ORIGINAL ARTICLE

Comparison of CD-138 Expression in Different Grades of Oral Squamous Cell Carcinoma

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ABSTRACT

Objective: To compare the expression of CD-138 (Syndecan-1) in different morphological grades of oral squamous cell carcinoma.

Study Design: Cross-sectional study.

Place and Duration of Study: The study was conducted at the Department of Histopathology, Rehman Medical Institute Peshawar, Pakistan from June 2022 to February 2023.

Methods: A total of 110 biopsy specimens from patients of either gender suspected as cases of oral squamous cell carcinoma (OSCC) underwent histopathological examination in the laboratory of Rehman Medical Institute. After eosin and hematoxylin staining, the diagnosis of oral squamous cell carcinoma and the grade were confirmed. Thereafter, immunohistochemical analysis for CD-138 was performed. Age, gender, biopsy site intensity, and positivity for CD-138 expression in different grades of OSCC were the parameters recorded.

Results: Mean age of the patients included in the study was 58.91 ± 6.42 years while the gender distribution showed a male predominance with 98 (89.1%) males and 12 (10.9%) females. The most common affected site in these patients was buccal mucosa of the oral cavity in 50 (45.5%) participants followed by 43 (39.1%) participants in which the affected site was tongue. The intensity of CD-138 (Syndecan-1) expression decreased significantly with a change in the histological appearance of the tissue samples from well-differentiated to poorly differentiated morphology with a *p*-value of <0.01.

Conclusion: Loss of CD-138 expression in oral squamous cell carcinoma is associated with a higher histological grade, tumor aggressiveness and poor prognosis.

Keywords: *Head and Neck Neoplasms, Immunohistochemistry, Squamous Cell Carcinoma, Syndecan-1.*

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Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common types of malignant oral cancers comprising more than 90% of head and neck carcinomas. Despite recent advances in the field of medical science, the high rate of morbidity and mortality associated with OSCC poses a great challenge to healthcare workers.[1] Tobacco and alcohol consumption are the most common modifiable factors that can lead to OSCC.[2] In addition genetic predisposition, ultraviolet light exposure, human papillomavirus, antioxidant deficient diet, immunosuppression, and oral potential malignant lesions are the factors predisposing to OSCC.[3]

The pathogenesis of OSCC is multifactorial which may comprise genetic, environmental, or other modifiable factors leading to alteration of the normal cellular homeostasis. These in turn can lead to dysregulation of cell division, inhibition of growth

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suppression, inhibition of apoptosis, ability to invade surrounding structures, angiogenesis, and evasion of immune surveillance.[4] The increasing incidence of OSCC in our country is alarming and factors like poor hygiene, use of tobacco, naswar (smokeless tobacco product) addiction, poor diet, and other factors are contributory. Male predominance, low socioeconomic status, and altered chewing habits e.g. frequent use of betel nuts or prolonged insertion of naswar (smokeless tobacco product) in the mucobuccal folds have been observed in most of the patients presenting with OSCC to a tertiary care hospital in Pakistan.[5] Prediction of the invasive/malignant nature of the cancerous cells early in the course of the disease can help in early interventions. Modern diagnostics has led to the use of several markers that can be utilized for screening, early diagnostics, prognosis, and predictive and monitoring markers. Factors and markers with a prognostic value include grade and size of the tumor, vascular invasion, nodal metastasis and biomarkers like CD44, ALDH 1, CD-138 (syndecan-1), NANOG and SAX2.[6,7]

CD-138, also called syndecan-1, is a glycoprotein present on the surface of the cells which can be normal or cancerous. CD-138 is known for its role in cell differentiation, division, and tumorigenesis. Expression of CD-138 in several tumor types has been studied including gastrointestinal, hematopoietic, head and neck neoplasms, and other tumors. Depending on the site of the tumor, the expression of CD-138 can lead to ample information on predicting the nature and behavior of the cancerous cells.[8,9] The rationale of this paper is to compare the expression of CD-138 in different histological grades of oral squamous cell carcinoma which can contribute towards establishing a better understanding of the utilization of CD-138 as a potential prognostic marker and a possible therapeutic target.

Methods

The cross-sectional study was conducted at the Department of Histopathology, Rehman Medical Institute Peshawar, Pakistan from June 2022 to February 2023 after obtaining approval from the Ethical Review Board of the hospital held on 16th June 2022 vide letter no: RMI/RMI-REC/Article Approval/41. A sample size of 80 patients was estimated with a 5% level of significance and 80% power of test when the reported prevalence of oral cell carcinoma in the Pakistani population was 14%.[10] Biopsy specimens of patients suspected of having squamous cell carcinoma of the oral cavity were received in the histopathology department of Rehman Medical Institute, Peshawar. All the specimens underwent formalin fixation and paraffinization followed by preparation of slides for eosin and hematoxylin staining. After confirmation of diagnosis and establishment of the grade of oral cancer as well, as moderately or poorly differentiated, immunohistochemical staining was performed as per the laboratory protocol using Biocare CD138 (B-A38), Biocare Mach 2™ Universal HRP-Polymer Detection (M2U522) and Cell Marque DAB Substrate Kit (957D). Using a non-randomized convenience sampling technique, a total of 110 specimens of patients diagnosed with oral cell carcinoma underwent immunohistochemical analysis.

Inclusion Criteria: Patients of either gender diagnosed with cases of oral carcinoma were included in the research study.

Exclusion Criteria: Patients who were not willing to submit samples for research, inadequate tissue specimens, and diagnosed with carcinoma other than oral cancer were excluded.

The intensity of the staining was recorded as negative for specimens without any staining, tumors with 1+ staining intensity in ≤70% of cells and 2+ intensity in ≤30% of cells were considered weakly positive (Figure.1), tumors with 1+ staining intensity in >70% of cells, 2+ intensity in 30% to 70%, or 3+ intensity in ≤30% were considered moderately positive and tumors with 2+ intensity in >70% or 3+ intensity in >30% of cells were considered strongly positive (Figure.2).[9]

Data analysis wasperformed using SPSS version 23. For categorical parameters, frequency and percentages were calculated and continuous variables were presented with mean and standard deviation (mean ± S.D). For comparison, a chi-square test was employed for categorical variables keeping a *P*-value of ≤0.05 as significant.

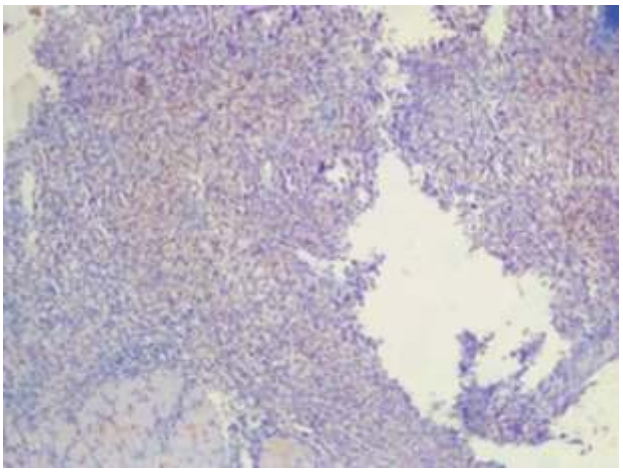

Fig.1: Weakly positive CD-138 expression is seen in poorly differentiated Oral Squamous Cell Carcinoma

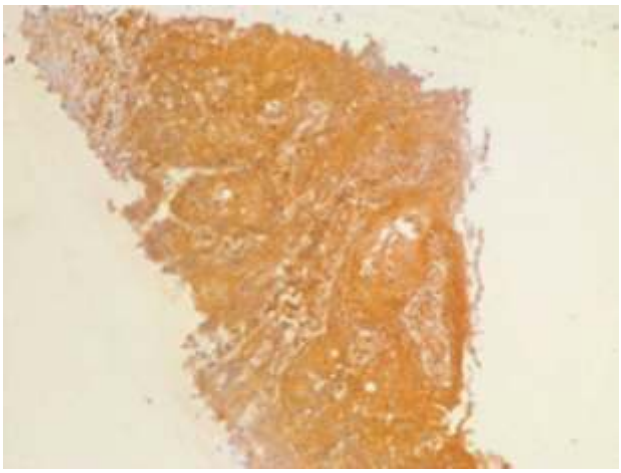

Fig.2: Well differentiated Oral Squamous Cell Carcinoma is strongly positive for CD-138

Results

The demographic characteristics of the sample population showed a male-to-female ratio of 8.16:1 (Table-1). The most common site of biopsy in these patients was buccal mucosa of the oral cavity in 50 (45.5%) participants. (Table-1) (n-110).

Table-1: Demographics and cancer sites among participants

Variables	
Age in years (Mean ± S.D)	58.91 ± 6.42
Gender	**n (%)**
Male	98 (89.1%)
Female	12 (10.9%)
Sites of Biopsy	
Buccal Mucosa	50 (45.5%)
Tongue	43 (39.1%)
Floor of Mouth	04 (3.6%)
Retromolar	07 (6.4%)
Hard Palate	03 (2.7%)
Lip	03 (2.7%)

Out of 110 specimens, 46 were well differentiated, 34 were moderately differentiated while 30 specimens had a poorly differentiated histological grade. Immunohistochemical analysis for CD-138 (syndecan-1) concluded strong intensity of staining for well-differentiated tumors as seen in 42 (38.18%) tissue samples. However, none of the poorly differentiated tissue samples revealed CD-138 positivity. The intensity of CD-138 (syndecan-1) expression decreased with a change in the histological appearance of the tissue samples from well-differentiated to poorly differentiated morphology with a *P*-value of <0.01 (Table-2).

Table-2: CD-138 (syndecan-1) expression in different grades of oral squamous cell carcinoma

Grades of differentiation	**Intensity of staining**			***P*-value**
	Strong positive	**Weak positive**	**No staining**	
Well-differentiated	42 (38.18%)	04 (3.63%)	00	<0.01
Moderately differentiated	06 (5.45%)	28 (25.45%)	00	
Poorly differentiated	00	08 (7.27%)	22 (20%)	
Total	48 (43.63%)	40 (36.36%)	22 (20%)	

Discussion

The cross-sectional comparative trial study was conducted to compare the expression of CD-138 (syndecan-1) in patients with different morphological grades of squamous oral cell carcinoma. Immunohistochemical analysis concluded that out of 46 (41.18%) well-differentiated tumor specimens, 42 (38.18%) revealed strong CD138 expression while 04 (3.63%) tissue specimens revealed weak CD-138 expression. Moderately differentiated morphology was observed in 34 (30.90%) specimens of which 06 (5.45%) revealed strong CD-138 expression and 28 (25.45%) specimens had a weak expression of CD-138. Similarly, the histological grade of 30 (27.2%) specimens was poorly differentiated and none of these specimens revealed strong CD-138 expression. However, 08 (7.27%) specimens had weak CD-138 intensity of staining and 22 (20%) specimens revealed no staining on immunohistochemical analysis. It is also important to note that the majority of the cases showing weak or negative staining presented with a higher stage of disease as well indicating greater invasion and spread of the tumor and possibly a graver prognosis. The intensity of staining decreased with the change in morphology from well-differentiated to poorly differentiated histological grade of tumor.

CD-138 is a membranous proteoglycan expressed by normal or cancerous cells. Increased expression of the membranous biomarker can be used as an indicator for prediction of the behavior and course of cancerous cells. Possible mechanisms of syndecan-1 leading to OSCC include its binding to growth factors and cytokines, promotion of angiogenesis around the cancerous cells, inhibition of the inflammatory response to tumor invasion, and gene regulation leading to adhesion and migration of tumor cells.[11,12] Several normal or cancerous cells express CD-138 and the frequency of CD138 expression was observed to be 100% in esophageal carcinomas followed by 79.5% in uterine carcinoma.[13]

Results of our study concluded that males were predominantly affected with OSCC with a mean age of 58.91 ± 6.42 years while the most commonly affected site was buccal mucosa 50 (45.5%) followed by tongue in 43 (39.1%) participants. However, in another pilot study on a similar topic, results showed male predominance in OSCC with an age range of 50-70 years but the most common site was the tongue followed by buccal mucosa.[14]

Syndecan-1 is a proteoglycan heparan sulfate that helps in cellular adhesion, growth of cells, and differentiation of B cells. Loss of expression of CD138 is observed in squamous cell carcinomas of the head neck and larynx of aggressive nature. Such loss of expression predicts the potential to invade surrounding structures and aggressiveness of the tumor cells in OSCC however SCC in situ does not exhibit loss of CD138 expression.[15,16] In a trial conducted by Lakkam et al. the number of cases with normal histological pattern of oral mucosa was 10 cases while 11 specimens showed a severe dysplastic morphology. Immunohistochemistry of specimens with normal mucosal morphology revealed strong expression of CD-138 however out of 11 dysplastic tissue specimens, 10 revealed no staining while only 01 revealed intermediate syndecan-1 expression.[17]

CD-138 has been established as a reliable prognostic marker in several other tumors. The prognostic ability of Syndecan-1 was assessed in renal cell carcinoma and nasopharyngeal carcinoma. In renal cell carcinoma, the loss of expression of syndecan-1 was associated with a more invasive and aggressive tumor predicting a poor prognosis. Similarly, in nasopharyngeal carcinoma, the expression was observed in 21% of cases and an association with the advanced stage of the disease leading to poor prognosis was noticed.[18,19]

The study implies that the down-regulation of CD-138 is associated with advanced stages of oral squamous cell carcinoma. However, the exact mechanism of the association remains unclear. There is a requirement to conduct further research of following up with patients for a prolonged period to get an insight into the progression of the disease, rate of recurrence, and morbidity. Further research is warranted to observe the effect of treatment on the expression of CD-138 in oral squamous cell carcinoma.

Better prognosis in patients with OSCC requires a multidimensional approach which includes the patients, health-care workers, and health-care facilities. Nonetheless, the dilemma of late

presentation or late diagnosis leads to increased vulnerability to worse outcomes.[20] Innovations in diagnostics have led to early prediction of prognosis and behavior of malignant cells in patients diagnosed with neoplasms by the use of CD-138.[21] Hence our study proves the prognostic ability of CD-138 which is lost in aggressive tumors indicating poor prognosis. Immunohistochemistry of CD-138 in OSCC can become a useful indicator in the future for CD-138 targeted treatment.

This was a single-centered study. A multicentered study of the same would make the results more applicable to the general population. The expression of CD-138 and its association with prognosis of the patients with oral squamous cell carcinoma remains unclear which emphasizes the need for longitudinal studies. Similarly, the correlation between the expression of CD-138 markers and over or under-expression of other associated biomarkers was not observed in the study which may affect the disease progress and outcome. Furthermore, there is a need for ongoing research to assess the effect of CD-138 on disease recurrence rates and distant metastasis.

Conclusion

Loss of CD-138 expression in oral squamous cell carcinoma is associated with a higher histological grade and tumor aggressiveness. Furthermore, this expression profile could be used as an indicator of poor prognosis.

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Authors Contribution

SB: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing and proofreading

FJ: Data collection, data analysis, results and interpretation

AS: Data collection, data analysis, results and interpretation

MTK: Data collection, data analysis, results and interpretation

NA: Data collection, data analysis, results and interpretation, manuscript writing and proofreading

MK: Data collection, data analysis, results and interpretation, manuscript writing and proofreading