Impact of lymphovascular invasion in oral squamous cell carcinoma: A meta-analysis

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Objective. Lymphovascular invasion (LVI) has been reported as a predictor of prognosis in multiple cancers. The aim of this meta-analysis was to investigate the potential value of LVI as a prognostic predictor of oral squamous cell carcinoma (OSCC).

Study Design. To identify relevant studies, PubMed, Embase, Web of Science, and Cochrane Library database were searched from inception to October 2020. All studies exploring the association of LVI with overall survival (OS), disease-specific survival (DSS), or disease-free survival (DFS) and lymph node metastasis (LNM) were identified.

Result. Pooled odds ratios for LNM and hazard ratios for survival were calculated using fixed effects or random effects models. Thirty-six studies involving 17,109 patients with OSCC were included and further analyzed. The results showed that positive LVI was significantly associated with LNM and worse survival in patients with OSCC. Moreover, positive LVI was correlated with LNM in patients with early stage OSCC.

Conclusions. These findings indicate that LVI may serve as a prognostic predictor for the metastasis and prognosis of OSCC and could be considered a routine pathologic examination in clinical work. (Oral Surg Oral Med Oral Pathol Oral Radiol 2021;131:319–328)

Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the oral cavity, accounting for most head and neck squamous cell carcinomas. According to the International Agency for Research on Cancer, approximately 354,000 new cases of oral cancer were diagnosed globally in 2018, which led to 177,000 cancer-related deaths.[1] Despite advances in cancer diagnosis and treatment, the overall 5-year survival rate for OSCC is about 60%, and there has been no significant improvement in the last 20 years.[2,3] Furthermore, the incidence of OSCC in younger populations is on rise.[4] Therefore, exploring potential valuable markers in OSCC is worthy and necessary for risk evaluation. Many studies have shown that tumor budding,[5] depth of invasion,[6] and lymphovascular invasion (LVI)[7] are predictors of lymph node metastasis (LNM) and prognosis.

Importantly, LVI is known as a pathologic phenomenon in which tumor cells invade an endothelium-lined space of vascular or lymphatic vessels without underlying muscular walls.[8] Penetration of tumor cells into lymphovascular spaces through the endothelial cell layer is considered to be a significant step in the process of tumor metastases and has been reported as a promising prognostic feature in many cancers, such as prostate cancer[9] and colorectal cancer.[10] However, meta-analyses and systematic reviews have not demonstrated consensus regarding the question of whether LVI is a statistically significant prognostic factor in OSCC. Therefore, this meta-analysis aims to determine whether patients affected by OSCC with LVI have a worse prognosis and lymph node metastasis than those not presenting LVI.

MATERIALS AND METHODS

Search strategy

This analysis was performed according to Meta-analysis of Observational Studies in Epidemiology Recommendations for study reporting and Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines.[11] We searched PubMed, Web of Science, Embase, and the Cochrane Library to study the association between LVI and OSCC. The following keywords and their combinations were used: (oral OR tongue OR buccal OR mucous OR gingiva OR gum OR "hard palate" OR mouth) AND ("lymphovascular infiltration" OR "lymphovascular invasion" OR "lymphovascular space invasion" OR "lymphovascular space infiltration") AND squamous AND (cancer OR carcinoma). No restrictions were applied. A manual check of the references in the articles was performed to find more relevant citations. The initial search of the

Statement of Clinical Relevance

According to current evidence, lymphovascular invasion potentially serves as a poor prognostic predictor for lymph node metastasis and prognosis of patients with oral squamous cell carcinoma.

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databases included all articles published up to October 2020.

Inclusion criteria

Inclusion criteria included the following: (1) articles that evaluated the prognostic potential of LVI in patients with OSCC; (2) articles that reported adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) for disease-free survival (DFS), disease-specific survival (DSS), or overall survival (OS) in a multivariate analysis with Cox proportional hazard regression; (3) the number of node-negative (N0) and node-positive (N+) patients was reported clearly or calculated; (4) OSCC and LVI were diagnosed through pathologic examination.

Exclusion criteria

Exclusion criteria included the following: (1) reviews, meeting abstracts, short reports, communications, and letters; (2) studies developed in animal models or laboratory cell cultures; (3) studies in which no data were available on lymphovascular invasion in OSCC; and (4) articles not published in the English.

The primary outcome measures were OS, DFS, DSS, and LNM. To avoid duplication of data, only the largest sample size or the latest paper was included when studies overlapped the same patient pool. Nevertheless, if different results were measured, the publication was retained.

Data extraction and quality assessment

Two reviewers (S.J. Huang and Y. Zhu) extracted data from selected articles independently. The extracted information included the following:

Study characteristics: First author's last name, year of publication, number of cases, original country, and follow-up duration.

Patient characteristics: Age, gender, location site, tumor-node-metastasis (TNM) classification, association of LVI with LNM, survival outcomes (OS, DFS, or DSS), LVI measure, and the proportion of patients with positive LVI.

Data characteristics: Outcomes of multivariate analysis to extract HRs, 95% CIs, and covariates. Two adjudicating senior authors (Y.D. Zhang and J.S. Hou) resolved any disagreement after discussion.

The quality of the eligible studies was evaluated according to the Guidelines for Assessing Quality in Prognostic Studies on the Basis of Framework of Potential Biases[12] by 2 reviewers (S.J. Huang and Y. Zhu). The total score ranged from 0 to 12 for each study. According to the standards of the Centre for Evidence-Based Medicine in Oxford, England, all of these retrospective research studies are considered to have low and dissatisfactory levels of evidence.[55] Considering the features and sample size, we included 36 eligible articles. The quality assessment scores for these articles ranged from 6 to 11 (Supplemental Table S1, available at [URL/link]).

Statistical analysis

In our study, the effect measures for the outcomes of OS, DSS, and DFS were calculated using HRs and 95% CIs. An HR > 1 with a 95% CI exceeding 1 indicated a poor survival outcome for patients with OSCC with LVI. To summarize the correlation between LVI and LNM, the odds ratio (OR) and corresponding 95% CI for the combined studies was estimated, and $I^2 >$ 50% and $P < .10$ indicated heterogeneity. If the I^2 value was ≤50%, a fixed effects model was used to pool the HRs. Otherwise, a random effects model was selected.[13] Sensitivity analysis was performed by omitting each study in turn to verify the stability of the results of the meta-analysis. Furthermore, publication bias was identified by the Egger's linear regression and Begg's rank correlation and funnel plots. A trim-and-fill method was performed when the publication bias was significant. All reported P values are for 2-tailed tests and statistical significance was set as $P < .05$. The meta-analysis was conducted using Review Manager version 5.3 (Cochrane Collaboration, Oxford, UK) and Stata version 12.0 (Stata Corporation, College Station, TX).

RESULTS

Search results

Our search strategy yielded 546 papers from the electronic databases: 121 from PubMed, 180 from Web of Science, 229 from Embase, and 4 from the Cochrane Library. Of these, 248 duplicates were removed. After the first screening of titles and abstracts, 167 records were excluded for inappropriate publication types, insufficient data, or unrelated to OSCC, leaving 131 articles for full-text review. Finally, 36 articles that meet the inclusion criteria were chosen for the meta-analysis. Among these studies, 3 included overlapping patient cohorts, but they were enrolled by different study groups and therefore these studies were not excluded. The process for selection of relevant articles is shown in Figure 1.

Study characteristics of included studies

Overall, 36 studies were published between 1991 and 2019 involving 17,109 patients with OSCC were included. Among them, 20 studies focused on LNM and 17 studies reported prognostic effects (OS, DSS, and DFS). The characteristics of eligible research studies are provided in Tables I and II. The number of patients in each research study ranged from 33 to 9852. Twenty-six studies originated from Asian countries

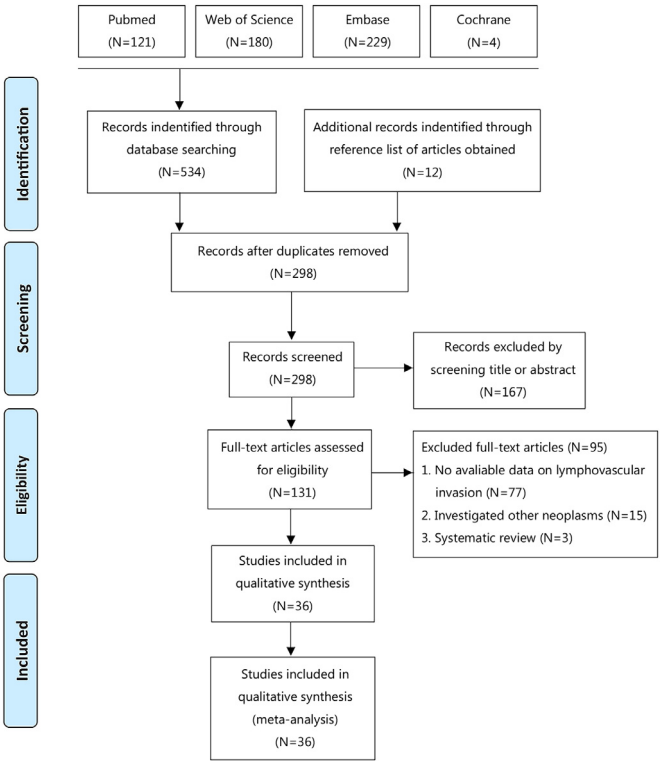

Fig. 1. Flowchart of the literature search and article selection adapted from Preferred Reporting Items for Systematic Review and Meta-Analyses.[11]

(China, India, Korea, Japan, and Pakistan), and the remaining 10 studies were from non-Asian countries (United States, Europe, Australia, Italy, and Brazil). Among 36 included studies, 11 studies reported oral tongue SCC[14-24] and 1 study each focused on floor of mouth cancer[7] and buccal mucosa SCC.[25] The tumor site of OSCC was mixed in the other 23 studies.[26-48]

With regard to the diagnostic modality for LVI, only 1 study used immunohistochemistry (IHC), 11 used hematoxylin and eosin (H&E) staining, and the other 24 did not mention the staining method for LVI. LVI positive was defined as the presence of tumor cell aggregates within an endothelial-lined space.[7] Because of the absence of specific immunohistochemical markers of lymphatic vessels, the detection of LVI was mainly assessed by H&E staining. However, H&E staining could not easily detect lymphatic invasion and one major challenge is to distinguish lymphatic channels from vessels.[19] Recently, a new selective immunohistochemical marker of lymphatic vessels, monoclonal antibody D2-40, was found to demarcate tumor cells in lymphatics and has been reported to be more sensitive compared with H&E staining for the detection of lymphatic invasion.[19] Nevertheless, an article on lung cancer indicated that using D2-40 immunostaining to diagnose LVI is unnecessary in practical settings, and H&E staining may be sufficient to diagnose LVI.[49] Because the use of D2-40 immunostaining to evaluate LVI status still remains controversial and most studies have not distinguished blood vessels and lymphatic vessels, we did not separate blood vessels and lymphatic vessels in our meta-analysis. The analysis of the 36 articles revealed a relative frequency of LVI that ranged from 1.9% to 89.2%, including 20 studies focused on LNM, 10 studies focused on OS, 7 studies reporting DSS, and 3 studies reporting DFS. In studies that focused on the prognostic potential of LVI, the length of follow-up was provided by all 17 studies. The HRs and corresponding 95% CIs of these studies were obtained by multivariate analysis.

Correlation between LVI and LNM

As shown in Figure 2A, our meta-analysis of 18 studies involving 2161 patients indicated that positive LVI was significantly associated with the presence of LNM (OR = 5.34; 95% CI, 3.44-8.30; $P < .00001$, random effects). Two studies[16,23] of LNM in early stage OSCC were excluded because their patient pool overlapped with those of Chen et al.[14] and Chung et al.[15] We observed moderate heterogeneity among the included studies of LNM ($I^2 = 51\%$, $P_h = .006$). In the positive LVI group, 271 out of 433 cases showed LNM

Table I. Characteristics of the included studies evaluating LVI and LNM in OSCC

*Authors**	*Year*	*Region*	*Site*	*No. of cases*	*No. of LVI+ (%)*	*Recruitment period*	*Age (years)*	*Follow-up (months)*	*TNM stage*	*Outcome*
Arora et al.[27]	2017	India	Oral	336	140 (41.7)	NA	55.4 ± 14.3	72.4 ± 11.5	cT1/T2	LNM
Bae et al.[48]	2020	Korea	Oral	130	20 (15.4)	2010-2016	52 (20-84)	46 (4-100)	cT1-4	LNM
Chang et al.[28]	2019	Taiwan	Oral	341	41 (12.0)	2002-2015	52.1 (23-84)	43 (0-143)	pT1-4	LNM
Chatterjee et al.[29]	2019	India	Oral	126	23 (18.3)	2012-2017	47.2 (22-78)	28.7 (12-78)	pT1-4	LNM
Chen et al.[14]	2008	Taiwan	Tongue	94	5 (5.3)	2000-2003	50 (26-82)	NA	pT1-4	LNM
Chung et al.[15]	2010	Korea	Tongue	62	13 (21.0)	1996-2005	55 (23-73)	43 (5-100)	pT1-4	LNM
Chung et al.[16]	2009	Korea	Tongue	43	4 (9.3)	2003-2008	55.0 (22-76)	33 (10-59)	cT1/2	LNM
Faisal et al.[18]	2018	Pakistan	Tongue	179	11 (6.1)	2006-2015	57.92 ± 11.93	NA	T1/2	LNM
Jardim et al.[33]	2015	Brazil	Oral	142	58 (40.8)	1998-2009	57	31.2 (2-176)	T1-4	LNM
Jones et al.[34]	2009	UK	Oral	69	24 (34.8)	1999-2003	60.7 ± 13.1	NA	T1-4	LNM
Kane et al.[35]	2006	India	Oral	48	1 (2.1)	2004-2005	21-90	NA	T1/T2	LNM
Kim et al.[36]	1993	Japan	Oral	90	2 (2.2)	1973-1990	61.0 (24-90)	NA	T1/T2	LNM
Michikawa et al.[19]	2012	Japan	Tongue	63	16 (25.4)	1999-2008	57.9 (20-89)	41.5 (8.3-60.0)	pT1-3	LNM
Nomura et al.[40]	2009	Japan	Oral	33	19 (57.6)	1999-2006	63.0 ± 13.4	NA	T1-4	LNM
Vishak and Rohan[21]	2014	India	Tongue	57	4 (7.0)	2006-2007	44.89 (25-65)	NA	T1	LNM
Sahoo et al.[42]	2019	India	Oral	150	35 (23.3)	2014-2016	NA	NA	cT1-4	LNM
Shimizu et al.[43]	2018	Japan	Oral	91	15 (16.4)	2004-2013	68 (33-88)	90 (6-164)	cT1/2	DFS, LNM
Sparano et al.[44]	2004	United States	Oral	45	4 (8.9)	1995-2001	55 (17-86)	NA	T1/T2	LNM
Suresh et al.[45]	2015	India	Oral	105	2 (1.9)	2006-2011	50.9 (25-70)	NA	cT1-4	LNM
Tai et al.[23]	2012	Taiwan	Tongue	190	41 (21.6)	2001-2009	50.8 (22-84)	42.4 (7-112)	T1/2	LNM

LVI, lymphovascular invasion; *LNM*, lymph node metastasis; *OSCC*, oral squamous cell carcinoma; *TNM*, tumor-node-metastasis; *NA*, not available; *DFS*, disease-free survival.
*Chen et al.[14] and Tai et al.[23] overlap. Chung et al.[16] and Chung et al.[15] overlap.

Table II. Characteristics of the included studies evaluating LVI and prognostic effects in OSCC

*Authors**	*Year*	*Region*	*Site*	*No. of cases*	*No. of LVI+ (%)*	*Recruitment period*	*Age (years)*	*Follow-up (months)*	*TNM stage*	*Outcome*
Al Feghali et al.[26]	2019	United States	Oral	163	38 (23.3)	2005-2015	60 (25-93)	44.5	T1-4	OS
Chen et al.[30]	2013	Taiwan	Oral	442	36 (8.1)	2004-2009	52 (25-91)	46 (4-105)	pT1/2	OS, DFS
Chen et al.[31]	2014	Taiwan	Oral	618	216 (35.0)	2007-2012	NA	29.9 (1.7-73.4)	T1-4	OS
Durr et al.[17]	2013	United States	Tongue	120	107 (89.2)	1999-2010	57.5 ± 15.3	41 ± 32	T1-4	OS
Fives et al.[7]	2016	Ireland	FOM	54	10 (18.5)	2000-2013	NA	33.5 (1-183)	pT1-4	OS
Heiduschka et al.[32]	2016	Australia	Oral	501	69 (13.8)	1987-2014	63.6 (53.2-72.9)	27.6 (1.2-223.2)	pT1-4	DSS
Lee et al.[37]	2018	Korea	Oral	231	19 (8.2)	2000-2012	57 (23-88)	113 (24-199)	pT1-4	OS, DSS
Lin et al.[38]	2015	Taiwan	Oral	554	81 (14.6)	2006-2008	51.95 (23-85)	42.84 ± 23.4	T1-4	OS, DSS
Liu et al.[39]	2017	Taiwan	Oral	1383	360 (26.0)	2004-2014	52.9 ± 11.1	42.8 ± 28.3	pT1-4	DSS
Mascitti et al.[47]	2020	Italy	Oral	66	20 (30.3)	1991-2018	32.1 ± 6.2	60	pT1-4	DSS
Oliver et al.[20]	2018	United States	Tongue	9852	1566 (15.9)	2004-2015	56 ± 10.7	45 (IQ 23-77)	T1-4	OS
Padma et al.[25]	2017	India	BM	198	136 (68.7)	2013-2015	54.16 ± 17.25	24 (3-34)	pT1-4	DFS
Quinlan-Davidson et al.[41]	2017	United States	Oral	233	56 (24.0)	2000-2012	58.9 (20-88)	35 (1-179)	cT1-4	OS
Sharma et al.[22]	2019	India	Tongue	202	53 (26.2)	2010-2016	54.19 ± 14.16	35.2 (1.2-99.9)	pT1-4	OS
Shimizu et al.[43,†]	2018	Japan	Oral	91	15 (16.4)	2004-2013	68 (33-88)	90 (6-164)	cT1/2	DFS, LNM
Subramaniam et al.[24]	2020	India	Tongue	425	104 (24.5)	2004-2015	45 (18-86)	27	pT1-4	DSS
Wei et al.[46]	2019	Taiwan	Oral	314	65 (20.7)	2001-2009	54.0 (22-85)	63.2 (29-130)	T1/2	OS, DSS

LVI, lymphovascular invasion; *OSCC*, oral squamous cell carcinoma; *TNM*, tumor-node-metastasis; *OS*, overall survival; *DSS*, disease-specific survival; *NA*, not available; *FOM*, floor of mouth; *IQ*, interquartile; *BM*, buccal mucosa; *DFS*, disease-free survival; *LNM*, lymph node metastasis.

*Wei et al.[46] and Chen et al.[31] overlap.

†The study was also included in the LNM group.

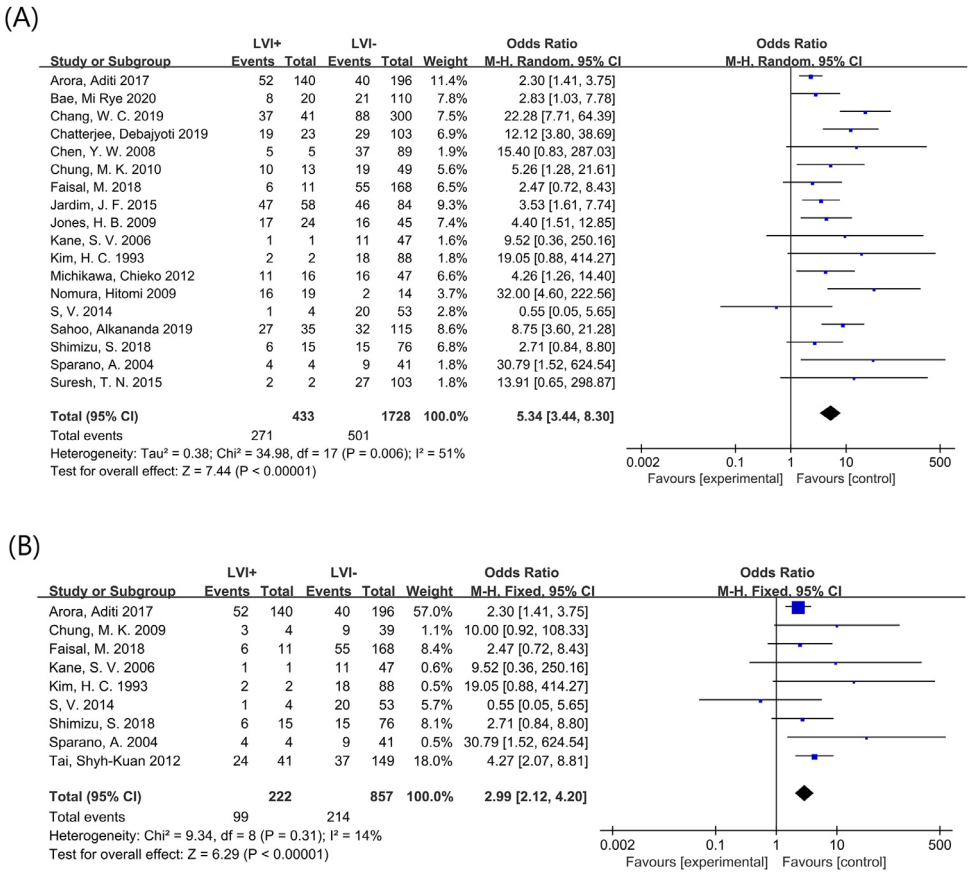

Fig. 2. Forest plots of the overall outcome for **(A)** lymph node metastasis and **(B)** lymph node metastasis in early stage OSCC.

(62.6%), and among the negative LVI group 501 of 1728 cases showed LNM (29.0%).

To furtherly clarify the role of LVI in predicting LNM, we assessed the effect of LVI in patients with early stage OSCC (Figure 2B). Nine studies including 1079 patients explored the relationship between LVI and LNM in patients with early tumor stages. Pooled analysis of the 9 studies revealed that LVI was significantly positively associated with LNM in early stage OSCC (OR = 2.99; 95% CI, 2.12-4.20; $P < .00001$, fixed effects), with low heterogeneity ($I^2 = 14\%$, $P_h = .31$).

Begg's test and Egger's test showed no significant evidence of publication bias for the studies included in the meta-analysis for LNM (Begg's test, $P = .363$; Egger's test, $P = .050$) and LNM in early stage OSCC (Begg's test, $P = .348$; Egger's test, $P = .181$). Furthermore, the funnel plots showed no significant asymmetric results. Therefore, the outcomes of the meta-analysis were reliable.

Prognostic value of LVI in OSCC

In total, 17 studies were used for the analysis of long-term survival in OSCC. OS, DSS, and DFS were the identified end points. Of these, 11 studies including 12,783 patients reported OS[7,17,20,22,26,30,31,37,38,41,46] (Figure 3A), 7 studies including 3470 patients reported DSS[24,32,37-39,46,47] (Figure 3B), and the other 3 studies with 731 patients reported DFS[25,30,43] (Figure 3C). Among studies focused on OS, Wei et al.'s study[46] from DSS group was excluded because their patient pool overlapped with that of Chen et al.'s study.[31]

There was no significant heterogeneity in the studies for OS ($I^2 = 43\%$, $P_h = .07$), DSS ($I^2 = 10\%$, $P_h = .35$), and DFS ($I^2 = 0\%$, $P_h = .45$) and a fixed effects model was used for all 3 groups. The results showed that positive LVI predicted poor OS (HR = 1.55; 95% CI, 1.43-1.69; $P < .00001$) and DSS (HR = 1.76; 95% CI, 1.48-2.09; $P < .00001$). Nevertheless, the result showed that positive LVI was not related to poor DFS (HR = 1.20; 95% CI, 0.89-1.62; $P = .24$).

In terms of publication bias, DSS and DFS had no obvious asymmetry in funnel plots. Because a small number of studies were included in DSS and DFS groups, approaches for detecting publication bias would have exhibited limited efficacy; therefore, Begg's test and Egger's test were not assessed. However, studies of OS had statistically significant publication bias (Begg's test: $P = .592$; Egger's test: $P = .023$). Four potential missing studies were identified by performing a trim-and-fill method. The results showed

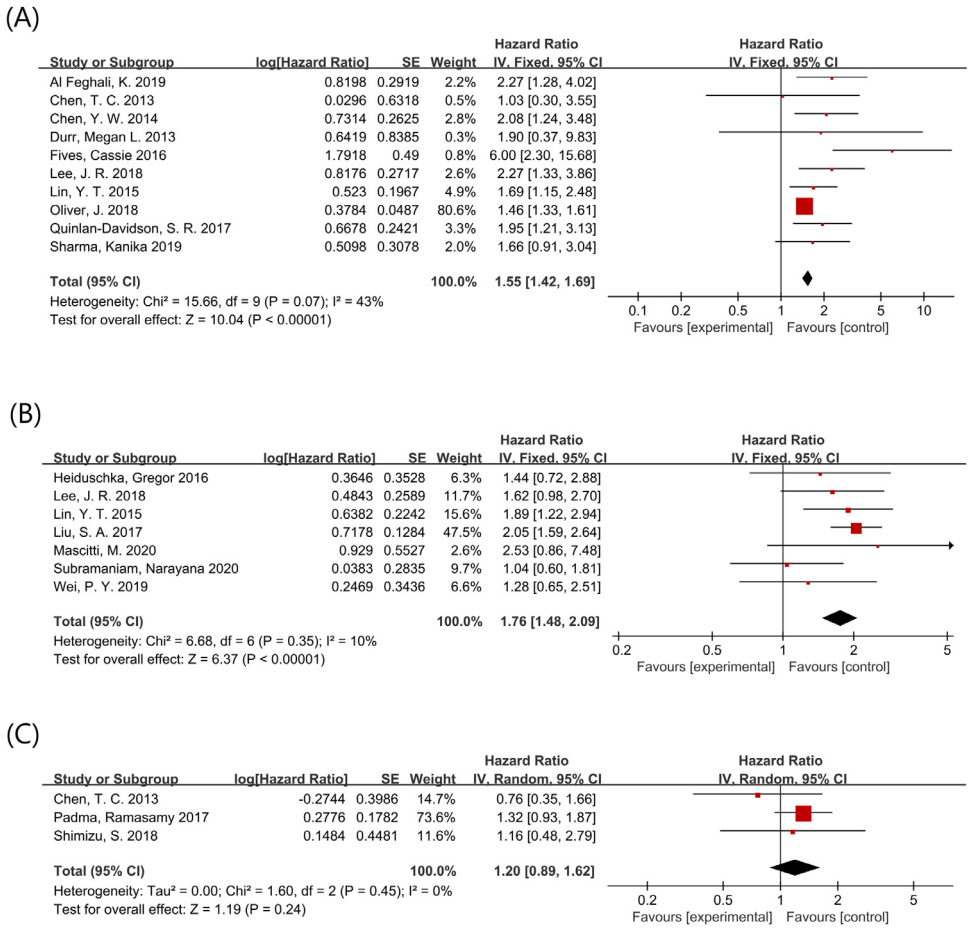

Fig. 3. Forest plots of the overall outcome for **(A)** overall survival, **(B)** disease-specific survival, and **(C)** disease-free survival.

that the recalculated pooled HR was 1.54 (95% CI, 1.25−1.90; $P < .00001$; random effects) for OS. It showed that even with publication bias, a similar result was obtained (Supplemental Figure S1, available at [URL/link]).

Sensitivity analysis

As shown in Figure 4, sensitivity analysis indicated that based on the pooled HR for LNM, the point estimate of the single omitted data set did not exceed the 95% CI. These outcomes showed that no individual study could possibly affect the pooled risk estimate and the results were robust and reliable.

Discussion

The current prognostic means based on TNM staging is the most common and practical method for clinically predicting the prognosis of patients with OSCC. Nevertheless, the TNM staging system did not achieve sufficient accuracy to help us make a clinical decision, especially for patients with early stage cancer. The latest version of the National Comprehensive Cancer Network Guidelines for the treatment of head and neck squamous cell carcinomas incorporated depth of invasion and extranodal extension into oral cancer TNM staging.[50] Furthermore, LVI has been added to the TNM staging system for liver tumors for improved tumor staging.[51] The aim is to accurately classify patients through the adverse feature in the process of tumorigenesis and to aid in diagnosis and prognosis. Although the prognostic value of LVI in patients with OSCC has been appraised by a number of studies, the results remain controversial. In the context of previous studies on OSCC, the prognostic significance of LVI has been increasingly recognized.[7,34] Nevertheless, some researchers reported that the presence of LVI did not have an association with poorer patient survival.[23,30] These discrepancies might be due to the different sample size, study design, or source of controls or patients involved.

Tumor cell invasion into peritumor tissue has long been postulated to be a significant pathologic factor, and its biological mechanism can explain its prognostic significance in OSCC. Studies have shown that the initial entry of neoplastic cells into the circulation occurs through blood vessels or lymphatic vessels.[52] The existence of LVI means that a considerable number of tumor cells are entering the vascular compartment, which is in turn one of the first steps for the potential

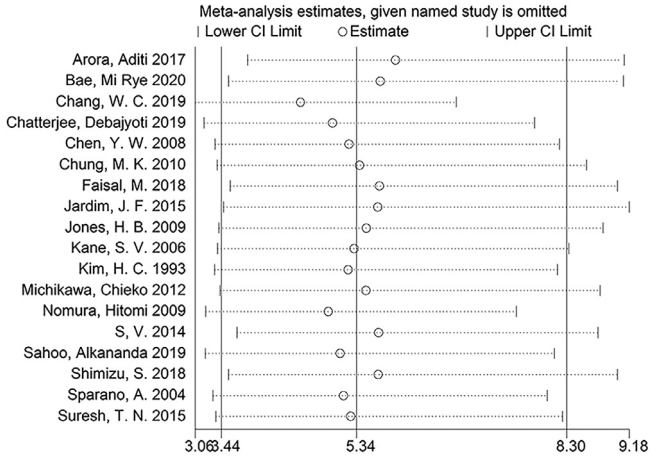

Fig. 4. Results of sensitivity analysis of lymph node metastasis showing the effect of each study on the overall estimate by sequentially excluding one study in one turn.

development of metastasis.[53] Sometimes it is difficult to differentiate between lymphatic vessels and blood vessels because the identification of lymphatic vessels is usually unclear. Many studies have not distinguished blood vessels and lymphatic vessels well.[7,28,30] Furthermore, if separate estimates of lymphatic vessel or blood vessel invasion were reported as prognostic indicators, the outcomes might have been confused due to overlapping populations. Therefore, we did not separate lymphatic invasion and vascular invasion in LVI in our meta-analyses.

The methods of distinguishing blood vessels from lymphatic vessels are usually conventional H&E and IHC staining. H&E staining is the most commonly used for detection of LVI in these studies. Interestingly, an article on oral tongue SCC indicated that evaluation of LVI by H&E staining had worse reproducibility of outcomes than IHC with D2-40 antibodies and resulted in increased interobserver discrepancies.[19] However, the use of IHC staining to evaluate LVI status remains controversial and is not practical for everyday clinical use, and establishing a standardized staining protocol is required to reveal the clinical significance of LVI in patients with OSCC.[8,19] To provide more evidence of the prognostic importance of LVI in patients with OSCC, more randomized controlled studies are required.

In this meta-analysis, we analyzed data from 17 eligible studies comparing OSCC survival according to LVI of the primary tumor region. The individual data were organized according to OS, DSS, and DFS. The results showed that positive LVI was associated with poor OS in OSCC. When the analysis was restricted to the survival outcome of DSS, a positive result was also observed in the present meta-analysis. Thus, LVI could be an independent predictor of OS and DSS in patients with OSCC.

It is noteworthy that our research also analyzed the association between LVI and lymph node metastases in OSCC and early stage OSCC, respectively. There are 2 choices for treatment of patients with cT1-2 OSCC: elective neck dissection and close follow-up (wait and watch), for which there is no consensus or guideline. Therefore, we need more clinical or pathologic and molecular biological markers to assist in the accurate and individualized treatment plans. Lymphatic metastasis is a continuous and complicated process in which cancer cells acquire the ability to leave the primary tumor site through the bloodstream and/or the lymphatic system. Furthermore, an important step in this process occurs when tumor cells penetrate into lymphovascular spaces through the endothelial cell layer. In OSCC, studies have indicated that the identification of LVI may be associated with the presence of LNM at the primary tumor,[19,29,34] thus constituting a significant marker for disease progression. We conducted a meta-analysis including 9 studies that only reported early stage OSCC, and the outcome shows that LVI has prognostic value to predict the occurrence of LNM in early stage OSCC. Because early detection of positive LVI has significant implications in the prognosis, it is significant to identify the patients with early stage OSCC with high risk of LNM for whom elective neck dissection or more adjuvant therapies may be required. For early stage patients whose pathologic sections are regarded as negative LVI, radical local tumor excision and close follow-up can be performed.[54]

Though our meta-analysis reported a positive conclusion, it has several limitations that must be considered. Firstly, all articles were retrospective studies despite the large number of samples used. Therefore, because of a lack of patient information, many confounding factors could not be corrected. Secondly, only published studies written in English were included, which may lead to selection bias. Thirdly, in the studies on LNM, moderate heterogeneity was

reported. This could be related to discrepancies in the patient characteristics, research protocol, and quality of the literature. Therefore, a random effects model was used to minimize the effect of heterogeneity and a sensitivity analysis was performed to support the strength of our outcomes. We are looking forward to further randomized controlled studies on LVI to compare the predictive value of this indicator using different evaluation methods and measurement standards.

We conclude that LVI is correlated with LNM in OSCC and has predictive value for patients with early stage OSCC. Positive LVI indicated poor survival and LNM trends, which indicates that LVI might be used as a prognostic biomarker for patients with OSCC in addition to the TNM staging system. Based on the above conclusions, we suggest that for patients with positive LVI, elective neck dissection or more aggressive therapies such as postoperative radiotherapy, chemotherapy, and biological therapy can be used to achieve better results.

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Table S1. Quality assessment of included studies.

Authors	*Year*	*Participation*	*Attrition*	*LVI detection*	*Outcome*	*Confounders*	*Analysis*	*Total*
Al Feghali et al	2019	☆	☆	☆	☆☆	☆	☆☆	8
Arora et al	2017	☆	☆	☆☆	☆☆	☆	☆☆	9
Bae et al	2020	☆☆	-	☆	☆	☆	☆☆	7
Chang et al	2019	☆☆	☆	☆☆	☆☆	☆☆	☆☆	11
Chatterjee et al	2019	☆	☆	☆☆	☆	☆	☆☆	8
Chen, T. C. et al	2013	☆☆	☆	☆	☆☆	☆☆	☆☆	10
Chen, Y. W. et al	2014	☆☆	☆	☆	☆☆	☆	☆☆	9
Chen, Y. W. et al	2008	☆☆	-	☆☆	☆	☆	☆☆	8
Chung et al	2010	☆	☆☆	☆	☆	☆	☆☆	8
Chung et al	2009	☆	☆	☆	☆☆	☆	☆☆	8
Durr et al	2013	☆☆	☆	☆	☆☆	☆☆	☆☆	10
Faisal et al	2018	☆☆	-	☆	☆☆	☆☆	☆☆	9
Fives et al	2016	☆☆	☆☆	☆☆	☆	☆	☆☆	10
Heiduschka et al	2016	☆☆	☆☆	☆☆	☆	☆☆	☆☆	11
Jardim et al	2015	☆☆	☆	☆☆	☆	☆	☆☆	9
Jones et al	2009	☆☆	-	☆	☆☆	☆	☆☆	8
Kane et al	2006	☆☆	☆	☆☆	☆☆	☆	☆☆	10
Kim et al	1993	☆	-	☆☆	☆	☆	☆☆	7
Lee et al	2018	☆☆	☆	☆	☆	☆	☆☆	8
Lin et al	2015	☆☆	-	☆	☆	☆	☆☆	8
Liu et al	2017	☆☆	-	☆☆	☆☆	☆	☆☆	9
Mascitti et al	2020	☆☆	-	☆	☆☆	☆☆	☆☆	9
Michikawa et al	2012	☆☆	-	☆☆	☆☆	☆	☆☆	9
Nomura et al	2009	☆☆	☆	☆☆	☆	☆	☆☆	9
Oliver et al	2018	☆☆	☆	☆	☆	☆☆	☆☆	9
Padma et al	2017	☆☆	☆	☆	☆	☆	☆☆	8
Quinlan et al	2017	☆☆	☆	☆	☆☆	☆	☆☆	9
S, V. et al	2014	☆☆	-	☆	☆	☆	☆☆	7
Sahoo et al	2019	☆	☆	☆☆	☆☆	☆	☆☆	9
Sharma et al	2019	☆☆	☆	☆	☆	☆	☆☆	8
Shimizu et al	2018	☆☆	☆	☆	☆☆	☆☆	☆☆	10
Sparano et al	2004	☆☆	-	☆	☆☆	☆	☆☆	8
Subramaniam et al	2020	☆☆	-	☆	☆☆	☆	☆☆	8
Suresh et al	2015	☆	-	☆	☆	☆	☆☆	6
Tai et al	2012	☆☆	☆	☆☆	☆☆	☆	☆☆	10
Wei et al	2019	☆☆	-	☆	☆☆	☆	☆☆	8

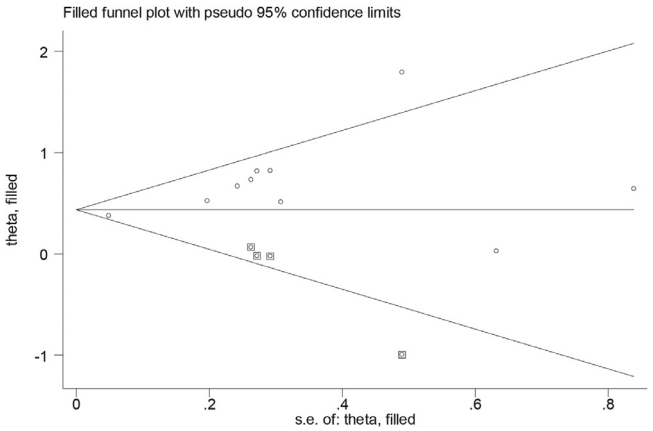

Figure S1. Trim-and-fill funnel plot on overall survival.