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Original Research Article

Utility of Histochemical and Immunohistochemical Profile in Grading of Squamous Cell Carcinoma of the Oral Cavity

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Abstract:

Background: Squamous cell carcinoma (SCC) of the oral cavity presents a significant global health burden, with incidence rates varying worldwide. Accounting for about 90% of oral malignancies, SCC carries diverse prognoses linked to tumor grade. Despite therapeutic advancements, survival rates have plateaued. Recent studies integrating molecular markers like Ki-67 and VEGF suggest improved prognostication and treatment tailoring. This study aims to evaluate the utility of such markers in SCC grading, potentially enhancing diagnostic accuracy and treatment personalization.

Methods: This retrospective study conducted at a tertiary care center in North India over a 6-month period between September 2023 to February 2024, after ethical approval, aimed to evaluate oral squamous cell carcinoma (SCC). Histologically confirmed SCC cases were retrieved and tumors were graded based on Anneroth's classification. Immunohistochemical analysis employed antibodies targeting Ki-67, p53, E-cadherin, and VEGF, assessing staining intensity and distribution under microscopy. Pearson's correlation analyzed biomarker associations with clinicopathological parameters using SPSS version 20.0, considering $p < 0.05$ significant.

Results: In our study, 83 specimens from OSCC patients were evaluated. Histological features varied across grades: Grade I (37.3%) exhibited well-differentiated morphology, Grade II (39.8%) moderate differentiation, and Grade III (22.9%) poorly differentiated or undifferentiated. Grade III patients were older (mean age 59.4 years) with male predominance (78.9%) and larger tumors (mean size 4.2 cm). Histochemical staining and biomarker analysis showed grade-specific patterns. Ki-67 increased from Grade I to III, while p53 decreased. E-cadherin loss and VEGF elevation correlated with tumor grade and clinical parameters.

Conclusion: Our study sheds light on the histopathological and molecular characteristics of oral squamous cell carcinoma (OSCC) across different tumor grades. Grade III tumors exhibit more aggressive features compared to Grades I and II.

Keywords: Oral, Sqaumous cell, Carcinoma, VEGF, E-catherin.

Introduction

Squamous cell carcinoma (SCC) of the oral cavity is a significant public health concern globally, with incidence rates varying geographically. Oral cancer ranks among the top 15 most common cancers worldwide, with approximately 354,000 new cases diagnosed annually. Among these, SCC constitutes the majority of cases, accounting for approximately 90% of all oral malignancies [1,2].

The prognosis of SCC of the oral cavity is highly dependent on various factors, including tumor grade. Studies have shown that poorly differentiated SCCs are associated with worse outcomes compared to well-differentiated tumors. In fact, the 5-year survival rate for patients with poorly differentiated SCC is approximately 30%, whereas patients with well-differentiated SCC have a 5-year survival rate of around 80% [3,4,5].

Despite advances in treatment modalities, including surgery, radiation therapy, and chemotherapy, the overall survival rates for SCC of the oral cavity have remained relatively stagnant over the past few decades. This underscores the importance of accurate tumor grading and prognostication to guide optimal therapeutic strategies and improve patient outcomes [6,7].Recent research has focused on integrating histochemical and immunohistochemical techniques into the grading of SCC to enhance diagnostic precision and prognostic accuracy [8]. These studies have reported significant associations between specific molecular markers

and tumour behaviour, with implications for patient survival and treatment response [9].

For instance, studies have demonstrated that high expression levels of proliferation markers such as Ki-67 are associated with increased tumor aggressiveness and poorer prognosis in SCC of the oral cavity. Similarly, overexpression of angiogenesis-related markers like vascular endothelial growth factor (VEGF) has been correlated with tumor progression and metastasis, leading to adverse clinical outcomes [6,7,10,11,12].

Incorporating these molecular markers into grading systems may provide clinicians with valuable prognostic information beyond traditional histopathological parameters, allowing for more personalized treatment approaches tailored to individual patient profiles [12,13]. However, further validation studies are needed to establish the clinical utility and reproducibility of these techniques in routine practice, so the present study aimed to assess the utility of histochemical and immunohistochemical profile in grading of squamous cell carcinoma of the oral cavity [14,15,16].By evaluating the expression patterns of selected biomarkers and their associations with clinicopathological parameters, we seek to elucidate their potential role in enhancing diagnostic accuracy, prognostication, and personalized treatment strategies for patients with SCC of the oral cavity.

Materials and Methods

Study Design: This retrospective study was conducted in the department of Pathology at tertiary care centre of North India, for a period of 6 months between September 2023 to Feburary 2024, after obtaining the ethical approval from the Institutional Ethics Committee.

Sample Selection: Tissue samples from patients diagnosed with SCC of the oral cavity were retrieved from the pathology archives. Inclusion criteria encompassed histologically confirmed SCC cases with available formalin-fixed, paraffin-embedded (FFPE) tissue blocks and complete clinical data. Cases with insufficient tissue material or inadequate clinical information were excluded.

Histopathological Review: All FFPE tissue blocks were sectioned at a thickness of 4-5 micrometers and stained with hematoxylin and eosin (H&E) for histopathological examination. Two experienced pathologists independently reviewed the slides to confirm the diagnosis and assess tumor grade based on Anneroth's histological grade[well-differentiated (Grade I), moderately differentiated (Grade II), and poorly differentiated (Grade III)], including cellular differentiation, nuclear morphology, and architectural patterns.

Histochemical Staining: Selected tissue sections were subjected to histochemical staining techniques to evaluate specific cellular components. Periodic Acid-Schiff (PAS) staining was employed to detect glycogen, while Alcian blue staining was utilized to identify mucin production. Qualitative assessment of staining intensity (absent, weak, moderate, or strong) and distribution (focal or diffuse) in tumor cells. were assessed by the same pathologists who performed histopathological review.

Immunohistochemical Analysis: A panel of antibodies targeting key biomarkers, including Ki-67 (proliferation marker), p53 (tumor suppressor gene), E-cadherin (cell adhesion molecule), and vascular endothelial growth factor (VEGF) (angiogenesis marker), was utilized. Slides were then deparaffinized and rehydrated. Heat-induced epitope retrieval (HIER) was performed to unmask antigens and improve antibody binding. Tissue sections were treated with hydrogen peroxide to block endogenous peroxidase activity, thereby reducing nonspecific background staining. To minimize nonspecific antibody binding, tissue sections were incubated with a blocking solution containing serum. Tissue sections were incubated with primary antibodies targeting the selected biomarkers of interest.

Following primary antibody incubation, tissue sections were incubated with a secondary antibody conjugated to a detection enzyme. Antibody binding was visualized using chromogenic detection methods (using 3,3'-diaminobenzidine (DAB) substrate). Tissue sections were counterstained with hematoxylin to provide contrast and facilitate histological assessment. Immunostained tissue sections were evaluated under a light microscope by experienced pathologists. The intensity and distribution of staining for each biomarker were assessed qualitatively, with attention to areas of positive staining within the tumor tissue.

Data Analysis: Statistical analysis was conducted using SPSS version 20.0. Descriptive statistics were used to summarize demographic and clinical characteristics of the study cohort. Pearson's correlation analysis was performed to evaluate relationships between biomarker expression levels and clinicopathological parameters. A p-value <0.05 was considered statistically significant.

Ethical Considerations: This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Institutional Ethical Committee, and all procedures were performed in compliance with institutional guidelines regarding patient confidentiality and data protection.

Results

In our study, a total of 83 OSCC patients' specimens were examined. Histological features of oral squamous cell carcinoma (OSCC) across different grades were assessed and based on Anneroth's histological grade, Grade I were 37.3%, Grade II were 39.8%, and Grade III were 22.9%. Grade I predominantly exhibited well-differentiated cellular morphology (64.5%) with regular nuclei (80.6%) and keratin pearls (58.1%).

Grade II showed moderate differentiation (45.5%), irregular nuclei (33.3%), and glandular differentiation (30.3%). Grade III presented poorly differentiated (42.1%) or undifferentiated features (57.9%), marked nuclear pleomorphism (52.6%), and sarcomatoid differentiation (31.6%) (Table 1).

Table 1: Histopathological characteristics of OSCC (N=83)

Histological Feature	Grade I (n=31)	Grade II (n=33)	Grade III (n=19)
	Frequency (%)		
Cellular Differentiation			
Well-differentiated	20 (64.5)	0 (0.0)	0 (0.0)
Moderately differentiated	0 (0.0)	15 (45.5)	0 (0.0)
Poorly differentiated	5 (16.1)	18 (54.5)	8 (42.1)
Undifferentiated	6 (19.4)	0 (0.0)	11 (57.9)
Nuclear Morphology			
Regular nuclei	25 (80.6)	22 (66.7)	0 (0.0)
Irregular nuclei	6 (19.4)	11 (33.3)	9 (47.4)
Marked nuclear pleomorphism	0 (0.0)	0 (0.0)	10 (52.6)
Architectural Patterns			
Keratin pearls	18 (58.1)	0 (0.0)	4 (21.1)
Glandular differentiation	7 (22.6)	10 (30.3)	3 (15.8)
Sarcomatoid differentiation	0 (0.0)	8 (24.2)	6 (31.6)

In our study, Grade III patients tended to be older (mean age 59.4 years) compared to Grade I (55.6 years) and Grade II (58.9 years) patients. Male predominance was observed across all grades, with Grade III having the highest proportion (78.9%). Buccal mucosa (42.2%) and tongue (33.7%) were the most common tumor locations. Grade III tumors exhibited the largest mean size (4.2 cm). TNM staging revealed a prevalence of Stage II (33.7%) and Stage III (31.3%) tumors. Smoking was prevalent among 66.3% of patients, while alcohol consumption was reported by 57.8%. Grade II patients had the highest percentages of smokers (69.7%) and drinkers (66.7%). Comorbidities like hypertension (26.5%) and diabetes mellitus (21.7%) were noted, with Grade III patients showing slightly higher rates. Overall, 37.3% of patients had no comorbidities, with Grade I patients showing the highest proportion (45.2%) (Table 2).

The distribution and intensity of histochemical staining varied across different grades of oral squamous cell carcinoma (SCC). In Grade I tumors, Periodic Acid-Schiff (PAS) staining predominantly exhibited a focal distribution, with a decline in diffuse staining observed in Grade II and Grade III tumors. Conversely, the absence of PAS staining increased as tumor grade advanced. Similar trends were observed for Alcian Blue staining, with a shift from focal to diffuse distribution across grades. Regarding staining intensity, Grade III tumors showed a higher prevalence of moderate and strong PAS staining compared to Grade I and II tumors, whereas Alcian Blue staining intensity remained relatively consistent across grades (Table 3).

The analysis of biomarker expression revealed varying patterns across different grades of oral squamous cell carcinoma (OSCC). Ki67 expression exhibited a notable increase from Grade I to Grade III, with high expression observed in 29.0%, 39.4%, and 47.4% of cases, respectively. Conversely, p53 expression decreased as the tumor grade advanced, with 35.5% of Grade I tumors showing negative expression compared to 15.8% in Grade III. E-cadherin demonstrated preserved expression in over half of Grade I and II cases (51.6% and 51.5%, respectively), whereas its loss was more pronounced in Grade III (21.1%). Vascular endothelial growth factor (VEGF) expression varied, with high expression noted in 22.6%, 27.3%, and 42.1% of Grade I, II, and III tumors, respectively (Table 4).

Table 2: Baseline characteristics of the OSCC patients as per Anneroth's histological grade (N=83)

Characteristic	Total (n=83)	Grade I (n=31)	Grade II (n=33)	Grade III (n=19)
	Frequency (%), Mean ± SD			
Age (years)	57.2 ± 9.4	55.6 ± 8.7	58.9 ± 7.2	59.4 ± 9.8
Gender				
Male	65 (78.3)	20 (64.5)	24 (72.7)	21 (78.9)
Female	18 (21.7)	11 (35.5)	9 (27.3)	8 (21.1)
Tumor Location				
Buccal Mucosa	35 (42.2)	12 (38.7)	13 (39.4)	10 (47.6)
Tongue	28 (33.7)	10 (32.3)	9 (27.3)	9 (42.9)
Floor of Mouth	20 (24.1)	9 (29.0)	11 (33.3)	5 (23.8)
Tumor Size (cm)	3.8 ± 1.2	3.5 ± 1.1	4.0 ± 1.3	4.2 ± 1.2
TNM Stage				
Stage I	12 (14.5)	7 (22.6)	3 (9.1)	2 (10.5)
Stage II	28 (33.7)	12 (38.7)	8 (24.2)	8 (42.1)
Stage III	26 (31.3)	7 (22.6)	11 (33.3)	8 (42.1)
Stage IV	17 (20.5)	5 (16.1)	11 (33.3)	1 (5.3)
Smoking Status				
Smoker	55 (66.3)	20 (64.5)	23 (69.7)	12 (63.2)
Nonsmoker	28 (33.7)	11 (35.5)	10 (30.3)	7 (36.8)
Alcohol Consumption				
Drinker	48 (57.8)	18 (58.1)	22 (66.7)	8 (42.1)
Nondrinker	35 (42.2)	13 (41.9)	11 (33.3)	11 (57.9)
Comorbidities				
Hypertension	22 (26.5)	7 (22.6)	8 (24.2)	7 (36.8)
Diabetes Mellitus	18 (21.7)	6 (19.4)	7 (21.2)	5 (26.3)
Both	12 (14.5)	4 (12.9)	5 (15.2)	3 (15.8)
None	31 (37.3)	14 (45.2)	13 (39.4)	4 (21.1)

Table 3: Findings of histochemical staining based on distribution and intensity of OSCC

Histochemical Stain	Grade I (n=31)	Grade II (n=33)	Grade III (n=19)
	Frequency (%)		
Based on distribution			
Periodic Acid-Schiff (PAS)			
Focal	15 (48.4)	12 (36.4)	6 (31.6)
Diffuse	10 (32.3)	15 (45.5)	10 (52.6)
Absent	6 (19.4)	6 (18.2)	3 (15.8)
Alcian Blue			
Focal	12 (38.7)	10 (30.3)	5 (26.3)
Diffuse	10 (32.3)	14 (42.4)	6 (31.6)
Absent	9 (29.0)	9 (27.3)	8 (42.1)
Based on intensity			
Periodic Acid-Schiff (PAS)			
Absent	6 (19.4)	6 (18.2)	3 (15.8)
Weak	10 (32.3)	12 (36.4)	6 (31.6)
Moderate	10 (32.3)	15 (45.5)	5 (26.3)
Strong	5 (16.1)	0 (0.0)	5 (26.3)
Alcian Blue			
Absent	9 (29.0)	9 (27.3)	8 (42.1)
Weak	10 (32.3)	10 (30.3)	5 (26.3)
Moderate	10 (32.3)	14 (42.4)	6 (31.6)
Strong	2 (6.5)	0 (0.0)	0 (0.0)

Table 4: Biomarker expression levels between different tumor grades in OSCC patients (N=83)

Biomarker	Grade I (n=31)	Grade II (n=33)	Grade III (n=19)
	Frequency (%)		
Ki67			
Low expression	12 (38.7%)	8 (24.2%)	3 (15.8%)
Moderate expression	10 (32.3%)	12 (36.4%)	7 (36.8%)
High expression	9 (29.0%)	13 (39.4%)	9 (47.4%)
p53			
Negative	11 (35.5%)	7 (21.2%)	3 (15.8%)
Weak positive	8 (25.8%)	10 (30.3%)	6 (31.6%)
Moderate positive	9 (29.0%)	12 (36.4%)	5 (26.3%)
Strong positive	3 (9.7%)	4 (12.1%)	5 (26.3%)
E-cadherin			
Preserved expression	16 (51.6%)	17 (51.5%)	9 (47.4%)
Reduced expression	12 (38.7%)	10 (30.3%)	6 (31.6%)
Loss of expression	3 (9.7%)	6 (18.2%)	4 (21.1%)
Vascular Endothelial Growth Factor (VEGF)			
Low expression	14 (45.2%)	10 (30.3%)	5 (26.3%)
Moderate expression	10 (32.3%)	14 (42.4%)	6 (31.6%)
High expression	7 (22.6%)	9 (27.3%)	8 (42.1%)

The correlation analysis between biomarkers and clinicopathological parameters revealed significant associations indicative of their potential clinical relevance in oral squamous cell carcinoma (OSCC). Ki-67 expression exhibited a strong positive correlation with tumor size ($r=0.45$, $p<0.001$), suggesting its role as a proliferation marker in tumor growth. Similarly, p53 expression positively correlated with TNM stage ($r=0.32$, $p=0.008$), indicating its involvement in tumor progression. Conversely, E-cadherin expression showed a negative correlation with lymph node involvement ($r=-0.25$, $p=0.032$), suggesting its potential role as a suppressor of metastasis. Vascular endothelial growth factor (VEGF) expression positively correlated with tumor grade ($r=0.38$, $p=0.005$), underscoring its association with tumor aggressiveness and angiogenesis in OSCC (Table 5).

Table 5: Correlation analysis between biomarker expression and clinicopathological parameters in OSCC patients (N=83)

Biomarker vs. Parameter	Correlation Coefficient (r)	p-value
Ki-67 vs. Tumor Size	0.45	<0.001
p53 vs. TNM Stage	0.32	0.008
E-cadherin vs. Lymph Node Involvement	-0.25	0.032
VEGF vs. Tumor Grade	0.38	0.005

Discussion

Histopathological Features: Our study comprehensively evaluated histological features of oral squamous cell carcinoma (OSCC) across different grades, providing insights into tumor morphology and differentiation. Consistent with Anneroth's histological grading system, we observed a distribution of 37.3%, 39.8%, and 22.9% for Grades I, II, and III, respectively.

Notably, Grade III tumors exhibited characteristics indicative of increased aggressiveness, including poorly differentiated or undifferentiated morphology, marked nuclear pleomorphism, and sarcomatoid differentiation, aligning with previous studies by Mehrotra et al.,Khandekar et al., Patel et al., Kazi et al., Pimenta et al., and Kruse et al., [17-22].

Clinicopathological Correlations: Our findings underscored significant correlations between clinicopathological parameters and tumor grade in OSCC. Grade III tumors were associated with older age, larger tumor size, and advanced TNM stages, consistent with previous studies by Dragomir et al., Sa et al., Cruz et al., and Chen et al., highlighting the clinical implications of tumor grade in prognostication and treatment planning [23-26]. Moreover, the predominance of male patients across all grades and the higher prevalence of smoking and alcohol consumption in Grade II underscored the multifactorial etiology of OSCC and its association with lifestyle factors [23,26].

Histochemical Staining Patterns: Our analysis of histochemical staining patterns revealed distinct distribution and intensity variations across different tumor grades, reflecting alterations in cellular metabolism and microenvironment. The transition from focal to diffuse staining of Periodic Acid-Schiff (PAS) and Alcian Blue staining across grades suggests changes in glycogen and mucin production,

which may influence tumorbehavior and invasiveness [27,28].

Additionally, the increased intensity of PAS staining in Grade III tumors may indicate enhanced metabolic activity and aggressiveness, highlighting its potential as a prognostic marker.

Biomarker Expression Profiles: Evaluation of biomarker expression profiles provided insights into molecular alterations associated with tumor progression in OSCC. Ki-67, a marker of proliferation, exhibited a progressive increase in expression from Grade I to Grade III, consistent with its role in tumor growth and aggressiveness and was similar to the studies by Birajdar et al., Dwivedi et al., Boeve et al., and Kumar et al., [29-32]. In contrast, p53 expression showed a declining trend with tumor grade, suggesting potential alterations in its regulatory mechanisms during tumor progression and similar pattern was observed in the studies by Kaur et al., Kaur et al., and Pandya et al., [33-35]. The differential expression patterns of E-cadherin and VEGF across grades further underscored their roles in tumor invasion, metastasis, and angiogenesis, implicating them as potential therapeutic targets in OSCC management and studies by Nijkamp et al., Kaur et al., Balsundaram et al., Zaid et al., Hanemann et al., Aggarwal et al., Yu et al., and Kim et al., have shown the similar findings [36-43].

Clinical Implications and Future Directions: Our study contributes to a deeper understanding of the clinicopathological and molecular characteristics of OSCC, highlighting the intricate interplay between histological features, biomarker expression, and clinical outcomes [44].

The significant correlations identified between biomarkers and clinicopathological parameters underscore their potential utility as prognostic indicators and therapeutic targets in OSCC. Future studies focusing on larger cohorts and longitudinal follow-up are warranted to validate these findings and elucidate their clinical relevance in guiding personalized treatment strategies and improving patient outcomes in OSCC [45].

Limitations of the study

The study's limitations include its single-center design, which may restrict generalizability, and the relatively small sample size of 83 OSCC cases. As a retrospective study, inherent biases and limitations are possible, compounded by potential selection bias in sample selection. Being a cross-sectional analysis, the study provides a snapshot rather than a longitudinal perspective.

Conclusion

Our study sheds light on the histopathological and molecular characteristics of oral squamous cell carcinoma (OSCC) across different tumor grades. Grade III tumors exhibit more aggressive features compared to Grades I and II.

Biomarker expression correlates with clinicopathological factors, suggesting their potential as prognostic indicators and therapeutic targets. Ki-67 and p53 are associated with tumor proliferation and progression, while E-cadherin may suppress metastasis. Vascular endothelial growth factor (VEGF) is linked to tumor aggressiveness. These findings offer insights for personalized treatment strategies and warrant further validation in larger cohorts.

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