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Association of Human Papillomavirus with Oropharyngeal Malignancies - A Cross-Sectional Study in a Tertiary Care Hospital of North-East India

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ABSTRACT

Background: Oropharyngeal squamous cell carcinoma (OPSCC) is traditionally linked to tobacco and alcohol use, but human papillomavirus (HPV), particularly type 16, has emerged as a major etiological factor. Data from northeastern India remains limited despite the rising incidence globally.
Objective: To determine the association of HPV DNA in oropharyngeal carcinoma biopsies and assess its association with demographic, lifestyle, and clinical variables.
Methods: A cross-sectional study was conducted at Agartala Government Medical College and Govind Ballabh Pant Hospital over two years. Seventy-five histologically confirmed OPSCC patients were enrolled consecutively. Tissue biopsies underwent histopathological examination and nested PCR for HPV DNA detection. Associations between HPV status and clinical/demographic factors were analysed using Chi-square tests.
Results: HPV DNA was detected in 20% (15/75) of OPSCC cases. Positivity was highest among younger patients (67% in 20–29 years) and was significantly associated with multiple sexual partners ($p = 0.033$). No significant associations were observed with smoking (males vs females; $p = 0.91$ vs 0.53), alcohol consumption ($p = 0.71$), or socioeconomic status ($p = 0.32$). HPV positivity was most frequent in tonsillar (9.6%) and base-of-tongue carcinomas (5.8%) and was relatively higher in proliferative lesions (9.6%) compared to ulceroproliferative growths (5.2%). All HPV-positive cases were squamous cell carcinoma, predominantly moderately differentiated.
Conclusion: HPV contributes substantially to OPSCC in northeastern India, with sexual behaviour emerging as a key determinant. Routine HPV testing, improved vaccination coverage, and awareness programs are essential to reduce the burden of HPV-associated OPSCC.

Keywords: Human papillomavirus; Oropharyngeal squamous cell carcinoma; Prevalence; Public health; Northeastern India

INTRODUCTION

Oropharyngeal malignancies represent a growing public health challenge worldwide. Traditionally, tobacco chewing, smoking, and alcohol consumption were considered the dominant risk factors for oropharyngeal squamous cell carcinoma (OPSCC).[1] However, in the past two decades, human papillomavirus (HPV) infection has emerged as a major etiological agent, particularly HPV type 16, which is strongly implicated in carcinogenesis of the oropharynx.[2] The recognition of HPV as a causative factor has reshaped the epidemiological and clinical landscape of head and neck cancers.

HPV Biology and Mechanism of Carcinogenesis

HPV is a double-stranded DNA virus comprising more than 200 identified genotypes, among which the high-risk types, particularly HPV16 and HPV18, are most strongly implicated in malignant transformation. These oncogenic variants are frequently associated with neoplasms arising in the oral cavity, pharynx, larynx, esophagus, lungs, breasts, bladder, ovaries, colon, anus and anal canal, prostate, vulva, stomach, and skin, underscoring their broad oncogenic potential across diverse epithelial tissues.[3] The viral oncoproteins E6 and E7 play a pivotal role by inactivating tumour suppressor proteins p53 and retinoblastoma (pRb), leading to genomic instability and uncontrolled cellular proliferation.[4] This molecular pathway distinguishes HPV-positive OPSCC from carcinogen-driven cancers, which typically involve mutagenic DNA damage from tobacco or alcohol exposure.

Epidemiological Trends

Globally, the incidence of HPV-associated OPSCC has risen sharply, particularly in North America and Europe, where HPV-positive cases now outnumber HPV-negative ones.[5] Across epidemiological studies, the age-standardized incidence rate (ASIR) of HPV-associated OPSCC has been reported to be highest in North America, Europe, and Oceania. Although the disease affects both sexes, men consistently exhibit higher ASIRs than women. Globally, the attributable fraction (AF) of OPSCC linked to HPV infection has been estimated to range between 30.8% and 42.7%. This pattern is also evident in densely populated Asian countries, with India showing HPV attribution rates of approximately 15–23% and China reporting proportions between 26–32%.[6]

In India, regional variations exist, with northeastern states reporting increasing HPV prevalence in oropharyngeal cancers.[7] This trend is influenced by sexual practices, poor oral hygiene, and limited HPV vaccination coverage. Recent Indian studies have documented HPV DNA in a significant proportion of OPSCC biopsies, underscoring the need for systematic surveillance and molecular testing.[8]

Clinical and Prognostic Implications

HPV-positive OPSCC exhibits distinct clinical features compared to HPV-negative tumours:

- Patients are often younger and have fewer traditional risk factors.[9]
- Tumours demonstrate better prognosis, with higher responsiveness to radiotherapy and chemotherapy.[10]

Surrogate biomarkers such as p16 immunohistochemistry are widely used to infer HPV status, though direct HPV DNA detection remains the gold standard.[11] These differences necessitate tailored treatment strategies and highlight the importance of HPV testing in routine diagnostic workflows.

Public Health Significance

HPV vaccination is the cornerstone of prevention. While vaccination programs have achieved success in reducing cervical cancer incidence, their impact on

oropharyngeal cancers remains underexplored, particularly in low- and middle-income countries. In India, gender-neutral vaccination policies are inconsistently implemented, and coverage remains limited.[12] Raising awareness, strengthening vaccination programs and improving diagnostic infrastructure (e.g., PCR-based HPV DNA detection) are essential to reducing the burden of HPV-associated OPSCC.

Rationale for the Present Study

Given the rising incidence of HPV-associated OPSCC and the paucity of regional data from northeastern India, this study aims to determine the association of HPV DNA in oropharyngeal carcinoma biopsies and assess its association with demographic, lifestyle, and clinical variables. Establishing the prevalence of HPV in this population will provide critical insights into disease etiology, guide treatment decisions, and inform public health strategies for prevention and control.

MATERIALS AND METHODS

Study Design and Type

This investigation was conducted as a cross-sectional observational study.

Study Setting

The study was carried out in the Department of Pathology at Agartala Government Medical College (AGMC) and Govind Ballabh Pant Hospital (GBPH), in collaboration with the Departments of Otolaryngology and Microbiology at the same institution.

Study Period

The study spanned two years, comprising 18 months of sample collection followed by 6 months of data analysis.

Study Population

The study population consisted of patients with histologically confirmed oropharyngeal carcinoma diagnosed in the Department of Pathology, AGMC & GBPH.

Inclusion Criteria

- Patients newly diagnosed with oropharyngeal carcinoma on histopathological examination.
- Patients willing to provide informed written consent to participate in the study.

Exclusion Criteria

- Patients with oropharyngeal carcinoma who had previously received any form of cancer treatment (chemotherapy, radiotherapy, surgery, immunomodulation, etc.).
- Patients unwilling or unable to provide informed written consent.

Sample Size

Hospital records indicated 50, 47, and 55 cases of oropharyngeal carcinoma in the years 2019, 2020, and 2021, respectively, yielding a total of 152 cases (≈4.1 cases per month). Based on this incidence, the expected number of cases over 18 months was calculated to be 75 patients, which constituted the study sample size.

Sampling Technique

A time-frame census sampling technique was employed. All patients with oropharyngeal carcinoma who attended the Department of Otolaryngology during the study period and fulfilled the inclusion and exclusion criteria were enrolled consecutively.

Study Tools

Patient information was recorded using a structured proforma, and tissue biopsy specimens served as the primary material for analysis. Standard histopathological processing was performed using 10% neutral buffered formalin, ethanol, xylene, paraffin wax, and related laboratory consumables. Haematoxylin and eosin (H&E) staining was carried out on formalin-fixed, paraffin-embedded tissue blocks using hematoxylin solution (Harris), eosin Y solution 1%, acid alcohol, alkaline water for bluing, glass slides, an incubator, and staining jars.

For viral DNA purification, appropriate biosafety measures were followed, including

the use of protective equipment and QIAcube HT instrumentation, along with reagents such as proteinase K and isopropanol/ ethanol.

Nested PCR was performed using a thermal cycler, electrophoresis unit, gel documentation system, and PCR consumables (agarose, primers, nuclease-free water, and master mix). Standard molecular biology equipment, such as pipettes, a vortex mixer, a thermocycler, and a hot-plate magnetic stirrer, was used to ensure accurate amplification and detection of HPV DNA.

Study Procedure

All eligible patients were provided with detailed information regarding the study objectives and procedures. Written informed consent was obtained prior to participation. A comprehensive history was recorded for each patient, including age, place of residence, socio-economic status, ethnicity, lifestyle habits, sexual practices, and HPV vaccination history. Clinical examination was performed in the Department of Otolaryngology (ENT), AGMC & GBPH, and all routine investigations were completed.

Biopsy specimens were collected in the Department of ENT. Each specimen was divided into two parts: one portion was fixed in 10% neutral buffered formalin and submitted to the Department of Pathology for histopathological examination, while the other portion was preserved in phosphate buffer solution at −20 °C and transferred to the Department of Microbiology (Viral Research and Diagnostic Laboratory) for molecular detection of HPV DNA.

METHODOLOGY

All clinically diagnosed cases of oropharyngeal carcinoma presenting to the Department of ENT were considered for inclusion, with strict adherence to the predefined inclusion and exclusion criteria.

Histopathological Processing

Tissue specimens obtained from biopsy were received in the Department of Pathology and processed according to standard histopathological protocols. The procedure involved fixation in 10% neutral buffered formalin, followed by sequential steps of dehydration, clearing, and infiltration. The tissues were then embedded in paraffin, and sections were prepared using microtomy.

Following deparaffinization with xylene, the slides were rehydrated through descending grades of alcohol.[13] One representative slide was stained with H&E for histopathological evaluation.

Diagnostic Classification

Histopathological diagnosis was established according to the World Health Organization (WHO) classification of oropharyngeal malignancies. Cases were categorised into:

- Squamous cell carcinoma, HPV-positive
- Squamous cell carcinoma, HPV-negative

All slides were independently reviewed by two pathologists, and a consensus diagnosis was recorded to ensure accuracy.

Molecular Analysis

A portion of the biopsy specimen collected in the Department of ENT was fixed in 10% neutral buffered formalin and forwarded to the Department of Pathology for histopathological evaluation. The remaining tissue sample was preserved in phosphate-buffered saline at −20 °C and subsequently transferred to the Department of Microbiology (Viral Research and Diagnostic Laboratory) for molecular detection of HPV DNA. Genomic DNA extraction and PCR were performed as per standard protocols.

Consent

Written informed consent was obtained from all study participants prior to enrolment. The consent forms were provided in the local languages (Bengali or Kokborok) or in English for those not conversant with the regional languages. Each form was duly signed by the respondents. Strict

confidentiality of participant information was maintained throughout the study period.

Funding
This research did not require any specific external funding support.

Data Management and Statistical Analysis
All collected data were systematically entered and analysed using SPSS version 30. The Chi-square test and Fisher-Freeman-Halton test were applied wherever appropriate to assess associations between categorical variables. A p-value <0.05 was considered statistically significant.

Ethical Considerations
The study protocol was reviewed and approved by the Institutional Ethics Committee of AGMC & GBPH (Ref. No. F.4 (6-13)/AGMC/Medical Education/IEC Approval/2022/18837). The research commenced only after formal written approval was obtained.

RESULTS

In the present study, 75 cases were included; 33 (44%) were from urban areas and 42 (56%) from rural settings. Among the urban participants, 11 (14.7%) tested positive for HPV, while 22 (29.4%) were negative. In contrast, only 4 (5.3%) of the rural participants were HPV-positive, whereas the majority, 38 (50.6%), were HPV-negative. Overall, HPV negativity was more prevalent across both groups (60/75, 80%), while HPV positivity accounted for 20% of the total cases (Figure 1).

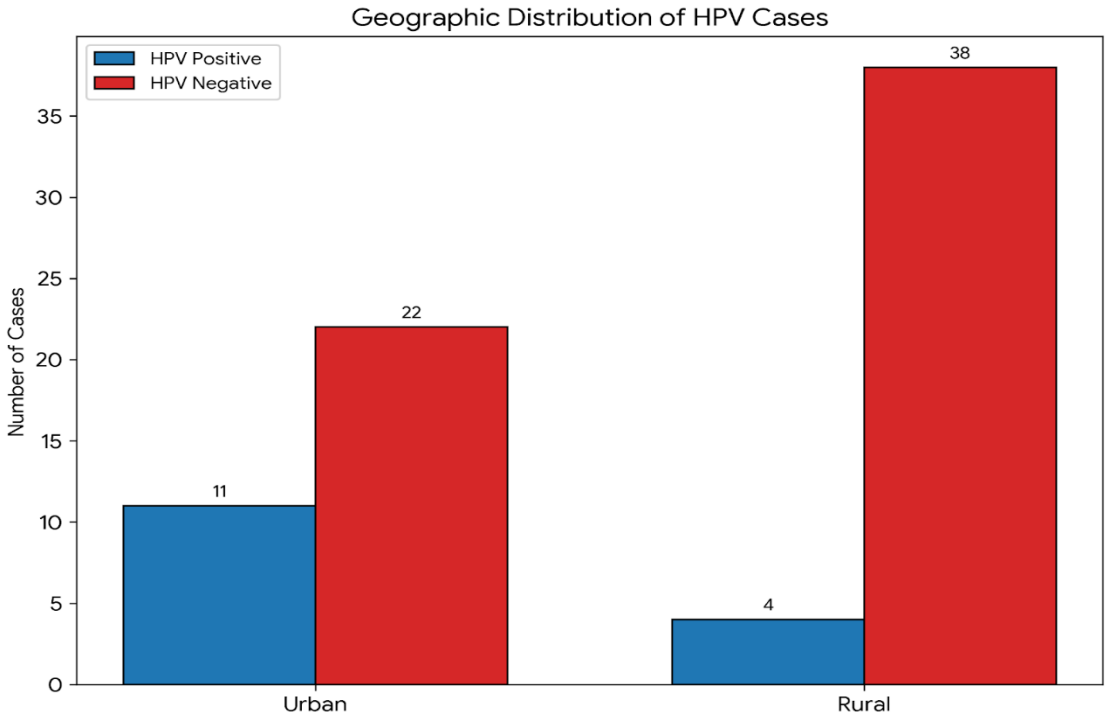

Figure 1: Distribution of HPV-Positive and HPV-Negative Cases by Residential Area (N=75)

Table 1 demonstrates the age distribution of the 75 study participants and their HPV status. The majority of cases were concentrated in the 50–69-year age range, which together accounted for more than half of the study population, while younger adults (20–39 years) and the elderly (70 years and above) comprised smaller proportions. Interestingly, HPV positivity was highest among the youngest group (20–29 years, 67%), despite their small sample size, whereas middle-aged groups (40–59 years) showed moderate positivity rates of 25–31%. In contrast, older participants (60 years and above) exhibited markedly lower HPV positivity, ranging from 0–11%. Overall, 15

cases (20%) were HPV-positive, and 60 cases (80%) were HPV-negative.

Table 1: Age Distribution of HPV Status (N=75)

Age Group	No. of cases	Percentage (%) of no. of cases	HPV Positive	HPV Negative
< 20 YEARS	0	0%	0(0%)	0(0%)
AGE 20-29	3	4.0%	2(67%)	1(33%)
AGE 30-39	10	13.3%	1(10%)	9(90%)
AGE 40-49	12	16.0%	3(25%)	9(75%)
AGE 50-59	19	25.3%	6(31%)	13(69%)
AGE 60-69	20	26.7%	2(10%)	18(90%)
AGE 70-79	9	12.0%	1(11%)	8(89%)
AGE 80-89	2	2.7%	0(0%)	2(100%)
TOTAL	**75**	**100**	**15**	**60**

Figure 2 outlines the sex distribution of the study participants and their HPV status. Of the total cases, males constituted the majority (69%, 52/75), while females accounted for 31% (23/75). Among males, 12 cases (23%) were HPV-positive and 40 (77%) were HPV-negative, whereas among females, 3 (13%) were HPV-positive and 20 (87%) were HPV-negative.

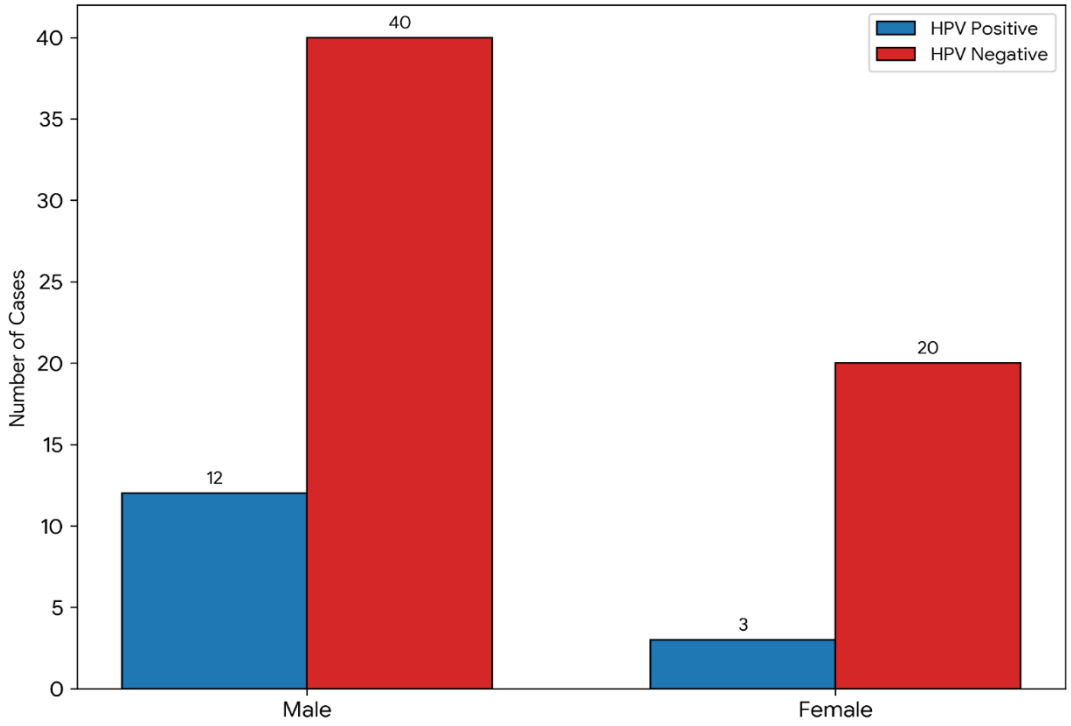

Figure 2: Sex Distribution and HPV Status (N=75)

Figure 3 presents the distribution of the study participants by religion and HPV status. The majority of cases were Hindu (76%, 57/75), among whom 10 (18%) were HPV-positive and 47 (82%) were HPV-negative. Muslims accounted for 9% (7/75) and had a relatively high positivity rate of 43% (3/7). Christians comprised 12% (9/75), showing 22% positivity (2/9), while Buddhists represented the smallest group (3%, 2/75), all of whom were HPV-negative.

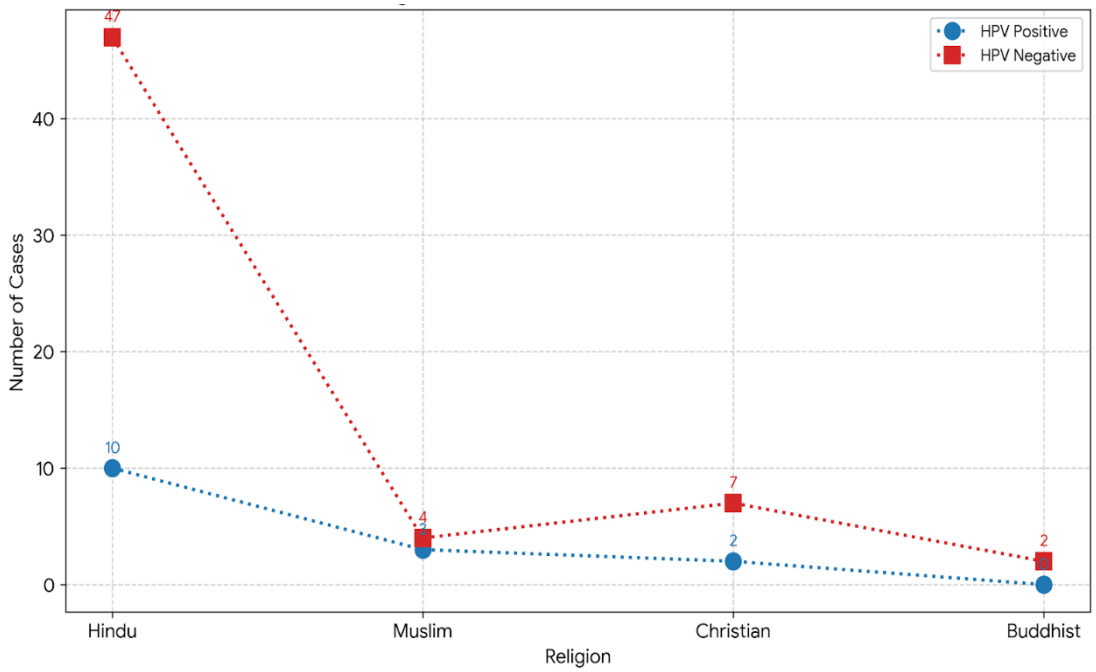

Figure 3: Distribution of HPV Status According to Religious Distribution

In Table 2, the distribution of HPV status across socioeconomic strata is presented for 75 participants. The majority belonged to the lower-middle class (51%), followed by the lower class (22%), middle class (15%), upper-middle class (8%), and upper class (4%). HPV positivity was highest among the middle class (36%) and upper/upper middle strata (33% each), while lower middle and lower strata showed comparatively lower positivity rates (16% and 13%, respectively). Overall, 15 participants (20%) were HPV-positive and 60 (80%) were HPV-negative. To assess the association between socioeconomic status and HPV infection, Fisher-Freeman-Halton test was applied, yielding a p-value of 0.32 with 4 degrees of freedom.

Table 2: HPV Status Across Different Socioeconomic Strata (N=75)

	Total no. of cases	%	HPV Positive	%	HPV Negative	%	Degrees of freedom	p-value
Upper	3	4%	1	33%	2	67%	4	0.32
Upper Middle	6	8%	2	33%	4	67%		
Middle	11	15%	4	36%	7	64%		
Lower Middle	38	51%	6	16%	32	84%		
Lower	16	22%	2	13%	14	87%		
Total	75	100%	15		60			

Figure 4 illustrates the distribution of study participants according to the number of sexual partners and their HPV status. The majority reported having a single partner (74.7%, 56/75), while a smaller proportion had multiple partners (25.3%, 19/75). HPV positivity was significantly higher among those with multiple partners, with 7 cases (37%) testing positive compared to 12 (63%) testing negative. In contrast, among participants with a single partner, only 8 cases (14%) were HPV-positive, while 48 (86%) were HPV-negative. The chi-square test yielded a p-value of 0.033, indicating statistical significance.

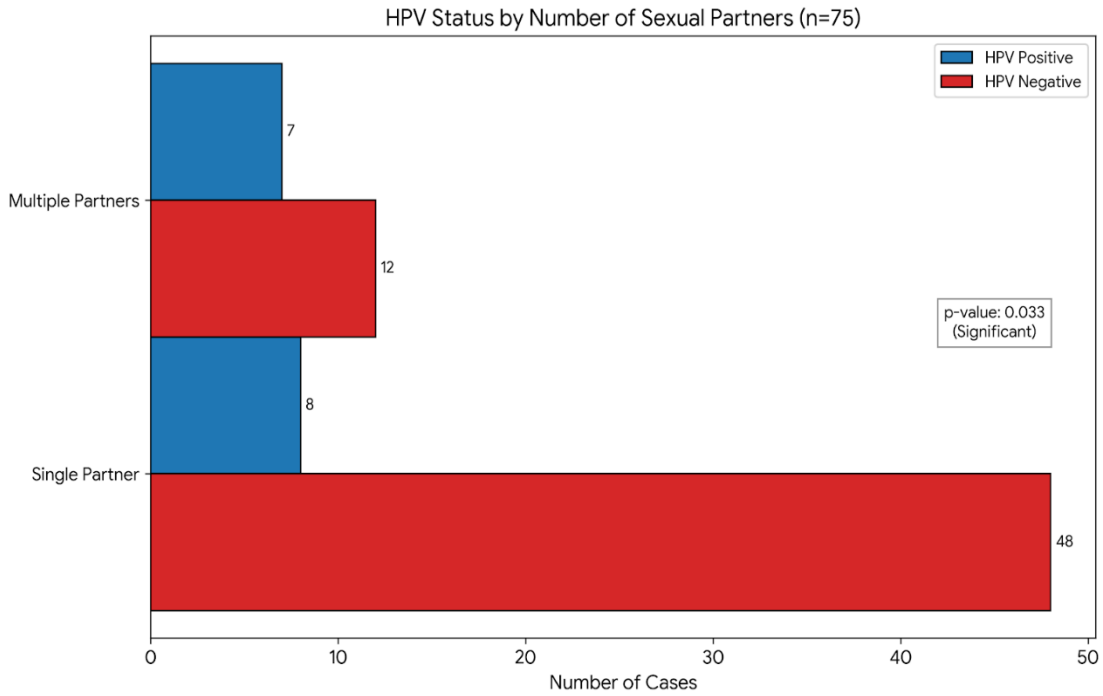

Figure 4: Distribution of Sexual Partners and HPV Status

Table 3 presents the distribution of HPV status according to smoking habit and sex among 75 participants. Among male smokers (41% of the cohort), 7 were HPV-positive and 24 HPV-negative, while male non-smokers (28%) showed 5 positives and 16 negatives. In females, 2 of 9 smokers were HPV-positive compared to 7 negatives, whereas only 1 of 14 non-smokers tested positive, with 13 negatives. The calculated odds ratios suggest that male smokers had nearly the same risk of HPV infection as male non-smokers (OR = 0.93, 95% CI: 0.25–3.46, p = 0.91), while female smokers appeared to have a higher relative risk compared to female non-smokers (OR = 3.71, 95% CI: 0.28–8.54, p = 0.53). However, both p-values are well above 0.05, indicating that these differences were not statistically significant.

Table 3: Distribution of HPV Status by Smoking Habit and Sex (N=75)

	HPV Positive	**HPV Negative**	**TOTAL**	**Odds ratio (95% CI)**	**p-value**
Male (smoker)	7(9.3%)	24(32%)	31(41%)	0.93 (0.25- 3.46)	0.91
Male (non-smoker)	5(6.7%)	16(21%)	21(28%)		
Female (smoker)	2(2.6%)	7(9%)	9(12%)	3.71 (0.28 – 8.54)	0.53
Female (non-smoker)	1(1.4%)	13(18%)	14(19%)		
Total	15	60	75		

Table 4 summarises the distribution of HPV cases in relation to alcohol consumption among the study population. Out of 75 participants, 27 (36%) reported consuming alcohol, among whom 6 (22%) were HPV-positive and 21 (78%) were HPV-negative. The remaining 48 participants (64%) did not consume alcohol, with 9 (19%) HPV-positive and 39 (81%) HPV-negative. The chi-square test value of 0.13 with a p-value of 0.71 indicates that there was no statistically significant association between alcohol consumption and HPV infection.

Table 4: Association of Alcohol Consumption with HPV Status in the Study Population (N=75)

	Total no. of cases	HPV Positive	HPV Negative	χ^2 value	p-value
Alcohol Consumed	27(36%)	6(22%)	21(78%)	0.13	0.71
Alcohol Not Consumed	48(64%)	9(19%)	39(81%)		
Total	75	15	60		

Table 5 summarises the clinical presentations of oropharyngeal carcinomas in the study population and their association with HPV status. The predominant presentation was Ulceroproliferative growth, observed in 30 cases (40%). Among these, only 4 cases (5.2%) were HPV-positive, while the majority, 26 cases (34.7%), were HPV-negative. The second most frequent presentation was proliferative growth, accounting for 24 cases (32%), with a relatively higher HPV positivity (7 cases, 9.6%) compared to 16 HPV-negative cases (21.5%).

Ulcerative lesions were seen in 12 cases (16%), of which 2 (2.6%) were HPV-positive and 10 (13.9%) were HPV-negative. Patch lesions were less common, comprising 7 cases (9.3%), with only 1 case (1.5%) HPV-positive and 6 (8%) HPV-negative. The least frequent presentation was infiltrative growth, noted in 2 cases (2.7%), with equal distribution between HPV-positive and HPV-negative cases (1 case each, 1.5%).

Table 5: Clinical Presentations of Oropharyngeal Carcinomas and HPV Status in the Study Cohort

Presentation	Total no. of case n (%)	HPV Positive n (%)	HPV Negative n (%)
Ulceroproliferative Growth	30(40%)	4(5.2%)	26(34.7%)
Proliferative Growth	24(32%)	7(9.6%)	16(21.5%)
Patch	7(9.3%)	1(1.5%)	6(8%)
Ulcerative Lesion	12(16%)	2(2.6%)	10(13.9%)
Infiltrative Growth	2(2.7%)	1(1.5%)	1(1.5%)

Table 6 presents the distribution of primary tumour sites among patients with oropharyngeal carcinoma, stratified by HPV status. The base of the tongue was the most common site, accounting for 41 cases (55%), with 4 cases (5.8%) HPV-positive and 37 cases (50%) HPV-negative. The tonsil was the second most frequent site, observed in 18 cases (24%), of which 7 (9.6%) were HPV-positive and 11 (15%) HPV-negative.

The soft palate contributed 10 cases (13%), with 2 HPV-positive (2.7%) and 8 HPV-negative (11%). The least common site was the lateral wall of the pharynx, comprising 6 cases (8%), with 2 HPV-positive (2.7%) and 4 HPV-negative (5.8%).

Table 6: Distribution of Primary Tumour Sites and HPV Status in the Study Population

Primary Site	HPV Positive	HPV Negative	Total no. of cases	Percentage
Base of Tongue	4(5.8%)	37(50%)	41	55%
Soft Palate	2(2.7%)	8(11%)	10	13%
Tonsil	7(9.6%)	11(15%)	18	24%
Lateral wall of the pharynx	2(2.7%)	4(5.8%)	6	8%

Table 7 outlines the histopathological spectrum of oropharyngeal carcinomas in the study cohort and their HPV status. The overwhelming majority of cases were diagnosed as squamous cell carcinoma (SCC), comprising 71 cases (95%). All 15 HPV-positive cases (100%) fell within this category, while 56 cases (93%) were HPV-negative.

Other histological variants were rare. Verrucous carcinoma was identified in only 1 case (1%), which was HPV-negative. Squamous carcinoma in situ accounted for 2 cases (3%), both of which were HPV-

negative. Similarly, severe dysplasia was observed in 1 case (1%), also HPV-negative.

Table 7: Histological Diagnosis of Oropharyngeal Carcinomas and HPV Status in the Study Population

	Total no. of Cases n (%)	HPV Positive n (%)	HPV Negative n (%)
SCC	71(95%)	15(100%)	56(93%)
Verrucous Carcinoma	1(1%)	0(0%)	1(2%)
Squamous Carcinoma in-situ	2(3%)	0(0%)	2(3%)
Severe Dysplasia	1(1%)	0(0%)	1(2%)

Table 8 details the histological grading of oropharyngeal carcinomas across different primary sites and their HPV status. The majority of cases were moderately differentiated carcinomas, comprising 52 cases (72%). Within this group, HPV positivity was distributed across sites: 6 cases in the tonsil, 2 in the soft palate, 4 in the base of the tongue, and 1 in the lateral pharyngeal wall, while the remainder were HPV-negative.

Well-differentiated carcinomas accounted for 19 cases (26%), with HPV positivity observed in 1 tonsillar case and 1 lateral pharyngeal wall case. The rest were HPV-negative, predominantly involving the base of the tongue and soft palate. Only 1 case (1%) was classified as poorly differentiated carcinoma and was HPV-negative.

Table 8: Histological Grading of Oropharyngeal Carcinomas and HPV Status by Primary Site

Histological Grade	Total	Tonsil (HPV+VE)		Soft Palate (HPV +VE)		Base of Tongue (HPV +VE)		Lateral wall of the pharynx (HPV +VE)		Total
		HPV +	HPV -	HPV +	HPV -	HPV +	HPV -	HPV +	HPV -	
Moderately Differentiated	52	6	7	2	6	4	24	1	2	52
Well-Differentiated	19	1	3	0	2	0	10	1	2	19
Poorly Differentiated	1	0	1	0	0	0	0	0	0	1
Total	72	18		10		38		6		72

Figures 5–7 illustrate the histopathological spectrum of SCC. Figure 5 depicts a well-differentiated squamous cell carcinoma with a characteristic squamous pearl, observed under light microscopy at ×400 magnification with H&E staining. Figure 6 shows a pictomicrograph of a moderately differentiated SCC, where nests of neoplastic squamous cells with hyperchromatic nuclei (red arrow) are evident at ×100 magnification (H&E). Figure 7 highlights a poorly differentiated SCC, characterised by bizarre nuclear morphology (red arrow) and frequent mitotic figures (black arrow), seen at ×400 magnification (H&E). The inset further demonstrates immunohistochemical staining, revealing cytoplasmic cytokeratin (CK) positivity in neoplastic squamous cells (blue arrow) with Pan-CK at ×400 magnification.

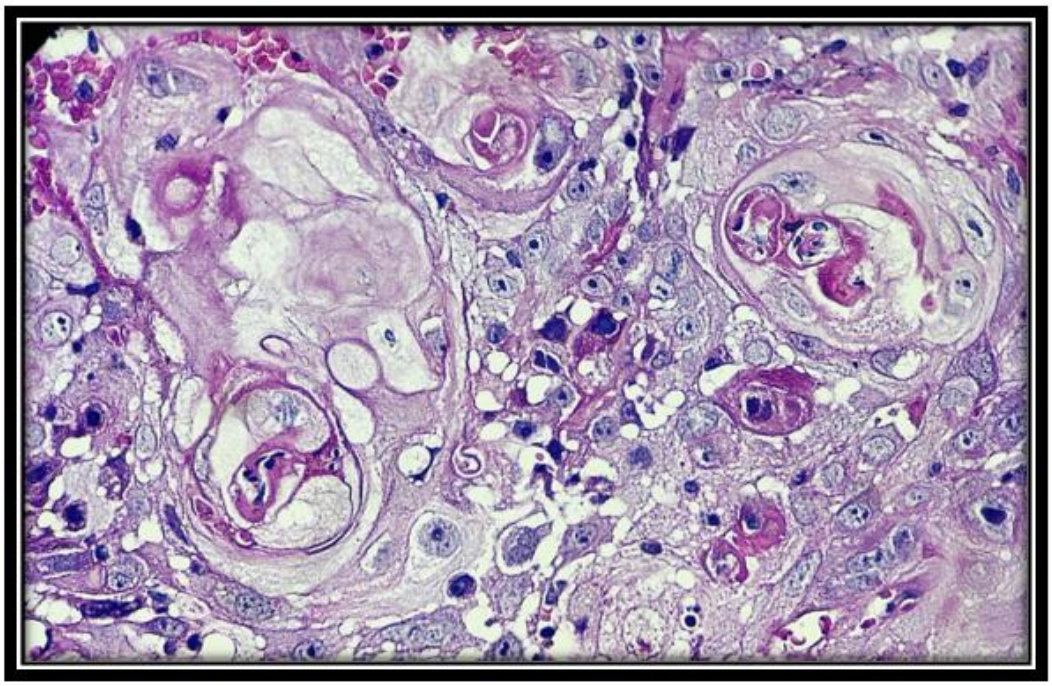

Figure 5: Light microscopy showing a squamous pearl in a well-differentiated squamous cell carcinoma (H&E), 400x magnification.

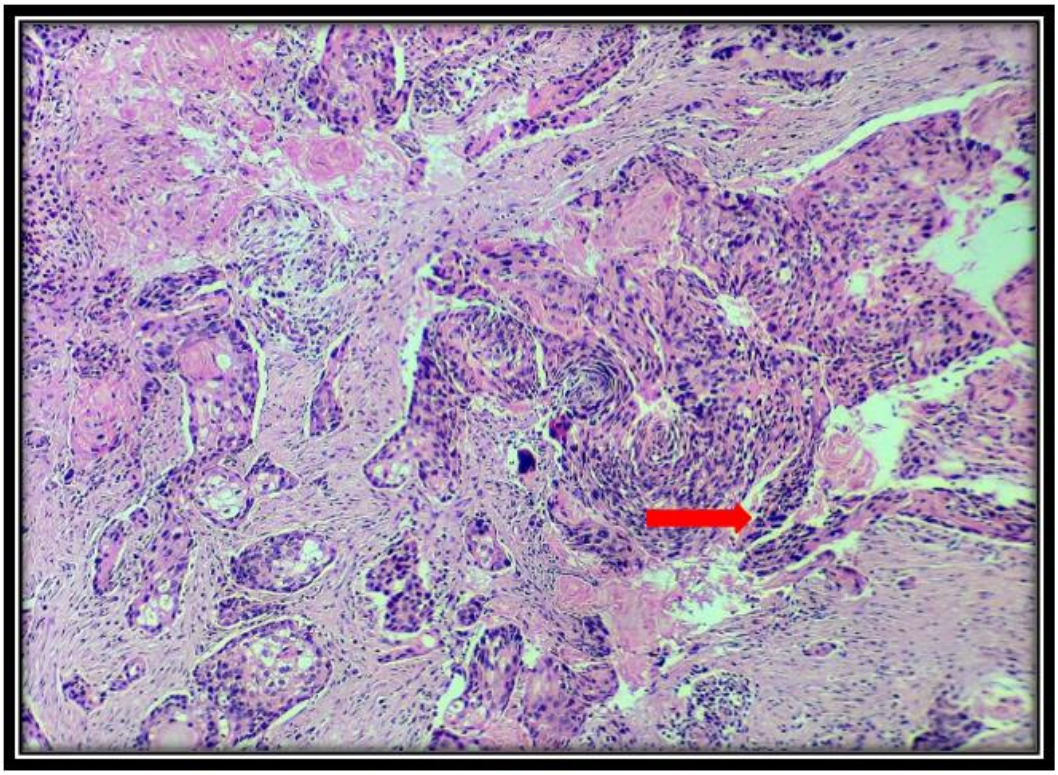

Figure 6: Pictomicrograph of moderately differentiated squamous cell carcinoma showing neoplastic squamous cells with a hyperchromatic nucleus (red arrow) arranged in nests (H&E), 100x magnification.

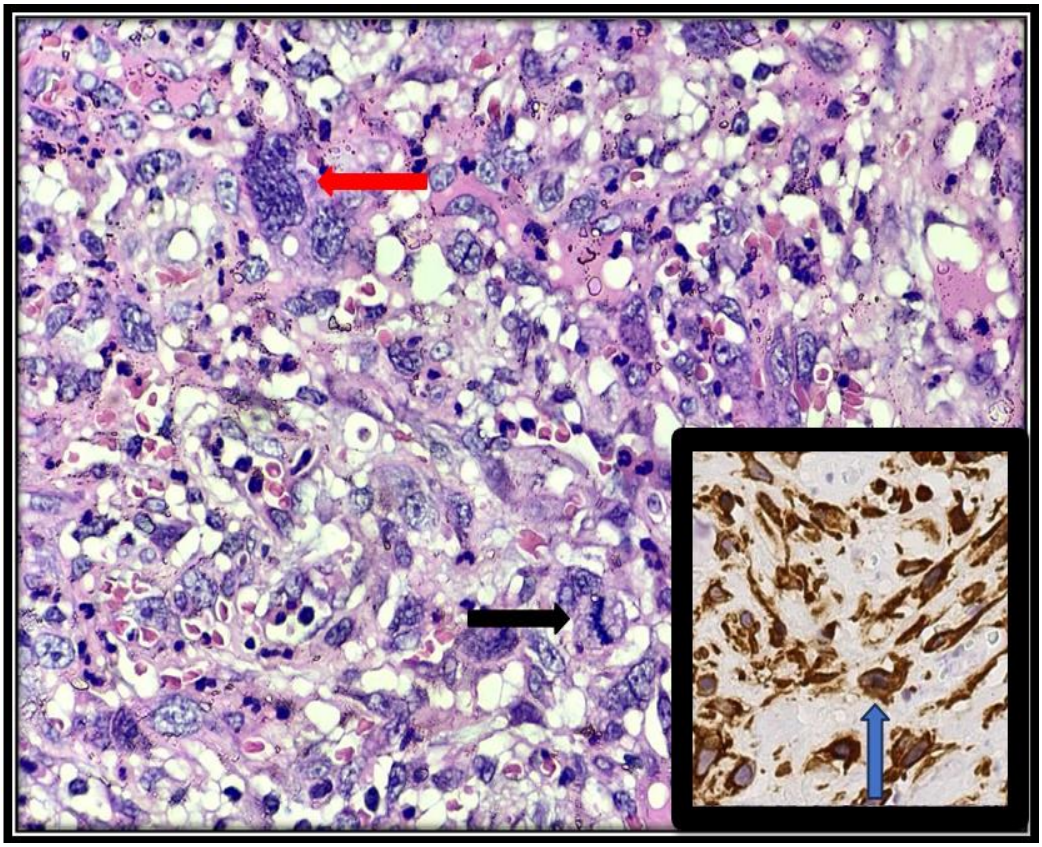

Figure 7: Poorly differentiated squamous cell carcinoma exhibiting pleomorphic nuclei (red arrow) with frequent mitotic figures (black arrow) on H&E staining at 400x magnification. The inset shows an immunohistochemical analysis of cytoplasmic cytokeratin positivity in neoplastic squamous cells (blue arrow) using PanCK at 400x magnification.

DISCUSSION

This study was undertaken to determine the association of HPV DNA in oropharyngeal carcinoma biopsies and assess its association with demographic, lifestyle, and clinical variables.

Epidemiological Significance of HPV in Oropharyngeal Carcinoma

The present study demonstrated that 20% of OPSCC cases were HPV-positive, with a statistically significant association between multiple sexual partners and HPV infection ($p = 0.033$). This finding aligns with recent epidemiological reports indicating that sexual behaviour, particularly oral sex and multiple partners, is a critical determinant of HPV transmission in oropharyngeal cancers.[14] Younger patients (20–29 years) exhibited the highest HPV positivity (67%), suggesting that early sexual exposure and lifestyle practices may contribute to the rising burden of HPV-associated OPSCC in India. Comparable age-related trends have been documented in global cohorts, with HPV positivity peaking among younger adults.[15]

Clinical and Prognostic Implications

HPV-positive OPSCC is increasingly recognised as a distinct clinical entity with better prognosis compared to HPV-negative tumours. In our cohort, HPV positivity was most frequently observed in tonsillar (9.6%) and base-of-tongue (5.8%) carcinomas, consistent with international evidence that these sites are highly susceptible to HPV-driven oncogenesis.[16] Importantly, HPV-positive tumours are known to respond more favourably to radiotherapy and chemotherapy, prompting ongoing trials of treatment de-escalation to minimise toxicity while maintaining survival outcomes.[17] These findings underscore the need for routine HPV testing in diagnostic workflows to guide personalised treatment strategies.

Clinical Presentations and HPV Association

In our cohort, ulceroproliferative growth was the most common clinical presentation (40%), followed by proliferative growth (32%). Interestingly, HPV positivity was relatively higher in proliferative lesions (9.6%) compared to ulceroproliferative growths (5.2%). This observation is consistent with recent reports that HPV-positive OPSCC often presents with exophytic or proliferative lesions, whereas HPV-negative tumours more frequently manifest as ulcerative or infiltrative growths.[18] Recognising these distinct clinical patterns may aid in early suspicion of HPV-driven disease and guide diagnostic testing.

Lifestyle Factors and HPV Association

Although smoking and alcohol consumption are established risk factors for OPSCC, our study did not demonstrate statistically significant associations between these habits and HPV positivity. This observation supports the growing consensus that HPV-positive OPSCC arises through viral oncogenesis rather than mutagenic damage from tobacco or alcohol.[19] Nevertheless, the slightly higher HPV positivity among smokers (11.6%) compared to nonsmokers (8.1%) suggests that lifestyle factors may still modulate susceptibility, warranting further investigation in larger cohorts.

Public Health Implications

The findings highlight the urgent need to strengthen HPV vaccination programs in India. Despite the proven efficacy of gender-neutral vaccination in preventing oral HPV infections,[20] coverage remains limited, particularly in northeastern states. Public health campaigns should emphasise the role of sexual behaviour in HPV transmission and promote vaccination among both sexes. Furthermore, integrating molecular diagnostics, such as PCR-based HPV DNA detection and p16 immunohistochemistry, into routine practice will improve surveillance and enable early identification of HPV-associated OPSCC.

Comparison with Global Trends

Globally, HPV-positive OPSCC has surpassed HPV-negative OPSCC in incidence, particularly in the United States and Europe.[21] While India reports lower attribution rates (15–23%), regional variations are evident, with northeastern states showing increasing HPV prevalence. Our study contributes to this growing body of evidence, confirming that HPV is a significant etiological factor in OPSCC in northeastern India. These results reinforce the importance of region-specific surveillance and targeted interventions to address the evolving epidemiology of head and neck cancers.

Study Limitations

- **Sample size constraint:** The study included 75 patients, which may limit generalizability to the wider population.
- **Single-centre design:** Conducted in one tertiary hospital; findings may not reflect regional variations across northeastern India.
- **Cross-sectional nature:** The design precludes causal inference; associations observed cannot establish temporal relationships.
- **Limited molecular profiling:** Only nested PCR was used for HPV DNA detection; additional methods (e.g., genotyping, viral load quantification) could provide deeper insights.
- **Vaccination history underreported:** Self-reported vaccination status may be subject to recall bias, limiting accurate assessment of preventive coverage.

CONCLUSION

This study establishes that HPV infection is a significant contributor to OPSCC in northeastern India, with sexual behaviour emerging as a key determinant. The distinct clinical profile and better prognosis of HPV-positive tumours highlight the importance of routine HPV testing and

tailored treatment strategies. Strengthening vaccination coverage, raising awareness, and integrating molecular diagnostics are essential steps to reduce the burden of HPV-associated OPSCC in India.

Declaration by Authors

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