Original Article

Assessment of immune infiltrate in oral cancer: An immunohistochemical study

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Abstract

Background: The biomarkers of antitumour immune response provide valuable prognostic information and aid in the stratification and treatment of cancer. Tumour microenvironment (TME) defines the cancer biology, and assessment of tumour-infiltrating lymphocytes (TILs) in oral squamous cell carcinoma is an arena of vigorous research.

Aims and Objectives: The present study is designed to determine the association of $CD8^+$ and $CD3^+$ T lymphocytes with clinicopathological parameters and their role as prognostic biomarkers.

Materials and Methods: This is an observational and institution-based study. Tissue blocks of histologically proven oral squamous cell carcinoma patients were retrieved from archives, and all clinicopathological parameters were noted. The semiquantitative and quantitative methods of TILs assessment were meticulously applied both in the stromal and intratumoural regions using immunohistochemistry. The standard statistical methods were employed for data analysis.

Results: A significant association of $CD8^+$ T lymphocytes with clinical tumour size ($P = 0.012$), clinical ($P = 0.011$), and pathological ($P = 0.048$) staging was observed. $CD3^+$ T lymphocytes were significantly associated with clinical node involvement. However, no survival benefits were observed with both biomarkers.

Conclusion: $CD8^+$ T lymphocytes showed a significant association with clinical tumour size, clinical, and pathological staging. However, the study did not provide evidence for the prognostic value of the presence of $CD3^+$ and $CD8^+$ T lymphocytes in the tumour epithelium and stroma of oral cancer patients.

Keywords: Immune biomarker, immunology, lymphocytes, oral squamous cell carcinoma, prognosis

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INTRODUCTION

The conventional treatment modalities for oral squamous cell carcinoma (OSCC) are dictated by tumour size, node, and metastasis (TNM) systems that reflect the disease burden and are used to inform prognostic estimates and treatment planning.[1] The overall 5-year survival rate in OSCC patients is dismal around 50%, and recurrence occurs in about 35% of cases.[2] The accumulating evidence shows that the predictive capability of the 8th American

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Joint Committee on Cancer (AJCC) staging system needs improvement to obtain a more robust prognostic stratification as same-stage tumour reveals varying biological and clinical behaviours as well as therapeutic and prognostic outcomes.[3] Recent scientific exploration has substantiated the synergistic role of the tumour microenvironment (TME) and immune contexture in the advancement of aggressive tumours, treatment resistance, and adverse prognosis.[4] So a better understanding of the inflammatory milieu and immune subversion would pave the way for the rationale design of new immunomodulatory approaches with immunotherapeutic agents endowed with the capacity to thwart immunoevasion mechanisms.[5]

Tumour-infiltrating lymphocytes (TILs) underscore the role of the immune system in the biology of various solid malignancies.[6] Currently, enormous efforts have been made in unravelling the spatial organization of different TIL phenotypes in tumour areas to determine their predictive and prognostic value. This would help to determine clinical decisions in oral cancer patients not based on average population estimates but individual bio-clinical characteristics.[1,7] Attempts have been made to derive immune score which can be used as an additional parameter in TNM staging for prognostic stratification and treatment personalization.[8] Many studies have been published in the last few decades evaluating $CD3^+$, $CD4^+$, $CD8^+$, and Treg $FOXP3^+$ T lymphocytes subsets with varied and contradictory results.[9] However, $CD3^+$ and $CD8^+$ TILs have been reported to represent strong biomarkers to identify a subgroup of head and neck squamous cell carcinoma (HNSCC) and OSCC patients with lower probability of disease progression, better prognosis, longer survival and also associated with improved outcome.[10,11] Therefore, the present study is designed with the rationale that evaluation, subtyping and location of TILs may differ significantly by tumour site and extent thus reflecting tumour immune signature in OSCC patients. The current research work intends to establish a feasible standardized procedure and criterion to assess $CD3^+$ and $CD8^+$ TIL levels using immunohistochemistry (IHC) supported with semiquantitative and quantitative analysis that can aid in diagnostic and prognostic adjudication.

MATERIALS AND METHODS

The present observational and institutional study was carried out in full accordance with ethical principles with the approval of the Institutional Review Board. The study protocol was approved by the Research Ethics Committee of the hospital (BMHR-IECCMCL/0622-213). The patient's medical data was anonymized prior to access and analysis. The Ethics Committee waived the need for written informed consent from the study subjects because all potentially patient-identifying information was removed before data analysis. From the given prevalence of oral cancer by GLOBCAN 2018 Indian data,[12] the minimum sample size was calculated to be $n = 70$. However, a complete clinical, histopathological, and immunohistochemical analysis of 103 patients was performed. Patients with histopathologically confirmed squamous cell carcinoma without any prior treatment and with tumours located in the oral cavity (tongue, gingiva, buccal mucosa, alveolus, floor of the mouth, or hard palate) were included in the study. The medical records of 103 OSCC patients who met the inclusion criteria were retrospectively retrieved and evaluated. Demographic details, clinicopathological characteristics (lymph node status, staging, histological grade, and primary treatment information), and follow-up information (survival data and death) were extracted from medical records. The patients were staged according to the AJCC 8th edition staging system.[13] Patients with any previous treatment (chemotherapy/radiotherapy) for the present malignancy, without sufficient formalin-fixed and paraffin-embedded (FFPE) tissue for immunostaining and with insufficient clinical data were excluded from the study.

Methodology

FFPE blocks of all patients were obtained from the department archives. Biopsies were reviewed in a blind manner and independently by two senior pathologists. The pretherapeutic biopsy samples were analyzed and investigated for the presence of CD3 + and CD8 + TILs in tumour tissues. Hematoxylin and eosin-stained sections were reviewed to check the quality and the representativity of the tissue selected for supplementary analysis with IHC. In addition, the differentiation status and grade of the tumours were also noted (WHO grading system).[13]

IHC staining procedure (according to the manufacturer's instructions)

Four μ sections were picked up on poly-L-lysine coated slides and incubator at 60°C for 30 min. Sections were then de-waxed in xylene and brought to water through descending grades of alcohol. High-temperature antigen retrieval was performed in citrate buffer at pH 6.0. After cooling, the slides were washed in deionized water. Endogenous peroxide was neutralized using peroxidase block for 5 min. The slides were then washed in Tris-buffered saline (TBS) for 5 min followed by a protein block for 5 min. Again washing with TBS was done for 5 min. Primary antibody [anti-CD3 (Biocare Medical) and anti-CD8 (Biocare Medical)] was added, and the slides

were incubated in a moist chamber overnight at 2–4°C in a refrigerator. The next day morning, the slides were washed in TBS for 5 min and incubated with post-primary block for 30 min. The slides were washed again with TBS for 5 min and incubated with polymer for 30 min. Peroxidase activity was developed with diaminobenzedine (DAB) working solution for 5 min. The slides were rinsed in tap water and counter-stained with hematoxylin for 1 min. The slides were then rinsed in water again for 5 min followed by dehydration, clearing, and mounting in dibutylphthalate polystyrene xylene (DPX). Appropriate dilutions were standardized, and positive and negative controls were also run for each batch of staining. Sections from tonsils served as positive controls, while the omission of primary antibodies served as negative controls.

Semiquantitative and quantitative analysis

TILs that were positively stained for designated markers (CD3 and CD8) were studied using a ×20 objective lens. TILs were evaluated in the two tumour regions, i.e., intraepithelial compartment (cells within tumour cell nests) and the stroma (cells within the intratumoural stroma) excluding the necrotic and degenerated areas. TIL scoring of tumours was performed semiquantitatively (on a scale of 1–4) by measuring the densities of CD3- and CD8-positive cells as previously described by Dahlin *et al*., 2011:[14]

1. No or sporadic cells; 2. Moderate number of cells; 3. Abundant occurrence of cells; and 4. Highly abundant occurrence of cells [Figures 1 and 2].

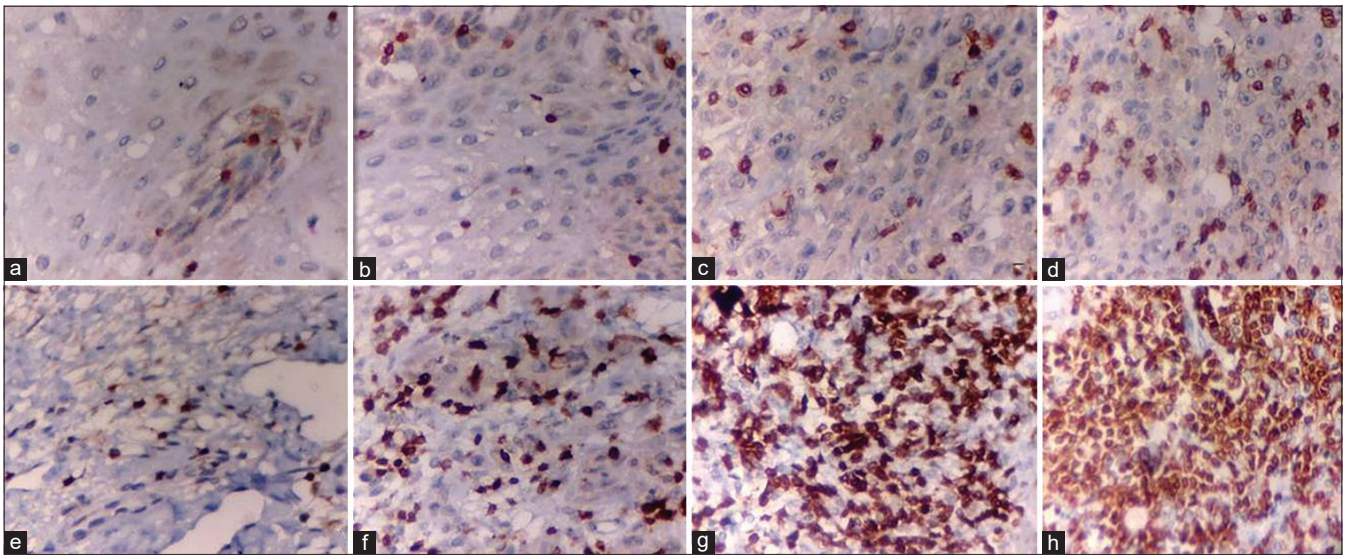

Figure 1: Photomicrographs (×20) representing tumour with intraepithelial (a-d) and stromal (e and f) CD3 score (a and e) 1, no, or sporadic cells; (b and f) 2, moderate numbers of cells; (c and g) 3, abundant occurrence of cells; and (d and h) 4, highly abundant occurrence of cells

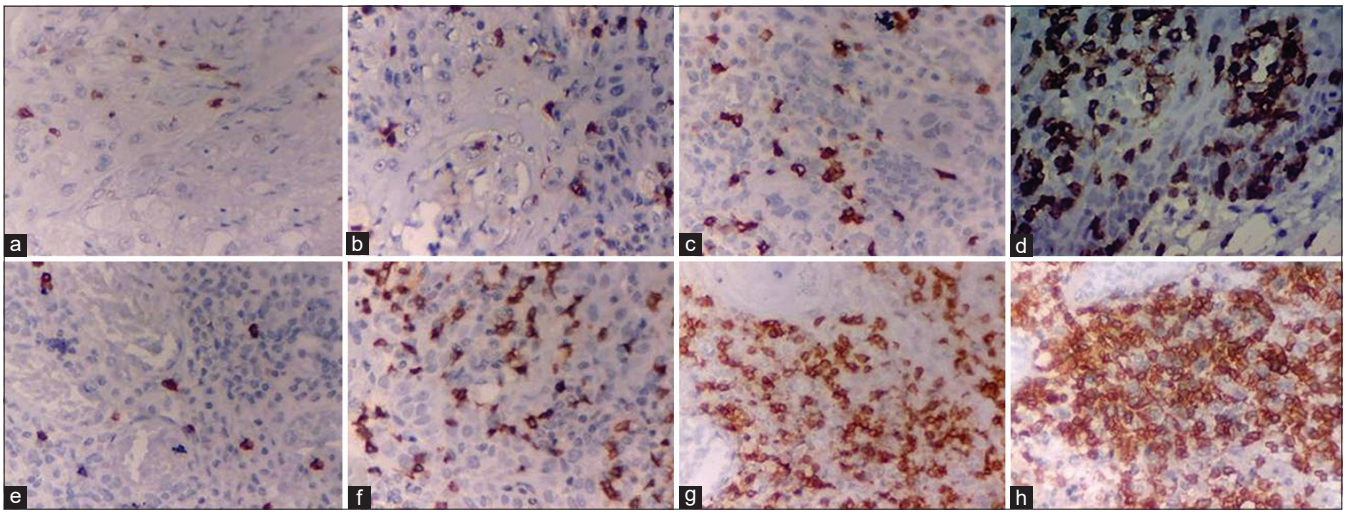

Figure 2: Photomicrographs (×20) representing tumour with intraepithelial (a-d) and stromal (e-f) CD8 score (a and e) 1, no, or sporadic cells; (b and f) 2, moderate numbers of cells; (c and g) 3, abundant occurrence of cells; and (d and h) 4, highly abundant occurrence of cells

Average score was given after assessing multiple microscopic fields (Five ×20 hot spot areas). The total score for $CD3^+$ and $CD8^+$ TILs was measured as the sum of the individual scores from the two tumour areas (intraepithelial compartment, and stroma), respectively. Photographic records were kept for all the cases. Cases were scored blindly with respect to patient history, clinical, and pathological details by two independent observers. In case of disagreement, a final decision was made upon further re-examination of the slides based on consensus by both pathologists. The median TIL score of each case was measured and used as the cutoff point to separate the patient cohort into two groups with either low or high $CD3^+$ and $CD8^+$ TIL expression.

By the use of a standard light microscope, images were acquired with a CCD-camera (Labovison) using a ×40 objective, transferred to a computer, and semiautomatically evaluated using the ImageJ software (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation [LOCI, University of Wisconsin]).[15,16] Digitized images of sections, for $CD3^+$ and $CD8^+$ TILs, were analyzed using the two compartments per biopsy, the intraepithelium, and the stromal. Five different areas of most abundant cell infiltration under ×40 were examined, whereas necrotic areas were excluded from the measurements. Positively stained nucleated cells were counted, and results were expressed as a percentage of positively stained cells/count of cells per high power field.

All statistical analyses were performed using IBM SPSS version 25 (IBM Corp., Armonk, N.Y., USA). The descriptive statistics used were frequencies, percentages, and means ± standard deviations. Fischer exact test was performed to find the association between $CD3^+$ and $CD8^+$ TILs and clinicopathologic variables. Overall survival (OS) and recurrence-free survival (RFS) were evaluated using the Kaplan–Meier estimate, and the differences between survival curves were tested for statistical significance using the log-rank test. A comparison of clinicopathological variables and TIL density scores with clinical and pathological staging was performed using Kruskal-Wallis test. One-way ANOVA was used to find the relation between intraepithelial and stromal cell counts among different staging groups.

RESULTS

Patient characteristics

A total of 103 patients were included, and of the total participants, 74 (71.8%) were males, and 29 (28.2%) were females. The average age of the participants was 55 ± 12.6 years. The clinicopathological characteristics of the patient cohort are summarized in Table 1.

Associations of TIL markers (CD3 and CD8) with pathological characteristics

In semiquantitative analysis, the total score of $CD3^+$ and $CD8^+$ TILs ranged from 2 to 10, and the median value was used as a cutoff point to separate the patient cohort into two groups with either low or high CD3 and CD8 expressions. As a dichotomous variable, CD3 expression was defined as 'low' (median score ≤3) in 85 and 'high' (median score ≥3) in 18 patients. Similarly, CD8 expression was defined as 'low' (median score ≤4) in 82 and 'high' (median score ≥4) in 21 patients. As determined by Fisher's exact test, no significant association of designated markers CD3 and CD8 was observed with age, gender, site, tumour grading, and distant metastasis.

Total CD8 score revealed a significant association with clinical tumour size (cT) ($P = 0.012$), clinical (cTNM) ($P = 0.011$), and pathological (pTNM) ($P = 0.048$) staging. [Table 2] Stromal CD8 density also revealed a significant association with clinical node involvement (cN) ($P = 0.046$). There was a trend of association of more advanced stage cases with low CD8 TIL density; however, these differences only approached with no statistical significance. There was no significant association of total CD3 score with tumour size and distant metastasis. However, CD3 score was significantly associated with clinical node involvement (cN) ($P = 0.047$). No significant findings were observed with CD3 stromal/intraepithelial score and clinicopathological variables. [Table 3] CD3 and CD8 expression in the tumour stromal compartment was consistently higher as compared to intraepithelial compartment. Weighted kappa showed a poor agreement for CD3 ($K = 0.014$) and CD8 ($K = 0.003$) expression in tumour intraepithelial and stromal compartments with weighted kappa being 0.014 and 0.00326 for CD3 and CD8 biomarkers in these compartments. Kruskal wall is test revealed no statistical significant differences in $CD3^+$ and $CD8^+$ percentage of cells among clinical and pathological staging groups 2–4 (for analysis purposes, stages 1 and 2 were combined together).

Prognostic role of TIL markers and clinicopathological characteristics for survival

Log-rank test showed a significant difference in OS among stage 2, 3, and 4 patients (clinical and pathological) with survival time in months being 7456 and 51 for the pathological staging groups and being 7550 and 52 for the clinical staging groups. A significant difference was observed for RFS also [Figure 3]. However, no survival benefits were observed in low and high CD3/CD8 TIL

Table 1: Clinicopathological characteristics of the oral squamous cell carcinoma patients

Clinicopathological variables	No of cases (frequency)	Percentage
Number of patients	103	
Age	Mean age±SD – (55±12.6) years M-54.9±12.42, F-55±13.28 years	
Gender	M=29 (28.2%)	F=74 (71.8%)
Site		
Buccal mucosa	40	38.8
Floor of the mouth	2	1.9
Hard palate	1	0.9
Hard palate and buccal mucosa	2	1.9
Lateral border of tongue	39	37.8
Lower alveolus and gingiva	13	12.6
Upper alveolus and gingiva	6	5.8
Tumour size, node involvement, and metastasis (TNM-classification, AJCC 8th edition)		
cT		
T1	2	1.9
T2	35	34.0
T3	37	35.9
T4	29	28.2
pT		
T1	2	1.9
T2	30	29.1
T3	32	31.1
T4	39	37.9
cN		
0	43	41.7
1	39	37.8
2	17	16.5
3	4	3.8
pN		
N0	34	33.0
N1	36	35.0
N2	21	20.4
N3	12	11.7
Metastasis	0	101
	x	2
pTNM		
I	1	1.0
II	17	16.5
III	30	29.1
IV	55	53.4
cTNM		
I	2	2.0
II	19	0.18
III	42	40.7
IV	40	38.8
Tumour grade		
Well differentiated	25	24.2
Moderately differentiated	67	65
Poorly differentiated	11	1.6
Type of biopsy	Incisional - 46	44.6
	Excisional - 57	55.3

cT=Clinical tumour size, pT=Pathological tumour size, cN=Clinical node involvement, pN=Pathological node involvement, pTNM=Pathological staging, cTNM=Clinical staging, M=Metastasis, SD=Standard deviation

density groups, and scores were 63.716, 28.809 (CD3) and 64.969, 27.505 (CD8) in months. These differences only approached but did not reach statistical significance. None of the T-cell markers showed a correlation with OS or RFS [Figure 4].

DISCUSSION

The tumour immune microenvironment and its impact on clinical decision-making and therapeutic prediction are becoming ever more significant. A decisive prerequisite to comprehend the patient's benefit from immunomodulation is the characterization of immune status.[17] Tumour lymphocytic infiltrate with T cells being the predominant cell typerepresents important immune signatures and is considered as marker of patient outcomes, but evidence in support of this hypothesis has been limited.[18] HNSCC patients with a high TIL density have been significantly

Table 2: Association of Total CD8 expression (high/low) with clinicopathological variables

Parameters		Low CD8	High CD8	Number of subjects	*P**
cT	1	2	0	2	
	2	32	3	35	0.012
	3	23	14	37	
	4	25	4	29	
	Total	82	21	103	
pT	1	2	0	2	0.080
	2	28	2	30	
	3	22	10	32	
	4	30	9	39	
	Total	82	21	103	
cN	0	34	9	43	0.369
	1	33	6	39	
	2	13	4	17	
	3	2	2	4	
pN	0	28	6	34	0.914
	1	29	7	36	
	2	16	5	21	
	3	9	3	12	
	Total	82	21	103	
M	0	80	21	101	0.632
	x	2	0	2	
	Total	82	21	103	
pStaging	I	1	0	1	0.048
	II	17	0	17	
	III	21	9	30	
	IV	43	12	55	
Total		82	21	103	
cStaging	I	2	0	2	0.011
	II	19	0	19	
	III	28	14	42	
	IV	33	7	40	
Total		82	21	103	

*Fisher's Exact Test. cT=Clinical tumour size, pT=Pathological tumour size, cN=Clinical node involvement, pN=Pathological node involvement, M=Metastasi

Table 3: Association of Total CD3 expression (high/low) with clinicopathological variables

Parameters		Low CD3	High CD3	Number of subjects	*P*
cT	1	2	0	2	
	2	30	5	35	0.537
	3	28	9	37	
	4	25	4	29	
	Total	85	18	103	
pT	1	2	0	2	0.302
	2	26	4	30	
	3	23	9	32	
	4	34	5	39	
	Total	85	18	103	
cN	0	32	11	43	0.047
	1	35	4	39	
	2	16	1	17	
	3	2	2	4	
	Total	85	18	103	
pN	0	26	8	34	0.122
	1	31	5	36	
	2	20	1	21	
	3	8	4	12	
	Total	85	18	103	
M	0	83	18	101	0.680
	x	2	0	2	
	Total	85	18	103	
pTNM	I	1	0	1	0.418
	II	15	2	17	
	III	22	8	30	
	IV	47	8	55	
	Total	85	18	103	
cTNM	I	2	0	2	0.597
	II	16	3	19	
	III	32	10	42	
	IV	33.0	7.0	40.0	
	Total	85	18	103	

*Fisher's exact test. cT=Clinical tumour size, pT=Pathological tumour size, cN=Clinical node involvement, pN=Pathological node involvement, pTNM=Pathological staging, cTNM=Clinical staging, M=Metastasis

associated with favourable prognosis and improved survival; however, the predictive and prognostic value of TILs remains ambiguous in OSCC.[19]

T lymphocyte subsets; $CD8^+$(cytotoxic T lymphocytes) and $CD3^+$ TILs (pan T-cell marker) that previously were thought to be positively prognostic for several malignant tumours were considered in the analysis. Both are markers of adaptive and antigen-specific antitumour immune responses.[9,20] The current research evaluated the localization and density of $CD3^+$ and $CD8^+$ T cells with clinicopathological parameters, disease progression and outcome. CD8 score revealed a significant association with tumour size and clinical as well as pathological staging. A trend of high $CD8^+$ TIL density in stage 1 and 2 tumours was observed, but we could not establish these as markers of disease progression as no statistically significant differences were seen. It was observed that CD3 and CD8 expressions in the tumour stromal region were consistently higher as compared to the intraepithelial compartment, and CD8 stromal density revealed significant association with cTNM/pTNM with a trend towards higher stage showing less CD8 lymphocyte infiltration. Ahuja *et al.* (2021)[21] reported that stromal TILs showed a significant association with tumour size and lymphovascular invasion, but no significant association was found with age, gender, nodal status, grade, perineural invasion, and site in the oral cavity. Zhou *et al.*[22] showed that $CD3^+$ and $CD8^+$ lymphocyte quantities differed inside the tumour centre, and the invasion margin in OSCC and more number of cells were found in the tumour centre. Mukherjee *et al.* (2020)[11] observed that in gingivo-buccal oral squamous cell carcinoma (GBOSCC), high infiltrates of $CD3^+$ and $CD8^+$ cells in the stroma were associated with significantly improved survival and were predictive of disease progression. It has been postulated that based on tumour compartmentalization, the prognostic significance of intraepithelial, stromal, and invasive front TILs varies.[10] In a few studies, stromal and invasive margin TILs are considered as better prognostic predictors. Caruntu *et al.* (2021)[18] supported the independent character of

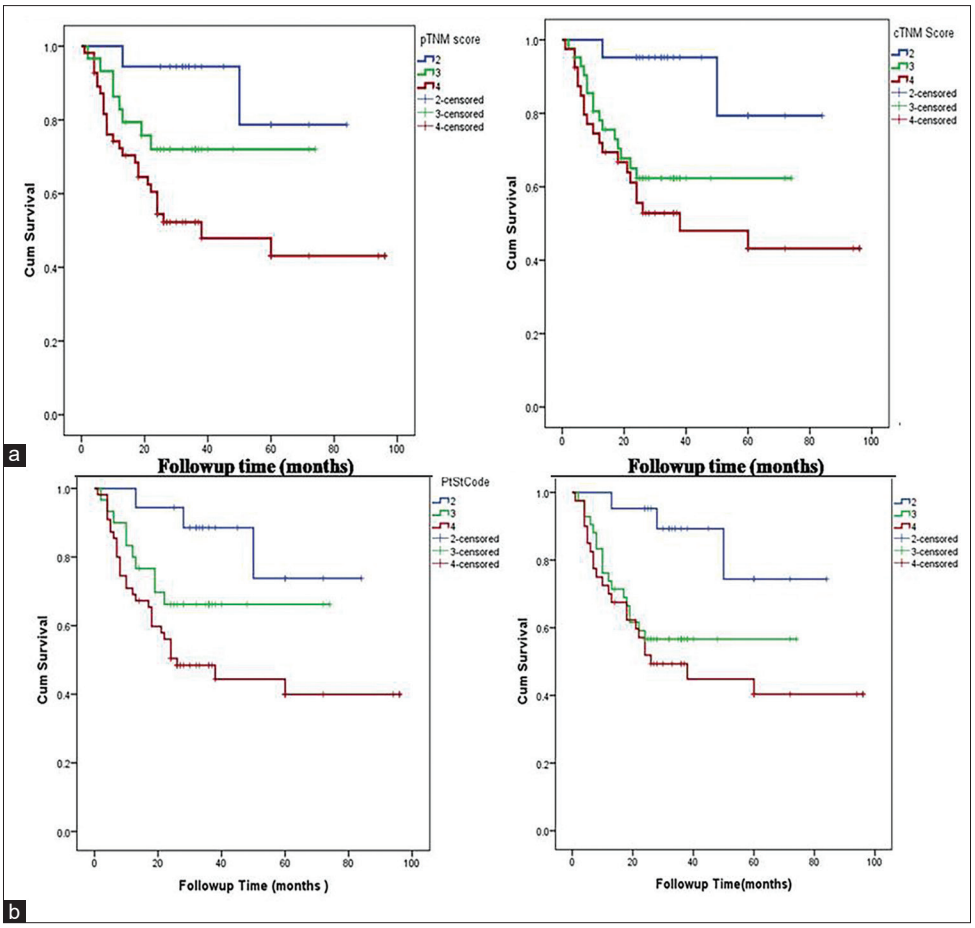

Figure 3: Survival distributions for the different levels of pTNM and cTNM (a). Overall survival (b). Recurrence-free survival

$CD8^+$ lymphocyte infiltrate at the invasive front as a prognostic biomarker in OSCC. One of the reasons for variation in our results could be the non-inclusion of invasive margin assessment. However, only a few studies deal with the spatial distribution of immune cells within the tumour and large knowledge gaps still remain concerning their comprehensive spatiotemporal characterization.[23,24]

Ruiter *et al.* (2020)[25] could not establish evidence for the prognostic value of $CD3^+$ and $CD8^+$ T lymphocytes in the tumour epithelium of advanced stage, human papilloma virus (HPV)-negative HNSCC patients treated with primary chemoradiotherapy with digital quantification. Mamilos *et al.* (2023)[26] also showed that high and low immune cell counts in the tumour centre and invasion front were not associated with OS. We also did not observe survival benefits in patients with high CD8/CD3 density. Quan *et al.* (2020)[6] reported that number of $CD8^+$ T cells were not associated with any clinical factors or OS. Moreover, it has been suggested that primary solid tumours may be ignored by the immune system and that a meaningful immune response is only mounted in regional lymph nodes. Pretscher *et al.* (2009)[27] did not observe an association of tumour-infiltrating immune cells at the primary site with outcome.

In contrast to our findings, Shimizu *et al.* (2019)[28] observed that previously untreated patients with OSCC with high tumour-infiltrating $CD8^+$ T cells had significantly better disease-specific survival (DSS), OS, and RFS. Also in their study, high stromal $CD8^+$ T-cell density at the tumour periphery and high parenchymal $CD8^+$ T-cell density at the invading edge were independent prognostic markers for the clinical endpoints. Tabachnyk *et al.* (2012)[29] showed that a high density of tumour-infiltrating $CD8^+$ T cells observed in OSCC patients had a better DFS after concurrent chemoradiotherapy. Watanabe *et al.* (2010)[30] first examined TILs in 87 OSCC patients based on their spatial distribution. Low numbers of $CD8^+$ cells in the tumour centre and the invasion margin were associated with worse survival, while this association only remained significant in early-stage OSCC. Fang *et al.* (2017)[31] also reported that OSCC patients with strong CD8 expression had a significantly better clinical outcome. Kakkar *et al.* (2023)[20] observed no impact of $CD3^+$ T-cell density on prognosis; however, high $CD8^+$ count was associated with longer DFS and OS.

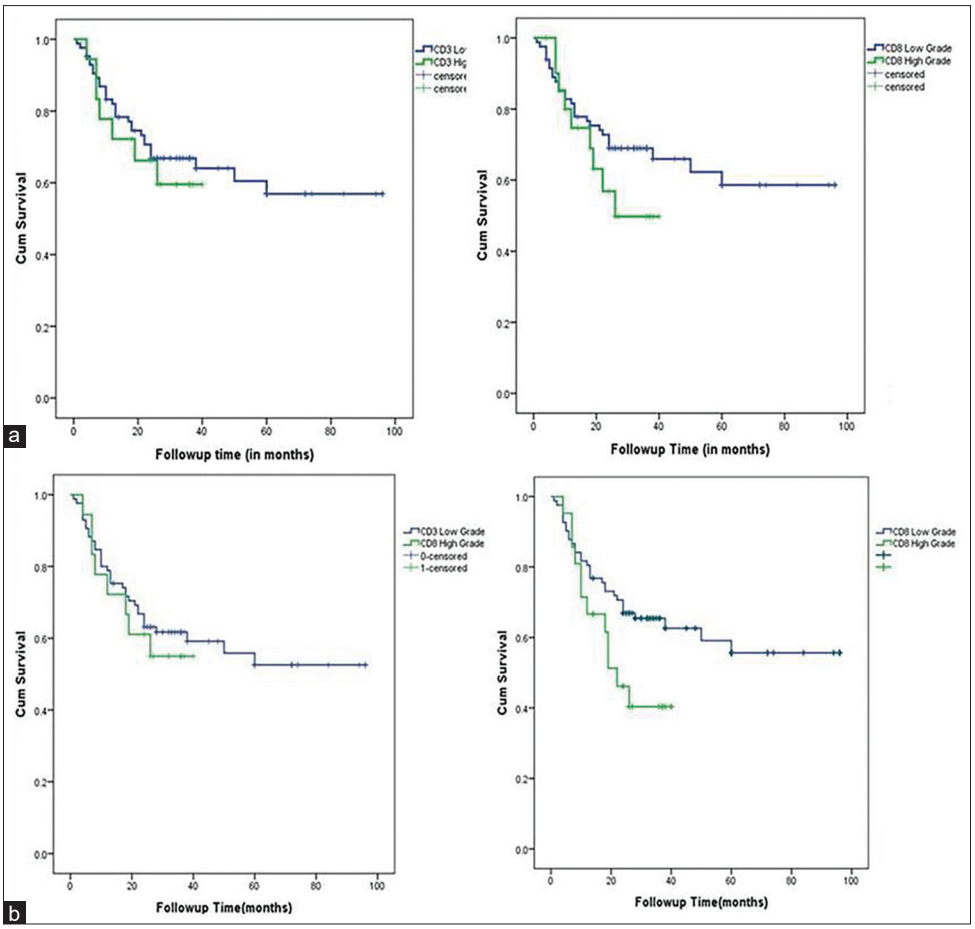

Figure 4: Survival distributions for the different levels of low/high CD3 and CD8 expression (a). Overall survival (b). Recurrence-free survival

We did not observe statistically significant association between high/low CD3 and CD8 cell counts and clinicopathological factors in digital analysis. It is suggested that for digital quantification, whole slide image analysis might give better results. Digital evaluation and machine learning approaches though promising are not yet validated.[31,32]

Despite taking best care of clinical and all technical optimizations in our study design, we could not find a prognostic role for T-cell markers CD3 and CD8 in tumour epithelium and stroma, an observation that is in contrast with the previous studies. This could imply that the pre-treatment composition of the TME is of less importance than the antitumour immune response induced by the (chemo) radiotherapy. However, there are evidences supporting and negating this hypothesis as studies do show an association between the pre-treatment presence of TILs and treatment outcome more in HNSCC than OSCC.[28,29,33,34] Inconsistent results with literature might also be attributed to not assessing the functional status of CD8 cells with PD-1/PD-L1 antibodies,[35] HPV status; as evidence is there that $CD3^+/CD8^+$ TIL density has more significant outcomes in HPV positive tumours.[36] Also, there are many grey areas like tumour mutation burden, other immunesuppressive cell subsets, TME hypoxia, and inflammatory mediators.[37]

Due to the retrospective nature, the fixation method of biopsies was not uniform, which could potentially affect the IHC evaluation results. There are chances of potential cross-reactivity with other cellular components and some background staining, thus compromising immunohistologic interpretation. Heterogeneous patient cohorts with respect to treatment modality, tumour stage, biopsy type, and subsite can be other confounding factors.[34,38] Methods of assessing TILs varied strongly and that make the results of different studies incomparable.[39] IHC evaluation scoring methodology, semiquantitative or digital is not yet standardized and validated by International Immuno-Oncology Biomarker Working Group as has been done with H&E staining. Furthermore, consensus on robust cutoffs is lacking, and accordingly, the clinical implementation of TIL analysis in the routine practice is far from reached yet. Moreover, integrative analyses of multiple subsets of TILs and combined TIL scores

could be clinically more useful predictive and prognostic factors.[40] Thus, a more accurate analysis of the potential benefit of assessing T-cell infiltrates requires a homogenous sample, multicentric approach, large numbers of patients with tumours of similar site with detailed information regarding other prognostic clinical characteristics, treatment regimens, and specific outcomes.

Cancer immunotherapy has shown unprecedented success but yielded substantial benefits only in a small proportion of patients and selected cancer types, highlighting the need to characterize immune landscape of individual cancer and identify potential responders and design rational therapeutic strategies.[41] The need of the hour is to establish a standardized, cost-effective, reproducible, and reliable method of TIL assessment using immune biomarkers which specifically predict treatment outcome and direct personalized medicine.

CONCLUSION

In conclusion, this study did not provide evidence for a prognostic value of the presence of $CD3^+$ and $CD8^+$ T lymphocytes in the tumour epithelium and stroma of OSCC patients. The results revealed the compositions, heterogeneity, and patterns of TIL infiltration in OSCC samples. However, a method to assess TILs was described, and future studies are needed to explore how these results can be leveraged. Our work would add value to the literature as retrospective evaluation and prospective studies are warranted to standardize methodology, scoring criteria and also to develop validated cutoff values.

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Conflicts of interest

There are no conflicts of interest.

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