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Role of high-risk human papillomavirus in oropharyngeal squamous cell carcinoma: A clinicopathological and molecular study from a tertiary centre in India.

Dr. Amod Kumar[1], Dr. Aashish Gupta[2], Dr. Monica[3], Dr. Payal Kumari[4]*, Dr. Sunil Kumar[5]
[1]Associate Professor, Department of Pathology, Nalanda Medical College, Patna, Bihar, India
[2]Associate Professor, Department of Pathology, Nalanda Medical College, Patna, Bihar, India
[3]Associate Professor, Department of Microbiology, Motilal Nehru Medical College, Prayagraj, Uttar Pradesh, India
[4]Department of Pathology, Mahavir Cancer Sansthan, Patna, Bihar, India
[5]Professor, Department of Pathology, Nalanda Medical College, Patna, Bihar, India

Abstract

Background
High-risk human papillomavirus (hrHPV) has emerged as a major etiological agent in a biologically distinct subset of oropharyngeal squamous cell carcinoma (OPSCC), associated with improved clinical outcomes. However, Indian data on HPV-associated OPSCC remain heterogeneous and limited.

Objective
To determine the prevalence of hrHPV-driven OPSCC and evaluate its clinicopathological, molecular, and prognostic correlates.

Methods
A retrospective cross-sectional study was conducted at a single tertiary care teaching hospital in eastern India. Consecutive primary OPSCC cases diagnosed between January 2020 and December 2023 were analyzed. p16 immunohistochemistry was used as a screening tool, followed by hrHPV DNA polymerase chain reaction (PCR). Discordant cases underwent E6/E7 mRNA analysis when tissue permitted. Clinicopathological parameters and progression-free survival were evaluated.

Results
Among 180 OPSCC cases, hrHPV positivity was identified in 52.2%. HPV-positive tumors were significantly associated with younger age, lower tobacco exposure, non-keratinizing morphology, lower T stage, higher nodal stage, and superior two-year progression-free survival (81% vs. 65%, p=0.03).

Conclusion
hrHPV-driven OPSCC constitutes a substantial proportion of OPSCC in India. Integrated p16 and molecular testing offers reliable classification with important prognostic implications.

Recommendations
Routine p16 screening with confirmatory molecular testing should be incorporated into OPSCC diagnostic protocols. Prospective multicentric Indian studies are recommended.

Keywords: *Human papillomavirus; Oropharyngeal squamous cell carcinoma; p16 immunohistochemistry; Polymerase chain reaction; E6/E7 oncoproteins*
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Corresponding Author: *Dr. Payal Kumari*
Email: ***drpayal1302@yahoo.com***
Department of Pathology, Mahavir Cancer Sansthan, Patna, Bihar, India.

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Introduction

Oropharyngeal squamous cell carcinoma (OPSCC) accounts for a significant proportion of head and neck malignancies worldwide and represents a major public health challenge in developing countries [1]. Traditionally, OPSCC in India has been closely associated with tobacco use, areca nut chewing, and alcohol consumption [2]. These carcinogens induce chronic epithelial injury, genomic instability, and malignant transformation.

Over the past two decades, a distinct etiological pathway involving high-risk human papillomavirus (hrHPV) infection has been recognized, particularly in cancers of the tonsil and base of tongue [3]. HPV-16 is responsible for the majority of HPV-driven OPSCC, followed by less common high-risk subtypes [4]. Viral E6 and E7 oncoproteins cause cancer by turning off p53 and retinoblastoma proteins, which makes p16 overexpress [5].

HPV-positive OPSCC is very different from HPV-negative disease in terms of how it spreads, how it looks under a microscope, and how likely it is to get better [6]. Patients are usually younger, have little exposure to tobacco, and have tumours that don't keratinise and spread through cystic nodal metastasis [7]. HPV-positive OPSCC responds better to radiation therapy and chemoradiation, which leads to better survival rates [8].

Even though more people around the world are aware of it, the rate of HPV-related OPSCC in India varies widely from study to study because of differences in geography, diagnostic methods, and patient characteristics [9–11]. It is therefore important to have reliable institutional data that uses standardised diagnostic algorithms. This study looks at the clinicopathological and molecular features of hrHPV-associated OPSCC at a tertiary care centre in India and compares HPV status to clinical outcomes.

Materials and Methods

Study Design and Setting

This retrospective cross-sectional study was conducted in the Department of Pathology at Nalanda Medical College & Hospital, Patna, a government-run tertiary care referral centre serving eastern India.

Study Period

January 1, 2020, to December 31, 2023. Data analysis was performed between January and March 2024.

Inclusion Criteria

- Primary OPSCC cases
- Adequate formalin-fixed paraffin-embedded tissue
- No prior treatment

Exclusion Criteria

- Recurrent or metastatic disease
- Prior head and neck malignancy
- Inadequate tissue for molecular testing

Histopathology and Immunohistochemistry

Tumors were classified as keratinizing or non-keratinizing. p16 immunohistochemistry was considered positive when ≥70% of tumor cells showed strong nuclear and cytoplasmic staining.

Molecular Testing

hrHPV DNA PCR targeted HPV-16, 18, 31, 33, 35, 45, 52, and 58. p16-positive/DNA-negative cases underwent E6/E7 mRNA in-situ hybridization when feasible.

Statistical Analysis

Data were analyzed using SPSS v26. Chi-square test, Mann-Whitney U test, and Cox regression were applied. $p<0.05$ was considered statistically significant.

Results

A total of 180 cases of primary oropharyngeal squamous cell carcinoma (OPSCC) were included in the present study. All cases fulfilled the inclusion criteria and had adequate tissue for histopathological and molecular evaluation. The results are presented under clinicodemographic characteristics, HPV status and molecular findings, clinicopathological correlations, and survival outcomes, with appropriate citation of figures and tables.

Clinicodemographic Characteristics

The median age of the study population was 56 years (interquartile range: 49–62 years). A marked male predominance was noted, with males accounting for 86% of cases. A history of tobacco smoking and/or areca nut chewing was present in 58% of patients, while alcohol consumption was reported in 31%. The tonsil (39%) and base of tongue (35%) were the most frequently involved anatomical subsites, followed by soft palate (9%) and other oropharyngeal regions (17%). Baseline clinicodemographic

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and pathological characteristics stratified by HPV status are summarized in Table 1.

HPV Status and Molecular Findings

High-risk HPV-driven OPSCC was identified in 94 out of 180 cases, yielding an overall HPV positivity rate of 52.2%. HPV detection was based on combined p16 immunohistochemistry and high-risk HPV DNA polymerase chain reaction (PCR). Among HPV-positive tumors, HPV-16 was the predominant genotype, detected in 88% of cases, followed by HPV-18 in 7% and other high-risk subtypes in 5%.

Eight cases showed diffuse p16 positivity but were negative for hrHPV DNA by PCR. Of these, seven cases had adequate tissue for E6/E7 mRNA in-situ hybridization, and six cases demonstrated transcriptionally active HPV infection. These findings highlight the value of a tiered diagnostic approach. The molecular testing algorithm and results are detailed in Table 2.

Histopathological and Anatomical Distribution

Histopathological examination of HPV-positive tumors predominantly revealed a non-keratinizing morphology, characterized by sheets and nests of basaloid tumor cells with a high nuclear-to-cytoplasmic ratio and minimal keratinization (Figure 1).

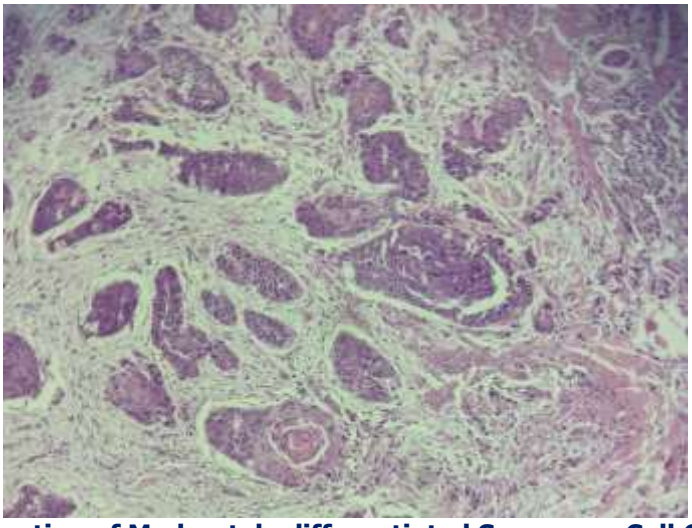

Figure 1: H&E section of Moderately differentiated Squamous Cell Carcinoma, 10X

Analysis of anatomical subsite distribution demonstrated that HPV positivity was highest in tumors arising from the tonsil and base of tongue, whereas tumors from the soft palate and other oropharyngeal sites showed lower HPV prevalence (Figure 2).

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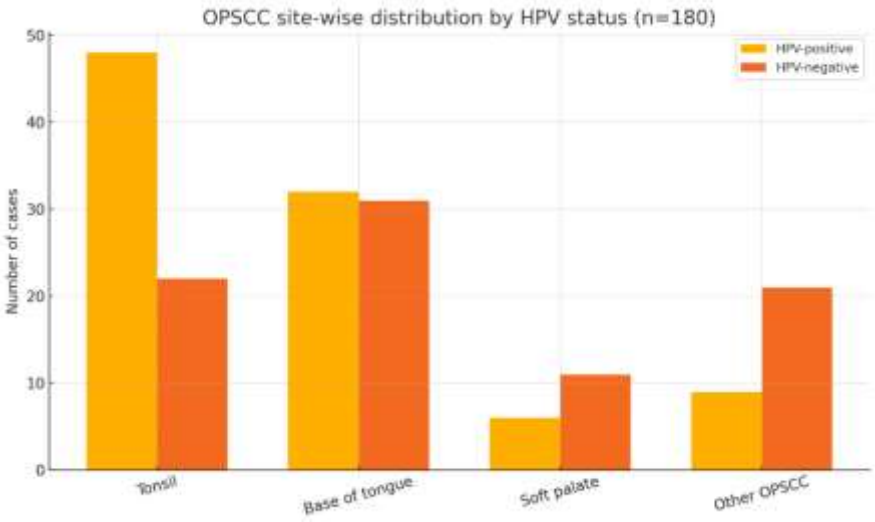

Figure 2. Site-wise distribution by HPV status (n=180)

Clinicopathological Correlation with HPV Status

HPV-positive OPSCC cases were significantly younger than HPV-negative cases (median age: 53 years vs. 59 years; p = 0.01). A significantly lower proportion of HPV-positive patients reported tobacco or areca nut use compared to HPV-negative patients (41.5% vs. 75.6%; p < 0.001).

From a histopathological perspective, non-keratinizing morphology was strongly associated with HPV positivity (72.3% in HPV-positive tumors vs. 18.6% in HPV-negative tumors; p < 0.001). About tumor staging, HPV-positive tumors more frequently presented with early primary tumor stage (T1–T2: 68.1% vs. 44.2%; p = 0.002) but showed a higher nodal stage at presentation (N2–N3: 60.6% vs. 38.4%; p = 0.004). These clinicopathological correlations are summarized in Table 1.

Table 1: Baseline Clinicopathological Characteristics by HPV Status

Parameter	HPV Positive (n=94)	HPV Negative (n=86)	p-value
Median age (years)	53	59	0.01
Male sex (%)	85	87	0.69
Tobacco/areca use (%)	41.5	75.6	<0.001
Non-keratinizing morphology (%)	72.3	18.6	<0.001
T1–T2 stage (%)	68.1	44.2	0.002
N2–N3 stage (%)	60.6	38.4	0.004

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Table 2: Molecular Testing Cascade and Results

Test Performed	Number Tested	Positive (%)
p16 Immunohistochemistry	180	102 (56.7)
hrHPV DNA PCR (p16-positive cases)	102	94 (92.2)
E6/E7 mRNA ISH (discordant cases)	7	6 (85.7)

Treatment Outcome and Survival Analysis

Among 132 patients treated with definitive intent, the median follow-up duration was 28 months. HPV-positive OPSCC patients demonstrated significantly superior two-year progression-free survival compared to HPV-negative patients (81% vs. 65%; log-rank p = 0.03). On multivariate Cox proportional hazards regression analysis adjusting for age, T stage, N stage, and treatment modality, HPV positivity remained an independent favorable prognostic factor (hazard ratio: 0.58). Survival outcomes are summarized in Table 3.

Table 3: Two-Year Progression-Free Survival by HPV Status

Group	Two-year PFS (%)	Adjusted Hazard Ratio
HPV Positive	81	0.58
HPV Negative	65	Reference

Discussion

This study shows that more than half of OPSCC cases were caused by hrHPV, which supports the changing pattern of disease spread that has been reported around the world [12–14]. The fact that HPV-16 is the most common type fits with what molecular profiles show in both Western and Asian groups [15].

Younger patients with less exposure to tobacco had HPV-positive OPSCC, which is in line with previous studies in India and other countries [16–18]. The strong link with non-keratinizing morphology makes histopathological pattern recognition even more useful for diagnosis [19].

Even though they had a higher nodal stage, HPV-positive tumours had a much better progression-free survival. This positive result has been linked to increased radiosensitivity and tumour clearance mediated by the immune system [20–22].

The strategy of using both p16 and molecular testing in this study reduced the chances of misclassification and made sure that HPV attribution was biologically relevant, as international guidelines suggest [23–25]. These results advocate for regular HPV testing in OPSCC within Indian clinical practice.

Limitations

- Retrospective design
- Limited data on long-term survival
- All discordant cases had incomplete E6/E7 testing

Recommendations

Routine p16 screening with confirmatory molecular testing should be incorporated into OPSCC diagnostic protocols. Prospective multicentric Indian studies are recommended.

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Conflict of Interest

The authors declare no conflict of interest.

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List of Abbreviations

OPSCC – Oropharyngeal squamous cell carcinoma
HPV – Human papillomavirus
PCR – Polymerase chain reaction

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