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Original Research Article

Identification of tertiary lymphoid structures in oral squamous cell carcinoma and its clinico-pathological associations

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ABSTRACT

Background: Tertiary lymphoid structures (TLS) are ectopic, vascularised lymphoid structures that may be found in and around tumor tissue. The role of TLS has been studied in few solid tumors in which high TLS expression was found to be favourable prognostic biomarker.
Methods: The present study was a retrospective and prospective study for a period of one year. Resected specimen of Oral squamous cell carcinoma (OSCC) with no history of previous treatment were included. The presence of TLS was assessed in tumor proper, invasive front as well as in adjoining areas of tumor. The association of TLS with size of the tumor, degree of differentiation of the tumor, pattern of invasion/tumor budding, tumor infiltrating lymphocytes (TIL) and lymph node pattern have been studied.
Results: 52 resected specimens of OSCC were evaluated in present study, out of which 24 (46.15%) showed presence of TLS. Amongst the tumor with TLS, 54.16% had size <2 cm, 62.5% were well differentiated carcinoma and 8.3% had worst pattern of invasion. TILs were distributed equally amongst three sites- tumor proper, invasive front and the adjacent tissue, in the TLS positive cases. The lymphocyte predominant pattern of lymph node showed a strong association with the expression of TLS (37.5%).
Conclusions: In the present study, TLS has been found to be associated with positive prognostic factors. Also, it has been found that TLS is not a proxy of the TILs. Identification and evaluation of TLS has a great potential as prognostic marker and needs evaluation.

Keywords: Oral squamous cell carcinoma, Tertiary lymphoid structure, Tumor infiltrating lymphocytes

INTRODUCTION

Head and neck cancer comprises approximately 30% of all cancers in India. Oral squamous cell carcinoma comprises a large proportion of all head and neck cancers in different cancer registries in India. The eitiology has been linked to both genetic predisposition as well as exposure to carcinogens due to lifestyle behaviour. A male predominance has been found in most of the studies conducted in India, which could again be related to lifestyle behavioural patterns in males.[1-3]

There are many prognostic indicators studied for head and neck squamous cell carcinoma which includes the size of the tumor, depth of invasion, lymphovascular/ perineural invasion, pattern of invasion and the involvement of draining lymph nodes. Recent studies have focussed on the role of several components of the tumor microenvironment (TME) in the progression of tumor. These includes the tumor stroma ratio/ stromal cells, microvessel density, extracellular matrix and the immune cells of the host body. The primary tumors, when exposed to the interaction of the factors in the TME, alter or adapt its metabolic and genetic composition.[4-6]

The immune cells of the host body are either in the form of tumor infiltrating lymphocytes (TILs) or the tertiary lymphoid structure (TLS). TILs are a subset of lymphocytes having higher targeted immunological response against tumor cells- a higher number of TILs indicative of a better response.[7,8] TLS are lymphoid aggregates composed of central B lymphocytes with active germinal centres and peripheral T lymphocytes along with dendritic cells and high endothelial venules. Since TLS are directly exposed to TME, it is postulated that it has a role in the immune response of the body against the infiltrating tumor cells.[8-10]

METHODS

The present study was a retrospective and prospective descriptive study for a period of one year (December 2023 to November 2024) in Barpeta Cancer Centre, Barpeta, Assam, India. Resected specimen of Oral squamous cell carcinoma without history of previous treatment were included in present study. The specimen are grossed and evaluated using standard protocol with submission of adequate representative tissue for processing.

The hematoxylin and eosin stained slides prepared from the representative submitted tissue were examined for the presence of TLS. Any lymphoid aggregate with germinal centre formation was evaluated with CD20, CD3, CD5, CD23 and BCL-6 for categorizing the TLS (Figure 1). The site of occurrence of TLS (tumor proper/infiltrating front/adjoining tissue) was examined along with typing/ grading of TLS (classical/non-classical, complete/ incomplete, grade 0/grade 1/grade 2). The cases were further evaluated for the prognostic characteristics including size of the tumor, degree of differentiation, pattern of invasion/ tumor budding, TIL and the lymph node immune-architectural pattern, with reference to the presence of TLS.

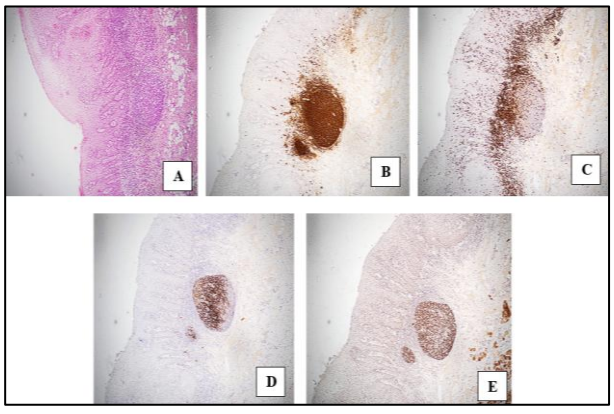

Figure 1: TLS in the tissue adjacent to the tumor: A) H and E stained section showing TLS; B) CD20 expressed by the central zone of lymphocytes; C) CD3 expressed by the peripheral T-lymphocytes; D) CD23 expressed by the dendritic cell meshwork; E) BCL-6 expressed by the activated germinal centre B-lymphocytes.

For determining relationship with the size of tumor, three arbitrary size ranges were considered (<2 cm, 2-4 cm, >4 cm) in which the tumors were categorized based on presence or absence of TLS. Similarly, tumors were categorized on degree of differentiation (well differentiated/ moderately differentiated/ poorly differentiated) and pattern of invasion (pattern 1-4/ pattern 5).

In the cases showing TLS, the density of TILs and the architectural pattern of the draining lymph nodes were evaluated. TILs were categorized as low density (<20 TIL/high power field), moderate density (20-40 TIL/high power field) and high density (>40 TIL/high power field), and the assessment of TIL was done in the three zones- tumor proper, invasive front of the tumor and the tissue just adjacent to the tumor. The "invasive front" of the tumor was considered the field at 40X in which half of the field of vision was occupied by the tumor and the other half by the stroma. The "tumor proper" was defined as the field of vision in the tumor adjacent to the field of invasive front. "tissue adjacent to the tumor" was the field beyond the tumor wherein no tumor cells were visible.

Six immunoarchitectural patterns of the draining lymph nodes (germinal centre formation- GC, lymphocyte predominant- LP, lymphocyte depleted- LD, sinus histiocytosis- SH, vascular transformation of lymph node structure- VTS, unstimulated- US) were identified and the category having the highest number of lymph nodes was assigned to the corresponding case.

RESULTS

A total of 52 resected specimen of OSCC were included in the present study, out of which 24 (46.15%) showed the presence of TLS. All the TLS detected in the present study were complete/classical/grade 2, where there was a central B-zone highlighted by CD20, peripheral T-cell zone highlighted by CD3, CD5, and reticular follicular cells highlighted by CD23 (Figure 1). In the present study, the highest number of cases were found in the buccal mucosa (40.38%) (Table 1).

Table 1: Incidence of TLS and distribution of the cases under the present study.

Total number of cases	(n=52)
Total number of TLS positive cases (%)	24 (46.15)
Sex ratio (Male/Female)	0.925 (25/27)
Distribution of cases according to site of tumor (%)	
Buccal mucosa	21 (40.38)
Tongue	08 (15.38)
Gingivo-buccal mucosa	15 (28.84)
Retromolar triangle	05 (9.61)
Hard palate	02 (3.84)
Floor of mouth	01 (1.92)

Table 2: Presence of TLS with respect to size of tumor, degree of differentiation and pattern of invasion/ tumor budding.

	Size of tumor				Degree of differentiation				Pattern of invasion/tumor budding		
	<2 cm	>2 cm but ≤4 cm	>4cms	Total	Well-differentiated	Moderately differentiated	Poorly differentiated	Total	Pattern 1-4	Pattern 5	Total
TLS present	13	10	01	24	15	6	3	24	22	02	24
TLS absent	07	19	02	28	9	17	2	28	21	07	28
Total	20	29	03	52	24	23	05	52	43	09	52

Table 3: Distribution of TIL in the tumor proper, invasive front of the tumor and the tissue adjacent to the tumor (in TLS positive cases).

	Tumor proper	Invasive front	Adjacent tissue
TIL (low density)	11	07	08
TIL (moderate density)	08	08	07
TIL (high density)	05	09	09
Total	24	24	24

In the present study, the highest number of TLS (54.16%) was found in tumors of small size (<2 cm). Also, TLS was more common in tumors which were well differentiated (62.5%) as compared to moderately differentiated (25%) and poorly differentiated tumors (12.5%). On the other hand, it was the moderately differentiated tumors (60.71%) which was most commonly encountered in the TLS negative cases. With respect to the pattern of invasion, 8.3% of TLS positive cases showed worst pattern of invasion- pattern 5, whereas this pattern 5 invasion was seen in 25% of TLS negative cases (Table 2).

Table 4: Association of TLS positive cases with different immunoarchitectural patterns of draining lymph nodes.

Immunoarchitectural type	Percentage
Germinal centre- GC	3/24 (12.5)
Lymphocyte predominant- LP	9/24 (37.5)
Lymphocyte depleted- LD	0
Sinus histiocytosis- SH/ vascular transformation of lymph node structure- VTS	4/24 (16.67)
Unstimulated- US	8/24 (33.34)

TILs were assessed in the tumor proper, invasive front of the tumor and the tissue adjacent to the tumor tissue and it was found that TIL density was relatively uniform in the different zones of the tumor (Table 3).

In the present study, the lymphocyte predominant pattern had the highest occurrence (37.5%) amongst the TLS positive cases, whereas lymphocyte depleted pattern did not occur in any of the TLS positive cases under the study (Table 4).

DISCUSSION

TLS are aggregates of lymphoid cells in a definite structure composed of central zone of B-lymphocytes, peripheral rim of T-lymphocytes and containing central meshwork of dendritic cells and high endothelial venules. The structure resembles an activated germinal centre in a lymph node. However, it lacks the capsule that is present in a lymph node. TLS is exposed directly to the tumor microenvironment and is presumed to be formed as a response to the infiltrating tumor cells.[8,11-13]

The presence of TLS in the tumor microenvironment has been associated with a favourable prognosis in many solid tumors, with respect to the optimisation of immunotherapy. This has been predominantly researched in lung carcinoma, but carcinomas of urinary bladder and cholangiocarcinomas also show similar outcome in presence of TLS.[14-18]

The present study has attempted to identify the TLS in OSCC and study its prognostic importance. In the present study, 24/52 (46.15%) of the cases showed the presence of TLS which was higher than that described by Qunxing et al (26.8%) and Wirsing et al (21%) in oral squamous cell carcinoma.[8,19] This might be due to the variation in the response to the tumor in mixed ethnic groups in the geographical location of the study.

The presence of TLS has been found to be more associated with good prognostic factors- 54.16% of TLS positive cases had size of tumor <2 cm, 62.5% of TLS positive cases were well-differentiated squamous cell

carcinoma and 91.67% of TLS positive cases had worst pattern of invasion 1-4. The studies of Wirsin et al (58.8%) and Sun et al (76.7%) had found a high percentage of TLS positive cases being well-differentiated.[19,20] However, the study of Qunxing et al [3] had found 32% TLS positive cases to be well differentiated. These studies have found a positive predictive value and presentation at an early stage (pT1/pT2) which is also seen in the present study.

The distribution of TILs in different zones (tumor proper, invasive front of the tumor and the adjacent tissue) have been studied in the TLS positive cases and it was not found to be significant, indicating that the formation of TLS could be an independent positive prognostic factor in the oral squamous cell carcinoma. The study of Zhang et al draws in a conclusion that the lymph node pattern of the draining lymph node with presence of TLS and TILs indicate good prognosis.[21] However, the same study showed that TILs were more frequently detected in the invasive front/peritumoral zone of the tumor, unlike the present study.

In the present study, the lymphocyte predominant pattern of the lymph node (37.5%) was the most commonly associated immunoarchitectural pattern in TLS positive cases and none of the cases showed lymphocyte depleted pattern. The studies of Raj et al and Mady et al found lymphocyte predominant/germinal centre predominant patterns to have good prognosis.[22,23] Although a study showing direct association of lymph node pattern and presence/absence of TLS has not been done, the present study draws an indirect association between the good prognostic patterns of lymph node with the presence of TLS. The presence of germinal centre and lymphocyte predominant patterns are an indication of active cell mediated immune response to the invading tumor cells.[24] On the other hand, the lymphocyte depleted pattern indicates a poor response with distant metastasis.[25,26]

The study has been carried out in a limited number of samples. Since the incidence of TLS is low, a higher sample size will help further find a strong association with other prognostic factors.

CONCLUSION

TLS has found to be an important component of tumor microenvironment. The identification of TLS as a good independent prognostic factor will help in directing the course of treatment in future, particularly re-routing from conventional treatment modalities to exploring immunomodulator treatment options.

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