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Research Article

Association of Oral Biological and Immunohistochemical Expression of P53 and Ki-67 with Histopathological Grade and Clinical Outcomes in Oral Squamous Cell Carcinoma

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is a highly aggressive and poorly prognostic cancer. The present study assessed the immunohistochemical expression of p53 and Ki-67 in OSCC and their relationship with the histological grade and clinical outcome.

Methods: A cross-sectional observational study was made of 80 cases of histologically confirmed OSCC in the Department of Oral Biology, Khyber Medical University (KMU), Peshawar from September, 2025 to February, 2026. Formalin-fixed paraffin-embedded tissues were stained with p53 and Ki-67. The expression patterns were evaluated and correlated with the histological grades and available clinical parameters. A significance level of $p < 0.05$ was used.

Results: The majority of cases were moderately differentiated (45%), followed by well differentiated (35%) and poorly differentiated (20%) OSCC, with p53 overexpression increasing with increasing grade (36% in well differentiated, 67% in moderately differentiated, and 81% in poorly differentiated). Ki-67 labeling index also showed a progressive rise (18.4%, 32.7%, and 56.9% respectively; $p < 0.05$). There was a significant association between high expression of both markers and advanced clinical stage, lymph node metastasis, and increased recurrence.

Conclusion: The study shows that there is a strong positive correlation between increased expression of p53 and increased expression of Ki-67 and higher histological grades and aggressive clinical behavior in OSCC. These biomarkers could be useful as additional tools to traditional histopathology for better prognosis evaluation and risk stratification in oral cancer patients.

Keywords: Oral Squamous Cell Carcinoma, P53, Ki-67, Immunohistochemistry, Histological Grade, Prognosis.

INTRODUCTION

Despite recent advances in the therapeutic approach to oral squamous cell carcinoma (OSCC), its aggressive clinical course, delayed diagnosis and poor prognosis make it a major global disease burden. Local invasion and regional metastasis are common features of this disease, and early diagnosis and accurate prognosis are important to enhance patient survival. Thus, molecular markers are of interest in this regard to enhance the accuracy of diagnosis and to elucidate the biology of the tumor. Immunohistochemical markers, particularly those related to cell proliferation and tumor suppression, have gained significant importance in the evaluation of OSCC, as they also provide prognostic information in addition to the traditional histopathological analysis (1). The molecular alterations highlight the need to study markers associated with both proliferation and genetic instability in oral cancer (2). The most important area to determine tumor behavior is at the tumor's

invasive front, where the tumor cells have the most aggressive biological characteristics.

The up-regulation of proliferation-related and tumor suppressor markers at this site is a sign of increased activity and invasiveness of the tumor (3). The abnormal expression of p53 and Ki-67 at the invasive tumour front has been associated with greater cell proliferation, as well as changes in the mechanisms controlling tumour growth, suggesting that they may be important for the assessment of tumour aggressiveness and prediction of disease behaviour. Ki-67 is well known as a good marker for cellular proliferation because it is expressed in all active phases of the cell cycle but not in resting cells. The expression level is a direct measure of growth fraction of tumor tissues and is therefore an ideal measure to assess the biological aggressiveness of oral lesions. OPDs have significantly higher Ki-67 expression than invasive carcinoma and hence it is a valuable marker for the grading and diagnostic evaluation of oral epithelial lesions (4). High Ki-67 expression has also been closely associated with high tumor grade and advanced clinical stage in OSCC, indicating that there is a close correlation between Ki-67 expression and tumor progression and aggressiveness.

The increased proliferative activity (as reflected by the Ki-67 expression) suggests rapid tumor growth and more aggressive clinical course, which makes it a potential prognostic marker for the management of oral cancer (5). The expression of Ki-67 is significantly different between OED and OSCC, with higher expression in OSCC, implying an increase in Ki-67 expression with malignant transformation. The gradual increase supports the significance of Ki-67 as a marker for the development of disease and its diagnostic value to distinguish different stages of oral epithelial disease (6). Histological grading continues to be an essential part of the assessment of OSCC and is a common method to assess tumor differentiation and clinical behavior (7). However, this morphological method cannot completely capture the biological diversity of tumors. There is also further evidence of the usefulness of Ki-67 in prognosis, since it is well correlated with the histological progression of oral lesions (8).

High Ki-67 expression is typically associated with poorly differentiated tumors and high proliferation and aggressive tumor behavior. These results further validate its usefulness as a grading and prognostic tool for OSCC. In addition to molecular changes in tumor cells, there are also viral factors that are linked to oral carcinogenesis such as human papillomavirus (HPV). It has been suggested that the expression of HPV proteins is associated with some clinicopathological features of OSCC, suggesting that viral oncogenesis may play a role in alterations in tumor biology and disease progression. The results presented here demonstrate the multi-factorial nature of OSCC initiation and how the viral and cellular mechanisms interact in oral malignancy. The expression of Ki-67 has also been shown to be variable between various histological grades and tumour differentiations. In poorly differentiated tumors it is typically found and is associated with a high proliferative activity and high biological aggressiveness.

The association of Ki-67 expression with histological grade indicates that it could serve as a marker of tumor severity and biological aggressiveness in OSCC (10). The progressive up-regulation of Ki-67 expression from a benign oral lesion to dysplastic oral lesions to invasive carcinoma further underscores its importance in the multistep process of oral carcinogenesis (11). This form of expression could be a progressive increase in the ability to multiply in malignant transformation. It could be a useful aid in diagnosis for lesions of higher malignant potential. Immune regulatory pathways have also been associated with oral cancer along with markers of proliferation. The immune system evasion mechanisms have been identified in OSCC and OPDs, including alterations in immune checkpoints (PD-1 and PD-L1) (12). The results indicate that immunological biomarkers are also emerging as important tools to study oral carcinogenesis.

The study of biomarkers in OSCC has resulted in the identification of many histological and serological markers that have been found to be diagnostic and prognostic. These biomarkers offer valuable data regarding the biology of the tumour and may help to guide personalised treatment, such as risk stratification and treatment planning, in patients with oral cancer (13). The function of p53 in tumour suppression is well known and is crucial for the regulation of DNA repair, cell cycle arrest and apoptosis (14). Genomic instability and tumour progression is due to mutations or abnormal expression of p53. The dual-marker approach also helps in early detection of malignant changes and in better evaluation of tumor aggressiveness, which are beneficial for routine histopathological evaluation (15). Histological and immunohistochemical studies of OSCC

samples in recent years have shown that there is a significant difference in the expression of p53 and Ki-67 between different histological grades.

The markers are usually expressed at higher levels in tumors of higher grade and more aggressive clinical behavior, indicating their possible role in prognosis and follow-up of the disease (16). Systematic reviews have also identified immunohistochemical markers as being important for early detection and prognosis of OSCC. The markers p53 and Ki-67 are specifically useful because they are associated with the suppression of the tumor and cellular proliferation, respectively. The evaluation they provide provides valuable information about tumour biology and can help in the early diagnosis and prediction of prognosis in oral cancer (17).

Objective: To assess the immunohistochemical expression of p53 and Ki-67 in oral squamous cell carcinoma and their relationship to histological grading and clinical outcome for prognosis.

MATERIALS AND METHODS

Study Design: Cross-sectional observational study

Study Setting: Department of Oral Biology, Khyber Medical University (KMU), Peshawar

Study Duration: September, 2025 to February, 2026

Sample Size: There were 80 cases of the disease confirmed by histological examination

Sample Technique: Non-probability consecutive sampling

Inclusion Criteria: The study included patients who had histopathologically confirmed OSCC. Formalin-fixed paraffin-embedded (FFPE) tissue blocks were selected only if they contained sufficient material for immunohistochemical analysis. To achieve a reliable correlation between biomarker expression and clinicopathological parameters, cases with complete clinical records, including patient demographics, tumor site, and histological grading, were also included.

Exclusion Criteria: Samples with inadequate or poorly preserved tissue that might affect the quality of immunohistochemical staining were excluded. Furthermore, patients who had undergone radiotherapy or chemotherapy prior to biopsy were excluded because of the possible change in biomarker expression. Additionally, patients with incomplete clinical information and those with recurrent tumors were excluded to ensure data uniformity and minimize bias in the analysis.

METHODS

The formalin fixed paraffin embedded tissue blocks of histopathologically confirmed OSCC were collected from the departmental archives. Representative sections were cut and stained with hematoxylin and eosin for diagnosis confirmation and histological grading was carried out based on conventional criteria. Immunohistochemical staining was carried out using a primary antibody against p53 and Ki-67, with the usual procedure of deparaffinization, antigen retrieval, endogenous peroxidase blocking and incubation with primary and secondary antibody. The visualization was carried out with a chromogenic detection system and hematoxylin was used for counterstaining the slides. The stained areas were examined by light microscopy and the immunoreactivity was assessed on the basis of the percentage of tumor cells that stained positively for p53, and for Ki-67 by counting the percentage of nuclei stained positively. The collected and analyzed data included the available clinical parameters and histological grade. Associations between biomarker expression and clinicopathological variables were evaluated by appropriate statistical tests and a p value < 0.05 was considered statistically significant.

RESULTS

The study included a total of 80 cases of oral squamous cell carcinoma (SCC) confirmed by histopathology. Most patients were male, and most lesions were found on the buccal mucosa and tongue. Histological grading showed that OSCCs were moderately differentiated, followed by well-differentiated and poorly differentiated.

Table 1: Histological Grade Distribution

Grade	Frequency	Percentage
Well differentiated	28	35%
Moderately differentiated	36	45%
Poorly differentiated	16	20%

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The moderately differentiated OSCC (45%) were the most numerous, with most cases having a mid-spectrum biological behavior.
The expression patterns of p53 and Ki-67 were variable across histological grades, as determined by immunohistochemical analysis. There was an increase in expressions with the progression of the tumor grade.

Table 2: p53 Expression

Grade	Low	High
Well differentiated	18 (64%)	10 (36%)
Moderately differentiated	12 (33%)	24 (67%)
Poorly differentiated	3 (19%)	13 (81%)

p53 expression was significantly higher in poorly differentiated tumors, suggesting greater genetic instability in aggressive OSCC cases.

The Ki-67 labeling index showed a similar increase with increasing tumor grade, indicative of increased proliferation.

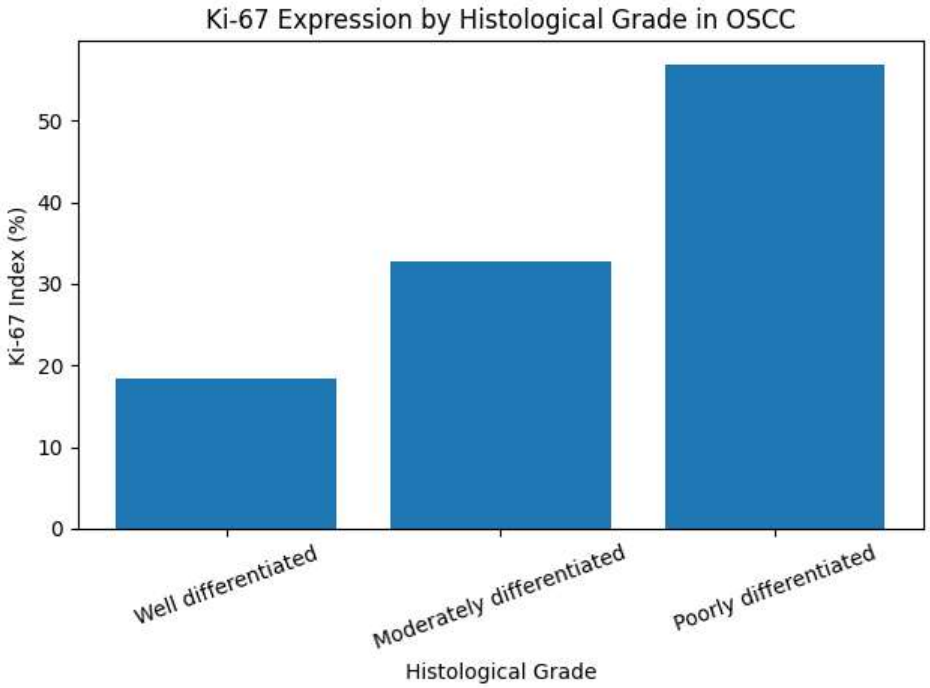
Table 3: Ki-67 Expression

Grade	**Ki-67 Index (%)**
Well differentiated	18.4
Moderately differentiated	32.7
Poorly differentiated	56.9

There was a statistically significant increase in Ki-67 expression with increasing histological grade ($p < 0.05$), reflecting a strong association with tumor aggressiveness.

Correlation analysis showed a positive correlation between p53 and Ki-67 expression, indicating that tumors with high genetic instability also exhibit high proliferation activity.

Figure 1: Ki-67 Expression Graph



The clinical outcome analysis revealed that patients with high p53 and Ki-67 expression had more advanced-stage disease, lymph node metastasis, and recurrence at follow-up. Tumors with poor differentiation had the poorest prognosis, and those with good differentiation had a relatively good prognosis. Overall, both biomarkers showed significant correlation with histological grade and clinical

aggressiveness, which supports their use as potential prognostic markers in OSCC.

DISCUSSION

In the present study, the immunohistochemical expression of p53 and Ki-67 was analysed in OSCC and its correlation with histological grade and clinical behaviour was studied. There was a high correlation between the expression of both markers and the histological grade, with higher expression of p53 and Ki-67 as the grade of the tumour increased. The results are in agreement with the previous studies that showed that IHC markers could be useful to understand the biology of the tumor and to stratify the prognosis of OSCC (1). The findings support the hypothesis that molecular changes are also significant in tumor progression, as well as the traditional histopathological assessment. Alteration of key pathways involved in regulating cellular proliferation and apoptosis is involved in the molecular mechanisms of OSCC. In this study, increased expression of both markers is correlated with increased genetic instability and proliferation dysregulation with increasing tumor grade.

This is consistent with the earlier evidence that highlighted the deregulation of the proliferative signaling and the changes in the tumor suppressor genes as key components of oral carcinogenesis (2). These pathways can be linked together to provide a comprehensive understanding of tumor behavior and progression. The present analysis revealed that expression of both p53 and Ki-67 was more intense in more aggressive histological grade, thus confirming their association with tumor invasion and biological activity. This is in line with earlier reports of increased expression at the invasive tumour front where tumour cells are more proliferative and invasive (3). The invasive front is a part of the tumour that is known to be the most biologically active and the expression of markers in this region may be directly linked to the aggressiveness of the disease. This study confirmed the role of Ki-67 as an indicator of cell growth activity as it was found to be significantly higher in the grade of the tumour.

Previous studies have been conducted systematically, and they have demonstrated that the expression of Ki-67 gradually increases from potentially malignant disorders towards invasive carcinoma, which proves the diagnostic and grading value of Ki-67 (4). This progressive increase demonstrates its importance in the identification of biologically aggressive lesions that should be more closely monitored in the clinic. Such a correlation between Ki-67 expression and histopathological severity has also been observed in earlier studies, where higher expression was observed in advanced tumors and poorly differentiated tumors (5). The present results confirm this correlation, indicating that Ki-67 could be a valuable marker to detect tumor progression and could be useful for clinical staging and prognosis. Moreover, comparative study of OED and OSCC has revealed a gradual increase in Ki-67 expression in malignant transformation (6). The present study is in agreement with this continuum model, and the higher the expression of Ki-67 was, the poorer the differentiation was, which is linked to the higher proliferative potential of the lesions as they become more malignant.

Although histological grading remains a basic method in the assessment of OSCC, it has interobserver variability and is not always a reflection of tumor biology. The findings of this study are consistent with previous studies that have shown that using immunohistochemical markers in addition to histological grading enhance diagnostic accuracy and prognosis (7). This approach could give a more objective assessment of tumor aggressiveness. The present study also demonstrated a significant difference in Ki-67 expression as a function of the histological grade, as previous studies have demonstrated a correlation between Ki-67 expression and tumour differentiation (8). The results underscore the importance of cell proliferation as a key player in tumor behavior and clinical outcomes. In addition to molecular factors, viral oncogenesis is also associated with oral cancer. HPV has been suggested to impact the clinicopathological characteristics of OSCC through its influence on cellular pathways (9).

While the status of the virus was not assessed in the present study, these results indicate that oral carcinogenesis is a complex process. This association between Ki-67 expression and histological grade has been confirmed by other studies, which show significant differences across levels of tumor differentiation (10). The current results confirm this trend and suggest that Ki-67 is a marker of tumor aggressiveness. In addition, progressive up-regulation of Ki-67 expression from benign to carcinoma has been reported previously, which corroborates its involvement in the multistep carcinogenesis model (11). This concept is supported by the present study, which demonstrates that a

higher proliferative activity is associated with more advanced disease. In addition to markers of proliferation, immune regulatory pathways have also been shown to play a role in tumor progression. Immune checkpoint molecules have been studied and identified to be involved in tumor immune evasion and progression in oral malignancies (12). The results indicate that genetic changes not only play a role in the development of OSCC but also interact with the immune microenvironment.

Recent literature has stressed the need for combining several biomarkers for better prognosis evaluation, and the importance of the use of histological and serological markers for personalized cancer management has been emphasized (13). The present study corroborates this view by showing that the combination of p53 and Ki-67 is meaningful in prognosis. p53 is important in maintaining genomic stability, and its alteration is associated with uncontrolled cellular proliferation. A previous study showed that p53 expression is abnormal and linked to tumor progression and biological aggressiveness (14). This is in line with the present findings, which show that low p53 expression is associated with well-differentiated tumors.

The combined analysis of p53 and Ki-67 has been shown to enhance the diagnostic and prognostic value of squamous cell carcinomas (SCC) (15). In the present study, this notion was confirmed, as co-expression of both markers was highly correlated with tumor grade and clinical aggressiveness. Histological examination of OSCC has shown that there is a marked difference in the expression of these biomarkers depending on the grade of differentiation, which has led to their use for tumor classification and prognosis (16). The present results confirm this association, suggesting that immunohistochemical analysis can complement the histopathological assessment. Lastly, immunohistochemical markers have been highlighted as playing a key role in early detection and prognosis of OSCC (17). In the present study, we found that both p53 and Ki-67 were highly correlated with histological grade and clinical behavior, suggesting their potential as routine prognostic biomarkers in OSCC.

CONCLUSION

The present study showed that immunohistochemical expression of p53 and Ki-67 was significantly increased with increasing histological grade of OSCC. The expression levels of both markers were generally higher in poorly differentiated tumors, suggesting that they are correlated with increased cellular proliferation and genetic instability, and aggressive tumor behavior. The positive association between p53 and Ki-67 indicates that changes in cell cycle regulation and proliferation are important to the development of OSCC. Moreover, higher levels of these biomarkers were correlated with poor clinical parameters, further confirming their prognostic value. The results underscore the importance of integrating immunohistochemical evaluation of p53 and Ki-67 into routine histopathological evaluation for more precise risk stratification and prognosis. The analysis of these markers can help clinical care to detect high-risk patients and tailor treatment more effectively. They should be supported by further large-scale prospective studies to confirm their clinical usefulness and predictive value.

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