patients met the eligibility criteria during the study period. Of these, tissue blocks for two patients were missing. TMA was successfully constructed for all the remaining cases. Finally, 200 patients were included in subsequent analyses. Table 1 summarizes the clinicopathological characteristics of patients. Patient age was 24–88 (mean, 61) years. The most common site of malignancy was the tongue (47.5%), followed by the floor of the mouth (18%). All stages of disease were observed, and two-thirds of the patients received postoperative radiotherapy or chemoradiotherapy.

Table 1 Clinicopathological characteristics of patients ($n = 200$)

Variable	Category	Number (%)
Age	Years, mean (range)	61.2 (24–88)
Sex	Female	73 (36.5)
	Male	127 (63.5)
Tumor site	Tongue	95 (47.5)
	Floor of the mouth	36 (18)
	Buccal mucosa	22 (11)
	Gums	22 (11)
	Other	18 (9)
T stage	T1-T2	129 (64.5)
	T3-T4	71 (35.5)
N stage	N0	132 (66)
	N1	29 (14.5)
	N2	39 (19.5)
Clinical stage	I	50 (25)
	II	38 (19)
	III	32 (16)
	IVA	80 (40)
Treatment	Surgery alone	67 (33.5)
	Surgery with RT	100 (50)
	Surgery with RT & CMT	33 (16.5)
Tumor differentiation	Well	147 (73.5)
	Moderate	47 (23.5)
	Poor	6 (3)
Lymphovascular invasion	Absent	185 (92.5)
	Present	15 (7.5)
Perineural invasion	Absent	179 (89.5)
	Present	21 (10.5)
Margin status	Free	166 (83)
	Not free	34 (17)

RT, radiotherapy; CMT, chemotherapy

Association of E-cadherin and Vimentin Expression with Clinicopathological Characteristics

Two tissue cores were successfully obtained from 190 patients, whereas only one core could be obtained from the remaining 10 patients because of tissue loss during processing ($n = 6$) or inadequate tumor cell number ($n = 4$). E-cadherin expression was localized in the cell membrane, and vimentin expression was localized in the cytoplasm. Tumors with negative E-cadherin expression exhibited predominant vimentin expression at the tumor–stromal interface. E-cadherin and vimentin immunoreactivity scores were significantly and negatively correlated (Spearman's rank correlation, $r = -0.16$, $p = 0.018$) (Fig. 1).

Patients were then stratified into groups based on the expression status of E-cadherin and vimentin, as guided by Kaplan–Meier curves and log-rank test, as follows: negative or positive E-cadherin expression (immunostaining scores ≤60 or > 60, respectively) and negative or positive vimentin expression (immunostaining score ≤ 10 or > 10, respectively). The results showed that E-cadherin expression was positive in 172 of 200 (86%) tumors, vimentin expression was positive in 87 of 200 (43.5%) tumors. p16 expression can be evaluated in 183 cases. Positive p16 expression (diffuse nuclear and cytoplasmic staining in ≥70% of tumor cells [18]), was observed in only six of 183 (3.3%) cases.

Table 2 summarizes the associations of E-cadherin and vimentin expression with clinicopathological characteristics. Tumors with negative E-cadherin or positive vimentin expression tended to be more aggressive in terms of tumor size (T3-T4) and nodal metastasis, albeit without significance. None of the clinicopathological characteristics was significantly associated with either E-cadherin or vimentin expression.

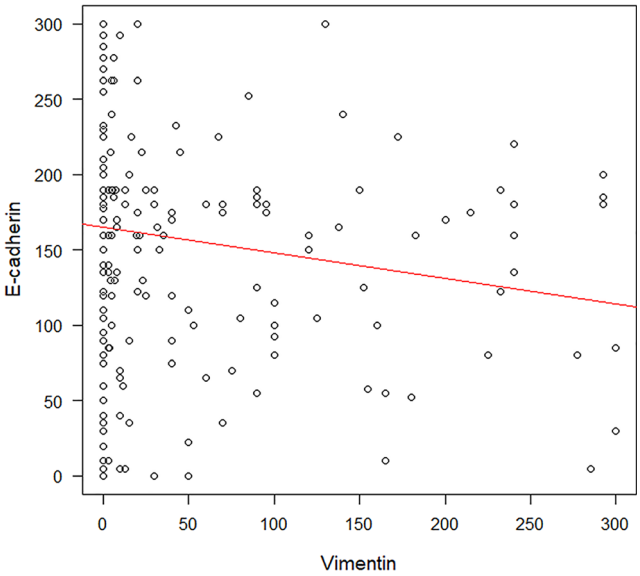


Fig. 1 Scatter plot showing the correlation between the E-cadherin and vimentin immunoreactivity scores (intensity × percentage of positively stained cells)

EMT Status and its Association with Clinicopathological Characteristics

A combined evaluation of the expression of both proteins was performed to assess the EMT status, and patients were classified into three groups as follows: (1) no EMT, defined as positive E-cadherin and negative vimentin expression; (2) complete EMT, defined as negative E-cadherin and positive vimentin expression; and (3) partial EMT, defined as positive E-cadherin and positive vimentin or negative E-cadherin and negative vimentin expression. Representative immunostaining features of each EMT status were shown in Fig. 2. Based on this categorization, 99 (49.5%) tumors exhibited no EMT, 87 (43.5%) exhibited partial EMT, and 14 (7.5%) exhibited complete EMT. The EMT status was not significantly associated with any clinicopathological characteristic (data not shown).

Associations of E-cadherin, Vimentin and p16 Expression and the EMT Status with 5-Year Disease-Specific Survival

The median survival time of the entire cohort was 48 months. Kaplan–Meier analysis revealed significant differences in survival according to E-cadherin and vimentin expression and the EMT status ($p = 0.006$, $p = 0.001$, and $p < 0.001$, respectively) (Fig. 3). In univariate Cox regression, negative E-cadherin expression, positive vimentin expression, partial EMT, and complete EMT were significantly associated with poor survival (Table 3). The other

Table 2 Association of E-cadherin and vimentin expression with clinicopathological characteristics

Variable	E-cadherin, number (%)			Vimentin, number (%)		
	Positive	Negative	P value	Negative	Positive	P value
Age						
≤65 years	105 (61.0)	13 (46.4)	0.211	70 (61.9)	48 (55.2)	0.412
>65 years	67 (39)	15 (53.6)		43 (38.1)	39 (44.8)	
Sex						
Female	63 (36.6)	10 (35.7)	>0.99	34 (30.1)	39 (44.8)	0.056
Male	109 (63.4)	18 (64.3)		79 (69.9)	48 (55.2)	
T stage						
T1-T2	113 (65.7)	16 (57.1)	0.380	75 (66.4)	54 (62.1)	0.528
T3-T4	59 (34.3)	12 (42.9)		38 (33.6)	33 (37.9)	
N stage						
N0	116 (67.4)	16 (57.1)	0.286	78 (69.0)	54 (62.1)	0.303
N1-N2	56 (32.6)	12 (42.9)		35 (30.9)	33 (37.9)	
Clinical stage						
I-II	78 (25.0)	10 (35.7)	0.341	54 (47.8)	34 (39.0)	0.218
III-IV	94 (75.0)	18 (64.2)		59 (52.2)	53 (60.9)	
Differentiation						
Well	39 (22.7)	8 (28.6)	0.465	27 (23.9)	20 (23.0)	0.936
Moderate-Poor	133 (77.3)	20 (71.4)		86 (76.1)	62 (77.0)	
LVSI						0.285
Absent	159 (92.4)	26 (92.9)	>0.99	107 (94.7)	78 (89.7)	
Present	13 (7.6)	2 (7.1)		6 (5.3)	9 (10.3)	
PNI						
Absent	156 (90.7)	23 (82.1)	0.184	101 (89.4)	78 (89.7)	>0.99
Present	16 (9.3)	5 (17.9)		12 (10.6)	9 (10.3)	
Margin						
Free	142 (82.5)	24 (85.7)	0.680	93 (82.3)	73 (83.9)	0.764
Not free	30 (17.4)	4 (14.3)		20 (17.7)	14 (16.1)	

LVSI, lymphovasular invasion; PNI, perineural invasion; RT, radiotherapy; CMT, chemotherapy

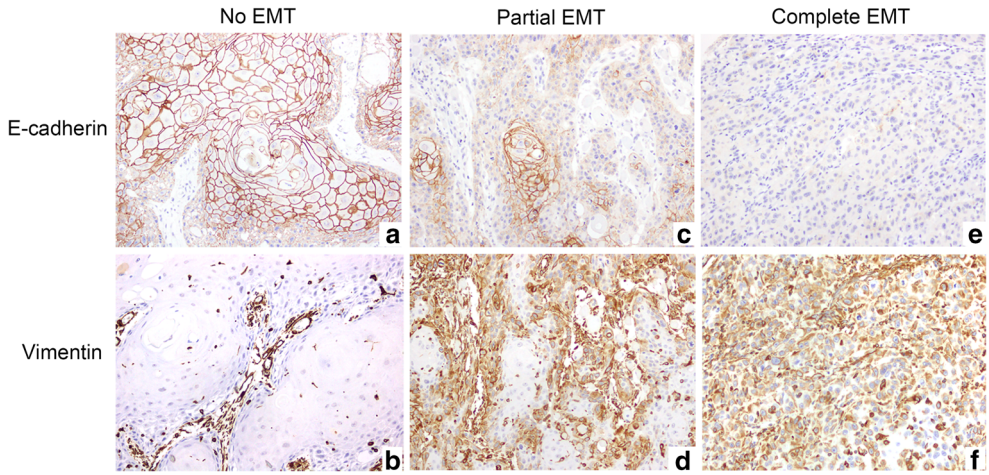


Fig. 2 Immunohistochemical staining of E-cadherin and vimentin of representative cases of epithelial–mesenchymal transition (EMT). Note gradual decreased E-cadherin and increased vimentin expression from No EMT *(***a**, **b***)*, partial EMT (**c**, **d**) and complete EMT status (**e**, **f**).*400*x magnification

significant factors included T stage, N stage, clinical stage, and treatment. p16 expression showed no significant association with survival. Because of the potential collinearity between individual protein expression and the EMT status as well as between T or N stage and clinical stage, we assessed these variables separately in multivariate models (Tables 4 and 5, respectively). All variables significantly associated with survival in univariate analysis remained significant in multivariate Cox regression analysis. Notably, the hazard ratio (HR) increased with EMT progression. In addition, complete EMT status was associated with higher HR [HR, 2.88, 95% confidence interval (CI), 1.44–5.79] than individual protein expression [negative E-cadherin, HR, 1.74, 95% CI, 1.04–2.93; positive vimentin, HR, 1.64, 95% CI, 1.12–2.41].

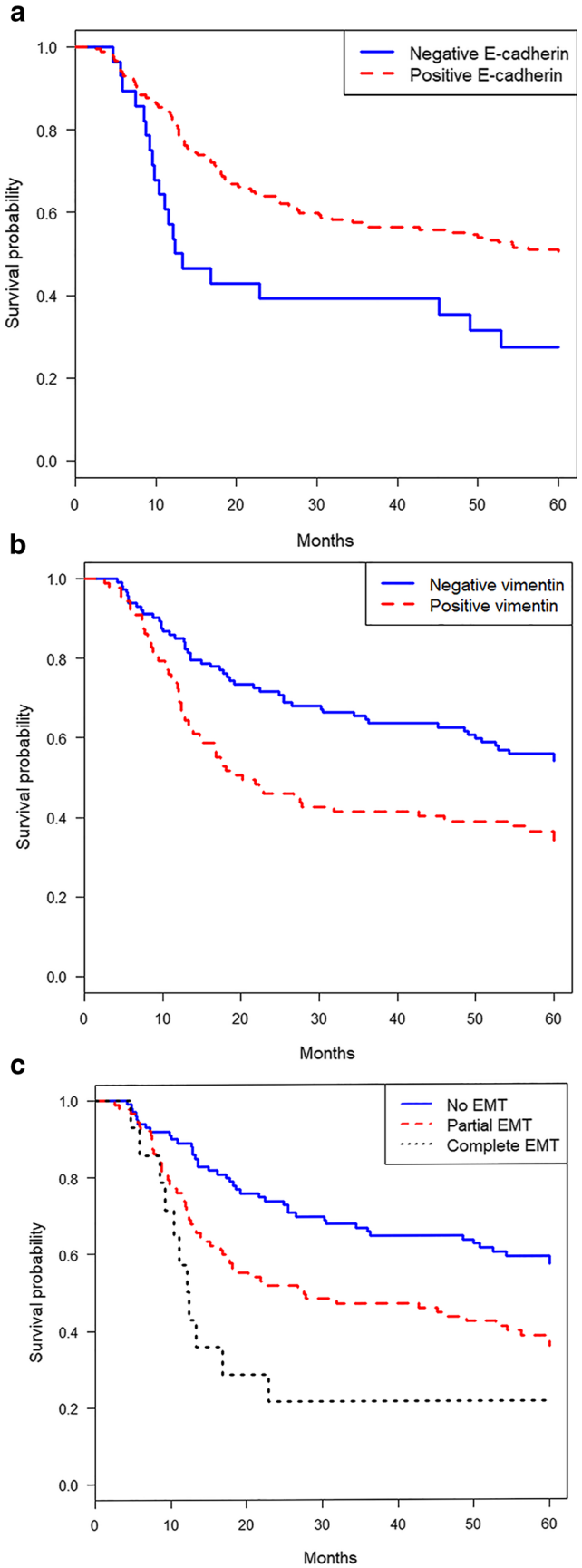
Discussion

EMT is a process through which immotile epithelial cells converts to motile mesenchymal-like cells. It enhances invasion and metastasis of epithelial cancer cells [7]. Suppression or loss of expression of adhesion molecules and acquisition of expression of mesenchymal markers are the hallmarks of EMT. In this study, we evaluated the prognostic significance of E-cadherin and vimentin expression as individual and combined factors representing the EMT status. Our results revealed that evaluation of the combined protein expression had a stronger prognostic value than evaluation of individual protein expression. In addition, mortality risk increased in a stepwise manner with EMT progression.

E-cadherin, a calcium-dependent cell surface protein, is a major molecule responsible for cell-to-cell adhesion in epithelial tissues [6]. Vimentin is a major constituent of the intermediate filament family of proteins, which is typically expressed in mesenchymal cells, such as fibroblasts, endothelial cells, and glial cells [19]. Alterations of expression of these two proteins occurs consistently during EMT and confer a migratory phenotype in epithelial cancer cells. Similarly, such alterations at the tumor invasive front or tumor–stromal interface in OSCC have been demonstrated by many studies [10, 20, 21]. Therefore, we sampled tissues at the tumor–stromal interface as representative of the tumor invasive front. Consistent with most other reports, we observed negative E-cadherin and positive vimentin expression, predominately at the tumor–stromal interface. This observation was further confirmed by the significant and negative correlation between E-cadherin and vimentin expression, which is also consistent with previously reported observations [15, 16]. However, all these reports, including our study, revealed considerably weak correlation (correlation efficient, $r \leq 0.2$). This may due to the complexity of EMT as multiple regulatory factors and signaling pathways have been reported to involve in process [7].

Previous studies have reported significant associations of E-cadherin and vimentin expression with clinicopathological characteristics, particularly tumor differentiation and nodal metastasis [12–14, 22]. However, no association of either E-cadherin or vimentin expression with any clinicopathological characteristic was demonstrated in this study, which corroborates the findings of Liu *et aI* [10] and Ukpo et al [23]. Although E-cadherin and vimentin expression was not associated with any clinicopathological characteristic, we found that both proteins showed a strong prognostic significance. Although our finding is consistent with most previous reports [10–12], some studies did not identify the prognostic significance of these proteins [13–15].This discrepancy may be attributable to various factors, specifically differences in the immunohistochemical evaluation of protein expression. Although most studies performed semi-quantitative evaluation involving the multiplication of the intensity score by the proportion of positively stained cells, different cutoffs were used to define the expression categories.

Gradual loss of E-cadherin and simultaneous upregulation of vimentin expression are anticipated during EMT; therefore, combined evaluation of E-cadherin and vimentin in any tumor would represent the actual EMT status rather than evaluation



◀ **Fig. 3** Kaplan–Meier curves for disease-specific survival according to E-cadherin expression (**a**), vimentin expression (**b**), and the epithelial–mesenchymal transition (EMT) status (**c**)

of individual protein expression. In addition, tumors with more EMT features are expected to be more aggressive than those with fewer EMT features; our results support this spec-

Table 3 Univariate Cox regression analysis of disease-specific survival

Variable	HR (95% CI)	P value
Age		
≤65 years	1	
>65 years	1.4 (0.96–2.05)	0.079
Sex		
Male	1	
Female	0.72 (0.49–1.05)	0.089
Tumor differentiation		
Moderate	1	
Well	0.83 (0.54–1.3)	0.422
Poor	0.69 (0.21–2.27)	0.539
T stage		
T1-T2	1	
T3-T4	3.04 (2.07,4.45)	<0.001
N stage		
N0	1	
N1-N2	1.99 (1.35,2.92)	<0.001
Clinical stage		
I		
II	1.54 (0.76–3.11)	0.230
III	2.17 (1.07–4.4)	0.031
IV	4.48 (2.54–7.91)	<0.001
Lymphovascular invasion		
Absent	1	
Present	1.75 (0.94–3.27)	0.079
Perineural invasion		
Absent	1	
Present	1.22 (0.67–2.22)	0.519
Surgical margin		
Free	1	
Not free	1.38 (0.85–2.24)	0.195
Treatment		
Surgery alone	1	
Surgery with RT	3.15 (1.92–5.15)	<0.001
Surgery with RT & CMT	2.53 (1.36–4.71)	0.003
Recurrence		
Yes	1	
No	0.56 (0.31–1.02)	0.06
E-cadherin expression		
Positive	1	
Negative	1.94 (1.19–3.16)	0.008
Vimentin expression		
Negative	1	
Positive	1.85 (1.26–2.7)	0.002
p16 expression		
Negative	1	
Positive	0.6 (0.15–2.42)	0.469
EMT status		
No	1	
Partial	1.88 (1.26–2.81)	0.002
Complete	3.33 (1.71–6.51)	<0.001

RT, radiotherapy; CMT, chemotherapy; EMT, epithelial–mesenchymal transition

Table 4 Multivariate Cox regression analysis of disease-specific survival using individual protein expression

Variable	HR (95% CI)	P value
Age		
≤65 years	1	
>65 years	1.94 (1.28–2.95)	0.002
Stage		
I	1	
II	1.38 (0.67–2.84)	0.377
III	2.21 (1.07–4.57)	0.032
IV	3.41 (1.86–6.25)	<0.001
Treatment		
Surgery alone	1	
Surgery with RT	2.27 (1.34–3.86)	0.002
Surgery with RT & CMT	2.33 (1.16–4.69)	0.017
E-cadherin expression		
Negative	1	
Positive	1.74 (1.04–2.93)	0.036
Vimentin expression		
Negative	1	
Positive	1.64 (1.12–2.41)	0.011

HR, hazard ratio; CI, confidence interval; RT, radiotherapy; CMT, chemotherapy

ulation. Combined evaluation of these two proteins in association with clinical outcomes has been reported in some cancers including lung squamous cell carcinoma [16] and penile cancer [17], however it has rarely been reported in head and neck cancer. In head and neck squamous cell carcinoma, a small-scale study ($n = 26$) has reported a significant higher frequency of distant metastasis in patients with low E-cadherin and high vimentin (100%) compared to other patients (44%) [24]. To the best of our knowledge, this is the first study to analyze combined E-cadherin and vimentin expression as a measure of EMT in association with survival outcomes of patients with OSCC.

Table 5 Multivariate Cox regression analysis of disease-specific survival using the EMT status

Variable	HR (95% CI)	P value
Age		
≤65 years	1	
>65 years	1.95 (1.29–2.97)	0.002
Stage		
I	1	
II	1.38 (0.67–2.83)	0.38
III	2.21 (1.07–4.59)	0.033
IV	3.43 (1.86–6.33)	<0.001
Treatment		
Surgery alone	1	
Surgery with RT	2.26 (1.32–3.86)	0.003
Surgery with RT & CMT	2.32 (1.16–4.64)	0.018
EMT		
No	1	
Partial	1.64 (1.09–2.49)	0.019
Complete	2.88 (1.44–5.79)	0.003

HR, hazard ratio; CI, confidence interval; RT, radiotherapy; CMT, chemotherapy; EMT, epithelial–mesenchymal transition

HPV infection is currently known to be an important etiologic factor of OPSCC and that HPV-related OPSCC has a better prognosis than non HPV-related ones [18]. However, in non-OPSCC including OSCC, the role of HPV infection is not promising. Many published papers report p16 expression (as a surrogate marker of HPV infection) in only 9–15% of OSCC and the prognosis is independent of HPV status or p16 expression [25–28]. In the present study, we found a remarkably low frequency of p16 expression (3.3%) and it was not associated with survival. A previous study from Thailand also demonstrated a very low prevalence of HPV-DNA in OSCC and oral premalignant lesions (one from 32 cases) [29]. This imply that HPV infection does not play important role in OSCC in our population.

As a retrospective study, information regarding lifestyle habits such as smoking or alcohol and betel consumption, which may be the confounding factors, was not available. We cannot undermine the potential effects of these risk factors on protein expression and outcomes. The other possible limitation is that we may have missed cases of tumor recurrence because patients may have visited different hospitals for treatment.

In conclusion, our study demonstrated that although both E-cadherin and vimentin expression could serve as independent prognostic factors, combined evaluation of the expression of these proteins as a measure of EMT may provide a better indication of prognosis in patients with surgically resected OSCC.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Abbreviations *ASR,* Age-standardized incidence rate; *CI,* Confidence interval; *CMT,* chemotherapy; *EMT,* Epithelial–mesenchymal transition; *HR,* Hazard ratio; *LVSI,* lymphovasular invasion; *OSCC,* Oral squamous cell carcinoma; *PNI,* perineural invasion; *RT,* radiotherapy; *TMA,* Tissue microarray

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