Clinical significance of lymphatic and blood vessel invasion in oral tongue squamous cell carcinomas

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SUMMARY

Although vascular invasion (VI) is recognized as an important predictor of lymph node metastasis and a significant prognostic factor in head and neck squamous cell carcinoma (HNSCC), there is currently no common definition for the pathological evaluation of VI status. We reviewed the medical records of 63 consecutive resected primary oral tongue SCCs (OTSCCs) without preoperative treatment between June 1999 and April 2008, and evaluated VI status by investigating lymphatic vessel invasion (LVI) and blood vessel invasion (BVI) by using immunohistochemistry (IHC) with monoclonal antibody D2-40 (D2-40) and Elastica van Gieson (EVG) staining, respectively. Subsequently, we analyzed their correlations with cervical lymph node metastasis and prognosis. LVI was found in 16 of the 63 tumors (25.4%) and BVI was in 32 tumors (50.8%). Univariate analysis revealed that the presence of LVI is statistically correlated with lymph node metastasis. Moreover, multivariate logistic regression analysis revealed that LVI is an independent risk factor of nodal metastasis (odds ratio = 4.262, 95% confidence interval = 1.262–14.397, p = 0.020). In contrast, Kaplan–Meier survival analysis revealed that patients with BVI had a significantly shorter disease-free survival (DFS) and overall survival (OS) rates than those without BVI (68.6% versus 90.3%, p = 0.028 and 68.6% versus 93.5%, p = 0.013, respectively). The present study clearly demonstrated that LVI at primary OTSCC had significant correlation with lymph node metastasis, and that BVI was significantly associated with recurrence and poor prognosis. Evaluation of VI status, as LVI and BVI status separately, using IHC with D2-40 and EVG staining may be useful in predicting lymph node metastasis and poor prognosis in OTSCCs.

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Introduction

It is generally accepted that tumor invasion to vascular is the first step in the development of a metastatic focus in general solid malignant tumors. Vascular invasion (VI) includes lymphatic vessel invasion (LVI) and blood vessel invasion (BVI), both of which are thought to be the beginning of lymphogenous and hematogenous metastasis, respectively.[1,2]

The importance of LVI in lymph node metastasis and prognosis in various kinds of solid tumor is clearly recognized.[1,3–9] However, little attention has been placed upon VI in relation to oral malignancies and the development of lymph node metastasis and prognosis. One of the main reasons for this may be that interobserver variability in the evaluation of LVI and BVI cannot be neglected because of the difficulties in recognizing lymphatic channels and veins using standard hematoxylin and eosin (HE) staining alone.[10–13]

Recently, immunohistochemistry (IHC) and special staining for identifying lymphatic channels and vessels have been widely used to determine VI in various tumors.[10–15] For example, IHC with the monoclonal antibody D2-40 (D2-40) not only highlights lymphatic channels, but this antibody also reacts with O-linked sialoglycoprotein (MW: 40 kda) on the surface of the lymphatic endothelium, making it a new selective marker that enables us to distinguish lymphatic channels from vessels.[12–14,16] Identification of vessels has been further aided by Elastica van Gieson (EVG) staining,

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which stains elastic fibers located in the venous wall dark violet. Thus, EVG staining significantly improve the accuracy of BVI evaluation.[15,17–19]

In this study, we therefore attempted to evaluate the VI status of oral tongue squamous cell carcinomas (OTSCCs) by investigating LVI status using IHC with D2-40 and BVI status using EVG staining of primary OTSCCs, and we subsequently examined the association between VI status and cervical lymph node metastasis and prognosis.

Materials and methods

Materials

The medical records of 63 consecutive OTSCC patients who had undergone curative surgery at Maxillofacial Surgery, University Hospital of Dentistry, Tokyo Medical and Dental University (Tokyo, Japan), between June 1999 and April 2008 were reviewed for this study. No patients underwent preoperative treatment. All protocols were reviewed and approved by the Research Ethics Committee of Tokyo Medical and Dental University, and informed consent was obtained from all patients in accordance with our Institutional guidelines. Detailed patient characteristics are described in Table 1. Primary tumors were resected then immediately fixed in 10% (v/v) formalin and embedded in paraffin. Serial 4-μm sections were prepared from each sample for routine HE staining to obtain pathological information (Tables 1). Pathological T staging, cellular differentiation and the mode of invasion of primary tumors were defined on the basis of the International Union Against Cancer TNM classification,[20] the World Health Organization (WHO) classification,[21] and the modification of the criteria of Jakobsson et al.,[22,23] respectively. Pathological data included LVI and BVI in primary tumors. One or two (mean: 1.6) additional paraffin blocks of aggressive sections were used for IHC with D2-40 and EVG staining to evaluate LVI and BVI, respectively.

Table 1
Correlation between LVI/BVI and clinicopathological parameters.

Clinicopathological parameters	LVI+	LVI−	P value	BVI+	BVI−	P value
Gender						
Male	12	37	NS	25	24	NS
Female	4	10		7	7	
Age (yrs)						
⩽58	6	22	NS	15	13	NS
>58	10	25		17	18	
Growth pattern						
Superficial	1	9	NS	3	7	NS
Exophytic	7	19		11	15	
Endophytic	8	19		18	9	
Pathological T stage						
1–2	13	41	NS	26	28	NS
3	3	6		6	3	
Cellular differentiation						
Well/ Moderate	15	39	NS	26	28	NS
Poor	1	8		6	3	
Mode of invasion						
1–3	9	31	NS	15	25	0.005
4C–4D	7	16		17	6	
Pathological lymph node metastasis						
Negative	5	31	0.015	16	20	NS
Positive	11	16		16	11	
Recurrence/Metastasis						
Negative	13	37	NS	22	28	0.034
Positive	3	10		10	3	
Survival						
Alive	12	39	NS	22	29	0.012
Dead	4	8		10	2	

LVI: lymphatic vessel invasion, BVI: blood vessel invasion, NS: not significant.

Immunohistochemistry and Elastica van Gieson staining

Paraffin sections measuring 4 μm in thickness were dewaxed and hydrated, then after inactivation of endogenous peroxidase with 3% hydrogen peroxidase for 20 min, they were reacted with anti D2-40 monoclonal antibody (prediluted) (COVANCE, CA) overnight at 4 °C. This was followed by incubation with secondary antibodies (Envision+; Dako, CA). The reaction was visualized using 3,3-diaminobenzidine, and nuclei were lightly counterstained with hematoxylin. LVI positive was defined as the presence of tumor cell aggregates within the D2-40 stained lymphatic lumen or invasion of the media of a vessel with ulceration of the intima (Fig. 1B). It is easier and more accurate to detect LVI using D2-40 than HE staining (Fig. 1A and B).

EVG staining was performed according to the standard protocol. BVI was easily identified with EVG staining because the elastic fiber of the veins was stained dark violet (Fig. 1C and D). BVI positive was defined as the presence of tumor cell aggregates within the dark violet stain or invasion of the media of a vessel with ulceration of the intima (Fig. 1D).

All slides were evaluated independently by two authors (C.M. and K.K.), neither of whom had knowledge of the clinical data. LVI and BVI were considered positive only when both observers agreed.

Statistical analysis

VI status was compared with the patient's clinicopathological information using the chi-square test. After initial screening by univariate analysis, multivariate logistic regression analysis was applied to analyze the significant risk factors of lymph node metastasis. The disease-free survival (DFS) rate and overall survival (OS) rate were estimated using the Kaplan–Meier method and statistical significance was determined using the log-rank test. DFS time was defined as the interval between the date of first visit and the date of development of local, regional recurrence and distant metastasis after surgery. OS time was also calculated from the date of initial examination to the date of death, or to the date of the five-year follow-up. All statistical analyses were performed using SPSS for Windows (version 17.0, SPSS, Inc., Chicago, IL). P values less than 0.05 were considered statistically significant.

Results

Clinicopathological data

Patients included 49 males (77.8%) and 14 females (22.2%) with a mean age of 57.9 years (range, 20–89). The median follow-up period was 41.5 months (range, 8.3–60.0). The growth pattern of the primary tumor was classified as superficial (n = 10, 15.9%), exophytic (n = 26, 41.3%), or endophytic (n = 27, 42.8%). Of the 63 patients, 23 (36.5%) presented with T1, 31 (49.2%) with T2, 9 (14.3%) with T3 tumors. Of the 63 patients, 27 (42.9%) had positive nodes.

Staining

To evaluate the presence of LVI and BVI in primary OTSCCs, IHC with D2-40 and EVG staining were preformed (Fig. 1). LVI is found in 16 of the 63 tumors (25.4%), and BVI is found in 32 (50.8%).

Association with clinicopathological parameters

The association between LVI/BVI status and the clinicopathological parameters in the OTSCC patients are summarized in Table 1. There were no significant correlations between LVI and gender,