Stromal Expression of CD10 in Oral Squamous Cell Carcinoma and its Association with Histological Grade

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is one of the most frequently encountered malignancies of the oral cavity worldwide. Identification of reliable molecular markers may improve prognostic assessment and individualized management of affected patients.

Methods: This cross-sectional observational study was performed in the Department of Pathology, Dhaka Medical College, Dhaka, between September 2022 and August 2024. A total of 56 histopathologically confirmed OSCC cases, including 40 resected specimens and 16 mucosal biopsies, were enrolled according to predefined inclusion and exclusion criteria. Hematoxylin and eosin-stained slides and paraffin-embedded tissue blocks were reviewed in all cases. Immunohistochemical staining for CD10 and Masson trichrome staining were carried out on formalin-fixed paraffin-embedded tissue sections. Stromal CD10 expression was assessed and correlated with histological grade. Statistical analysis was performed using SPSS version 23, and a p-value below 0.05 was considered statistically significant.

Results: Most patients belonged to the 41–60 years age group (46.4%). The mean age was 61.0 ± 12.84 years, and males were more frequently affected than females with a ratio of 2.1:1. Positive stromal CD10 immunoreactivity was detected in 45 (80.4%) cases. The highest positivity was observed in poorly differentiated tumors (100%), followed by moderately differentiated tumors (96.3%) and well differentiated tumors (44.4%). The association between CD10 expression and histological grade was statistically significant (p=0.00001).

Conclusion: The present study demonstrated a significant relationship between stromal CD10 expression and increasing histological grade of OSCC. Therefore, CD10 may serve as a useful adjunctive marker for predicting aggressive biological behavior in oral squamous cell carcinoma.

[Journal of Histopathology and Cytopathology, 2026 Jul; 10 (2):96-103]
DOI: https://www.doi.org/10.69950/jhc.2026.10.2.3

Keywords: Oral Squamous Cell Carcinoma, Immunohistochemistry, CD10

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Introduction

Oral cancer remains a major health burden worldwide and is considered one of the most common malignant neoplasms affecting the head and neck region.[1] According to recent GLOBOCAN data, oral cancer accounts for a substantial number of newly diagnosed cancer cases and cancer-related deaths every year across the globe.[2] The disease is particularly prevalent in South and Southeast Asian countries, where tobacco smoking, smokeless tobacco use, betel nut chewing, and alcohol intake are common lifestyle-related risk factors.[3]

Among oral malignancies, oral squamous cell carcinoma (OSCC) is the predominant histological subtype. The pathogenesis of OSCC is multifactorial and involves progressive genetic and molecular alterations that may arise either de novo or from pre-existing potentially malignant disorders such as leukoplakia and erythroplakia.[4] These precursor lesions may gradually progress to epithelial dysplasia and invasive carcinoma over time. OSCC may involve different anatomical regions of the oral cavity, including the tongue, buccal mucosa, gingiva, floor of the mouth, and palate.[5]

The molecular mechanisms underlying oral carcinogenesis include alterations in oncogenes and tumor suppressor genes such as p16, p53, H-ras, cyclin D1, and epidermal growth factor receptor (EGFR).[6] Besides tumor cell–related genetic abnormalities, increasing evidence indicates that the tumor microenvironment also has a crucial influence on tumor development and progression. The stromal compartment surrounding malignant epithelial cells contains fibroblasts, inflammatory cells, endothelial cells, and extracellular matrix proteins that actively participate in tumor growth and invasion.[7]

Tumor-associated stromal cells release different enzymes and signaling molecules, including matrix metalloproteinases, which facilitate degradation of the extracellular matrix and support local invasion and metastasis.[7] Consequently, interactions between neoplastic cells and stromal elements are now recognized as important contributors to tumor aggressiveness.

Cluster of Differentiation 10 (CD10), also known as common acute lymphoblastic leukemia antigen (CALLA) or neutral endopeptidase, is a membrane-associated zinc-dependent metalloproteinase involved in the degradation of several bioactive peptides.[8] Increased stromal CD10 expression has been documented in different epithelial malignancies including breast, gastric, and colorectal carcinomas, where it is associated with aggressive tumor behavior.[9] In OSCC, stromal fibroblasts and cancer-associated fibroblasts expressing CD10 are believed to promote tumor progression by altering the extracellular environment and enhancing invasive potential.[10] Furthermore, elevated CD10 expression has been linked with tumor recurrence, poor prognosis, and resistance to therapy.[11]

While stromal CD10 expression has been investigated in oral squamous cell carcinoma in different populations, evidence from Bangladesh remains scarce. To the best of our knowledge, this is the first study evaluating stromal CD10 immunoexpression and its association with histological grade of OSCC in a Bangladeshi population. The findings of this study provide baseline local evidence that may improve understanding of tumor biology and facilitate future prognostic research in Bangladesh.

Although advances in surgery and adjuvant treatment have improved patient management, the prognosis of OSCC remains unsatisfactory

in many patients. Therefore, identification of additional prognostic markers related to tumor stromal interaction may contribute to better risk stratification and future targeted therapy. Based on these observations, the present study was undertaken to evaluate stromal CD10 expression in OSCC and determine its association with histological grade.

Methods

This cross-sectional observational study was conducted in the Department of Pathology, Dhaka Medical College, Dhaka, from September 2022 to August 2024. Ethical approval was obtained from the Institutional Review Board (IRB) of Dhaka Medical College prior to commencement of the study. Written informed consent was obtained from all participants.

A total of 56 histopathologically confirmed cases of oral invasive squamous cell carcinoma were included using purposive sampling. Among them, 40 were resected specimens and 16 were mucosal biopsy samples. Cases were selected irrespective of age and sex. Patients who had received chemotherapy or radiotherapy before tissue sampling were excluded from the study.

Previously prepared hematoxylin and eosin-stained slides were reviewed to confirm the diagnosis and assess tumor grade. Histological grading was performed according to the WHO (2022) classification of squamous cell carcinoma into well differentiated, moderately differentiated, and poorly differentiated categories.[12] Relevant demographic and clinical information were recorded using a structured data collection sheet.

Following gross examination, tissue specimens were fixed in 10% buffered formalin and processed routinely. Paraffin-embedded tissue blocks were sectioned at 5 µm thickness and stained with hematoxylin and eosin.

Masson trichrome staining was performed at Bangladesh Medical University to facilitate visualization of stromal collagen fibers surrounding tumor islands. Microscopic evaluation was carried out under 100× magnification. Stromal collagen appeared blue, cytoplasm and keratin stained red, and nuclei stained black.[13]

For immunohistochemistry, 4 µm sections were placed on poly-L-lysine coated slides. Antigen retrieval was performed using heat-induced epitope retrieval solution followed by peroxide blocking to inhibit endogenous peroxidase activity. Sections were incubated with monoclonal mouse anti-human CD10 antibody (clone 56C6, Dako Denmark). Subsequently, secondary antibody conjugated with horseradish peroxidase was applied. Diaminobenzidine tetrahydrochloride was used as chromogen, and Mayer's hematoxylin was used for counterstaining.

Stromal CD10 expression was evaluated independently by two experienced histopathologists who were blinded to the clinicopathological data. Brown cytoplasmic staining of peritumoral stromal fibroblasts was considered positive. Ten representative high-power fields (×400) were examined for each case, and only the percentage of positively stained stromal fibroblasts was recorded. Cases showing immunoreactivity in <10% of stromal fibroblasts were considered negative, whereas those with staining in ≥10% of stromal fibroblasts were regarded as positive according to the criteria described by Patankar et al.[10] In cases of discrepant interpretation, a consensus diagnosis was reached through joint microscopic review. Staining intensity and combined immunohistochemical scoring systems were

not evaluated in the present study.[10,14] Tonsillar tissue served as positive control.[15]

All statistical analyses were carried out using IBM SPSS version 23. Numerical variables were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. The Chi-square test was used to evaluate associations between categorical variables. Statistical significance was considered at $p < 0.05$.

Results

Table I shows that the age of the oral SCC patients in the present study ranged from 27 to 90 years with a mean age of 61.0 (± 12.84) years. Among 56 cases of OSCC, 38 (68%) cases were male and 18 (32%) cases were females with male-to-female ratio 2.1:1.

Figure 1 presents most of the cases were moderately differentiated (Grade 2) tumors, 27 (48.3 %) followed by well differentiated (Grade 1) 18 (32.1%) and poorly differentiated (Grade 3) 11 (19.6%) tumours.

Table II illustrates that CD10 immunoexpression was positive in 45 (80.4%) study cases of OSCC and rest 11 (19.6%) cases were CD10 negative. Masson trichrome staining clearly demonstrated the stromal collagen surrounding tumor nests and facilitated the identification of peritumoral stromal fibroblasts for subsequent immunohistochemical evaluation of CD10 (Figure 2).

In this study positive CD10 expression increased with higher histological grade, being highest in Grade 3 tumour (100%) followed by 96.3% in Grade 2 and 44.4% in Grade 1 tumour. Table III shows the association of CD10 expression with histological grade of OSCC, which was statistically significant. (P value = 0.00001).

Table I: Demographic distribution of the study cases (n=56)

Age (years)	Frequency	%
21-40	4	7.1%
41-60	26	46.4%
61-80	24	42.9%
81-100	2	3.6%
Gender		
Male	38	68%
Female	18	32%
Total	56	100%

Table II: Immunohistochemical expression of CD10 in oral SCC (n=56)

Characteristics	Frequency	%
Positive	45	80.4
Negative	11	19.6
Total	56	100.0

Table III: Association of CD10 expression with histological grade of OSCC (n=56)

Histological grading of OSCC(n=56)	Expression of CD10		P Value
	Positive N (%)	Negative N (%)	
Well differentiated (Grade 1) (18)	8 (44.4)	10 (55.6)	0.00001[s]
Moderately differentiated (Grade 2) (27)	26 (96.3)	1 (3.7)	
Poorly differentiated (Grade 3) (11)	11 (100.0)	0 (0.0)	

s –significant

Chi-square test was done to measure the level of significance

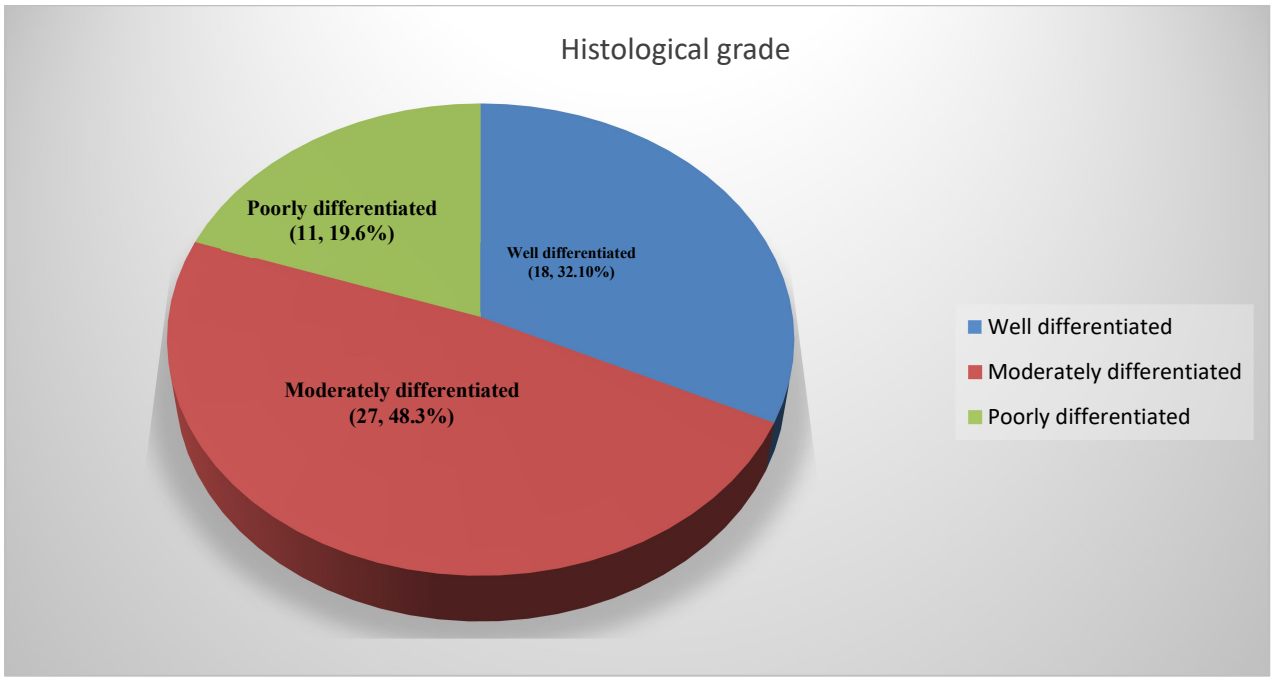

Figure 1. Pie chart showing distribution of the study cases by histological grade of oral SCC (n=56)

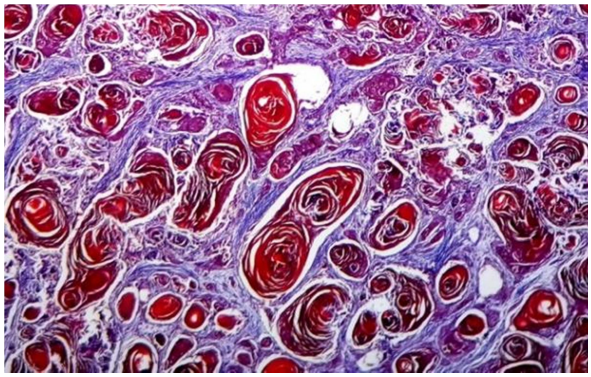

Figure 2. Photomicrograph shows well differentiated oral squamous cell carcinoma (Masson Trichrome, 10X)

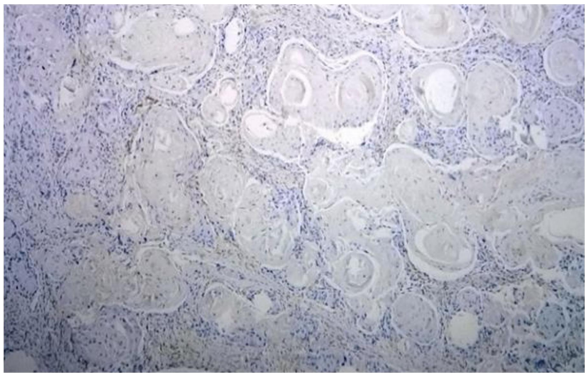

Figure 3. Photomicrograph shows well differentiated oral squamous cell carcinoma showing negative staining of CD10 (IHC, 10X)

Figure 4: Photomicrograph shows moderately differentiated oral squamous cell carcinoma positive staining of CD10 (IHC, 4X)

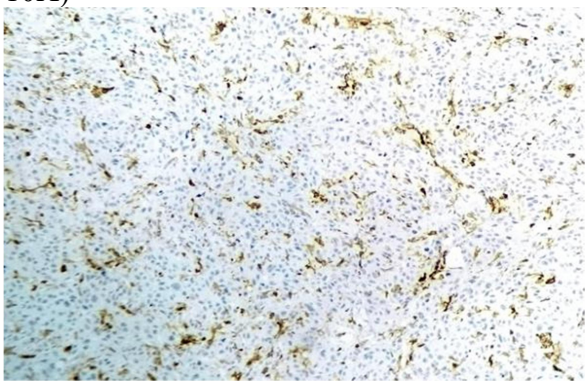

Figure 5: Photomicrograph of poorly differentiated oral squamous cell carcinoma showing positive staining of CD 10 (IHC, 10X)

Discussion

The present study evaluated stromal CD10 expression in oral squamous cell carcinoma and analyzed its association with histological grade. Increasing attention has recently been directed toward the role of the tumor microenvironment in cancer progression, particularly the contribution of stromal fibroblasts and extracellular matrix remodeling in facilitating invasion and metastasis.[7] Masson trichrome staining was primarily used to improve visualization of stromal collagen and peritumoral fibroblasts during assessment of CD10 immunoreactivity rather than as an independent study variable.

In this study, positive CD10 immunoexpression was identified in 80.4% of OSCC cases. Similar findings were reported by Patankar et al.[10] and Piattelli et al.[14] who observed increased stromal CD10 expression in a large proportion of oral squamous cell carcinoma cases. These observations support the hypothesis that stromal CD10 expression may contribute to the biological aggressiveness of OSCC. CD10 is a zinc-dependent cell surface metalloproteinase capable of degrading several biologically active peptides within the extracellular matrix. Increased stromal CD10 expression may facilitate degradation of extracellular matrix components, thereby promoting local tumor invasion and enhancing metastatic potential.[14] Furthermore, CD10-positive cancer-associated fibroblasts contribute to remodeling of the tumor microenvironment through secretion of matrix metalloproteinases, cytokines and growth factors that support tumor proliferation, angiogenesis and invasion.[17,18] These biological mechanisms may explain the CD10 expression observed in the present study.

The patients included in the present study ranged in age from 27 to 90 years, with a mean age of 61 years. Most cases occurred in the 41–60 year age group. Comparable age distribution patterns have been described by Li et al.[11] and Akhter et al.[3] The predominance of middle-aged and older patients may reflect prolonged exposure to carcinogenic substances such as tobacco, betel nut, and alcohol.

Male predominance was observed in the present study, with a male-to-female ratio of 2.1:1. Similar male predominance has been documented in previous studies on head and neck squamous cell carcinoma.[11] This finding may be explained by increased exposure of males to established environmental and lifestyle-related risk factors.

Most tumors in the present study were moderately differentiated, followed by well differentiated and poorly differentiated carcinomas. Similar distributions have been documented in previous studies evaluating histopathological grading patterns in OSCC.[4,5]

A statistically significant association was observed between stromal CD10 expression and histological grade. Positivity increased progressively with higher tumor grade and reached 100% in poorly differentiated tumors. Similar associations between elevated stromal CD10 expression and aggressive tumor grade were also reported by Piattelli et al.[14], Manolea et al.[16], and Helmy et al.[17] These findings indicate that stromal CD10 expression may be related to increased invasive and metastatic potential.

However, not all investigators have reported similar findings. Fukusumi et al.[18] observed no significant association between CD10 expression and histological differentiation in head and neck squamous cell carcinoma. Such discrepancies may be explained by differences in sample size, immunohistochemical protocols, scoring

criteria and ethnic variation among study populations.

The present study has several limitations. It was conducted at a single tertiary care center with a relatively small sample size using purposive sampling, which may limit the generalizability of the findings. Furthermore, the absence of long-term follow-up, survival analysis and clinicopathological correlation with TNM stage and lymph node metastasis limited the assessment of the prognostic significance of stromal CD10 expression.

Conclusion

The present study demonstrated a significant association between stromal CD10 expression and histological grade of oral squamous cell carcinoma. Increased CD10 expression was observed in higher-grade tumors, suggesting that CD10 may serve as a useful adjunctive biomarker for assessing tumor aggressiveness. Further multicenter studies with larger sample sizes and long-term follow-up are required to establish its prognostic significance.

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