RESEARCH ARTICLE

IMMUNOLOGICAL STATUS OF METASTATIC AND NON METASTATIC LYMPH NODES IN ORAL SQUAMOUS CELL CARCINOMA

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ARTICLE INFO	ABSTRACT
	Aim: Lymph node morphology does reflect certain parameters of immune responses. The study was aimed to evaluate, compare, and correlate the morphological changes and the type of immune responses in paraffin embedded lymph node sections of patients diagnosed with metastatic and non-metastatic oral squamous cell carcinoma. **Methods**: This study constituted a total of 29 histopathologically proven metastatic and 31 non-metastatic cases obtained from 60 oral squamous cell carcinoma patients. These 60 cases yielded a total of 429 lymph node sections which were subjected to haematoxylin and eosin stain. All the 429 sections were analyzed for the type of immune response (cell mediated or humoral response) based on the morphological changes. **Results:** Data analysis using chi-square test yielded a highly significant result in immunological status in the lymph node sections of metastatic oral squamous cell carcinoma in contrast to non-metastatic oral squamous cell carcinoma. **Conclusion:** The study highlights the status of the immune system related to the prognosis of the patient and a comprehensive treatment protocol over the conventional methods of examination of lymph node. Thus the recent study concluded that the histopathological lymph node pattern may serve as a surrogate marker in predicting the metastasis and thus enhancing the survival of the patients.

INTRODUCTION

Oral cancer holds the eighth position in the cancer incidence ranking worldwide, with epidemiologic variations between different geographic regions (it is the third most common malignancy in south-central Asia) (Peterson, 2003). The World Health Organization expects a worldwide rise in oral squamous cell carcinomas (OSCC) incidence in the next few decades. In India, oral cancer is one of the most common cancers and constitutes a major public health problem. Oral cancers have a significant impact on the patient's quality of life, because of the functional loss that results with the treatment modalities even with the highest care rendered nowadays.

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Oral squamous cell carcinoma has a remarkable incidence worldwide and a fairly onerous prognosis, encouraging further research on factors that might modify disease outcome (Massano, 2006). Squamous cell carcinoma of the head and neck (SCCHN) frequently metastasizes to the regional lymph nodes; hence the status of the cervical lymph nodes is the most important prognostic factor in squamous cell carcinoma of the upper aerodigestive tract. The number of positive nodes and the presence of extranodal (or extracapsular) spread are the two most commonly used prognostic factors. Other characteristics of prognostic importance are the size of the node, level of positive nodes and histologic response to the tumour in the node. The extent of nodal disease in the neck has prognostic significance not only for failure in the neck but also for the development of distant metastases and possibly for recurrence at the primary site (Vikram, 1984; Leemans, 1994 and Leemans, 1994). Histopathologic examination of neck

dissection specimens therefore provides basic information for diagnosis, staging and prognosis (Detection of nodal micrometastasis in head and cancer, 1996). Lymph node morphology does reflect certain parameters of immune responses associated with humoral antibody production or cell mediated immunity, as the lymph node is one of the tissues most frequently examined by histopathologists. Correlation between the lymph node immune response and other parameters such as site, size and histopathological grade of the primary tumour, could assist the surgeons in their final assessment and treatment planning for the patient.[7] There are studies on the clinical and histopathological features of the primary tumour and its metastases to lymph nodes, but very few studies have been conducted to know other concurrent changes occurring in the lymph nodes due to metastases. It may be assumed that the histopathological analysis of the regional tumour draining lymph nodes could elucidate the immunological host tumour relationship and render additional information on patient survival.

The relative common presentation, delay in diagnosis is also frequent which could be correlated to patient delay (in looking for professional care), professional delay (in reading the diagnosis), or both. Thus, knowledge of the varied presentation and an experienced eye can go a long way in preventing the high morbidity and mortality associated with oral cancers (Yadav, 2012 and Vidyadevi, 2014). A pertinent issue for consideration is rich lymphatic supply of the oral cavity, which results in many cases being detected first in the advanced stage itself. Late presentation may be attributed to patient with ignorance of symptoms and lack of concern for the disease. It is a well known fact that the microscopic pattern of a regional lymph node can represent the immunologic reactivity in that node. Based on this, several investigators have attempted to formulate morphologic-immunologic analysis of lymph nodes draining a variety of malignant tumours based on the knowledge of the distribution of lymphoid cells of different classes in the node. Although analysis of the histological lymph node reactivity has been studied in various types of cancers including breast, larynx, lungs, stomach and uterine cervix, few studies have focused on head and neck cancer.[8] Therefore the present study was done to observe the morphological changes occurring in the lymph nodes, to correlate these changes with the presence or absence of metastases and various other clinicopathological characteristics.

MATERIAL AND METHODS

This retrospective study constituted a total of 60 patients who have undergone radical neck dissection for primary oral squamous cell carcinoma of the oral cavity in our Institution. Twenty nine patients of metastatic and thirty one patients of non-metastatic oral squamous cell carcinoma constituted our study subjects. Paraffin embedded tissue sections of the lymph nodes of these 60 patients were retrieved from the archives of our institution. These 60 patients yielded a total of 429 node sections which were haematoxylin and eosin stained. The clinical data with respect to age, gender, location & type of lesion were retrieved from clinical records already existing in our institution. Nodal sections which were folded or which could not be analyzed were not included in the study. Cases with incomplete clinical data and patients with recurrent squamous cell carcinoma who have received radiotherapy were excluded from the study. These nodes were analyzed with

respect to immune response for which five histologic patterns of lymph node reactivity were considered, as follows:

- Lymphocytic predominance (LP), lymph nodes with an increased number of small lymphocytes throughout the cortex, the paracortical area and the medullary region.
- Germinal centres (GC), lymph nodes with germinal centres containing large lymphoid cells and mitotic figures.
- Sinus histiocytosis (SH), lymph nodes with distention and prominence of lymphatic sinusoids; the sinuses are engorged with histiocytes.
- Normal lymph nodes (NL), without significant changes in architectural and cell distribution; cortical lymphoid follicles are present, without germinal centre or fibrosis.
- Lymphocytic depletion (LD), lymph nodes without germinal centres and depleted of lymphocytes; fibrosis and hyaline deposits are present.

When lymph nodes showed more than one reactivity pattern, the case was classified according to the pattern of the majority of lymph nodes. Nodal sections which revealed lymphocytic predominance & sinus histiocytosis were categorized to have cell mediated immune response; germinal centre formation was considered to exhibit humoral immune response. The total number of cell mediated and humoral immune response were calculated from all the lymph nodes analyzed and were correlated with metastatic and non-metastatic lymph nodes. In metastatic nodal sections, the degree of differentiation was also noted. In addition, each metastatic lymph node was evaluated for pattern of invasion in which they were categorized as total replacement, invasion in the form of cords and invasion in the form of islands.

Statistical Analysis

Chi-square test was applied to find an association between Immune response of lymph nodes with metastatic deposit of metastatic oral squamous cell carcinoma and lymph nodes of non-metastatic oral squamous cell carcinoma. Logistic regression analysis was applied to check the overall prediction rate of the type of immunity by the morphological changes in the lymph nodes. IBM-SPSS-STATISTICS-VERSION 20 was used for statistical analysis.

RESULTS

A total of 60 patients who had undergone radical neck dissection in our institution for primary oral squamous cell carcinoma of the oral cavity constituted our study group. Resected lymph nodes of 60 patients, who have undergone radical neck dissection, were analyzed in our study. It consisted of 29 patients with lymph node metastasis (pN+) and 31 patients free of metastases (pN0). Haematoxylin and eosin stained lymph node sections of each of these above study subjects were analyzed for immune response. Thus, a total of 429 paraffin embedded haematoxylin and eosin stained lymph node sections were harvested from 60 patients. Thirty one cases of non-metastatic oral squamous cell carcinoma yielded 200 lymph node sections while twenty nine cases of metastatic oral squamous cell carcinoma yielded 229 nodal sections. Of the 229 lymph node section in the metastatic oral squamous cell carcinoma group, 70 sections were positive for metastatic deposit while the remaining 159 lymph node sections did not

show metastatic deposit. The study subjects, combining both metastatic and non-metastatic oral squamous cell carcinoma were predominantly males (76.6 %; n=46), above 30 years of age (80%; n= 48), with primary lesions occurring predominantly in the buccal mucosa (65%; n=39) constituting smokeless tobacco use as the predominant habit (68.3%; n= 41) (Table 1).

The division of 270 numbers of lymph nodes (70 + 200) polarized across the metastatic & non-metastatic cases showcases the contrast between the cell mediated & humoral immunity as shown in the contingency table (Table 2). In the contingency table (Table 2), the bipolar properties of 270 nodes were represented demonstrating the association of attributes.

Table 1. Clinical parameters of patients with metastatic and non-metastatic oral squamous cell carcinoma

Clinical Parameters	Non-metastatic OSCC	Metastatic OSCC
Age		
Less than 30 yrs	4	4
More than 30 yrs	27	25
Gender		
Male	25	22
Female	6	7
Habit		
Smokeless Tobacco	18	24
Smoking	05	01
Mixed habit	05	03
No habit	03	01
Location		
Buccal Mucosa	23	16
Retromolar Trigone	03	04
Tongue	04	09
Floor of the mouth	1	0

Table 2. The contingency table shows the division of 270 lymph nodes polarized across the metastatic cases & non-metastatic cases which showcases the contrast between the cell mediated & humoral immunity

Contingency Table

	Cell Mediated Immunity	Humoral Immunity	Total
Metastatic cases	57	13	70
Non-Metastatic cases	79	121	200
Total	136	134	270
$x^2 = 36.46$	df = 1	p < 0.001	

Table 3. The classification table shows that 28 out of 29 metastatic cases and 31 out of 31 non-metastatic cases have been correctly classified giving an overall prediction rate of 98.3%

Classification Table

			MET or NONMET CASE		Percentage Correct
			1	2	
Step 1	MET or NONMET CASE	1	28	1	96.6
		2	0	31	100.0
	Overall Percentage				98.3

MET: Metastatic case
NONMET: Non-metastatic case

Table 4. The variables in the equation table indicate that cell mediated immunity predicts the classification into the metastatic group

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	gender(1)	-2.534	2.196	1.332	1	.249	.079
	Age	.140	.092	2.339	1	.126	1.151
	MetLNImm			.699	2	.705	
	MetLNImm(1)	-1.793	2.144	.699	1	.403	.167
	MetLNImm(2)	-25.689	18090.098	.000	1	.999	.000
	NonmetLNImm			.000	3	1.000	
	NonmetLNImm(1)	25.632	8837.234	.000	1	.998	135501720016.986
	NonmetLNImm(2)	.623	14423.354	.000	1	1.000	1.865
	NonmetLNImm(3)	1.643	28892.559	.000	1	1.000	5.172
	Constant	-26.430	8837.235	.000	1	.998	.000

The primary tumour was predominantly well differentiated oral squamous cell carcinoma (76.6 %; n= 46). With respect to immune response and metastasis, statistical analysis was done using chi-square test. In order to apply the above test, two extremes of values were taken (i.e. 70 sections of positive metastatic lymph node and 200 sections of non-metastatic lymph nodes. 159 sections were excluded in this test because it could not be applied in either of the extremes.

Table 2 exhibited a very strong chi square value of 36.36, degree of freedom (df) = 1, with a highly significant p value of <0.001. Thus the cell mediated immunity appeared to be strongly associated with metastasis positive lymph nodes. Further statistical analysis was done using the logistic regression analysis which signifies the proper classification of metastatic and non-metastatic cases using the type of immunity retrospectively. The classification table (Table 3) showed that

28 out of 29 metastatic cases and 31 out of 31 non-metastatic cases have been correctly classified giving an overall prediction rate of 98.3%.
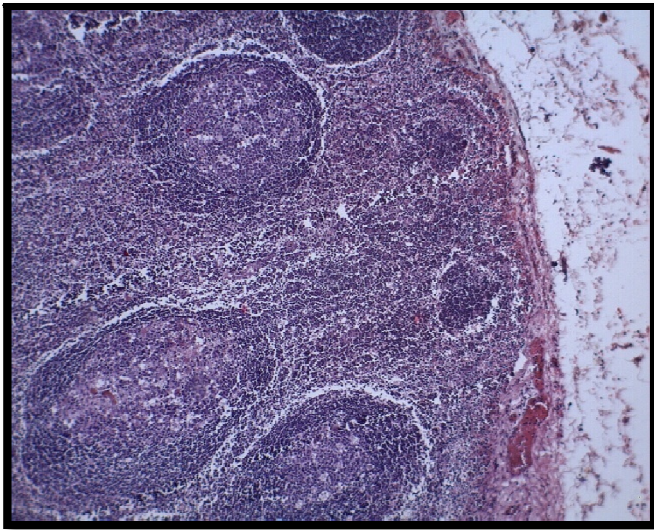

Fig. 1. Microphotograph reveals lymph node with numerous secondary follicles with central germinal centre formation (H&E stain, 4x)
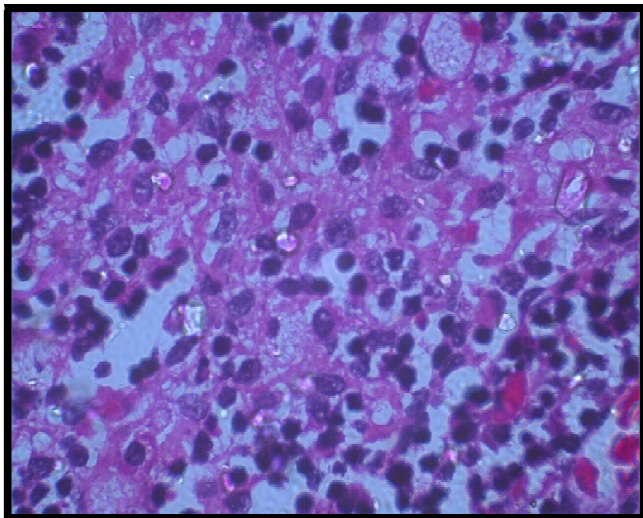

Fig. 2. Microphotograph of lymph node showing sinus histiocytosis characterized by the presence of large cells with foamy cytoplasm (H&E, stain, 40x)

Even the Hosmer and Lemeshow. Test yielded a p value of 0.817 signifying that the above mentioned prediction is nearly correct. In order to give the quantitative weightage to all the 429 lymph nodes, a logistic regression was done by including all the lymph node characteristics by deciding the predominant type of immunity exhibited by the lymph nodes of individual cases (Table 4). A monstrously large odds was obtained for one variable which may be just because there are missing values of metastatic type of lymph nodes in respect of the non-metastatic cases as there is no defect in entering of the results of such a good number of lymph nodes analyzed. This is also known to be a feature of outcome of logistic regression when there is "Complete Separation". So, it can be reasonably concluded that the cell mediated immunity strongly predicts the outcome of metastasis in contrast to the same event among the non-metastatic cases. The odds of this event for every lymph node increase of this type among the metastatic cases indicate that the quantitative contribution of cell mediated immunity is also high in predicting the outcome of metastasis in the patient.

DISCUSSION

Despite optimal treatment, the prognosis of the advanced squamous cell carcinoma remains poor. This poor survival is primarily due to a high rate of locoregional failure and secondarily to distant metastases (Yadav, 2012 and Vidyadevi, 2014). Incidence of regional lymph node metastases in head and neck cancer is high and the presence of lymph node metastases is the single most important prognostic factor. An understanding of the mechanism whereby tumor cells are prevented from developing lymph node metastases becomes essential to understand the unpredictable clinical behaviour of patients with malignant disease has led many investigators to search for factors that may be used as an index of prognosis (Makesh, 2014; Crissman, 1989; Crissman, 2001). Even as the stage and grade of the primary neoplasm have been shown to have prognostic significance, the biological variability of individual patients has led investigators to search for other factors that might refine the clinician's ability to predict survival. Epidemiological and experimental evidence in the past two decades have implicated the immune system in the etiopathogenesis of cancers (Crissman, 1989).

The morphological and immunological patterns are lymphocyte predominance and sinus histiocytosis, which supposedly reflect the active response of the thymus-dependent cells linked to cellular immunity. Germinal centre predominance, indicates an active response in the thymus-independent germinal centres related to humoral immunity (Crissman, 1989; Gnepp, 2001 and Anneroth, 1987). Although very few studies are available that correlate the role of immune response in cervical lymph nodes and the occurrence of metastasis, conflicting results are noted. Tsakralides V *et al* in their study found that metastases to the cervical lymph node occurred twice more frequently in patients showing germinal centre predominance than in patients with lymphocyte predominance pattern (Berlinger, 1976). Similarly in a study done by Di Giorgio and Tosi P *et al* on lung carcinoma and Patt BS in laryngeal carcinoma they found that cell mediated immune response inhibits metastasis (DiGiorgio, 2000). Similar observations were made by Malicka in patients with cancer of the larynx and by Carter and Gershon and Edwards *et al*., in experimental tumours. Several investigators have found that germinal centres are found early in the humoral response to antigenic stimuli and that they are largely related to the production of plasma cells and humoral antibodies. Thus, these data support the hypothesis that lymph nodes with germinal centre predominance pattern are responding to antigenic stimulation with the production of humoral antibodies. In contrast to above results our study showed that most of the tumour free nodes of non-metastatic and metastatic oral squamous cell carcinoma showed humoral immunity. However, the lymph nodes with metastatic deposit exhibited predominantly cell mediated immune response and it was statistically significant ($p<.05$) (Table 2). While sinus histiocytosis also pointed towards cell mediated immune response (Figure 2) Humoral immunity was evident by the presence of numerous secondary follicles with central pale area, the germinal center (Figure 1). Similar result was obtained by Berlinger NT *et al* in their study on patients with head and neck carcinoma in which they found that both germinal centre predominance and the lymphocyte

predominant pattern experienced less incidence of metastases, 23 and 31%, respectively (Berlinger, 1976; DiGiorgio, 2000; Manfro, 2010; Tsakraklides, 1973; Nagata, 2004 and Bennett, 1971). This was attributed to the evidence of concomitant lymphoblastic activity in the thymus-dependent deep cortex. Several other researches were conducted in past substantiating the fact, germinal centre predominance and lymphocyte together or independently constitute humoral response thereby prolonging the survival rate. The current study was also explained by another school of thought suggesting that accumulation of macrophages in sinus histiocytosis was thought to ward off tumour and was believed to be the first morphological response of the host immune system against the tumour. Nagata *et al.*, (2004) in an experimental study of reactivity patterns in carcinoma induced in rats showed that follicular hyperplasia and lymphocyte proliferation were induced earlier than sinus histiocytosis in response to carcinoma (Black, 1972). This evidence that sinus histiocytosis develops only during the late stages of tumour growth in experimental animals, supports the fact that the presence of SH indicates more advanced stage of carcinoma thus correlating with the poorer patient prognosis (Hunter, 1975; Layland, 2005; Dire, 1963; Dire, 1963 and Okura, 2005).

Conclusion

This study concludes that the histopathological lymph node pattern may serve as a surrogate marker in predicting the metastasis and survival of the patients. Patients with lymph node showing germinal centre predominance showed a better prognosis compared to lymphocytic predominance. Even presence of sinus histiocytosis may indicate a more advanced stage of carcinoma or complete lack of immune stimulation thus having a fatal outcome. Thus, node related parameters should be assessed for every oral squamous cell carcinoma patient and according to the findings; the patients should be categorized into the relevant risk categories. Assessment of these parameters would help the pathologist send the referring surgeon a more informative report for assessing patient prognosis and formulating a more comprehensive treatment protocol.

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