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AN ASSESSMENT OF P16 EXPRESSION IN PATIENTS WITH VARYING GRADES OF ORAL SQUAMOUS CELL CARCINOMA: A SINGLE-CENTER EVALUATION

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ABSTRACT

Objective

To assess the expression of p16 in different grades of OSCC to elucidate its prevalence and prognostic significance in this carcinoma type.

Methodology

This prospective observational study investigated p16 expression in 69 cases of oral Squamous Cell Carcinoma spanning in multi centers including **Teaching Hospitals of Bolan Medical College / City International Hospital Quetta, Pakistan and Ayub Medical College Abbotabad, Pakistan in the duration from October, 2022 to December, 2023**. Formalin-fixed paraffin-embedded tissue blocks were utilized in the study for Hematoxylin and Eosin (H&E) staining and immunohistochemical (IHC) analysis, cases having inadequate formalin fixation and those receiving chemotherapy were excluded. Staining intensities and the proportion of stained cells were used to grade p16 expression; values ≥4 indicated positive. The indirect IHC method involved de-paraffinization, antigen retrieval, peroxidase blocking, and antibody incubation. Data analysis was done with SPSS (version:20.0) and involved Chi-square tests for categories related variables, with significance set at a p-value of <0.05.

Results

The study analyzed 69 patients with Oral Squamous Cell Carcinoma (OSCC), predominantly male (66.7%), with an age range of 26 to 85 years, most commonly 61-70 years (43.5%). The tongue was the most frequent OSCC site (30.4%), followed by the buccal mucosa (26.1%). Well-differentiated OSCC was most prevalent (63.8%), followed by moderately (21.7%) and poorly differentiated cases (14.5%). p16 expression was positive in 72.5% of cases and negative in 27.5%. Among smokers, 72.5% were p16 positive; among snuff users, 73.7% were p16 positive; and among chewable tobacco users, 72.0% were p16 positive.

Conclusion
The tongue was the most frequently observed site for OSCC, with a predominance of well-differentiated lesions. Notably, there was a significant association between tobacco consumption (smoking & snuff usage) and p16 expression, even though there was no discernible relationship within p16 expression and OSCC grades. These findings emphasize the need to consider regional and demographic variations in OSCC research.

Keywords: Oral squamous cell carcinoma, smoking, p16.

Introduction

Worldwide, oral squamous cell carcinoma (OSCC) is a significant cause of cancerous lesions, with notable prevalence in regions such as South Asia, Melanesia, and Central and Eastern Europe. According to GLOBOCAN 2020 estimates, Head and Neck Squamous Cell Carcinoma (HNSCC), which includes OSCC, ranks as the seventh most frequent carcinoma globally. It is responsible for 4.6% of all carcinoma-related fatalities, accounting for approximately 450,000 deaths annually. Each year, about 890,000 new cases of HNSCC are diagnosed, representing 4.5% of all global carcinoma cases. In South Asia, countries such as India, Pakistan, and Bangladesh experience significant health burdens due to OSCC. For instance, in Karachi, OSCC constitutes 8.8% of all carcinoma cases.

Men are affected by OSCC at a ratio of 2:1 more often than women, and the condition is usually identified in people between the ages of 60 and 80.[4] The tongue is the most prevalent site worldwide, but the buccal mucous membrane is particularly vulnerable in South Asia. The carcinoma can also appear in other regions of the mouth, such as the palate, the floor of the oral cavity, lip, gingiva, and buccal mucosa.[5] Established risk factors for OSCC include nicotine use, chewing of pan, prolonged sun exposure, smokeless tobacco (snuff), and Human Papillomavirus (HPV).[6]

A crucial factor in understanding the pathophysiology of OSCC is the tumor suppressor p16INK4A (p16), a member of the INK4 class of cell cycle inhibitors. p16 expression is vital for retaining Rb-family proteins in a hypo-phosphorylated state, which facilitates the binding of E2F and results in G1 cell-cycle arrest.[7] Hyper methylation of the p16 promoter is associated with gene silencing and is more common in OSCC than genetic alterations. Studies have shown that p16 hyper methylation frequently occurs in pre-cancerous oral lesions, and lesions with a hyper methylated p16 promoter are prone to transform into oral cancers.[8-9]

The expression of p16 serves as a marker for oral mucosal dysplasia and malignant transformation. Both increased and decreased expressions of p16 have been reported in oral premalignant and malignant lesions.[10] Notably, p16 is consistently found in areas of microinvasion and at the superficial margins of OSCC, whereas its expression is heterogeneous in moderate to severe epithelial dysplasia and carcinoma in situ (CIS).[11]

The aim of this study was to recognize the expression of p16 in various grades of oral squamous cell carcinoma and establish its association with variables like age, gender, tobacco usage and its histopathological grades.

Objective

To assess the expression of p16 in different grades of OSCC to elucidate its prevalence and prognostic significance in this carcinoma type.

Methodology

Study Design:

This study uses a prospective observational methodology.

Duration and Setting:

This study was conducted at Teaching Hospitals of Bolan Medical College / City International Hospital Quetta, Pakistan and Ayub Medical College Abbotabad, Pakistan in the duration from October, 2022 to December, 2023.

A total of 69 previously diagnosed cases of OSCC are included in the study. Those patients receiving chemotherapy and those with inadequate formalin fixation, which could impair antigen retrieval, weren't included. Convenient sampling techniques are employed to select eligible cases meeting the inclusion criteria.

Ethical Considerations:

Prior to starting the clinical study, ethical approval was acquired from the hospital's Institutional Review Board (IRB). Before registration, everyone who participated or their legal representatives provided informed consent.

Immunohistochemical Staining:

From the hospital's pathology archive, tissue blocks embedded in paraffin and treated with formalin were taken from patients with OSCC that had already been diagnosed. These blocks were sectioned into 4-5μm thin slices for immunohistochemistry (IHC) and staining with hematoxylin and eosin (H&E).

Interpretation of p16 Expression:

A scale of 0 to 3 was used to score the degree of p16 staining, with mild, moderate, or intense expression indicated, accordingly. Furthermore, the proportion of cells that were stained was evaluated using a 4-point scale that extended from 81–100% of stained cells to no staining. Both the intensity and percentile scores were multiplied to arrive at the overall p16 expression score, which ranged from 0 to 12. Positive p16 expression is indicated by a score of 4 or higher.

Immunohistochemistry Protocol:

The technique of indirect immunohistochemistry was utilized. After deparaffinizing the tissue slices, heat treatment was used to extract antigens employing a citrate buffer solution. After that, slides were treated with p16 antibody and a peroxidase blocking solution. Slides were incubated, then color developed using a peroxidase substrate solution, dehydrated, and counterstained with hematoxylin.

Data Analysis:

Software for statistics was used to analyze the data (SPSS version 20.0). Gender and p16 expression are examples of categorical variables for which frequency and percentages were computed. Categorical data were analyzed using chi-square tests, with a threshold for significance of $P < 0.05$.

Results

Among the 69 patients, the majority were males 46 (66.7%), with females accounting for 23 cases (33.3%). The patients' ages ranged from 25 to 85 years, with 61–70 years being their most common age group (30 cases, 43.5%). Regarding the primary sites of OSCC, the tongue was the most prevalent location, identified in 21 cases (30.4%), followed by the buccal mucosa with 18 cases (26.1%), and other sites (floor of mouth, gums, etc.) making up 30 cases (43.5%). Based on histopathological grading, 44 cases (63.8%) with well-differentiated OSCC was found to be the most prevalent type.
Moderately differentiated OSCC accounted for 15 cases (21.7%), and poorly differentiated OSCC for 10 cases (14.5%). Table-1 illustrate these findings.

Table-1: Demographic, Clinical Characteristics, and Lesion Grading of Patients with OSCC Based on p16 Expression Status

Variable		Positive p16 (N=50)	Negative p16 (N=19)	Total (N=69)	p-value
Gender	Male	34 (49.28%)	12 (17.39%)	46 (66.7%)	0.701
	Female	16 (23.19%)	7 (10.14%)	23 (33.3%)	
Age Group (years)	26-40	8 (11.59%)	5 (7.25%)	13 (18.8%)	0.099
	41-60	17 (24.64%)	7 (10.14%)	24 (34.8%)	
	61-70	16 (23.19%)	14 (20.29%)	30 (43.5%)	
	71-85	9 (13.04%)	3 (4.35%)	12 (17.4%)	
Site of Lesion	Tongue	17 (24.64%)	4 (5.80%)	21 (30.4%)	0.23
	Buccal mucosa	13 (18.84%)	5 (7.25%)	18 (26.1%)	
	Other sites	20 (28.99%)	10 (14.49%)	30 (43.5%)	
Histopathological Differentiation Grade	Well	32 (46.38%)	12 (17.39%)	44 (63.8%)	0.34
	Moderate	11 (15.94%)	4 (5.80%)	15 (21.7%)	
	Poor	7 (10.14%)	3 (4.35%)	10 (14.5%)	

P16 expression was evaluated among the 69 OSCC cases, particularly focusing on the correlation with smoking, snuff use, and chewable tobacco use (Table-2). The findings indicated that positive p16 expression was detected in 50 cases (72.5%), while 19 cases (27.5%) exhibited negative p16 expression. Among the smokers (40 cases, 58.0%), positive p16 expression was noted in 29 cases (72.5%) and negative p16 expression in 11 cases (27.5%). Among the snuff users (38 cases, 55.1%), positive p16 expression was detected in 28 cases (73.7%) and negative p16 expression in 10 cases (26.3%). In contrast, among non-snuff users (31 cases, 44.9%), positive p16 expression was found in 22 cases (71.0%) and negative p16 expression in 9 cases (29.0%). For chewable tobacco users (25 cases, 36.2%), positive p16 expression was seen in 18 cases (72.0%) and negative p16 expression in 7 cases (28.0%).

Table-2: Correlation of tobacco use and P16 expression

Tobacco Form		Positive p16	Negative p16	Total
Smoking	Smokers	29 (42.03%)	11 (15.94%)	40 (57.97%)
	Non-smokers	21 (30.43%)	8 (11.59%)	29 (42.03%)
Snuff	Users	28 (40.58%)	10 (14.49%)	38 (55.07%)
	Non-users	22 (31.88%)	9 (13.04%)	31 (44.93%)
Chewable Tobacco	Users	18 (26.09%)	7 (10.14%)	25 (36.23%)
	Non-users	32 (46.38%)	12 (17.39%)	44 (63.77%)
Overall		50	50 (72.46%)	19 (27.54%)

Discussion

Our comprehensive study on Oral Squamous Cell Carcinoma (OSCC) provides a multifaceted exploration into the complex background of this carcinoma, covering tumor site distribution, histopathological differentiation, and p16 expression. Through meticulous analysis and comparison with existing literature, our findings enrich the understanding of OSCC epidemiology, biology, and clinical implications, paving the way for more effective management strategies.

Firstly, our investigation reveals that out of 69 patients diagnosed with OSCC, 46 (66.7%) were males, while 23 (33.3%) were females. Notably, the tongue emerged as the primary site for OSCC development, with 21 cases (30.4%) identified in this location. However, remarkable disparities emerge when juxtaposed with researches by Ehtesham et al., and Anwar et al., which highlight the buccal mucosa or other sites as predominant locations.[12-13] These discrepancies may reflect regional variations, genetic predispositions, or differing exposure profiles to environmental carcinogens, underscoring the complex interplay of factors shaping OSCC tumorigenesis.

Furthermore, our study illuminates the histopathological panorama of OSCC, revealing a preponderance of well-differentiated cases, with 44 cases (63.8%) falling into this category. This distribution is consistent with observations by Sarfaraz et al., suggesting a potentially less aggressive disease phenotype and more favorable clinical outcomes among these patients.[14] Yet, contrasting distributions across differentiation grades reported by Mehdi et al. and others emphasize the need for standardized grading criteria and highlight the heterogeneous nature of OSCC biology.[15]

In our exploration of molecular markers, our study elucidates an interesting association between p16 expression and tobacco usage. Among the 69 OSCC cases, positive p16 expression was noted in 29 smokers (42.03%) and 28 snuff users (40.58%). This observation underlines the potential influence of lifestyle factors on OSCC pathogenesis and draw attention to the need for comprehensive risk assessment strategies integrating genetic, environmental, and behavioral variables. However, the lack of a significant correlation between p16 expression and OSCC grades challenges prevailing paradigms and features the complexity of molecular pathways driving tumor progression.

Moreover, our analysis of p16-positive OSCC cases from our locality offers unique insights into regional variations in disease biology. The high prevalence of p16 positivity compared to Western populations suggests potential ethnic or geographic disparities in OSCC etiology and accentuates the importance of contextualizing research findings within diverse population settings.

Overall, our study represents a significant contribution to the nascent field of OSCC research, providing an understanding of disease epidemiology, histopathology, and molecular foundations. By answering the obscure complexities of OSCC, our findings lay the basis for more targeted and personalized approaches to disease management, ultimately advancing our quest for improved patient outcomes and enhanced strategies for combating this formidable carcinoma.

Conclusion

Our research provides important new understandings of the genetic markers, histological features, and epidemiology of oral squamous cell carcinoma (OSCC). With a preponderance of well-differentiated lesions, we discovered that the tongue was the most often diagnosed site for OSCC. Notably, there was no significant link found between p16 expression and OSCC grades, however a significant relationship between tobacco consumption (smoking and snuff use) and p16 expression was identified. These results highlight how important it is for OSCC research to take demographic and regional differences into account. All things considered, our findings advance knowledge about OSCC and highlight the significance of individualized treatment plans for bettering patient outcomes.

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