Section: Pathology

Original Research Article

EVALUATION OF IMMUNOHISTOCHEMICAL EXPRESSION OF P16 PROTEIN IN THE ORO-PHARYNGEAL SQUAMOUS CELL CARCINOMA

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Abstract

Background: Oropharyngeal squamous cell carcinoma (OPSCC) is the sixth most common cancer in the world. Tobacco use, alcohol consumption & Human Papilloma Virus (HPV) infection are main causative factors of OPSCC. p16 positivity correlates strongly with HPV positivity and is associated with favorable prognosis. p16 expression is independent prognostic marker for OPSCC. The aim of study was to evaluate pattern of expression of p 16 in oropharyngeal squamous cell carcinoma. **Materials and Methods:** Total 65 cases of OPSCC were included in study over a period of one year. Histopathological & Immunohistochemical staining with p16 was done followed by microscopic examination. **Result:** Most common age group of presentation was 41-50 years of age. There was male preponderance. Gingiva and buccal mucosa were most common sites to get involved in 36.92% cases followed by tongue in 26.69% cases. History of Addiction was found in 35.38% cases. Histologically 72.30% cases were of well differentiated Squamous cell carcinoma. P16 positivity was found in 60% of cases. **Conclusion:** Squamous cell carcinoma was most common malignancy in oropharynx and well differentiated SCC being most common histologic grade. No significant association was found in p16 expression and histopatholgic grade. However p16 positivity depicts association with HPV. However in present study HPV was not detected due to resource limiting setting.

INTRODUCTION

Oropharyngeal squamous cell carcinoma (OPSCC) is the sixth most common cancer in the world.[1] Majority of the cases are seen in 5th and 6thdecade of life. It is more common in males as compared to female. Tobacco use, alcohol consumption & Human Papilloma Virus (HPV) infection are main causative factors of OPSCC.[2] Although tobacco and alcohol are the main etiologic factors in three-fourth of these cases, infection with high-risk Human Papilloma Virus (HPV) is now the primary cause ofsquamous cell carcinoma of the oropharynx.[3]

In Tobacco, Chemical carcinogen (like Benzopyrene), damage DNA by interacting with a particular DNA sequence or base. After exposure of a cell to a mutagen or an initiator, tumorigenesis can be enhanced, which stimulate proliferation of mutated cells leads mutation and eventually carcinoma.[4] High alcohol consumption leads to alteration of mucosal membrane which renders the mucosal membrane susceptible to Human Papilloma Virus (HPV) infection.[5] The combination of tobacco and alcohol use has been reported as increased risk of OPSCC.[6]

The protein p16 is a cellular protein involved in cell cycle regulation. In normal cells, p16 protein is expressed in very low levels and is almost undetectable by Immunohistochemistry (IHC). Due to the transforming activity of E7 oncogene, p16 is strongly expressed in tumor cells affected by HPV and may be easily detected by IHC. The oncogenic proteins E6 and E7 released by the high-risk virus subtype(HPV16andHPV18) interruptthe p53 and Retinoblastoma gene (Rb gene)tumour suppressing pathway respectively, which leads to increased cell proliferation andgenomic in stability leading to carcinogenesis. Thus reduced or lost Rb gene functionresult in enhanced p16 level, as a result of negative feedback control. Hence, p16 positively correlates strongly with HPV positivity and associated with favorable prognosis.[7] p16

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expression is independent prognostic marker for OPSCC.

This study aims to evaluate the pattern of IHC expression of p16 in OPSCC and the histomorphological grades of OPSCC.

Aim & Objectives:

Aim- To study the pattern of expression of p16 protein in Oro-pharyngeal squamous cell carcinoma.

Objectives:

Primary Objective- To study the pattern of expression of p16 protein in Oropharyngeal squamous cell carcinoma by Immunohistochemistry

Secondary Objectives-

- To study the various patterns and scoring of p16 expression in oro-pharyngeal squamous cell carcinoma.
- To evaluate, histopathological features of oro-pharyngeal squamous cell carcinoma.
- To correlate the association between p16 protein expression with histological grades of oro-pharyngeal squamous cell carcinoma.

MATERIALS AND METHODS

Study Design- The present study was observational cross sectional descriptive type.

Study Setting- The present study was conducted in Department of Pathology

Study Duration- The present study was conducted from September 2022 to May 2024.

Study Samples- All biopsies and histopathological specimen reported as OPSCC in all age groups and both the gender were included in the study.

Inclusion Criteria

Biopsies and histopathological specimen diagnosed as Oropharyngeal squamous cell carcinoma on histopathological evaluation.

Exclusion Criteria

Patients on chemotherapy and radiotherapy.

Inadequate/ poorly preserved specimen.

Sample Size - Sample size was 65 and was calculated by using Prevalence

$n = z21-\alpha/2P(1-p) / ME2$

Where $z1\text{-}\alpha/2$ = 1.64 at 90% Confidence Interval

P= Prevalance of HPV in Oro-pharyngeal malignancies i.e 0.60q=(1-p)= 1-0.60 = 0.40(8)

M.E.= Margin of error = 0.10 (10%) (relative error)

n= 1.64x1.64x 0.60x0.40/0.10x 0.10 = 64.55

n= 65 (minimum) at 90% Confidence Interval

Methods

1. 1.Gross Examination - The specimens were received in 10% formalin as biopsy and surgically excised specimens. Gross morphology of small biopsy and specimen was described in terms of amount or weight, size, color and consistency. Representative bits were taken post 24 hours fixation in formalin.
2. 2.Microscopic Examination - Tissues were processed in automated tissue processor (Thermo automatic tissue processor 120) and embedded in paraffin wax. By using rotary microtome, 4-5-micron thick sections were cut and stained with Haematoxylin& Eosin (H&E). H&E stained slides were examined under light microscope for microscopic evaluation.
3. Immunohistochemistry (IHC)- IHC will be done by using p16 marker on paraffin block sections. Scoring will be done for intensity of expression and for number of positive cells.

- p16 – Show nuclear and cytoplasmic staining
- IHC Grading-

IHC Grading	% of cell positive for p16 IHC protein
Grade 0	0%
Grade 1	1%-25%
Grade 2	26%-50%
Grade 3	51%-75%
Grade 4	76%-100%

RESULTS

In present study, total 65 cases of Oropharngyeal SCC were included.

Age of the patients varied from 20-80 years with the maximum number of cases belonging to 41-50 years (35.38%) followed by 31-40 years (20%), 51-60 years (18.46%), 61-70 years (12.30%), 20-30 years (10.76%) and 71-80 years (3.07%).

In the present study maximum numbers of patients 78.46% were males where as 21.53 % of patients were females.

In the Present study, Gingiva & Buccal mucosa were the most common site involved in the OPSCC (36.92%), followed by Tongue (27.69%), Alveolus (23.07%), Lip (7.69%). Palate (3.07%) and Tonsil (1.53%).

In the Present study 64.61% patients were not addicted and 35.38% patients were having addiction like Smoking (35.38%), Alcohol (24.61%) Tobacco (24.61%), Gudakhoo (15.38%).

In the present study, Of the 65 cases 47 cases (72.30 %) were well differentiated squamous cell carcinoma (WDSCC), 17 cases (26.15%) were moderately differentiated squamous cell carcinoma (MDSCC) and rest 1 case (1.5 %) was Poorly Differentiated Squamous Cell Carcinoma (PDSCC).

In the present study, maximum numbers of cases 60% were positive for p16 and 40% cases were negative to p16 immunohistochemistry.

p16 immunohistochemistry grading in study population (n= 65) is shown in [Table 1].

Diverse histopathological Findings were seen in OPSCC. Hemorrhage being most common type found in 90.76% followed by necrosis. [Table 2]

Statistical Analysis

1. Chi-SquareCalculation:

- $\chi 2$=1.66
- Degrees of Freedom=2p-value=0.436
- Interpretation: Thep-valueis 0.436, which is greater than 0.05, indicating that there is no statistically significant association between histological grade and p16 protein expression.

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2. Logistic Regression Results

Logistic Regression Model: logit (p)=β0+β1×Moderate+β2×Poor

Interpretation:

- The intercept(log-oddsforwell-differentiated) is not statistically significant.
- The coefficient for moderately differentiated is not statistically significant(p-value0.710).

The coefficient for poorly differentiate dis not statistically significant, and the estimate is extremely uncertain due to the very small sample size(p-value0.991). Based on both the Chisquare test and logistic regression analysis, the reisnostatistically significant association between histological grade and p16 protein expression in oropharyngeal squamous cell carcinoma within the dataset. The logistic regression analysis further confirms this lakh of significant association, with all coefficients showing non-significantp-values.

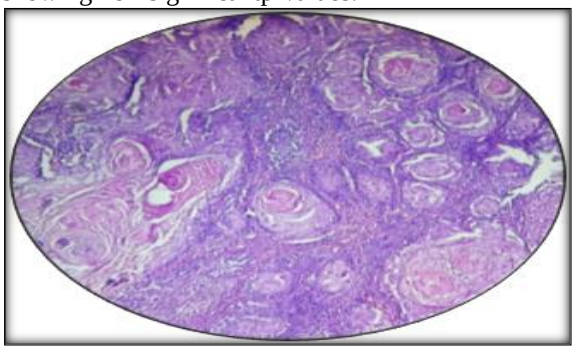

Figure 1: Histopathology section of Well Differentiated Squamous Cell Carcinoma showing multiple keratin pearls and intracytoplasmic keratinization. (H&Estain,100x)

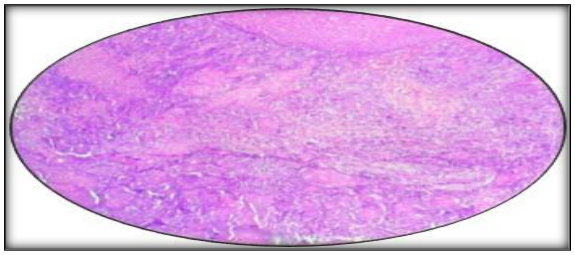

Figure 2: Histopathology section of Moderately Differentiated Squamous Cell Carcinoma showing tumour in diffuse sheets. (H&E stain, 100X)

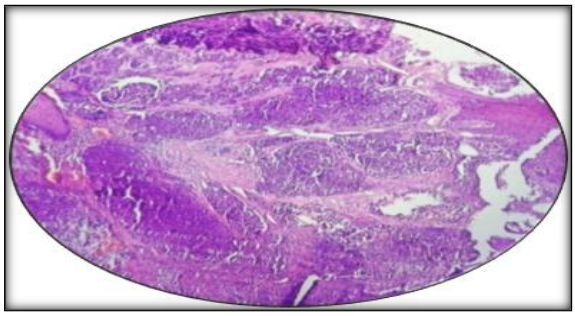

Figure 3: Histopathology section of Poorly Differentiated Squamous Cell Carcinoma showing clusters of highly pleomorphic tumour cells with hyperchromatic nucleus and fair number of mitotic figures. (H&E stain, 100X)

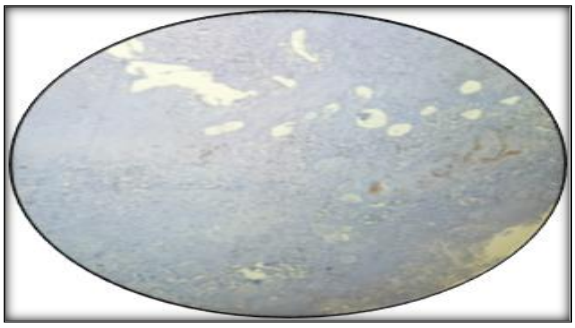

Figure 4: Histopathology section of Moderately Differentiated Squamous Cell Carcinoma., IHC p16 score-1, 1%-25%of nuclei and cytoplasm positive (IHC,100X)

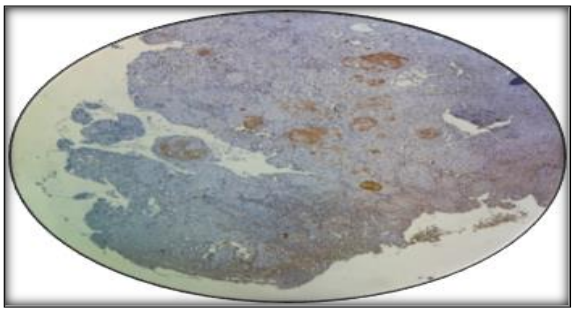

Figure 5: Histopathology section of Moderately Differentiated Squamous Cell Carcinoma. IHC p16 score- 2, 26%- 50% of nuclei and cytoplasm positive (IHC,100X)

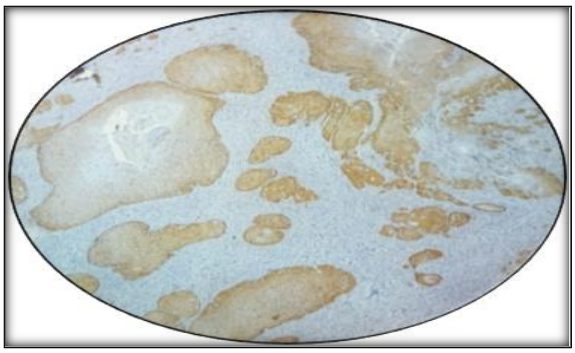

Figure 6: Histopathology section of Well Differentiated Squamous Cell Carcinoma. IHC p16 score- 3, 51%-75% of nuclei and cytoplasm positive (IHC,100X)

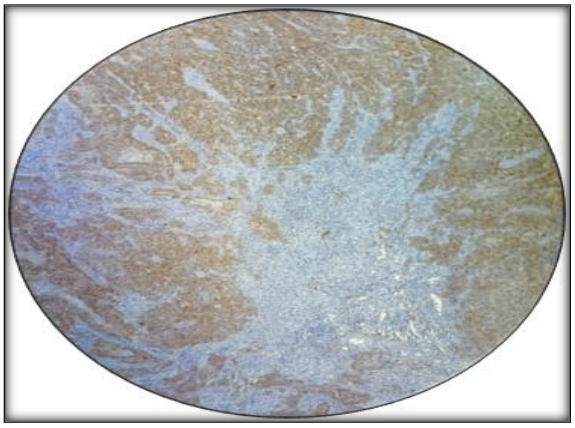

Figure 7: Histopathology section of Well Differentiated Squamous Cell Carcinoma. IHC p16 score- 4, 76-100% of nuclei and cytoplasm positive (IHC,100X)

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Table 1: Grading of p16 Immunohistochemistry Study Population (n=65)

p16 Immunohistochemistry Grading	No. of cases (n=65)	Percentage of cases(%)
Grade 0	26	40%
Grade 1	16	24.61%
Grade 2	6	9.23%
Grade3	6	9.23%
Grade4	11	16.92%
Total	65	100%

Table 2: Various Histopathological findings in OPTIC (n65)

Histopathological Findings	Number (n=65)	Percentage (%)
Haemorrage	59	90.76%
Necrosis	53	81.53%
Lymphovascularinvasion	04	6.15%
Perineuralinvasion	02	3.07%
Tissue Eosinophilia	09	13.84%
Giant Cell Reaction	13	20%
Keratin Pearls	45	70.76%
Inter-cellular Desmosomes	45	70.76%
Calcification	02	3.07%
Mitosis		
<5/10HPF	32	49.2%
5-10/HPF	29	44.61%
>10/10HPF	04	6.15%

Table 3: Correlation with p16 Expression with Histopathologic Grade (n=65)

Histologic Grade	P16 Positivity
WDSCC	59.17%
MDSCC	64.7%
PDSCC	00

Table 4: Model Summary

Coefficient	Estimate	Std.Error	zvalue	p-value
(Intercept)	0.3878	0.2972	1.305	0.192
Histological_grademoderate	0.2184	0.5882	0.371	0.710
Histological_gradepoor	-15.9538	1455.3976	-0.011	0.991

Table 5: Odds Ratios and Confidence Intervals.

Coefficient	OddsRatio	95%CI(Lower)	95%CI (Upper)
(Intercept)	1.47	0.81	2.67
Histological_grade (moderate)	1.24	0.38	4.07
Histological_grade (poor)	0.00	0.00	Inf

DISCUSSION

The present study included 65 cases of OPSCC which were subjected to theImmunohistochemicalforp16.

In present study the mean age of the study participants was 45.5 years which was comparatively younger as compared to study done by J.S polling et. Al,[8] Premalatha S,[9] and Zeyi Dengetal,[10] where mean age of presentation was more than 50 years.

In our study male preponderance was noted as seen in study done by Hong-Xue Meng et al,[11] Pandey P et al,[12] and Justin. R.Shinn et. al.[13]

In present study GBS & BuccalMucosa region was found as the most common locationof OPSCC. Alveolus Region Was 2nd Most Common Site, followed by Tongue, Lip, Palate and Tonsil. However, studies done by Prakash Pet al,[14] J.Spollingetal,[8] Patil S,[15] and Premalatha S,[9] concluded, tongue as the most commonlocation. This may be because of addiction with Gudhakhu rubbed at buccal mucosa & GBS creating mechanical irritation.

In the present study,72.30% of total cases were WDSCC. Similar Results Were Found In Study doneby Pandey P et al.[12] Of all cases of WDSCC, 59.17% case show edp16 positivity comparable to the studies done by Patil S. et al,[15] and Pandey P et al.[12] 26.15% of total cases were MDSCC and 64.7% of MDSCC cases showed p16 positivity. Similar resultswere found in studies done by Saxena P et al,[16] and Pandey P et al.[12]

CONCLUSION

In the present study, we concluded that OPSCC is most common malignant tumour of oral cavity commonly presenting in GBS and more commonly found in males as compared to females. Most common histologic grade is well differentiated squamous cell carcinoma. p16 expression is independent prognostic marker for OPSCC. No Significant Correlation was Found between histopathological grades of OPSCC andp16 expression. However p16 positivity indicate favorable prognosis and depict its association

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between HPV. More studies recommended to be conducted to determine association between p16and OPSCC.

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