

# The Relationship between Perineural Invasion, Peritumoural Inflammation, and Cervical Lymph Node Metastases in Laryngeal Squamous Cell Carcinoma

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## ABSTRACT

**Introduction:** Amongst all the malignant tumours, laryngeal cancer is found in 2.2% of males and 0.4% of females. Histopathologically, 90-95% of all laryngeal cancer is Squamous Cell Carcinoma (SCC). Tumour stage and cervical lymph node metastases are the most influential factors in determining a worsening prognosis.

**Aim:** To investigate the relationship between perineural invasion, peritumoural inflammation, other commonly used prognostic parameters such as tumour stage, and cervical lymph node metastases in laryngeal SCC which contribute to the follow-up and treatment of patients.

**Materials and Methods:** The pathology archive at Istanbul Sisli Hamidiye Etfal Education and Research Hospital was utilised for this research. Retrospective analysis of data on 110 cases of laryngectomy and neck dissection specimens

was performed. Cases diagnosed as SCC were included in the study. Histopathologic analysis was conducted on the paraffin-embedded tissue sections of 110 cases. The data were encoded, transmitted to a computer, and analysed using SPSS® 20.0. The Kruskal-Wallis H test was used to compare the groups. Statistical significance was considered to be  $p\text{-value} \leq 0.050$ .

**Results:** The rate of cervical lymph node metastases was higher in the presence of perineural invasion, mild peritumoural inflammation, and advanced tumour stage. A statistically significant relationship was identified between perineural invasion, peritumoural inflammation, tumour stage, and the rate of cervical lymph node metastases in case of SCC of the larynx.

**Conclusion:** Cervical lymph node metastases should be carefully investigated in the presence of perineural invasion and mild peritumoural inflammation in case of SCC of the larynx.

**Keywords:** Cervical lymph node metastases, Larynx, Perineural invasion, Peritumoural inflammation

## INTRODUCTION

Amongst all the malignant tumours, laryngeal cancer is found in 2.2% of males and 0.4% of females [1]. Approximately 1,30,000 new cases and 3,660 deaths were associated with cancer of the larynx in 2017 [2]. Histopathologically, 90-95% of laryngeal cancer is SCC, which commonly affects people in the sixth and seventh decades of life [3,4].

There is a significant relationship between laryngeal cancer and smoking [2,4]. The incidence of laryngeal cancer has decreased by 2.4% annually over the last 10 years owing to increased awareness that tobacco and alcohol use are major predisposing factors in the development of laryngeal cancer [2].

The stage and symptoms of laryngeal cancer varies depending on the physical site at which it is located [5]. A change in voice is the first symptom of glottic cancer. Dysphagia and hemoptysis are commonly associated with cancer of the supraglottis. Dyspnea is the most common symptom in cancer of the subglottis [1,3]. The cure rate for patients diagnosed with limited disease (i.e., T1 and T2) is excellent, with survival rates of 80-90%. However, the majority of patients are diagnosed with advanced-stage (i.e., T3 and T4) and regional lymph node metastases. The survival rate for these patients is <50% [6].

There is a significant relationship between lymph node metastases and prognosis in laryngeal tumours as in other head and neck tumours. Tumour stage and cervical lymph node metastases are the most important influential factors in determining a worsening prognosis. Increased lymphatic vessel density in the primary tumour area is associated with a greater possibility of metastases [6,7]. The rate of cervical lymph node metastases is

approximately 5% in early-term glottic tumours but may increase up to 40% in advanced-stage tumours. The rate of cervical lymph node metastases is roughly 15-20% for subglottic and 40% for supraglottic tumours [1,8].

Thus, the current study objective was to investigate the relationship between perineural invasion, peritumoural inflammation, other commonly used prognostic parameters such as tumour stage, and cervical lymph node metastases in laryngeal SCC. It was hypothesised that the study findings for the 110 laryngectomy and neck dissection samples will contribute to the findings in the literature on the subject and contribute to the follow-up and treatment of patients.

## MATERIALS AND METHODS

The pathology archive of Istanbul Sisli Hamidiye Etfal Education and Research Hospital was accessed for this research. Retrospective analysis of the data was conducted on 110 cases of laryngectomy and neck dissection. Ethical approval to conduct the study was obtained from the Sisli Hamidiye Etfal Education and Research Hospital ethics committee (Istanbul-SEEAH2007/1).

Cases diagnosed as SCC were included in the study. Histopathologic analysis was performed of the paraffin-embedded tissue sections obtained from 110 cases. Four micron sections prepared from paraffin blocks were stained with haematoxylin and eosin staining. The preparations were evaluated by histopathologic examination.

The relationship between perineural invasion, peritumoural inflammation, and cervical lymph node metastases was investigated in SCC of the larynx. In addition, the association between other common clinical and histopathologic parameters (i.e., age, gender,

T stage, maximum tumour size, tumour invasion depth, tumour localisation, degree of tumour differentiation, and vascular invasion) with cervical lymph node metastases was explored.

The patients were classified as males (n=104) and females (n=6) and grouped according to their age (i.e., ≤39 years, 40-49 years, 50-59 years, and ≥60 years). The groups were further classified according to the cancer site (i.e., supraglottic, glottic, and subglottic) and T stage (i.e., T1, T2, T3, and T4). Perineural and vascular invasion was classified as either present or absent. Peritumoural inflammation was divided into three categories of mild (1-5 inflammatory cells per field×40 ocular magnification), moderate (6-20 inflammatory cells per field×40 ocular magnification), and severe (≥20 inflammatory cells per field×40 ocular magnification). The tumours were described as well, moderately, or poorly differentiated and were classified according to size (i.e., ≤9 mm, 10-19 mm, 20-29 mm, and ≥30 mm). Invasion depth was described as ≤4 mm, 5-9 mm, 10-14 mm, or ≥15 mm. The cervical neck dissection material was grouped according to the presence or absence of metastases of SCC of the larynx in the cervical lymph nodes.

### STATISTICAL ANALYSIS

The data were encoded, transmitted to a computer, and analysed using SPSS® 20.0. The Kruskal-Wallis H test was used to compare the groups. Statistical significance was considered to be a p-value of ≤0.050.

### RESULTS

One hundred and ten laryngectomy and cervical neck dissection specimens were evaluated. The patients ranged in age from 35-80 years. The median age of the study subjects was 55.7 years. The majority of the participants were aged 50-60 years. Cervical lymph node metastases was identified in 46 cases (41.8%).

Perineural invasion was identified in 23 cases (20.9%) and not found in 87 cases (79.1%). Lymph node metastases was present in 15 (65.2%) and 31 (35.6%) of these cases, respectively [Table/Fig-1].

		Perineural Invasion		
		+	-	Total
Lymph Node Metastases	+	15 (65,2%)	31 (35,6%)	46 (41,8%)
	-	8 (34,8%)	56 (64,4%)	64 (58,2%)
Total		23	87	110

[Table/Fig-1]: The rate of cervical lymph node metastases according to perineural invasion.

A statistically significant relationship was identified between perineural invasion and the rate of cervical lymph node metastases (p≤0.050).

Mild, moderate, and severe peritumoural inflammation was found in 49 (44.5%), 38 (34.5%), and 23 cases (21%). Cervical lymph node metastases was seen in 25 (51.0%), 17 (44.7%), and 4 (17.4%) of these cases, respectively [Table/Fig-2].

		Peritumoural Inflammation			
		Mild	Moderate	Severe	Total
Lymph Node Metastases	+	25 (51,0%)	17 (44,7%)	4 (17,4%)	46 (41,8%)
	-	24 (49,0%)	21 (53,3%)	19 (82,6%)	64 (58,2%)
Total		49	38	23	110

[Table/Fig-2]: The rate of cervical lymph node metastases according to peritumoural inflammation.

A statistically significant reverse relationship was observed between peritumoural inflammation and the rate of cervical lymph node metastases (p≤0.050).

Of all the cases, 6 (5.5%) were classified as T1, 30 (27.2%) as T2, 65 (59.1%) as T3, and 9 (8.2%) as T4. Lymph node metastases was identified in one T1-classified case (16.7%), in nine T2-classified cases (30%), in 29 T3-classified cases (44.6%), and in seven T4-classified cases (77.8%) [Table/Fig-3].

		Tumour Stage				
		T1	T2	T3	T4	TOTAL
Lymph Node Metastases	+	1 (16,7%)	9 (30%)	29 (44,6%)	7 (77,8%)	46 (41,8%)
	-	5 (83,3%)	21 (70%)	36 (55,4%)	2 (22,2%)	64 (58,2%)
Total		6	30	65	9	110

[Table/Fig-3]: The rate of cervical lymph node metastases according to tumour stage.

A statistically significant relationship was found between tumour stage and the rate of cervical lymph node metastases in SCC of the larynx (p≤0.050).

Of the study subjects, 104 (94.5%) were males and 6 (5.5%) were females. Cervical lymph node metastases was present in 42 (40.4%) and 4 (66.7%) of these cases, respectively [Table/Fig-4].

		Gender		
		Male	Female	Total
Lymph Node Metastases	+	42 (40,4%)	4 (66,7%)	46 (41,8%)
	-	62 (59,6%)	2 (33,3%)	64 (58,2%)
Total		104	6	110

[Table/Fig-4]: The rate of cervical lymph node metastases according to gender.

The difference between gender and the rate of cervical lymph node metastases was not found to have statistical significance (p>0.050).

Vascular invasion was present in 90 cases (81.8%) and absent in 20 (18.2%). Lymph node metastases was observed in 38 (42.8%) and 8 (40%) of these cases, respectively [Table/Fig-5].

		Vascular Invasion		
		+	-	Total
Lymph Node Metastases	+	38 (42,8%)	8 (40%)	46 (41,8%)
	-	52 (57,2%)	12 (60%)	64 (58,2%)
Total		90	20	110

[Table/Fig-5]: The rate of cervical lymph node metastases according to vascular invasion.

The difference between vascular invasion and the rate of cervical lymph node metastases did not have statistical significance (p>0.050).

Of all the cancer sites, the supraglottis was involved in 54 cases (49.1%), the glottis in 55 cases (50%), and the subglottis in one case (0.9%). Cervical lymph node metastases was present in 26 cases involving the supraglottis (48.1%), in 20 cases involving the glottis (36.3%), and was absent in the case affecting the subglottis [Table/Fig-6].

		Tumour Localisation			
		Glottic	Supraglottic	Subglottic	Total
Lymph Node Metastases	+	20 (36,3%)	26 (48,1%)	0 (0%)	46 (41,8%)
	-	35 (63,7%)	28 (51,9%)	1 (100%)	64 (58,2%)
Total		55	54	1	110

[Table/Fig-6]: The rate of cervical lymph node metastases according to tumour localisation.

The difference between tumour localisation and the rate of the cervical lymph node metastases was not found to have statistical significance (p>0.050). Nevertheless, the rate of lymph node metastases was higher in tumours in the supraglottic region compared to the glottic region.

The tumours were poorly differentiated in 46 cases (41.8%), moderately differentiated in 52 cases (47.3%), and well-differentiated in 12 cases (10.9%). Lymph node metastases was present in 23 (50%), 19 (36.5%), and 4 (33.3%) of these cases, respectively [Table/Fig-7].

		Tumour Differentiation Degree			
		Well	Moderately	Poorly	Total
Lymph Node Metastases	+	4 (33.3%)	19 (36.5%)	23 (50%)	46 (41.8%)
	-	8 (66.7%)	33(63.5%)	23 (50%)	64 (58.2%)
Total		12	52	46	110

**[Table/Fig-7]:** The rate of cervical lymph node metastases according to tumour differentiation degree.

The difference between the degree of tumour differentiation and the rate of cervical lymph node metastases was not found to be statistically significant ( $p>0.050$ ). Nevertheless, a correlation was found between an escalation in the rate of lymph node metastases and a reduction in the degree of tumour differentiation.

Seven of the patients (6.4%) were aged  $\leq 39$  years, 26 (23.6%) were aged 40-49 years, 39 (35.5%) were aged 50-59 years, and 38 (34.5%) were aged  $>60$  years. Lymph node metastases was identified in 3 (42.9%), 13 (50%), 13 (33.3%), and 17 (44.7%) of these cases, respectively. The difference between age and the rate of cervical lymph node metastases was without statistical significance ( $p>0.050$ ).

The difference between tumour size, tumour invasion depth, and the rate of the cervical lymph node metastases was also without statistical significance ( $p>0.050$ ). Nevertheless, the rate of lymph node metastases was higher with large tumour size and higher tumour invasion depth.

## DISCUSSION

A significant relationship has been established between lymph node metastases and prognosis in laryngeal tumours, as is the case with other head and neck tumours. Cervical lymph node metastases has been identified as the most influential factor in determining a worsening prognosis. Histopathologic and clinical factors have been effectively used to determine a prognosis in laryngeal cancer [7].

Sixty to sixty-five per cent of all laryngeal cancer occurs in the glottic region, 30-35% in the supraglottic region, and  $\leq 5\%$  in the subglottic region [1]. There is a close correlation between tumour location and cervical lymph node metastases in laryngeal cancer. The rate of lymph node metastases in the glottic area in laryngeal cancer is lower than that in other regions. The most likely reason for this is that the lymphatic network in this region is not large. In addition, tumours in this site tend to be diagnosed promptly owing to voice-related indications that present earlier. The rate of cervical lymph node metastases is roughly 5% for early-term tumours in the glottic region of the larynx but can increase up to 40% for advanced-stage tumours. The rate of Cervical lymph node metastases is roughly 15-20% for subglottic and 40% for supraglottic tumours [1,8]. The rate of cervical lymph node metastases was seen to be higher in supraglottic cancer in the current study.

Tumour stage is the most influential factor in determining the rate of cervical lymph node metastases and prognosis. Tumour stage and size, invasion depth, and histopathologic grade are frequently used as prognostic parameters in head and neck tumours [7,10]. These parameters play an important role in determining prognosis [1,7,10]. An association was identified in the current study between an increased rate of cervical lymph node metastases and advanced tumour stage.

Perineural invasion and peritumoural inflammation are other important, albeit less frequently used prognostic parameters. Perineural invasion refers to tumour cell invasion in, around, and through the nerve. It is under-recognised as a means of metastatic spread that occurs via tumoural invasion of the nerves and is an important prognostic determinant of poor survival in laryngeal cancer [11,12]. A significant correlation has been found between perineural

invasion and lymph node metastases in the SCC of the larynx in several studies [11-16]. The present results on the relationship between perineural invasion and lymph node metastases in SCC of the larynx were compatible with those in the literature.

Goldsmith MM et al., examined the relationship between the number of eosinophils in tumour tissue and prognosis in 82 cases of head and neck cancer, and observed that prognosis was more hopeful in cases of excessive inflammation [17]. Ercan I et al., did not find a meaningful association between eosinophilic infiltration of the larynx and cervical lymph node metastases in 78 patients undergoing resection for laryngeal SCC when its impact on prognosis was evaluated [9]. They reported that more research involving a wider array had to be done in this regard [9]. Keberle M et al., found a significant correlation between peritumoural lymphocytic infiltration and regional lymph node metastases in the head-neck region and the larynx [18]. The rate of cervical lymph node metastases was demonstrated to be higher in the current study in cases of mild peritumoural inflammation [18]. The present results on the relationship between peritumoural inflammation and lymph node metastases in SCC of the larynx were compatible with those in the literature.

It is our considered opinion that perineural invasion and peritumoural inflammation need to be investigated carefully, in addition to other commonly used prognostic parameters (i.e., tumour stage and size, and the degree of tumour differentiation) when evaluating laryngectomy specimens. These parameters, in conjunction with other prognostic clinical and histopathologic parameters, should be exactly noted in pathology reports.

## LIMITATION

In this study, there was only one tumor in subglottic area. For this reason, it is necessary to support this result with more homogeneous and larger series.

## CONCLUSION

Cervical lymph node metastases should be carefully investigated in the presence of perineural invasion and mild peritumoural inflammation in case of SCC of the larynx.

## REFERENCES

- [1] Rosai J, Ackerman LV. Larynx and Trachea, In: Rosai and Ackerman's Surgical Pathology 11<sup>th</sup> edition, Elsevier, China: 2011. Pp. 319-33.
- [2] National Cancer Institute. Surveillance, Epidemiology, and End Results. Cancer Stat facts: Larynx Cancer, 2017. Available from: <https://seer.cancer.gov/statfacts/html/larynx.html>. [Last access date 10 February 2018].
- [3] Kumar V, Abbas AK, Fausta N. Carcinoma of the larynx, In: Pathologic Basis of Disease, 7<sup>th</sup> edition, China: 2005. Pp. 786.
- [4] Thompson LDR: Malignant neoplasm of the larynx, In: Head and Neck Pathology, Elsevier, China: 2006. Pp. 51-88.
- [5] American Cancer Society. Laryngeal Cancer Stages. 2018. Available from: <https://www.cancer.org/cancer/laryngeal-and-hypopharyngeal-cancer/detection-diagnosis-staging/staging.html> [Access date 15 February 2018]
- [6] Megwalu UC, Sikora AG. Survival outcomes in advanced laryngeal cancer. JAMA Otolaryngol Head Neck Surg. 2014;140:855-60.
- [7] Tomik J, Skladzien J, Modrzejewski M. Evaluation of cervical lymph node metastases of 1400 patients with cancer of the larynx. Auris Nasus Larynx. 2001;28(3):233-40.
- [8] Siczka J, Zietek E. The problem of cervical metastases in vocal cord carcinoma. Otolaryngol. 2001;55(3):259-61.
- [9] Ercan I, Cakir B, Basak T, Ozdemir T, Sayin I, Turgut S. Prognostic significance of stromal eosinophilic infiltration in cancer of the larynx. Otolaryngol Head Neck Surg. 2005;132:869-73.
- [10] Remmert S, Rottmann M, Reichenbach M, Sommer K, Friedrich HJ. Lymph node metastases in head-neck tumours. Laryngorhinootologie. 2001; 80(1):27-35.
- [11] Liebig C, Ayala G, Wilks JA, Berger DH, Albo D. Perineural invasion in cancer: A review of the literature. Cancer. 2009;115:3379-91.
- [12] Frunza A, Slavescu D, Lascar I. Perineural invasion in head and neck cancers-a review. Journal of Medicine and Life. 2014;7(2):121-23.
- [13] Fagan JJ, Collins B, Barnes L, D'Amico F, Myers EN, Johnson JT. Perineural invasion in SCC of the head and neck. Arch Otolaryngol Head Neck Surg. 1998;124(6):637-40.
- [14] Yilmaz T, Hoşal AŞ, Gedikoglu G, Onerci M, Gürsel B. Prognostic significance of vascular and perineural invasion in cancer of the larynx. Am J Otolaryngol. 1998;19(2):83-88.

- [15] Yao M, Roebuck JC, Holsinger FC, Myers JN. Elective neck dissection during salvage laryngectomy. *Am J Otolaryngol.* 2005;26(6):388-92.
- [16] Magnano M, De Stefani A, Lerda W, Usai A, Ragona R, Bussi M, Cortesina G. Prognostic factors of cervical lymph node metastases in head and neck SCC. *Tumori.* 1997;83(6):922-26.
- [17] Goldsmith MM, Cresson DH, Askin FB. The importance of stromal eosinophilia in head and neck cancer. *Otolaryngol Head Neck Surg.* 1987;106:27-33
- [18] Keberle M, Strobel P, Marx A, Hahn D, Hoppe F. CT determination of lymphocytic infiltration around head and neck SCCs may be a predictor of lymph node metastases. *Eur Arch Otorhinolaryngol.* 2003;260:558-64.

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