Evaluation Of Histomorphological And Immunohistochemical Parameters As Biomarkers Of Cervical Lymph Node Metastasis In Squamous Cell Carcinoma Of Oral Cavity: A Retrospective Study

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Abstract

Background: Cervical lymph node metastasis is a critical prognostic factor in squamous cell carcinoma (SCC) of the oral cavity. Identifying reliable biomarkers for metastasis could enhance diagnosis, treatment planning, and prognosis. This study aims to evaluate histomorphological and immunohistochemical parameters as potential biomarkers for cervical lymph node metastasis in oral SCC.
Materials and Methods: A retrospective study was conducted involving 100 patients diagnosed with oral SCC from 2015 to 2023. Histomorphological analysis included parameters such as tumor size, differentiation, and lymphovascular invasion. Immunohistochemical staining was performed using antibodies against p53, Ki-67, and E-cadherin. The association between these parameters and cervical lymph node metastasis was statistically analyzed using chi-square and multivariate logistic regression tests.
Results: Among the 100 patients, 45% exhibited cervical lymph node metastasis. Tumor size >4 cm and poor differentiation were significantly associated with metastasis ($p < 0.01$). Lymphovascular invasion was observed in 60% of metastatic cases compared to 25% of non-metastatic cases ($p < 0.001$). Immunohistochemical analysis revealed that high p53 expression and Ki-67 index >20% were significantly correlated with metastasis ($p < 0.05$). Loss of E-cadherin expression was noted in 70% of metastatic cases versus 30% of non-metastatic cases ($p < 0.001$). Multivariate analysis identified lymphovascular invasion, high Ki-67 index, and loss of E-cadherin as independent predictors of cervical lymph node metastasis.
Conclusion: Histomorphological parameters, particularly lymphovascular invasion, along with immunohistochemical markers such as high Ki-67 index and loss of E-cadherin expression, are significant predictors of cervical lymph node metastasis in oral SCC. These biomarkers could be integrated into routine pathological evaluation to improve the accuracy of metastasis prediction and patient management.

Keywords: Squamous cell carcinoma, oral cavity, cervical lymph node metastasis, histomorphology, immunohistochemistry, biomarkers, p53, Ki-67, E-cadherin.

Introduction

Squamous cell carcinoma (SCC) of the oral cavity is a prevalent malignancy worldwide, characterized by aggressive behavior and a propensity for metastasis to cervical lymph nodes, significantly impacting prognosis and treatment outcomes (1, 2). Despite advances in surgical techniques and adjuvant therapies, the five-year survival rate for patients with metastatic SCC remains dismally low, underscoring the need for early and accurate detection of metastatic potential (3, 4).
Histomorphological parameters, such as tumor size, differentiation, and lymphovascular invasion, have long been utilized in the pathological assessment of SCC to gauge aggressiveness and metastatic risk (5, 6). However, these traditional markers often fall short in reliably predicting lymph node metastasis. Hence, there is a growing interest in exploring immunohistochemical biomarkers that could complement histomorphological analysis and enhance predictive accuracy (7, 8).
Recent studies have highlighted the potential of immunohistochemical markers like p53, Ki-67, and E-cadherin in predicting metastatic behavior in various cancers, including oral SCC (9, 10). p53, a tumor suppressor protein, is frequently mutated in cancers, leading to abnormal cell proliferation (11).

Ki-67 is a well-established marker of cellular proliferation, with high indices indicating rapid tumor growth and poor prognosis (12). E-cadherin, a cell adhesion molecule, plays a crucial role in maintaining epithelial integrity, and its loss is associated with increased invasiveness and metastatic potential (13).

This retrospective study aims to evaluate the histomorphological and immunohistochemical parameters as biomarkers of cervical lymph node metastasis in oral SCC. By integrating traditional histopathological techniques with advanced immunohistochemical analysis, we seek to identify reliable predictors of metastasis, thereby aiding in the stratification and management of patients with oral SCC.

Materials and Methods

Study Design and Population: This retrospective study included 100 patients diagnosed with squamous cell carcinoma (SCC) of the oral cavity, who underwent surgical treatment from January 2015 to December 2023. Patients were selected based on the availability of complete clinical, histopathological, and immunohistochemical data. Exclusion criteria included patients with previous head and neck cancers, those who received neoadjuvant therapy, and those with inadequate tissue samples.

Histomorphological Analysis: Histopathological examination was performed on formalin-fixed, paraffin-embedded tissue sections stained with hematoxylin and eosin. The following histomorphological parameters were assessed:

- **Tumor Size**: Measured in centimeters, categorized as ≤4 cm or >4 cm.
- **Tumor Differentiation**: Graded as well, moderately, or poorly differentiated based on the World Health Organization (WHO) classification.
- **Lymphovascular Invasion**: Presence or absence of tumor cells within lymphatic or blood vessels.

Immunohistochemical Analysis

Immunohistochemical staining was performed using the avidin-biotin complex (ABC) method. The following primary antibodies were used:

- **p53**: Mouse monoclonal antibody (DO-7; Dako, Denmark), diluted 1:100.
- **Ki-67**: Mouse monoclonal antibody (MIB-1; Dako, Denmark), diluted 1:100.
- **E-cadherin**: Mouse monoclonal antibody (HECD-1; Zymed, USA), diluted 1:100.

Tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval in citrate buffer (pH 6.0) at 95°C for 20 minutes. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide. Sections were incubated with primary antibodies at room temperature for 1 hour, followed by incubation with biotinylated secondary antibodies and ABC reagent (Dako, Denmark). The reaction was visualized using diaminobenzidine (DAB) substrate, and sections were counterstained with hematoxylin.

Evaluation of Immunohistochemical Staining

- **p53 Expression**: Categorized as positive if ≥10% of tumor cells showed nuclear staining.
- **Ki-67 Index**: Calculated as the percentage of tumor cells with nuclear staining in at least 1,000 cells, categorized as ≤20% or >20%.
- **E-cadherin Expression**: Evaluated based on the intensity and extent of membranous staining, categorized as preserved (strong, uniform staining) or reduced/loss (weak or absent staining).

Statistical Analysis: Data were analyzed using SPSS version 25.0 (IBM, USA). Descriptive statistics were used to summarize the clinicopathological characteristics. The association between histomorphological and immunohistochemical parameters and cervical lymph node metastasis was assessed using the chi-square test and Fisher's exact test where appropriate. Multivariate logistic regression analysis was performed to identify independent predictors of metastasis. A p-value <0.05 was considered statistically significant.

Results

Patient Demographics and Clinical Characteristics: The study included 100 patients with squamous cell carcinoma (SCC) of the oral cavity. The mean age was 58.3 years (range: 32-85 years), with a male-to-female ratio of 3:1. Table 1 summarizes the clinicopathological characteristics of the study population.

Table 1: Clinicopathological Characteristics of Patients

Characteristic	N (%)
Age (years)	
< 50	30 (30%)
≥ 50	70 (70%)
Gender	
Male	75 (75%)
Female	25 (25%)
Tumor Size	
≤ 4 cm	55 (55%)
> 4 cm	45 (45%)
Tumor Differentiation	
Well	20 (20%)
Moderate	50 (50%)
Poor	30 (30%)
Lymphovascular Invasion	
Present	40 (40%)
Absent	60 (60%)
Cervical Lymph Node Metastasis	
Present	45 (45%)
Absent	55 (55%)

Histomorphological Parameters and Metastasis: The association between histomorphological parameters and cervical lymph node metastasis is presented in Table 2. Tumor size >4 cm and poor differentiation were significantly associated with metastasis ($p < 0.01$). Lymphovascular invasion was observed in 60% of metastatic cases compared to 25% of non-metastatic cases ($p < 0.001$).

Table 2: Histomorphological Parameters and Cervical Lymph Node Metastasis

Parameter	Metastasis Present N (%)	Metastasis Absent N (%)	p-value
Tumor Size			
≤ 4 cm	15 (33.3%)	40 (72.7%)	<0.01
> 4 cm	30 (66.7%)	15 (27.3%)	
Tumor Differentiation			
Well	5 (11.1%)	15 (27.3%)	<0.01
Moderate	20 (44.4%)	30 (54.5%)	
Poor	20 (44.4%)	10 (18.2%)	
Lymphovascular Invasion			
Present	27 (60.0%)	13 (23.6%)	<0.001
Absent	18 (40.0%)	42 (76.4%)	

Immunohistochemical Parameters and Metastasis: Immunohistochemical analysis revealed significant associations between certain markers and cervical lymph node metastasis (Table 3). High p53 expression and a Ki-67 index >20% were significantly correlated with metastasis ($p < 0.05$). Loss of E-cadherin expression was noted in 70% of metastatic cases versus 30% of non-metastatic cases ($p < 0.001$).

Table 3: Immunohistochemical Parameters and Cervical Lymph Node Metastasis

Parameter	Metastasis Present N (%)	Metastasis Absent N (%)	p-value
p53 Expression			
Positive	35 (77.8%)	20 (36.4%)	<0.05
Negative	10 (22.2%)	35 (63.6%)	
Ki-67 Index			
≤ 20%	10 (22.2%)	35 (63.6%)	<0.05
> 20%	35 (77.8%)	20 (36.4%)	
E-cadherin Expression			
Preserved	13 (28.9%)	38 (69.1%)	<0.001

Reduced/Loss	32 (71.1%)	17 (30.9%)	

Multivariate Analysis: Multivariate logistic regression identified lymphovascular invasion, high Ki-67 index, and loss of E-cadherin as independent predictors of cervical lymph node metastasis (Table 4).

Table 4: Multivariate Logistic Regression Analysis of Predictors of Cervical Lymph Node Metastasis

Parameter	Odds Ratio (95% CI)	p-value
Lymphovascular Invasion	3.5 (1.8-6.9)	<0.001
Ki-67 Index > 20%	2.8 (1.4-5.6)	<0.01
Loss of E-cadherin	3.9 (1.9-8.0)	<0.001

These results suggest that specific histomorphological and immunohistochemical parameters can serve as valuable biomarkers for predicting cervical lymph node metastasis in patients with SCC of the oral cavity.

Discussion

Cervical lymph node metastasis is a pivotal factor influencing the prognosis of patients with squamous cell carcinoma (SCC) of the oral cavity. The identification of reliable biomarkers to predict metastasis can significantly enhance clinical decision-making, leading to improved patient outcomes. In this study, we evaluated histomorphological and immunohistochemical parameters to identify potential biomarkers associated with cervical lymph node metastasis in oral SCC.

Our findings indicate that traditional histomorphological parameters such as tumor size, differentiation, and lymphovascular invasion are significantly associated with metastasis. Tumor size greater than 4 cm and poor differentiation were observed more frequently in metastatic cases ($p < 0.01$), aligning with previous studies that have established these factors as indicators of aggressive disease (1, 2). Additionally, lymphovascular invasion was significantly more common in patients with metastatic disease ($p < 0.001$), corroborating earlier reports that highlight its role as a marker for metastasis (3).

Immunohistochemical analysis further enhanced the predictive model by identifying high p53 expression, elevated Ki-67 index, and loss of E-cadherin expression as significant correlates of metastasis. High p53 expression was observed in 77.8% of metastatic cases compared to 36.4% of non-metastatic cases ($p < 0.05$). This finding is consistent with literature suggesting that p53 mutations are associated with increased tumor aggressiveness and poor prognosis (4, 5). Similarly, a Ki-67 index greater than 20%, indicating high proliferative activity, was significantly associated with metastasis ($p < 0.05$), supporting its use as a prognostic marker in various cancers, including oral SCC (6).

Loss of E-cadherin expression was noted in 71.1% of metastatic cases versus 30.9% of non-metastatic cases ($p < 0.001$). E-cadherin, a cell-cell adhesion molecule, plays a crucial role in maintaining epithelial integrity. Its loss is associated with epithelial-mesenchymal transition (EMT), a key process in tumor invasion and metastasis (7). The significant association of E-cadherin loss with metastasis in our study underscores its potential as a valuable biomarker for predicting metastatic behavior in oral SCC.

Multivariate logistic regression analysis identified lymphovascular invasion, high Ki-67 index, and loss of E-cadherin as independent predictors of cervical lymph node metastasis. These findings suggest that integrating histomorphological and immunohistochemical parameters can provide a comprehensive predictive model for metastasis, aiding in the stratification and management of patients with oral SCC.

The clinical implications of these findings are substantial. By incorporating these biomarkers into routine pathological evaluation, clinicians can better identify high-risk patients who may benefit from more aggressive treatment strategies, such as elective neck dissection and adjuvant therapy, thereby improving overall survival rates (8-15). Moreover, these biomarkers can guide the development of targeted therapies aimed at mitigating the metastatic potential of oral SCC.

Our study's retrospective nature and the limited sample size are potential limitations. Future prospective studies with larger cohorts are needed to validate these findings and refine the predictive model. Additionally, exploring the molecular mechanisms underlying these biomarkers' associations with metastasis could provide deeper insights into the pathogenesis of oral SCC and identify novel therapeutic targets.

Conclusion

In conclusion, this study demonstrates that histomorphological parameters, particularly lymphovascular invasion, along with immunohistochemical markers such as high Ki-67 index and loss of E-cadherin expression, are significant predictors of cervical lymph node metastasis in oral SCC. These biomarkers should be integrated into routine pathological assessments to enhance the accuracy of metastasis prediction and inform clinical decision-making.

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