



(RESEARCH ARTICLE)



Step serial sectioning of lymph node in oral squamous cell carcinoma for micrometastasis and extra nodal extension: A cross-sectional study

Mahima Jaiswal *, Mithila Bisht, Anjana Arya and Cheena Garg

Department of Pathology, Rohilkhand Medical College & Hospital, Bareilly.

International Journal of Science and Research Archive, 2025, 16(02), 343-348

Publication history: Received on 27 June 2025; revised on 04 August 2025; accepted on 06 August 2025

Article DOI: <https://doi.org/10.30574/ijrsra.2025.16.2.2320>

Abstract

Oral cavity carcinomas, categorized under head and neck cancers, are among the most common malignancies worldwide. India has one of the highest incidences worldwide and accounts for nearly 30% of annual global incidence. Occult metastasis (also known as micrometastasis) is defined as proliferation within the node of tumor tissue measuring > 0.2mm and <2mm in diameter and is often a potential pitfall for nodal metastasis. Extranodal extension (ENE) refers to growth of tumour outside the encapsulated lymph nodes that typically acts as natural barrier for tumor progression.

The aim of the study was to study step serial sectioning of lymph node in oral squamous cell carcinoma for detection of micrometastasis and extranodal extension

Objectives: The objective of the study was to classify oral squamous cell carcinomas based on pathological tumor(pT) and nodal stage(pN) and to determine the percentage increased nodal positivity in the node negative(pN0) cases of oral SCC on applying step serial sectioning and also calculate the percentage of increased ENE positive status in cases of ENE negative and nodal positive SCC with use of step serial sectioning.

Methodology: Study was cross sectional and was carried out at tertiary care centre ,Bareilly, UP for one year which included all histopathologically diagnosed node negative(N0) and pN stageN1-N3 with ENE negative status cases of oral SCC. 80 lymph nodes from diagnosed cases of oral squamous cell carcinoma were retrieved and out of these 46 negative lymph nodes were examined to look for micrometastasis. 34 out of 80 lymph nodes were positive that were analysed for extranodal extension. All paraffin embedded tissue blocks of these cases were further trimmed at 100micrometer interval after the initial evaluation, 2-3micron sections were obtained till the block was completely exhausted to look for micro metastasis and extra nodal extension.

Results: Out of 46 node negative cases, which yielded 704 sections , micrometastasis was detected in one node after step serial sectioning (2.2%) while 45 nodes (97.8%) were negative for micrometastasis. Nodal status was upstaged in one case(2.2%).However ENE status remained unchanged even after step serial sectioning.

Conclusion: Step serial sectioning can play an important role in diagnosing occult metastasis in node negative cases and spares the low risk patient from unnecessary treatment.

Keywords: Neck Nodes; Micrometastasis; Serial Sectioning; Oral cancer

* Corresponding author: Mahima Jaiswal

1. Introduction

Oral cancer is considered to be serious global public health issue accounting for more than 11,000 deaths annually. It is the 16th most common malignancy in the world.¹ The oral cancers incidence is estimated to be 73.6 per 100,000 population cases in India. India has one of the greatest rates worldwide and accounts for nearly 30% of annual global incidence.¹

Oral cavity carcinomas consist of neoplasms arising from the buccal mucosa, floor of mouth, alveolar ridges, retromolar trigone, hard palate, tongue and inner aspect (mucosal surface) of lips. Oral cavity cancers have a tendency to frequently metastasize to regional lymph nodes predominantly to cervical nodes which is the primary site for capture of these tumor cells that have invaded the lymphatic system and therefore establishes itself as the strongest predictor of disease prognosis and outcome².

Occult metastasis (also known as micrometastasis) is defined as proliferation within the node of tumor tissue measuring > 0.2mm and <2mm in diameter and is often a potential pitfall for nodal metastasis. Micrometastases cannot be easily detected preoperatively by any currently available staging technique. Along with palpation, the overall error is high in assessing the occult metastasis. In order to identify occult metastatic deposits, the serial sectioning (SS) approach was developed. This technique entails dissecting the LN block at various intervals until the block is entirely drained.³

Another parameter, i.e. Extranodal extension (ENE) refers to growth of tumour outside the encapsulated lymph nodes that typically acts as natural barrier for tumor progression. Its presence reflects the aggressiveness of tumor, and creates anatomical challenges for clearance of disease. It also increases the risk of metastasis to distant area.⁴

The aim of the study was to study step serial sectioning of lymph node in oral squamous cell carcinoma for detection of micrometastasis and extranodal extension.

2. Material and methods

A cross-sectional study carried out at tertiary care centre Bareilly for a duration of one year. All histopathologically diagnosed cases of oral squamous cell carcinoma undergoing neck dissection and showing: Node negative (N0) on pN staging and pN stage N1-N3 with ENE negative status were included in the study. Cases showing both nodal positivity and ENE positivity on H&E staining, cases of oral squamous cell carcinoma who have undergone pre-operative chemotherapy and radiotherapy and cases with recurrence of disease and any concurrent malignancy were excluded from the study group.

3. Methodology

The study was conducted, at tertiary care centre Bareilly after taking approval from Institutional Ethics Committee. The received specimen was fixed in 10% neutral buffered solution and was subjected to gross and microscopic examination as per standard protocol. The largest lymph node was identified and measured. Lymph node <5mm was submitted as whole and those with size >5mm was bisected into two halves and submitted in one cassette. Paraffin embedded tissues (4-5 micron) were cut and stained by Haematoxylin and Eosin stain. After staining slide was subjected to microscopic examination and decision for step serial sectioning was taken as under: -Those lymph node which were identified as node negative on routine H&E staining i.e. N0 was further taken up for step serial sectioning to look for micrometastasis and largest lymph node identified as positive but ENE negative on microscopy was subjected to step serial sectioning to identify ENE. Serial microsections each measuring 4-5 micro meter was retained every 100 micrometer till the tissue was completely exhausted.

4. Results

Total 80 cases of major biopsy specimen were included in the study. Step serial sectioning method was used in largest lymph nodes of oral SCC patients for detection of micrometastasis and extranodal extension. In the present study, out of 80 cases of OSCC, 72 cases (90.0%) were males and 8 cases (10.0%) were females. So male preponderance was observed in our study. Maximum patients 27 (34.0%) belonged to the age group of 41-50 years. Buccal mucosa was the most common site involved in our study accounting for 38 (48.0%) followed by alveolus. Elderly age group more (>50 years) developed predominantly higher histological grade of oral squamous cell carcinoma i.e. moderately differentiated. The commonest level of largest lymph node involved in our present study was level II nodes. The maximum number of cases in our study were in pathological N0 46 (58%) stage and 34 (42%) were in pT4a stage. In N0

cases serial sectioning was performed to look for micrometastasis since they were found to be strongly associated with patients prognosis. Table 1 and Table 2 shows that out of 46 node negative cases, serial sectioning upstaged the nodal status in one case. In all node positive cases step serial sectioning was performed to look for extranodal extension, however there was no change in the ENE status even after step serial sectioning.

Table 1 Distribution Of Data Based On Detection Of Micrometastasis

Total cases=46	Micrometastasis in one section	Micrometastasis after step serial section	P value
Yes	0	1	<0.001
No	0	45	

Table 2 Distribution Of Cases According To Change In N Stage From N0 To N1

Before Step Serial Sectioning	Node Positive	Node Negative	Total
	34	46	80
After Step Serial Sectioning	35	45	80
P Value		0.8748	

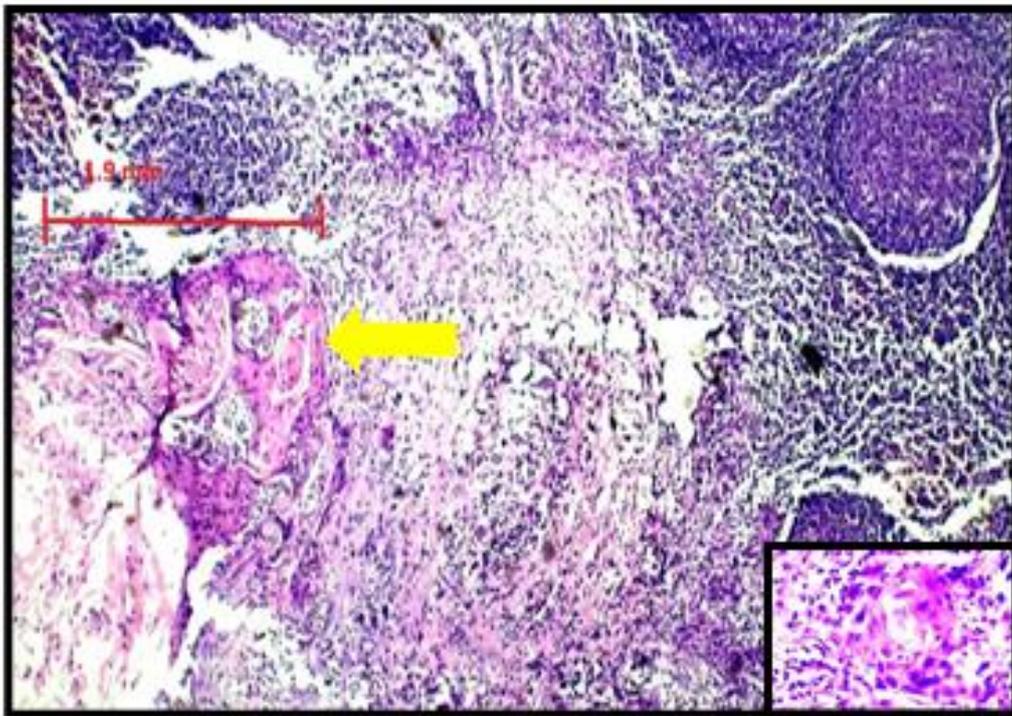


Figure 1 Image showing Micrometastasis In Lymph Node Of OSCC In Low Power View(H&E Stain)

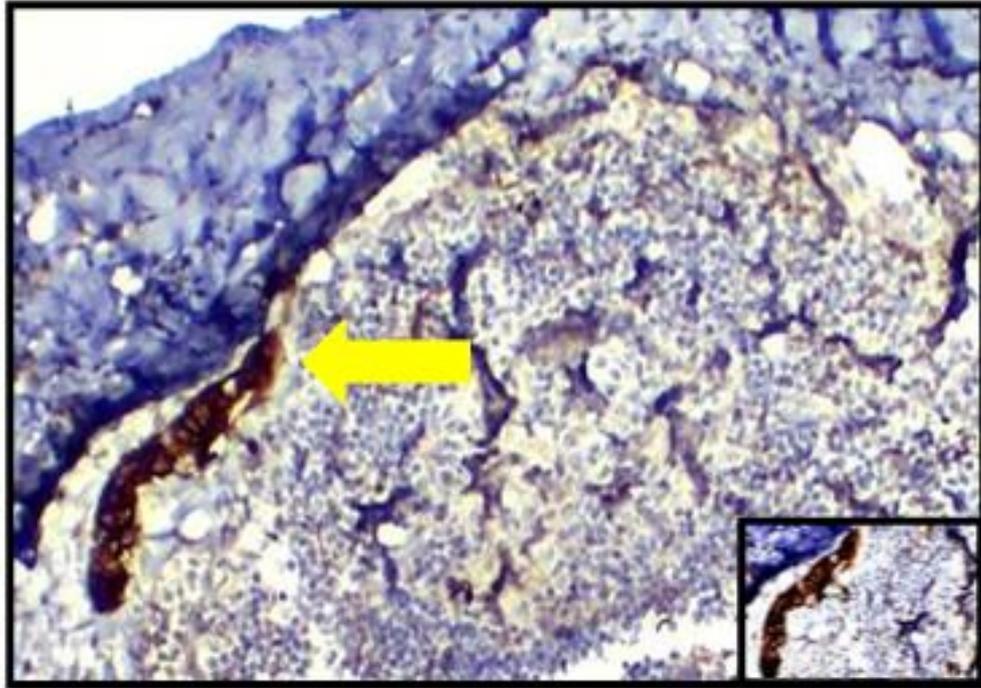


Figure 2 IHC Image Showing Micrometastasis In A Lymph Node Of OSCC In Low Power

5. Discussion

Oral cavity cancers frequently metastasize to the cervical group of nodes. The tumor cells harbour in these nodes therefore its detection becomes the primary concern of treating doctor and the pathologist as they play an essential role in the prognosis and survival of the patients.

Various procedures such as grossing, embedding, sectioning and staining are employed in processing of specimens. However deeper sections are required in certain situations to arrive at a final diagnosis for which expertise is required. Serial and step sections are other terminologies for deeper sections that are also used interchangeably. Every section collected is serial section and specimens collected at a specified depth forms a step section.⁵

In our study, distribution of patient according to gender was done. The majority of the patients were males (90%) with M:F ratio of 9:1, this was concordant with the study done by Elaiwy O et al.⁶ in which 91.6% of study subjects were male and 8.6% were females with male to female ratio being 10.9:1 and Feller L et al.⁷ in which squamous cell carcinoma of oral cavity was seen mainly affecting men than women(1.5:1). L Cases ranged from 29 years to 75 years of age with a median age of 50 years. Majority (63.7%) of the study participants were below 50 years of age. Our study results were similar to the study conducted by Sinha G M et al.⁸ in which majority of the study subjects were below 50 years of age and this was concordant with our study conducted. This is consistent with the Surveillance, Epidemiology, and End Results database maintained by the National Cancer Institute which offers proof of rise in proportion of oral cancers that develops in younger adults. 77 cases out of 80 (96.2%) had history of tobacco chewing and 69 (86.2%) out of 80 cases had history of smoking. Our study results were also similar to the study of Smitha T et al.⁹ in which tobacco was considered a major etiological agent in causing oral cancer. In our study, buccal mucosa being the commonest site of involvement (48.5%), followed by alveolus (21%) and tongue being the third commonest site (20%). . This study was similar to Jayasooriya PR et al.¹⁰ in which out of 896 cases of oral squamous cell carcinoma, buccal mucosa was the most prevalent site in 43% cases which clinically presented as ulcerative growth over mucosa. In our study out of 80 cases, 36 patients (45%) were in pathological T4 stage and , 46 (58%) patients were in pathological N0 stage which was similar to to study done by Varsha BK et al. and Patil S et al.

Table 3 Incidence of occult micrometastasis detected by step serial sectioning in this study in patients with OSCC compared with different studies

Studies	year	Sample size	Total number of lymph node sections yielded	Micrometastasis after step serial sectioning (%)
Prakash R et al ⁽¹¹⁾	2011	16	2269	2(2.03%)
Dhawan I et al ⁽³⁾	2016	10	461	1(2.25%)
Bhopal T et al ⁽¹²⁾	2018	20	-	1(5%)
Raut T et al ⁽¹³⁾	2023	30	-	2(6.6%)
Present study	2024	80	704	1(2.2%)

In the current study conducted, we retrieved 80 lymph nodes from diagnosed cases of oral squamous cell carcinoma. We examined 46 negative lymph nodes that after step serial sectioning yielded 704 sections in total, out of which micrometastasis was detected in one node after step serial sectioning (2.2%) while 45 nodes (97.8%) were negative for micrometastasis. Our study had similar findings with the study done by Prakash R et al.¹¹ in which micrometastasis was studied in 2269 sections corresponding to 119 nodes. Micrometastasis was found in 2 nodes in one section method that increased to 3 nodes in step serial sectioning method. No other lymph nodes were found to be positive by any of the above methods of sectioning in their study which was concordant with our study. The detection rate was 2.03% when compared with one section method.

According to a study done by Dhawan I et al.³ in which 133 lymph nodes (461 sections) from 10 patients were evaluated for micrometastasis, no micrometastasis was found by single section and 1 lymph node showed micrometastasis by step serial section method. Micrometastasis that was found in one lymph node was of size >1.0 cm, that was statistically non significant. This was concordant with our study.

According to a similar study done by Bhopal T et al.¹² 20 cases of oral squamous cell carcinoma were considered, out of which 16 sentinel nodes were identified and 10 were node negative. Only 1 out of 10 negative lymph nodes showed micrometastasis after step serial sectioning. The major limitation of this research was small sample size similar to our study.

In our study, 34 out of 80 lymph nodes were positive that were analysed for extranodal extension. 312 sections were obtained from 34 lymph nodes after step serial sectioning. None of the sections demonstrated extra nodal extension after step serial sectioning. Although nodal involvement and extra nodal extension has been well studied in head and neck squamous cell carcinoma, it appears that there is limited data on detection of extra nodal extension after step serial sectioning in initially ENE negative lymph nodes. However scarce literature is available on other organs lymph node ENE after serial sectioning.

Even after extensive database search, we found very few direct studies on ENE status after serial sectioning. This indicated the potential novelty of this study and demands further research. According to a study done by Bell RB et al.¹⁴ in which they enrolled 36 cases of early stage oral squamous cell carcinoma and found that step serial sectioning did not provide any additional finding in histopathological study of lymph nodes or carcinoma. However, their study was limited by small sample size and study only on early stage oral squamous cell carcinoma. These studies were concordant with our study.

6. Conclusion

In the present scenario, many treatment decisions are based on TNM staging, imaging studies and histological findings. In the light of this, the current study demonstrated the use of step serial sectioning in detection of micrometastasis and extranodal extension. From this study we conclude that the standard protocol which we follow for grossing of specimen received in our department is adequate and step serial sectioning was not much useful in our study as it did not bring any significant change in the results. Step serial sectioning alone is more expensive and labor intensive than the conventional method therefore step serial sectioning should be combined with other methods like IHC, special stains as well for the improved sensitivity and specificity of the detection of micrometastasis and extra nodal extension.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

- [1] Cancer Today [Internet]. Iarc.fr. 2024 [cited 2024 Dec 30]. Available from: <http://gco.iarcfr/en/dataviz/bars?types=01&mode=value1&populations>.
- [2] Kumar V, Abbas AK, Aster JC. Robbins and Cotran Pathological Basis of Disease 10th ed. Vol 2. Philadelphia: Elsevier; 2020:735.
- [3] Dhawan I, Sandhu SV, Bhandari R, Sood N, Bhullar RK, Sethi N. Detection of cervical lymph node micrometastasis and isolated tumor cells in oral squamous cell carcinoma using immunohistochemistry and serial sectioning. *J Oral Maxillofac Pathol* 2016;20(3):436-44.
- [4] Shaw RJ, Lowe D, Woolgar JA, Brown JS, Vaughan ED, Evans C, et al., Extracapsular spread in oral squamous cell carcinoma. *Head Neck-J SCI SPEC*. 2010;32(6):714-22.
- [5] Patil S, Rao R, Nagaraja A. Deep sections, a guiding path for accurate diagnosis in histopathology-a retrospective study. *J Adv Oral Res*. 2013;4(2):15-20.
- [6] Elaiwy O, El Ansari W, AlKhalil M, Ammar A. Epidemiology and pathology of oral squamous cell carcinoma in a multi-ethnic population: Retrospective study of 154 cases over 7 years in Qatar. *Ann Med Surg (Lond)*. 2020;60:195-200.
- [7] Feller L, Lemmer J. Oral squamous cell carcinoma: epidemiology, clinical presentation and treatment. *J Cancer Ther*. 2012;3(4):263-8.
- [8] Sinha GM, Hegde V. Detection of micro-metastasis using cytokeratins (AE1/AE3) in haematoxylin & eosin-stained N0 lymph nodes of oral squamous cell carcinoma. *Indian J Med Res*. 2023;157(4):316-21.
- [9] Smitha T, Sharada P, Girish H. Morphometry of the basal cell layer of oral leukoplakia and oral squamous cell carcinoma using computer-aided image analysis. *J Oral Maxillofac Pathol*. 2011;15(1):26-33.
- [10] Jayasooriya PR, Pitakotuwage TN, Mendis BR, Lombardi T. Descriptive study of 896 Oral squamous cell carcinomas from the only University based Oral Pathology Diagnostic Service in Sri Lanka. *BMC Oral Health*. 2016;16:1
- [11] Prakash AR, Kumar GS, Shetty P. A Study on the detection of micrometastasis in the cervical lymph nodes of oral squamous cell carcinomas by serial sectioning. *J Clin and Diag Res*. 2011;5(1):78-81.
- [12] Bhopal T, Vadla N, Mallikarjun S, Lakshmi S. Sentinel lymph node-a captain in the management of oral squamous cell carcinoma. *J Evol Med Dent Sci*. 2018;7(51):5452-5.
- [13] Raut T, Rath R, Das SN, Besra K, Mohanty S, Mohanty A. Evaluation of micrometastasis and isolated tumor cells in node-negative early-stage oral tongue squamous cell carcinoma: a cross-sectional study in tertiary-level Page | 79 References hospitals in eastern India. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2024;137(3):274-81.
- [14] Bell RB, Markiewicz MR, Dierks EJ, Gregoire CE, Rader A. Thin serial step sectioning of sentinel lymph node biopsy specimen may not be necessary to accurately stage the neck in oral squamous cell carcinoma. *J Oral Maxillofac Surg*. 2013;71(7):1268-77.