RESEARCH ARTICLE

Clinicopathological significance of perineural and lymphovascular invasion in oral and oesophageal squamous cell carcinoma: A retrospective study

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Abstract

Objective: To determine the presence and association of perineural and lymphovascular invasion with different stages of oral and oesophageal squamous cell carcinoma, and to assess their relationship with lymph node metastasis in both types of tumours.

Method: The retrospective study was conducted from December 1, 2023, to April 1, 2024, at the Department of Histopathology, Dow Diagnostic Research and Reference Laboratory, Dow University of Health Sciences, Karachi, and comprised diagnostic reports of histopathology along with haematoxylin and eosin slides of patients diagnosed with oral squamous cell carcinoma and oesophageal squamous cell carcinoma from June 1, 2022, to June 1, 2023. Data ws compared to explore the association of perineural and lymphovascular invasion with tumour stage and nodal metastasis. Data was analysed using SPSS 26.

Results: Of the 100 cases, 60(60%) had oral squamous cell carcinoma, with 41(68.3%) being males and 35(58.3%) being aged >40 years. There were 40(40%) cases with oesophageal squamous cell carcinoma, with 28(70.2%) being males and 27(68.4%) being aged >40 years. Among the oral cases, perineural invasion (p<0.001) and lymphovascular invasion (p=0.05) were significantly associated with nodal metastasis. Depth of invasion was significantly associated with both perineural (p=0.024) and lymphovascular (p=0.047) invasion. Tumour size was significantly associated with perineural invasion (p=0.05), but not with lymphovascular invasion (p=0.227). Among the oesophageal squamous cell carcinoma cases, a significant association was identified for tumour stage with perineural invasion (p=0.047) and lymphovascular invasion (p=0.006). Perineural invasion (p=0.002) and lymphovascular invasion (p<0.001) were significantly associated with nodal metastasis.

Conclusion: Perineural and lymphovascular invasion showed potential as diagnostic and therapeutic targets for managing oral squamous cell carcinoma and oesophageal squamous cell carcinoma.

Keywords: OSCC, ESCC, Perineural invasion, Lymphovascular invasion. (JPMA 76: 43; 2026)

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Introduction

Oesophageal cancer (EC), including oesophageal squamous cell carcinoma (ESCC), adenocarcinoma and rare subtypes, stands as the eighth most prevalent cancer worldwide, contributing to 500,000 deaths annually and accounting for 5.3% of all cancer-related fatalities.[1] Simultaneously, oral squamous cell carcinoma (OSCC), representing over 90% of oral cancer cases, prevails in Asia and is ranked the second most common cancer in Pakistan. In 2020, the Global Cancer Observatory (GLOBOCAN) reported 354,864 new cases of oral cancer and 177,384 deaths.[2]

Despite the potential curability of EC through radical surgery and the adoption of multidisciplinary treatments, the prognosis for many EC patients remains bleak, with a 5-year overall survival rate hovering around 60%.[3] OSCC also has a similar challenging scenario despite advances in diagnostic and therapeutic approaches.[4]

Both OSCC and ESCC exhibit a remarkable ability to invade the basement membrane, gaining access to the extracellular matrix and infiltrating the circulating lymphatics, nerves and blood vessels. This invasive trait not only challenges immune checkpoints and immune-surveillance mechanisms, but also presents a formidable obstacle to the removal of metastatic deposits, contributing substantially to the overall poor prognosis observed in these cancers.[5]

Various histopathological features, including lympho-vascular invasion (LVI), have been identified as the major route of metastasis for carcinomas characterised by the presence of tumour cells in the endothelium-lined spaces of vascular and lymphatic vessels.[6] This invasion pattern is

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defined by tumour cells infiltrating these channels without involving muscular layers. The prognostic significance of LVI is well-supported by studies across different solid tumours, including breast[7] and lung carcinoma(8), as well as gastrointestinal tract (GIT) tumours.[9]

Another main histopathological feature, perineural invasion (PNI), represents the infiltration of cancer cells around nerve fibres, serving as an important route for cancer spread to adjacent tissues and distant organs.[10] Tumours demonstrating PNI have been characterised as "neurotrophic tumours" and the significance of PNI has been recognised in various cancers, including EC.

The literature revealed a varied impact of PNI and LVI across different cancer types. While numerous studies supported their adverse prognostic significance, dissenting opinions and inconsistent data have persisted. Studies across various cancers, including prostate[11] and colon,[12] have highlighted the diverse roles and impacts of PNI.

Contrasting evidence has also emerged regarding the prognostic implications of LVI. Studies on breast cancer,[7] bladder carcinoma[13] and colorectal carcinoma[14] have indicated associations with poor prognosis, tumour upstaging, and decreased survival. In some studies, particularly in the case of non-small cell lung cancer (NSCLC),[15] LVI exhibited varied prognostic impact compared to PNI.

The current study was planned to determine the presence and association of PNI and LVI with different stages of OSCC and ESCC, and to assess of relationship of PNI and LVI with lymph node metastasis in both types of tumours.

Materials and Methods

The retrospective study was conducted from December 1, 2023, to April 1, 2024, at the Department of Histopathology, Dow Diagnostic Research and Reference Laboratory, Dow University of Health Sciences (DUHS), Karachi, and comprised histopathology reports along with haematoxylin and eosin (H&E) slides of patients diagnosed with OSCC and ESCC from June 1, 2022, to June 1, 2023. After approval from the institutional ethics review committee, all cases of complete tumour resection with simultaneous neck dissection of oral and oesophageal cancer in either gender were included. Patients who had received any form of prior treatment were excluded, and so were patients exhibiting recurrence and distant metastasis.

Demographic information regarding all the cases was collected through patient history forms. Clinico-pathological data, including tumour stage, size, presence of LVI and PNI along with lymph node metastasis, was collected from histopathology diagnostic reports. H&E slides of the cases were re-examined by a pathologist blinded to initial reports for the confirmation of stage and the presence of perineural and lymphovascular invasion.

Data was analysed using SPSS 26. Data was expressed as frequencies and percentages. Association of PNI and LVI with tumour size, depth of invasion and grade was determined using chi-square test. Univariate analyses was also performed. P<0.05 was considered significant.

Results

Of the 100 cases, 60(60%) had OSCC, with 41(68.3%) being males and 35(58.3%) being aged >40 years. There were 40(40%) cases with ESCC, with 28(70.2%) being males and 27(68.4%) being aged >40 years.

Among the OSCC patients, buccal mucosa 42(70%) and tongue 10(16.7%) were the most common sites of occurrence. Tumour stage 3 was identified in 20(33.3%) cases, while 4(6.7%) patients were classified as stage 1. There were 18(30%) cases each with stages 2 and 4. PNI was identified in 27(45%) patients, whereas LVI was observed in 18(30%) cases. Tumour size >4mm was found in 23(36.7%) individuals, and depth of invasion (DOI) >10mm was evident in 28(46.7%) (Figure 1).

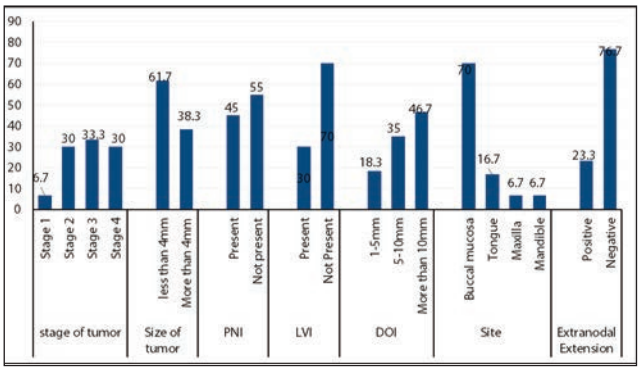

Figure-1: Clinico-pathological characteristics of oral squamous cell carcinoma (OSCC) patients.
PNI: Perineural invasion, LVI: Lymphovascular invasion, DOI: Depth of invasion.

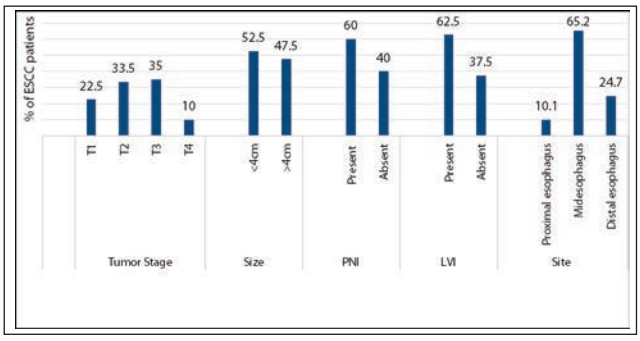

Figure-2: Clinico-pathological features of oesophageal squamous cell carcinoma (ESCC) patients.*PNI: Perineural invasion, LVI: Lymphovascular invasion.*

Table-1: Association of perineural invasion (PNI) and lymphovascular invasion (LVI) with tumour stage and nodal metastasis in oral squamous cell carcinoma (OSCC) cases.

Clinico-pathological Parameters	Perineural Invasion (PNI)		*p*-value	Lymphovascular Invasion (LVI)		*p*-value
	Present (n=27)	Absent (n=33)		Present (n=18)	Absent (n=42)	
Stage of Tumour						
Stage 1	0	4	0.207	0	4	0.182
Stage 2	7	11		3	15	
Stage 3	10	10		8	12	
Stage 4	10	8		7	11	
Nodal Metastasis						
Present	22	5	<0.001*	13	19	0.055*
Absent	5	27		5	23	

Table-2: Association of perineural invasion (PNI) and lymphovascular invasion (LVI) with tumour stage and nodal metastasis in oesophageal squamous cell carcinoma (ESCS).

Clinico-pathological Parameters	Perineural Invasion (PNI)		*p*-value	Lymphovascular Invasion (LVI)		*p*-value
	Present (n=24)	Absent (n=16)		Present (n=25)	Absent (n=15)	
Stage of Tumour						
Stage 1	3	6	0.047*	2	7	0.006*
Stage 2	6	7		7	6	
Stage 3	12	2		13	1	
Stage 4	3	1		3	1	
Nodal Metastasis						
Present	18	4	0.002*	21	1	0.001*
Absent	6	12		4	14	

Among the ESCC cases, depicted middle portion of the oesophagus was involved in 27(65.2%) patients as the primary site. Staging revealed that 14(35%) patients had stage 3, followed by 13(33.5%) having stage 2. Tumour size >4mm was found in 21(52.5%) cases. PNI and LVI was positive in 24(60%) and 25(62.5%) patients, respectively (Figure 2).

Among the OSCC cases, tumour stage was not significantly associated with PNI (p=0.207) and LVI (p=0.182). PNI and LNI were significantly associated with nodal involvement (Table 1).

In univariate analysis, DOI was significantly associated with both PNI (p=0.024) and LVI (p=0.047). Tumour size was significantly associated with PNI (p=0.05), but not with LVI (p=0.227).

Among the ESCC cases, tumour stage had a significant association with PNI (p=0.047) and LVI (p=0.006). PNI (p=0.002) and LVI (p<0.001) were significantly associated with nodal metastasis (Table 2)).

Discussion

Tumour stage and nodal metastasis are well-known indicators of cancer progression. The current study provided comprehensive evidence regarding the potential association of PNI and LVI with tumour stage and nodal metastasis in OSCC and ESCC cases. the assessment of PNI in combination with LVI has considerable significance since they are two pathological features that indicate interaction of tumour with its microenvironment.[16] Recent study has concluded that migration of tumour cells through nerves can be recognised as a possible route of metastasis.[17] On the other hand, LVI can be defined as the presence of tumour cells within blood or lymphatic vessels that has been recognised as an important step in tumour progression.[18]

Patients of OSCC exhibit significant PNI and LVI at the time of diagnosis, leading to poor survival. The current study showed that PNI and LVI increased in higher stages of OSCC. However, on statistical analysis, no significant association was detected for tumour stage with the presence of PNI and LVI. This was in alignment with a study[19] which concluded that the incidence of PNI and LVI was greater in oral cancer stages 3 and 4. The presence of these two histological features has been correlated with poor survival rate, highlighting their importance as independent prognostic factors. Contrasting evidence has been put forward in a study[20] illustrating that PNI and LVI could not be regarded as a reliable predictors of early-stage cancers of the oral cavity.

Several studies have cited an association between tumour stage and PNI in ESCC, and it has been considered an indicator for prognosis and recurrence. It is extremely plausible that neural invasion occurs as a concomitant consequence of tumour advancement.[6,9,10] The current study observed that the presence of PNI and LVI became substantially prominent in aggressive tumours and a notable association of tumour stage was observed with PNI and LVI. Similar findings were reported by Zhang et al.[21]

Both PNI and LVI have also been implicated in promoting nodal metastasis. Literature indicates that tumours of the oral cavity with PNI and LVI have an increased propensity of lymph node involvement.[22] The current study also indicated a strong association of nodal metastasis with PNI and LVI.

In addition, it was observed that patients with oesophageal cancer adhered to a similar pattern, and there was a significant association of both LVI and PNI with lymph node metastasis, highlighting the potential impact of these morphological characteristics on disease prognosis.

Contrary to the current observations, a study[3] identified LVI to be a prognostic marker for individuals without nodal involvement.

The current study has its limitations. First the data was collected from a single centre, limiting the generalisability of the findings. Second, the study design was retrospective which could have possibly introduced selection bias, and that is why standardised inclusion criteria and single-blinding were incorporated to improve internal validity. Third, the events per variable (EPV) shortfall (≤2 predictors in OSCC and ≤1 in ESCC), a small sample size and the retrospective design may not have fully accounted for potential confounding variables in multivariate analysis. As such, the data was subjected only to univariate analysis. Multi-centre, large-scale, prospective studies are needed to validate the current findings.

Despite the limitations, however, the current study represented a pioneering investigation, providing insight into the association of PNI and LVI with tumour stages and nodal metastasis in OSCC and ESCC cases.

Conclusion

There were significant correlations between the presence of PNI/LVI and cancer progression in ESCC. PNI/LVI exhibited notable association with nodal metastasis in OSCC, shedding light on their potential prognostic implications.

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Author Contribution:
AG: Formulated the study concept and proposal for IRB approval.
MS: Writing and data analysis.
UB: Supervision, proof reading and critical analysis.
SSZ: Writing.
SB & RA: Data collection and analysis.