DOI: 10.7860/JCDR/2026/86047.24035

Original Article

Pathology Section

Immunohistochemical Expression of CD57 in Oral Squamous Cell Carcinoma: A Cross-sectional Study

R RANJITH KUMAR[1], TN SURESH[2], D ASWATHAPPA[3]

ABSTRACT

Introduction: Over 90% of oral malignancies in India are squamous cell carcinomas, with oral cancer accounting for about 30% of the total number of cancers in this country. Tumour microenvironment is a critical factor in tumour progression and immunomodulation. The anti-tumour immune response and good prognosis of various cancers have been linked to the presence of CD57, which is a marker of migrated differentiated Natural Killer (NK) cells.

Aim: To evaluate the association between CD57 expression and TNM staging in Oral Squamous Cell Carcinoma (OSCC).

Materials and Methods: The present observational cross-sectional study conducted in Department of Pathology, Sri Devaraj Urs Medical College, Kolar, Karnataka, India, involving OSCC found in 60 histologically confirmed cases between January 2024 and December 2024. The immunohistochemical staining of the CD57 was done on the positive control of tonsil tissue using primary mouse monoclonal antibody. Stromal lymphocytes that were stained in cytoplasm were regarded as positive. The five stromal hotspots were assessed and the average result was considered to be the CD57 labelling index. Quartile-based cut-off values were used as a categorisation of CD57 expression. Parameters studied were tumour grade, T stage, Lymph node positive and negative cases and overall Tumour-Node-Metastasis (TNM) stage. Descriptive statistics were used to report the 25th, 50th (median), and 75th percentiles. Associations between categorical variables were assessed using the Chi-square test. A p-value <0.05 was considered statistically significant. The statistical analysis was done in Statistical Package for Social Sciences (SPSS) version 22.

Results: The male-to-female ratio was 1:1.8. Buccal mucosa was the most common site 41 (68.3%) cases. TNM staging revealed 5 (8.3%) cases in stage I, 21 (35%) in stage II, 16 (26.7%) in stage III, and 18 (30%) in stage IV. Lymph node metastasis was present in 18 cases (30%). High CD57 expression showed a statistically significant association with nodal-negative status (p=0.0001). Lymph node metastasis was identified in 18 of 60 (30.00%) cases, whereas 42 (70.00%) cases showed no nodal involvement.

Conclusion: The potential of CD57 as an immunological marker concerning tumour behaviour was demonstrated by a significant association of the nodal status with the expression of the CD57 in OSCC.

Keywords: Cytoplasmic staining, Natural killer cells, Stromal lymphocytes, Tumour microenvironment

INTRODUCTION

In most cases, OSCC occurs in the oral cavity. Oral malignancies comprise approximately one-third of all the cancers in India, and the squamous cell carcinomas constitute over 90% of the incidences [1]. The head and neck squamous cell carcinoma comprise a broad spectrum of such malignant conditions that involves oropharynx, larynx, nasal sinus and oral cavity malignancies. In men, oral cancer is at 8.7 and 3.5 cases per 100,000, and almost entirely tobacco-related, whereas the same is not explained by any smoking as in women [2]. Recent literature has shown that tumour cell communication and tumour microenvironment (immune cells, stromal cells, and endothelial cells) play a very important role in the tumour behaviour and treatment response.

Unlike other cells of the anti-tumour response, Natural Killer (NK) cells are involved in innate and adaptive responses to the immune system [3]. NK cells have anti-tumour immunity, acting independently of sensitisation by direct cytotoxicity and release of cytokines that recognise and kill stressed or malignant cells [4]. The major characteristics of NK cells include immunologic memory, production of cytokines, and cytotoxic activity. CD57 is a surface protein present on terminally differentiated NK cells and a sub-population of T lymphocytes and CD57-positive cells increase cytotoxic potential and interferon-γ [5].

NK-cell markers that indicate terminal differentiation and functional maturity include CD57, but most of the markers used, including CD16 and CD56, are more often indicators of activation or lineage markers than sustained cytotoxic potential. Thus, the choice of CD57 in the research was based on the aim to determine the prognostically applicable functioning active population of NK-cells in the tumour microenvironment. NK-cell receptor-based therapy has only had modest success, and, at this point, the increased expression of CD57 is often linked to positive outcomes [6,7]. The Chi-square test was used to determine associations (not correlation coefficients) between the categories of CD57 expression and the clinicopathological parameters which include TNM staging, nodal status (nodal positive vs nodal negative), tumour grade, and T stage.

Primary objective: To evaluate the association between CD57 expression and TNM staging in OSCC.

Secondary objectives:

1. To assess the association between CD57 expression and nodal status;
2. To evaluate the relationship between CD57 expression and tumour grade.

MATERIALS AND METHODS

The present cross-sectional observational cross-sectional study in a laboratory involving 60 histologically diagnosed cases of OSCC during the period of January 2024 to December 2024 at Department of Pathology, Sri Devaraj Urs Medical College, Kolar and selected via convenience sampling and underwent composite resection and nodal dissection. Before the start of the study, approval of the Institutional

Ethics Committee was received with IEC no: SDUAHER/R&D/CEC/SDUMC-PG/66/NE/-2025-26.

Sample size calculation: Sample size was calculated using the formula $n=Z^2\ pq/d^2$, taking Z=1.96 at 95% confidence interval, expected proportion of nodal metastasis (p) as 30% and allowable error (d) as 12%. The minimum sample size obtained was 56, and after accounting for tissue loss/non interpretable sections, the final sample size was rounded to 60 [8].

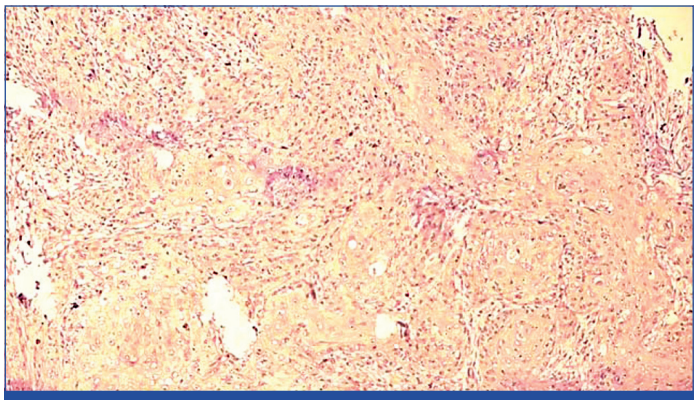
Inclusion and Exclusion criteria: Inclusion criteria included histopathologically proven primary OSCC cases in which the patient was treated with neck dissection. The exclusion criteria were the patients who had already experienced chemotherapy or radiotherapy and possessed inadequately preserved tissue samples. Slides were evaluated on the lymph node metastasis, and the TNM staging was done based on the 8th edition of the American Joint Committee on Cancer (AJCC) staging system [9].

Study Procedure

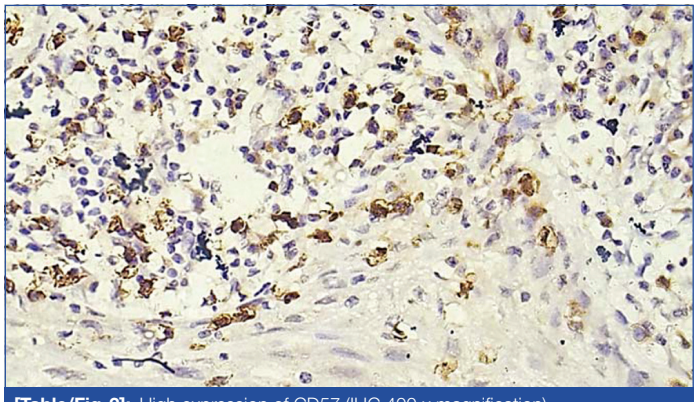
The CD57 immunohistochemical staining was done by using a primary mouse monoclonal antibody (Clone NK-1, Diagnostic Biosystems, USA) with tonsillar tissue as a positive control. The expression of CD57 was determined semi-qualitatively using the stromal lymphocytes via cytoplasm staining. Positivity was taken to be a cytoplasmic immunoreactivity with high power microscopy (x400). Five stromal hotspots on each case were picked, and the average number of CD57-positive lymphocytes was measured as the CD57 labelling index.

Quartile-based cut-off values (25th, 50th and 75th percentiles) were used in categorising CD57 expression to permit stratified comparison of the density of immune cells across clinicopathological parameters, since no universally acceptable absolute cut-off level is available in the literature [Table/Fig-1,2].

CD57 labelling index: Five high powered fields were selected randomly and the density of CD57 lymphocytes was counted and cytoplasm of the cells stained with CD57 was counted as positive. CD57 Labeling Index (LI) for each case is calculated as the average of the number of CD57 cells in the five HPFs. Interquartile Range (IQR) was taken as 13.3 by using 1st and 3rd Quartile.



[Table/Fig-1]: Low expression of CD57 (IHC 200 X magnification).



[Table/Fig-2]: High expression of CD57 (IHC 400 x magnification).

The 25, 50 and 75 percentiles for mean density were calculated and tested where cut-off is taken as mean value of (32.24) for dividing the tumour into below the mean considered as CD57 low expression and above the mean considered as CD57 high expression, using Chi-square test, p-value of ≤0.05 was taken as significant.

CD57 expression was evaluated in relation to tumour grade, T stage, nodal status (nodal-positive versus nodal-negative), and overall TNM stage.

STATISTICAL ANALYSIS

The data were coded and entered in Microsoft Excel after which statistical package each underwent statistical analysis using SPSS version 22. Means, medians and ranges were used to express quantitative data. The Chi-square test was used to determine associations between the categories of CD57 expression and the clinicopathological parameters which include TNM staging, nodal status (nodal positive vs nodal negative), tumour grade, and T stage. The p-value of 0.05 was taken as being statistically significant.

RESULTS

A total of 60 cases of OSCC were included in the study. The male-to-female ratio is 7:13, with 21 (35%) males and 39 (65%) females. The age of patients ranged from 31 to 80 years, with mean age of patients was 56.67 with standard deviation of 11.12 years where majority of cases occurring in the 51-60 year age group 23 (38.33%) cases. The most common anatomical site involved was the buccal mucosa 41 (68.33%) cases, followed by the tongue 16 (26.67%) cases.

Histopathologically, 51 (85.00%) cases were well differentiated, 8 (13.33%) cases were moderately differentiated, and one (1.67%) case was poorly differentiated. Based on tumour size, T2 stage was most frequent 26 (43.33%) cases, followed by T3 19 (31.67%) cases, T4 14 cases (23.33%), and T1 1 case (1.7%). Lymph node metastasis was present in 18 (30.00%) cases and absent in 42 (70.00%) cases. According to TNM staging, five (8.33%) cases were stage I, 21 (35.00 %) cases stage II, 16 (26.67%) cases stage III and 18 (30.00%) cases stage IV. The detailed distribution of clinicopathological parameters is summarised in [Table/Fig-3]. CD57 expression was categorised based on quartile cut-off values of the CD57 labelling index. A statistically significant association was observed between high CD57 expression and nodal-negative status, at both the IQR cut-off (IQR=13.3; p=0.0001) and the first quartile cut-off (Q1 mean=22.6; p=0.028). No statistically significant association was observed between CD57 expression and tumour grade, T stage, or overall TNM stage (p>0.05). The association between CD57 expression and clinicopathological parameters is shown in [Table/Fig-4].

Parameters	Category	Number of cases (%)
Age (years)	31-40	5 (8.33%)
	41-50	12 (20.00%)
	51-60	23 (38.33%)
	61-70	15 (25.00%)
	71-80	5 (8.33%)
Gender	Male	21 (35.00%)
	Female	39 (65.00%)
Site of tumour	Buccal mucosa	41 (68.33%)
	Tongue	16 (26.67%)
	Retromolar trigone	2 (3.33%)
	Floor of mouth	1 (1.67%)
Tumour grade	Well differentiated	51 (85.00%)
	Moderately differentiated	8 (13.33%)
	Poorly differentiated	1 (1.67%)

T stage	T1	1 (1.67%)
	T2	26 (43.33%)
	T3	19 (31.67%)
	T4	14 (23.33%)
Nodal status	Positive	18 (30.00%)
	Negative	42 (70.00%)
TNM stage	Stage-I	5 (8.33%)
	Stage-II	21 (35.00%)
	Stage-III	16 (26.67%)
	Stage-IV	18 (30.00%)

[Table/Fig-3]: Clinicopathological characteristics of Oral Squamous Cell Carcinoma (OSCC) cases (N=60).

CD57 cut-off (quartile)	Tumour grade (p-value)	T stage (p-value)	Nodal status (p-value)	TNM stage (p-value)
IQR - 13.3	0.576	0.8539	0.0001*	0.7481
Q1 - 22.6	0.732	0.5919	0.0280*	0.1735
Q2 - 33.4	0.980	0.3400	0.306	0.7500
Q3 - 35.9	0.732	0.3592	0.2709	0.4351

[Table/Fig-4]: Association of CD57 expression (quartile-based cut-off values) with clinicopathological parameters.
*-indicate statistically significant association ($p < 0.05$)}

DISCUSSION

The role of NK cells in innate anti-tumour immunity is significant in causing tumour cell death by releasing cytokines, antibody-dependent cellular cytotoxicity, as well as changing the recognition of major histocompatibility complex class I molecules [10]. CD57 is one of the NK-cell markers that are expressed by terminally differentiated and functionally mature NK cells with prolonged cytotoxic potential and, therefore, is useful in the determination of immune activity in the tumour microenvironment.

CD57 is an immunohistochemical marker expressed on mature, terminally differentiated NK cells and on a subset of cytotoxic T lymphocytes Functionally, CD57+ NK cells participate in tumour immune surveillance by mediating direct cytotoxicity, releasing immune-modulating cytokines (including IFN-γ), and contributing to antibody-dependent cellular cytotoxicity, particularly when tumour cells evade T-cell immunity through reduced MHC class I expression. The proportion of well-differentiated squamous cell carcinoma (85%) observed in the present study is comparatively higher than that reported in most OSCC studies (50-70%). This finding may be related to earlier diagnosis, where tumours are detected before undergoing significant dedifferentiation. Furthermore, local habits such as prolonged use of areca nut and tobacco could lead to gradual epithelial transformation, often resulting in well-differentiated histology and surgically manageable and less aggressive cases may have also influenced this distribution. Together, these factors may account for the higher frequency of well-differentiated tumours in this study.

Several studies in squamous carcinomas and other solid tumours suggest that increased CD57+ immune infiltration often accompanies a less aggressive tumour phenotype, supporting a protective role in limiting progression and metastasis. For example, Taghavi N et al., [11] reported prognostic relevance of CD57 in OSCC, and Cope Z et al., [12] demonstrated higher CD57+ cell counts in non metastatic lip cancers compared with intraoral SCC, implying a relationship between CD57+ immune presence and reduced regional spread. In addition, a meta-analysis across solid tumours reported that higher CD57+ lymphocyte infiltration was frequently linked with improved survival outcomes, reinforcing the concept that CD57 marks cytotoxic immune cells actively involved in tumour control [13].

High levels of CD57 expression and nodal-negative had a statistically significant relation with OSCC in the current research. The magnitude of the CD57-positive stromal lymphocytes was observed to be greater in the absence of lymph node metastasis cases. Fang J et al., have also reported the same observation at the increased CD57 expression in the cases that were lymph node-negative, as it supports the contribution of immune cells positive for CD57 in inhibiting the regional tumour progression [14].

Nevertheless, there was no statistically significant correlation between the expression of CD57 and tumour grade, T stage and overall TNM stage.

Similarly, Singh S et al., also reported enhanced immune cell infiltration in cases of enhanced CD57 expression, but the results were not consistent among tumour stages. The insignificance of TNM stage in the current research might be mediated through the composite nature of the TNM staging in which tumour size and nodal status are combined; though nodal status alone was significant, it might have been masked when incorporated within the general overall staging [15].

Oliveira Maciel TA et al., analysed 32 cases of lower lip squamous cell carcinoma and assessed CD57+ NK cells at the invasion front in relation to stage, nodal status and tumour grade. On TNM staging, 15/32 (46.90%) cases were stage I/II and 17/32 (53.10%) were stage III/IV, although CD57+ counts were higher in early-stage tumours, the association was not significant (p=0.576). For nodal status, 16/32 (50.00%) were non metastatic and 16/32 (50.00%) were metastatic, CD57+ cells were numerically higher in nodal-negative cases but the difference was not significant (p=0.622). For histologic grade, 16/32 (50.00%) were low-grade and 16/32 (50.00%) were high-grade tumours; CD57+ infiltration showed no significant association with grade (p=0.936) [16].

Kumar S et al., found that there were high levels of CD57 positivity found in majority of patients in their study, (91%). Grading was classified in OSCC cases according to the histopathological grade of differentiation. When looking at CD57 expression, this research found that Group-II had the greatest rate of positive at 96.6%, followed by Group-III at 88.9%, and Group-I at 81.8% tumours of various grades with statistically significant correlations being found between CD57 and tumour differentiation. Conversely, the current research was unable to establish a strong correlation between the CD57 expression and tumour grade, which points to the inconsistency between populations and study designs [17].

Rathipriya A et al., evaluated the immunohistochemical expression of CD57 in 30 cases of OSCC, which were evenly distributed among well-differentiated, moderately differentiated, and poorly differentiated tumours (10 cases each). CD57 positivity was identified in 28 out of 30 cases (93.33%), while two cases (6.67%) did not demonstrate immunoreactivity. Among the positive cases, 17 cases (56.67%) exhibited moderate to strong staining intensity and 11 cases (36.67%) showed mild staining. Statistical analysis revealed a highly significant association between CD57 expression and histological grade, with a Chi-square value of 23.01 and a p-value of 0.001, indicating that CD57 expression varied significantly according to tumour differentiation [18].

Limitation(s)

The lack of a large sample size and the inclusion of one Institution in this study are also limitations of the research because it could cause selection bias. Also, the lack of survival analysis does not allow the evaluation of the prognostic impact of CD57 expression. To prove these findings, future multi-center studies involving larger populations with follow-up of these populations and assessing them over time are needed.

CONCLUSION(S)

The levels of CD57 expression were found to have a significant association with the nodal-negative status in OSCC and there was no significant association with the tumour grade, T stage, or

the overall TNM stage. These results imply that the expression of CD57 could portray local immune response regarding the nodal involvement of the regions. There is a need to conduct more studies involving a survival analysis in order to establish the survival value of the presence or absence of CD57 in OSCC.

Acknowledgement

The authors express their sincere gratitude to Professor and Head, Department of Pathology, for continuous guidance and valuable feedback throughout the study. The authors also thank the technical staff of the histopathology laboratory for their assistance during specimen processing and slide preparation.

REFERENCES

[1] Das H, Motghare S. India as "the oral cancer capital of the world": The rising burden of oral malignancies across the nation. Int J Sci Healthcare Res. 2021;6(2):99-107.
[2] Hu M, Chen H, Wang R, Zhang R, Yao J, Qiu X, et al. Global, regional, and national burden of lip and oral cavity cancer and its attributable risk factors from 1990 to 2021: An analysis of the Global Burden of Disease study 2021. Int J Clin Pharm. 2026;48(1):93-106.
[3] De Ruiter EJ, Bisheshar SK, de Roest RH, Wesseling FWR, Hoebers FJP, van den Hout MFCM, et al. Assessing the prognostic value of tumour-infiltrating CD57+ cells in advanced-stage head and neck cancer using QuPath digital image analysis. Virchow's Arch. 2022;481(2):223-31.
[4] Luo N, Chen C, Zhou W, Hao J, He S, Liu Y, et al. Natural killer cell-mediated antitumour immunity: Molecular mechanisms and clinical applications. Med Comm. 2025;6(9):e70387.
[5] Arellano-Ballestero H, Zubiak A, Dally C, Orchard K, Alrubayyi A, Charalambous X, et al. Proteomic and phenotypic characteristics of memory-like natural killer cells for cancer immunotherapy. Journal for Immunotherapy of Cancer. 2024;12(7):e008717.
[6] Ferreira KDM, Tramontani Ramos R, Stofela Sodre C, NivoloniTannure P, Armada L, de Souza Gonçalves L, et al. Pro- and anti-inflammatory biomarkers as potential prognostic markers in oral squamous cell carcinoma. Int J Odontostomatol. 2022;16(2):258-66.
[7] Giordano C, Carlomagno S, Falco M, Cantoni C, Vitale M, Caruana I, et al. CD94-driven in vitro expansion of highly functional adaptive NKG2C+ NKG2A-CD57+ NK cells from CMV+ healthy donors. Frontiers in Immunology. 2025;16:1481745.
[8] Lwanga SK, Lemeshow S. Sample size determination in health studies: A practical manual. Geneva: World Health Organization. 1991.
[9] Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging: The Eighth Edition AJCC Cancer Staging Manual. CA Cancer J Clin. 2017;67(2):93-99.
[10] St-Pierre F, Bhatia S, Chandra S. Harnessing natural killer cells in cancer immunotherapy: A review of mechanisms and novel therapies. Cancers (Basel). 2021;13(8):1988.
[11] Taghavi N, Bagheri S, Akbarzadeh A. Prognostic implication of CD 57, CD 16, and TGF-β expression in oral squamous cell carcinoma. Journal of Oral Pathology & Medicine. 2016 Jan;45(1):58-62.
[12] Zancope E, Costa NL, Junqueira-Kipnis AP, Valadares MC, Silva TA, Leles CR, Mendonça EF, Batista AC. Differential infiltration of CD8+ and NK cells in lip and oral cavity squamous cell carcinoma. Journal of oral pathology & medicine. 2010 Feb;39(2):162-7.
[13] Hu G, Wang S. Prognostic role of tumor-infiltrating CD57-positive lymphocytes in solid tumors: A meta-analysis. Oncotarget. 2017;9(8):8111.
[14] Fang J, Li X, Ma D, Liu X, Chen Y, Wang Y, et al. Prognostic significance of tumor infiltrating immune cells in oral squamous cell carcinoma. BMC Cancer. 2017;17(1):375. doi:10.1186/s12885-017-3317-2.
[15] Singh S, Urs AB, Kumar P. Expression and analysis of CX3CL1 chemokine and CD57+ lymphocytes in oral squamous cell carcinoma and their correlation with clinicopathologic features. J Cancer Res Ther. 2024;20(3):770–775.
[16] Oliveira Maciel TA, Serpa MS, Mafra RP, Gonzaga AK, Souza LB, Pinto LP. Immunohistochemical analysis of natural killer cells and CD8+ T lymphocytes in lower lip squamous cell carcinoma. J Clin of Diagn Res. 2017;11(12):EC22-EC25.
[17] Kumar S, Chandra A, Agrawal R, Singh P, Pallavi M. Prognostic implications of CD57 expression in oral squamous cell carcinoma cases. Indian J Pathol Microbiol. 2023;66(4):815-18.
[18] Rathipriya A, Ramesh M, Prem D, Sakthivel VS, Murugesan A, Varrshana V. Immunohistochemical expression of CD57 in different histopathological grades of oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2025;29(4):578-83.

PARTICULARS OF CONTRIBUTORS:
1. Postgraduate Student, Department of Pathology, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India.
2. Professor and Head, Department of Pathology, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India.
3. Professor, Department of Surgical Oncology, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. TN Suresh,
Professor and Head, Department of Pathology, SDUMC, Kolar,
Karnataka-563103, India.
E-mail: Sureshtn.path@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Dec 28, 2025
- Manual Googling: Mar 23, 2026
- iThenticate Software: Mar 25, 2026 (6%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Dec 24, 2025**
Date of Peer Review: **Jan 22, 2026**
Date of Acceptance: **Mar 27, 2026**
Date of Publishing: **Aug 01, 2026**