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Evaluation of Cancer Stem Cell Marker CD44 Expression by Immunohistochemistry in Oral Squamous Cell Carcinoma and its Clinicopathological Correlations

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is a major public health burden in India, with high rates of recurrence and metastasis. Cancer stem cells (CSCs) marked by CD44 are implicated in tumour initiation, progression, and therapy resistance. This study evaluated CD44 immunoexpression in OSCC and its correlation with clinicopathological parameters.

Methods: A cross-sectional study of 70 consecutive histologically confirmed OSCC resection specimens (mandibulectomy and related procedures with neck dissection) received at a tertiary centre was performed. CD44 immunohistochemistry (Scytek mouse monoclonal antibody) was scored for percentage positivity (0–3) and intensity (0–3); a composite total score was categorized as weak (1), low (2–4), or high (5–9). Associations with age, gender, tumour size, grade, depth of invasion (DOI), lymph-node status, margins, and time to presentation were analysed by chi-square test ($p < 0.05$ significant).

Results: Mean age was 48.93 ± 12.24 years; 82.9% were male. Most tumours were Grade 1 (57.1%), measured 2–4 cm, and had DOI >10 mm (80%). Lymph-node involvement was present in 35.7%. All cases showed some CD44 positivity; 60% had 11–50% positive cells and 55.7% showed weak intensity. Composite scores were high in 28.6%, low in 18.6%, and weak in 52.9%. Higher CD44 expression correlated significantly with lower histological grade ($p = 0.003$) and lymph-node involvement ($p = 0.03$). No significant associations were found with tumour size, DOI, or time to presentation.

Conclusions: CD44 is widely expressed in OSCC. Higher expression is associated with better differentiation yet also with nodal metastasis, suggesting a dual role in adhesion and dissemination. CD44 IHC may serve as a useful adjunct prognostic marker; larger prospective studies with survival data are warranted.

Keywords: Oral Squamous Cell Carcinoma; CD44; Cancer Stem Cells; Immunohistochemistry; Prognostic Marker; Lymph-Node Metastasis

Abbreviations: OSCC: Oral Squamous Cell Carcinoma; CSCs: Cancer Stem Cells.

Introduction

Oral squamous cell carcinoma (OSCC) constitutes approximately 95% of oral malignancies in developing countries and accounts for nearly one-third of the global burden, with India bearing a disproportionately high share [1-3]. The five-year survival rate remains around 50%, largely because of late presentation, local recurrence, and regional or distant metastasis [4]. Traditional clinicopathological parameters (TNM stage, grade, depth of invasion, nodal status) guide treatment but are imperfect predictors of

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outcome.

An emerging concept attributes tumour heterogeneity, therapy resistance, and metastatic potential to a subpopulation of cancer stem cells (CSCs) [5,6]. CSCs possess self-renewal capacity, multipotency, and the ability to initiate tumours. Among surface markers, CD44-a transmembrane glycoprotein involved in cell-matrix adhesion, migration, and signalling-has been extensively studied in head-and-neck squamous cell carcinoma [7-9]. High CD44 expression has been linked to aggressive behaviour and poorer survival in some series, yet other studies report inverse correlations with grade, reflecting loss of adhesion in dedifferentiated tumours [10-12].

Data from the Indian subcontinent remain limited. The present study therefore evaluated CD44 immunoexpression in a consecutive series of surgically treated OSCC patients at a tertiary centre in central India and correlated expression with standard clinicohistopathological parameters.

Materials and Methods

Study Design and Setting

Cross-sectional observational study conducted in the Department of Pathology, Mahatma Gandhi Memorial Medical College and Maharaja Yashwantrao Hospital, Indore, after Institutional Ethics Committee approval (EC/MGM/NOV-29/23). The study period was 12 months following approval.

Inclusion and Exclusion Criteria

Patients >20 years of age with histopathologically confirmed OSCC who underwent resection (mandibulectomy or related procedures ± neck dissection) were included. Poorly preserved tissue, refusal of consent, and non-OSCC oral lesions were excluded.

Sample size

Seventy consecutive eligible specimens were analysed (calculated using the formula $n = Z^2pq/d^2$ with 5% margin of error).

Tissue Processing and Immunohistochemistry

Specimens fixed in 10% neutral buffered formalin were grossed according to CAP protocol, processed routinely, and stained with haematoxylin–eosin for histological grading (WHO/Broders), staging (AJCC 8th edition), DOI measurement, margin assessment, and nodal evaluation.

Four-micrometre sections were subjected to CD44 immunohistochemistry using a mouse monoclonal antibody (Scytek Laboratories, catalogue RA0055-C.5). Antigen retrieval was performed in citrate buffer (pH 6.4) by microwave. The polymer-HRP/DAB detection system was employed. Positive staining was defined as membranous ± cytoplasmic reactivity in tumour cells. Two independent pathologists scored the most representative tumour-centre areas:

- Percentage positivity: 0 (<10%), 1 (11-50%), 2 (51-80%), 3 (>80%).
- Intensity: 0 (none), 1 (weak), 2 (moderate), 3 (strong).

Total score = percentage score × intensity score. Categories: weak (1), low (2-4), high (5–9) [9,13].

Statistical Analysis

Data were entered in Microsoft Excel and analysed with SPSS version 25. Descriptive statistics (frequencies, means ± SD) were calculated. Associations between categorical variables and CD44 categories were tested by chi-square test; ANOVA was used for continuous variables. A two-sided p-value <0.05 was considered statistically significant.

Results

Demographics and Clinical Features

Of 70 patients, 58 (82.9%) were male and 12 (17.1%) female. Mean age was 48.93 ± 12.24 years (range 21–80); the largest group was 51–60 years (34.3%). Ulceroproliferative growth was the commonest presenting complaint (45.7%), followed by non-healing ulcer (18.6%). Time from symptom onset to presentation was <6 months in 35.7%, 6–12 months in 37.1%, and >1 year in 27.1%. Segmental mandibulectomy was the most frequent procedure (48.6%).

Tumour Characteristics

Tumour size was 2–4 cm in the largest subset; 80% of tumours had DOI >10 mm. Histological grade was Grade 1 in 40 (57.1%), Grade 2 in 27 (38.6%), and Grade 3 in 3 (4.3%). Nodal metastasis was absent in 45 (64.3%), present in 1–4 nodes in 22 (31.4%), and >4 nodes in 3 (4.3%). Surgical margins were free in 91.4%.

CD44 Immunoexpression

All cases showed CD44 positivity. Percentage scores: 1 (11–50%) in 60%, 2 (51–80%) in 22.9%, 3 (>80%) in 17.1%. Intensity was weak in 55.7%, moderate in 24.3%, and strong in 20%. Composite total scores: high 28.6%, low 18.6%, weak 52.9% (Figures 1-18).

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Clinicopathological Correlations

- **Grade:** Higher CD44 expression was significantly associated with lower grade (p = 0.003). Grade 1 tumours showed high expression in 40%, whereas Grade 2 and 3 tumours were predominantly weak (77.8% and 100%, respectively).
- **Lymph-Node Status:** High CD44 expression was significantly more frequent in cases with 1–4 involved nodes (50%) than in node-negative cases (17.8%) (p = 0.03).
- **Other Parameters:** No statistically significant associations were observed with tumour size (p ≈ 0.53), DOI (p = 0.529), or time to presentation (p = 0.556).



Figure 1: Gross Image of Hemimandible.

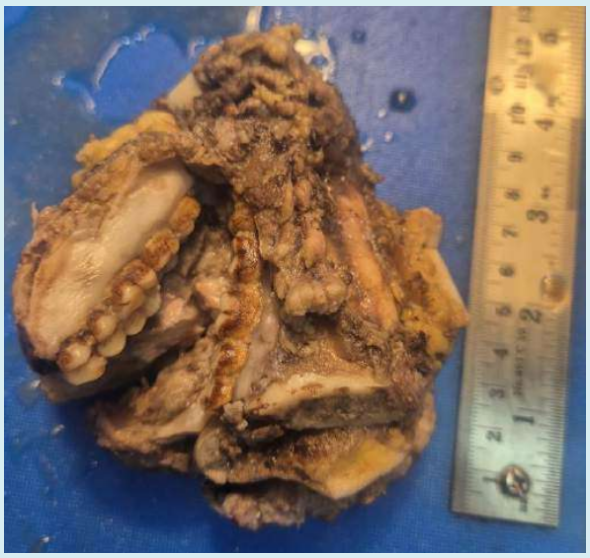


Figure 2: Gross Specimen of Hemimandible.



Figure 3: Mandible with Ulcerproliferative Growth.



Figure 4: Friable Growth on Cut Section.

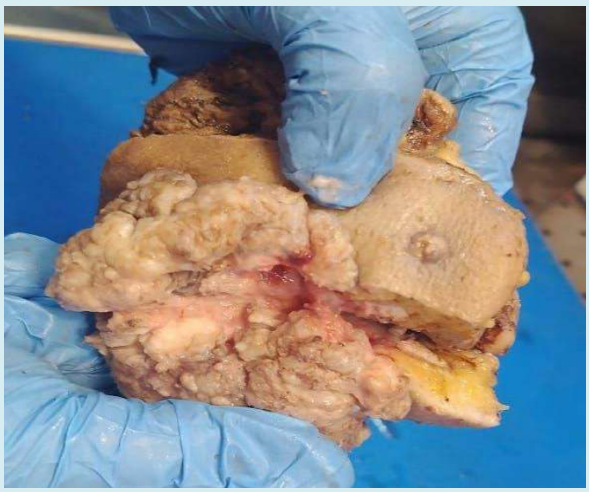


Figure 5: Friable Growth on Cut Section.

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Figure 6: Sections of Mandible in Cassettes.

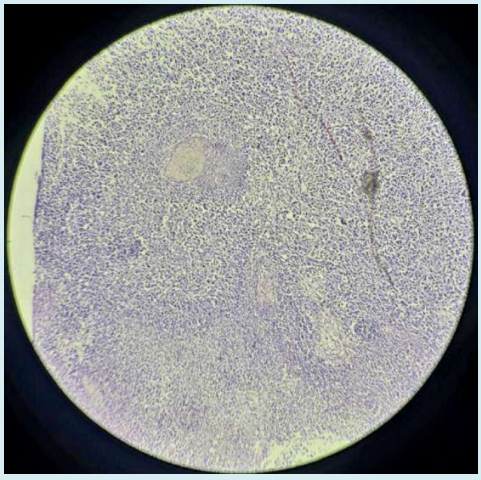


Figure 7: Microphotograph Shows Poorly Differentiated OSCC (10x).

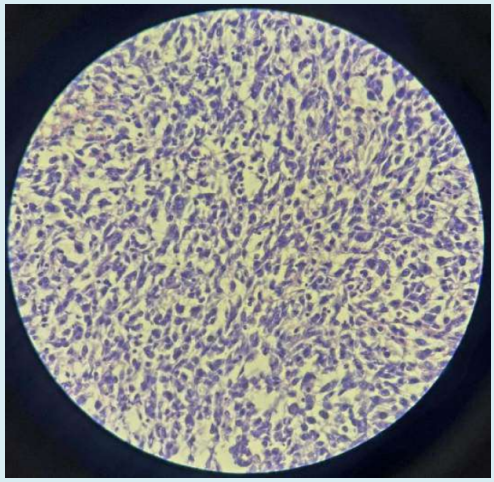


Figure 8: Microphotograph Shows Poorly Differentiated OSCC (40x)

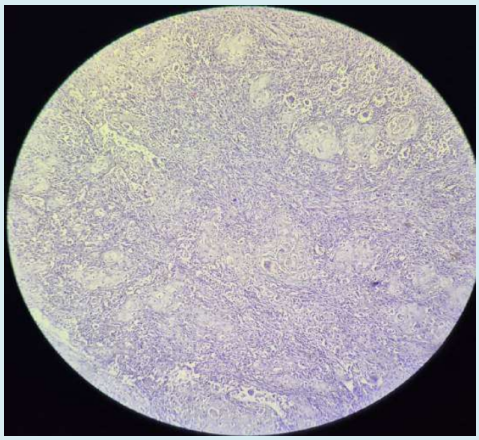


Figure 9: Microphotograph of Tumor Shows Moderately Differentiated OSCC (10x).

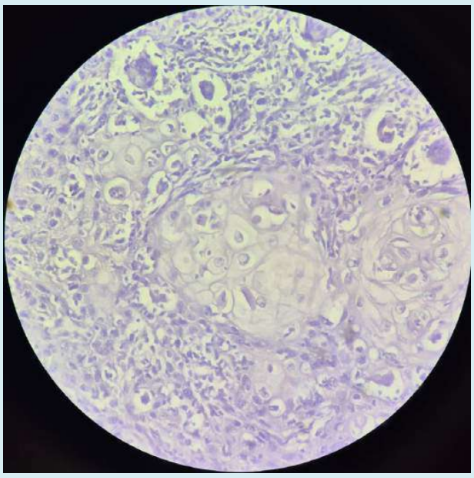


Figure 10: Microphotograph of Tumor Shows Moderately Differentiated OSCC (40x).

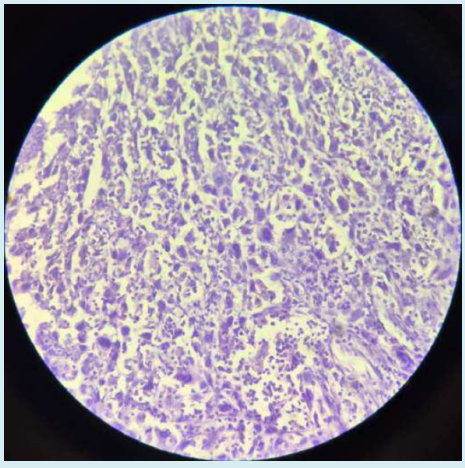


Figure 11: Microphotograph of Tumor Shows Singly Scattered Pleomorphic Cells (40x).

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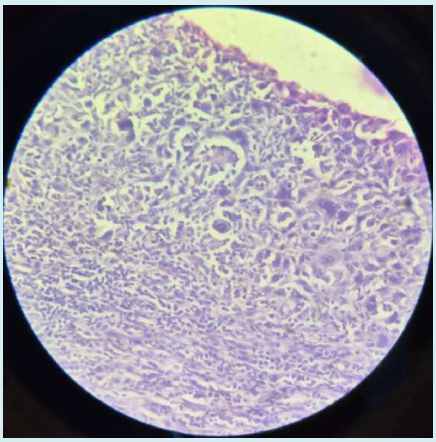


Figure 12: Microphotograph of Tumor Shows Mitotic Figures (40x).

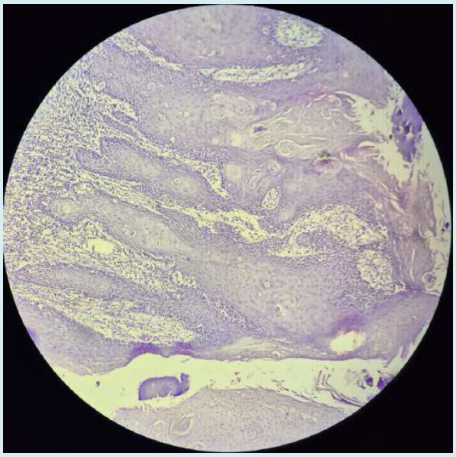


Figure 13: Microphotograph Shows Well Differentiated OSCC (10x).

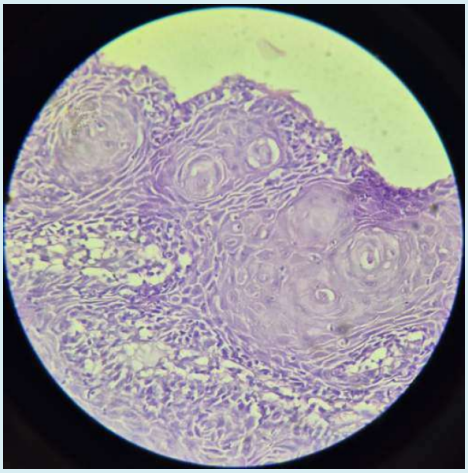


Figure 14: Microphotograph of Tumor Shows well Differentiated OSCC (40x).

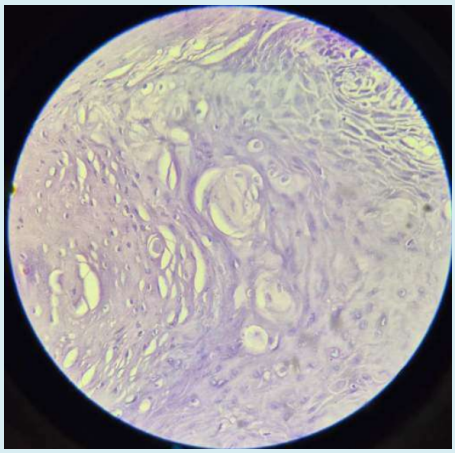


Figure 15: Microphotograph Shows Well Differentiated OSCC (40x).

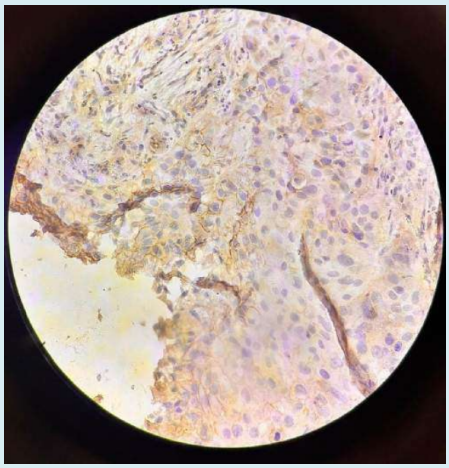


Figure 16: Microphotograph Shows Mild Staining on IHC (40x).

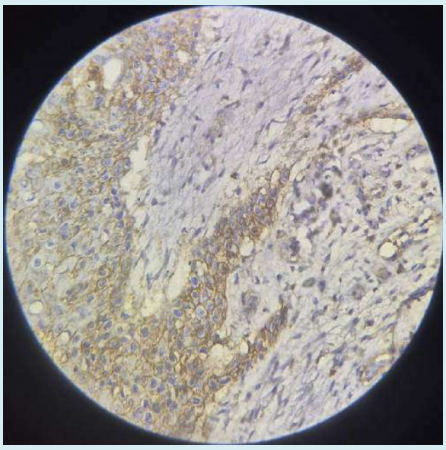


Figure 17: Microphotograph Shows Moderate Staining On IHC (40x).

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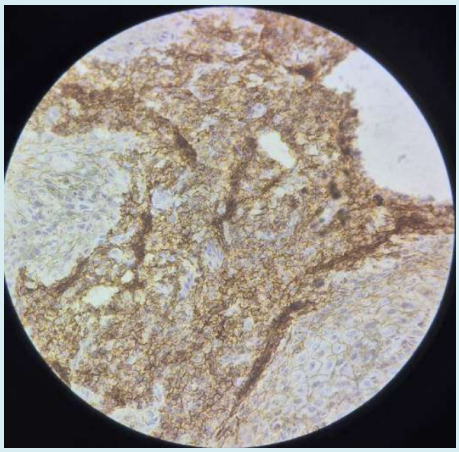


Figure 18: Microphotograph Shows Intense Staining on IHC (40x).

Discussion

OSCC remains a disease of middle-aged to elderly males in tobacco-using populations, consistent with previous Indian and global epidemiological data. The predominance of well-differentiated tumours and frequent deep invasion reflect the typical presentation pattern at tertiary centres in low- and middle-income countries, where delayed diagnosis is common.

CD44 was expressed in all cases, with a substantial proportion (approximately 47%) showing moderate-to-high composite scores. This prevalence is comparable to reports by Chen J, et al. [12] (49%) and Tandon A, et al. (48%) [8]. The significant inverse relationship between CD44 intensity/score and histological grade observed here aligns with findings of Koukourakis MI, et al. Hema KN, et al. and Chakraborty S, et al. who noted stronger CD44 labelling in well-differentiated tumours [6,7,9] One interpretation is that CD44 primarily marks more differentiated cells that retain cell–cell and cell–matrix adhesion; progressive loss of CD44 in higher-grade lesions may facilitate epithelial–mesenchymal transition and invasion.

Conversely, the positive association between higher CD44 expression and nodal metastasis ($p = 0.03$) supports the role of CD44 in promoting lymphatic dissemination, as reported by Ortiz RC, et al. Hendawy H, et al. and Ma C, et al. [10,13,14]. CD44 can engage hyaluronan and activate downstream pathways (Rho, PI3K–Akt) that enhance motility and survival in the metastatic niche. Thus, CD44 appears to play context-dependent dual roles: adhesion maintenance in differentiated tumours and facilitation of metastasis once the invasive phenotype is acquired.

Absence of correlation with tumour size, DOI, and time to presentation is consistent with several prior studies and may reflect the limited sample size or the multifactorial nature of these parameters. Limitations of the present work include modest sample size, single-centre design, lack of survival follow-up, and restriction to resection specimens (selection bias toward operable disease).

Conclusion

CD44 is consistently expressed in OSCC. Higher expression correlates with better histological differentiation yet also with lymph-node metastasis, underscoring its complex biological role. Immunohistochemical evaluation of CD44 may complement conventional prognostic parameters and help identify patients at higher risk of nodal spread. Multicentre studies with long-term clinical outcome data are needed to validate its independent prognostic value and potential utility as a therapeutic target.

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