

ORIGINAL ARTICLE

Immuno-reactivity of excised lymph nodes in neck dissections of squamous cell carcinomas of oral cavity

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Received: 26-11-2012

Accepted: 12-06-2014

ABSTRACT

Background: Regional lymph nodes play an important role in acting as anatomic barriers to systemic dissemination of tumor cells. This reflects in the host immunologic response. Oral squamous cell carcinoma, is known to be associated with early deficiencies of cell-mediated immunity, the pathology of which is reflected in the histology of the regional lymph nodes. **Aim:** The goal of this study was to study the different immunity reactions in the lymph nodes and to correlate it with the histopathology of tumor proper. **Materials and Methods:** Out of 40 head and neck dissections, 30 were male and 10 females within the age range of 21–72 years. According to Tumor Node Metastasis classification, there were 12 cases of stage II and stage III, respectively while 16 were of stage IV. A total of 372 nodes were histologically evaluated. The harvested lymph nodes were categorized into metastatic and non-metastatic nodes exhibiting four patterns as: Lymphocyte predominance pattern, germinal center predominance pattern, lymphocyte depleted pattern and unstimulated pattern. **Results:** The predominant pattern of lymph node reactivity was of lymphocyte predominance (199 nodes) followed by germinal center predominance (117 nodes); lymphocyte depleted (17 nodes) and unstimulated node pattern (39 nodes). Twenty-seven nodes were positive for metastasis. A statistically significant relationship ($P = 0.0019$ and $P = 0.0290$, chi square, respectively) was observed between the number of nodes harvested and stage and level of lymph nodes. A brief follow-up period of 3 years was carried out. **Conclusion:** Further studies relating the immuno-morphologic assessment of the lymph nodes in conjunction with other factors may be helpful in assessing the metastases risk of the individual.

Key words: Cervical lymph nodes, immuno-morphological pattern, oral squamous cell carcinoma

INTRODUCTION

Squamous cell carcinoma is the most common neoplasm and comprises approximately 80% of the cancers of the oral cavity and despite optimal treatment, the prognosis remains poor.^[1] Lymphatic metastasis is the most important mechanism in the spread of head and neck squamous cell carcinomas and is also the main determinant of the stage and prognosis.^[2] The

incidence of lymph node metastasis depends mainly on the size and site of the primary tumor.^[2] Survival decreases by 50% with the presence of regional lymphadenopathy.^[3] Staging systems are designed to compare similarly staged patients, to facilitate prognosis and to provide useful information for patient management.

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How to cite this article: Suchitra G, Puranik RS, Vanaki SS, Prasad BG, Malgaonkar NI. Immuno-reactivity of excised lymph nodes in neck dissections of squamous cell carcinomas of oral cavity. J Oral Maxillofac Pathol 2015;19:128-33.

Access this article online

Quick Response Code:



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www.jomfp.in

DOI:

10.4103/0973-029X.164520

The prognosis depends on factors relating to tumor, treatment and patient.^[4] With the remarkably rapid advances in modern immuno-biology in the last decade, it has come to realization that there is indeed a dynamic inter-relationship between the immunologic capacities of the lymphoid system and malignancy. With modern knowledge of the distribution and function of the various classes of lymphocytes within the lymph nodes, the analysis of changes within lymph nodes draining an antigenic challenge has regained its rightful prominence.^[5] Ever-growing evidence indicates that an important relationship exists between competence of the immunologic apparatus and the incidence of certain kinds of neoplasia. Further, active immunologic defenses can be detrimental to malignant tumor growth. In addition, it has been recognized that the microscopic pattern of a regional lymph node can represent an indicator of the immunologic reactivity in that node. On this basis, Tsakraklides^[6] has formulated a morphologic-immunologic analysis of lymph nodes regional to malignant tumors. This analysis based on the presence or absence of the reactive proliferation of lymphocytes within the regional lymph nodes has been employed to associate with the prognosis of the patient. Thus, the present study has been undertaken as an attempt to correlate the clinical and histological features of primary lesion with pattern of lymph node metastases and also to study the architectural changes in lymph nodes with or without metastases and thus elucidating the immunologic host-tumor relationship and its correlation with the histopathology and staging of primary tumor.

MATERIALS AND METHODS

A retrospective research investigation with a total of 40 neck-dissection cases was obtained. The selection criteria for the cases included those cases with a total nodal yield of six or more. Case history was obtained on a proforma and the follow-up period of 3 years was observed. The primary tumor area of the specimen was selected for histopathological examination and the lymph nodes at all levels were carefully harvested and fixed in 10% formalin for 48 hours, routinely processed and the paraffin blocks prepared. Serial tissue sections of 4–5µm thickness were obtained and the slides were stained with routine hematoxylin and eosin stain and further evaluated for the histopathologic changes.

The primary tumor grading was categorized according to World Health Organization grading as well differentiated, moderately differentiated and poorly differentiated based on the squamous differentiation, degree of keratinization and presence of intercellular bridges.

The lymph nodes were assessed using the criteria of Tsakraklides *et al.*^[6] and categorized as:

Lymphocyte predominance

- Lymph nodes are moderately enlarged

- Increased number of small lymphocytes throughout the cortex, the para-cortical area and the medullary regions
- Cortex shows lymphoid follicles without germinal centers
- Expanded paracortical area
- Prominent endothelial cells in capillaries and postcapillary venules
- Sinusoids show sinus histiocytosis and/or large numbers of large and small lymphoid cells.

Germinal center predominance

- Lymph nodes larger than in lymphocyte predominance
- Germinal centers contain large lymphoid cells and mitotic figures
- Para cortical areas unremarkable
- Enlarged medullary cords with increased number of plasmablasts and plasma cells.

Lymphocyte depletion

- Hypocellular and fibrotic
- Small and often show small hyaline deposits on the wall of blood vessels of paracortical area and larger patches of hyaline in the medullary cords and extensive amounts of hyaline could be found deposited in any part of the lymph node
- Relative increase in the number of plasma cells.
- Germinal centers are absent
- Lymphocytes markedly depleted throughout the lymph node.

Unstimulated node

- Cortical lymphoid follicles without germinal centers
- Paracortical areas are small and hypocellular
- Medullary cords are unremarkable
- No significant fibrosis and no hyaline deposition.

Observation was also carried out for the presence or absence of metastasis within the nodes. The results were statistically analyzed using the Chi-square test.

RESULTS AND OBSERVATIONS

The study included lymph nodes from 40 patients of oral squamous cell carcinoma in whom the treatment rendered was neck dissection of any type. Of these patients, 30 cases (75%) were male and 10 cases (25%) were female patients. The age range of these patients was from 21 to 72 years, with a mean age range of 48.93 for male patients and 50.60 for female patients. The site of the lesion included areas from retromolar region, buccal mucosa, tongue, gingivobuccal sulcus and alveolar mucosa. Thirty-three cases were of well-differentiated squamous cell carcinoma out of which 25 cases (75.76%) were male and 8 (24.24%) were female patients. Seven cases

were of moderately differentiated squamous cell carcinoma, out of which 5 (71.43%) were male patients and 2 (28.57%) were female patients. The relationship of the tumor grade to sex of the patient had no statistical significance.

The stage distribution of the tumor was also compared to the sex of the patient. Among stage II (12 cases), 11 cases (91.67%) were male and 1 case (8.33%) was female. Among stage III (12 cases), 6 (50%) were male and 6 (50%) were female. Among stage IV (16 cases), 13 (81.25%) were male patients and 3 (18.75%) were female. Chi-square test revealed a statistically significant result with *P* value of 0.0471 [Table 1].

The total number of nodes harvested among these 40 neck dissections was 380. Out of these, 8 nodes were excluded from histological interpretation due to technical faults and a total of 372 nodes were considered for the study [Table 2]. Lymphocyte predominance pattern was seen in 199 nodes (53.49%) [Figure 1], germinal center predominance pattern was noticed in 117 nodes (31.45%) [Figure 2], lymphocyte depleted pattern in 17 nodes (4.57%) [Figure 3] and unstimulated node pattern in 39 nodes (10.48%) [Figure 4].

The nodal yield in each level was Level I-116, Level II-101, Level III-97, Level IV-31, Level V-27 nodes. Correlation depicted that the number of nodes harvested decreased with increasing levels of lymph nodes. (*P* value = 0.0019) [Table 3].

The distribution of number of lymph nodes compared to the stage of the tumor was found to be statistically significant with *P* value of 0.029, implying that as the stage of the tumor increased from stage II to IV, the number of nodes harvested increased, but the distribution of lymph node patterns as regards to the age of the patient and sex of the patients had no significant correlation. Apart from these basic four histological patterns, other histological features such as follicular hyperplasia, sinus histiocytosis and prominent

Table 1: Stage-wise distribution of cases

| Staging | Male | % | Female | % | Total |
|---------|------|-------|--------|-------|-------|
| II | 11 | 91.67 | 1 | 8.33 | 12 |
| III | 6 | 50.00 | 6 | 50.00 | 12 |
| IV | 13 | 81.25 | 3 | 18.75 | 16 |
| Total | 30 | 75.00 | 10 | 25.00 | 40 |

Chi-square=6.1110, df (degree of freedom)=2, *P*=0.0471, Significant

Table 2: Distribution of total nodes harvested

| Category | Number of nodes | % |
|----------|-----------------|--------|
| LP | 199 | 53.49 |
| GCP | 117 | 31.45 |
| LD | 17 | 4.57 |
| US | 39 | 10.48 |
| Total | 372 | 100.00 |

LP: Lymphocyte predominant pattern, GCP: Germinal center predominant pattern, LD: Lymphocyte depleted pattern, US: Unstimulated node

vascularity within the lymph nodes were also observed. Fifteen nodes showed follicular hyperplasia, 21 with sinus histiocytosis and 43 nodes had prominent vasculature. The lymph nodes positive for metastases were 13 (48.15%) in level I, 7 (25.93%) in level II, 3 (11.11%) in level III, 2 (7.41%) in level IV, 2 (7.41%) in level V [Table 4]. The result was statistically significant implying decreased rate of positive nodes with increasing levels.

DISCUSSION

To date, very few studies have been conducted relating the immuno-morphologic assessment of the excised lymph nodes. Although in recent years numerous biological and molecular factors have been proposed as prognosticators in oral and oropharyngeal squamous cell carcinomas, these are yet to have an impact on routine clinical care and detailed histopathological staging of surgical resection specimens remains an important determinant of post-operative management and prognosis prediction.^[7] This study was an attempt to correlate the immune-reactivity with the tumor proper. The study showed increased number of male patients with advanced stage which was in accordance with the literature^[8,9] and that may be attributed to the relatively increased frequency of habits related to tobacco usage in male patients over female patients. Lower mean age range in males also reflects increased exposure of the young population to the deleterious habits rendering them more vulnerable to the development of debilitating diseases such as precancerous or cancerous lesions or various other disorders.

In our study, buccal mucosa was the most common site (25 cases) followed by alveolar mucosa and tongue which was in accordance to few studies^[10], but contrasts with others.^[9] This discrepancy may be related to the few number of cases undertaken in the present study and also to the site of placement of smokeless tobacco in the buccal vestibule which is more prevalent in Indian subcontinent than in other geographical areas. Findings similar to that of the previous studies regarding the size of the primary tumor were observed in the present study with 16 (40%) of T2; 9 (22.5%) of T3, 15 (37.5%) of T4 cases and absence of T1 cases.^[8,9] The less number of T1 cases in other studies and the absence of T1 cases in our study may reflect the delayed reporting of the patients to the clinics due to late presentation of the symptoms and subsequent diagnosis. Our study comprised more of clinically N0 stage which differs from previous studies wherein more of N2 stage was observed. This could be attributed to the difference in the selection criteria of the cases. The distribution of the number of nodes harvested at different levels corresponds to the topographical distribution of the lymph nodes.^[11]

There were 33 well-differentiated cases and 7 cases of moderately differentiated squamous cell carcinoma. In the present study, poorly differentiated cases were unavailable and hence this limitation hindered the effective comparison

between the tumor grades. But metastatic nodes were more evident in moderately differentiated tumor cases and thus histological differentiation remains a useful prognostic indicator.^[12] But future work should analyze the influence of this factor involving all the grades. Also the metastatic nodes were more in stage IV cases, suggesting that survival and recurrence of patients with neck dissection cases correlates significantly with the stage of the disease.^[13] With a 3 year follow-up in our study out of the 16 cases with stage IV disease, 4 patients died within a span of 2 years.

Metastasis followed a decreasing frequency of order from levels I to V as in accordance with previous studies.^[2,11,14] A higher incidence of metastasis positive lymph nodes in level I affirm the fact that nodal involvement is primarily determined by locoregional spread from primary anatomical site. But, this is in contrast to patterns in tongue region, the nodal involvement frequently is in level II^[15] reflecting the pattern of lymphatic drainage. Metastases negative nodes also exhibit spectrum of histopathological changes which may provide more information in assessing the impact of tumor on the host or individual's immunity. Thus, the assessment

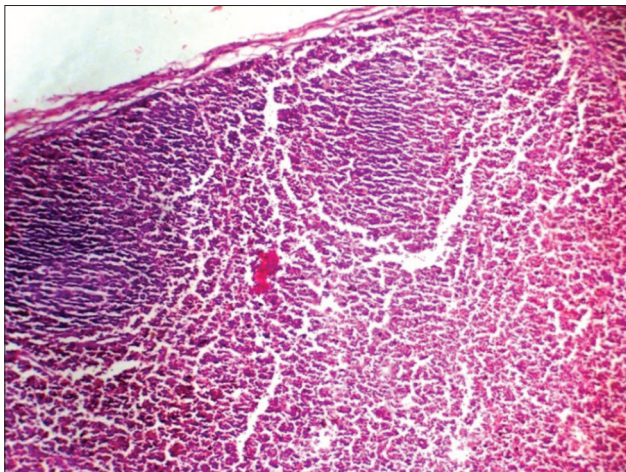


Figure 1: Photomicrograph showing lymphocyte predominant pattern (H&E stain, x100)

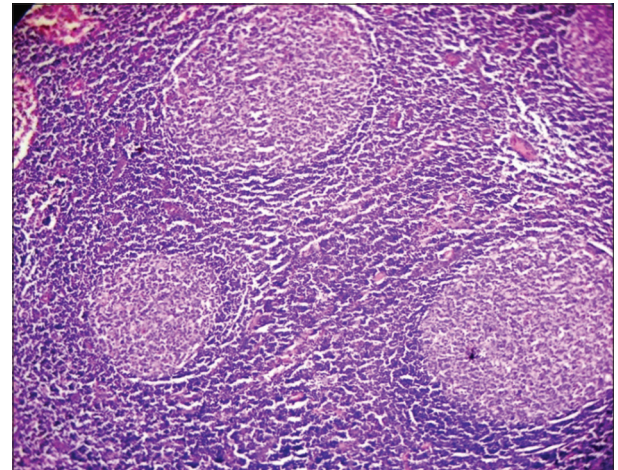


Figure 2: Photomicrograph showing germinal center predominant pattern (H&E stain, x100)

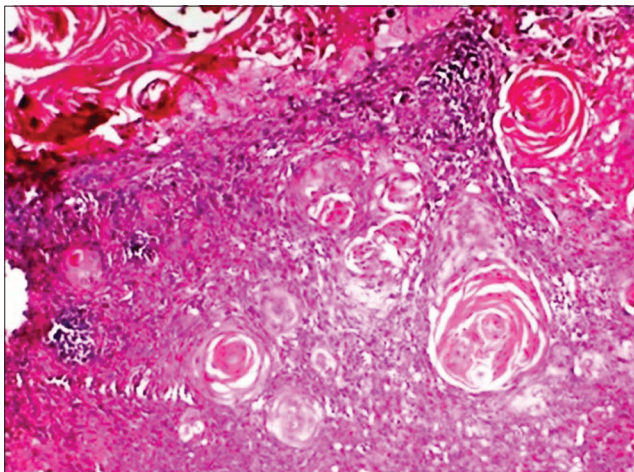


Figure 3: Photomicrograph showing lymphocyte depleted pattern (H&E stain, x100)

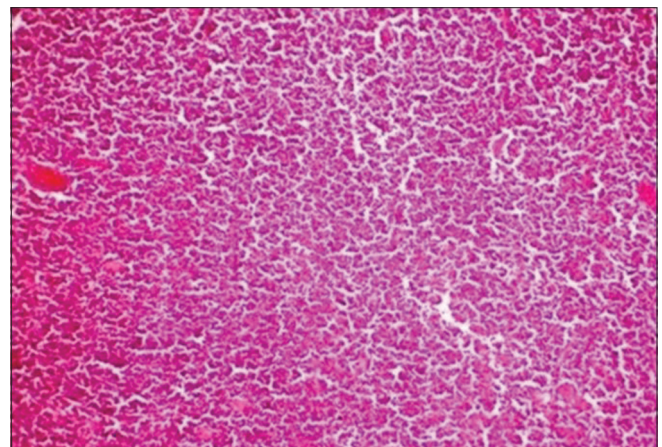


Figure 4: Photomicrograph showing unstimulated lymphnode (H&E stain, x100)

Table 3: Total nodes present in each category

| | Level I | % | Level II | % | Level III | % | Level IV | % | Level V | % | Total |
|-------|---------|------|----------|------|-----------|------|----------|------|---------|------|-------|
| LP | 58 | 29.2 | 53 | 26.6 | 51 | 25.6 | 19 | 9.55 | 18 | 9.05 | 199 |
| GCP | 45 | 38.5 | 35 | 29.9 | 25 | 21.4 | 9 | 7.69 | 3 | 2.56 | 117 |
| LD | 2 | 11.8 | 6 | 35.3 | 7 | 41.2 | 2 | 11.8 | 0 | 0.00 | 17 |
| US | 11 | 28.2 | 7 | 17.9 | 14 | 35.9 | 1 | 2.56 | 6 | 15.4 | 39 |
| Total | 116 | 31.2 | 101 | 27.2 | 97 | 26.1 | 31 | 8.33 | 27 | 7.26 | 372 |

Chi-square=31.0936, df (degree of freedom)=12, P=0.0019, Significant, LP: Lymphocyte predominant pattern, GCP: Germinal center predominant pattern, LD: Lymphocyte depleted pattern, US: Unstimulated node

Table 4: Distribution of positive nodes at various levels

| Levels | Number of positive nodes | % |
|--------|--------------------------|-------|
| I | 13 | 48.15 |
| II | 7 | 25.93 |
| III | 3 | 11.11 |
| IV | 2 | 7.41 |
| V | 2 | 7.41 |
| Total | 27 | 7.26 |

of lymph node reactivity aims to indirectly assess the host immune response facing a malignant tumor. Lymphocyte predominant pattern was the most constant finding in lymph nodes which was similar to previous study^[6], wherein it is suggested that this pattern may be actively engaged in a cell-mediated immune response. Further, it is also suggested that sinus histiocytosis and lymphoid hyperplasia are manifestations of cellular immunity and thus may indicate a favorable prognosis. Paracortical hyperplasia is considered as one of the feature of lymphocyte predominant pattern in our study. This feature has been characterized as a separate cellular response in earlier studies^[16] and it has been found to have a statistically significant association with recurrence rate and mortality and improved 5-year survival. Germinal center predominance pattern, the second common finding in this study was the common feature of metastatic nodes in earlier study.^[6] It has been suggested that germinal centers are formed early in the humoral response to antigenic stimuli and that they are largely related to the production of plasma cells and humoral antibodies. In the present study, this may also be due to the reactive lymphadenitis found in some patients with malignant tumors and the active immune response of the host against the malignant tumor. Also the lymph node reactivity can vary according to the distance between the tumor and the lymph nodes, infection, patient nutrition and subjectivity in analyzing the lymph nodes. The concept of germinal center predominance and follicular hyperplasia is not clear. It appears that the word “germinal center predominance” is used in relation to the prominence of germinal centers in dissected lymph nodes associated with or without metastasis. But in few studies, the predominance of germinal centers within the lymph nodes has been characterized as follicular hyperplasia that indicated humoral stimulation and corresponded with decreased survival.^[16] Thus, it appears that standardization and reproduction of these results in larger samples may be essential in order to indicate the exact lymph node reactivity assessment for prognostic evaluation.

In the present study, the lymphocyte depleted pattern was observed in only few nodes (17; 4.57%). This pattern has been attributed to the exhaustion of immunologic response and observed in cases with metastases and had unfavorable prognosis.^[6] But due to short follow-up period, this factor could not be assessed. The uncommon unstimulated node pattern more frequently observed in levels IV and V implies that as the levels of the nodes moved farther apart from the primary site the

nodal histological pattern was unaltered suggesting decreased antigenic stimulus. This pattern describes the peripheral node from individuals without cancer or other evidence of antigenic stimulation and indicated good prognosis. Apart from these aforementioned primary histological patterns, it was interesting to observe in the present study that most of the nodes (11.56%) showed prominent vascularity within the lymph nodes. Although the role of angiogenesis is unclear, it is suggested that because of the rich native vascularity of lymph nodes, neoangiogenesis is redundant for the growth of metastatic tumor.^[17] Other histological findings such as cystic degeneration; extra capsular spread and fibrosis were not observed in the present study. These observations also provide insight to the reactivity of the lymph nodes and hence larger sample size, different histological grades and stages of the tumor should be considered in further studies.

CONCLUSION AND SCOPE

The histopathology of lymph nodes harvested in neck dissections exhibit distinct metastatic and non-metastatic features. The proposed immuno-morphological subtypes coupled with other factors may aid in assessing the prognostic index of carcinoma patients. Studies correlating the immuno-morphological features of the resected lymph nodes with the survival rate have been reported. Previous studies state that the Lymphocyte predominant pattern which was more common in non metastatic cases had better 5-year survival rate while the lymphocyte depleted pattern more common in metastatic cases presented with a unfavorable prognosis. Germinal center predominance had an intermediate survival rate. A short follow-up period of about 3 years in the present study made this finding inconclusive. Future studies of similar kind correlating lymph node histology, staging and survival rates may provide better insight in predicting prognosis of patients with carcinoma.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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