

Expression of P16 in Various Grades of Oral Squamous Cell Carcinoma of Tertiary Care Hospitals of Peshawar

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ABSTRACT

Introduction: Oral squamous carcinoma (OSCC) is one of the ten most common carcinomas in the world. The survival rate of OSSC is poor due to its diagnosis at advanced stages. The main function of p16 in the body is to halt the tumor process, but genetic mutation may result in the development of OSCC.

Objectives: The aim and objective was to compare the immunohistochemical expression of p16 in various grades of oral squamous cell carcinoma.

Materials and Methods: This study was a retrospective comparative cross-sectional (Analytical) study. It was conducted at the Department of Pathology, Peshawar Medical College from August 2020 to August 2021. The sample size was calculated through G Power and 103 patients were included in the study. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 20.0. Chi-Square test was performed for categorical variables.

Results: It was observed in our study that the mean age of OSCC was 59.16 ± 13 SD. The commonest effected age was 61-70 years (29%). The male to female ratio was approximately 1.2:1. The most common site involved by OSCC was buccal mucosa (n=49, 47%) followed by the tongue (n = 29, 29%).

The p16 expression was positive in most OSCC were well differentiated (n=50, 92.5%), followed by moderately differentiated cases (n=26, 92.8%) and poorly differentiated (n=81, cases, 85.7%). There was no significant difference for p16 expression and grades of OSCC cases (P- value =0.601).

Conclusion: The expression of p16 was more common in Well-differentiated oral squamous cell carcinoma, followed by moderately and poorly differentiated OSCC.

Keywords: Oral Squamous cell carcinoma, P16, Human Papillomavirus

INTRODUCTION

Oral squamous carcinoma (OSCC) is one of the ten most common carcinomas in the world. The survival rate of OSSC is poor due to its diagnosis at advanced stages (1). Worldwide, 58% of head and neck malignancies occur in South East and South Asia. A study was conducted in Karachi from 1995 – 2002 which showed that OSCC constitutes 8.8% of all cases of carcinomas (2). Globally, OSSC occur most frequently in males than in females i-e 2:1. Initially, OSCC was thought to be more prevalent at the age of 60 to 80 years. Recently, the incidence of OSCC is getting more common in younger individuals (3)

OSCC can involve any part of the oral cavity which includes buccal mucosa, tongue gingiva, palate, floor of the mouth and even lip. Worldwide, tongue is the most common anatomical location while in South Asia it is the buccal mucosa (4).

Tobacco, alcohol, betel quid chewing and prolonged sun exposure are some of the most commonly reported etiological factors of OSCC. Besides these risk factors, smokeless tobacco (snuff) and Human Papilloma Virus (HPV) increases the risk of OSCC (5).

HPV is another emerging risk factor. HPV belongs to papillomaviridae family. Among high-risk types, HPV16 and 18 play a major role in the pathogenesis of OSCC. Patients with HPV-associated OSCC have a 90% better prognosis than those without HPV associated OSCC(6).

p16 is a protein which suppresses tumor and is involved in many malignant processes of the body. The main function of p16 in the body is to halt the tumor process, but genetic mutation may result in the development of OSCC. p16 could be useful as a biomarker to predict HPV high-risk cancers (7).

P16 is expressed at low levels in normal cells which is almost undetected by IHC. Because of the transformational activity of the E7 gene, p16 is highly expressed in HPV-infected tumor cells and can be easily detected. As a result, p16 expression is closely linked to HPV positivity (6).

This study is designed to evaluate the immunohistochemical expression of P16 in various grades of OSCC in our population.

MATERIAL AND METHODS

This descriptive cross-sectional study was conducted at the department of Pathology, Peshawar Medical and Dental College, Peshawar. Sample size was measured by using G power software version (3.1.9.7), with an effective size of 0.5, alpha 0.05, power 80% and degree of freedom 2%. Sampling was done with non-probability convenient sampling technique. The research was conducted following the approval by the Institutional Review Board (IRB), Peshawar and IRB approval number is Prime/IRB/2020/235. Already diagnosed 103 cases of OSCC were included. Cases with poor formalin fixation where antigen retrieval issues may arise were excluded. Blocks of the patients receiving chemotherapies were also excluded. Cases with previous records were collected at Department of Oral Pathology, Peshawar Medical College (PMC), Department of Oral and Maxillofacial Surgery, Peshawar Dental College and Hospital (PDC), Khyber College of Dentistry, Peshawar and stained for immunohistochemistry at "Department of Pathology, Peshawar Medical College, Riphah International University" Laboratory procedures were done in Department of Pathology, Peshawar Medical and Dental College, Peshawar. Already Diagnosed formalin fixed, paraffin embedded OSCC tissue blocks were taken and sections were cut for both H&E staining and IHC procedure. Four to five microns (μ m) thin paraffin embedded OSCC tissue sections were stained with Heamatoxylin and Eosin (H&E) by using standard protocols. Microscopic examination of H&E slides was done for the confirmation of clinical diagnosis of OSCC and selection of IHC staining using p16 antibody. P16 expression was done as Interpretation of staining:

Histopathological grading of OSCC was performed in accordance with World Health Organization (WHO) guidelines: Grade i: (well-differentiated) Grade ii: (moderately differentiated) Grade iii: (poorly differentiated)

The intensity of P16 was scored follow as:

Weak =1, Moderate = 2, strong = 3

The percentage of stained cells was scored as follows: No staining = 0, 0-10% cells stained = 1, 11-50% cells stained = 2, 51 - 80% cells stained = 3 and 81 - 100% cells stained = 4. And the

P16 final scores: Final score of "0-12" was obtained by multiplying the intensity and percentage of cells with 2 variables. A score of 4 or higher was considered positive.

Immunohistochemistry (IHC): Indirect immuno-histochemistry technique was adopted. Formalin-fixed and paraffin embedded tissues were deparaffinized. Antigen retrieval was accomplished by soaking the samples in citrate buffer solution and then heating them in a microwave oven at 95-100 degrees Celsius for 20 minutes. The slides were allowed to cool at ambient temperature, for 15 to 20 minutes. Phosphate buffer saline (PBS) and distilled water were used to wash the slides. Peroxidase blocking solution (PBS) was applied to the sections of the slides and incubated at room temperature for 10 minutes. Slides have been rinsed in PBS for 6 mins. To expose the color of the antibody, chromogen substrate was added and slides were incubated in peroxidase substrate solution. Slides were cleaned again after allowing color to develop for less than 5 minutes. After that, slides were submerged in Haematoxylin counterstaining solution for 1-2 minutes. Slides were cleaned for another 15 minutes under running tap water. Alcohol was used to dehydrate tissue slides for 5 minutes. The slides were cleaned with three changes of xylene and a cover slip was applied with mounting solution before being kept at room temperature.

RESULTS

Data has been observed and analyzed using SPSS software (version 25). Descriptive analysis has been performed. Chi square test was used to analyze categorical data. The P-value less than or equal to 0.05 is considered as significant. The quantitative variables like age were measured as range, mean and standard deviation. The qualitative/ categorical variables like gender and p16 expression were measured as frequency and percentages and compared among three categories of OSCC.

In 103 cases of OSCC, average age of patients was 59 ± 13 years. Over all age range of patients was 26 - 85 years (min – max). Male to female ratio was 1.2:1. The most common site of development of OSCC was Buccal mucosa ($p=0.973$). Most OSCC were well differentiated ($n=54$, 53%), followed by moderately differentiated cases ($n=28$, 27%) and poorly differentiated ($n=21$ cases, 20%) (Table I).

The p value showed no significant difference for p16 expression and grades of OSCC cases (P- value =0.601) (Table II).

Figure-I shows the Hematoxylin and Eosin staining of well differentiated OSCC. Strong staining has been seen in well differentiated OSCC cases (Figure-II).

Table 1: Clinico-pathologic Parameters for Oral squamous Cell Carcinoma Cases

Parameters	(n)	Percentage (%)
Age Groups		
21-30 years	4	4%
31-40 years	3	3%
41-50 years	20	20%
51-60 years	25	24%
61-70 years	31	29%
71-80 years	11	11%
>80 years	9	9%
Mean Age	59 years \pm 13 SD	
Gender		
Male	56	54%
Female	47	46%
M: F	1.2:1	
Site of lesion		
Tongue	30	29%
Buccal mucosa	49	47%
Cheek	3	3%
Alveolar Ridge	16	15%
Lip	5	6%
Grades of OSCC		
Well differentiated	54	53%

Moderately differentiated	28	27%
Poorly differentiated	21	20%

Table 2: Frequency of cases in relation to scores of p16 Expression in OSCC:

p16 Expression	Positive	Negative	Total
	94 (91.3%)	9 (8.7%)	103 (100%)

Table 3: Relation of p16 expression in OSCC cases with Grades of OSCC:

Grades	n (%)	p16 Expression		p- value (0.601)
		Positive	Negative	
Well differentiated	54 (52.4%)	50 (92.5%)	4 (7.5%)	
Moderately differentiated	28 (27.2%)	26 (92.8%)	2 (7.2%)	
Poorly differentiated	21 (20.4%)	18 (85.7%)	3 (14.3%)	
Total	103 (100%)	94 (91.3%)	9 (8.7%)	

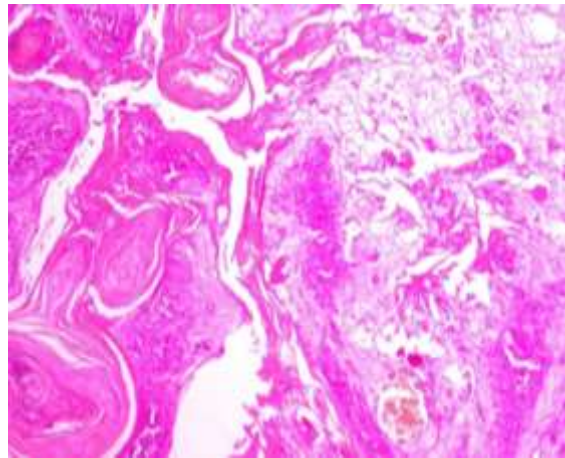


Figure 1: Well Differentiated OSCC (H&E Mag 10X)

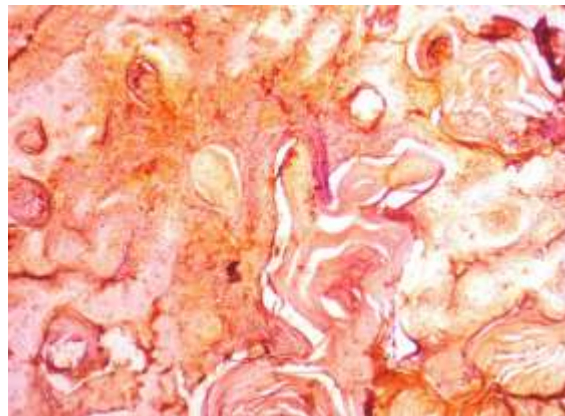


Figure-2: p16 Expression (IHC Mag 10x)

DISCUSSION

Demographics of Oral Squamous Cell Carcinoma: The mean age in our population was 59 years \pm 13 SD which is in accordance with a study conducted by Sahaf et al; in 2017 in Pakistan which showed the mean age 53.13 years in OSCC patients (8). Another study conducted by Sairat et al; 2019 from Pakistan showed the mean age of 55.0 years which is in accordance to our results (9). In contrast to our findings a study conducted by Singh et al in 2016 in India showed patient age ranging from 20-85 years with the mean age of 48.3 years (10). A study done in Japan by Hasegawa in 2017 also showed an age range between 40-86 years with the mean age of 63.7 years (11).

A study by Sahaf et al., 2017 from Pakistan showed a slight male predominance in OSCC (M: F 1.3:1) (12). A study conducted by Asif et al., 2020 in Pakistan also showed a slight male predominance (M: F 1.4:1) (13). In contrast to our study, an international study by Troeltzsch et al., 2017 from Germany reported equal number of cases (M: F 1:1) (14).

In our study males were slightly more affected by OSCC than females with a M: F ratio 1.2:1. In Accordance to our study Ferlay J et al; in 2015 also reported a male predominance with a M:F ratio 2:1 (Ferlay et al., 2015). This is because males are more likely to be at risk because they are more often exposed to the risk factors including snuff, gutka, areca nut, betel quid and smoking (15). Another study from Pakistan implicated the role of snuff as the cause of male predominance in OSCC (16).

In our study the commonest site of OSCC was buccal mucosa 49 (47%) cases in our study population. In accordance to our findings a study conducted in Pakistan claimed that OSCC was twice as common in the buccal mucosa (68.8%) (Anwar et al., 2020). Similarly, studies conducted by Mehdi et al in 2018 and Akram et al in 2019 showed buccal mucosa as the most frequently involved site in OSCC patients (16)(Akram, Shabbir, & Mirza, 2019). In contrast to our study, Zhang et al in 2014 showed tongue as a most frequent site for OSCC (17). Contrary to this Ehtesham et al., 2017 reported that OSCC mostly affects the border of tongue and floor of mouth in males, while buccal sulcus is the most commonly affected anatomical site in females (18).

In this research had found that the majority of the cases were well differentiated (52.4%), followed by Moderately Differentiated cases (27.2%) and then poorly differentiated cases (20.4%). In accordance with our study Sarfaraz et al., 2020 showed well differentiated to be the most common grade for OSCC cases (19). Contrary to our Results Mehdi et al., 2019 from Pakistan showed same number of moderately differentiated and poorly differentiated cases and slightly less number of well differentiated cases (20). Rehman et al., in 2019 from Pakistan also showed moderately differentiated grade as the most common grade for OSCC (21).

p16 expression and Oral Squamous Cell Carcinoma: In our study, majority of the cases showed maximum expression of p16 between the age group of 61-70 years. There was no statistically significant association found between age and p16 expression (p-value = 0.0695). In contrast to our results there was a statistically significant association found between age and expression of p16 (p = 0.001) (22).

In our study, P16 positivity among males was more than females, i.e 54.4% and 45.6% respectively. In accordance to our results, a study from Pakistan showed more p16 positivity in males than females (Ali et al., 2018). A study conducted by Belobrov et al in 2018 also showed that male (57%) had more p16 positivity than females (43%) (23). In contrast to our results Naz et al in 2022 found no significant association of p16 expression with gender of the patients (24).

In our study, buccal mucosa showed maximum cases with positive p16 expression. The p value was not statistically significant between site of the tumor and expression of p16 (p value = 0.973). In accordance to our results Naz et al, in 2022 found no significant association between site and expression of p16. In contrast to our results, a study conducted by Gupta et al found a statistically significant association between p16 with site of the tumor (22).

In our study most of the cases with p16 positivity were found in well differentiated OSCC cases (n=50, 92.5%) followed by moderately differentiated OSCC cases (n= 28, 90.9%) and poorly differentiated OSCC cases (n= 18, 85.7%). There was no statistically significant association between p16 expression and grades of OSCC (p-value = 0.601). In accordance to our results, a study conducted by Agarwal et al, in 2021 found no significant association between grades of OSCC and p16 expression (25). In contrast to our results, a study conducted by Naz et al in 2022 showed that the most of the OSCC cases with p16 positivity were

found in the moderately differentiated (79.5%) followed by well and poorly differentiated OSCC (24).

CONCLUSION

1 p16 expression in various grades of OSCC showed no significance association.

2 Although a stronger staining was found in well differentiated OSCC patients but it was not statistically significant.

Limitations: A comparatively small sample size.

Limited time available.

Financial constraints.

Recommendations for further work: Future studies with a larger sample size including follow up may be designed to substantiate our findings.

P16 can be used as a prognostic marker and can help in the management of OSCC patients who are negative for p16 expression by regular follow ups.

Conflict of Interest: Authors declare no conflict of interest.

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