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THE PROGNOSIS, PREOPERATIVE S-1 TREATMENT RESPONSE, AND CLINICOPATHOLOGICAL CHARACTERISTICS ARE ASSOCIATED WITH TERTIARY LYMPHOID FORMATIONS IN TONGUE CANCER

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Abstract

Objective: The purpose of this study was to assess the connection between TLSs and prognosis in tongue squamous cell carcinoma (TSCC) patients following preoperative S-1 treatment.

Study Design: Clinical Randomized trial

Place and Duration: Department of Oral Pathology, Baqai Medical University, Karachi. Nov 2022-Aug 2023.

Methods: Total 165 TSCC patients were presented in this study. Following informed written consent, comprehensive demographics were recorded. 90 patients received S-1 chemotherapy in group I and 75 patients were included in group II without S-1 treatment. We looked into TLS occurrence in both resected and preoperative biopsy specimens. All of the data were analysed using SPSS 23.0.

Results: Thirty-two (18.2%) resected specimens and twenty-seven (16.4%) biopsy specimens had TLSs. TLSs were linked to lymphatic invasion positive and instances that were clinicopathologically progressed. TLSs were absent from every instance with pStage 0, or benign cancer. TLSs were substantially more prevalent in preoperative S-1 chemotherapy instances in patients treated with S-1 for longer than 21 days, as well as in patients with treatment effects, stage (1,2). When compared with preoperative S-1 treatment, TLSs may be a prognostic factor, however they may not be one by themselves.

Conclusion: When evaluating the indication for preoperative S-1 treatment, the existence of TLSs was proposed as a factor indicating a favorable prognosis. Patients with TLSs who have TSCC may have a better prognosis due to the synergistic effect of S-1, which activates antitumor immunity.

Keywords: Tertiary lymphoid structure, squamous cell carcinoma, S-1, preoperative chemotherapy

INTRODUCTION

Interactions between tumour cells and their surroundings have a significant impact on the clinical course of cancer. Tumours are organs that behave like normal tissues and can even outcompete them, as has become more and more clear in the last several decades. From this perspective, research on the distinct cell types that make up a tumour and the tumour microenvironment (TME) that is created during tumour growth is the only way to fully understand tumour biology. The term "immune contexture" was coined to further emphasise the importance of immune infiltration quantity and quality into the tumour microenvironment (TME) in determining the medical condition of cancer patients. Tumor-associated immune infiltrates are defined by their type, density, immunity, and position [1-3].

Known by several names such as ectopic lymphoid tissues, tertiary lymphoid organs, or tertiary lymphoid tissues, secondary lymphoid structures (TLSs) arise in non-lymphoid organs under chronic inflammatory situations such as cancer, infection, autoimmune illnesses, and aging-related ailments.[4,5] TLSs are well-organized lymphoid aggregates that have a network of specialised fibroblasts. They resemble lymphoid secondary organs (SLOs), especially lymph nodes, in many anatomical and functional aspects. For instance, high endothelial venules (HEVs), specialised blood arteries that aid in the migration of lymphocytes from the circulation into lymphoid tissues, are present in TLSs and SLOs and can stimulate antigen-specific immune responses.[6]

These cells called effector cells then leave the local lymph node in cancer to enter the tumour microenvironment, where they locate and destroy cancer cells. Several researchers have shown that, in addition to this pathway, an adaptive immune response that mimics the pattern often associated with SLO is created in situ in certain tumours. This takes place inside structures known as tertiary lymphoid structures (TLS), which are spatially ordered structures. Tumor-associated de novo TLS creation necessitates specialised immune cell types as well as appropriate concentrations of chemokines and cytokines.[7] Both the tumour cores and their margins may produce TLS. Mature TLS are made up of germinal centres, T and B cell zones, and other components similar to SLOs. Both innate and adaptive immune cells, including B cells, plasmablasts, dendritic cell types, neutrophils, macrophages, helper CD4+ or lethal CD8+ T lymphocytes, and plasma cells, are found within these compartmentalised structures. Furthermore, HEV frequently binds to Tes in tumours, which encourages the early recruitment of immune cells as well as the release of activated immune cells into the bloodstream via TLS.[8,9]

The field of tumour immunology has focused a great deal of research attention on the local immune system within the tumour microenvironment (TME). Tertiary lymphoid structure (TLSs) are characterised as accumulating areas (or aggregation) of ectopic cells found in nonlymphoid tissues throughout irritation and carcinogenesis. TLSs have been noticed in the TME and discovered to have an important part in the anticancer immune response, as well as associate with greater survival in many tumours [10]. Histologically speaking TLSs appear as organ-like formations of cells that can be evaluated simply using eosin and hematoxylin (HE) slides that were stained or using immunohistochemical [11,12].

TLSs are becoming more and more interesting due to their association with ageing and chronic inflammatory diseases. TLSs can have a positive or negative impact on various disorders, depending on the circumstances. For instance, in certain cancer types, but not all of them, the existence of TLSs is linked to an enhanced response to therapy. Antitumour immune responses are produced within TLSs that are situated close to tumors[13]. Similarly, TLSs brought on by infections help the host by producing

anti-pathogen immune responses. On the other hand, TLSs in autoimmunity stimulate autoreactive lymphocyte activation, which leads to the formation of autoantibodies, and their existence is linked to a poor prognosis. Acute kidney injury (AKI) in the elderly is one kidney illness that is accelerated in progression by the formation of TLSs. In [14] Chronic inflammatory illnesses associated with ageing may be influenced by age-dependent TLS development, which has been documented in the kidney, lung, and other organs. In [15] These results indicate that TLSs are crucial sites for regulating local immunity and should be taken into account when developing treatment approaches to stop the advancement of chronic illnesses.

The objective was to evaluate the relationship between TLSs and prognosis in patients with preoperative S-1 treatment for tongue squamous cell carcinoma (TSCC).

MATERIALS AND METHODS

This Clinical Randomized trial was conducted at Department of Oral Pathology, Baqai Medical University, Karachi from Nov 2022-Aug 2023.and comprised of 165 patients. Patients undergoing endoscopic submucosal dissection, non-radical resection cases, preoperative radiation patients, patients who passed away during the intraoperative phase, and stomach intramural metastases were among the instances that were excluded.

To find out the tumor's stage, grade, and location, comprehensive pathology reports were examined. Patients provided direct answers to a structured questionnaire on sociodemographic information such as marital status, age, location, educational attainment, occupation, status in society, chewing habits, history of cancers in the family, frequency of chewing, and type of chewing substance.

The oesophageal cancer treatment guidelines served as the basis for both the specifics of the regimens and the indications for preoperative chemotherapy. The preoperative protocol consisted of 5-FU+cisplatin (the FP), 5-FU+nedaplatin (FGP), and 5-FU+cisplatin+docetaxel (the DCF). Based on the patient's condition and side effects, the chemotherapy dose was lowered as necessary. Until their surgeries, all of the patients had immunotherapy. Based on the Japanese Classification of Oesophageal Cancer, 11th Edition, tumours were histologically diagnosed.

All patients had upper gastrointestinal endoscopy, CT scanning, and fuorodeoxyglucose-positron emission tomography imaging as part of their clinical diagnosis. All of the oesophageal surgeons at our facility agreed on the diagnosis. 90 patients received S-1 chemotherapy in group I and 75 patients were included in group II without S-1 treatment. We looked into TLS occurrence in both resected and preoperative biopsy specimens. All of the data were analysed using SPSS 23.0.

RESULTS

There were 103 (62.4%) males and 62 (37.65) females in this study. Included cases had mean age 65.9±8.24 years with mean BMI 24.7±5.22 kg/m^2. There were 88 (53.3%) smokers and 77 (46.7%) non-smokers. Mostly cases were married and 73 (44.2%) cases were educated. 115 (69.7%) cases had poor socio-economic status. Chewing habits was found in 95 (57.6%) cases.(table 1)

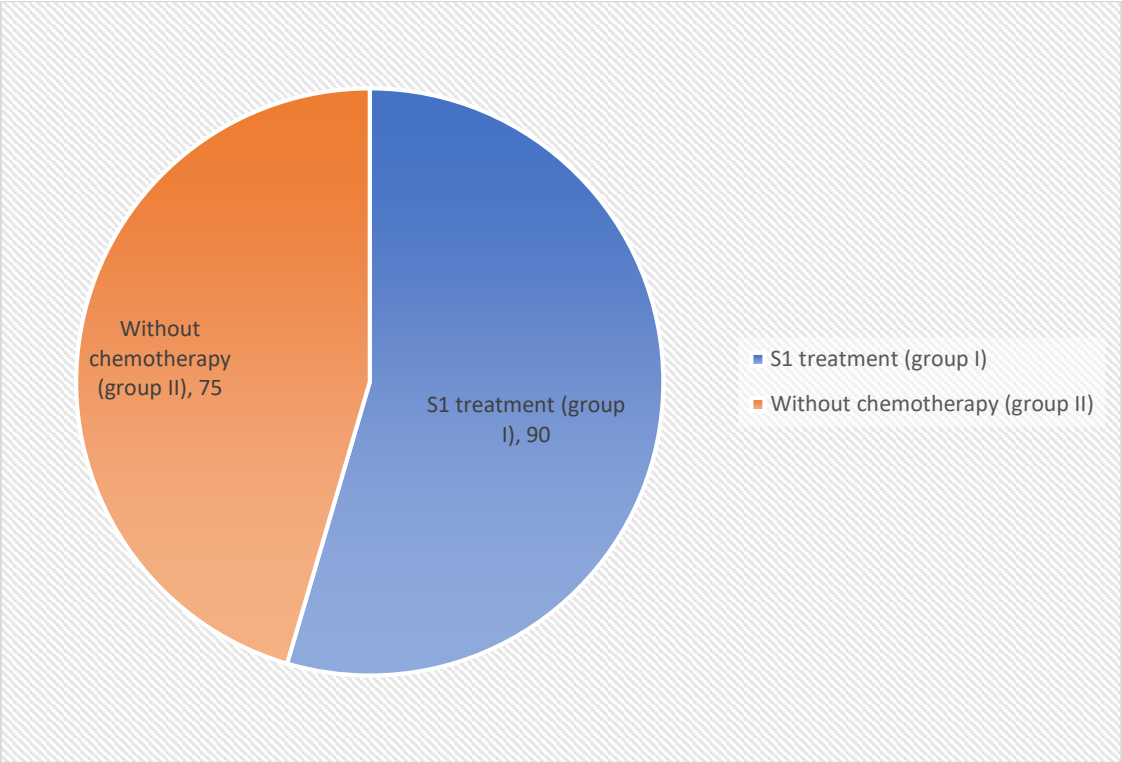
Table-1: Baseline information for each enrolled case

Variables	Frequency (165)	Percentage
Gender		
Male	103	62.4
Female	62	37.6
Age (mean)	65.9±8.24	
BMI (mean)	24.7±5.22	
Smokers		
Yes	88	53.3
No	77	46.7
Marital status		
Yes	130	78.8

No	35	21.2
Education Status		
Educated	73	44.2
Un-educated	92	55.8
Socio-economic status		
Poor	115	69.7
Middle/high	50	30.3
Chewing Habits		
Yes	95	57.6
No	70	42.4

In current study, 90 patients received S-1 chemotherapy in group I and 75 patients were included in group II without S-1 treatment.(figure 1)

Figure-1: Patients distribution with respect to S1 treatment



Thirty-two (18.2%) resected specimens and twenty-seven (16.4%) biopsy specimens had TLSs.(table 2)

Table-2: Frequency of TLSs among presented cases

Tertiary lymphoid structure	**Frequency**	**Percentage**
Resected Specimens	32	18.2
Biopsy Specimens	27	16.4
Total	59	34.6

TLSs were linked to lymphatic invasion positive and instances that were clinicopathologically progressed. TLSs were absent from every instance with pStage 0, or benign cancer. TLSs were substantially more prevalent in preoperative S-1 chemotherapy instances in patients treated with S-1 for longer than 21 days, as well as in patients with treatment effects, stage (1,2). When compared with preoperative S-1 treatment, TLSs may be a prognostic factor, however they may not be one by themselves.(Table 3)

Table-3: Correlation of TLSs with chemotherapy

Variables	**Group I (90)**	**Group II (75)**
TLSs		
Yes	42 (46.7%)	17 (22.7%)
No	48 (53.3%)	63 (87.35)
Stage		
0	0	0
1	25	10
2	15	4
3	2	3

DISCUSSION

Modulating TLS or TLS-forming immune system cells, such as B lymphocytes, is one promising way to: (i) increase endogenous immune responses; (ii) induce de novo local immunity towards tumours in poorly immunogenic tumours; and (iii) reroute a suppressive immune microenvironment towards effective antitumor immunity. Anti-cancer therapies include chemotherapy, radiation therapy, and immune checkpoint inhibitors activate the immune system to fight cancer cells. Improved disease-free and overall survival rates have been associated with TLS in patients receiving adjuvant a drug known as (anti-HER2 antibody), post and neoadjuvant drug regimens, and immune checkpoint medications. [16,17]

The current investigation revealed that the S1 treatment detected in ESCC tissue were frequently seen in aggregated forms. T cells that were identified as TLS were also present in this cluster along with follicular DCs. Additionally, in cases of early-stage oesophageal cancer, the high-TLS subgroup had a better prognosis; however, in cases with a grade of three or higher, there was no difference in the prognosis. The increased quantity of M2 macrophages and Treg cells, which function as immunosuppressors near TLSs or locally within the tumour, could be the cause of this. For instance, in mouse studies, TLSs rose in response to Treg suppression in TLSs, while CD4 and CD8 cells also rose concurrently [18].

Additional study is needed on subjects such as the ability of macrophages in cancer-associated TLSs to produce cytokines and the condition of T and B cell proliferation and activation in TLSs with a higher percentage of tingle-body macrophages [19]. The current study focused on TLS maturation, or germinal centre establishment, since S1 therapy is not enough to assess functional TLSs. B cells differentiate into memory B cells and plasma cells in the germinal centre. There was increased clonality in B cells in GC-TLS-rich tumour regions (Barros et al., 2015).[20] The prognosis was positive for patients in stages 2 and 3 of the current study who had a substantial number of matured GC-TLSs. Moreover, individuals who received NAC and saw a therapeutic response had a higher density of GC-TLSs. Notably, the density of CD20-TLSs did not correlate with the prognosis of individuals with NAC. In those receiving preoperative treatment for lung cancer, Silina showed that the existence of TLSs was not associated with a better prognosis [21].

As a result, the available evidence suggests that B-cell maturation induced by chemotherapy and the formation of germinal center-containing TLSs (GC-TLSs) may result in an anticancer immune response and a positive prognosis over the long term when treated with NAC. Recently, reports have emerged regarding the relationship between TLS maturation and the efficacy of ICI therapy. For example, patients with renal cell carcinoma and melanoma who participated in clinical trials of immune checkpoint blocking medication had their tissues subjected to RNA-seq analysis by Helmink et al. In addition to having higher amounts of memory B cells and germination center-like B cells, they discovered that the treatment response group had considerably higher expressions of genes connected to changes in TLS density and B cell function [22]. The observation of GC-TLS in a higher proportion of patients exhibiting a clinical response to ICI therapy in our investigation implies a connection between

B cells, or TLS, and the response to ICI treatment. However, we think that determining a TLS's phenotype is crucial to determining its usefulness.

CONCLUSION

When evaluating the indication for preoperative S-1 treatment, the existence of TLSs was proposed as a factor indicating a favorable prognosis. Patients with TLSs who have TSCC may have a better prognosis due to the synergistic effect of S-1, which activates antitumor immunity.

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