Original Article

Assessment Of Ki-67 Proliferation Index In Relation To Histological Grades Of Oral Squamous Cell Carcinoma

Aliya Muzafar[1], Ifrah Ahmed[2], Hamna Ahmad[3], Samreen Hameed[4], Rabia Altaf[5], Numrah Bilal Butt[6]

Abstract

Objective: To evaluate the Ki-67 proliferation index and its correlation with different histological grades of Oral squamous cell carcinoma.

Methods: This retrospective immunohistochemical study was conducted at the Pathology Department of Mayo Hospital, Lahore, from January 2024 to May 2025. A total of 30 histopathologically confirmed cases of OSCC were selected. Hematoxylin and eosin (H&E) stained slides were used for grading tumors, followed by immunohistochemical staining for Ki-67. The staining intensity and percentage of positively stained tumor cells were evaluated and scored.

Results: Among the 30 cases, 6 (20%) showed no immunoreactivity for Ki-67, 1 case (3%) exhibited mild staining, 13 cases (43.3%) demonstrated moderate staining, and 10 cases (33.3%) displayed intense Ki-67 expression. A significant positive correlation was found between higher Ki-67 expression and poor tumor differentiation ($p < 0.05$). High-grade tumors (moderately and poorly differentiated OSCC) exhibited markedly increased Ki-67 labeling index as compared to well-differentiated tumors.

Conclusion: This study reinforces the utility of Ki-67 as a reliable proliferative marker in oral squamous cell carcinoma. Its expression correlates significantly with histological grade, indicating its potential role in prognostication and guiding therapeutic strategies.

Keywords: Carcinoma Squamous Cell; Cell Proliferation; Immunohistochemistry; Ki-67 Antigen; Mouth Neoplasms; Neoplasm Grading; Tumor Cells Malignant.

Introduction

Oral squamous cell carcinoma (OSCC) remains a major public health issue globally, particularly in South Asia, where it ranks among the most common malignancies and exhibits high morbidity and mortality rates despite therapeutic advances.[1,2] In Pakistan, OSCC is especially prevalent, with many patients presenting at advanced stages and poor differentiation, underscoring the need for reliable prognostic biomarkers.[3]

Cell proliferation is a fundamental hallmark of cancer, and the Ki-67 antigen, expressed in cycling cells but absent in resting (G0) cells, is a widely accepted marker of proliferative activity. Elevated Ki-67 labeling index (LI) has been shown to correlate with tumor grade, stage, and poorer clinical outcomes in diverse cancers.[4,5] Specifically in OSCC, a large prospective cohort study identified significantly higher Ki-67 LI in poorly differentiated tumors ($p < 0.001$) and an association with reduced three- and five-year survival rates.[4]

Local studies in Pakistan offer additional insights: An investigation at AFIP Rawalpindi compared Ki-67 expression in OSCC versus pseudoepitheliomatous hyperplasia, finding markedly higher Ki-67 positivity in OSCC lesions and an increasing trend with histological grade.[5] Another Rawalpindi-based study assessing Cyclin D1 and Ki-67 in primary and metastatic OSCC found that although Ki-67 did not significantly differ between primary and nodal metastases, it correlated modestly with histological progression.[6]

Globally, meta-analyses and systematic reviews confirm Ki-67's value: elevated expression correlates positively with higher histologic grade, nodal metastasis, and worse prognosis in head and neck squamous cell carcinoma.[7,8] Even beyond OSCC, studies in breast and colorectal cancers support a robust positive association between the Ki-67 index and tumor grade.[9]

These cumulative findings justify further evaluation of Ki-67 in OSCC tumor grading and prognostic stratification. However, inconsistency in scoring methods and cutoff thresholds remains a challenge, especially in resource-limited settings.[7]

We aimed to determine the correlation between Ki-67 expression levels and histological grade, and to evaluate its utility as a proliferation-based marker in predicting tumor aggressiveness and guiding management of OSCC in our local clinical context.

Materials And Methods

This retrospective observational study was conducted at the Pathology Department of Mayo Hospital, Lahore, from January 2024 to May 2025. Calculation of the sample was carried out using the statistical tool provided by the WHO. Parameters included a 95% confidence level, a proportion of 0.80 based on the frequency of Ki-67 positivity in OSCC, and an alpha error of 0.5.[3]

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

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HA, SH, RA, NBB - Acquisition, Analysis, Interpretation
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OSCC, or inadequate/autolyzed tissue were excluded. Patient's 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 3% hydrogen peroxide solution was used to quench intrinsic peroxidase activity. The tissue slides were incubated with an anti-Ki-67 primary antibody (clone MIB-1, Dako, Denmark, and dilution of 1:100) for 30 minutes at room temperature. After washing, a secondary antibody and DAB (diaminobenzidine) chromogen were applied for visualization. Hematoxylin was used as a counterstain before dehydration and mounting.

Immunoreactivity for Ki-67 was evaluated by two independent pathologists blinded to clinical and histological data. The percentage of positively stained nuclei in 1000 tumor cells was counted in five high-power fields (HPFs), and the final proliferation index was calculated. Ki-67 expression was categorized semi-quantitatively as: negative (0–5%), mild (6–25%), moderate (26–50%), and strong (> 50%) nuclear staining. Discrepancies between observers were resolved by joint review using a multi-head microscope.

Data were recorded and analyzed using SPSS v 25. Frequencies and percentages were calculated for qualitative variables, while mean and SD were determined for quantitative variables. The association between Ki-67 expression and tumor grade was assessed using the chi-square test, and a p-value < 0.05 was considered significant.

Results

A total of 30 cases of histologically confirmed OSCC were included. The patients comprised 19 males (63.3%) and 11 females (36.7%), with a mean age of 54.2±11.6 years. The most common tumor site was the buccal mucosa (40%), followed by the tongue (33.3%), alveolar ridge (16.7%), and floor of the mouth (10%). Based on histological grading, cases were categorized as well-differentiated, moderately differentiated, and poorly differentiated tumors (Table 1). Immunohistochemical staining with Ki-67 revealed nuclear positivity in varying proportions. Cases showed either negative, mild, moderate, or strong expression patterns (Table 2).

A significant association was observed between Ki-67 expression levels and the histological grade of OSCC. Strong Ki-67 expression was predominantly observed in moderately and poorly differentiated tumors, while negative or mild expression was mostly seen in well-differentiated cases. The relationship between tumor grade and Ki-67 expression was significant ($p = 0.021$), as shown in Table 3.

Table 1: Distribution of cases according to histological grade of OSCC

Histological grade	Frequency	Percentage
Well differentiated	10	33.3
Moderately differentiated	13	43.4
Poorly differentiated	7	23.3

Table 2: Ki-67 immunohistochemical expression in OSCC cases

Ki-67 expression level	Frequency	Percentage
Negative (0–5%)	6	20.0
Mild (6–25%)	1	3.3
Moderate (26–50%)	13	43.4
Strong (> 50%)	10	33.3

Table 3: Correlation between Ki-67 expression and histological grade of OSCC

Tumor grade	Negative	Mild	Moderate	Strong	Total	p-value
Well differentiated	4	1	4	1	10	
Moderately differentiated	2	0	6	5	13	0.021
Poorly differentiated	0	0	3	4	7	
Total	6	1	13	10	30	

Discussion

This study demonstrates a significant association between Ki-67 expression and histological grade in OSCC, with higher Ki-67 indices observed in moderately and poorly differentiated tumors. This aligns with prior literature indicating Ki-67's capacity to reflect tumor aggressiveness and proliferative activity.

A multicenter retrospective study of OSCC patients found that Ki-67 labeling index (LI) correlated strongly with adverse clinicopathological features, including higher tumor grade, advanced TNM stage, and nodal metastasis; higher Ki-67 was linked to poorer three- and five-year survival ($p < 0.001$).[10] This supports our finding of elevated Ki-67 expression in higher-grade tumors.

A systematic meta-analysis reinforced Ki-67 as a negative prognostic marker in OSCC, particularly within Asian populations. Subgroup analysis revealed that ethnic background, Ki-67 cut-off values, and antibody clone (MIB-1) affected outcomes.[11] The significance of Ki-67 in our local population is thus consistent with meta-analytic evidence and reinforces its impact in resource-limited settings like Pakistan.

In contrast, a local Rawalpindi/Islamabad study comparing Ki-67 expression between primary and metastatic OSCC reported no significant difference in Ki-67 levels between metastatic and primary tumors ($p = 0.715$), although Cyclin D1 varied significantly ($p = 0.003$).[5] While not

directly addressing grade, this highlights the heterogeneity in proliferation marker expression and the need to interpret Ki-67 alongside other markers.

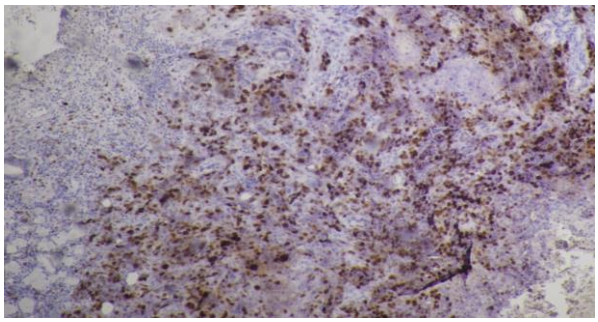

Figure 1: Ki-67 "strong" positive expression in OSCC (H&E x 400)

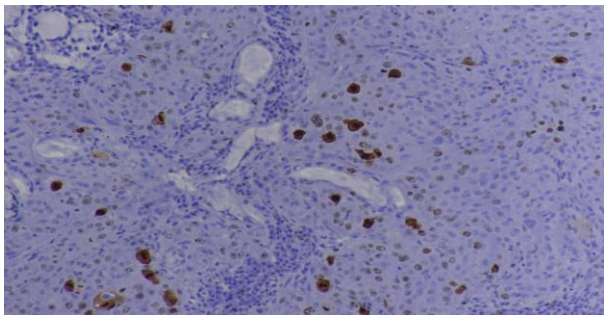

Figure 2: Ki-67 showing a moderate pattern of intensity

Another Indian study found a clear gradient in Ki-67 LI across OSCC grades: well-differentiated tumors exhibited a mean LI of 28%, escalating to 69% in poorly differentiated tumors ($p < 0.05$).[12] These quantitative results parallel our semi-quantitative scoring categories and support the grading relevance of Ki-67.

A Syrian study evaluating Ki-67 in normal mucosa, dysplasia, and OSCC found that Ki-67 expression progressively increased from normal to dysplasia to carcinoma, and that its localization extended from basal (normal) to diffuse (poorly differentiated OSCC).[13] Such spatial patterns reinforce our observation of strong nuclear staining in high-grade tumors.

In head and neck squamous cell carcinoma (HNSCC) beyond the oral cavity, a study predicted Ki-67 expression preoperatively using MRI-based machine learning models with high accuracy (AUC = 0.9), and confirmed that higher Ki-67 was associated with advanced T stage and nodal metastasis.[14] This further establishes Ki-67 as a surrogate marker for proliferative potential and invasive behavior.

Another recent immunohistochemical investigation evaluated USP22 alongside Ki-67 in OSCC and normal mucosa, revealing significantly elevated Ki-67 expression in carcinoma tissues compared to normal controls ($p < 0.001$).[15] Though not grading-specific, this underscores the broad utility of Ki-67 in distinguishing malignant from benign tissue.

Finally, a deep-prognostic modeling study found that Ki-67 is an independent predictor of recurrence, metastasis, and overall survival in OSCC, with multivariate hazard ratios > 1.8 even after adjusting for tumor differentiation and nodal status [16]. This confirms its role not only as a grading marker but also as a prognostic tool.

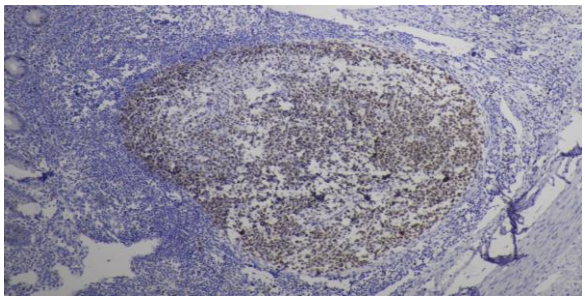

Figure 3: Ki-67 showing a weak pattern

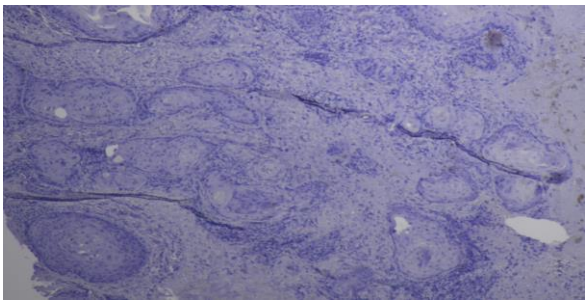

Figure 4: Ki-67 "negative" staining expression (H&E x 400).

Limitations of our study include its retrospective design, modest sample size, and semi-quantitative rather than digital scoring of Ki-67, which may introduce observer variability. Future research using digital image quantification and larger cohorts would enhance reproducibility and allow exploration of optimal Ki-67 cut-off thresholds for prognostication.

In summary, our findings corroborate robust evidence that Ki-67 expression increases with declining differentiation in OSCC. Incorporating Ki-67 labeling into routine histopathological evaluation may enhance prognostic stratification and inform treatment planning in OSCC, especially in our local clinical context, where advanced-stage presentations are common.

Conclusions

This study demonstrates a significant correlation between Ki-67 expression and the histological grade of OSCC. The Ki-67 immunostaining intensity increased with higher tumor grades, reflecting greater proliferative activity in less differentiated tumors. These findings reinforce the potential utility of Ki-67 as a reliable adjunctive biomarker for assessing tumor aggressiveness and predicting biological behavior in OSCC. Routine assessment of the Ki-67 proliferation index in histopathological evaluation may aid in risk stratification and therapeutic decision-making, particularly in resource-constrained settings.

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Author Information

1. Associate Professor, Fatima Jinnah Medical University, Lahore 2,5. Demonstrator, King Edward Medical University, Lahore 3. Undergraduate Student, Fatima Jinnah Medical University, Lahore 4. Associate Professor, King Edward Medical University, Lahore 6. Postgraduate Resident, King Edward Medical University, Lahore
Corresponding author: Dr. Aliya Muzafar aliya.muzafar.222@gmail.com

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