ORIGINAL ARTICLE

EXPRESSION OF CD68 IN ORAL SQUAMOUS CELL CARCINOMA

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Background: Oral Squamous Cell Carcinoma (OSCC) is the most common malignant cancer of oral cavity. Objective of this study was to evaluate the immunohistochemical expression of CD68 in OSCC and to assess CD68 immunohistochemical expression in various grades of OSCC. **Methods:** Records of OSCC resections were retrieved from computerized laboratory data of Peshawar Medical College, Peshawar, and Pakistan Institute of Medical Sciences, Islamabad. Blocks of their tissue preserved in formalin and paraffin were also extracted. A 5-micron segment stained with haematoxylin and eosin was analysed using a light microscope. Patient's age, sex, location of the lesion, and TNM staging were received from biopsy request forms, while the histological diagnosis, excision margin status, and lymph node involvement were retrieved from the biopsy reports. **Results:** As per intensity of CD68, 32 (32%) patients had weak CD68 expression of OSCC, 46 (46%) patients had moderate CD68 expression and 22 (22%) patients had strong CD68 expression. **Conclusion:** While there was no link between patient age or gender and CD68 expression in OSCC, there was a significant association with tumour site and histological grades. Due to its significant association with histological grades, CD68 may serve as a marker for OSCC aggressiveness and prognosis. Tumour location is important for predicting CD68 levels, and it may have a role in tumour behaviour.

Keywords: CD68, Squamous cell carcinoma, Staining, Immunohistochemical expression
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INTRODUCTION

A frequent malignancy of the head and neck region is oral squamous cell carcinoma (OSCC).[1] It is the 6th most prevalent cancer in the world.[2] In South Asia OSCC is one of the main causes of cancer deaths and this high prevalence is mainly because of epidemiological factors like tobacco and betel quid chewing.[3] There are numerous aetiological causes for OSCC which develop in the oropharynx and oral cavity. Nevertheless, smoking and alcohol continue to be the most prominent risk factors, particularly in the West.[4] The main aetiological factors for OSCC in South Asian nations include smokeless tobacco use and products made from areca nuts.[5] The 5-year survival rate has remained below 50% for the past 30 years despite recent breakthroughs in the molecular biology, diagnosis, and therapy of OSCC, primarily because of recurrence or metastasis.[6] An increasing amount of research contributes to the tumour microenvironment as being important for the growth and metastasis of cancer.[7] The use of antibodies provides the foundation for the identification of Tumour Associated Macrophages (TAMs) in humans, i.e., CD68. The CD68 antibody recognizes both M1 and M2 macrophages. In recent studies, it was demonstrated that the CD68 and positive macrophages are related to histological grading and poor prognosis.[8] The CD68 antigen of macrophages is expressed in cancer cells. Cancer cells' characteristics that resemble macrophages are correlated with low survival rates.[9] Tumour cells over express the protein CD68 and high levels of CD68 are associated with high tumour grade, big tumour size, and malignant characteristics, which reflect tumour development and aggressiveness. Increasing evidence has indicated that CD68 is an important biomarker predicting tumour aggressiveness and progression.[10]

The objective of this study was to evaluate immunohistochemical reaction of CD68 in different grades of Oral Squamous Cell Carcinoma, to determine the aggressiveness and progression of the tumour.

METHODOLOGY

The Board of Advanced Studies and Research at Riphah International University granted its approval for the research project. The study was done from Aug 2022 to Feb 2023. Sample size was calculated using the G power as 80%. Total sample size was 94 rounded as 100. Non-probability sampling was used, and 100 most recent samples (paraffin blocks) were retrieved for this study and labelled as 1 to 100.

Cases of OSCC resections were retrieved from the electronic laboratory data of Peshawar Medical College, Peshawar and Pakistan Institute of Medical Sciences, Islamabad. The blocks of tissue that had been preserved in paraffin were retrieved. The tissue slices, 5 microns in thickness, were stained with haematoxylin and eosin and then viewed under a microscope. The biopsy reports were used to extract and document the histological diagnosis, excision margin status, and lymph node involvement, using established processes.

CD68 expression levels were assessed immunohistochemically. The classification of CD68 was expressed as weak, moderate, or strong. Low intensity signified a low or mild level of staining intensity detected in the cells and indicated a reduced

intensity of CD68 markers or a diminished immunological response. Moderate indicated a moderate degree of staining intensity, suggesting a noticeable and somewhat widespread presence of CD68 markers inside the cells or tissue being analysed. High indicated a robust and concentrated staining detected in the cells, indicating a substantial presence and extensive dispersion of CD68 markers. This indicated a strong immunological response or an increased expression of CD68 in the cells or tissue.

The OSCC grading system evaluates differentiation, nuclear pleomorphism, mitotic count, and tumour architecture, assigning values from 1 to 4 for each category. The overall score ranges from 4 to 16. A 'High (>4 score)' implies a higher level of differentiation and less aggressive traits, while a 'Low (1–4 Score)' predicts a lower level of differentiation and a more aggressive tumour phenotype within the scoring range. This grading system facilitated the categorization of patients according to their histological characteristics.

Demographic information included data such as age, gender, and location of the lesion, which was gathered via the paperwork requesting a biopsy. The TNM Staging categorization method was used to evaluate the size of the tumour, involvement of lymph nodes, and presence of metastasis to determine the degree and spread of cancer.

Data entry and analysis were conducted using SPSS-20. For statistical analysis, chi-square test and the Fisher exact test, assuming $p \leq 0.05$ as statistically significant.

RESULTS

This study involved biopsy samples from 100 OSCC patients, and was conducted at the Department of Pathology, Peshawar Medical College, Peshawar and Pakistan Institute of Medical Sciences, Islamabad. Twenty-seven percent patients fell in age group of ≤50 years while 73% patients were in age group >50 years. Mean age of the patients was 58.16±14.348 years.

Thirty-two (32.0%) patients had weak CD68 expression, 46 (46.0%) had moderate, and 22 (22.0%) patients exhibited strong CD68 expression. Among the 100 cases, 27 (27.0%) were labelled as 'High', 65 (65.0%) were classed as 'Low', and 8 (8.0%) cases were 'Negative'. (Table-1).

Within age group of ≤50 years, 10 cases were classified as 'High', 13 as 'Low', and 4 cases as 'Negative'. Among those aged >50 years, 17 cases were classified as 'High', 52 as 'Low', and 4 cases were classified as 'Negative'. (Table-2).

Among men, there were 11 instances classified as 'High', 36 cases as 'Low', and 4 cases as 'Negative'. Among females, there were 16 cases classified as 'High', 29 cases as 'Low', and 4 cases as 'Negative'. There was no statistically significant correlation between gender and final assessment results in OSCC patients (Table-3).

The final rating 'High' across tumour sites was: 7 instances in gingiva, 0 in hypo-pharyngeal area, 2 in labia commissure, and 18 in maxilla/mandible. A strong association was seen between the tumour site and the final assessment results (p=0.026) (Table-4).

Grade I OSCC did not earn a 'High' final grade, however, Grade II OSCC had 5 instances and Grade III had 22 cases. There were 24 instances of Grade I, 41 instances of Grade II, and no instances of Grade III OSCC that were given a 'Low' final assessment. A statistically significant correlation (p<0.001) was seen between the histological grade of OSCC and the classified final assessments (Table-5).

Table-1: Frequencies of intensity of CD68 expression and final evaluation (n=100)

	Frequency	Percent
Intensity of CD68		
Weak	32	32
Moderate	46	46
Strong	22	22
Final Evaluation		
High (>4 score)	27	27
Low (1–4 Score)	65	65
Negative (0 score)	8	8

Table-2: Final evaluation in age groups

Final Evaluation	Age ≤50 Years	Age >50 Years	Total	*p*
High	10	17	27	0.076
Low	13	52	65	
Negative	4	4	8	
Total	27	73	100	

Table-3: Association of final evaluation with gender

Final Evaluation	Male	Female	Total	*p*
High	11	16	27	0.440
Low	36	29	65	
Negative	4	4	8	
Total	51	49	100	

Table-4: Association final evaluation with tumour site

Site of tumour	'High'	'Low'	'Negative'
Gingiva	7	30	0
Cheek	0	2	0
Hypopharyngeal Region	0	2	1
Labia Commissure	2	1	2
Tongue	0	1	0
Maxilla/Mandible	18	29	5
Total	27	65	8
p	0.026		

Table-5: Histological grades of final evaluation

Final Evaluation	Grade I	Grade II	Grade III	Total	*p*
High	0	5	22	27	<0.001
Low	24	41	0	65	
Negative	8	0	0	8	
Total	32	46	22	100	

Grade I= (Well differentiated OSCC), Grade II= (Moderately differentiated OSCC), Grade III= (Poorly differentiated OSCC)

DISCUSSION

In our study, most (73.0%) patients were above 50 years of age. Studies conducted by Siddiqui *et al*[11], Qureshi *et*

al[12], and Shah *et al*[13], show that most of the cases were above the age of 50 years. However, studies conducted by Devendra *et al*[4], Perdomo *et al*[14], and Huque *et al*[15] yielded different results, as most of their cases were below the age of 50 years. This difference may be due to early detection of OSCC in elderly patients. Since the turn of the century, patient awareness in young patients has increased. Nevertheless the underlying process is still unknown, oral microbiota dysbiosis is a carcinogenesic factor, and it is suggested that young patients have a particular bacterial makeup that promotes the development of OSCC. In our study, male and female genders were almost equally affected by OSCC with male to female ratio of 1.04:1. This is consistent with the studies conducted by Siddiqui *et al*[11]. Mori *et al*[16] also reported similar results. However, this contradicts the study by Hu Y *et al*[5] who reported, Male to Female ratio as 3:2, Zhao X *et al*[17] reported M:F= 2:1, and Troiano *et al*[18] reported M:F= 3:1. These differences in gender distribution can be attributed to multiple reasons such as lifestyle and geographical location. The use of tobacco, and genetic predisposition might also be a reason. Occupational exposures to certain chemicals like nickel compounds, rubber manufacturing, employment in beauty salons, woodworking, and asbestos mining are considered causes of the development of OSCC.

The histological grading of oral squamous cell carcinoma (OSCC) in our study revealed that 32% patients had Grade I, 46% had Grade II, and 22% had Grade III carcinoma. The most common histological grade in our cohort was Grade II (moderately differentiated). This finding is consistent with studies by Lin *et al*[19] and Zhou *et al*[20], who reported a predominance of moderately differentiated OSCC in their patients. In their studies, moderate differentiation was also observed to correlate with a higher risk of metastasis and poorer clinical outcomes. However, our results are in contrast with the findings of Huang *et al*[21] who reported a higher prevalence of well-differentiated (Grade I) OSCC in their cohort, suggesting regional or cohort-specific variations in histological grading.

The most frequent tumour site in our study was gingiva, followed by the labial commissure, hypopharyngeal region, cheeks, and tongue. These results are aligned with Huang *et al*[22] and Lin *et al*[19], who also identified the gingiva as the most common site for OSCC. This suggests a regional consistency in the distribution of OSCC across different studies. Zhou *et al*[20] found that the tongue is more frequently affected by OSCC, highlighting the potential influence of regional risk factors and patient demographics on tumour localization.

Our analysis of CD68 expression in OSCC specimens revealed a statistically significant relationship between CD68 intensity and both tumour location and histological grade. These findings are in line with Zhou *et al*[20] and Huang *et al*[21] reporting a strong association between CD68 expression and tumour differentiation. Both studies suggested that CD68-positive macrophages within the tumour microenvironment are linked to more aggressive tumour behaviour, and increased CD68 expression correlates with a poorer prognosis. In particular, Zhou *et al*[20] found that higher CD68 expression was associated with increased lymph node metastasis and advanced tumour stages in OSCC. Huang *et al*[21] demonstrated that CD68 could serve as a prognostic marker, with higher expression levels correlating with reduced overall survival.

Our findings contrast with those of Huang *et al*[22] who reported no significant correlation between CD68 expression and tumour aggressiveness. This discrepancy may be due to differences in sample size, methodology, or patient demographics and further studies with larger cohorts and standardized protocols may clarify the role of CD68 in OSCC progression.

In contrast to the significant correlations observed with tumour grade and location, our study found no significant association between CD68 expression and patients' age or gender. This is consistent with Lin *et al*[19] who reported that CD68 expression did not vary significantly between age groups or gender. It is worth noting that while CD68 was present in nearly all cases of OSCC, with only 8% of cases showing no expression (0 score), its intensity and distribution appeared to be more strongly influenced by histological grade and tumour site rather than demographic factors.

Our study supports the growing body of evidence that CD68 is a promising biomarker for predicting OSCC aggressiveness, particularly in relation to histological grading and tumour location. The findings are consistent with those of Zhou *et al*[20], Huang *et al*[21], and Lin *et al*[19], further highlighting the potential of CD68 expression as a prognostic tool in OSCC management. Further work is needed to resolve discrepancies in the literature and to better understand the mechanisms by which CD68 influences tumour behaviour and outcomes.

CONCLUSION

There was a strong correlation between CD68 expression and tumour location as well as histological grades. This emphasizes the significance of tumour site in forecasting CD68 expression and its possible impact on tumour behaviour. CD68 expression may serve as a marker for aggressiveness and prognosis of OSCC.

LIMITATIONS & RECOMMENDATIONS

The sample size was small, and this study was limited to immunohistochemical analysis only. Further work is needed to explore functional linkages and corroborate these results on a broader scope.

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REFERENCES

1. Stasikowska-Kanicka O, Wągrowska-Danilewicz M, Danilewicz M. Association of infiltrating cells with microvessel density in oral squamous cell carcinoma. Pol J Pathol 2017;68(1):40–8.
2. Almangush A, Mäkitie AA, Triantafyllou A, de Bree R, Strojan P, Rinaldo A, *et al*. Staging and grading of oral squamous cell carcinoma: An update. Oral Oncol 2020;107:104799.
3. Ali A, Manzoor MF, Ahmad N, Aadil RM, Qin H, Siddique R, *et al*. The burden of cancer, government strategic policies, and challenges in Pakistan: A comprehensive review. Front Nutr 2022;9:940514.
4. Devendra P, Vinita T, Mohite Deepali P, Manjiri T. The expression of CD68+ Macrophages in Oral Squamous Cell Carcinoma a Clinicopathologic Correlation. Ann Romanian Soc Cell Biol 2021;25(3):5552–61.
5. Hu Y, He MY, Zhu LF, Yang CC, Zhou ML, Wang Q, *et al*. Tumor-associated macrophages correlate with the clinicopathological features and poor outcomes via inducing epithelial to mesenchymal transition in oral squamous cell carcinoma. J Exp Clin Cancer Res 2016;35:12.
6. Suárez-Sánchez FJ, Lequerica-Fernández P, Suárez-Canto J, Rodrigo JP, Rodriguez-Santamarta T, Domínguez-Iglesias F, *et al*. macrophages in oral carcinomas: relationship with cancer stem cell markers and PD-L1 expression. Cancers (Basel) 2020;12(7):1764.
7. Heusinkveld M, van der Burg SH. Identification and manipulation of tumor associated macrophages in human cancers. J Transl Med 2011;9:216.
8. Jablonski KA, Amici SA, Webb LM, Ruiz-Rosado JD, Popovich PG, Partida-Sanchez S, *et al.* Novel markers to delineate murine M1 and M2 macrophages. PloS one 2015;10(12):e0145342.
9. Weber M, Iliopoulos C, Moebius P, Büttner-Herold M, Amann K, Ries J, *et al.* Prognostic significance of macrophage polarization in early stage oral squamous cell carcinomas. Oral Oncol 2016;52:75–84.
10. Zhang QW, Liu L, Gong Cy, Shi HS, Zeng Yh, Wang XZ, *et al*. Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. PloS One 2012;7(12):e50946.
11. Siddiqui IA, Khan H, Siddiqui R, Hafeez M, Dogar MR, Shahid W, *et al*. Oral cancer frequency at different sub sites presenting at a tertiary care hospital in Karachi Pakistan. Glob J Otolaryngol 2017;6(3):49–53.
12. Qureshi ZM, Qamar S. Association of programmed death ligand-1 overexpression with the grade and stage of oral squamous cell carcinoma. J Coll Physicians Surg Pak 2020;30(6):662–4.
13. Shah S, Mahajan A, Thiagarajan S, Chidambaranathan N, Sutar M, Sable N, *et al*. Importance and implications of neurovascular bundle involvement and other MRI findings of oral tongue squamous cell carcinoma (OTSCC) on prognosis. Oral Oncol 2021;120:105403.
14. Perdomo S, Roa GM, Brennan P, Forman D, Sierra MS. Head and neck cancer burden and preventive measures in Central and South America. Cancer Epidemiol 2016;44 Suppl:S43–52.
15. Huque R, Zaman MM, Huq SM, Sinha DN. Smokeless tobacco and public health in Bangladesh. Indian J Public Health 2017;61(Suppl 1):S18–24.
16. Mori K, Hiroi M, Shimada J, Ohmori Y. Infiltration of M2 tumor-associated macrophages in oral squamous cell carcinoma correlates with tumor malignancy. Cancers (Basel)) 2011;3(4):3726–39.
17. Zhao X, Ding L, Lu Z, Huang X, Jing Y, Yang Y, *et al*. Diminished CD68+ cancer-associated fibroblast subset induces regulatory T-cell (Treg) infiltration and predicts poor prognosis of oral squamous cell carcinoma patients. Am J Pathol 2020;190(4):886–99.
18. Troiano G, Caponio VCA, Adipietro I, Tepedino M, Santoro R, Laino L, *et al.* Prognostic significance of CD68+ and CD163+ tumor associated macrophages in head and neck squamous cell carcinoma: A systematic review and meta-analysis. Oral Oncol 2019;93:66–75.
19. Lin LH, Lin JS, Yang CC, Cheng HW, Chang KW, Liu CJ. Overexpression of platelet-derived growth factor and its receptor are correlated with oral tumorigenesis and poor prognosis in oral squamous cell carcinoma. Int J Mol Sci 2020;21(7):2360.
20. Zhou J, Chen C, Chen X, Fei Y, Jiang L, Wang G. Vitamin C promotes apoptosis and cell cycle arrest in oral squamous cell carcinoma. Front Oncol 2020;10:976.
21. Huang S, Zhu Y, Cai H, Zhang Y, Hou J. Impact of lymphovascular invasion in oral squamous cell carcinoma: A meta-analysis. Oral Surg Oral Med Oral Pathol Oral Radiol 2021;131(3):319–28.
22. Huang F, Xin C, Lei K, Bai H, Li J, Chen Q. Noncoding RNAs in oral premalignant disorders and oral squamous cell carcinoma. Cell Oncol (Dordr) 2020;43:763–77.

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