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Orginal Article

CLINICOPATHOLOGICAL ANALYSIS AND CD44 EXPRESSION IN ORAL AND OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Objectives: *To assess the relationship between CD44 expression in malignant cells, clinicopathological findings, and prognostic outcomes in oral squamous cell carcinoma.*

Materials and Methods: *This was a retrospective comparative study utilized excisional and incisional biopsy specimens from oral and oropharynx, collected at Akbar Niazi Teaching Hospital, Islamabad, between September 2022 and September 2024. Among 40 specimens, 13 were well-differentiated, 19 were moderate, and 8 were poor squamous cell carcinomas, with or without nodes metastasis. These specimens were randomly selected for CD44 immunohistochemical staining.*

Results: *The majority of patients were between 50 and 75 years old (mean was 62.5±7.2 years), and males were 77.5%. The mean tumor depth was 0.7±0.4 mm. T1 tumors were the most common (55%), with the majority exhibiting N0 nodal status (62.5%). Grade 2 tumors were the most frequent (47.5%), while stage I tumors had the highest prevalence (40%). Strong CD44 expression was detected in 72.5% cases, with basal cell invasion present in 55%. Lesions were most frequently located on the tongue (30%) and vocal cords (25%). Insignificant difference was observed in age, gender, tumor size, nodal status, or basal cell invasion across expression levels ($p > 0.05$). A notable variation in tumor grades was identified across different expression levels ($p = 0.022$).*

Conclusion: *Reduced CD44 expression in aggressive OSCC, characterized by poor differentiation and lymph node involvement, indicates a potential association with disease progression. CD44 may help preserve tissue structure, and its loss could serve as a potential marker of poor prognosis.*

Key words: *DCarcinoma, Basal cell; Hyaluronan receptors; Mouth neoplasms; Oropharyngeal neoplasms; Carcinoma, Squamous cell.*

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INTRODUCTION

Oral cancer is increasingly prevalent in developing countries compared to developed ones. It can originate as prime malignancy in any oral tissue. It encompasses tumors originating from oral cavity, with lips, tongue, buccal mucosa, soft and hard palate, larynx, oropharynx, and hypopharynx,

while without salivary gland tumors[1]. In Pakistan, age-standardized incidence of oral cancer is 14 / 100,000 individuals[2]. Oral cancer has a high prevalence among men, listed among three most frequent cancers. The incidence is rising due to population aging and growing prevalence of cancer risk factors. The rising prevalence is attributed to factors such as tobacco consumption, poor nutrition, sedentary lifestyles, and infections[3].

The International Agency for Research on Cancer recently confirmed that increased use of smokeless tobacco contributes to oral cancer[4]. Oral squamous cell carcinoma (OSCC) accounts over 90% of oral malignancies. Although advances in therapeutics, the rate of 5 years survival for OSCC remains around 55% and is significantly lower for tumors located more posteriorly[5]. The WHO has recently adopted resolutions on diet, health, and physical activity to aid in the prevention and control of oral malignancies[6].

Numerous studies have explored prognostic indicators for OSCC at molecular level, identifying several tumor suppressor genes involved in etiology of oral and oropharyngeal SCC[7,8]. CD44 is a key human cell surface receptor for hyaluronate, playing a role in various physiological processes. CD44 contributes to tumor aggressiveness in vivo by interacting with hyaluronate rich stroma[9].

This study objectives to assess the relationship between CD44 expression in tumor cells, clinicopathological findings, and prognostic outcomes in OSCC.

MATERIALS AND METHODS

This was a retrospective comparative study utilized excisional and incisional biopsy specimens from oral cavity, collected from Pathology Department at Akbar Niazi Teaching Hospital, Islamabad, between September 2022 and September 2024. Prior to initiation, the study received approval from ethics committee of the institute (Ref No. IMDC/DS/IRB/219), and consent was exempted. All oral and oropharyngeal SCC diagnosed through incisional or excisional biopsy were received from the Department of Oral Pathology, and all histopathological variants of OSCC reported in the Department of Pathology were included. Excluded from the study were benign neoplasms of oral and oropharynx mucosa, primary tumors other than SCC of oral and oropharynx, as well as recurrent and metastatic tumors in these regions.

All cases were selected through simple randomization. A computer-generated random number list was used to avoid selection bias. A total of 40 specimens were randomly chosen, including 13 well-differentiated, 19 moderately, and 8 poorly differentiated SCC, with or without nodal metastasis, for CD44 immunohistochemical staining. By histopathological examination, group 1, 2 and 3 were diagnosed as malignant (Grade I, II and III).

Comprehensive patient data, including age, gender, tumor site, and personal history, were obtained from pathology. Hematoxylin and eosin-stained tissue samples were examined to verify the histopathological diagnosis of SCC. The specimens underwent incisional or excisional biopsy.

For histopathological evaluation, 4-micrometer-thick sections were prepared from Paraffin blocks sectioned with a semi-automated microtome equipped with disposable blades and subsequently stained with Hematoxylin and Eosin. Histological features of OSCC were categorized in well, moderate, and poor differentiated grades (G1–G3), with or without nodes metastasis, based on WHO criteria.

Since 2018, American Joint Committee on Cancer staging is utilized to classify for oral and oropharyngeal tumors. It utilizes the TNM staging, assessing tumor extent (T), regional lymph node involvement (N), and distant metastasis (M). Tissue sections were analyzed and placed on chrome alum-coated slides, antigen retrieval in a pressure cooker with TRIS buffer (pH 9.2), and processed with horseradish peroxidase (HRP).

Tumor size, stage, depth, and nodal status were analyzed as outcomes data, while expression levels served as the explanatory data. All quantitative data, including age and tumor depth, were assessed for normal data distribution. Shapiro-Wilk test was employed for normal data distribution. For quantitative data with a normal distribution (age), independent t test was applied to compare mean values across different expression levels. For quantitative data without normal distribution (depth), Mann-Whitney U test was applied to compare median and interquartile range (IQR) across different expression levels.

Categorical variables, including gender, tumor stage, size and nodal status, Fisher's exact test was applied to compare across expression levels. A p value ≤ 0.05 was set as significant. Data analysis was conducted using SPSS v 23.

RESULT

The patient's mean age was 62.5±7.2 years. Among 40 patients, 14 were 35–55 years, while 26 were 56–75 years old. Among 40 cases, 31 (77.5%) were males, while 9 (22.5%) were females. The median tumor depth was 0.7 mm (IQR: 0.5–1.1 mm).

Among the patients, 55% (n=22) had T1 tumors, 37.5% (n=15) had T2 tumors, and 7.5% (n=3) had T3 tumors. The majority of patients had N0 nodes (62.5%, n=25), while 17.5% (n=7) had N1 nodes and 10% (n=4) had N2b nodes. Among the patients, 32.5% (n=13) were in Grade 1, 47.5% (n=19) in Grade 2, and 20% (n=8) in Grade 3. Among patients, 40% (n=16) were in Stage I, 20% (n=8) in Stage II, 30% (n=12) in Stage III, and 10% (n=4) in Stage IV. Table 1 presents additional parameters, including expression levels, basal cell invasion, and lesion site among patients.

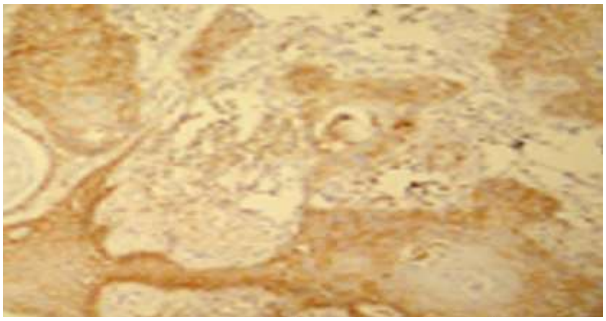

Fig 1: Well differentiated SCC CD44 expression (H stain × 100)

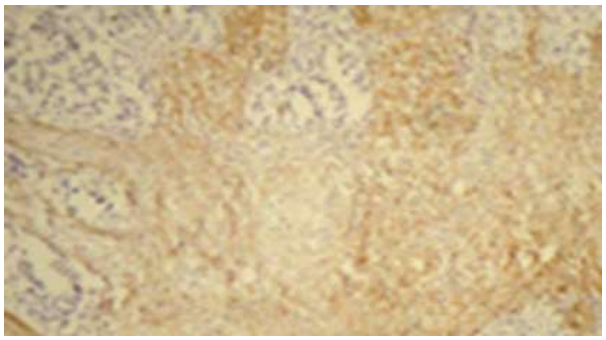

Fig 2: Moderate differentiated SCC CD44 expression (H stain × 100)

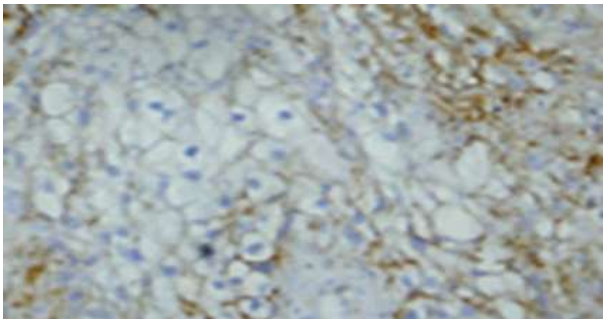

Fig 3: Poorly differentiated SCC CD44 expression (H stain × 100)

No difference was found in age, gender, nodal status, tumor size, or basal cell invasion across expression levels ($p > 0.05$). In the weak expression, 9.1% (n=1) was Grade 1, while 45.5% (n=5) were Grade 2, and 45.4% (n=5) were Grade 3. In the strong expression, 41.4% (n=12) were Grade 1, 48.3% (n=14) were Grade 2, and 10.3% (n=3) were Grade 3. A significant difference in tumor grades was observed between expression levels ($p = 0.022$), (Table 2). The post-hoc analysis (Bonferroni) revealed that the significant difference was observed in Gade 3.

Table 3 presents the association between CD44 expression levels (strong vs. weak) and key clinicopathological features. OR < 1 indicates a negative association with strong CD44 expression; OR > 1 indicates a positive association. No results were

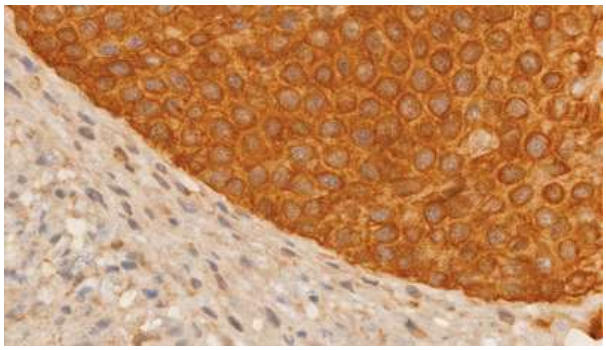

Fig 4: Strong SCC CD44 expression (H stain × 100)

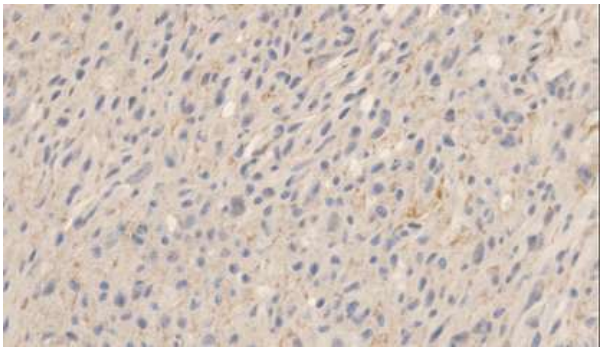

Fig 5: Weak SCC CD44 expression (H stain × 100)

significant at $p < 0.05$, though a trend was observed for histologic grade ($p = 0.080$).

DISCUSSION

This study conducted immunohistochemical and clinicopathological evaluations on 40 cases of oral and oropharyngeal SCC. This study evaluated CD44 expression, its prognostic significance, association with basal invasion, grading, and its potential role in targeted therapy for better patient outcomes. The highest incidence was observed in 56 to 75 years ages. The average age at presentation was 62.5 years, with males accounting 77.5% of cases. Selvamani et al reported average age at presentation of 55.8 years, with a higher incidence in males[10]. Singh et al reported average age at presentation of 52.8 years[11].

In this study, the tongue was frequent site (30%), then prevalent in vocal cords, buccal mucosa, pharynx, and lips. Selvamani et al reported a 32% incidence of cases occurring on the tongue[10]. Rai et al reported the buccal mucosa predominant site (53%), then prevalent in tongue (18%) and lip tumors (14%)[12]. In Smitha et al study tongue, buccal mucosa, and lip tumors accounted for 12%, 31%, and 4% of cases, respectively[13].

In this study, out of 40 cases, 35% (n=14) had nodal metastasis, while 65% (n=26) showed no nodal involvement. This finding aligns with Xia et al who reported that most cases lacked nodal involvement, with 10 cases showing nodal metastasis and 34 without. This suggests that patients are aware of OSCC, leading to early-stage clinical presentation[14].

In this study, most cases were classified as T1 stage. This finding contrasts from other studies, which identified T2 was most commonly reported stage at diagnosis. The association was found insignificant across tumor stages and CD44 immuno-expression. While, in stage T2, nearly all cases exhibited strong CD44 immuno-expression. The association was observed insignificant across nodes status and CD44 immuno-expression. Strong CD44 expression was noted in N0 and N1 cases. This is consistent with Hema et al who found insignificant variation in tumor stages or nodes status concerning CD44 immuno-expression[15].

In this study, all 13 cases of well-differentiated SCC exhibited CD44 expression, with 12 showing strong expression and 1 displaying weak expression. Among the 19 cases of moderately differentiated SCC, 14 exhibited strong expression, while 5 had weak expression. Among 8 cases of poorly differentiated SCC, 5 exhibited weak expression, while 3 demonstrated strong expression. Therefore, well-differentiated carcinomas exhibit higher CD44 expression compared to poorly differentiated carci-

Table 1: Demographic characteristics, n=40

Variables		n	%
Ages (years)	35 – 55	14	35.0
	56 – 75	26	75.0
Gender	Male	31	77.5
	Female	9	22.5
Tumor size	T1	22	55.0
	T2	15	37.5
	T3	3	7.5
Nodes (pN)*	N0	25	62.5
	N1	7	17.5
	N1a	2	5.0
	N1b	1	2.5
	N2	1	2.5
	N2b	4	10.0
Grade	1	13	32.5
	2	19	47.5
	3	8	20.0
Stage	I	16	40.0
	II	8	20.0
	III	12	30.0
	IV A	4	10.0
Expression level	Weak	11	27.5
	Strong	29	72.5
Basal cell invasion	Absent	18	45.0
	Present	22	55.0
Lesion site	Tongue	12	30.0
	Buccal mucosa	7	17.5
	Lip	5	12.5
	Pharynx*	16	40.0

Pharynx cases were as oropharyngeal based on clinical records (base of tongue, tonsillar region, soft palate). pN: pathological nodal staging

Table 3: CD44 expression and clinicopathological parameters

Variable	OR	95% CI	p-value
Histologic grade 1	0.14	0.02 – 1.26	0.080
Nodal involvement (N1/ N2 vs N0)	1.58	0.39 – 6.50	0.524
Tumor size (T1 vs T2/T3)	2.86	0.63 – 12.98	0.174

Table 2: Compare the expression levels with clinical and pathological findings, n=40

Variables		Expression levels		P-value
		Weak (n=11)	Strong (n=29)	
Ages (years)	35 – 55	4 (36.4%)	10 (34.5%)	.702
	56 – 75	7 (63.6%)	19 (65.5%)	
Gender	Male	10 (90.9%)	21 (72.4%)	.121
	Female	1 (9.1%)	8 (27.6%)	
Tumor size	T1	8 (72.7%)	14 (48.3%)	.100
	T2	2 (18.2%)	13 (44.8%)	
	T3	1 (9.1%)	2 (6.9%)	
Nodes (pN)*	N0	6 (54.5%)	19 (65.5%)	.532
	N1	3 (27.3%)	7 (24.1%)	
	N2	2 (18.2%)	3 (10.4%)	
Grade	1	1 (9.1%)	12 (41.4%)	.022
	2	5 (45.5%)	14 (48.3%)	
	3	5 (45.4%)	3 (10.4%)	
Stage	I	6 (54.5%)	10 (34.5%)	1.00
	II	0	8 (27.6%)	
	III	4 (36.4%)	8 (27.6%)	
	IV A	1 (9.1%)	3 (10.4%)	
Basal cell invasion	Absent	7 (63.6%)	11 (37.9%)	.060
	Present	4 (36.4%)	18 (62.1%)	

*: pathological nodal staging

nomas. This result is similar with studies conducted by Hema et al and Kaza et al which demonstrated significant results[15,16].

Due to its association with histological differentiation and nodal status, CD44 could serve as a supplementary immunohistochemical marker in incisional biopsy specimens, aiding in risk stratification of OSCC cases. Its inclusion in diagnostic panels alongside markers such as p16, Ki-67, and p53 could help refine early-stage characterization, particularly in borderline lesions where tumor aggressiveness is uncertain. In maxillofacial oncology, incorporating CD44 into preoperative biopsy protocols may assist in planning the extent of surgical margins and neck dissection by identifying tumors with more aggressive profiles at an earlier stage[17].

Given the significant inverse relationship between CD44 expression and histological grade (p = .022), tumors with weak CD44 expression may represent a more biologically aggressive subtype. This suggests the potential value of incorporating CD44 status into preoperative planning, particularly regarding margin width and neck dissection decisions. Further studies with larger cohorts and survival endpoints are required to validate CD44 as a prognostic and surgical planning biomarker.

CONCLUSION

The study demonstrated a significant inverse association between CD44 expression and histologic grade in oral and oropharyngeal squamous cell carcinoma (OSCC), with stronger CD44 expression observed in well-differentiated tumors. These findings suggest that CD44 may play a role in maintaining epithelial integrity through enhanced cell adhesion. Conversely, reduced CD44 expression was more frequent in poorly differentiated tumors and those with advanced stage and nodal involvement. While these patterns imply a possible link with tumor progression, the absence of follow-up data limits definitive conclusions about its prognostic value.

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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	RR, MI, FF, ZN, AM RA
Acquisition, Analysis or Interpretation of Data:	RR, MI, FF, ZN, AM RA
Manuscript Writing & Approval:	RR, MI, FF, ZN, AM RA

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.