



EVALUATION OF CERVICAL NODAL MICROMETASTASIS IN ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

The presence of cervical lymph node metastasis in oral squamous cell carcinoma carries an universally grave prognosis. A single 5- μ m section from a 1-cm lymph node samples only 1/2000th of that node. So, micrometastasis present at the time of primary diagnosis is often missed by conventional method.

MATERIAL AND METHODS: Paraffin embedded blocks of lymph nodes of neck dissection specimen which were classified as pN0 on routine histopathological examination between the year 2014 to 2016 were included in the study. Serial step sectioning was done at 200 μ m interval, stained with H&E and screened by two pathologists for micrometastasis. IHC using PAN cytokeratin antibody was done for further confirmation.

Results: 497 lymph nodes from 30 patients classified as pN0 on routine histopathological examination were studied. A total of 2150 sections were examined for micrometastasis. Most cases were pTNM Stage III(50%). On microscopic examination 2 /497 lymph nodes showed micrometastasis. Statistically significant correlation was found between micrometastasis and pathological TNM staging ($p=0.036$) and also with bone invasion.

Conclusion: On serial step sectioning micrometastasis was observed in 6.6%(2/30) of cases. Serial step sectioning along with IHC helps in detection of micrometastasis especially in higher TNM stage cases.

KEYWORDS : Oral Squamous Cell Carcinoma, Micrometastasis , lymphnode, Serial Step Sectioning

INTRODUCTION:

Oral cancer is among the top three types of cancers in India.¹ The incidence of oral cancer is highest in India and Southeast Asian countries. In India, 90 -95% of the oral cancers is squamous cell carcinoma.² The presence of cervical lymph node metastasis in oral squamous cell carcinoma carries an universally grave prognosis and it decreases the survival by more than 50%.³ In patients with oral cancer 5year survival is around 90% in Stage I and 50% in Stage II and presence of lymphnode metastasis is considered as Stage III /IV which further decreases the 5year survival. In many cases it seems as metastasis is present but not detected.⁴ Routine histopathological examinations only detect metastases larger than 2mm. Small foci of metastatic cancer called micrometastasis are often missed because of sampling problems.

Micrometastasis is defined as individual tumor deposit measuring 0.2- 2.0 mm and are typically located in subcapsular sinuses of lymphnode. A single 5- μ m section from a 1-cm lymph node samples only 1/2000th of that node.⁵ So, micrometastasis present at the time of primary diagnosis is often missed by conventional method. Studies have shown micrometastasis in 5-58 % (mean 19.6 %) of patients who were reported to be negative for metastasis on routine histopathological examination.^{6,7} The demonstration of micrometastasis in neck dissection specimen is a marker of early tumor dissemination and it helps to identify patients at increased risk of early regional recurrence. The technique of serial step sectioning (SSS) involves cutting of the LN block at different intervals until the block is completely exhausted was introduced to detect occult metastatic deposits. Due to limitations of hematoxylin and eosin (H&E) alone, the adjunctive use of IHC and Serial step sectioning has been suggested for enhanced tissue interpretation and proper diagnosis of N₀ Oral squamous cell carcinoma patients. This combination has resulted in upstaging leading to adequate regional treatment and therefore improving diagnosis and prognosis of these patients.⁸

This study was done to determine the frequency of micrometastasis in cervical lymph node in oral squamous cell carcinoma patients

with pN0 disease with the help of serial step sectioning and correlate it with histopathological features.

MATERIAL AND METHODS:

STUDY DESIGN – Retrospective study.

SOURCE OF DATA – Neck dissection specimens of oral squamous cell carcinoma with histopathologically reported as pN₀ which were received in the Dept. of Pathology R.L.Jalappa Hospital and Research Center attached to Sri Devaraj Urs Medical College, Tamaka, Kolar from 2014 to 2016 were included in the study.

Ethical clearance was obtained from Institutional ethical committee to conduct the study Paraffin embedded blocks of lymph nodes of neck dissection specimen of 30 patients were included in the study. Neck node positive cases were excluded from the study.

Serial step sections, each measuring 3–5 μ m, were retained for every 200 μ m throughout the block. The number of sections obtained varied according to the size of lymphnode. The slides were stained with H&E and screened by two pathologists for micrometastasis. IHC using pancytokeratin antibody was performed in suspected cases of Micrometastasis for further confirmation..

The pathologic reporting of lymph nodes were as follows

Negative – reactive changes, sinus histiocytosis, lymphostasis, benign inclusions etc.

Isolated tumor cells – small clusters of cells not greater than 0.2 mm, or nonconfluent or nearly confluent clusters of cells not exceeding 200 cells in a single cross sections

Micrometastasis – individual tumor deposit larger than 0.2 mm but not exceeding 2.0 mm

Macrometastasis – individual tumor deposit larger than 2.0 mm
Tumour thickness is defined as the distance measured from the surface of the tumour including the keratin to the point of

maximum invasion in the underlying connective tissue stroma. In cases of ulcerated tumours, base of the ulcer serves as the reference point.

Depth of invasion was measured from the basement membrane to the deepest point of invasive tumor in paraffin-embedded sections.

Statistical analysis:

Data was entered into Microsoft excel data sheet and was analysed using SPSS 22 version software. Fischer exact test was used as test of significance. p value <0.05 was considered as statistically significant.

Results:

30 oral squamous cell carcinoma patients classified as pN₀ on routine histopathological examination were studied. Female predominance was noted with F:M ratio of 2.7:1. Common site was buccal mucosa (90%) and then by anterior 2/3rd of tongue [10%]. Mean age of distribution was found to be 54.83.

Clinical staging in most cases were TNM stage III [56.7%] followed by stage IV a [43.3%].

Tumor size in most cases were 2-4cm (50%), >4cm in 30% cases and <2cm in 20% cases.

Most cases were well differentiated (77%) followed by moderately differentiated (33%).

On Histopathological examination pTNM staging was III in 50% cases followed by Stage IVa (20%), Stage I (20%), Stage II (10%).

497 lymph nodes which were reported as negative for metastasis were serially sectioned. on an average 16 lymph nodes were retrieved from each case. A total of 2150 sections were examined for micrometastasis. Micrometastasis was observed in 2 out of 30 cases (6.6%) i.e. 2/497 lymph nodes showed micrometastasis (Fig.1). Diagnosis of Micrometastasis was confirmed by Pan cytokeratin antibody positivity by IHC (Fig.2).

On statistical analysis no significant association was found between clinical TNM stage and Micro metastasis.

Significant association was observed between Micro metastasis and Pathological staging. p=0.036

Significant association was observed between micrometastasis and bone invasion i.e. both the cases of micrometastasis had bone invasion.

There was no significant difference in mean tumor thickness and tumor depth with respect to micrometastasis status. No lymphovascular invasion was seen in the study population.

Discussion:

Lymphnode metastasis is an important prognostic factor for a patient with oral squamous cell carcinoma. Studies have shown that lymphnode measuring more than 2cm might have reactive hyperplasia without metastasis on histopathological examination. General policy of elective neck dissection based on clinical TNM staging exposes many OSCC patients to neck dissection that may not be necessary.⁹ There is absence of reliable preoperative assessment to confirm metastasis in lymphnodes prior to neck dissection. Sentinel lymphnode biopsy and superselective neck dissection have their pitfalls and has not become the standard of care. In a study a concordance rate of 52% was found for the cT- and pT-classification. Overstaging of the extent of the primary tumor (59%) was found more frequently than understaging of the size.¹⁰ Because of the impact of nodal status on treatment and survival in OSCC, accurate staging of cervical lymphnode is critical. Oral cancers are more common in males than females. However, there is a rise in the incidence of these malignancies in females.

Reverse gender ratio was observed in India (Bengaluru), where male to female ratio was 1:2.0 and Mumbai cancer registries study also reported with 1:1.^{11,12} However in India a higher incidence is seen in males. In a study by in Kerala, 57.8% were males and 42.2% were females.¹³ In a study in Gujarat 75% of oral cancers were reported in males.¹⁴ In our study female predominance was seen and they presented at advanced stage. This could be due to common habit of betel quid chewing in the study area. Women have substantially high level of chewing habits than men in many rural areas, as they believe that tobacco has magical and medicinal properties and also delayed seeking of medical care and lower acceptance of treatment is seen more in females.

Sankaranarayan *et al.* found that the peak-age frequency of occurrence of oral carcinoma is the fifth decade in India which is at least a decade earlier than that described in the western literature.¹⁵ In our study mean age of distribution was found to be 54 years.

Site of involvement in oral malignancy has a variable geographical distribution. In our study, buccal mucosa was the commonest site of oral cancer. In South India, buccal mucosa carcinoma is the most common oral subsite, and about 70% of the cases presents at an advanced stage of disease.¹⁶ In a study in Kerala buccal mucosa was reported as the most frequent site.¹³ Tongue cancer are the most common cancer in Mumbai, Ahmedabad and Kamrup district in Northeast. Tongue cancer is also found to have higher rates of cervical nodes metastasis.^{17,18} There was no significant relationship found between micrometastasis and primary site in a study by Jung Hae Cho *et al.*

Clinical TNM system is widely used by clinician for the management of OSCC patients. According to several studies, lymph node metastasis risk increases if tumor size exceed 2 cm. In a study in China it was reported that nodal metastasis risk is increased for the lesions of size larger than 3 cm.¹⁹ Shear *et al.* in nearly 900 patients found grade and size as predictive factors of lymph node metastasis.²⁰ In our study maximum cases were found to have tumor size between 2-4cm. The two cases with micrometastasis in our study showed size >4cm. Jung Hae Cho *et al.* found a significant positive correlation found between the presence of micrometastasis and the tumor size.

In the current study, maximum number of cases were well differentiated. Both the cases of micrometastasis were from moderately differentiated squamous cell carcinoma. Kurokawa *et al.*²¹ and Pimenta *et al.*²², found a significant correlation between histological grades of differentiation and occult nodal metastasis. In a study by Jung Hae Cho *et al.* no significant correlation was found between the presence of micrometastasis and the histological grade of differentiation.

Many studies on correlation of tumor thickness, tumor depth with lymphnode metastasis are available in literature. In a study by Shao hui hang *et al.*²³ concluded that the optimal cutoff point for tumor thickness is 4 mm for risk of cervical lymphnode metastasis and consideration for neck management. However most of these studies addressed carcinoma tongue and not buccal mucosa which is more common in our study area.

In a study by Almgush *et al.*²⁴, high recurrence and lymphnode metastasis risk was found to be present in tongue cancers with an invasion depth of 4 mm or more. While in a study in Tata memorial hospital Mumbai it was found that tumor depth more than 5 mm carries a high risk for cervical lymphnode metastasis in cancers of oral cavity.²⁵ In a landmark study in Korea a significant positive correlation was found between the presence of micrometastasis and the tumor depth of invasion. However there is no agreement regarding optimal cutoff point for tumor depth in buccal cancer for the risk of cervical node metastasis.

Many studies have found a positive correlation between lymphovascular invasion and lymphnode metastasis. However in

our study none of the cases showed lymphovascular invasion.²⁷

In a study by Ross GL et al. bone invasion was found to predispose to cervical metastases.²⁸ In our study both the cases of micrometastasis showed bone invasion.

Histopathological examination is highly sensitive and specific test for detection of metastasis but earliest stage of metastasis to lymph node especially whenever there is a small foci of metastatic cancer called micrometastasis are often missed because of sampling problems. Different methods were employed to detect micrometastasis in which serial sectioning was commonly used. Many controversies exist on whether serial sectioning is actually helpful or not especially because it is a tedious process and is difficult to implement on routine work.

Table 1; Comparison of studies using serial step sectioning

	Ambrosch et.al 1996 ⁶	Prakash et.al 2011 ⁷	Ferlito et.al 2002 ²⁹	Present Study 2016
Lymph nodes studied	1020	119	554	497
No. of cases	76	16	73	30
Positive cases	6	2	16	2
Percentage of micrometastasis	7.9%	12.5%	21.9%	6.6%

Due to limitations of the conventional methods, newer methods such as immunohistochemistry (IHC) and molecular assays were introduced for improved detection of micrometastasis within the lymph node. Immunohistochemistry is a sensitive & confirmatory test to detect occult metastasis, micrometastasis or isolated tumor cells in the dissected lymphnodes which show negative metastasis on histopathology.³⁰ In our study the cases which were suspected for micrometastasis were further confirmed by IHC.

Use of IHC can help in upstaging of clinically N₀ cases. Barrera et al reported the upstaging of 29% of N₀ cases by IHC⁸ and Hamakawa et al. found micrometastasis in 15.9% of N₀ cases by use of IHC.³¹ Kwon et al. reported 13.8% micrometastasis by IHC staining in cases negative on HPE.³²

In a study in Korea, micrometastasis was compared with various clinicopathological factors. Jung Haecho and his colleagues studied 54 neck dissection specimens and found that micrometastasis represents an adverse prognostic factor and provides a valuable prognostic information in patients. It was concluded that clinically node negative oral SCC patients with micrometastasis should be considered for additional adjuvant therapy especially in cases of deep invasion and large tumor size.³⁶ In our study micrometastasis was also compared with various clinicopathological parameters and micrometastasis was found to have a positive correlation with pTNM staging.

The International Breast Cancer Study which was one of the largest micrometastases studies, showed that both disease free interval and overall survival were significantly affected by the presence of micrometastatic disease at 5 and 6 years of follow-up.³³ The presence of lymph node metastases remains the most reliable prognostic predictor and the gold indicator for adjuvant treatment in colon cancer.³⁴ But more studies are required to evaluate the prognostic significance of micrometastasis in lymphnodes in oral cancer and its importance in clinical management.

CONCLUSION

Although more laborious and expensive than conventional methods, the combined application of IHC and serial step sectioning can be used for improved diagnosis, node negative oral squamous carcinoma patients especially in higher TNM stage cases.

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