Original Article

Immunohistochemical Evaluation of Cellular Retinol-Binding Protein-1 Expression Across Histological Grades of Oral Squamous Cell Carcinoma: A Retrospective Analysis

Ifrah Ahmed, Aliya Muzafar, Samreen Hameed, Safeena Sarfraz, Rabia Altaf & Numrah Bilal

ABSTRACT

Objective: To analyze the correlation between cellular retinol-binding protein-1 (CRBP-1) expression and tumor grade, differentiation, and invasiveness in oral squamous cell carcinoma.

Methodology: This retrospective immunohistochemical study was conducted at Pathology Department of Mayo Hospital, Lahore, from January 2024 to May 2025 after ethical approval. A total of 30 formalin-fixed, paraffin-embedded oral squamous cell carcinoma (OSCC) tissue specimens were retrieved and graded histologically. An anti-CRBP-1 antibody was used for immunohistochemical staining. Expression levels were semi-quantitatively scored and correlated with tumor grade (well, moderately, and poorly differentiated).

Results: Out of 30 OSCC cases, strong CRBP-1 positivity was observed in 14 cases (46.7%). Among these, 4 cases (28.6%) were well-differentiated, 8 cases (57.1%) were moderately differentiated, and 2 cases (14.3%) were poorly differentiated. Another 14 cases (46.7%) showed weak CRBP-1 expression, while 2 cases (6.7%) were negative. A statistically significant inverse correlation was found between CRBP-1 expression and tumor grade (Spearman's $\rho = -0.52$; $p < 0.01$), indicating reduced expression in less differentiated tumors.

Conclusion: CRBP-1 expression is inversely associated with histological grade in OSCC, suggesting its potential role as a differentiation marker and possible prognostic indicator.

KEYWORDS: Biomarkers, Carcinoma, Squamous Cell, Immunohistochemical, Retinol-Binding Protein, Tumor Grade

INTRODUCTION

More than 90% of oral cancers globally are oral squamous cell carcinoma (OSCC), which represents a significant health burden, especially in South Asia.[1] In Pakistan, OSCC ranks among the most common cancers, accounting for approximately 15% of all new cancer cases, an incidence markedly higher than the global average of 3%.[2,3] The predominance of risk factors such as tobacco, betel quid, gutka, and naswar in the region further exacerbates disease risk.[4]

Histopathological grading of OSCC (well, moderately, and poorly differentiated) is essential for prognostication and therapeutic planning.[5] Well-differentiated tumors tend to exhibit less aggressive behavior and better survival rates, while poorly differentiated variants are associated with poor prognosis.[6] Nevertheless, more specific molecular markers are needed to enhance grading accuracy and predict tumor behavior. Cellular retinol binding protein-1 (CRBP-1) plays a pivotal role in intracellular retinol transport and retinoic

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acid signaling, which is crucial for epithelial cell differentiation.[7] Loss or reduction of CRBP-1 has been implicated in tumorigenesis in various cancers such as endometrial, lung, and breast.[8] Despite this, CRBP-1 expression in OSCC remains understudied, particularly in high-burden regions like Pakistan.

Immunohistochemical (IHC) approaches allow for localized evaluation of protein expression in tissue and are ideal for assessing CRBP-1 in clinical specimens.[6] In Pakistan, recent immune-histochemical (IHC) studies of other markers such as Bcl-2, ZAG, EGFR, and p16 have demonstrated significant correlations between protein expression and histological grade or disease stage.[9-11] For instance, a study in Islamabad reported a significant association between Bcl-2 expression and OSCC grade ($p < 0.001$).[9] Similarly, research in Peshawar found higher EGFR expression in low-grade OSCC, while p16 expression linked to habits like snuff use.[10] These findings support the potential utility of differentiation-linked biomarkers in local OSCC cohorts.

Given the high prevalence of moderately to poorly differentiated OSCC in Lahore, evaluating CRBP-1 expression may offer a valuable adjunct to traditional grading.[2] Therefore, this retrospective IHC study was conducted to quantify CRBP-1 expression across histological grades. The study objective was to assess the correlation between CRBP-1 expression and tumor differentiation and invasiveness, and to evaluate its potential as a clinically relevant biomarker in OSCC.

METHODOLOGY

This retrospective observational study was conducted at Pathology Department of Mayo Hospital, Lahore, from January 2024 to May 2025 after ethical approval (letter number 647/RC/KEMU dated 28-04-2025). The sample size was calculated using a statistical tool provided by the WHO. Parameters included a 95% confidence level, a proportion of 0.80 based on significant biomarker expression in OSCC, and an alpha error of 0.5.[2]

A total of 30 formalin-fixed, paraffin-embedded (FFPE) tissue specimens with a confirmed histopathological primary OSCC were obtained from the pathology department's archival records using non-probability purposive sampling. Inclusion criteria comprised all cases diagnosed as primary OSCC with available clinical records and sufficient tissue for analysis. Cases with a history of prior chemotherapy or radiotherapy, recurrent OSCC, or inadequate/autolyzed tissue were excluded. Patients' demographics and clinical records, such as age, gender, and tumor location, were extracted from pathology reports and hospital records.

Each FFPE tissue block was sectioned at 3–5 µm thickness and stained with Hematoxylin and Eosin (H&E) for histopathological evaluation. The tumors were graded according to the WHO classification into well-, moderately, and poorly differentiated SCC. Histological evaluation and tumor grading were independently verified by two qualified histopathologists to ensure accuracy and consensus.

For immunohistochemical (IHC) evaluation, additional sections (3–4 µm thick) were cut and mounted on poly-L-lysine–coated slides. Tissue sections were deparaffinized with xylene and rehydrated through a series of graded alcohols, after which antigen retrieval was carried out in citrate buffer (pH 6.0) using microwave heating. A 10-minute incubation in 3% hydrogen peroxide was used to block endogenous peroxidase activity. The tissue slides were incubated with an anti-CRBP-1 primary antibody (specific clone and dilution as per manufacturer's guidelines, e.g., Abcam) for one hour at room temperature. A horseradish peroxidase (HRP)-linked secondary antibody was applied, followed by visualization using diaminobenzidine (DAB) chromogen. Hemato-xylin was used as a counterstain before dehydration and mounting. CRBP-1 expression was assessed semi-quantitatively by evaluating both staining intensity and the percentage of positive tumor cells

The intensity of staining was evaluated using a 4-point scale: 0 (absent), 1 (weak), 2 (moderate), and 3 (strong). The percentage of tumor cells showing positive staining was categorized as 0 (0–5%), 1 (6–25%), 2 (26–50%), and 3 (> 50%). The final immunoreactivity score (IRS) was obtained by multiplying the intensity and percentage scores. IRS values were categorized as negative (0), weakly positive (1–3), and strongly positive (≥ 4). All IHC slides were evaluated independently by two experienced pathologists who were blinded to clinical and histological data. Any discrepancies in scoring were resolved through mutual discussion and consensus.

Data were analyzed using SPSS v 25. Descriptive statistics were utilized to summarize demographic and categorical variables. The association between CRBP-1 expression and tumor grade was assessed using the chi square test. Spearman's rank correlation coefficient was used for correlation analysis between CRBP-1 expression scores and histological grade. A p-value < 0.05 was considered significant.

RESULTS

A total of 30 cases of histologically confirmed OSCC were included. The patients ranged in age from 35 to 75 years, with a mean age of 54.3±10.2 years. The majority of the cases were male (70%; n=21), while females accounted for 30% (n=9). The most common tumor site was the buccal mucosa, followed by the tongue and floor of the mouth. The distribution of demographic and clinicopathologic features is summarized in Table 1.

Histopathological grading revealed that 10 cases (33.3%) were well-differentiated, 14 cases (46.7%) were moderately differentiated, and 6 cases (20.0%) were poorly differentiated, as shown in Table 2. Immunohistochemical evaluation of CRBP-1 expression demonstrated a range of staining intensities and cellular distribution. Strong positivity (IRS ≥ 4) for CRBP-1 was observed in 14 out of 30 cases (46.7%), weak positivity (IRS 1–3) in 14 cases (46.7%), and negative expression (IRS = 0) in 2 cases (6.6%). The distribution of CRBP-1 expression intensity and percentage among OSCC grades is provided in Table 3.

Table 1: Demographic and clinical characteristics of patients, n=30

Characteristics	Frequency	Percentage
Age Group (years)		
31–40	4	13.3
41–50	9	30.0
51–60	10	33.3
> 60	7	23.4
Gender		
Male	21	70.0
Female	9	30.0
Tumor Site		
Buccal mucosa	14	46.7
Tongue	9	30.0
Floor of mouth	4	13.3
Others	3	10.0

Table 2: Distribution of histological grades of OSCC, n=30

Tumor grade	Frequency	Percentage
Well differentiated	10	33.3
Moderately differentiated	14	46.7
Poorly differentiated	6	20.0

Table 3: CRBP-1 expression pattern in OSCC cases, n=30

CRBP-1 expression	Frequency	Percentage
Strong positive	14	46.7
Weak positive	14	46.6
Negative	2	6.7

A significant inverse correlation was observed between CRBP-1 expression and tumor grade, with higher expression levels found in well-differentiated tumors and lower or absent expression in poorly differentiated tumors (Spearman's $\rho = -0.52$, $p = 0.004$). This relationship is detailed in Table 4.

Table 4: Correlation between CRBP-1 expression and histological grade of OSCC

Tumor grade	Spearman's ρ	P-value
CRBP-1 expression vs grade	–0.52	0.004

Significant p value: < 0.05 ↕ Pearson Chi square test.

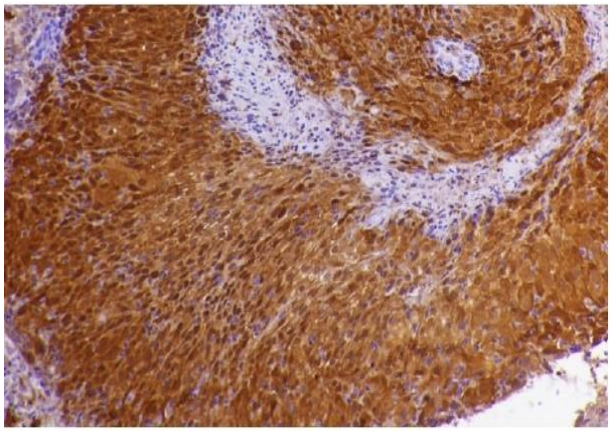
Chi square analysis also revealed a significant association between histological grade and CRBP-1 expression category (p = 0.031), suggesting that CRBP-1 expression may be linked to tumor differentiation. The categorical comparison is summarized in Table 5.

Table 5: Association between CRBP-1 expression category and histological grade

Tumor grade	Strong positive	Weak positive	Negative	Total	p-value
Well differentiated	4	6	0	10	0.031
Moderately differentiated	8	6	0	14	
Poorly differentiated	2	2	2	6	
Total	14	14	2	30	

Significant p value: < 0.05 ↕ Pearson Chi square test.

Figure 1: CRBP-1 "strong" positive expression in OSCC (H&E x 400).



Immunohistochemical evaluation of CRBP-1 expression in OSCC revealed variable staining intensity across different histological grades. Figure 1 illustrates a representative case showing strong CRBP-1 positivity, characterized by intense cytoplasmic staining in tumor cells.

Figure 2: CRBP-1 showing moderate pattern of intensity

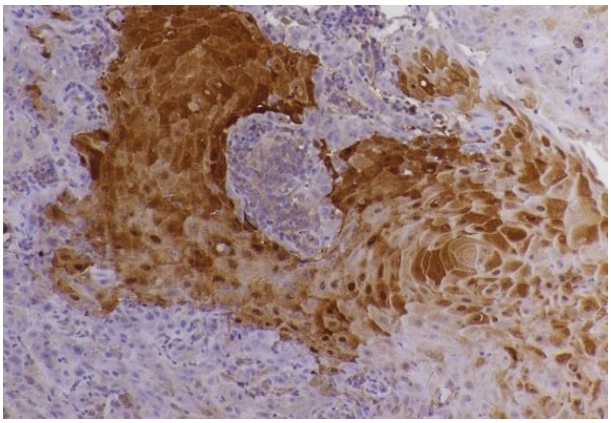


Figure 2 demonstrates moderate CRBP-1 expression, with noticeable but less intense cytoplasmic staining compared to strong cases.

Figure 3: CRBP-1 showing weak pattern

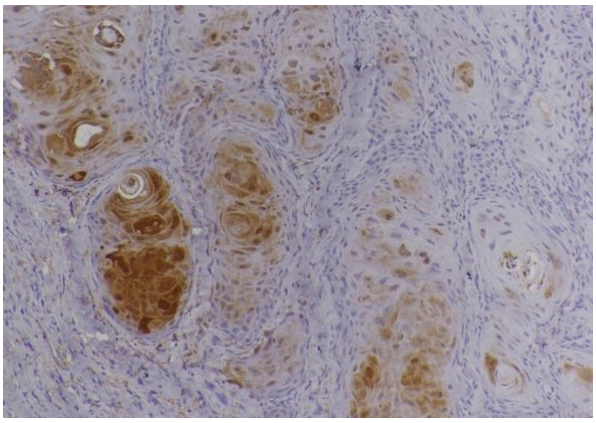


Figure 3 depicts weak CRBP-1 positivity, where only a few tumor cells show light cytoplasmic staining.

Figure 4: CRBP-1 "negative" staining expression (H&E x 400).

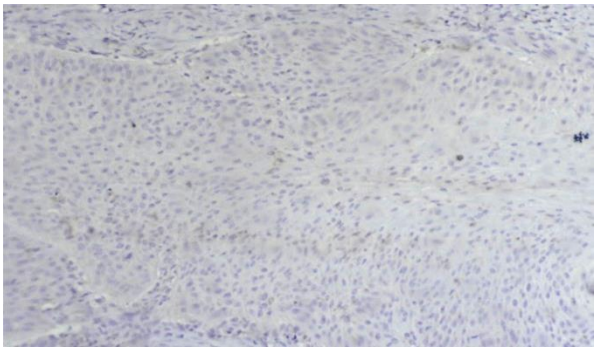


Figure 4 shows a case with negative CRBP-1 expression, indicating the absence of detectable immunoreactivity in tumor cells.

The figures visually reinforce the semi-quantitative scoring applied in this study and demonstrate the heterogeneous expression of CRBP-1 across OSCC cases of varying differentiation. Strong CRBP-1 expression was more commonly observed in well-differentiated tumors, whereas weak or negative staining was frequently noted in moderately and poorly differentiated tumors, supporting the correlation between CRBP-1 expression and tumor grade.

DISCUSSION

In this study, CRBP-1 expression demonstrated a significant inverse relationship with histological grade in OSCC, being highest in well-differentiated tumors and lowest or absent in poorly differentiated cases. This finding aligns with existing research indicating that CRBP-1 serves as a differentiation-associated marker in epithelial tumors.

Contrary to early reports of overexpression linked to aggressive oral cancers, this study results corroborate the hypothesis that CRBP-1 expression diminishes as differentiation worsens. In a comprehensive analysis of tongue squamous cell carcinoma (TSCC), Chen et al. reported increased CRBP-1 levels in tumor tissue compared to adjacent normal mucosa, correlating with differentiation status and overall survival.[12] Though increased tissue levels were documented, their TSCC cohort encompassed a wide histological spectrum, and subgroup analysis showed reduced CRBP-1 in higher grade tumors, parallel to our OSCC observations.

Similarly, in hepatocellular carcinoma (HCC), Fu et al. demonstrated that CRBP-1 overexpression suppressed proliferation and tumorigenesis, affirming its tumor-inhibitory role.[13] Though the tissue context differs, the consistent pattern high CRBP-1 correlates with lower proliferative activity, supports our conclusion of CRBP-1 functioning as a tumor suppressor in OSCC.

Mechanistically, suppression of retinoic acid (RA) signaling due to CRBP-1 downregulation may facilitate epithelial–mesenchymal transition (EMT) and loss of differentiation. While specific mechanistic studies in OSCC are limited, analogous findings in tongue cancer and other epithelial malignancies suggest a conserved role. One in vitro study noted CRBP-1 knockdown increased invasion and proliferation in TSCC cell lines, which mirrors decreased immunohistochemical expression in poorly differentiated tumors seen in our data.[14]

Comparisons with other IHC markers in OSCC suggest that CRBP-1 may serve as a reliable differentiation biomarker. For instance, Qureshi & Qamar reported significant correlation between PD-L1 expression and poor differentiation in a local OSCC cohort.[15] Though representing different molecular pathways, both markers show consistent grading associations. Additionally, elevated Cyclin D1 and CREPT expression correlated with higher grade and invasive behavior in a OSCC study.[16] These markers' grading associations parallel our CRBP-1 findings, reinforcing the validity of IHC-based stratification.

A limitation of this study is its retrospective nature and modest sample size. Additionally, semi-quantitative scoring may introduce observer bias, a limitation that we mitigated through blinded evaluation by two pathologists and consensus resolution.

Future investigations could explore CRBP-1 promoter methylation status, hypermethylation silencing has been documented in other epithelial cancers and may underlie expression loss in OSCC.[7,12] Functional assays, such as cell proliferation or migration studies in OSCC models, would also strengthen causal inferences drawn from our IHC data.

In summary, this study findings support CRBP-1 as a promising differentiation marker in OSCC, inversely correlated with tumor grade.

Incorporating CRBP-1 IHC into routine histopathological evaluation may enhance prognostic accuracy and guide therapeutic decisions. Prospective studies with diverse cohorts and mechanistic analyses are recommended to further validate CRBP-1's clinical utility.

CONCLUSION

This study demonstrates a significant inverse correlation between CRBP-1 expression and histological grade in OSCC. Strong CRBP-1 positivity was predominantly observed in well-differentiated tumors, while weak or absent expression was more common in moderately and poorly differentiated cases. These findings suggest that CRBP-1 may serve as a useful immune-histochemical marker for tumor differentiation and biological behavior in OSCC. Incorporating CRBP-1 expression analysis into routine diagnostic practice may aid in prognostic stratification and treatment planning.

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