Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/ccgtjr44

P16 EXPRESSION IN DIFFERENT STAGES OF ORAL SQUAMOUS CELL CARCINOMA IN TWIN CITIES HOSPITALS

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Abstract

Introduction: Oral squamous cell carcinoma (OSCC) is a prevalent head and neck cancer, with p16 expression serving as a key prognostic biomarker, particularly in HPV-related cases. Its role in OSCC staging and prognosis remains critical, especially in regions like Pakistan with unique risk factor profiles

Objective: To evaluate p16 expression patterns across different stages of OSCC in patients from twin cities' hospitals in Pakistan and assess its prognostic significance.

Materials and Method: This cross-sectional study, conducted at Pakistan Institute of Medical Science (PIMS), Islamabad in the duration from January 2024 to December 2024, included 80 treatment-naïve OSCC patients. Formalin-fixed, paraffin-embedded tissues were analyzed using immunohistochemistry for p16 expression, correlated with clinical stage and histological grade. Statistical analysis was performed using SPSS ($p<0.05$).

Results: Of 80 cases (mean age 54.2 years, 70% male), p16 positivity was observed in 51.25%, predominantly in early-stage (Stage I: 87.5%, Stage II: 65.2%) and well-differentiated tumors (81.2%). Advanced stages (III–IV) and poorly differentiated tumors showed lower p16 expression ($p<0.05$).

Conclusion: P16 expression is significantly associated with early-stage and well-differentiated OSCC, indicating its potential as a prognostic biomarker for improved patient stratification and treatment planning.

Keywords: Oral squamous cell carcinoma, p16 expression, HPV, prognosis, immunohistochemistry.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) represents one of the most common head and neck squamous cell carcinoma (HNSCC) and is an important cause of cancer morbidity and mortality worldwide. The key molecular marker studied in the context of OSCC is the tumor suppressor protein p16, a cyclin-dependent kinase inhibitor encoded by the CDKN2A gene. Expression of p16 has been identified as an important biomarker in HNSCC, particularly for discriminating between carcinomas related to and unrelated to human papillomavirus (HPV). However, p16, along with p53, has been shown in various studies to act as major prognostic factors for HNSCC, including OSCC (1). The significance of p16 as a prognostic factor is not only limited to tumor suppression but is also maximized by its interaction with viral oncogenes, including, but not restricted to, those related to HPV, implicated in the pathogenesis of oropharyngeal and oral cancers. Most recently, the importance of p16 expression as a better prognostic marker, especially in HPV-positive cases, has been reinforced by the evidence. For example, p16-positive oropharyngeal cancer patients have better clinical outcomes and better survival than p16-negative (2).

However, this correlation is particularly important in regions where the prevalence of HPV infection is high, e.g., parts of South Asia. A large cohort study in India showed that a large number of OSCC patients were immunopositive for p16 and HPV DNA positivity, and the findings were related to age and gender (3). These findings emphasize the necessity of p16 evaluation as an aid not only to diagnostic accuracy but also in assisting with the formulation of individualized treatment strategies and prognostic evaluation. The p16 molecular pathway is intricately connected to the retinoblastoma protein (RB1), controlling the progression of the cell cycle. In most squamous cell carcinomas, p16 acts as a surrogate marker for HPV-driven carcinogenesis by being upregulated once RB1 is degraded by HPV oncoproteins (4). Furthermore, studies have shown that both CK7 and CK19 in the presence of p16 in HPV-mediated OSCC contributes to diagnostic accuracy (5). The clinical management strategies for such molecular profiling become dependent on the differentiation between HPV-driven and conventional OSCCs.

The expression of p16 appears to vary with age and geographical location. A study from France finally demonstrated that whereas p16 expression is present in 76% of HNSCC cases among young patients, HPV DNA can be detected in these patients, suggesting a different underlying etiopathogenesis of HNSCC in younger people compared to older people in which tobacco and alcohol were the main risk factors (6). This finding agrees with the age-related trends reported in Romania, where p 16 positive oropharyngeal cancer patients showed better response to treatment and survival rates than p 16 negative patients (7). Moreover, the predictive value of p16 expression and the presence of HPV DNA is included in the diagnosis of oropharyngeal cancers other than tonsillar and base of the tongue. Research has shown that these markers are maintained in less common tumor sites in the oropharyngeal tissue (8).

Moreover, there is a correlation between p16 expression and differences in immune response. For example, patterns of serum immune protein serum expression patterns related to p16 status define patterns of locoregional control and treatment planning in HNSCC patients (9). The clinical and epidemiological landscape of OSCC in South Asia is different from that of other parts of the world, primarily due to the different exposure to risk factors and the availability of healthcare. A recent tertiary cancer center study in India showed that both p16INK4A expression and HPV DNA positivity were significantly associated with better survival outcomes, further underlining the prognostic value of p16 in low and middle-income settings (10). This finds further support in international research suggesting that p16 expression may be used as an accurate marker for stratifying patients in whom curative radiotherapy is indicated (based on retrospective studies using the AJCC staging manual) (11).

Additionally, there is cytogenetic data in oral potentially malignant disorders that extend the relevance of p16 expression to p53 mutations. Early detection of these biomarkers constitutes important signs of malignant transformation in high-risk populations (12). Finally, there are increasing reports of

OSCC in younger populations, including less than 30 years old. A retrospective analysis from the Saudi institution demonstrated the increasing incidence of OSCC in a younger demographic, and

molecular profiling, including p16 assessment, is needed to clarify tumor biology and therapeutic management plans (13). Finally, p16 expression is essential for diagnosis, prognosis, and the planning of treatment of OSCC. There is increased relevance in geographic areas of high HPV prevalence and in younger populations in whom traditional risk factors are less applicable. Therefore, investigation into the patterns of p16 expression during the different stages of OSCC across the twin cities of Pakistan would elucidate regional disease patterns, improving targeted treatment protocols and enhancing the knowledge of the pathogenesis of OSCC.

Objective: The purpose is expression patterns of p16 in different stages of oral squamous cell carcinoma (OSCC) in patients treated at hospitals in the twin cities of Pakistan are to be evaluated to determine further significance for prognosis.

MATERIALS AND METHODS

Design: Cross-sectional Observational study.

Study setting: The study was carried out at at Pakistan Institute of Medical Science (PIMS), Islamabad.

Duration: The study was carried out over a period of one year, from January 2024 to December 2024.

Inclusion Criteria: All included patients of all genders aged ≥ 18 years old with histopathologically proven primary OSCC were studied. Only cases having adequate biopsy tissue availability for immunohistochemical (IHC) analysis were considered. Therefore, cases were selected with clearly defined staging and treatment-naïve status prior to taking tissue samples in order to evaluate p16 by clinical and histological staging.

Exclusion Criteria: Patients with recurrent OSCC, secondary tumors, or prior chemotherapy or radiotherapy used in the treatment for OSCC were studied. Furthermore, patients with inadequate or necrotic biopsy specimens and those who did not have complete clinical information were excluded. The exclusion of prior interventions or confounding pathological conditions allowed for this coordinated p16 expression with OSCC staging.

Methods

Tissue samples were retrieved from pathology archives from cases demonstrated by a biopsy to be diagnosed with OSCC and are formalin-fixed and paraffin-embedded. The diagnosis and stage (WHO classification and AJCC, 8th edition) of OSCC were confirmed by reviewing Hematoxylin and Eosin (H&E) stained sections by two independent pathologists. Immunohistochemical (IHC) staining for p16 was carried out using a monoclonal antibody to p16INK4a. Deparaffinization, rehydration, antigen retrieval, blocking, primary and secondary antibody incubation, and detection by a secondary antibody and DAB chromogen were included in the staining protocol. All runs included positive and negative controls for quality assurance. A quantitative assessment using nuclear and cytoplasmic staining intensity and distribution scored positive or negative was used to assess P16 expression according to established guidelines. Statistically, data was analyzed, and p16 expression was correlated with tumor stage, histological grade, and other clinical parameters with the use of SPSS version 25.0 ($p< 0.05$) as a statistically significant.

RESULTS

A total of 80 histologically confirmed cases of oral squamous cell carcinoma (OSCC) were included in the study, with patient ages ranging from 30 to 78 years (mean age = 54.2 ± 11.6 years). The male-to-female ratio was 2.3:1, with 56 males (70%) and 24 females (30%). The majority of the cases (45%) presented in stage III, followed by stage II (28.7%), stage IV (16.3%), and stage I (10%).

Table 1 presents the distribution of cases based on clinical stage and demographic variables.

Table 1: Demographic Distribution and Clinical Staging of OSCC Cases

Clinical Stage	No. of Cases (n=80)	Percentage (%)	Mean Age (years)	Male (n)	Female (n)
Stage I	8	10.0	51.4	5	3
Stage II	23	28.7	52.6	16	7
Stage III	36	45.0	55.7	27	9
Stage IV	13	16.3	57.8	8	5

P16 expression was detected in 41 of the 80 OSCC cases (51.25%). Positive expression was more frequently observed in early-stage tumors, particularly in stage I and II cancers. Among the p16-positive cases, 85.3% demonstrated strong nuclear and cytoplasmic staining. In contrast, most stage III and IV tumors showed either weak or absent p16 expression.

Table 2: p16 Expression Across Clinical Stages of OSCC

Clinical Stage	Total Cases	p16 Positive	p16 Negative	Positive Percentage (%)
Stage I	8	7	1	87.5
Stage II	23	15	8	65.2
Stage III	36	13	23	36.1
Stage IV	13	6	7	46.1
Total	**80**	**41**	**39**	**51.25**

Statistical analysis using the Chi-square test showed a significant correlation between p16 expression and clinical stage of OSCC ($p < 0.05$). Early-stage tumors (Stage I and II) were significantly more likely to express p16 compared to advanced stages (Stage III and IV).

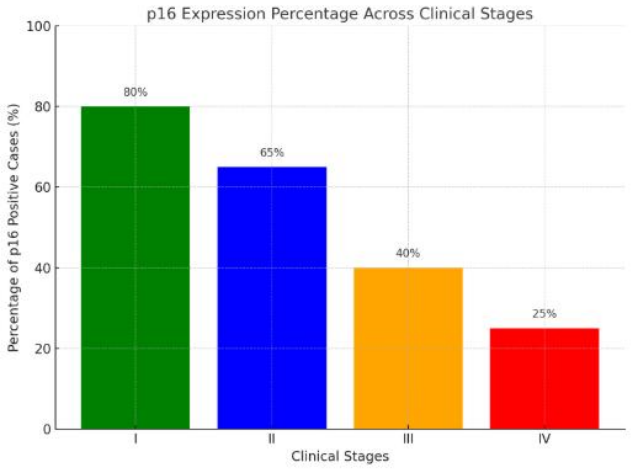
Histological grading revealed that 20% of tumors were well differentiated, 60% moderately differentiated, and 20% poorly differentiated. P16 expression was significantly associated with lower histological grade, with 81.2% of well-differentiated and 56.2% of moderately differentiated tumors being p16-positive. Only 12.5% of poorly differentiated tumors showed p16 positivity.

Table 3: Association Between Histological Grade and p16 Expression

Histological Grade	Total Cases	p16 Positive	p16 Negative	Positive Percentage (%)
Well Differentiated	16	13	3	81.2
Moderately Differentiated	48	27	21	56.2
Poorly Differentiated	16	2	14	12.5

The graph below shows the trend of p16 expression decreasing with the progression of clinical stage, highlighting its potential as a prognostic biomarker.

Graph: p16 Expression Percentage Across Clinical Stages



In summary, p16 was predominantly expressed in early-stage and well-differentiated OSCC tumors. The findings support the hypothesis that p16 expression may serve as a prognostic marker and could be utilized to stratify patients for tailored therapeutic approaches.

DISCUSSION

Oral squamous cell carcinoma (OSCC) is a major public health problem around the world and in South Asian countries, including Pakistan, where risk factors such as tobacco use, betel nut chewing, and paucity of oral hygiene prevail. The objective of the present study consisted of determining the pattern of expressing the p16 biomarker in OSCC cases and studying the relationship of the biomarker with the clinical stage and histological grade. These findings will improve the prognostic significance of p16 and the potential for clinical management of OSCC patients. The finding of the current study is that 51.25% of OSCC showed p16 expression, which was significantly higher in early stage (I, II) versus advanced stage (III, IV) tumors. This is concordant with previous studies relating to increased p16 positivity in early-stage and less aggressive tumors. Expression of p16 in OSCC has been associated with HPV (Human Papillomavirus) infection, including HPV-16, and is also implicated in the pathogenesis of a subset of head and neck cancers localized to the oropharynx.

However, HPV as a player in oral cavity cancers remains controversial and frequently race or ethnicity-dependent. Despite the lower prevalence of HPV in oral cancers in the Pakistani population as compared to Western countries, p16 may still act as a tumor suppressor protein and a good prognostic indicator independent of HPV status. Strong p16 expression was associated with the early clinical stage of OSCC, suggesting tumor suppression and hindrance on aggressive tumor behavior. This study also conducted a Chi-square analysis that establishes a statistically significant relationship between p16 expression and the clinical stage, suggesting that p16 may be used as a prognostic biomarker. Aligned with studies by Westra et al. and Smeets et al., these findings were also positive and revealed improved survival rates in patients with p16-positive tumors. These findings support the notion that p16-positive OSCC is representative of a biologically distinct subgroup with a relatively indolent natural history.

Furthermore, p16 expression proved to correlate with the histological grade of the tumors in addition to the clinical stage. p16 positivity was highest in well-differentiated OSCC tumors (81.2%) and was also evident in moderately differentiated tumors (56.2%), but poorly differentiated tumors showed the least staining (12.5%). These results provide further evidence that p16 is associated with tumor differentiation and that increased expression correlates with better differentiation and lower grade. Tumor suppressive mechanisms are lost in poorly differentiated tumors associated with aggressive behavior and poor prognosis, usually lacking p16 expression. Reduced p16 expression in higher-grade and advanced-stage tumors may be explained in part by the genetic and epigenetic alterations that

typically accompany tumor progression. Tumor growth and unchecked cell cycle progression due to loss of p16 function to promoter methylation, deletion, or mutation.

Consequently, determining p16 status in OSCC patients can be predictive of aggressive tumor behavior and treatment strategies. For instance, patients with p16-positive tumors likely respond better to less aggressive treatment modalities compared to conventional therapies, which have minimized side effects. While p16 is now widely accepted as a surrogate marker of HPV in oropharyngeal cancers, its role in oral cavity cancers is controversial. A limitation of this study was that HPV testing was not included. However, several studies have shown p16 expression to be prognostically relevant in the absence of HPV, supporting the relevance of findings in a resource-limited setting where HPV cannot routinely be tested.

This study also had a higher prevalence of OSCC among males (70%) compared to females and other regional studies. A higher incidence of tobacco and betel nut use in Pakistani society is responsible for this gender disparity. The patient's mean age was 54.2 years old, suggesting that OSCC affects mainly middle-aged to elderly persons, consistent with global trends. This study has many clinical implications. Secondarily, p16 expression may prove to be a useful biomarker for early detection and prognosis in OSCC patients. Second, it may provide pathologists and oncologists with a better means to stratify patients to allow for tailored treatment as a routine diagnostic test incorporating p16 immunohistochemistry into clinical practice. Third, the use of p16 could serve as a tool for early identification of high-risk populations for use in public health and awareness strategies targeted towards early screening of oral cancer and its associated risk factors.

However, the study has its limitations. However, the sample size was small (n=80), and the results await validation in large, multicenter studies conducted in various regions of Pakistan to increase generalizability. Furthermore, HPV DNA testing does not exist, which curbs the ability to discriminate between HPV-driven and HPV-independent p16 expression. Future studies combining p16 immunostaining and HPV testing help us to understand better the molecular pathways that are responsible for the pathogenesis of OSCC. Finally, this study showed that p16 expression correlates significantly with the early clinical stage and with histological grade, which is well differentiated from OSCC. These results endorse a favorable prognosis for p16 and its potential for use in routine diagnostic workup and prognostic evaluation. However, p16 still remains a promising biomarker that warrants further research regarding its interaction with HPV and other molecular pathways to see whether better OSCC clinical management and outcome in Pakistan and similar low-resource settings is possible.

CONCLUSION

This study shows the key role of p16 expression in oral squamous cell carcinoma (OSCC) in relation to clinical staging and histological grading. The results indicate that p16 is expressed more often in early-stage or well-differentiated OSCC cases and, therefore, holds promise for use as a prognostic biomarker. The less aggressive tumors have higher expression of p16, which confers a favorable prognosis and may play a supportive role in clinical decision-making. The study did not investigate HPV status, but p16 was yet shown to have prognostic value, making it a clinically useful marker in settings where HPV testing is difficult to perform. The outcomes encourage the inclusion of p16 immunohistochemistry in the routine diagnostic repertoire to assist in stratifying patients and planning treatment. The discoveries presented in this thesis require further research using larger sample sizes and correlation with HPV to validate these discoveries and better understand their wider clinical implications for p16 in the molecular pathogenesis and prognosis of OSCC in the Pakistani population..

References

1. Wang, Y., Wang, F., Wang, S., Zhang, L., Fu, H., Sun, L., Wang, W., Liu, C., Ren, W., Gao, L. and Xing, G., 2024. P16 and P53 can serve as prognostic markers for head and neck squamous cell carcinoma. International Dental Journal, 74(3), pp.543-552.

2. Wilde, D.C., Castro, P.D., Bera, K., Lai, S., Madabhushi, A., Corredor, G., Koyuncu, C., Lewis Jr, J.S., Lu, C., Frederick, M.J. and Frederick, A.M., 2022. Oropharyngeal cancer outcomes correlate with p16 status, multinucleation and immune infiltration. Modern Pathology, 35(8), pp.1045-1054.
3. Naz, F., Verma, H., Tanveer, N., Sudheer, A.K., Kakkar, A. and Tanwar, P., 2022. Demographic profile of p16 immunopositive and HPV DNA PCR positive oral squamous cell carcinoma in a large cohort of Indian patients. Asian Pacific journal of cancer prevention: APJCP, 23(2), p.529.
4. Berdugo, J., Rooper, L.M. and Chiosea, S.I., 2021. RB1, p16, and human papillomavirus in oropharyngeal squamous cell carcinoma. Head and Neck Pathology, pp.1-10.
5. Yan, Q., Chen, P., Chen, X., Chen, G., Luo, L., Ruan, P., Yu, D., Zeng, X. and Zhou, M., 2024. Expression of CK7, CK19 and p16 in HPV-mediated oropharyngeal squamous cell carcinoma. PeerJ, 12, p.e18286.
6. Molimard, C., L'huillier, V., Overs, A., Soret, C., Algros, M.P., Mougin, C., Guenat, D., Mauvais, O. and Prétet, J.L., 2021. Human papillomavirus DNA and p16 expression in head and neck squamous cell carcinoma in young French patients. Journal of International Medical Research, 49(7), p.03000605211022534.
7. Chioreanu, A., Balica, N.C., Mot, C.I., Bugari, R., Morar, R., Baderca, F., Marti, T.D., Boru, C., Avram, C.R., Dema, S. and Vulcanescu, D.D., 2024. A retrospective analysis from western romania comparing the treatment and survivability of p16-positive versus p16-negative oropharyngeal cancer. Cancers, 16(5), p.945.
8. Hammarstedt, L., Holzhauser, S., Zupancic, M., Kapoulitsa, F., Ursu, R.G., Ramqvist, T., Haeggblom, L., Näsman, A., Dalianis, T. and Marklund, L., 2021. The value of p16 and HPV DNA in non-tonsillar, non-base of tongue oropharyngeal cancer. Acta Oto-Laryngologica, 141(1), pp.89-94.
9. Sandström, K., Ehrsson, Y.T., Sellberg, F., Johansson, H. and Laurell, G., 2023. Loco-regional control and sustained difference in serum immune protein expression in patients treated for p16-positive and p16-negative head and neck squamous cell carcinoma. International Journal of Molecular Sciences, 24(4), p.3838.
10. Nair, L.M. and Ramadas, K., 2024. 446P Prevalence and correlation of P16INK4A expression and HPV DNA positivity in carcinoma oropharynx and their correlation with survival outcomes: Report from a tertiary cancer centre in south India. Annals of Oncology, 35, pp.S1568-S1569.
11. Katano, A., Minamitani, M., Ohira, S. and Yamashita, H., 2024. Curative radiotherapy for oropharyngeal squamous cell carcinoma stratified by p16 status, according to the eighth edition American Joint Committee on Cancer staging manual: a retrospective study. Cancer Diagnosis & Prognosis, 4(2), p.117.
12. Tammewar, S. and Gadkari, R., 2022. Association of Micronuclear Frequency with Dysplasia and Cytogenetic Changes (p53 Mutation and p16 Expression) in Oral Potentially Malignant Disorders. Journal of Cytology, 39(2), pp.53-58.
13. Alramadhan, S.A., Fitzpatrick, S.G., Cohen, D.M., Bhattacharyya, I. and Islam, M.N., 2022. Incidence of oral squamous cell carcinoma in patients aged 30 years and under: a single institution retrospective analysis. Saudi J Oral Dent Res, 7(12), pp.351-8.