

Histopathological Parameters predicting Occult Nodal Metastases in Tongue Carcinoma Cases: An Indian Perspective

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ABSTRACT

Background: It is a well-established fact that in squamous cell carcinoma cases, the presence of lymph node metastases decreased the 5-year survival rate by 50% and also caused the recurrence of the primary tumor with development of distant metastases. Till date, the predictive factors for occult cervical lymph nodes metastases in cases of tongue squamous cell carcinoma remain inconclusive. Therefore, it is imperative to identify patients who are at the greatest risk for occult cervical metastases. This study was thus performed with the aim to identify various histopathologic parameters of the primary tumor that predict occult nodal metastases.

Materials and methods: The clinicopathologic features of 56 cases of lateral tongue squamous cell carcinoma with cT1NoMo/cT2NoMo as the stage and without prior radiotherapy or chemotherapy were considered. The surgical excision of primary tumor was followed by elective neck dissection. The glossectomy specimen along with the neck nodes were fixed in formalin and 5 µm thick sections were obtained. The hematoxylin & eosin stained sections were then subjected to microscopic examination. The primary tumor characteristics

that were analyzed include tumor grade, invading front, depth of tumor, lymphovascular invasion, perineural invasion and inflammatory response. The nodes were examined for possible metastases using hematoxylin & eosin followed by cytokeratin immunohistochemistry.

Result: A total of 12 cases were found with positive occult nodal metastases. On performing univariate analysis, the histopathologic parameters that were found to be statistically significant were lymphovascular invasion ($p=0.004$) and perineural invasion ($p=0.003$) along with a cut-off depth of infiltration more than 5 mm ($p=0.01$).

Conclusion: Histopathologic assessment of the primary tumor specimen therefore continues to provide information that is central to guide clinical management, particularly in cases of occult nodal metastases.

Clinical significance The study highlights the importance of extensive histopathological screening, which holds the key for establishing occult metastases. Pathological upgrading of tumors is possible following histopathological studies similar to the present one. Presence of occult metastases justify neck dissection in these clinically N₀ cases. In an Indian setting, histopathological evaluation assumes a bigger role than other expensive and advanced techniques.

Keywords: Metastases, Occult, Tongue carcinoma.

How to cite this article: Jacob TE, Malathi N, Rajan ST, Augustine D, Manish N, Patil SG. Histopathological Parameters predicting Occult Nodal Metastases in Tongue Carcinoma Cases: An Indian Perspective. J Contemp Dent Pract 2016; 17(1):70-77.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Early detection and diagnosis are crucial in order to maximize the potential for successful outcomes in any disease condition. In no other disease process is this

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more imperative than in the detection, diagnosis and treatment of oral cancers. The treatment protocol for positive nodal disease is well standardized, but there is no clear agreement on the management of occult nodal metastasis. The incidence of occult metastasis is relatively high¹⁻⁴ and despite the advances in modern imaging technology, histopathologic examination of neck dissection specimen is still the only reliable way to detect the subclinical metastases. One approach is to treat the neck as 20% incidence of occult nodal metastasis in cases of oral squamous cell carcinoma. In this strategy, although of benefit for the patients with metastasis, about 80% of the patients without metastasis need to undergo neck dissection without any benefit. A staggering 20 to 50% of lymph node negative patients with occult nodal metastases go unidentified and these patients will eventually develop a clinically evident metastatic disease, usually within 2 years of follow-up.

This study was thus performed with the aim to identify various histopathologic parameters of the primary tumor that could predict occult nodal involvement, thus allowing appropriate treatment decisions and also by providing accurate indicator of patient outcome.

MATERIALS AND METHODS

The present study was carried out in the Department of Oral Pathology and Microbiology, Ramachandra Dental College, Chennai, and Head & Neck Institute Amrita Institute of Medical Science and Research Centre, Cochin.

A total of 56 patients with the inclusion criteria of lateral tongue carcinoma as the site, cT1NoMo/cT2NoMo as the stage and without prior radiotherapy or chemotherapy were considered. There was no clinical evidence of cervical nodal metastasis either by clinical palpation or by radiological imaging techniques. The preoperative clinical AJCC/UICC TNM stages were 40 cT1N0M0 and 16 cT2 N0M0 patients. An average of 11 nodes per case totaling to 616 nodes were obtained in the entire study. The glossectomy specimen along with the neck nodes were fixed in formalin and 5 µm thick sections were obtained. The hematoxylin & eosin stained sections were then subjected to microscopic examination. Further, the neck nodes were also subjected to AE1/AE3 (Pan Cytokeratin) staining, following a standardized protocol.

Histologic Variables

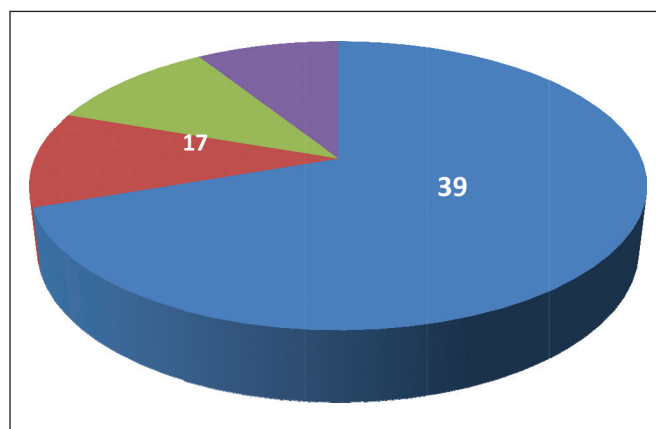
The primary tumor characteristics that were analyzed include tumor grade, invading front, depth of tumor,

lymphovascular invasion, perineural invasion and inflammatory response. The neck specimen was examined for positive occult nodal metastases. Grading of tumor was done according to the method originally described by Broder and adopted by World Health Organization (WHO). In tumor showing different grades, the higher grade determines the final categorization. The invading tumor front was grouped into pushing, infiltrative and a combination of both. Tumor depth was analyzed using computerized image analyzer. Measurement from surface of adjacent normal mucosa to the deepest point of invasion was considered. The deepest tumor was taken among all the blocks. Lymphovascular invasion was defined as the presence of aggregates of tumor cells within endothelial lined channels or invasion of media of vessel and infiltration of the perineural space of nerves was taken as perineural invasion. Host inflammatory response was graded as mild, moderate or severe.

RESULTS

On performing univariate analysis, the histopathologic parameters that were found to be statistically significant were lymphovascular invasion ($p=0.004$) and perineural invasion ($p=0.003$) along with a cut-off depth of infiltration more than 5 mm ($p=0.01$). A total of 17 cases were found with positive nodal metastases (Graph 1) (Figs 1 and 2).

There were 49 males and 7 female patients. The age range of patients was from 22 to 78 years, with a maximum number of patients seen in 5th and 6th decade. Duration of tumor varied from 1 to 24 months. In this study, maximum number of occult lymph nodes were from level II, followed by level III and level I (Graph 2).



Graph 1: The distribution of cases

In this study, there were a total of 17 positive lymph node biopsy cases and 39 negative cases without occult nodal metastasis

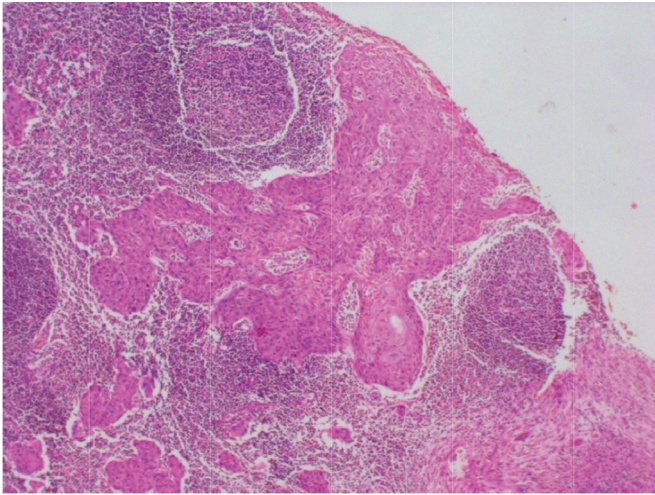
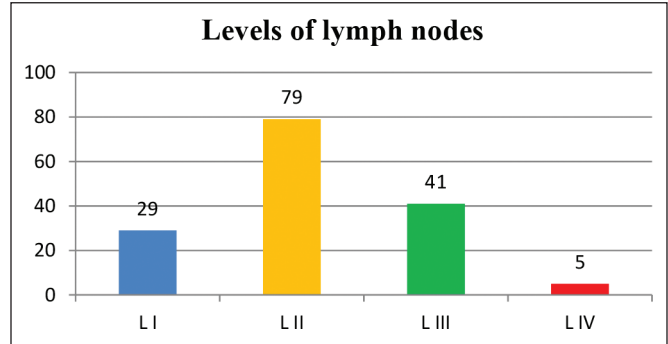


Fig. 1: Histologic section showing a lymph node positive for squamous cell carcinoma metastasis (H & E stain at 10 × magnification)



Graph 2: The levels of lymph nodes In this study, maximum number of lymph nodes were from level II (79 nodes), followed by level III (41 nodes), level I (29 nodes) and level IV (five nodes)

Table 1: Pearson Chi-square test for tumor grade

Group	Grade I	Grade II	Grade III	Total	p
Positive	10 (58.8%)	6 (35.3%)	1 (5.9%)	17 (100%)	0.249 (not significant)
Negative	28 (71.8%)	11 (28.2%)	0 (0%)	39 (100%)	
Total	38 (67.9%)	17 (30.4%)	1 (1.8%)	56 (100%)	

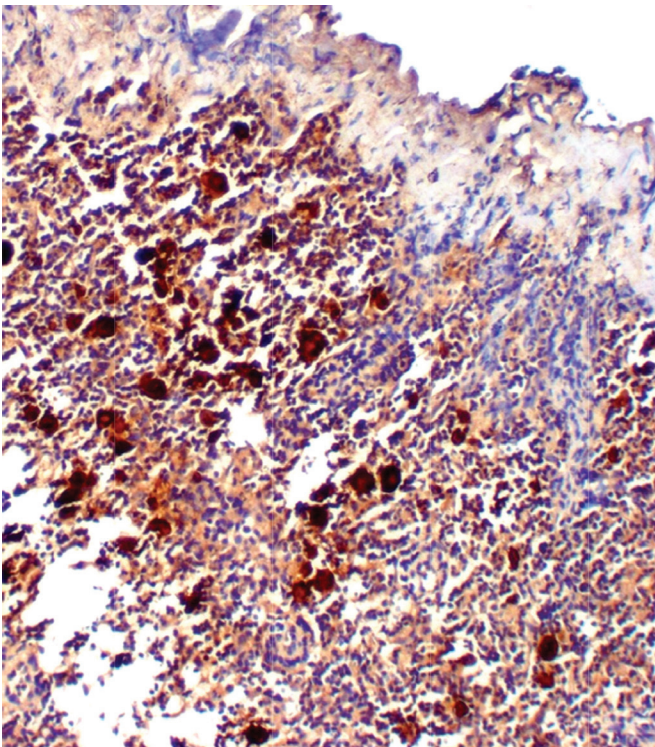


Fig. 2: Histologic section showing a lymph node positive for squamous cell carcinoma metastasis (IHC CK stain at 10 × magnification)

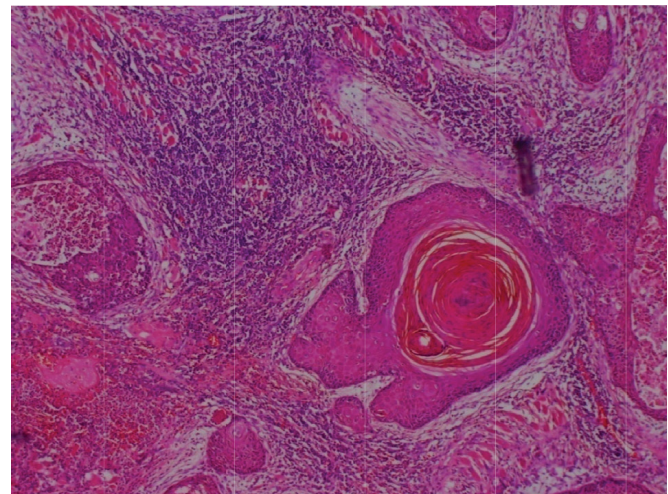


Fig. 3: Histologic section showing well differentiated squamous cell carcinoma (H & E stain at 10 × magnification)

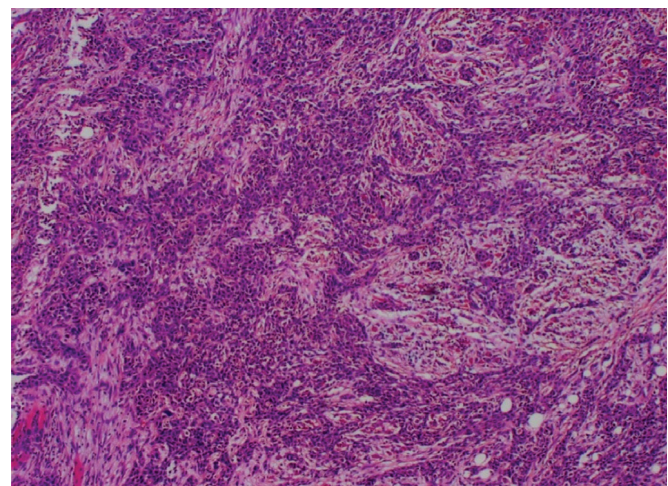


Fig. 4: Histologic section showing poorly differentiated squamous cell carcinoma (H & E stain at 10 × magnification)

In this study, there were 38 cases (67 .85%) in grade I tumor, 17 cases (30.35%) in grade II tumor and 1 case (1.78%) in grade III tumor. Tumor grade was statistically analyzed between the positive and negative node group using Pearson Chi-square test and was found to be statistically not significant (p=0.249) (Table 1, Figs 3 and 4).

The tumor front in the negative nodal biopsy group was found to be pushing in 17 cases (43.6%), infiltrative front in 17 cases (43.6%) and both fronts were seen in five cases (12.8%).

The tumor front in the positive nodal biopsy group was found to be pushing in four cases (37.5%) (Fig. 5) infiltrative front in 12 cases (70.6%) (Fig. 6) and both the fronts was seen in one case (5.9%). There was no statistical significance on using Pearson Chi-square test ($p=0.176$) (Table 2, Graph 3).

In the negative nodal biopsy group, maximum cases (26 cases) had depth of invasion in 0 to 5 mm range. Depth of invasion in positive nodal biopsy group was distributed as five cases in both 0 to 5 mm and 10 to 15 mm range and seven cases in 5 to 10 mm range showing a statistical significance of p equal to 0.01 (Graph 4, Table 3).

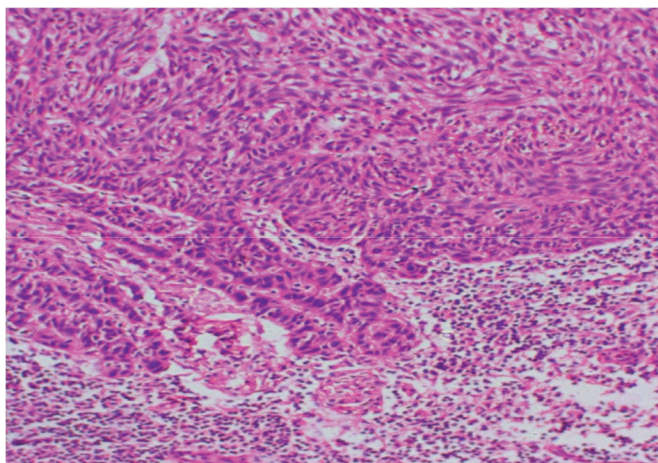


Fig. 5: Histologic section showing squamous cell carcinoma with pushing margins (H & E stain at 40 × magnification)

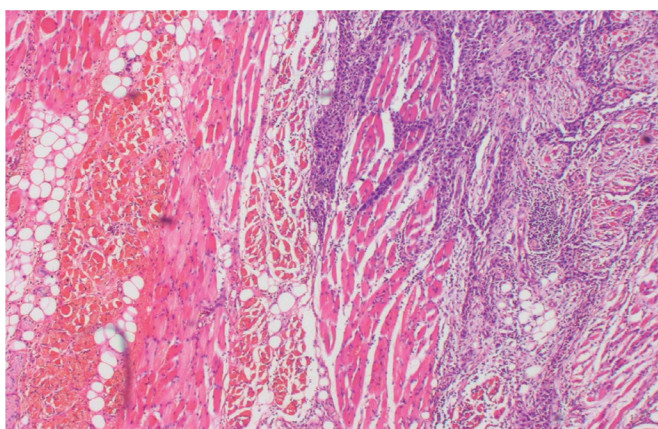
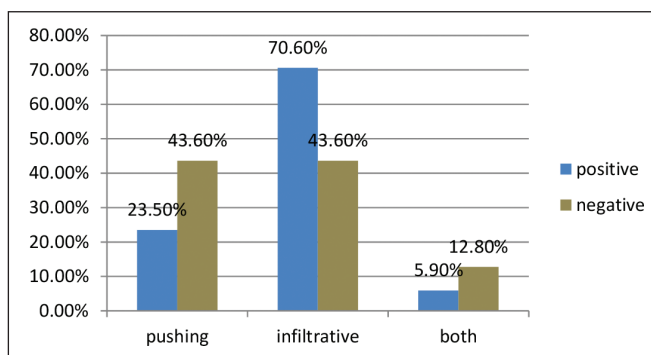
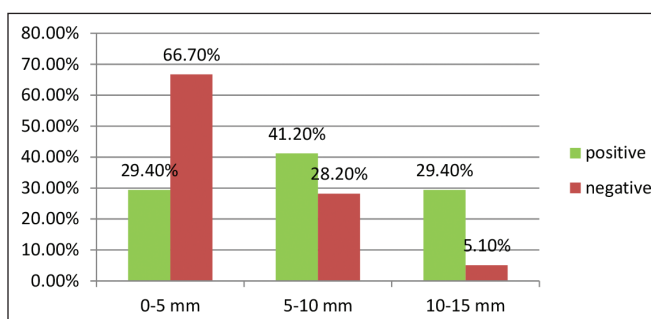


Fig. 6: Histologic section showing squamous cell carcinoma with infiltrative margins showing invasion into the muscle (H & E stain at 10 × magnification)



Graph 3: The distribution of tumor invasive front

In the positive lymph node biopsy group, there were four cases (23.5%) with pushing front, 12 cases (70.6%) with infiltrative front and one case (5.9%) with both infiltrative and pushing front. In the negative lymph node biopsy group, there were 17 cases (43.6%) with pushing front, 17 cases (43.6%) with infiltrative front and five cases (12.8%) with both infiltrative and pushing front



Graph 4: The depth of tumor in the study group

In the positive lymph node biopsy group, there were five cases (29.4%) in the range 0 to 5 mm, seven cases (41.2%) in the range 5 to 10 mm and five cases (29.4%) in the 10 to 15 mm range. In the negative lymph node biopsy group, there were 26 cases (66.7%) in the 0 to 5 mm range, 11 cases (28.2%) in the 5 to 10 mm range and two cases (5.1%) in the 10 to 15 mm depth range

Lymphovascular invasion was not evident in the negative node biopsy group and was seen in 35.3% of cases in the positive biopsy group (Fig. 7). There was statistical significance using Pearson Chi-square test ($p=0.000$) (Graph 5, Table 4).

Perineural invasion in the negative node biopsy group was seen in 10.3% of cases and 41.2% of cases in positive biopsy group (Fig. 8). There was statistical significance using Pearson Chi-square tests ($p=0.007$) (Graph 6, Table 5).

Table 2: Pearson Chi-square test for invasive front of tumor

Group	Pushing	Infiltrative	Both	Total	p
Positive	4 (23.5%)	12 (70.6%)	1 (5.9%)	17 (100%)	0.176 (not significant)
Negative	17 (43.6%)	17 (43.6%)	5 (12.8%)	39 (100%)	
Total	21 (37.5%)	29 (51.8%)	6 (10.7%)	56 (100%)	

There was no statistical significance on using Pearson Chi-square test ($p=0.176$)

Table 3: Pearson Chi-square test for depth of invasion of tumor

Group	0–5 mm	5–10 mm	10–15 mm	Total	p
Positive	5 (29.4%)	7 (41.2%)	5 (29.4%)	17 (100%)	0.010 (significant)
Negative	26 (66.7%)	11 (28.2%)	2 (5.1%)	39 (100%)	
Total	31 (55.4%)	18 (32.1%)	7 (12.5%)	56 (100%)	

Pearson Chi-square test was performed for statistical analysis showing a statistical significance of ($p < 0.01$)

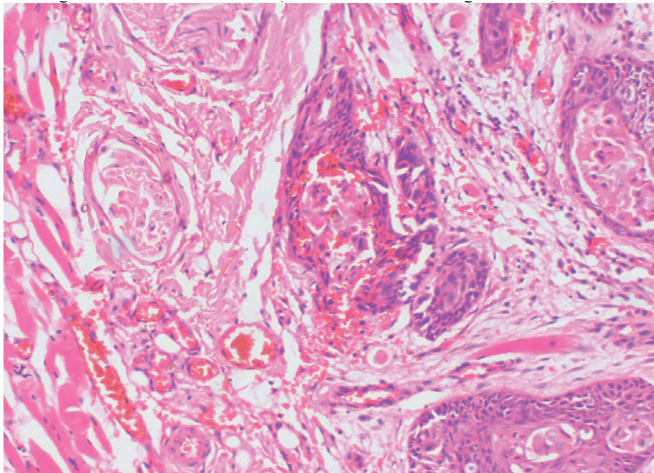


Fig. 7: Histologic section showing lymphovascular invasion in squamous cell carcinoma (H & E stain at 10 × magnification)

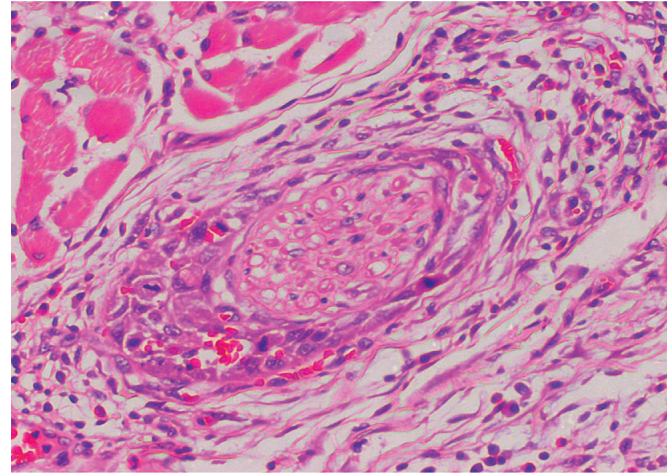
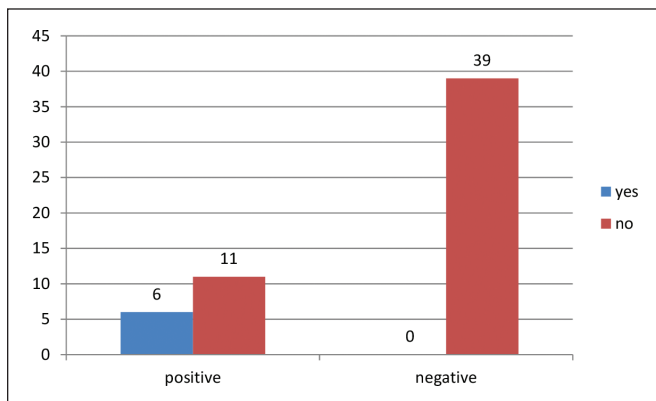
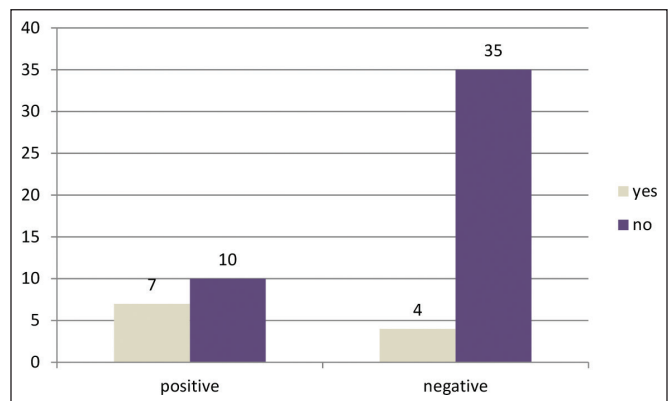


Fig. 8: Histologic section showing perineural invasion in squamous cell carcinoma (H & E stain at 40 × magnification)



Graph 5: The distribution of lymphovascular invasion

In the positive lymph node biopsy cases, there were six cases (35.3%) with lymphovascular invasion and 11 cases (64.7%) without lymphovascular invasion. In the negative lymph node biopsy cases, there were no positive cases (0%) and 39 cases (100%) were negative for lymphovascular invasion



Graph 6: The distribution of perineural invasion

In the positive node biopsy group, there were seven cases (41.2%) with perineural invasion and 10 cases (58.8%) without perineural invasion. In the negative node biopsy cases, there were four positive cases (10.3%) and 35 cases (89.7%) without perineural invasion

Table 4: Pearson Chi-square test for lymphovascular invasion

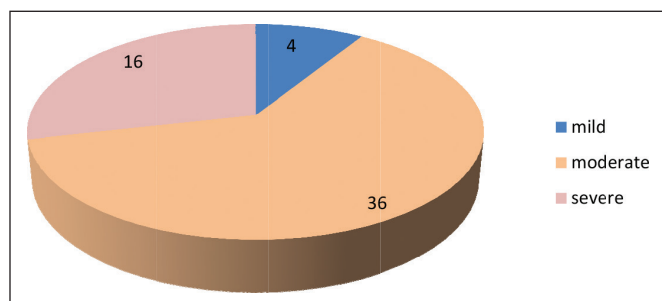
Group	Yes	No	Total	p
Positive	6 (35.3%)	11 (64.7%)	17 (100%)	0.000 (significant)
Negative	0 (0%)	39 (100%)	39 (100%)	
Total	6 (10.7%)	50 (89.3%)	56 (100%)	

There was statistical significance using Pearson Chi-square test ($p < 0.001$)

Table 5: Pearson Chi-square test for perineural invasion

Group	Yes	No	Total	p
Positive	7 (41.2%)	10 (58.8%)	17 (100%)	0.007 (significant)
Negative	4 (10.3%)	35 (89.7%)	39 (100%)	
Total	11 (19.6%)	45 (80.4%)	56 (100%)	

There was statistical significance using Pearson Chi-square tests ($p < 0.01$)



Graph 7: The inflammatory response to the invading tumor

In this study, the distribution of inflammatory response was found to be four cases (7.1%) with mild inflammatory response, 36 cases (64.3%) with moderate inflammatory response and severe inflammatory response was evident in 16 cases (28.6 %)

Table 6: Pearson Chi-square test for inflammatory response

Group	Mild	Moderate	Severe	Total	p
Positive	1 (5.9%)	11 (64.7%)	5 (29.4%)	17 (100%)	0.970 (not significant)
Negative	3 (7.7%)	25 (64.1%)	11 (28.2%)	39 (100%)	
Total	4 (7.1%)	36 (64.3%)	16 (28.6%)	56 (100%)	

There was no statistical significance on using the Pearson Chi-square test ($p=0.970$)

The inflammatory response in the negative nodal biopsy was mild in three cases (7.7%), moderate in 25 cases (64.1%) and severe in 11 cases (28.2%). The inflammatory response in the positive nodal biopsy was mild in one case (5.9%), moderate in 11 cases (64.7%) and severe in five cases (29.4%). There was no statistical significance on using the Pearson Chi-square (Graph 7, Table 6).

DISCUSSION

The clinical course of early squamous cell carcinoma of oral tongue is unpredictable and various histopathologic parameters of the primary tumor have been suggested as prognostic factors to be used in clinical decision-making and also as predictive factors for cervical metastasis.

The various histopathologic parameters considered were tumor grade, invading front, depth of tumor, lymphovascular invasion, perineural invasion and inflammatory response.

The prevalence of occult metastases can vary depending upon the primary tumor site. In 2007, Blanco and Chao⁵ explained the prevalence of occult neck metastases by site in their study as follows: oral tongue 60%, tongue base 55%, tonsil 36%, floor of mouth 25%, buccal mucosa 20%, retromolar trigone 20%, hard palate 15%, alveolus 15%.

The single most important factor that determines the survival of patients with head and neck cancer is nodal

metastatic status. Many studies confirm that 5-year survival rates decrease by approximately 50% once nodal metastasis is present.⁶ Furthermore, the presence of lymph node metastasis correlates with both recurrence of the primary tumor and the development of distant metastases.⁷ Thus, it becomes imperative in case of head and neck cancers to necessitate early detection and diagnosis in order to maximize the potential for successful outcome.

At least three mechanisms of nodal involvements are possible in advanced disease: embolic spread from node to node leading to multiple discrete positive nodes, or permeation of internodal lymphatics, or direct extracapsular spread resulting in fused (matted) nodes. The relative contribution of each mechanism in oral/oropharyngeal squamous cell carcinoma is uncertain. Some sites within the oral cavity may also drain directly to distant lymph nodes via long-range pathways or fast-tracks.⁸

The treatment protocol for positive nodal disease is well standardized, but considerable variation still exists in the management of clinically negative neck. At present, there is no accurate method to stage N0 neck, as there is no clear agreement on the management of occult nodal metastasis. One approach is to treat the neck as 20% incidence of occult nodal metastasis in cases of oral squamous cell carcinoma.^{9,10} In this strategy, although of benefit for the patients with metastasis, about 80% of the patients without metastasis need to undergo neck dissection without any benefit.²

A total of 56, T1-T2 N0, tongue squamous cell carcinoma cases included in this study were subjected to modified neck dissection. The primary tumor was assessed for various histopathologic parameters such as tumor grade, invading front, depth of tumor, lymphovascular invasion, perineural invasion and inflammatory response. The nodal status was correlated with the histologic parameters.

In this study, there were 38 cases (67.85%) of histological grade I tumor, 17 cases (30.35%) of grade II tumor and one case (1.78%) of grade III tumor. Tumor grade was also statistically analyzed between the positive and negative nodal group using Pearson Chi-square test and was found to be statistically not significant ($p=0.249$) as summarized in Table 1. The percentage of positive lymph node cases in grade I was 26.31%, in grade II was 35.29% and in grade III was 100%. As in our study, only T1&T2, No status cases were included and that there was only one case of poorly differentiated squamous cell carcinoma, statistically histological grading of tumor failed to show any significance. Yazdi and Khalili¹¹ in their study had similar results. The prognostic values of different grading classifications were studied. Statistical

analysis failed to detect any relationship between Broders' grades and lymph node metastasis.

The tumor front in the positive lymph node group was found to be pushing in four cases (23.5%), infiltrative front in 12 cases (70.6%) and both the fronts was seen in one case (5.9%). The tumor front in the negative lymph node group was found to be pushing in 17 cases (43.6%), infiltrative front in 17 cases (43.6%) and both fronts were seen in five cases (12.8%). There was no statistical significance on using Pearson Chi-square test (0.176) (Table 2). Wu-long et al¹² studied the histologic sections of tumor from 45 T1/T2N0 squamous cell carcinoma, clinically determined N0 patients and concluded that infiltrating-type invasion front was associated with increased occult metastases.

Depth of invasion in positive lymph node group was distributed as five cases in both 0 to 5 mm and 10 to 15 mm range and seven cases in 5 to 10 mm range. In the negative lymph node group, maximum cases (26 cases) had a depth of invasion in 0 to 5 mm range. Thus, beyond 5 mm depth, occult metastases can occur. There was statistical significance of $p=0.01$. Pearson Chi-square test was performed for statistical analysis as summarized in Table 3. This result is comparable with the study by Hideo Fukano et al¹³ carried out with the aim to use the depth of invasion as a predictive factor for cervical nodal metastasis in tongue carcinoma cases. Thirty-four primarily tongue carcinomas operated without any preoperative therapy were considered. The study concluded that there is a discerning point at 5 mm of tumor depth at which cervical metastasis is probable. Clark et al¹⁴ studied the distribution of occult metastases in 105 oral cancer patients with no clinical or radiological evidence of nodal disease. Occult neck metastases occurred in 34% of patients. Tumor thickness (tumor depth) was found to be the only independent predictor of occult metastases. Charoenrat¹⁵ also had similar result with tumor thickness exceeding 5 mm being statistically significant and was correlated with cervical metastases.

Lymphovascular invasion was seen in 35.3% of cases in the positive lymph node group and not evident in the negative lymph node group. There was statistical significance using Pearson Chi-square test ($p=0.000$) as shown in Table 4. This result correlated with the study by Chen et al¹⁶ wherein histopathological factors affecting nodal metastasis in 94 tongue cancer cases were assessed. One of the histological factors was lymphovascular permeation at the time of presentation that subjected to a higher incidence of neck nodal metastasis. Amarala et al¹⁷ analyzed the rates of occult metastasis and prognostic factors for clinical stages I and II squamous cell carcinoma of the tongue and floor of the mouth. For

tongue tumors, the presence of vascular embolization was found to be significant ($p=0.043$).

Similar results were seen in the study by Kurokawa et al¹⁸ wherein 50 previously untreated stage I or II squamous cell carcinoma of the tongue were reviewed for clinicopathologic factors associated with late cervical lymph node metastases and microvascular invasion was found to be a positive predictor.

Perineural invasion was seen in 41.2% of cases in positive lymph node biopsy group and 10.3% of cases in the negative lymph node biopsy group. There was statistical significance using Pearson Chi-square tests ($p=0.007$) as summarized in Table 5. Similar results were seen in a study by Fagan et al,¹⁹ which was performed to determine whether perineural invasion of small nerves affected the outcome of patients with squamous cell carcinoma of the upper aerodigestive tract and he found that perineural invasion of small nerves was associated with an increased risk of local recurrence and cervical metastasis. Chen et al¹⁶ have also concluded in their study that perineural invasion is a histopathologic parameter indicating higher incidence of neck nodal metastasis. Tumors that invade the perineural space are biologically more aggressive and are more likely to recur locally. Perineural invasion may be mediated by the presence of nerve cell adhesion molecule (NCAM) on the surface of squamous cell carcinoma, which engages in homophilic binding with NCAM expressed in neural and perineural tissue. The association between perineural invasion and local recurrence may result from either centrifugal or centripetal propagation of malignant cells along the perineural space and away from the primary tumor. Most primary tumors will only disseminate up to 2 cm along the perineural space, although there have been reports of perineural invasion up to 12 cm. Thus, allowing malignant cells to evade surgical excision or radiotherapy results in local recurrence.

The inflammatory response in the positive lymph node biopsy group was mild in one case (5.9%), moderate in 11 cases (64.7%) and severe in five cases (29.4%). The inflammatory response in the negative lymph node biopsy group was mild in three cases (7.7%), moderate in 25 cases (64.1%) and severe in 11 cases (28.2%). There was no statistical significance on using the Pearson Chi-square test as summarized in Table 6. Hiratsuka et al²⁰ in their study performed univariate and multivariate analyses for occult lymph node metastasis in 172 patients with clinically negative cervical lymph nodes to elucidate the clinical and histologic tumor risk factors that predict occult nodal metastases. In their results, they found lymphocytic infiltrate to be one of the factors predicting occult nodal status. The presence

of lymphocytic intratumoral and peritumoral infiltrate decreased the risk of concomitant cervical lymph node metastases, whereas a plasmocytic infiltrate increased the risk.

Effective management of patients with squamous cell carcinoma of the oral cavity depends on accurate staging to determine the prognosis and to select appropriate therapeutic strategies. The stage of the disease depends highly on the status of regional cervical lymph nodes at the risk of metastases from the primary tumor.

CONCLUSION

Clinically evident cervical metastases are found at presentation in approximately 30% of patients, whereas occult metastases occur in 20 to 30% of patients without any evidence of regional disease. Selecting the best management option for these patients requires accurately identifying subclinical cervical metastases. The accuracy of currently available means of detecting nodal disease such as imaging techniques has limitations. Therefore, histopathologic assessment of the primary tumor specimen continues to provide information that is central to guide clinical management, particularly in cases of occult nodal metastases.

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