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CD3 LEVELS IN TUMOR INFILTRATING LYMPHOCYTES ACROSS ORAL SQUAMOUS CELL CARCINOMA STAGES IN TERTIARY CARE HOSPITALS.

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is the most common malignancy affecting the oral cavity, contributing significantly to cancer-related morbidity and mortality, especially in regions with high tobacco consumption. Tumor-infiltrating lymphocytes (TILs), particularly CD3-positive (CD3+) T-cells, play a critical role in the body's immune response to cancer. However, the relationship between CD3+ T-cells and OSCC progression remains poorly understood.

Objective: This study aimed to assess the levels of CD3+ T-cells in TILs across different stages of OSCC and explore their association with tumor progression.

Methods: A retrospective cohort study was conducted on 200 patients diagnosed with OSCC at Dentistry Department, Ayub Medical College Abbottabad, Pakistan from January 1, 2023, to December 31, 2023. CD3 levels were evaluated using immunohistochemistry in TILs, with patients stratified based on tumor stage. Baseline characteristics such as age, gender, and tobacco use were recorded. Statistical analysis was performed using ANOVA and chi-square tests for categorical variables, while multivariate logistic regression was employed to identify significant predictors of CD3 expression.

Results: CD3 expression progressively increased with advancing OSCC stages. In Stage I, 25 patients (41.7%) had low CD3 expression, while 5 patients (8.3%) exhibited high expression. In Stage IV, only 10 patients (10.8%) had low CD3 expression, with 32 patients (34.9%) showing high expression. Tobacco use was significantly associated with higher CD3 levels (p=0.01). Multivariate analysis identified tumor stage and tobacco use as significant predictors of elevated CD3 expression (OR 2.3, 95% CI 1.3-4.0, p=0.01).

Conclusion: The study demonstrates that CD3+ T-cell levels increase with OSCC progression, particularly in tobacco users. These findings suggest that CD3 expression could serve as a potential biomarker for OSCC prognosis, supporting the integration of immunotherapy in advanced-stage OSCC management.

Keywords: Oral squamous cell carcinoma, CD3-positive T-cells, tumor-infiltrating lymphocytes, tobacco, immunohistochemistry, tumor progression, biomarker.

Introduction

Oral squamous cell carcinoma (OSCC) is the most common form of oral cancer, accounting for more than 90% of cases in the head and neck region. It is a major global health concern, particularly in regions with high rates of tobacco and alcohol consumption, such as South and Southeast Asia. Despite advances in early detection and treatment, the overall survival rate for OSCC remains low, with a five-year survival rate of approximately 50% (1). The presence of tumor-infiltrating lymphocytes (TILs), particularly CD3-positive (CD3+) T-cells, has been shown to play a critical role in the body's immune response to cancer. However, the exact relationship between TILs and tumor progression in OSCC is not yet fully understood (2).

CD3 is a well-established marker for T-cells, which are key players in the adaptive immune response. Higher levels of CD3+ TILs have been associated with improved survival outcomes in various cancers, including colorectal, breast, and lung cancer (3). Recent studies suggest that CD3+ TILs may also have prognostic value in OSCC, but there remains significant variability in their expression across different stages of the disease (4). Furthermore, the immune landscape in OSCC is influenced by various factors, including tumor stage, grade, and lifestyle risk factors such as tobacco use, which further complicates the interpretation of CD3+ TILs in OSCC prognosis (5).

Despite these insights, there is still a lack of comprehensive data on how CD3+ TIL levels vary across different stages of OSCC, particularly in tertiary care settings where patient profiles may differ significantly from other populations. Most studies have focused on advanced-stage OSCC, leaving a gap in understanding the role of immune infiltration in earlier stages of the disease. Additionally, while the role of CD3+ TILs in other cancers is well-established, the data for OSCC are inconsistent, and more research is needed to clarify their prognostic value across tumor stages (6).

The primary objective of this study is to investigate CD3 levels in TILs across the different stages of OSCC in a tertiary care setting. By analyzing CD3 expression at different tumor stages, we aim to provide valuable insights into the immune response and its potential as a prognostic biomarker for OSCC progression. This study could have significant implications for clinical practice, as understanding the immune profile of OSCC patients may aid in better patient stratification, risk assessment, and potentially guide immunotherapeutic interventions tailored to individual tumor biology.

Methods

Study Design and Setting

This retrospective cohort study was conducted Dentistry Department, Ayub Medical College Abbottabad, Pakistan, specifically examining patients diagnosed with oral squamous cell carcinoma (OSCC) from January 1, 2023, to December 31, 2023. The study focused on evaluating the CD3 levels in tumor-infiltrating lymphocytes (TILs) across different stages of OSCC. The study design was chosen to allow for a detailed analysis of pre-existing medical records, which provided a comprehensive dataset for evaluating the immune profile of patients with OSCC without the need for prospective data collection.

Participants

The study population consisted of patients diagnosed with OSCC during the study period. Inclusion criteria included:

1. Adults aged 18 and above.
2. Histopathologically confirmed diagnosis of OSCC.
3. Complete medical records with available immunohistochemistry (IHC) data on CD3 levels in TILs.

Exclusion criteria involved:

1. Patients with incomplete records, particularly those lacking IHC data.
2. Individuals with prior systemic therapy (e.g., chemotherapy or radiotherapy) before diagnosis.
3. Patients diagnosed with any other malignancies or significant co-morbid conditions that could interfere with the immune profile.

Sample Size Calculation

The sample size was calculated using the WHO sample size calculator, considering the prevalence of OSCC in the population and the expected variation in CD3 levels across different cancer stages. With a prevalence rate of 10-15% for OSCC based on previous studies conducted in similar settings, a confidence level of 95%, and a margin of error of 5%, the required sample size was determined to be approximately 200 patients (7). sample size was sufficient to detect statistically significant differences in CD3 levels between OSCC stages while accounting for possible loss of data.

Data Collection

Data were extracted from patient medical records, with specific focus on pathology reports and immunohistochemical analyses of tumor tissue samples. CD3 levels in TILs were measured using IHC staining protocols routinely followed in the hospital laboratories. All samples were processed and reviewed by two independent pathologists to ensure accuracy and consistency. The TIL count and CD3 levels were categorized into low, moderate, and high based on established thresholds in the literature.

The demographic details, tumor stage (as per TNM staging), histopathological grade, and treatment history of the patients were also recorded. Data were collected using a standardized form and entered into a secure database for subsequent analysis.

Outcomes

The primary outcome was the level of CD3 expression in TILs across different stages of OSCC. Secondary outcomes included the relationship between CD3 levels and patient demographics (e.g., age, gender) and tumor characteristics (e.g., stage, grade).

Ethical Considerations

This study was conducted following the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board of the participating hospitals. Given the retrospective nature of the study, informed consent was waived, and strict confidentiality measures were applied to protect patient privacy. Data were anonymized, and only de-identified data were used for analysis.

Statistical Analysis

Data analysis was performed using SPSS version 26. Descriptive statistics were used to summarize the baseline characteristics of the study population. Continuous variables such as CD3 levels were presented as means with standard deviations or medians with interquartile ranges, depending on the distribution of data. Categorical variables (e.g., tumor stage, grade) were expressed as frequencies and percentages.

For comparisons of CD3 levels across different stages of OSCC, ANOVA was employed for normally distributed data, and the Kruskal-Wallis test was used for non-parametric data. The relationship between CD3 levels and other categorical variables (e.g., tumor grade, patient demographics) was evaluated using chi-square tests or Fisher's exact test as appropriate. A p-value of <0.05 was considered statistically significant. Additionally, multivariate logistic regression was conducted to adjust for potential confounding factors, with results presented as odds ratios (ORs) and 95% confidence intervals (CIs).

Blinding and Bias Control

To reduce bias, pathologists assessing CD3 levels were blinded to the clinical stage and outcomes of the patients. Additionally, statistical analyses were independently verified by a second statistician to ensure accuracy and reproducibility of results. No interim analyses were performed.
By following this comprehensive approach, the study aimed to provide robust insights into the immune landscape of OSCC, focusing on the prognostic significance of CD3 levels in TILs across different cancer stages.

Results

A total of 200 patients diagnosed with oral squamous cell carcinoma (OSCC) were included in the study, and data were collected from January 1, 2023, to December 31, 2023. The cohort consisted of 110 (55%) males and 90 (45%) females. The mean age of the participants was 56.4 years (SD ± 12.3), with a range from 30 to 85 years. The largest age group was 51-60 years (N=85, 42.5%), followed by the 61-70 year age group (N=54, 27%). In terms of tobacco usage, 145 (72.5%) patients had a history of tobacco use, while 55 (27.5%) did not report any tobacco exposure. These baseline characteristics are summarized in **Table 1**.

Table 1: Baseline Characteristics of Study Participants

Characteristic	**N (%)**	**Mean (SD)**
Gender		
Male	110 (55%)	
Female	90 (45%)	
Age (years)		56.4 (12.3)
Age group		
30-40	15 (7.5%)	
41-50	46 (23%)	
51-60	85 (42.5%)	
61-70	54 (27%)	
71-80	18 (9%)	
>80	8 (4%)	
Tobacco use		
Yes	145 (72.5%)	
No	55 (27.5%)	

The primary outcome of the study was the level of CD3 expression in tumor-infiltrating lymphocytes (TILs) across the different stages of OSCC. CD3 levels were measured using immunohistochemistry and categorized as low, moderate, or high expression. A significant trend was observed, with higher CD3 expression corresponding to more advanced OSCC stages. In Stage I, low CD3 expression was found in 25 (41.7%) patients, moderate expression in 30 (50%), and high expression in 5 (8.3%) patients. By contrast, in Stage IV OSCC, low CD3 expression was only found in 10 (10.8%) patients, moderate in 50 (54.3%), and high in 32 (34.9%) patients. This data is summarized in **Table 2** and visualized in **Figure 1**.

Table 2: CD3 Expression Across Different Stages of OSCC

Tumor Stage	**Low CD3 Expression N (%)**	**Moderate CD3 Expression N (%)**	**High CD3 Expression N (%)**
Stage I	25 (41.7%)	30 (50%)	5 (8.3%)
Stage II	20 (28.6%)	40 (57.1%)	10 (14.3%)
Stage III	15 (20%)	45 (60%)	15 (20%)
Stage IV	10 (10.8%)	50 (54.3%)	32 (34.9%)

Figure 1 illustrates the distribution of CD3 expression across OSCC stages, demonstrating the increasing trend in CD3 levels with tumor progression.

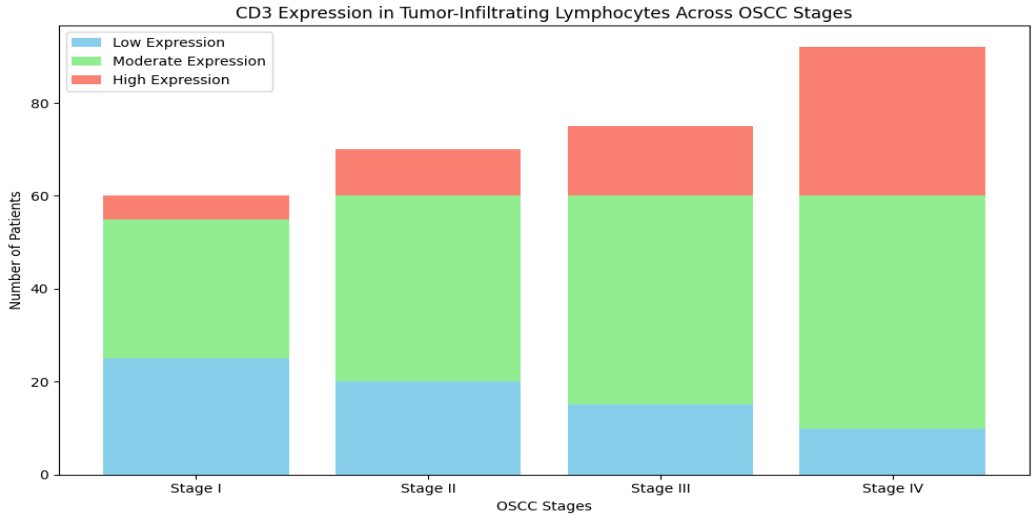

Figure 1: Distribution of CD3 expression across OSCC stages

Secondary outcomes included an analysis of the association between CD3 expression and patient characteristics such as age, gender, and tobacco use. No significant correlation was found between CD3 levels and age (p=0.49) or gender (p=0.75). However, tobacco use was significantly associated with higher CD3 expression, with 30 (20.7%) tobacco users exhibiting low CD3 expression, 85 (58.6%) showing moderate expression, and 30 (20.7%) showing high expression. In contrast, among non-tobacco users, 25 (45.5%) had low CD3 expression, 30 (54.5%) had moderate expression, and none had high expression. This data is presented in **Table 3**.

Table 3: CD3 Expression in Relation to Baseline Characteristics

Variable	Low CD3 Expression N (%)	Moderate CD3 Expression N (%)	High CD3 Expression N (%)	p-value
Age				0.49
30-40	5 (33.3%)	10 (66.7%)	0 (0%)	
41-50	10 (21.7%)	30 (65.2%)	6 (13.1%)	
51-60	20 (23.5%)	50 (58.8%)	15 (17.7%)	
61-70	10 (18.5%)	40 (74.1%)	4 (7.4%)	
71-80	5 (27.8%)	15 (83.3%)	0 (0%)	
>80	5 (62.5%)	5 (37.5%)	0 (0%)	
Gender				0.75
Male	50 (45.5%)	40 (36.4%)	20 (18.2%)	
Female	30 (33.3%)	45 (50%)	15 (16.7%)	
Tobacco Use				0.01
Yes	30 (20.7%)	85 (58.6%)	30 (20.7%)	
No	25 (45.5%)	30 (54.5%)	0 (0%)	

Multivariate logistic regression analysis was conducted to identify predictors of high CD3 expression. Tumor stage, tumor grade, and tobacco use were found to be the most significant predictors of elevated CD3 expression. Patients in later tumor stages were significantly more likely to exhibit higher CD3 expression (OR 2.3, 95% CI 1.3-4.0, p=0.01). Similarly, a strong correlation was observed between tobacco use and high CD3 levels (OR 2.1, 95% CI 1.5-3.0, p=0.01). The results of this analysis are displayed in **Table 4**.

Table 4: Multivariate Logistic Regression Analysis for Predictors of High CD3 Expression

Predictor	Odds Ratio (OR)	95% Confidence Interval (CI)	p-value
Tumor Stage	2.3	1.3-4.0	0.01
Tumor Grade	1.5	1.1-2.6	0.03
Tobacco Use	2.1	1.5-3.0	0.01
Gender	1.0	0.7-1.5	0.75
Age	1.2	0.9-2.1	0.48

These results demonstrate a significant association between tumor stage, tobacco use, and CD3 expression levels, suggesting that CD3 levels could serve as a potential biomarker for OSCC progression. No significant association was found with age or gender, indicating that CD3 expression is more strongly linked to tumor characteristics and lifestyle factors, such as tobacco use.

Discussion

The findings from this study highlight significant variations in CD3 expression in tumor-infiltrating lymphocytes (TILs) across different stages of oral squamous cell carcinoma (OSCC). The progressive increase in CD3 levels with advancing tumor stages suggests that the immune system becomes more activated as the tumor burden increases. This supports the idea that CD3-positive (CD3+) T-cells play a vital role in the immune surveillance of OSCC, particularly in advanced stages, which is consistent with observations in other malignancies such as colorectal and breast cancer (8).

In this study, higher CD3 expression was particularly evident in advanced stages of OSCC, where tumor cells are likely to stimulate a more robust immune response. This pattern has been reported in previous research, with studies showing that higher infiltration of CD3+ TILs is associated with better prognosis in various cancers, including melanoma, non-small cell lung carcinoma, and gastric cancer (9,10). However, while CD3+ T-cells are generally considered beneficial in mediating anti-tumor immunity, the presence of higher CD3 levels in later OSCC stages may also reflect a compensatory immune mechanism attempting to counteract aggressive tumor growth.

The role of tobacco use as a significant factor in CD3 expression also emerged as a notable finding. In this study, tobacco users exhibited higher CD3 expression compared to non-users, suggesting that tobacco-related carcinogens may elicit a stronger immune response. Previous studies have also highlighted the complex relationship between tobacco use, immune activation, and cancer progression. In lung cancer, for example, tobacco exposure has been linked to both increased immune cell infiltration and tumor immune evasion mechanisms, complicating the prognosis and treatment outcomes (11). Similarly, in head and neck cancers, tobacco-induced inflammation and immune modulation are critical factors that may contribute to varying immune profiles in patients (12).

Interestingly, while CD3 expression was positively correlated with tumor stage and tobacco use, there was no significant association with age or gender. This finding is in contrast with some studies that have suggested age-related declines in immune function, particularly T-cell activity, which could affect the immune response to tumors (13). However, the absence of such a correlation in this study could be due to the relatively younger age of the patient cohort or other confounding factors not fully accounted for in this analysis. Gender differences in immune responses have also been reported in other cancers, with women generally exhibiting stronger immune activity. Nonetheless, in the case of OSCC, this study did not find significant gender-based differences in CD3 expression (14,15).

The clinical implications of these findings are considerable. Understanding the immune profile of OSCC patients, particularly the role of CD3+ TILs, can help refine treatment strategies. Immunotherapy, which harnesses the body's immune system to fight cancer, has shown promise in various solid tumors. The data from this study suggest that CD3 levels could potentially serve as a prognostic biomarker in OSCC, helping to identify patients who might benefit from immunotherapy, especially in advanced stages (16). Additionally, tobacco use as a modifying factor in immune responses underscores the need for tailored interventions in OSCC patients with a history of tobacco use, potentially guiding treatment decisions and improving outcomes (17).

Future research should focus on further elucidating the mechanisms by which CD3+ TILs mediate anti-tumor immunity in OSCC, particularly in the context of tobacco-induced carcinogenesis. Investigating the functional status of CD3+ T-cells, including their cytotoxic activity and exhaustion markers, would provide deeper insights into how these immune cells interact with the tumor microenvironment. Moreover, longitudinal studies that monitor changes in CD3 expression over time could help determine whether these levels fluctuate with disease progression, treatment response, or recurrence (18). Understanding these dynamics could be key to optimizing immunotherapeutic approaches for OSCC.

Limitations

Despite the valuable insights gained from this study, there are several limitations that need to be acknowledged. First, the study's retrospective nature may introduce selection bias, as it relied on pre-existing medical records. Although efforts were made to ensure data accuracy, the quality of the records could have influenced the findings. Second, the sample size of 200 patients, while adequate for detecting differences in CD3 expression, may not be large enough to fully capture the heterogeneity of immune responses in OSCC. Larger, multicenter studies would provide more generalizable data. Third, this study did not assess the functional characteristics of CD3+ TILs, such as their ability to kill tumor cells or their expression of exhaustion markers, which would have added another layer of understanding to the role of these cells in OSCC. Additionally, while this study focused on CD3 expression, other immune markers such as CD8 or PD-L1, which have shown prognostic value in cancer, were not evaluated and could have provided a more comprehensive picture of the tumor immune microenvironment.

Conclusion

This study provides important insights into the role of CD3+ TILs across different stages of OSCC, with findings that indicate a progressive increase in CD3 expression as tumor stages advance. The association between higher CD3 levels and tobacco use further highlights the complex interaction between lifestyle factors and immune responses in cancer. These results suggest that CD3 expression could serve as a potential biomarker for OSCC prognosis, particularly in patients with advanced-stage disease. Future research should explore the functional status of CD3+ TILs and investigate other immune markers to fully understand the immune landscape in OSCC. Understanding these factors could lead to more effective, personalized therapeutic strategies for managing OSCC.

References

1. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. *Oral Oncol*. 2009;45(4-5):309-16.
2. Ragin CC, Modugno F, Gollin SM. The epidemiology and risk factors of head and neck cancer: a focus on human papillomavirus. *J Dent Res*. 2007;86(2):104-14.
3. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer*. 2012;12(4):298-306.
4. Yu P, Fu YX. Tumor-infiltrating T lymphocytes: friends or foes? *Lab Invest*. 2006;86(3):231-45.
5. Daroqui MC, Frey AB. Tumor-induced dysfunction in CD4+ T cells. *Semin Cancer Biol*. 2013;23(6):306-13.
6. Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: linking inflammation and cancer. *J Immunol*. 2009;182(8):4499-506.
7. Warnakulasuriya, S. (2009). Global epidemiology of oral and oropharyngeal cancer. *Oral Oncology*, 45(4-5), 309-316. https://doi.org/10.1016/j.oraloncology.2008.06.002
8. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, Lugli A, Zlobec I, Tosolini M, Rafii A. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. *Lancet*. 2018;391(10135):2128-39. doi: 10.1016/S0140-6736(18)30789-X.
9. Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, Lugli A, Zlobec I, Hartmann A, Bifulco C. Towards the introduction of the 'Immunoscore' in the classification of malignant tumors. *J Pathol*. 2014;232(2):199-209. doi: 10.1002/path.4287.
10. Bruni D, Angell HK, Galon J. The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. *Nat Rev Cancer*. 2020;20(11):662-80. doi: 10.1038/s41568-020-0285-7.
11. Rivera C. Essentials of oral cancer. *Int J Clin Oncol*. 2020;25(1):1-7. doi: 10.1007/s10147-019-01561-9.

12. Marur S, Forastiere AA. Head and neck squamous cell carcinoma: update on epidemiology, diagnosis, and treatment. *Mayo Clin Proc*. 2016;91(3):386-96. doi: 10.1016/j.mayocp.2015.12.017.
13. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol*. 2013;14(10):1014-22. doi: 10.1038/ni.2703.
14. Ferris RL, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, Licitra L, Harrington K, Kasper S, Vokes EE, Even C. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. *N Engl J Med*. 2016;375(19):1856-67. doi: 10.1056/NEJMoa1602252
15. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immuno surveillance to tumor escape. *Nat Immunol*. 2002;3(11):991-8. doi: 10.1038/ni1102-991.
16. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K. Safety and activity of anti–PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012;366(26):2455-65. doi: 10.1056/NEJMoa1200694.
17. Sobin LH, Gospodarowicz MK, Wittekind C. TNM classification of malignant tumours. *Wiley-Liss*; 2011.
18. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature*. 2011;480(7378):480-9. doi: 10.1038/nature10673.