



THE EVALUATION OF HUMAN PAPILLOMA VIRUS STATUS IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA USING P16 IMMUNOEXPRESSION: A DESCRIPTIVE OBSERVATIONAL STUDY AT TERTIARY CARE CENTRE, JAIPUR (RAJASTHAN)

Pathology

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ABSTRACT

Background and Objectives: Oropharyngeal squamous cell carcinomas (OPSCC) patients with human papilloma virus (HPV) infected tumours have more favorable prognosis, however data is very sparse in Indian literature. Our study aims to assess the HPV status in patients with OPSCC in our institution, to evaluate the diagnostic utility and prognostic significance, so that it can be utilized to guide the patient management. **Methods:** One hundred OPSCC cases were included in this study and analyzed for age, gender, habits, site, symptoms, histologic type, treatment, survival at the end of study. All tissue samples were subjected to p16 Immunohistochemistry. **Results:** Out of 100 cases 3 were found p16 positive. Significant difference was noted between the study variables among p16 positive and negative cases. Neck mass (58%) was the most common presenting symptom of OPSCC. All P16 positive cases were male patients and had median age of 48 years, presented with neck mass. History of smoking was present in one out of three HPV positive cases. P16 positivity is commonly seen with basaloid type of squamous cell carcinoma at tonsil followed by tongue. **Interpretation and Conclusion:** Results of this study provided evidence supporting p16 positive cases in younger age with less tobacco, alcohol, smoking habits. Neck mass was the initial presenting symptom. p16- negative cases had median age of 60 years with more frequent history of tobacco, alcohol and smoke use.

KEYWORDS

Human Papilloma Virus, Oropharyngeal squamous cell carcinoma, P16

INTRODUCTION

Oropharyngeal cancer is a disease of oropharynx, the middle part of throat which constitutes base of the tongue, palatine tonsils, soft palate and posterior pharyngeal wall.

Overall, 57.5% of global head and neck cancers (excluding esophageal cancers) occur in Asia especially in India, in both sexes. Over the past 10-15 years, the traditional paradigms of Head and neck squamous cell carcinoma (HNSCC) have been changing significantly. It has emerged as a heterogeneous group of diseases, with distinct molecular genetic changes. Oropharyngeal squamous cell carcinoma (OPSCC) comprises approximately a third of head and neck cancers in India.⁽¹⁾⁽²⁾ Studies have reported a changing trend in the incidence of oral and oropharyngeal squamous cell carcinoma. Rising trend is reported in many countries including India among the youngers, focusing the need of measures to reduce the incidence of and mortality from oral and oropharyngeal cancers.⁽³⁾

Despite of decreasing use of alcohol and tobacco, the incidence of oropharyngeal squamous cell carcinoma has trended steadily upwards for the last 10 to 15 years primarily due to increase in cancer related to Human papilloma virus (HPV) infection.⁽⁴⁾

These HPV associated squamous cell carcinomas have unique demographic characteristics, risk factors, biology, distinct morphology and clinical outcome as compared to HPV negative.

HPV-positive OPSCCs more commonly occur in male compared to female, at younger age than the typical age of presentation of head and neck carcinoma. These patients usually belong to higher socioeconomic status and less likely to have history of tobacco exposure. Man with multiple oral sex partners has higher chances of getting HPV infection from female as their genital mucosa carries high HPV viral load.⁽⁵⁾

Neck mass is most common presenting symptom in HPV-positive OPSCC whereas Sore throat with history of smoking is common among HPV-negative OPSCC.⁽⁶⁾

In HPV associated tumours, the transformation pathway leads to development of cancer. Carcinomas are caused by expression of E6

and E7 viral oncogenes that establish cellular conditions which inactivate tumour suppressor protein p53 and retinoblastoma protein pRb respectively, leading to malignant transformation of HPV infected cells.⁽⁷⁾

HPV associated oropharyngeal squamous cell carcinoma originates in the crypt epithelium and grows beneath the surface epithelial lining as nests and lobules of basaloid cells. Tumour nests are often embedded in lymphoid stroma, and may be penetrated by lymphoid cells.⁽⁸⁾

HPV-positive OPSCC carries a favorable prognosis compared to HPV-negative tumours. The better prognosis conferred by HPV positivity is reflected in the updated AJCC 8th edition TNM staging system, which for the first time separates staging for HPV(+) and HPV(-) OPSCCs and in general down-grades HPV(+) OPSCC staging.⁽⁹⁾⁽¹⁰⁾

The nuclear and cytoplasmic p16 overexpression correlate precisely with HPV positivity and are indicated to be specific for HPV positivity. p16 (p16INK4a) IHC has been widely adopted because it is cost effective, reliable, examines paraffin-embedded tissue and has high sensitivity.⁽¹¹⁾

p16 expression is now being used as a surrogate marker for HPV infection in OPSCCs. Patients with HPV positive OPSCC show a significantly improved prognosis when compared with HPV negative OPSCC, independent of treatment modalities chosen.⁽¹²⁾

Therefore, the purpose of study was to assess the HPV status in patients with OPSCC in our institution, to evaluate the diagnostic utility and prognostic significance, so that it can be utilized to guide the patient management.

MATERIAL AND METHODS

Hospital based Cross sectional descriptive type of observational study was conducted after ethical clearance from institutional ethics committee and written informed consent was obtained from all patients. A total of 100 oropharyngeal biopsies received in Department of Pathology from Department of ENT and Surgery, SMS hospital, Jaipur. All formalin fixed, adequate size biopsies with clinical details received during study period were included and biopsies of patients with second primary, patients with history of treatment, pregnant and

lactating women and patient who did not give consent were excluded.

The specimens were allowed to fix in 10% buffered formalin for 12-14 hours at room temperature and the gross features such as size, shape, colour, external surface, cut surface, consistency were noted. The margins were inked if possible.

Following adequate fixation, the tissues were routinely processed, following which the paraffin embedded serial sections of 3-4 μ thickness were cut. The sections were examined after staining with Haematoxylin and eosin (H&E) stain for histopathological confirmation and grading of the lesions was done. Immunohistochemistry (IHC) was performed with commercially available monoclonal antibody for p16INK4a protein (BioCare, USA).

Interpretation of p16 immunohistochemistry:

- p16 IHC positive (≥70% nuclear and cytoplasmic moderate to strong staining)
- p16 IHC negative (< 70% nuclear and cytoplasmic moderate to strong staining)

Data suggest that the correlation between HPV positivity and p16 overexpression is highest when the ≥70% staining for p16 overexpression is applied.(13)

Data obtained were expressed in percentage, proportion, graphs, tables and analyzed for proportion of various histomorphology of oropharyngeal SCCs, association of histo-morphological features with clinical profile and proportion of p16 Positive and p16 Negative cases. The cases were followed till the end of study.

RESULTS

Socio-demographic, clinical and histological tumour characteristics of cases are collated in [Table I] showing various characteristics of p16 positive and p16 negative cases. In total 100 cases of OPSCC were evaluated.70% patients were farmer followed by tailor 5%. 4% each as labour and housewife,3% each of welder, Driver, shopkeeper. Conventional squamous cell carcinoma 90% was the most common histologic type followed by basaloid SCC 09% and papillary SCC 01%. Moderate degree of differentiation was seen in 56(62.2%) of conventional squamous cell carcinoma [Figure1].

Chief complaints and site of involvement are elucidated in [Figure 2]. Majority of the patients 58% presented with neck mass followed by 39% with difficulty in swallowing. Almost equal frequency of change in voice 33% and an ulcer 32% was noted in the patients. Base of tongue was the most common affected site in 43% cases followed by tonsil in 32% and soft palate in 20% cases. The least affected site was posterior pharyngeal wall 5%.

The detailed description of three p16 positive cases is delineated in [Table II]. All p16 positive cases were presented with neck mass and showed basaloid histomorphology. Two p16 positive cases had shown strong and one moderate intensity of nuclear and cytoplasmic staining [Figure 3]. Only one had history of smoking. Others were non-user of smoke, alcohol and tobacco. They were alive till the end of study.

Treatment history of study population is collated in [Table III]. Majority of patients 69% underwent and completed concurrent chemotherapy and radiotherapy, 12%patients received only radiotherapy, while 5%patients discontinued the treatment due to side effects. On follow up, 14% patients expired till the end of study. All three p16 positive cases had received combined chemo and radiotherapy and were alive.

Table: I. Comparative profile of p16 positive and negative patients			
Variables	Total (%)	P16 negative (97/100)	P16 positive (3/100)
Median Age(years)	60	60	48
Gender			
Male	88	85	03
Female	12	12	00
Habits			
Smoking	64	63	01
Tobacco	21	21	00
Alcohol	10	10	00
Non-users	5	03	02

Sites of lesions			
Base of tongue	43	42	01
Tonsil	32	30	02
Soft palate	20	20	00
Posterior pharyngeal wall	05	05	00
Histologic variants of squamous cell carcinoma			
Conventional	90	90	00
Basaloid	09	06	03
Papillary	01	01	00

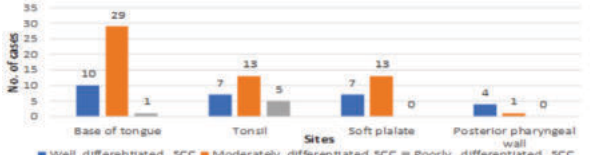


Figure 1: Conventional Squamous Cell Carcinoma (grades And Site Distribution)



Figure 2: Complaints Among Study Population

Table II: Detailed profile of p16 positive patients			
Age	47	48	50
Sex	Male	Male	Male
Site	Tonsil	Tonsil	Base of tongue
Lymph node involvement	Present	Present	Present
Smoke	No	No	Yes
Alcohol	No	No	No
Tobacco	No	No	No
Occupation	Painter	Welder	Driver
Clinical presentation	Ulcer, Neck mass	Ultero-proliferative lesion, Neck mass	Difficulty in swallowing, Change in voice, Bilateral neck mass
Variant of SCC	Basaloid SCC	Basaloid SCC	Basaloid SCC
Treatment	Chemo and Radio Therapy	Chemo and Radio Therapy	Chemo and Radio Therapy

Table III: Treatment history of study population		
Treatment	No. of cases	(%)
Chemo and radiotherapy	69	69%
Radiotherapy	12	12%
Ayurvedic treatment	06	06%
No treatment	08	08%
Discontinued treatment	05	05%
Total	100	100%

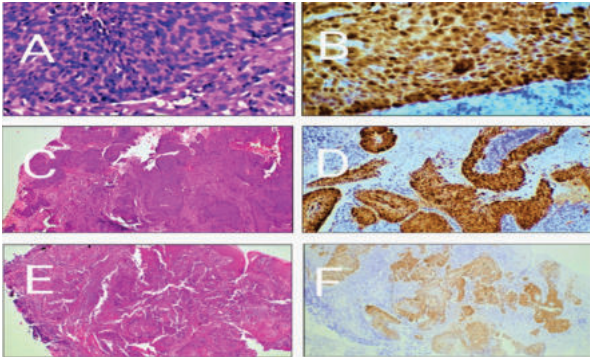


Figure 3: A) H & E-stained section (400x) Squamous cell carcinoma-

Cervix B) Corresponding p16 immunostained section (400x) Showing Strong and Diffuse nuclear and cytoplasmic staining (Positive control) C) H & E-stained section (200x) Basaloid squamous cell carcinoma D) Corresponding p16 immunostained section (400x) Showing Strong and Diffuse nuclear and cytoplasmic staining (Case) E) H & E-stained section (40x) Basaloid squamous cell carcinoma F) Corresponding p16 immunostained section (40x) Moderate and Diffuse nuclear and cytoplasmic staining (Case)

DISCUSSION

The data regarding p16 positive and negative squamous cell carcinoma and its correlation with demographic profile, risk factors and results from India are insufficient and conflicting. This is one of the initial studies from Jaipur, Rajasthan region of West India.

HPV-OPSCC has been shown to affect a younger population.^{—(14)} Our study also detected significant difference between median age of p16 positive and p16 negative cases with OPSCC (48 vs 60 years, respectively). The young population is prone to HPV infection as they practice unnatural sex pattern frequently.⁽¹⁵⁾

The incidence of oropharyngeal squamous cell carcinoma among males was significantly higher than females in the studies conducted by Y Saito et al⁽¹⁶⁾ and McIlwain et al^{—(14)} (90% vs 10% and 88% vs 12%, respectively). The results of our study were concordant with these studies. The male predominance is due to higher risk of infection with high-risk HPV, more addiction habits in male as compared to females. Some genetic factors and immune status of males also make them susceptible to HPV infection.^{—(17)}

Not a single female patient had shown p16 positivity in the study. Which was in contrast to the studies of C A Fischer et al⁽¹²⁾, McIlwain et al^{—(14)} (29% and 06% , respectively). They observed significant number of females showing p16 positivity. This can be explained by difference in the pattern of substance abuse and sexual behavior in females in India compared to foreign countries.⁽¹⁸⁾

The proportion of males with p16 positivity was higher in all the studies. Males commonly practice oral sex and have multiple sex partners than females, so they have higher chance of acquiring HPV infection. But such history was not given by the patients in the study. Either they hide it or acquired infection due to poor hygiene.⁽¹⁸⁾

Several studies have shown incidence of presenting symptoms in OPSCC. To best of our knowledge, no study has reported most common initial presenting symptoms. Neck mass (58%) was the commonest presenting complaint and base of tongue as most common site involved. Gorsky et al⁽¹⁹⁾ also found that the base of tongue OPSCC presented predominantly with neck mass (25.7%). M Pitchers et al⁽²⁰⁾ and McIlwain et al^{—(14)} also found more cases presented with neck mass (49.3% and 44%, respectively). The high frequency of neck mass as presenting symptom is due to rich lymphatic drainage from palatine tonsil and lingual tonsils to the cervical lymph nodes.⁽²¹⁾

The next common complaint was dysphagia (39%). This was comparable with the study of D Pandiar et al⁽²²⁾ (31.4%). M Pitcher et al⁽²⁰⁾ and McIlwain et al^{—(14)} found few cases presented with dysphagia (2.9% and 16%, respectively). The involvement of tongue frequently causes obstruction and dysphagia as was seen in the present study.⁽¹⁹⁾

M Pitcher et al⁽²⁰⁾ and McIlwain et al^{—(14)} found more cases presented with sore throat (33.3%) and (33%) respectively. In our study only (20%) patients had sore throat. Others might have ignored it considering sore throat as simple infection or allergy. Other complaints like change in voice, ulcer, loss of appetite, weight loss did not show concordance with other studies could be due to difference in sample size or an incidental finding.

Most patients in present study were farmers. The lack of awareness about initial nonspecific symptoms, their busy working schedules might resulted in delay in seeking medical advice. Sometimes delay in referral from primary health care centre result in progression of disease leading to obstructive symptoms.⁽²⁰⁾

The base of tongue (43%) was predominant affected site than the tonsil (32%). It was comparable with the study of C A Fischer et al⁽¹²⁾ and D Pandiar et al⁽²²⁾. The elderly patient (70%) from rural areas constituted

major proportion of study population. They commonly smoke bidi (64%) and consume tobacco (21%). Prolong and frequent exposure of tongue and other sites to harmful agents produce carcinogenic effect.⁽²³⁾

C A Fischer et al⁽¹²⁾, Y Saito et al⁽¹⁶⁾, McIlwain et al^{—(14)} and D Pandiar et al⁽²²⁾ found more cases affecting tonsil than our study. The tonsil is more likely to develop HPV associated SCC than the tongue because of structural difference. The tonsillar crypts act as reservoir for HPV, which is potent viral carcinogen. HPV acts by inducing activation of transformation pathway.^{—(14)}

In present study, 6.2% OPSCC from tonsil and 2.3% from base of tongue showed p16 overexpression. This is lower than studies done by C. A Fischer et al⁽¹²⁾, Y Saito et al⁽¹⁶⁾, McIlwain et al^{—(14)}. They found more cases showing p16 expression from tonsil and base of tongue. P16 expression for tonsillar area (6.2%) was comparable to the study of D Pandiar et al⁽²²⁾ (5.1%) but contrasts for base of tongue (12.2%) with our study (2.3%).

The overall prevalence of HPV infection and p16 expression is lower in India than western world and Japan is supported by D Pandiar et al⁽²²⁾.

The review of literature revealed that the prevalence of HPV infection varies across different parts of India. The studies from the East, North and Southern region found higher percentage of p16 positive OPSCC than our study suggesting relatively low prevalence of HPV in the West. It may be attributed to ethnicity, geographic variation, sample size and detection technique used.⁽²⁴⁾

Statistical analysis was limited due to small sample size, few p16 positive cases and lack of adequate data on the high-risk sexual behavior, So the results could not be generalized. Since it is a cross sectional, observational study, the survival could not be assessed.

INTERPRETATION AND CONCLUSION

The studies suggest that the prevalence of HPV associated HNSCC is increasing worldwide including India, largely involving oropharynx. The HPV-negative OPSCC is a devastating disease with high morbidity and mortality, While HPV-positive OPSCC behaves differently with better prognosis, hence it needs to accurately determine HPV as an etiological agent.

The infection with high-risk HPV subtype results in aberrantly increased expression of p16 protein, a surrogate marker of HPV.

The observations and their analysis in present study concluded that out of 100 cases, three male patients of OPSCC showed p16 overexpression. Though the percentage of p16 positivity was very low, statistically significant association for age, gender and site was found. The follow-up of these patients revealed that patient survived and were in good health till the end of study. This suggests that p16 immunohistochemistry can be implicated routinely to depict the HPV status and as biomarker for increased response to therapy.

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