Detection of Micrometastasis in Lymph Nodes of Oral Squamous Cell Carcinoma - IHC Study

Dinsha. K N[1], Priya N S[2], Kavita Rao[3]

ABSTRACT

Introduction: Squamous cell carcinoma (SCC) of the head and neck is the eighth most common malignancy in the world, which exhibits poor survival due to the high rate of loco regional LN metastasis. Malignant tumors of epithelial origin often metastasize to the RLNs. Micrometastasis (MM) represents tumor deposits measuring less than 2 mm in diameter. It is very difficult to diagnose MM by routine histopathological examination. So detection of MM by immunostaining using Pancytokeratin will yield a greater incidence of positive cervical lymph node MM. Aim: To detect the presence of MM in clinically negative lymph nodes.

Material and Methods: In this study clinically and histopathologically confirmed cases of SCC with neck dissection (n=30), retrieved from the department of oral and maxillofacial pathology. Demographic data and Grading of OSCC were collected .

Results: The cytokeratin expression was positive in 2 cases (11.8%) and negative in 28 cases (88.2%). The obtained 'p' value was 0.47, which is not statistically significant. Reactive changes in lymph nodes of different grades of OSCC were observed using H&E stain, among the nodes studied, the chief pattern noted was lymphocyte predominance. Thus, with this small sample size it is difficult to attain more reliable results. Further studies are required to be done, using more reliable methods and larger sample size for accurate detection of MM in lymph nodes of OSCC.

Keywords: Cervical Lymph Nodes, Micrometastasis, Oral Squamous Cell Carcinoma

INTRODUCTION

Squamous Cell Carcinoma (SCC) is the most common neoplasm of head and neck region, which exhibits poor survival due to the high rate of loco regional lymph node metastasis. Incidence of lymph node metastasis is high in head and neck cancer and is considered as the most important prognostic marker. OSCC of the oral mucosa and lips, however, comprises 90-95 per cent of all oral malignancies.[1] It has been reported that 21.9% of patients with oral cancer have micrometastasis with an average diameter of 1.36 mm.[2] OSCC is defined as 'a malignant epithelial neoplasm exhibiting Squamous differentiation as characterized by the formation of keratin and / the presence of intercellular bridges'. (Pindborg J et al 1997).[3] It accounts for 3% of all cancers in men and 2% in women. It ranks as the sixth most common cancer in men and twelfth in women. The account male/ female incidence ratio is approximately 1.8:1.[4] Most important mechanism for the spread of cancer cells in oral Squamous cell carcinoma is by lymphatic route. Tumor cells metastasize into the draining lymph nodes and these metastatic lymph nodes become visible and palpable, as a result of tumor cell entrapment. Clinical examination, radiological imagining and histopathological findings can assess lymph node status in OSCC.[5]

Micrometastasis represents tumor deposit measuring less than 2 mm in diameter. Histopathological examination is the highly sensitive and specific test for detection of metastasis but the earliest stage of metastasis to neck can be difficult to identify by light microscopy.[6]

Cervical lymph node status assumes a prime role and metastasis to lymph nodes have diminished the survival rate of patients in comparison with patients with negative metastasis. The presence of lymph node metastasis can be detected clinically, radiologically and ultrasound guided fine needle aspiration cytology. Current methods such as computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET)are widely used for preoperative detection of metastasis.[13]

It has been recognized that the microscopic pattern of a regional lymph node can represent an indicator of the immunologic reactivity in that node. Tsakraklides (1973) has formulated a morphologic- immunologic analysis of lymph nodes based on the presence or absence of the reactive proliferation of lymphocytes within regional lymph nodes. (6)

Microscopic examination of dissected lymph nodes still remains the gold standard for detection of lymph node metastasis. However, if a lymph node is examined only by one central section, micrometastases, which are typically localized in the sub capsular sinuses of lymph nodes, are likely to be missed. For this reason, it is very difficult to diagnose micrometastases by routine histopathological examination. So detection of micrometastases by immunostaining using Pancytokeratin will yield a greater incidence of positive cervical lymph node micrometastasis.[7]

Prognosis of OSCC is difficult to predict, despite the diagnosis

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and therapeutic progress in the field of oncology. There are various factors which influence the prognosis of a tumor. Among all the factors, development of lymphatic metastasis is considered as the single most important factor influencing the outcome of the patient with OSCC. The 5-year survival rate of patients with OSCC of the upper aerodigestive tract is reduced by almost 50% with the development of cervical metastases. [4] Thereby, the present study was done to find out the reactive changes in lymph nodes with routine H&E stain and presence of micrometastasis in negative nodes using the immunohistochemistry method.

At this juncture, it is important to understand the dynamics of micrometastasis and detect its presence in lymph nodes, as regional lymph nodes are the first site of areas of tumor cells and hence the strongest predictor of disease prognosis and outcome. Cervical lymph node status assumes a prime role and metastasis to lymph nodes have diminished the survival rate of patients in comparison with patients with negative metastasis.

MATERIAL AND METHODS

The present observational study includes archival samples of clinically and histopathologically confirmed cases of Oral Squamous Cell Carcinoma with neck dissection (n=30), retrieved from the department of oral and maxillofacial pathology.

30 negative lymph node sections were detected immunohistochemically for pancytokeratin expression.

30 negative lymph node sections were assessed for reactive changes in H&E stained sections using light microscopy.

Inclusion criterias were, Cases of primary Oral SCC with surgical neck dissection and lymph nodes irrespective of levels (Level I – Level V) were considered. Secondary or Metastatic cases of Oral Squamous cell carcinoma were excluded from the study.

H&E-stained sections were observed under light microscope. Lymph node reactivity patterns were established in accordance with a previous proposal for a standardized system of reporting human lymph node morphological characteristics in relation to immunological function given by WHO. The possible five patterns were assessed, (1). Lymphocyte predominance pattern (2). Germinal center predominance (3) Mixed pattern (sinus histiocytosis) (4.) Unstimulated pattern and (5) Lymphocyte depletion pattern. Depending upon the predominant lymph node immunomorphological pattern, the cases were then classified into the above five groups. Lymph nodes showing more than one reactivity pattern were classified into the most predominant type. [12]

RESULTS

The study included 30 negative lymph nodes from the diagnosed cases of OSCC with neck dissection. Of the 30 cases, 11 cases were of well differentiated grades, 16 were moderately differentiated and 3 were poorly differentiated grades of OSCC. The age ranged from 20-70 years and gender distribution was equal. Site distribution included

TABLE 1: Positivity of cytokeratin expression between different grades of OSCC

Positivity of Cytokeratin between different grades of SCC using Chi square test								
	PDSCC		MDSCC		WDSCC			
IHC Stain	n	%	n	%	n	%	χ^2 Value	P-Value
Positive	0	0.0%	2	11.8%	0	0.0%	1.516	0.47
Negative	3	100.0%	15	88.2%	9	100.0%		

TABLE 2a: Presence of reactive lymph node pattern in OSCC

Lymph node pattern	Number of cases
GCP	03
LP	16
SH	06
SH+GCP	01
UN	03

GCP-Germinal centre predominance, LP-lymphocyte predominant, SH-Sinus histiocytosis, UN-Unstimulated node.

Table 2b: Comparison of negative lymph node patterns between different grades of OSCC:

Comparison of Negative Lymph node patterns between different grades of OSCC using Chi square test								
	PDSCC		MDSCC		WDSCC			
LN Pattern	n	%	n	%	n	%	χ^2 Value	P-Value
GCP	0	0.0%	2	66.7%	1	33.3%	7.376	0.50
LP	3	18.8%	9	56.3%	4	25.0%		
SH	0	0.0%	5	83.3%	1	16.7%		
GCP+SH	0	0.0%	0	0.0%	1	100.0%		
UN	0	0.0%	1	33.3%	2	66.7%		

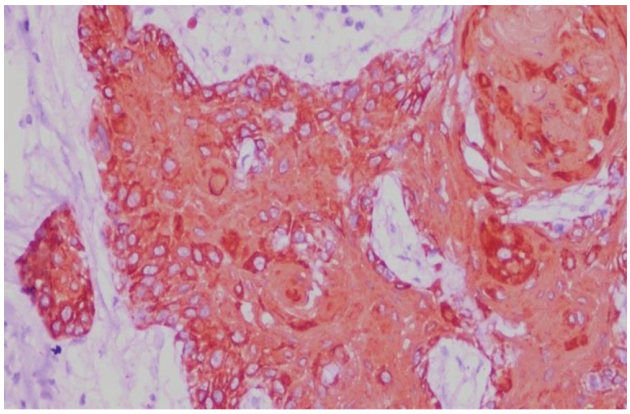
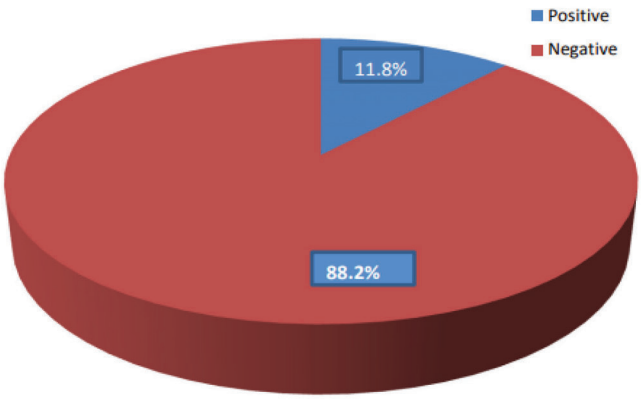
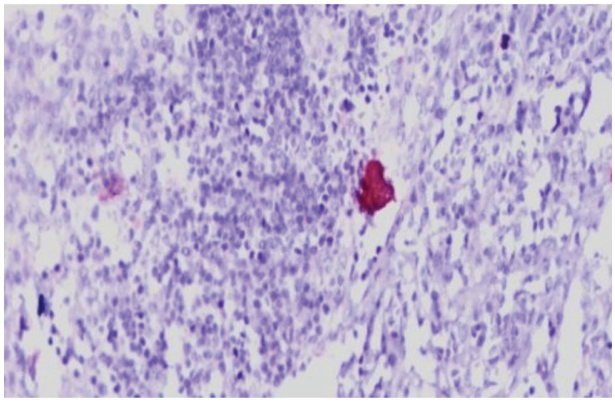


Figure-1: Positive Immunostaining with pan-cytokeratin (AE1/AE3) (x40) in control group.



Graph-1:cytokeratin expression in negative lymph nodes



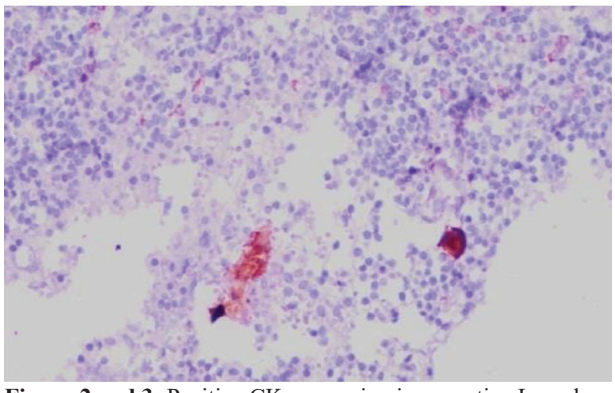
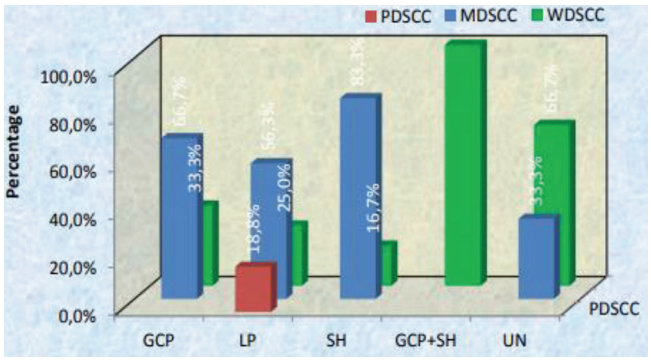


Figure-2 and 3: Positive CK expression in negative Lymph nodes (AE1/AE3) (x10)

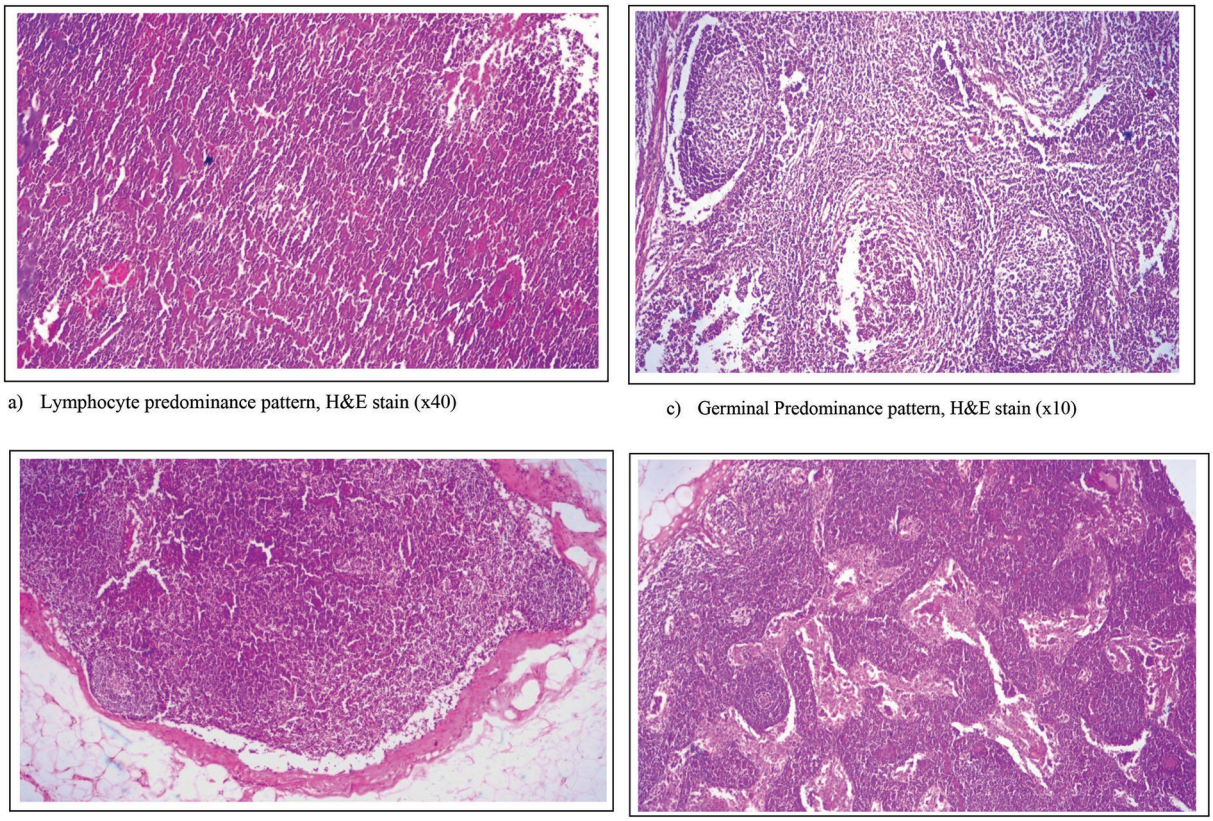


Graph-2: Histological patterns of negtaive lymph nodes with different OSCC grades.

alveolar mucosa, alveolar ridge, GB sulcus, anterior hard palate, floor of the mouth, tongue, and Buccal mucosa.

The present study was undertaken to detect the presence of micrometastasis from negative lymph nodes of OSCC using IHC for cytokeratin expression. All the 30 cases were assessed for the presence of micrometastasis, only 2 cases out of 30 showed positivity for CK expression. (Figure-1), (Graph-1)

The cytokeratin expression was positive in 2 cases (11.8%) and negative in 28 cases (88.2%). The obtained 'p' value was 0.47, which is not statistically significant. (Table-1) (Figure 2-3)



a) Lymphocyte predominance pattern, H&E stain (x40)

c) Germinal Predominance pattern, H&E stain (x10)

b) Unstimulated node, H&E stain (x10)

d) Sinus histiocytosis pattern, H&E stain (x10)

Figure-4: Patterns of lymph nodes in H& E stain

Reactive changes in lymph nodes of different grades of OSCC were observed using H&E stain and a correlation was made with the grades of OSCC and predominant patterns present in lymph nodes. Among the nodes studied, the chief pattern noted was LP . The least noted pattern was combination of SH+GCP .The other patterns noted were GCP, SH and UN. (Table-2a) (Figure-4) (Graph-2)

The predominant lymph node pattern in well, moderate and poorly differentiated OSCC was found to be lymphocyte predominant. The 'p' value obtained for comparison of LN patterns between different grades of OSCC was 0.50 which is not statistically significant. (Table 2b) (Graph 2)

DISCUSSION

Squamous cell carcinoma of the head and neck (HNSCC) is the eighth most common malignancy in the world, which exhibits poor survival due to the high rate of loco regional LN metastasis. [8] Regional lymph nodes (RLN) are considered to have their primary function not merely as anatomic barriers to the systemic dissemination of tumor cells but also as immunologic surveillance outposts. RLNs play a pivotal role in diagnosis, staging, and management of the disease. Malignant tumors of epithelial origin often metastasize to the RLNs. The presence of metastatic tumor in RLNs is the most important prognostic factor for patients with malignant tumors of epithelial origin. [9]

The incidence of lymph node metastasis depends mainly on the size and site of the primary tumor. The prognosis depends upon the factors relating to tumor, treatment and patient. Recent studies have noted a dynamic inter relationship between the immunologic capacities of lymphoid system and malignancy. The ever-growing evidence indicates that an important relationship exists between competence of the immunologic apparatus and the incidence of certain kinds of neoplasia.[14]

IHC has been introduced in hope to increase the detection rate of micrometastasis. CKs are applied most commonly for analysis of LN metastasis in OSCC patients. It is an important marker to differentiate epithelial tumors from the non-epithelial ones with a high degree of accuracy. Various studies were undertaken to check for the specificity of cytokeratin in the detection of micrometastasis.

Van den Brekel et al (1992) investigated the incidence of micrometastases in neck dissections of patients with head and neck cancer. Micrometastases were found in 66 lymph nodes in 41 of the 92 tumor-positive neck dissection specimens. [10]

In the present study out of 30 cases of lymph nodes of OSCC, 2 cases were positive for cytokeratin expression. H& E stained sections of these 2 cases showed a pattern of lymphocyte predominance and unstimulated node each. But the results were not statistically significant. Conventional methods of examining the lymph node in OSCC includes only the presence or absence of metastasis.

However, results of this study highlights the status of immune system which in turn is related to the prognosis of the patient. It would be prudent to include the immunomorphological evaluation of lymph node in routine basis which would give a fair idea for a comprehensive treatment plan. Based on

published data's, it appears that the node related parameters such as number of positive nodes, nodal immune response, percentage of the node replacement and extracapsular spread represents a significant prognostic indicator. [11]

The most commonly encountered problems with IHC staining are cross-reactivity with other antigens, false positivity, non-specificity and background staining. This may result in inaccurate results. These issues can overcome by doing serial sectioning of each portion. Serial sectioning of lymph nodes at different intervals gives a better result for detection of MM. However, this method was not employed in our study. A larger sample size would have given a better statistical value in the present study. Further studies with newer methods are needed for better understanding of detection of MM.

Combination of serial sectioning with IHC and RT-PCR methods gives better results than IHC. The combined application of these methods is justified for improved diagnosis and treatment planning of N0 OSCC patients. It should be recommended for diagnostic use in controlled studies of patients with negative LN metastasis on routine H&E stain, sparing low risk patients the morbidity of unnecessary treatment while appropriately identifying aggressive tumors that warrant adjuvant therapy despite their early stage. [6]

CONCLUSION

This study was undertaken to detect the micrometastasis in negative lymph nodes using cytokeratin and to study the reactive patterns of negative lymph nodes of OSCC and correlate it with the clinical parameters. The present study showed positive expression of cytokeratin in only 2 cases out of 30. Hence, IHC alone may not be the reliable diagnostic aid for detection of MM. Thus, combined methods of IHC with serial sectioning and newer methods like RT-PCR are needed for much better results for MM detection.

Reactive changes in 30 negative lymph nodes evaluated using H&E staining showed four immunologic patterns, among which the predominant pattern noted was lymphocyte predominance and a correlation was made with different grades of OSCC. Since the IHC expression was present in only 2 cases, this correlation was not statistically significant. To conclude, IHC may not be the reliable diagnostic aid for detection of MM as compared to molecular methods. Though, it is proved to be of high significant value in comparison with HPE. Further studies are required to be done, using more reliable methods and larger sample size for accurate detection of MM in lymph nodes of OSCC.

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